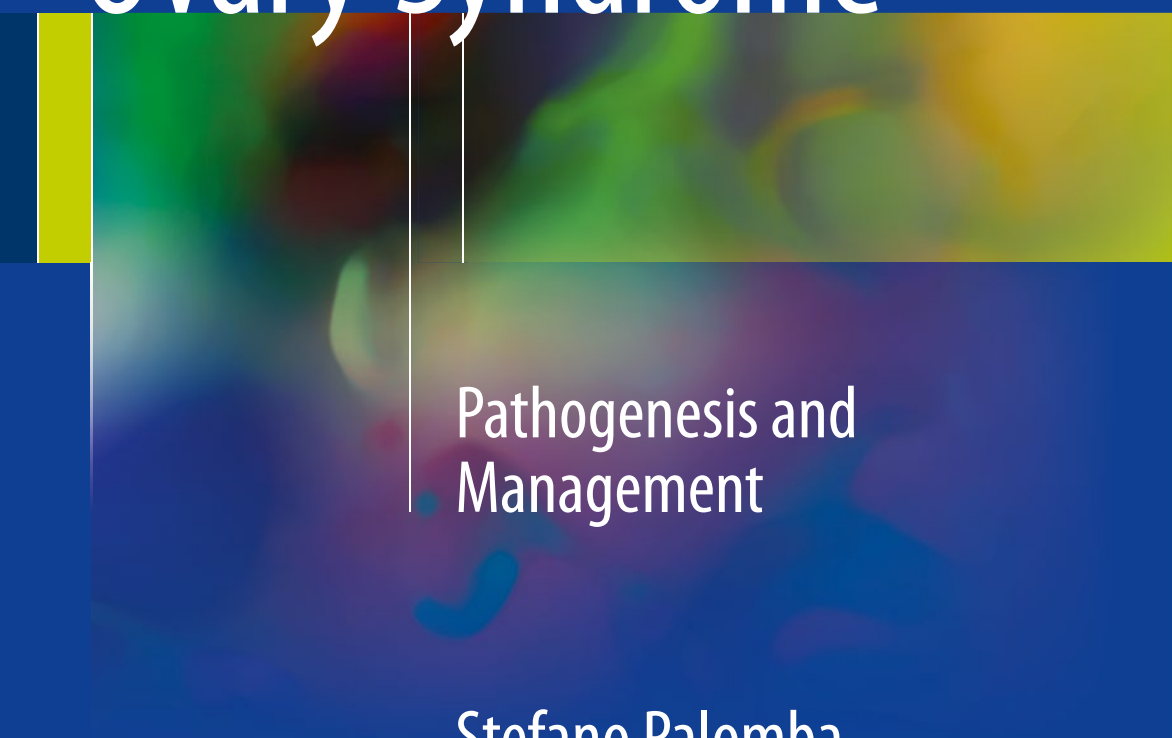
Infertility in Women with Polycystic Ovary Syndrome



Pathogenesis and Management

Stefano Palomba
Editor

Springer

Infertility in Women with Polycystic Ovary Syndrome

Stefano Palomba
Editor

Infertility in Women with Polycystic Ovary Syndrome

Pathogenesis and Management

Springer

Editor
Stefano Palomba
Unit of Reproductive Medicine and Surgery
ASMN-IRCCS of Reggio Emilia
Reggio Emilia
Italy

ISBN 978-3-319-45533-4 ISBN 978-3-319-45534-1 (eBook)
https://doi.org/10.1007/978-3-319-45534-1

Library of Congress Control Number: 2017955842

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Foreword

Dr. Palomba has done an outstanding job assimilating an exceptionally well-qualified and talented cohort of authors to write on the subject of fertility in women with polycystic ovarian syndrome (PCOS), which constitutes one of the most common endocrinopathies of reproductive aged women. The information provided is the most current evidence regarding the diagnosis and treatment of infertility in women with PCOS. In addition, there is in-depth discussion on new insights into the pathophysiology of this disease.

As so eloquently stated by Dr. Hatem Abu Hashim, who authored a chapter of this book outlining the role of laparoscopic ovarian drilling in women with PCOS, "Science, practice, and evidence are dynamic processes. A marvelous progress in the understanding of the pathophysiology and metabolic features of PCOS has been witnessed in the last two decades." As our understanding of this disease has evolved, so too has our clinical definitions and strategies for the treatment of infertility in patients affected with PCOS.

The first several chapters of the book are focused on providing analysis of the diagnostic criteria and pathophysiology underlying infertility associated with PCOS. These chapters include "Diagnostic criteria for PCOS" by Dr. Francesco Orio; "Anovulation in women with PCOS" by Dr. Ujvala Rao; "Oocyte quality in PCOS" by Dr. Christine Decanter; "Endometrial receptivity in PCOS" by Dr. Giuseppe Benagiano; and "Infertility and subfertility cofactors in women with PCOS" by Dr. Tal Shavit. These chapters are comprehensive, very well written, and provide a wealth of knowledge to readers interested in gaining a better understanding of the complexity of mechanisms underlying infertility in PCOS as well as the health implications associated with it. The subsequent chapters are extremely thorough with in-depth discussions on medical, surgical, and alternative treatment strategies of infertility in women with PCOS. These in turn are followed by a chapter, prepared by Dr. Coghlan, which distills down all the important information mentioned in the prior chapters. He does an excellent job describing evidence-based integrated strategies for enhancing fertility in PCOS.

Another very interesting chapter, written by Dr. John Nestler, discusses current evidence of inositol treatment and its role in improving fertility outcomes in women with PCOS. Dr. Nestler begins this chapter by outlining the intricate mechanisms underlying dysregulation of inositols in PCOS and the resulting consequences on insulin resistance, glucose uptake, ovarian androgen production, and glycogen

synthesis. This is followed by a detailed discussion on the metabolic and reproductive benefits of myo-inositol and D-Chiro-inositol treatment, alone or in combination, in PCOS.

Women with PCOS are at higher risk for developing ovarian hyperstimulation syndrome (OHSS). Dr. Melanie Walls, in her chapter "In vivo maturation" (IVM), introduces the utility of IVM for patients with PCOS, which can eliminate the potential risk of OHSS. She then very thoroughly summarizes different protocols and treatment regimens, as well as hormonal priming and culture conditions utilized in IVM, with a specific focus on their clinical outcomes.

Once conception occurs, adverse pregnancy and perinatal outcomes are more common in women with PCOS. These complications and their underlying pathophysiology are discussed in the final chapter "Complications of pregnancy" by Dr. Palomba.

To conclude, Dr. Palomba's book is an excellent contribution to our understanding of the complexities underlying the pathophysiology, diagnosis, and treatment of infertility in women with PCOS. The text is well organized and will serve as an excellent resource for both clinician and researcher alike.

Anthony M. DeAngelis
Resident Physician in Obstetrics and Gynecology
Danbury Hospital – Western Connecticut Health Network
24 Hospital Avenue, Danbury, CT, 06810, USA

Alan H. DeCherney, MD
Head Program in Reproductive and Adult Endocrinology
Eunice Kennedy Shriver National Institute of Child Health
and Human Development, National Institutes of Health
10 Center Drive, Bldg10, CRC, Rm 1-3140
Bethesda, MD, 20892, USA

Preface

Although the study of polycystic ovary syndrome (PCOS) was "my topic" for more than 15 years, the idea to write a book about infertility in women with PCOS came to me during 2015 when, participating in many meetings, courses, and congresses on the treatment of infertility, I realized that there was a lack of awareness of the syndrome among medical staff specialized in reproductive medicine, particularly with regard to assisted reproduction technologies (ARTs).

At first glance, the current book could be considered to consist merely of evidence-based guidance about the pathogenesis of infertility in women with PCOS and its treatment. However, it should not only be considered a technical tool to employ in clinical practice, but also as a cultural basis for approaching and understanding the new and future basic and clinical studies on infertility related to PCOS.

Anovulation, oocyte quality, and endometrial competence in women with PCOS are discussed in depth, along with almost all aspects of the infertility and subfertility cofactors potentially present in infertile patients with PCOS, including the impact and the interaction of PCOS phenotypes with regard to the reproductive outcome. From a therapeutic point of view, the book includes chapters on the classical medical treatments for treating PCOS-related ovulatory dysfunction (such as clomiphene citrate, letrozole, metformin, and gonadotrophins), in addition to new and potential therapeutic approaches, such as natural insulin sensitizers (i.e., inositol), acupuncture, dietary supplements, and traditional Chinese medicine. Strong emphasis is placed on the nonpharmacological approach (i.e., diet and physical activity), which is crucial for obese and overweight patients, and on the use of a more invasive approach, including controlled ovarian stimulation for in vitro fertilization with or without in vitro maturation of oocytes. Significant effort has been made to clarify that reproductive success can be achieved not by evaluating the available treatments individually, but as a concert of options to modulate in specific strategies tailored to patient characteristics.

Finally, the acknowledgements. I would like to thank all the authors who agreed to participate in the preparation of the chapters for the immeasurable help they gave me, for the many things they taught me, and for the patience they have had in following my comments and suggestions. I would also like to thank my family, and especially my son Francesco, whom I have denied so much precious time.

Reggio Emilia, Italy — Stefano Palomba

Contents

Part I Diagnosis, Pathophysiology, and Pathogenesis

1 Introduction 3
Stefano Palomba

2 Diagnostic Criteria for PCOS 11
Francesco Orio and Giovanna Muscogiuri

3 Anovulation in Women with PCOS 23
Ujvala Rao and Roy Homburg

4 Oocyte Quality in PCOS 31
Christine Decanter

5 Endometrial Receptivity in PCOS 41
Giuseppe Benagiano, Paola Bianchi, and Ivo Brosens

6 Infertility and Subfertility Cofactors in Women with PCOS 63
Tal Shavit and Togas Tulandi

7 PCOS Phenotypes: Impact on Fertility 81
Enrico Carmina

8 Follicle Excess and Abnormalities in Women with PCOS: Pathophysiology, Assessment and Clinical Role 89
Agathe Dumont, Pauline Plouvier, and Didier Dewailly

Part II Medical Treatments

9 Antiestrogens 109
Richard S. Legro

10 Aromatase Inhibitors 119
Nivin Samara and Robert F. Casper

11 Insulin-Sensitising Drugs 135
Stefano Palomba, Angela Falbo, and Giovanni Battista La Sala

12 Gonadotrophins 153
Sophie Christin-Maitre

Part III Lifestyle Management and Other Treatment Approaches

13 Lifestyle Interventions and Natural and Assisted Reproduction in Patients with PCOS 169
Renato Pasquali

14 Dietary Supplements, Phytotherapy and Chinese Herbal Medicine in PCOS 181
Xiao-Ke Wu and Ernest HY Ng

15 Laparoscopic Ovarian Drilling 195
Hatem Abu Hashim

16 Inositols 213
John E. Nestler and Antonio Simone Laganà

17 Acupuncture 227
Elisabet Stener-Victorin, Anna Benrick, Romina Fornes, and Manuel Maliqueo

Part IV Controlled Ovarian Stimulation and In Vitro Oocyte Maturation

18 Intrauterine Insemination 249
Madelon van Wely

19 Controlled Ovarian Stimulation for In Vitro Fertilisation Cycles 259
Raoul Orvieto

20 In Vitro Oocyte Maturation 271
Melanie L. Walls

Part V Integrated Strategies, Complications of Pregnancy, and Outlook

21 Integrated Strategies for Enhancement of Fertility in PCOS 289
Edwina Coghlan and Roger J. Hart

22 Complications of Pregnancy 305
Stefano Palomba and Bart C.J.M. Fauser

23 Conclusive Remarks and Future Perspectives 325
Stefano Palomba

Index 331

Contributors

Giuseppe Benagiano Department of Obstetrics, Gynaecology and Urology, Sapienza, University of Rome "La Sapienza", Rome, Italy

Anna Benrick Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

School of Health and Education, University of Skövde, Skövde, Sweden

Paola Bianchi Department of Medico-Surgical Sciences and Translational Medicine, Sant'Andrea Hospital, Faculty of Medicine and Psychology, University of Rome "La Sapienza", Rome, Italy

Ivo Brosens Faculty of Medicine, Catholic University of Leuven, Leuven, Belgium

Enrico Carmina Department of Health Sciences and Mother and Child Care, University of Palermo, Palermo, Italy

Robert F. Casper Division of Reproductive Sciences, University of Toronto, Toronto, ON, Canada

Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada

Toronto Centre for Advanced Reproductive Technology Fertility Partners, Toronto, ON, Canada

Sophie Christin-Maitre Reproductive Endocrine Unit, Hôpital St. Antoine, AP-HP, University Pierre and Marie Curie, Paris, France

Unité INSERM U933, Paris, France

Edwina Coghlan King Edward Memorial Hospital, Perth, WA, Australia

Christine Decanter Centre d'Assistance Médicale à la Procréation et de Préservation de la Fertilité, Hôpital Jeanne de Flandre, EA 4308 "Gamétogénèse et Qualité du Gamète", Centre Hospitalier Universitaire de Lille, Lille, France

Didier Dewailly Service de Gynécologie Endocrinienne et de Médecine de la Reproduction, Hôpital Jeanne de Flandre, Lille, France

Agathe Dumont Service de Gynécologie Endocrinienne et de Médecine de la Reproduction, Hôpital Jeanne de Flandre, CHRU Lille, Lille, France

Angela Falbo Unit of Gynaecology and Obstetrics, IRCCS–Arcispedale Santa Maria Nuova, Reggio Emilia, Italy

Bart C.J.M. Fauser Department of Reproductive Medicine and Gynecology, University Medical Center Utrecht, Utrecht, The Netherlands

Romina Fornes Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Roger J. Hart School of Women's and Infants' Health, University of Western Australia, Perth, WA, Australia

Bethesda Hospital, Perth, WA, Australia

School of Women's and Infants' Health, King Edward Memorial Hospital, Perth, WA, Australia

Hatem Abu Hashim Department of Obstetrics and Gynaecology, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Roy Homburg Homerton Fertility Centre, Homerton University Hospital NHS Foundation Trust, London, UK

Antonio Simone Laganà Unit of Gynecology and Obstetrics, Department of Human Pathology in Adulthood and Childhood "G. Barresi", University of Messina, Messina, Italy

Richard S. Legro Department of Obstetrics and Gynecology and Public Health Sciences, Penn State College of Medicine, Hershey, PA, USA

Manuel Maliqueo Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Endocrinology and Metabolism Laboratory, West Division, School of Medicine, University of Chile, Santiago, Chile

Giovanna Muscogiuri Ios and Coleman Medicina Futura Medical Center, Naples, Italy

John E. Nestler Department of Internal Medicine, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA, USA

Ernest HY Ng Department of Obstetrics and Gynaecology, First Affiliated Hospital, Heilongjiang University of Chinese Medicine, Harbin, China

Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong, China

Francesco Orio Department of Sports Science and Wellness, "Parthenope" University Naples, Naples, Italy

Raoul Orvieto Department of Obstetrics and Gynecology, Chaim Sheba Medical Center (Tel Hashomer), Ramat Gan, and Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Infertility and IVF Unit, Department of Obstetrics and Gynecology, Chaim Sheba Medical Center (Tel Hashomer), Ramat Gan, Israel

Stefano Palomba Unit of Gynaecology and Obstetrics, IRCCS–Arcispedale Santa Maria Nuova, Reggio Emilia, Italy

Renato Pasquali Division of Endocrinology, Department of Medical and Surgical Sciences, University Alma Mater Studiorum, S. Orsola-Malpighi Hospital, Bologna, Italy

Pauline Plouvier Service de Gynécologie Endocrinienne et de Médecine de la Reproduction, Hôpital Jeanne de Flandre, CHRU Lille, Lille, France

Ujvala Rao Homerton Fertility Centre, Homerton University Hospital NHS Foundation Trust, London, UK

Giovanni Battista La Sala Unit of Gynaecology and Obstetrics, IRCCS–Arcispedale Santa Maria Nuova, Reggio Emilia, Italy

University of Modena and Reggio Emilia, Modena, Italy

Nivin Samara Division of Reproductive Sciences, University of Toronto, Toronto, ON, Canada

Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada

Toronto Centre for Advanced Reproductive Technology Fertility Partners, Toronto, ON, Canada

Tal Shavit Department of Obstetrics and Gynecology, McGill University, Montreal, QC, Canada

Elisabet Stener-Victorin Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Togas Tulandi Department of Obstetrics and Gynecology, McGill University, Montreal, QC, Canada

Melanie L. Walls School of Women's and Infant's Health, The University of Western Australia, Perth, WA, Australia

Madelon van Wely Center for Reproductive Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Xiao-Ke Wu Department of Obstetrics and Gynaecology, First Affiliated Hospital, Heilongjiang University of Chinese Medicine, Harbin, China

Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong, China

Part I

Diagnosis, Pathophysiology, and Pathogenesis

Introduction

1

Stefano Palomba

Polycystic ovary syndrome (PCOS) is a very heterogeneous and complex disorder characterized by oligo-anovulation, hyperandrogenism and/or hyperandrogenemia, and polycystic ovarian morphology (PCOM) [1–3]. Moreover, throughout the years, its definition remains controversial. In 2012, an expert panel from the NIH Evidence-Based Methodology Workshop on PCOS [4] recommended that clinicians use the Rotterdam criteria for diagnosis of PCOS [2]; the same recommendation was also given subsequently in the practical guidelines of the Endocrine Society [5].

The syndrome affects a considerable but variable proportion of women in reproductive age. Specifically, the prevalence of PCOS according to the 1990 National Institutes of Health (NIH) criteria is 6–10% but it is at least double using broader Rotterdam or Androgen Excess-PCOS Society criteria [6].

The interest in PCOS has covered several peculiar aspects, including those reproductive, cosmetic, and medical [7, 8]. Similarly, several task forces, committees, and groups of special interest have produced many papers on the PCOS, its diagnostic criteria, its short- and long-term health consequences, and its therapeutic management [1–3, 5, 9–12]. Moreover, during the years, less interest and space have been given to the fertility concerns associated to the syndrome and even more interest have been observed for its metabolic and cardiovascular long-term health consequences [12]. For example, the last consensus document on infertility treatment in women with PCOS has been published more than 8 years ago [11]. This is partially due, as detailed below, to the difficulty to characterize the infertile patient with PCOS and to integrate specific PCOS-related features with largely accepted strategy for treating infertility. On the other hand, for example, many interventions in the assisted reproductive technologies (ARTs) are translated to patients with PCOS considering simplistically that they are “high-responder” patients, whereas the

S. Palomba
Unit of Gynecology and Obstetrics, IRCCS–Arcispedale Santa Maria Nuova,
Viale Risorgimento 80, 42123 Reggio Emilia, Italy
e-mail: stefanopalomba@tin.it

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_1

clinical practice highlights that many obese patients with PCOS with non-PCOM phenotype have frequently a poor response to gonadotrophin administration [13, 14].

An issue particularly important for approaching infertility in PCOS is the definition of the specific PCOS phenotypes since the variability in hormonal and metabolic abnormalities among PCOS phenotypes could influence the reproductive outcome. It has been also proposed provocatively to distinguish the syndrome in a "metabolic phenotype" and in a "reproductive phenotype" [15]. However, it is very probable that severe metabolic phenotypes are closely related to worst reproductive outcomes and vice versa [7]. International guideline [5] underlines no need to define formally the PCOS phenotype in the clinical practice and that PCOS is a risk factor for infertility only in the presence of oligo-anovulation. Conversely, the precise knowledge of PCOS phenotype, and its comorbidities (i.e., obesity, insulin resistance, etc.), is crucial in infertile patients in order to optimize and personalize the management of the patient [7]. In addition, even if the presence of ovarian dysfunction has a clear "weight" on the reproductive potential in patients with PCOS, other subclinical dysfunctions, including alterations in endometrial (Table 1.1) and oocyte [16] competence, cannot be leave out.

Table 1.1 Main endometrial abnormalities observed in patients with PCOS

Finding	Proposal mechanism
Reduced endometrial expression of SHBG	Abnormal steroid milieu for increased free androgens
Reduced serum IGFBP-1/ glycodelin levels	Abnormal mitotic activity for IGF-1 action/decreased immune-suppression (Th1)
Reduced endometrial expression of GLUT-4	Abnormal metabolic activity of the endometrial cell for hypoglycemia
Reduced Rabs and WASP proteins	Impaired cell surface GLUT-4 vesicle exposure and the consequent glucose uptake in the endometrium
Increased AR/ORalpha or no downregulation in luteal phase	Abnormal steroid milieu
Decreased endometrial αvβ3 integrin expression	Impairment of the cell-cell and cell-extracellular matrix interactions during the window of implantation
Decreased endometrial HOXA-10 expression	Reduced pinopode number/upregulation of the integrin expression (β subunit)
Reduced endometrial IGFBP-1 expression	Endometrial epithelial and stromal dysfunction for increased mitotic activity for IGF-1 action
Over-expression of steroid receptor coactivators	Increased endometrial proliferation
Abnormal gene expression pattern in luteal phase	Progesterone resistance and elevated estrogen activity with reduced decidualization
Abnormal gene expression pattern in the window period	Impaired window of implantation with abnormal blastocyst-endometrium interaction
Abnormal vascularization	Impaired metabolism and chromosomal alterations related to hypoxia

AR androgen receptor, *OR* estrogen receptor, *GLUT-4* glucose transmembrane protein 4, *IGFBP-1* insulin growth factor binding protein 1, *SHBG* sex hormone-binding globulin

A secondary analysis [17] of the data from the Pregnancy in Polycystic Ovary Syndrome I and II (PPCOS-I and PPCOS-II) trials [18, 19] on a total of 1376 infertile women with PCOS demonstrated that a younger age, lower baseline free androgen index and insulin, shorter duration of attempting conception, and higher baseline sex hormone-binding globulin (SHBG) significantly predict at least one pregnancy outcome. This study underlines that the prognosis of infertile women with PCOS is a combination of the classical and general prognostic factors with specific factors related to PCOS. Thus, a good clinician should always consider both in case of infertility in women with PCOS.

When the impact on infertility of the dyads of PCOS features is formally considered, no difference is showed between anovulation plus hyperandrogenism and anovulation plus PCOM [6]. Moreover, in the infertility field, "PCOM" means a very high antral follicle count (AFC, more than 12 per ovary). Interesting data demonstrated that the pregnancy and live birth rates are significantly and clinically better in patient with PCOM without clinical manifestation of PCOS in comparison with normal controls [20]. Also recent clinical data [21] demonstrated a better ongoing pregnancy rate in women with PCOM compared to non-PCOM controls. Interesting in vitro fertilization (IVF) data [22] demonstrated a significant relationship between AFC and reproductive outcomes with the high odds of pregnancy between 11 and 23 follicles, very close to cutoff used for PCOM diagnosis (Fig. 1.1). In 2013, Wiser et al. [23] demonstrated a different trend in the decline of the antral follicles in PCOS patients when compared to controls. That data seems to suggest an extended fertile window in women with PCOS. Moreover, a large cohort study

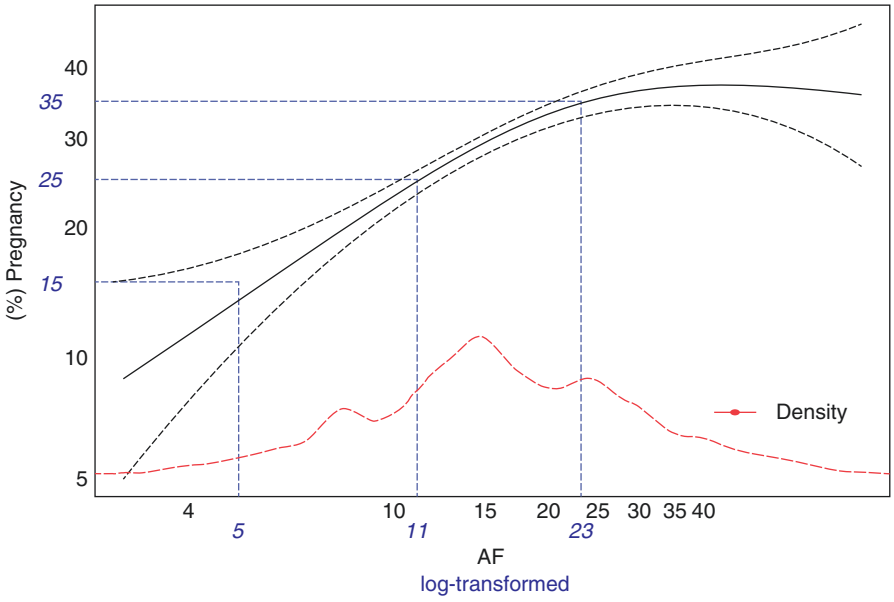


Fig. 1.1 Relationship between antral follicular count (AFC) and reproductive outcome. The high odds of pregnancy are observed between 11 and 23 antral follicles. From Holte et al. [22]

[24] of the Society of Assisted Reproductive Technology (SART) showed an overall difference in pregnancy rate between infertile patients with PCOS and with tubal factor of 5%. That difference resulted clinically significant even between patients aged 38 and 40 years [adjusted odd rate (aOR) 1.24, 95%CI 1.08 to 1.43] [24]. The evaluation of biological and clinical outcomes in women with PCOS treated with IVF categorized according to PCOS phenotypes demonstrated that PCOM phenotypes had better outcomes in comparison with non-PCOM phenotype, although that differences disappeared after adjusting data for women's age and body mass index (BMI) [25].

More and more studies in literature are aimed to assess the role of the anti-Mullerian hormone (AMH) in the pathophysiology of PCOS [26] and as effective tool to diagnose the antral follicular excess in women with and without PCOS and PCOM [27]. A role of the AMH assay has been also suggested as criterion of PCOS [28]. Moreover, AMH concentrations seem to be an effective predictor of pregnancy and live birth rates only in women with PCOS without PCOM [25].

It is crucial for the clinicians to understand that also in women with PCOS, the ovarian reserve evaluation is important for the strategy of management and for the results. All patients with PCOS should be not considered always patients with a "high reserve," and even in these infertile patients the role of a high AFC and of high concentrations of AMH can be important predictors of the number of oocyte yield in patients, as demonstrated in in vitro maturation (IVM) cycles [29].

Epidemiological findings about the fertility in women with PCOS are also controversial. No long-term data demonstrating that PCOS patients are more infertile than general population are actually available. A recent large register study [30] showed that women with PCOS at hospital admission have a diagnosis of infertility and require fertility investigation ten- and eightfold, respectively, more frequently than non-PCOS controls. However, cohort studies suggest that women with PCOS have, at the end of their reproductive life, the same potential of non-PCOS women [31]. In particular, after a long-term follow-up, no difference between PCOS and healthy controls was observed in the proportion of women with at least one child (86.7% vs. 91.6%, respectively) and, surprising, with at least one spontaneous pregnancy (67.5% vs. 73.6%) [31]. A meta-analysis of randomized controlled trials (RCTs) demonstrated that subfertile patients with PCOS who received IVF cycles have not significantly difference in terms of reproductive performance when compared to non-PCOS controls [32].

The primary endpoint in reproductive medicine is a healthy mother with a healthy baby in arm, and all other clinical and/or biological outcomes would be considered only a surrogate [33, 34]. This concept is true also for infertile patients with PCOS. Moreover, only one published document [2] underlines the increased risk for adverse pregnancy and neonatal outcomes in women with PCOS and that the obstetric risk may be exacerbated by obesity and/or insulin resistance and suggest a closer follow-up during pregnancy. A recent systematic review [35] confirms that women with PCOS exhibit a clinically significant increased risk of pregnancy and perinatal complications compared with non-PCOS controls. Even if not adjusted for BMI or other confounders, available data demonstrate at least a

twofold increased risk of pregnancy-induced hypertension and preeclampsia, gestational diabetes, and premature delivery [35]. More limited and sparse data suggest also an increased risk of neonatal morbidity [35]. At the moment, the exact etiopathogenesis for explaining that risk in women with PCOS is unknown, and it involves potentially relationship with genetic, environmental, clinical, and biochemical factors [35]. However, longitudinal data [36] demonstrated that the incidence of adverse events in women with PCOS varies according to the features and phenotypes of PCOS. Specifically, the risk of pregnancy complications resulted nonsignificant in non-hyperandrogenic and ovulatory phenotypes, whereas it was twofold increased in non-PCOM phenotype [36]. In other words, the presence of PCOM seems to be a protective factor in terms of incidence of pregnancy complications. This hypothesis seems to be confirmed since women with a full-blown PCOS phenotype showed a risk of adverse pregnancy complication slightly lower than those observed in non-PCOM/PCOS patients [36]. The analysis of each specific PCOS features confirmed that PCOM was not related to an increased risk, whereas oligo-amenorrhea and hyperandrogenemia were related to a risk four- to fivefold higher for pregnancy complications [36].

One of the main effectors of the increased risk of pregnancy complications in PCOS and non-PCOS patients is the placenta. Placenta function is crucial for the fetal growth and for the physiological metabolic changes of pregnancy. Data demonstrated that the process of decidual trophoblast invasion is impaired in women with PCOS [37] and that the rate and the extent of abnormal macroscopic and microscopic findings in the placenta from uncomplicated pregnancies were increased [38]. Significant and indirect relationships between the incidence and extent of lesions and markers of biochemical hyperandrogenism and insulin resistance suggest that these two factors may be the main determinants of the effects of PCOS on trophoblastic and placental tissue [37, 38]. In addition, that alterations vary among PCOS phenotypes [39]. A higher rate of abnormalities, both on the trophoblastic and on placental tissue, was observed in patients with full-blown and non-PCOM phenotypes, suggesting the importance of the role played by hyperandrogenism and high follicular excess (i.e., PCOM) as negative and positive predictors, respectively, of favorable outcome [39].

On the other hand, a retrospective large analysis [40] showed an association between the number of oocytes retrieved and adverse obstetric outcomes of preterm delivery and children born with a low birth weight after IVF treatment. Specifically, women with more than 20 oocytes retrieved have a higher risk of adverse obstetric outcomes suggesting potential causative relationship with PCOS/PCOM [40]. Moreover, because no data were available about the gonadotrophin doses and protocol used, it is possible to conclude that an excessive ovarian stimulation is related with adverse pregnancy outcomes.

Based on these considerations, the importance of a transverse knowledge about the infertility and the PCOS in order to consider the specificities of the syndrome in the field of infertility/subfertility is clear. A recent online survey on the diagnosis and management of infertile patients with PCOS in IVF centers (http://www.ivf-worldwide.com/survey/pcos-results.html) demonstrated that experts in infertility

treatments and in ART procedures have heterogonous and frequently wrong ideas and conceptions on the PCOS definition, diagnosis, and management. Thus, an up-to-date knowledge on infertility in the context of the PCOS is probably needed and will be provided in the current book, and evidence-based guidance on its treatment will be also suggested. Anovulation, oocyte quality, and endometrium competence in women with PCOS will be deeply discussed in the next chapters. The aspects of the infertility and subfertility cofactors in infertile patients with PCOS, as well as the impact and the interaction of PCOS phenotypes on the reproductive outcome, will be approached. Not only the classical medical treatments for treating the PCOS-related ovulatory dysfunction, including clomiphene citrate, letrozole, metformin, inositol, and gonadotrophins, but also other potential therapeutic approaches, such as laparoscopic ovarian drilling and acupuncture, will be reviewed. Careful and critical attention will be also devoted not only to the non-pharmacological approach such as the lifestyle interventions but also to the use of controlled ovarian stimulation undergoing intrauterine insemination or IVF with or without IVM of oocytes. Finally, having in mind all potential and available treatments, it is particularly important that the strategy of treating infertility in women with PCOS should be considered as the simultaneous and/or sequential association of one or more interventions in order to maximize the chances to achieve a single healthy baby in the shorter time and in the safer manner for the mother and the child.

References

1. Zawadzki J, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens HR, Haseltine FP, Merriam GR, editors. Polycystic ovary syndrome. Boston: Blackwell Scientific; 1992. p. 377–84.
2. Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Consensus on women's health aspects of polycystic ovary syndrome (PCOS). Hum Reprod. 2012;27:14–24.
3. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE, Witchel SF, Androgen Excess Society. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. J Clin Endocrinol Metab. 2006;91:4237–45.
4. NIH Office of Disease Prevention. Evidence-based Methodology Workshop on Polycystic Ovary Syndrome. Expert Panel Guidelines on PCOS. 2012, https://prevention.nih.gov/docs/programs/pcos/FinalReport.pdf.
5. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, Welt CK. Diagnosis and treatment of polycystic ovary syndrome: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2013;98:4565–92.
6. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. Endocr Rev. 2015;36:487–525.
7. Orio F, Palomba S. Reproductive endocrinology: new guidelines for the diagnosis and treatment of PCOS. Nat Rev Endocrinol. 2014;10:130–2.
8. Jayasena CN, Franks S. The management of patients with polycystic ovary syndrome. Nat Rev Endocrinol. 2014;10:624–36.

9. Yildiz BO, Azziz R, Androgen Excess and PCOS Society. Ovarian and adipose tissue dysfunction in polycystic ovary syndrome: report of the 4th special scientific meeting of the Androgen Excess and PCOS Society. Fertil Steril. 2010;94:690–3.
10. Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, Gambineri A, Kelestimur F, Macut D, Micic D, Pasquali R, Pfeifer M, Pignatelli D, Pugeat M, Yildiz BO, ESE PCOS Special Interest Group. The polycystic ovary syndrome: a position statement from the European Society of Endocrinology. Eur J Endocrinol. 2014;171:1–29.
11. Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Fertil Steril. 2008;89:505–22.
12. Goodman NF, Cobin RH, Futterweit W, Glueck JS, Legro RS, Carmina E. American Association of Clinical Endocrinologists, American College of Endocrinology, and Androgen Excess and PCOS Society disease state clinical review: guide to the best practices in the evaluation and treatment of polycystic ovary syndrome—part 2. Endocr Pract. 2015;21:1415–26.
13. Palomba S, Falbo A, Zullo F. Management strategies for ovulation induction in women with polycystic ovary syndrome and known clomifene citrate resistance. Curr Opin Obstet Gynecol. 2009;21:465–73.
14. Palomba S, Falbo A, Di Cello A, Cappiello F, Tolino A, Zullo F. Does metformin affect the ovarian response to gonadotropins for in vitro fertilization treatment in patients with polycystic ovary syndrome and reduced ovarian reserve? A randomized controlled trial. Fertil Steril. 2011;96:1128–33.
15. Dunaif A, Fauser BC. Renaming PCOS-a two-state solution. J Clin Endocrinol Metab. 2013;98:4325–8.
16. Palomba S, Daolio J, La Sala GB. Oocyte quality and competence in women with polycystic ovary syndrome. Trend Endocrinol Metab. 2017;28:186–98.
17. Kuang H, Jin S, Hansen KR, Diamond MP, Coutifaris C, Casson P, Christman G, Alvero R, Huang H, Bates GW, Usadi R, Lucidi S, Baker V, Santoro N, Eisenberg E, Legro RS, Zhang H, Network RM. Identification and replication of prediction models for ovulation, pregnancy and live birth in infertile women with polycystic ovary syndrome. Hum Reprod. 2015;30:2222–33.
18. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, Steinkampf MP, Coutifaris C, McGovern PG, Cataldo NA, Gosman GG, Nestler JE, Giudice LC, Leppert PC, Myers ER, Cooperative Multicenter Reproductive Medicine Network. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. N Engl J Med. 2007;356:551–66.
19. Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P, Christman GM, Huang H, Yan Q, Alvero R, Haisenleder DJ, Barnhart KT, Bates GW, Usadi R, Lucidi S, Baker V, Trussell JC, Krawetz SA, Snyder P, Ohl D, Santoro N, Eisenberg E, Zhang H, NICHD Reproductive Medicine Network. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. N Engl J Med. 2014;371:119–29.
20. Engmann L, Maconochie N, Sladkevicius P, Bekir J, Campbell S, Lin TS. The outcome of in-vitro fertilization treatment in women with sonographic evidence of polycystic ovarian morphology. Hum Reprod. 1999;14:167–71.
21. Sigala J, Sifer C, Dewailly D, Robin G, Bruyneel A, Ramdane N, Lefebvre-Khalil V, Mitchell V, Decanter C. Is polycystic ovarian morphology related to a poor oocyte quality after controlled ovarian hyperstimulation for intracytoplasmic sperm injection? Results from a prospective, comparative study. Fertil Steril. 2015;103:112–8.
22. Holte J, Brodin T, Berglund L, Hadziosmanovic N, Olovsson M, Bergh T. Antral follicle counts are strongly associated with live-birth rates after assisted reproduction, with superior treatment outcome in women with polycystic ovaries. Fertil Steril. 2011;96:594–9.
23. Wiser A, Shalom-Paz E, Hyman JH, Sokal-Arnon T, Bantan N, Holzer H, Tulandi T. Age-related normogram for antral follicle count in women with polycystic ovary syndrome. Reprod Biomed Online. 2013;27:414–8.

24. Kalra SK, Ratcliffe SJ, Dokras A. Is the fertile window extended in women with polycystic ovary syndrome? Utilizing the Society for Assisted Reproductive Technology registry to assess the impact of reproductive aging on live-birth rate. Fertil Steril. 2013;100:208–13.
25. Ramezanali F, Ashrafi M, Hemat M, Arabipoor A, Jalali S, Moini A. Assisted reproductive outcomes in women with different polycystic ovary syndrome phenotype: the predictive value of anti-Mullerian hormone. Reprod Biomed Online. 2016;32:503–12.
26. Garg D, Tal R. The role of AMH in the pathophysiology of polycystic ovarian syndrome. Reprod Biomed Online. 2016;33:15–28.
27. Christiansen SC, Eilertsen TB, Vanky E, Carlsen SM. Does AMH reflect follicle number similarly in women with and without PCOS? PLoS One. 2016;11:e0146739.
28. Dewailly D. Diagnostic criteria for PCOS: is there a need for a rethink? Best Pract Res Clin Obstet Gyneacol. 2016;37:5–11.
29. Guzman L, Ortega-Hrepich C, Polyzos NP, Anckaert E, Verheyen G, Coucke W, Devroey P, Tournaye H, Smitz J, De Vos M. A prediction model to select PCOS patients suitable for IVM treatment based on anti-Mullerian hormone and antral follicle count. Hum Reprod. 2013;28:1261–6.
30. Hart R, Doherty DA. The potential implications of a PCOS diagnosis on a woman's long-term health using data linkage. J Clin Endocrinol Metab. 2015;100:911–9.
31. Hudecova M, Holte J, Olovsson M, Sundström PI. Long-term follow-up of patients with polycystic ovary syndrome: reproductive outcome and ovarian reserve. Hum Reprod. 2009;24:1176–83.
32. Heijnen EM, Eijkemans MJ, Hughes EG, Laven JS, Macklon NS, Fauser BC. A meta-analysis of outcomes of conventional IVF in women with polycystic ovary syndrome. Hum Reprod Update. 2006;12:13–21.
33. Barnhart KT. Live birth is the correct outcome for clinical trials evaluating therapy for the infertile couple. Fertil Steril. 2014;101:1205–8.
34. Legro RS, Wu X, Barnhart K, Niederberger C, Ng EH, Palomba S, Maria AS, Emilia R, Zhang H, Farquhar C, Rebar RW, Pellicer A, Reindollar R, Fauser BC, Tapanainen JS, Evers H, Shankaran S, Silver RM, Mol B, Norman RJ, Silver RM, Bhattacharya S, Vanderpoel S, Bhattacharya S, Evers JL, Ng EH, Niederberger C, Norman RJ, Palomba S, Pellicer A, Reindollar R, Rebar R, Shankaran S, Silver RM, Tapanainen JS, Vanderpool S, Zhang H. Improving the reporting of clinical trials of infertility treatments (IMPRINT): modifying the CONSORT statement. Hum Reprod. 2014;29:2075–82.
35. Palomba S, de Wilde MA, Falbo A, Koster MP, La Sala GB, Fauser BC. Pregnancy complications in women with polycystic ovary syndrome. Hum Reprod Update. 2015;21:575–92.
36. Palomba S, Falbo A, Russo T, Tolino A, Orio F, Zullo F. Pregnancy in women with polycystic ovary syndrome: the effect of different phenotypes and features on obstetric and neonatal outcomes. Fertil Steril. 2010;94:1805–11.
37. Palomba S, Russo T, Falbo A, Di Cello A, Amendola G, Mazza R, Tolino A, Zullo F, Tucci L, La Sala GB. Decidual endovascular trophoblast invasion in women with polycystic ovary syndrome: an experimental case-control study. J Clin Endocrinol Metab. 2012;97:2441–9.
38. Palomba S, Russo T, Falbo A, Di Cello A, Tolino A, Tucci L, La Sala GB, Zullo F. Macroscopic and microscopic findings of the placenta in women with polycystic ovary syndrome. Hum Reprod. 2013;28:2838–47.
39. Palomba S, Falbo A, Chiossi G, Tolino A, Tucci L, La Sala GB, Zullo F. Early trophoblast invasion and placentation in women with different PCOS phenotypes. Reprod Biomed Online. 2014;29:370–81.
40. Sunkara SK, La Marca A, Seed PT, Khalaf Y. Increased risk of preterm birth and low birthweight with very high number of oocytes following IVF: an analysis of 65868 singleton live birth outcomes. Hum Reprod. 2015;30:1473–80.

Diagnostic Criteria for PCOS

2

Francesco Orio and Giovanna Muscogiuri

2.1 Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous and complex disorder that has both metabolic and hormonal implications and that represents one of the major causes of infertility in women. Great efforts have been made in the last two decades to identify diagnostic criteria for this syndrome. Besides the hormonal aspects, metabolic issues such as insulin resistance and obesity and the susceptibility to develop earlier than expected glucose intolerance states have encouraged the notion that these aspects should be included in the diagnostic criteria to plan potential therapeutic strategies in affected women. Further, PCOS clusters in families and both female and male relatives can show stigmata of the syndrome thus suggesting a genetic background. Genome-wide association studies have identified a number of candidate regions that should be further investigated in order to identify their role in the development of the syndrome.

In this chapter, the main criteria to diagnose PCOS will be summarised and discussed highlighting the potential strengths and limitations.

2.2 Historical Viewpoint

In 1935, Stein and Leventhal described several cases presenting with oligomenorrhoea/amenorrhoea combined with the presence at operation of bilateral ovaries with polycystic ovary morphology (PCOM) [1]. Of these patients, three also presented obesity and five showed signs of hirsutism. Only one patient was both obese

F. Orio (✉)
Department of Sports Science and Wellness, "Parthenope" University Naples, Naples, Italy
e-mail: francescoorio@virgilio.it

G. Muscogiuri
Ios and Coleman Medicina Futura Medical Center, Naples, Italy

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_2

and showed hirsutism. These findings were important to demonstrate that not all the clinical features associated with PCOS must be present along with PCOM proven by morphology [2–4].

An elevated luteinising hormone/follicle-stimulating hormone (LH/FSH) ratio has been used as a diagnostic test for PCOS for many years. In fact, women with PCOS have been reported to have defects of gonadotrophin secretion, including an elevated LH level, elevated LH to FSH ratio, and an increased frequency and amplitude of LH pulsations [5, 6]. Despite the large use of this parameter to diagnose PCOS, concerns about the clinical utility of the ratio have led to the Rotterdam European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) consensus statement on PCOS recommending against its inclusion.

The introduction of transvaginal ultrasonography was of paramount importance to demonstrate that patients with oligomenorrhoea, obesity, and hirsutism do not necessarily have the typical PCOM on ultrasound [7, 8]. Since the aetiology of PCOS is far from well understood, diagnostic criteria for PCOS have been revised several times [2, 9]. Specialty groups may still differ in their use of diagnostic criteria and diagnostic workup, as well as in their choice of first- and second-line treatment [9].

2.3 Available Diagnostic Criteria

Three principal set of criteria of PCOS are in widespread use today.

2.3.1 National Institutes of Health (NIH) Criteria

The first set of criteria comes from the proceedings of an expert conference sponsored in part by the National Institute of Child Health and Human Disease (NICHD) of the US National Institutes of Health (NIH) on April 16–18, 1990. During this meeting, all participants were surveyed regarding their perception of what features formed part of PCOS, and Drs Zawadski and Dunaif summarised the findings in the meeting proceedings [10]. They concluded that the major criteria for PCOS should include (1) hyperandrogenism (defined as excessive terminal hair that appears in a male pattern, acne or androgenic alopecia) and/or hyperandrogenemia (elevated serum androgen level and typically includes an elevated total, bioavailable or free serum testosterone), (2) anovulation or oligo-ovulation (anovulation may manifest as frequent bleeding at intervals <21 days or infrequent bleeding at intervals >35 days; a midluteal progesterone lower than 3–4 ng/mL may help with the diagnosis) and (3) exclusion of other known disorders (Table 2.1).

Hirsutism can be assessed using the Ferriman-Gallwey score or its validated modifications (Fig. 2.1).

Table 2.1 Diagnostic criteria for PCOS according to the 1990 NIH conference, the revised criteria from the ESHRE/ASRM sponsored consensus meeting (2003) and the criteria of the Androgen Excess Society (2006)

NIH criteria (1990)	Rotterdam criteria (2003)	Androgen Excess and PCOS Society (2006)
Must include all the following: – Hyperandrogenism and/or hyperandrogenemia – Anovulation or oligo-ovulation	*Must include two of the following*: – Anovulation or oligo-ovulation – Clinical and/or biochemical signs of hyperandrogenism – PCOM	*Requires all the following*: – Hirsutism and/or hyperandrogenemia – Oligo-ovulation and/or PCOM

Exclusion of other possible related disorders: ovarian or adrenal androgen-secreting tumours, thyroid disease, hyperprolactinemia, nonclassical congenital adrenal hyperplasia

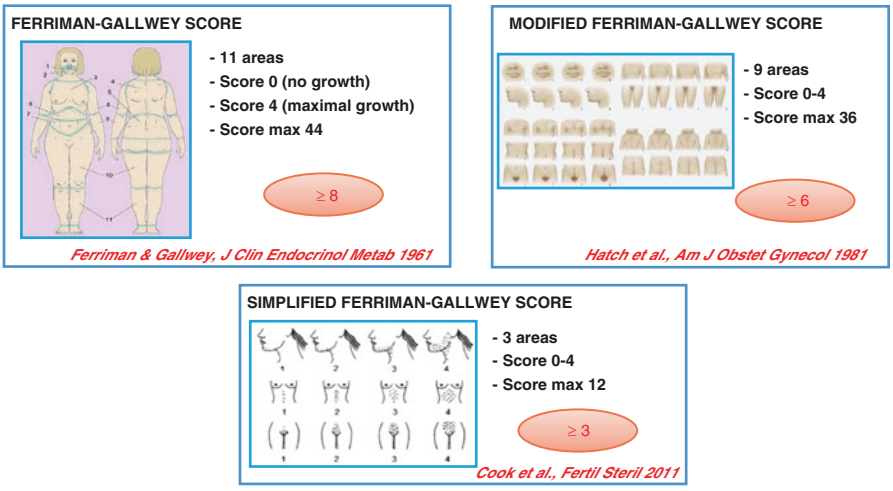


Fig. 2.1 The Ferriman-Gallwey scoring system to diagnose hirsutism. In the classical Ferriman-Gallwey score, each of the 11 body areas is rated from 0 (absence of terminal hairs) to 4 (extensive terminal hair growth), and the numbers in each area are added to obtain the total score. A score ≥8 generally defines hirsutism. In the modified Ferriman-Gallwey score, each of the nine body areas is rated from 0 (absence of terminal hairs) to 4 (extensive terminal hair growth), and the numbers in each area are added to obtain the total score. A score ≥6 generally defines hirsutism. In the simplified Ferriman-Gallwey score, each of the three body areas is rated from 0 (absence of terminal hairs) to 4 (extensive terminal hair growth), and the numbers in each area are added to obtain the total score. A score ≥3 generally defines hirsutism

The differential diagnosis for PCOS includes adrenal congenital hyperplasia (post-ACTH stimulation 17 hydroxyprogesterone higher than 300 ng/dL), hyperprolactinemia (prolactin levels higher than 12 mg/L), hypothyroidism (TSH higher than 4.5 mLU/L), Cushing's syndrome (11 pm salivary cortisol

higher than 0.15 ug/dL, 24-h urine free cortisol higher than 50 ug/d, overnight 1 mg dexamethasone suppression higher than 1.8 ug/dL), premature ovarian insufficiency (FSH higher than 30 mUI/mL and oestradiol lower than 20 pg/mL) and virilising adrenal and ovarian cancer. The virilising cancers are associated with high levels of androstenedione (>3 ng/mL) and/or DHEA-S (>3500 ng/mL) and/or DHEA (>9 ng/dL). However, most of the signs that are common to PCOS are often not specific for PCOS. In fact, oligomenorrhoea is common after menarche during normal puberty and is therefore not specific to adolescents, as well as acne is common, although transitory during adolescence.

The survey of the NIH had the advantage to identify PCOS as a diagnosis of exclusion of other androgen disorder along with ovarian consequences. The NIH/NICHD criteria interpreted clinical hyperandrogenism as hirsutism, since more than 70% of hirsute women are hyperandrogenemic [2]. Consequently, three principal phenotypes are generally identified: (a) women with hirsutism, hyperandrogenemia, and oligo-ovulation, (b) women with hirsutism and oligo-ovulation or (c) women with hyperandrogenemia and oligo-ovulation (Table 2.1). Thanks to the NIH/NICHD criteria, it has been begun to understand the enormous high prevalence of the disorder [2, 3, 7, 8] and the high frequency of insulin resistance [9, 11] with increased risk of developing type 2 diabetes mellitus that often accompanied this syndrome [12, 13]. The broadening of the diagnostic criteria has led to a large body of research comparing the PCOS phenotypes. However, an unintended consequence of the broadening of the diagnostic criteria has been the inclusion in studies of multiple PCOS phenotypes without stratification. This failure to investigate precisely defined PCOS phenotypes has resulted in confusion in the literature because the metabolic features of the syndrome vary by phenotype. Based on this issue, Drs. Dunaif and Fauser proposed provocatively to distinguish the syndrome in a *metabolic phenotype* and a *reproductive phenotype* [14]. The main debate raised by these criteria was around the fact that PCOM were very commonly associated with hirsutism and hyperandrogenemia in women with regular, ovulatory, cycles [15, 16]. This is because anovulation is not necessarily chronic, and that intermittent, or even prolonged, episodes of regular, ovulatory, cycles could punctuate the pattern of anovulatory vaginal bleeding or amenorrhoea [1, 17, 18].

2.3.2 ESHRE/ASRM Criteria

The burgeoning issue for including ovulatory women with PCOM and hyperandrogenism in the definition of PCOS was a major determinant in motivating the ESHRE/ASRM workshop. This consensus conference among expert of PCOS was convened in Rotterdam, the Netherlands, on May 1–3, 2003, sponsored in part by the ESHRE and the ASRM [19, 20]. The meeting proceedings recommended that PCOS be defined when at least two of the following three features were present: (1) oligo- and/or anovulation, (2) clinical and/or biochemical signs of hyperandrogenism and (3) PCOM. These criteria again highlighted that PCOS is a diagnosis of exclusion (see above and Table 2.1).

Table 2.2 Threshold of sonographic ovarian characteristics proposed at the 2003 Rotterdam consensus for the diagnosis of PCOM

	2003 Rotterdam criteria
Number of follicles	>12 follicles
Measures of follicles	2–9 mm in diameter
Ovarian size	>10 mL^3

PCOM as defined by the 2003 Rotterdam criteria referred to the presence of at least one ovary exhibiting 12 or more follicles measuring 2–9 mm in diameter, regardless of location, and/or a total volume > 10 mL^3, as determined by transvaginal ultrasound (Table 2.2) [19, 20]. This definition differs somewhat from that originally proposed by Adams and colleagues [21] using transabdominal ultrasound that defined PCOM as those containing at least ten follicles between 2 and 8 mm in diameter in one plane, arranged either peripherally around a dense core of ovarian stroma or scattered throughout an increased amount of stroma. These latter investigators have more recently modified their definition to consider as PCOS those containing at least eight follicles 2–8 mm in diameter [22]. The number of women who were misclassified by using the modified Adams et al. [22] criteria versus the Rotterdam criteria is negligible.

The recent use of three-dimensional sonography enables the assessment of the volume of the ovary and ovarian follicles. Using the difference between these two parameters, the volume of the ovarian stroma can be assessed. The use of stromal volume to ovarian volume ratio as a diagnostic feature of PCOS that correlates with androgen concentration has been demonstrated of value [23]. However, stromal volume is a variable that is strictly correlated with the volume of the entire ovary. That is why its assessment is of little use in clinical practice.

It should be noted that the 2003 Rotterdam criteria defined a population of patients that is inclusive of those women previously diagnosed as having PCOS according to the 1990 NIH/NICHD criteria. In fact, the 2003 Rotterdam criteria have expanded but not replaced the NIH (1990) criteria. The 2003 Rotterdam criteria added two new phenotypes of PCOS, namely, patients who have PCOM, hirsutism and/or hyperandrogenemia but have normal ovulation and women who have PCOM and irregular ovulation but no sign of androgen excess [24–26]. As consequence of accepting these two phenotypes, the Rotterdam 2003 criteria increase the phenotypic heterogeneity of the disorder thus decreasing the ability of genetic and other molecular studies to detect a common underlying abnormality. However, a finding of PCOM can predict the response to ovulation induction, because women with this ovarian morphology are more sensitive to gonadotrophin stimulation than spontaneously cycling women, possibly as a result of the larger pool of small antral follicles available for recruitment [27].

A positive correlation has been found between anti-Müllerian hormone (AMH) levels and the number of small follicles as well as ovarian volume. The results of published studies indicated that the level of AMH is higher in patients with PCOS, which can be helpful in the diagnosis in this syndrome [28–30]. An AMH cut-off value of 20 pmol/L has been suggested for diagnosis of PCOS [31]. Further, it has also been shown that there is a correlation between higher AMH concentration, rare

menstruation and hyperandrogenism. However, due to the usage of various methods to analyse plasma AMH levels, it is difficult to compare previous studies and to identify cut-offs for PCOS patients.

2.3.3 Androgen Excess (AE)-PCOS Society Criteria

The most recent criteria were defined by a task force of the Androgen Excess (AE)-PCOS Society in 2006, which recommended the following diagnostic criteria for PCOS: (1) hirsutism and/or hyperandrogenemia, (2) oligo-ovulation and/or PCOM and (3) exclusion of other androgen excess or related disorders (Table 2.1). The AE-PCOS (2006) attempts to make a balance between the NIH (1990) and the Rotterdam (2003) definitions, using a careful review of the literature to substantiate their criteria [32]. In this definition, ovulatory women with hirsutism and/or hyperandrogenemia, and PCOM, are defined as having PCOS owing to their increased risk of metabolic dysfunction, albeit less than core PCOS patients. However, the AE-PCOS definition does not include patients who solely demonstrate ovulatory dysfunction and PCO on ultrasound, without evidence of androgen excess, as having PCOS. The AE-PCOS (2006) criteria identify individuals with PCOS who have an increased risk of metabolic dysfunction, albeit less than the 1990 NIH criteria.

2.4 Actual Limitations and Future Perspectives

The main limit of the current guidelines for the diagnosis of PCOS is that it is considered only as a fertility and cosmetic disorder without mention to long-term risks [33]. An expert panel from the 2012 NIH Evidence-Based Methodology Workshop on PCOS recommended that clinicians use the more recent Rotterdam criteria for diagnosis [34]. Consequently, the prevalence of PCOS has doubled after starting the broader Rotterdam or AE-PCOS Society criteria with 1990 NIH-defined PCOS being the most common phenotype. Evaluation of women with PCOS should exclude alternate androgen excess disorders and risk factors for endometrial cancer, mood disorders, obstructive sleep apnoea, diabetes, and cardiovascular disease [35].

However, important and specific limitations and weaknesses of each of the three cardinal features in PCOS diagnosis also exist and are detailed in Table 2.3.

The European Society of Endocrinology suggested to implement the diagnostic criteria of PCOS with the use of new biomarkers of androgen excess and ovarian dysfunction and in particular the development of a more objective method to define and quantify hirsutism in the different parts of the body. Further, they suggest to pay more attention to the impact of androgen on metabolism as key point for the prevention of type 2 diabetes and cardiovascular events not only during adult age but extended to well after the menopause [36]. Recently, it was highlighted the notion that corrects diagnosis of PCOS impacts on the likelihood of associated metabolic and cardiovascular risks and leads to appropriate intervention, depending upon the woman's age, reproductive status and her own concerns. Further, management of infertility in women with PCOS requires an understanding of the pathophysiology of

Table 2.3 Diagnostic strengths and weaknesses of the main diagnostic features of PCOS

Diagnostic criteria	Strength	Limitation
Hyperandrogenism	Included as a component in all major classifications A major clinical concern for patients	Measurement is performed only in blood Concentrations differ during time of day and age Normal data/values are not clearly defined Assays are not standardised across laboratories Clinical hyperandrogenism is hard to quantify and may vary by different ethnic
Ovulatory dysfunction		Normal ovulations vary in lifespan Ovulatory dysfunction is difficult to measure objectively
PCOM	Historically associated with syndrome	Technique dependent Difficult to obtain standardised measurement May be present in other diseases (low specificity)

Modified by Legro et al. [35]

Table 2.4 New diagnostic tools (and thresholds) for PCOM

(1) The threshold for FNPO defining PCOM should be ≥25 follicles per whole array
(a) This threshold applies to use of newer imaging technology (essentially transducer frequency ≥8 MHz)
(b) FNPO is recommended over OV since FNPO has been shown to have greater predictive power for PCOS and less variability among populations aged 18–35 years
(c) Real-time methods should follow recently proposed standardisation. Offline methods, with either 2D or 3D ultrasound, must be applied after completion of a learning curve and standardisation
(2) The threshold for OV should remain at ≥10 mL OV may have a role in instances when image quality does not allow for reliable estimates of FNPO
(3) The use of the AMH assay as a surrogate to ultrasound is for research purpose only at the present time. Only in-house AMH threshold for PCOM can be used until there is standardisation of the assay techniques

From Dewailly et al., Hum Reprod Update 2014

anovulation [37]. Lastly, it is mandatory to highlight that PCOS diagnosis does not mean PCOM. In fact, PCOM may be part of a wider PCOS spectrum, where only a minority of patients may show hyperandrogenism and most of whom have no hormonal dysfunctions [4]. A task force report from the AE-PCOS Society recommends using follicle number per ovary for the definition of PCOM setting the threshold at ≥25, but only when using newer technology that affords maximal resolution of ovarian follicles (i.e. transducer frequency ≥ 8 MHz). If such technology is not available, they recommend using ovarian volume rather than follicle number per ovary for the diagnosis of PCOM for routine daily practice but not for research studies that require the precise full characterisation of patients (Table 2.4). The appropriateness of

proposed thresholds for follicle number per ovary can be influenced by several factors as described in Table 2.5 [38]. As reported in Table 2.2, the recommended criteria of the ASRM/ESHRE consensus meeting recommended to fulfil at least one of the following criteria to diagnose PCOM: either 12 or more follicles measuring 2 ± 9 mm in diameter or increased ovarian volume (>10 cm^3). If there is a follicle >10 mm in diameter, the scan should be repeated at a time of ovarian quiescence to calculate volume and area. The presence of a single PCOM ovary is sufficient to

Table 2.5 Parameters contributing to variations in thresholds for follicle number in polycystic ovaries

	Inconsistent parameter among studies	Considerations
Clinical populations	Definition of PCOS	Potential to yield heterogeneous cohorts
		PCO as an inclusion criterion is controversial
	Inclusion criteria for controls	Recruitment methods for controls often not specified
		Appropriateness of subfertile women as controls
		PCO as an exclusion criterion is controversial
	Age	Thresholds do not apply to women <18 and >35 years
	Ethnicity	Follicle counts may vary among ethnic populations
Statistical approach	Arbitrary cut-offs	Biased by the interpreter
	Based on 100% specificity	Biased at the expense of test sensitivity
	ROC curve analysis with Youden's Index	Balances test sensitivity and specificity
	95th percentile of control population	Concedes a false negative rate
		Concedes a false positive rate
Technical issues	Newer versus older technology	More follicles can be visualised using newer ultrasound technology
	TA versus TV ultrasound	TA approaches are indicated for certain clinical populations
		Visualisation is poorer using low frequency TA approaches, particularly with obesity
	Real-time versus offline counts	Increased duration for post hoc analyses
		Offline methods yield higher counts
		Potential for increased precision in follicle counts made offline
	2D versus 3D follicle counts	Increased cost of 3D equipment
		3D affords shorter scan time for patients
		3D allows for multi-planar and volume based assessments of follicle counts from stored image files
		3D multi planar view has highest reliability in follicle counts
		3D methods yield lower follicle counts
		Automated assessment of follicle counts by reconstructed volumes requires further validation

provide the diagnosis. The distribution of follicles and a description of the stroma are not required in the diagnosis [39].

A role of the AMH assay has been also suggested as criterion of PCOS and specifically of PCOM or antral follicular excess [40]. Moreover, AMH concentrations seem to be an effective predictor of pregnancy and live birth rates only in women with PCOS without PCOM [41]. This issue will be discussed more in deep in Chap. 8.

Conclusion

Although it is clear that PCOM are a frequent feature of PCOS, this controversy highlights the immediate and considerable need for additional investigation into PCOS and its associated phenotypes and morbidities. It is crucial to establish the diagnostic criteria for PCOS because the long-term consequences of PCOS are still unclear, and the early treatment, including infertility management, may play a role in the prevention of metabolic and cardiovascular diseases. The new generation of ultrasonography as well as the measurement of anti-Müllerian hormone and genetics of PCOS may contribute in developing tailored therapeutic strategies to treat women with PCOS.

References

1. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol. 1935;29:181–91.
2. Azziz R, Sanchez LA, Knochenhauer ES, Moran C, Lazenby J, Stephens KC, Taylor K, Boots LR. Androgen excess in women: experience with over 1000 consecutive patients. J Clin Endocrinol Metab. 2004;89:453–62.
3. Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG, Zapanti ED, Bartzis MI. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. J Clin Endocrinol Metab. 1999;84(11):4006.
4. Orio F, Palomba S, Carbone M, Muscogiuri G. Prevalence of polycystic ovary morphology in a region of South Italy. J Ultrasound. 2016;19:301–2.
5. Fauser BC, Pache TD, Lamberts SW, Hop WC, de Jong FH, Dahl KD. Serum bioactive and immunoreactive luteinizing hormone and follicle-stimulating hormone levels in women with cycle abnormalities, with or without polycystic ovarian disease. J Clin Endocrinol Metab. 1991;73:811–7.
6. Taylor AE, McCourt B, Martin KA, Anderson EJ, Adams JM, Schoenfeld D, Hall JE. Determinants of abnormal gonadotropin secretion in clinically defined women with polycystic ovary syndrome. J Clin Endocrinol Metab. 1997;82:2248–56.
7. Michelmore KF, Balen AH, Dunger DB, Vessey MP. Polycystic ovaries and associated clinical and biochemical features in young women. Clin Endocrinol (Oxf). 1999;51:779–86.
8. Asunción M, Calvo RM, San Millán JL. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. J Clin Endocrinol Metab. 2000;85:2434–8.
9. Carmina E, Koyama T, Chang L, Stanczyk FZ, Lobo RA. Does ethnicity influence the prevalence of adrenal hyperandrogenism and insulin resistance in polycystic ovary syndrome? Am J Obstet Gynecol. 1992;167:1807–12.
10. Zawadski JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens JR, Haseltine F, editors. Polycystic ovary syndrome. Boston, MA: Blackwell Scientific; 1992. p. 377–84.

11. Legro RS, Finegood D, Dunaif A. A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 1998;83:2694.
12. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care. 1999;22:141–6.
13. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metab. 1999;84:165–9.
14. Dunaif A, Fauser BC. Renaming PCOS-a two-state solution. J Clin Endocrinol Metab. 2013;98:4325–8.
15. Adams J, Polson DW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. Br Med J (Clin Res Ed). 1986;293:355–9.
16. Conway GS, Honour JW, Jacobs HS. Heterogeneity of the polycystic ovary syndrome: clinical, endocrine and ultrasound features in 556 patients. Clin Endocrinol (Oxf). 1989;30:459–70.
17. Goldzieher JW, Green JA. The polycystic ovary. I. Clinical and histologic features. J Clin Endocrinol Metab. 1962;22:325–8.
18. Baird DT, Corker CS, Davidson DW, Hunter WM, Michie EA, Van Look PF. Pituitary-ovarian relationships in polycystic ovary syndrome. J Clin Endocrinol Metab. 1977;45:798–801.
19. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81:19–25.
20. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19:41–7.
21. Adams J, Franks S, Polson DW, Mason HD, Abdulwahid N, Tucker M, Morris DV, Price J, Jacobs HS. Multifollicular ovaries: clinical and endocrine features and response to pulsatile gonadotropin releasing hormone. Lancet. 1985;2:1375–9.
22. Adams JM, Taylor AE, Crowley WF Jr, Hall JE. Polycystic ovarian morphology with regular ovulatory cycles: insights into the pathophysiology of polycystic ovarian syndrome. J Clin Endocrinol Metab. 2004;89:4343–50.
23. Fulghesu AM, Angioni S, Frau E, Belosi C, Apa R, Mioni R, Xamin N, Capobianco GP, Dessole S, Fruzzetti F, Lazzarini V, Minerba L, Melis GB, Lanzone A. Ultrasound in polycystic ovary syndrome—the measuring of ovarian stroma and relationship with circulating androgens: results of a multicentric study. Hum Reprod. 2007;22:2501–8.
24. Ferriman D, Gallwey JD. Clinical assessment of body hair growth in women. J Clin Endocrinol Metab. 1961;21:1440–7.
25. Hatch R, Rosenfield RL, Kim MH, Tredway D. Hirsutism: implications, etiology, and management. Am J Obstet Gynecol. 1981;140:815–30.
26. Cook H, Brennan K, Azziz R. Reanalyzing the modified Ferriman-Gallwey score: is there a simpler method for assessing the extent of hirsutism? Fertil Steril. 2011;96:1266–70.
27. van der Meer M, de Boer JA, Hompes PG, Schoemaker J. Cohort size rather than follicle-stimulating hormone threshold level determines ovarian sensitivity in polycystic ovary syndrome. J Clin Endocrinol Metab. 1998;83:423–6.
28. Iliodromiti S, Kelsey TW, Anderson RA, Nelson SM. Can anti-Mullerian hormone predict the diagnosis of polycystic ovary syndrome? A systematic review and meta-analysis of extracted data. J Clin Endocrinol Metab. 2013;98:3332–40.
29. Pigny P, Merlen E, Robert Y, Cortet-Rudelli C, Decanter C, Jonard S, Dewailly D. Elevated serum level of anti-mullerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest. J Clin Endocrinol Metab. 2003;88:5957–62.
30. Laven JS, Mulders AG, Visser JA, Themmen AP, De Jong FH, Fauser BC. Anti-Müllerian hormone serum concentrations in normoovulatory and anovulatory women of reproductive age. J Clin Endocrinol Metab. 2004;89:318–23.

31. Eilertsen TB, Vanky E, Carlsen SM. Anti-Mullerian hormone in the diagnosis of polycystic ovary syndrome: can morphologic description be replaced? Hum Reprod. 2012;27:2494–502.
32. Task Force on the Phenotype of the Polycystic Ovary Syndrome of the Androgen Excess Society. Position statement: the Androgen Excess Society evidence-based criteria for defining the polycystic ovary syndrome as a predominantly hyperandrogenic syndrome. J Clin Endocrinol Metab. 2006;91:9237–45.
33. Orio F, Palomba S. Reproductive endocrinology: new guidelines for the diagnosis and treatment of PCOS. Nat Rev Endocrinol. 2014;10:130–2.
34. NIH Office Disease Prevention. Evidence based methodology workshop on polycystic ovary syndrome. 2012 expert panel guidelines on PCOS.
35. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, Welt CK. Diagnosis and treatment of polycystic ovary syndrome: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2013;98:4565–92.
36. Goodman NF, Cobin RH, Futterweit W, Glueck JS, Legro RS, Carmina E. American Association of Clinical Endocrinologists, American College of Endocrinology, and Androgen Excess and PCOS Society disease state clinical review: guide to the best practices in the evaluation and treatment of polycystic ovary syndrome–part 2. Endocr Pract. 2015;21:1415–26.
37. Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, Gambineri A, Kelestimur F, Macut D, Micic D, Pasquali R, Pfeifer M, Pignatelli D, Pugeat M, Yildiz BO, ESE PCOS Special Interest Group. The polycystic ovary syndrome: a position statement from the European Society of Endocrinology. Eur J Endocrinol. 2014;171:1–29.
38. Dewailly D, Lujan ME, Carmina E, Cedars MI, Laven J, Norman RJ, Escobar-Morreale HF. Definition and significance of polycystic ovarian morphology: a task force report from the Androgen Excess and Polycystic Ovary Syndrome Society. Hum Reprod Update. 2014;20:334–52.
39. Balen AH, Laven JS, Tan SL, Dewailly D. Ultrasound assessment of the polycystic ovary: international consensus definitions. Hum Reprod Update. 2003;9:505–14.
40. Dewailly D. Diagnostic criteria for PCOS: is there a need for a rethink? Best Pract Res Clin Obstet Gyneacol. 2016;37:5–11.
41. Ramezanali F, Ashrafi M, Hemat M, Arabipoor A, Jalali S, Moini A. Assisted reproductive outcomes in women with different polycystic ovary syndrome phenotype: the predictive value of anti-Mullerian hormone. Reprod Biomed Online. 2016;32:503–12.

Anovulation in Women with PCOS

3

Ujvala Rao and Roy Homburg

3.1 Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory subfertility and affects up to 10% of the female population. Given this significant burden, there has been much research into its aetiology and management. Despite many attempts, it has been difficult to define what exactly is the cause of anovulation in PCOS. There are various features of PCOS which could contribute to the disruption of ovulation and these will be discussed in this chapter. Before we launch into this discussion, we will revise the process of normal ovulation.

3.2 Ovulation

The hypothalamus secretes gonadotrophin-releasing hormone (GnRH), a decapeptide that is conveyed into the portal circulation between the hypothalamus and the pituitary. Here, relatively low doses of GnRH, which cannot be easily detected in the peripheral circulation, stimulate the pulsatile secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH pulses are difficult to measure due to its long half-life. The frequency of LH pulses varies during the cycle from every 60–90 min in the follicular phase to 8–12 hourly in the late luteal phase. The amplitude of the pulses also varies and is greater preceding ovulation and again in the luteal phase [1]. This pulsatile release is key to the process of ovulation as sustained release of GnRH has an inhibitory effect on the hypothalamic-pituitary-ovary (HPO) axis.

U. Rao (✉) • R. Homburg
Homerton Fertility Centre, Homerton University Hospital NHS Foundation Trust, London, UK
e-mail: ujvalarao1@gmail.com

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_3

As it develops, the oocyte is enveloped by granulosa cells and theca cells. As the dominant follicle approaches maturity, the theca cells differentiate into the theca interna and externa layers. Towards the end of the menstrual cycle as the corpus luteum degenerates, secretion of progesterone, oestrogen and inhibin A decreases, bringing to an end the negative feedback of these substances upon the hypothalamus. As a result, FSH is released and recruits a cohort of small antral follicles and then induces differentiation of granulosa cells. FSH also has the effect of sensitizing the dominant follicle to the effect of LH, a key factor in producing ovulation approximately 36 h after the LH surge. The pulses of LH act on the theca cells and stimulate the production of the androgens androstenedione and testosterone. These are then conveyed to the granulosa cells where they are converted to oestradiol by the action of aromatase. The activity of aromatase is stimulated by FSH. During this process, oestradiol levels rise, eventually providing negative feedback upon FSH secretion.

Mid-cycle, the large surge of LH secretion is brought about by several factors. Oestradiol levels which have been slowly rising and have a negative feedback effect upon the pituitary suddenly switch to a positive feedback situation. There is also increased sensitivity of the pituitary GnRH receptors. The LH surge has the effect of causing ovulation, stimulates oocyte maturation by inducing resumption of meiosis and produces luteinization of the granulosa cells leading to the formation of the corpus luteum.

The selection of the dominant follicle occurs due to the fact that one follicle usually develops greater sensitivity to FSH stimulation. Therefore, as FSH concentrations fall under the negative feedback mechanism, this follicle continues to grow and produces a greater concentration of oestradiol and inhibin.

3.3 Anovulation in PCOS

In 1935, Stein and Leventhal first described PCOS from their observations of seven women who had enlarged ovaries, amenorrhoea, infertility and hirsutism. Their hypothesis was that the sclerocystic thickening of the ovarian cortex prevented the expulsion of the oocyte and hence led to disturbance of ovulation [2]. This appeared to be supported by the finding that ovarian wedge resection restored ovulation.

As time has gone by, it has become apparent that the basic lesion of PCOS is an endocrinological disturbance within the ovary itself—an excessive production of androgens. This is associated with various extra-ovarian hormonal abnormalities including insulin resistance, hyperinsulinaemia and raised concentration of LH [3]. Despite these many endocrinological associations with PCOS, none of these individually serve to explain the pathogenesis of the condition.

PCOS has been found to be more common in female relatives of affected women, leading to a hypothesis that the condition is genetically inherited though perhaps risk is modified by environmental factors. One study found that 22% of the sisters of women with PCOS fulfilled the diagnostic criteria themselves [4]. It was earlier

thought that the condition exhibited an autosomal dominant pattern of inheritance but this has not been confirmed by more contemporary studies, which have suggested a more complex genetic pattern. Various genes have been implicated such as the fibrillin-3 gene and those that code for insulin receptors such as IRS-1. However, these findings have not been reproducible, at least partially due to small sample sizes and incomplete examination of the genes in question. Pregnant women with PCOS have been found to have elevated testosterone levels. Hence, another hypothesis for the aetiology of PCOS has been intrauterine exposure to androgens. It is, however, not clearly documented that the foetus is exposed to these elevated androgen levels. Cord blood androgen studies have shown mixed results. Increased sex hormone-binding hormone activity as well as the aromatase activity of the placenta may serve to reduce the effective concentration of androgen to which the foetus is exposed. Studies looking to confirm the level of intrauterine androgen exposure are ongoing [5].

In recent years, anti-Mullerian hormone (AMH) has emerged as a key player in the natural history of PCOS. We will explore the role of AMH and various other substances below (see also Chap. 8).

3.3.1 Abnormalities of gonadotrophin Release

PCOS is associated with an increase in pulse frequency and amplitude of LH and a normal or dampened frequency of FSH pulsatility. Studies in the daughters of PCOS patients around the time of puberty have shown that hypothalamic-pituitary abnormalities are apparent this early in a PCOS patient's life. Instead of the usual increase in pulsatility of LH release seen overnight, there is an increase LH pulsatility from the late afternoon. Hence, it is apparent that the GnRH pulse generator is altered very early in the course of PCOS [6]. LH pulse frequency in PCOS women does not exhibit the cyclic variation seen in women with ovulatory cycles. LH pulses are observed approximately hourly throughout the cycle. It is unclear whether the cause for this lies in the hypothalamus, pituitary or peripheral feedback mechanisms.

3.3.2 Hyperandrogenism

The ovaries produce all three classes of sex steroids, namely, oestrogens, progestins and androgens. As distinct from the adrenal gland, the ovary does not have 21α-hydroxylase or 11β-hydroxylase reactions; hence, there is no production of glucocorticoids or mineralocorticoids. The two androgens secreted by the ovary are androstenedione and dehydroepiandrosterone (DHEA). Androstenedione is produced by the stromal and thecal cells of the ovaries under the influence of LH. Around half of the androstenedione production in the female is from the ovary with the other half originating from the adrenal gland. DHEA derives mainly from the adrenal gland. Androstenedione is usually converted into oestradiol by the FSH-driven aromatase enzyme but as described below, aromatase activity is reduced in women with

PCOS. The surplus of androstenedione within the ovary is converted into oestrone and also into testosterone. The ovary also secretes androstenedione into the circulation, and this is partially converted in the peripheral tissues to testosterone. Increased concentrations of androstenedione, testosterone, oestrone and DHEA are seen in women with PCOS [7].

In vitro experiments have found that hyperandrogenism accelerates the development of follicles from primordial follicles to small antral follicles [8]. As a result, the density of pre-antral and small antral follicles in the polycystic ovary is six times that of the normal ovary. These follicles do not appear to undergo the expected progression into ovulatory follicles and also undergo a reduced rate of apoptosis [9]. This explains the typical appearance of the polycystic ovary.

3.3.3 AMH

More details regarding AMH will be given in Chap. 8. Briefly, AMH belongs to the transforming growth factor-beta (TGF-B) family and is produced exclusively by the gonads [10]. It is secreted during early intrauterine life by the Sertoli cells of the developing testes to induce regression of the Mullerian ducts in the male foetus. In females, it is secreted throughout life by the granulosa cells of the early primordial follicles. Its secretion increases and peaks in small antral follicles, and as the follicles progresses to a preovulatory state, the secretion of AMH recedes. Once a follicle reaches 10 mm in size, the AMH secreted by that follicle becomes undetectable. There is a strong correlation between the serum AMH and the number of small antral follicles, and hence it is widely used as a marker of ovarian reserve [11].

Given that women with PCOS have increased numbers of small pre-antral and antral follicles, their AMH concentrations are markedly increased compared to those women with normal ovaries. This has been substantiated by several studies [12, 13]. However, the mere number of follicles is not the only explanation for raised AMH levels in PCOS patients. It has been shown that the granulosa cells of anovulatory ovaries produce up to 75 times more AMH than those of women with regular cycles and normal ovaries and up to twice as much AMH as PCOS patients with ovulatory cycles [14]. Laven et al. also showed that AMH concentrations correlate positively with levels of LH, testosterone, mean ovarian volume as well as the number of ovarian follicles. Perhaps as a result of all of these correlations, the higher the AMH, the more severe the PCOS.

The exact function of AMH beyond foetal life has been the subject of various studies and remains unclear. In studies involving both rodent and human granulosa cells, exposure to high levels of AMH decreased the expression of aromatase messenger RNA [15, 16]. This leads to a lower than expected concentration of oestradiol within follicles [17]. Pellatt et al. proved this finding and further confirmed that AMH also has the effect of reducing the expression of FSH

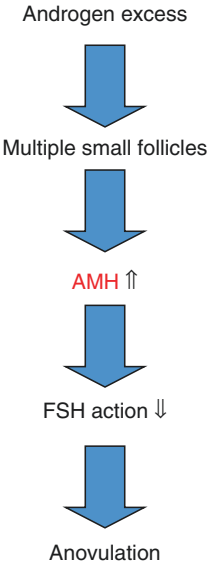


Fig. 3.1 A possible role for AMH in the anovulation of PCOS

receptor mRNA. As a result, it has been hypothesized that AMH inhibits the effect of FSH on follicles until they reach a size greater than 10 mm after which AMH concentrations fall [18].

The concentration of AMH in PCOS patients is demonstrably higher in those women with amenorrhoea compared to those with oligomenorrhoea who, in turn, have higher AMH levels than those with regular menstrual cycles [13]. The corollary to this finding is that the higher the AMH, the greater the ovulatory disturbance.

AMH also appears to interact with the other hormones within the HPO axis, further perpetuating the cycle of anovulation. These mechanisms are described in Fig. 3.1.

3.3.3.1 FSH and AMH

A finding of low FSH concentrations in PCOS patients would help to make sense of the anovulation characteristic of the condition. However, serum FSH levels are usually within normal limits albeit at the lower end of this range. There is however evidence of the fact that there is endogenous inhibition of the action of FSH, likely as a result of high AMH concentrations within the antral follicles [18].

It is apparent that this inhibitory function of AMH can be overcome by exogenous FSH or by stimulating a surge of FSH as in clomiphene ovulation induction. Clomiphene treatment restores ovulation in approximately 80% of patients (see Chap. 9). Even low doses of exogenous FSH have been shown to stimulate dominant follicular development [19]. This is a reassuring finding for those faced with the common clinical scenario of the subfertile PCOS patient.

3.3.3.2 LH and AMH

AMH and LH concentrations show a positive correlation as demonstrated by numerous authors [17, 20, 21]. The exact mechanism of this association is yet to be described but there are various plausible explanations for it. As described earlier in this chapter, disturbance of LH pulse frequency is an early lesion of PCOS and leads to an increased concentration of LH in the circulation. LH receptors are only found on theca cells of the follicles. LH acts on these cells to stimulate the conversion of cholesterol into androstenedione and testosterone. These androgens promote the progression of primordial to pre-antral follicles, which then produce an abundance of AMH.

Extrapolating from these associations, a gross method of reinstating ovulation would be to reduce the number of follicles in the ovaries, which would then result in lower AMH concentrations. This marries up well with the initial findings of Stein and Leventhal, who proved that a wedge resection of ovarian tissue restores ovulation as does the destruction of follicles in laparoscopic ovarian drilling. Beyond the age of 40, there is an accelerated loss of follicles, and hence it is not unusual for PCOS women to resume regular menstrual cycles as they cross this age [22].

However, waiting for a woman to reach the age of 40 or removing part of her ovary would not be high on the list of therapeutic options for the subfertile PCOS patient. There exist several methods of ovulation induction which are discussed in later chapters of this book. AMH concentrations appear to predict the response to these treatments in many cases. Weight loss of less than 5% of body weight has been shown to restore ovulation in up to 60% of PCOS patients (see also Chap. 13). It does however seem that women with higher AMH levels before weight loss are less likely to resume regular menstrual cycles after weight loss [23]. A study comparing AMH values in PCOS patients undergoing laparoscopic ovarian drilling found that women who had a higher AMH pre-procedure were less likely to resume spontaneous ovulation post-operatively and further identified a cut-off AMH of 7.7 ng/mL above which spontaneous ovulation was unlikely [24].

Conclusion

Although the exact aetiology of anovulation in PCOS has not yet been defined, the various factors outlined above, particularly the role of AMH, help us understand the ovulatory disturbance and provide parameters upon which to make treatment decisions.

References

1. Al-Azemi M, Omu FE, Omu AE. The effect of obesity on the outcome of infertility management in women with polycystic ovary syndrome. Arch Gynecol Obstet. 2004;270:205–10.
2. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol. 1935;29:181–91.
3. Homburg R. Androgen circle of polycystic ovary syndrome. Hum Reprod. 2009;24:1548–55.
4. Legro RS, Driscoll D, Strauss 3rd JF, Fox J, Dunaif A. Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. Proc Natl Acad Sci U S A. 1998;95:14956–60.

5. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. Endocr Rev. 2015;36:487–525.
6. Marx TL, Metha AE. Polycystic ovary syndrome: pathogenesis and treatment over the short and long term. Cleve Clin J Med. 2003;70:31–45.
7. Goodman NF, Cobin RH, Futterweit W, Glueck JS, Legro RS, Carmina E. American Association of Clinical Endocrinologists, American Collefe of Endocrinology, and Androgen Excess and PCOS Society Disease state clinical review: guide to the best practice in the evaluation and treatment of polycystic ovary syndrome. Part 1. Endocr Pract. 2015;21:1291–300.
8. Hillier SG, Tetsuka M. Role of androgens in follicle maturation and atresia. Ballière's Clin Obstet Gynaecol. 1997;11:249–60.
9. Beloosesky R, Gold R, Almog B, Dantes A, Land-Bracha A, Hirsh L, Itskovitz-Eldor J, Lessing JB, Homburg R, Amsterdam A. Induction of polycystic ovary by testosterone in immature female rats: modulation of apoptosis and attenuation of glucose/insulin ratio. Int J Mol Med. 2004;14:207–15.
10. Pepinsky RB, Sincalir LK, Chow EP, Mattaliano RJ, Manganaro TF, Donahoe PK, Cate RL. Proteolytic processing of Mullerian inhibiting substance produces a transforming growth factor beta like fragment. J Biol Chem. 1988;263:18961–4.
11. van Rooij IA, Broekmans FJ, Scheffer GJ, Looman CW, Habbema JD, de Jong FH, Fauser BJ, Themmen AP, te Velde ER. Serum antimullerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. Fertil Steril. 2005;83:979–87.
12. Cook CL, Siow Y, Brenner AG, Fallat ME. Relationship between serum Mullerian-inhibiting substance and other reproductive hormones in untreated women with polycystic ovarian syndrome and normal women. Fertil Steril. 2002;77:141–6.
13. Pigny P, Jonard S, Robert Y, Dewailly D. Serum anti-mullerian hormone as a surrogate marker for antral follicle count for definition of the polycystic ovarian syndrome. J Clin Endocrinol Metab. 2006;91:941–5.
14. Pellatt L, Hanna L, Brincat M, Galea R, Brain H, Whitehead *S. granulosa* cell production of anti-Mullerian hormone (AMH) is increased in polycystic ovaries. J Clin Endocrinol Metab 2007;92:240–245.
15. di Clemente N, Goxe B, Remy JJ, Cate R, Josso N, Vigier B. Inhibitory effect of AMH upon the expression of aromatase and LH receptors by cultured granulose cells of rat and porcine immature ovaries. Endocrine. 1994;2:553–8.
16. Grossman MP, Nakajima ST, Fallat ME, Siow Y. Mullerian-inhibiting substance inhibits cytochrome P450 aromatase activity in human granulosa lutein cell culture. Fertil Steril. 2007;89:1364–70.
17. Laven JS, Mulders AG, Visser JA. Anti-Müllerian hormone serum concentrations in normoovulatory and anovulatory women of reproductive age. J Clin Endocrinol Metab. 2004;89:318–23.
18. Pellatt L, Rice S, Dilaver N, Heshri A, Galea R, Brincat M, Brown K, Simpson ER, Mason HD. Anti-Müllerian hormone reduces follicle sensitivity to follicle-stimulating hormone in human granulosa cells. Fertil Steril. 2011;96:1246–51.
19. Homburg R, Howles CH. Low-dose FSH therapy for anovulatory infertility associated with polycystic ovary syndrome: rational, results, reflections refinements. Hum Reprod Update. 1999;5:493–9.
20. Piouka A, Famakiotis D, Katsikis I. Anti-Müllerian hormone levels reflect severity of PCOS but are negatively influenced by obesity: relationship with increased luteinizing hormone levels. Am J Physiol Endocrinol Metab. 2009;296:238–43.
21. Homburg R, Ray A, Bhide P, Gudi A, Shah A, Timms P, Grayson K. The relationship of serum anti-Mullerian hormone with polycystic ovarian morphology and polycystic ovary syndrome: a prospective cohort study. Hum Reprod. 2013;28:1077–83.
22. Elting MW, Kwee J, Korsen TJ. Aging women with polycystic ovary syndrome who achieve regular menstrual cycles have a smaller follicle cohort than those who continue to have irregular cycles. Fertil Steril. 2013;79:1154–60.

23. Moran LJ, Noakes M, Clifton PM, Norman RJ. The use of anti-Müllerian hormone in predicting menstrual response after weight loss in overweight women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2007;92:3796–802.
24. Amer SA, Li TC, Ledger WL. The value of measuring anti-Müllerian hormone in women with anovulatory polycystic ovary syndrome undergoing laparoscopic ovarian diathermy. Hum Reprod. 2009;24:2760–6.

Oocyte Quality in PCOS

4

Christine Decanter

4.1 Introduction

Polycystic ovarian morphology (PCOM) and polycystic ovary syndrome (PCOS) are very common findings in an in vitro fertilization (IVF) centre population, explaining why the concept of oocyte quality is so challenging. Indeed, 18–25% of the infertile couples meet the diagnosis criteria for PCOS ([1]; http://www.ivf-worldwide.com/survey/pcos-results.htm), whereas the prevalence of PCOM has been estimated as high as 33% in asymptomatic patients [2–4]. With the advent of highly sensitive ultrasound machines, the PCOM, mainly based on the antral follicle count, has become one of the main diagnosis criteria of PCOS and is now well recognized to be the common base of the wide spectrum of clinical, hormonal and metabolic phenotypes of the PCOS. PCOM is characterized by a significantly enlarged cohort of early growing and recruitable follicles. This excessive follicle number is linked to disturbances in folliculogenesis which are thought to be the consequence of intra-ovarian hyperandrogenism [5–7] (see also Chaps. 2 and 8). During controlled ovarian hyperstimulation (COH), the cohort of growing follicles is frequently heterogeneous in size, with mature, intermediary and small follicles. In parallel, the number and quality of mature oocytes has been proposed as being poor [6, 8, 9] leading to lower pregnancy rates and higher abortion rates. Furthermore, recent data suggested that oocyte competence could be impaired in PCOS patients due to an inadequate dialogue between the cumulus cells and the oocyte and an impairment of the follicular microenvironment [10, 11].

Despite these assumptions, the paucity of clinical studies focusing on oocyte quality in PCOS women does not allow to make definitive conclusions. Most of

C. Decanter
Centre d'Assistance Médicale à la Procréation et de Préservation de la Fertilité, Hôpital Jeanne de Flandre, EA 4308 "Gamétogénèse et Qualité du Gamète", Centre Hospitalier Universitaire de Lille, Lille, France
e-mail: christine.decanter@chru-lille.fr

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_4

these studies are retrospective, concerning PCOS patients diagnosed with heterogeneous criteria and with various clinical and metabolic phenotypes with a historical control group [12–17] (see also Chaps. 2 and 7).

Mostly, oocyte quality was only extrapolated through fecundation, implantation and pregnancy rates according to the number of mature oocytes, i.e. metaphase II oocytes [12–17]. Consequently, the results provided by these studies are conflicting, reporting either a better oocyte/embryo quality and pregnancy rates or vice versa.

The concept of oocyte quality represents the oocyte developmental competence, meaning the intrinsic ability of oocytes to undergo meiotic maturation, fertilization, proper embryonic development and successful pregnancy [18]. These competences are progressively acquired during the follicular development through the cross-talk between oocyte and somatic/surrounding granulosa cells (GCs) [19]. As follicular growth disturbances have been widely shown in PCOS, especially during COH, it has been postulated that the consecutive endocrine and/or paracrine follicular microenvironment modifications could have detrimental effects on the oocyte quality.

In this review, we will firstly focus on the main approaches proposed to investigate oocytes from PCOS, and then we will look over the clinical relevance of all the compiled results from these studies by examining the final oocyte competence during the IVF process in PCOS patients.

4.2 Evaluation of Oocyte Quality

Evaluating the oocyte quality is obviously complicated because of the few number of oocytes retrieved during the IVF process. Hence, there is various ways, invasive or non-invasive, direct or indirect, to investigate the oocyte competence. Three main approaches, morphological, genetic and OMICS, have been proposed to investigate oocytes from PCOS.

4.2.1 Morphological Approach

After having removed the cumulus-corona cells in preparation for intracytoplasmic injection (ICSI), oocyte evaluation is based on the nuclear maturation status, the morphology of the cytoplasm and on the appearance of the extracytoplasmic structures. A higher number but a same rate of metaphase II oocytes between PCOS patients and controls were highlighted in various retrospective studies [12–14], as well as a higher number but a same rate of top-quality embryos [13, 14]. These results were confirmed in the meta-analysis of Heijnen et al. and in a recent prospective study comparing PCOM patients versus non-PCOM [20, 21]. Despite being the main morphological indicator, nuclear maturity examination alone is not enough to determine the quality of an oocyte: the nucleus and the cytoplasm have to mature in synergy in order to reach the conditions for an optimal fertilization rate. In addition, it is now well recognized that some specific morphological oocyte abnormalities,

such as the presence of a wide perivitelline space (PVS) or a granular cytoplasm, must be given attention since it has been reported that they are associated with a significant decrease in the chance of fertilization [22, 23]. Only three studies are interested in oocyte morphology in PCOS patients. Sahu et al. [13] reported similar oocyte morphology in isolated PCOM, PCOS and age-matched controls. Sigala et al. [21] performed a prospective comparative study with a systematic examination of oocyte morphology in PCOM versus non-PCOM patients. Nuclear maturation, extra- and intracytoplasmic oocyte abnormalities were assessed, i.e. fragmented or abnormal first polar body (IPB), abnormal zona pellucida (ZP), presence of an enlarge perivitelline space (PVS) or material in the PVS and an abnormal shape of the oocyte [21]. No specific morphological abnormalities as well as no difference regarding the incidence of these abnormalities were observed in the PCOM versus non-PCOM patients. In this study, PCOM was defined according to the threshold of 19 follicles per ovary. Among the PCOM group, there were 52.5% of PCOS and 47.5% of PCOM only. No difference was observed between oocytes from PCOS and PCOM-only patients, but it has to be mentioned that obese PCOM patients were excluded [21]. Piomboni et al. [24] compared the oocyte quality based on the precited morphologic criteria in three groups of nonobese PCOS patients: PCOS treated by D-chiro-inositol, PCOS treated by metformin and non-treated PCOS. They showed a significantly higher number of good-quality oocytes in the groups treated by insulin-sensitizing agents, as well as they highlighted in parallel a significant reduction of reactive oxygen species production in the follicular fluid [24]. Data on the effect of inositol on oocyte quality are extensively reviewed in Chap. 16.

A few studies using polarized light microscopy (PLM) have shown, by highlighting meiotic spindle abnormalities, that some of metaphase II oocytes may still be immature [18, 25]. Indeed, meiotic spindle, when detectable with polscope microscope, is not always aligned with the first polar body (PB1) in fresh metaphase II oocytes, which may adversely affect the fertilizing ability and in vitro/in vivo developmental competence [25]. In addition, it has recently been shown that oocytes with normal spindle morphology are significantly more likely to produce euploid embryos [26]. Only two studies are interested in the spindle and chromosome configurations in oocytes from PCOS patients. Li et al. [27] compared the incidence of abnormality in spindle and chromosomal configurations in both in vitro and in vivo-matured oocytes. A higher rate of abnormalities was found in the group of in vitro-matured oocytes [27]. Vieira et al. compared in vitro-matured oocytes from PCOS versus non-PCOS patients and didn't find any difference [28]. To the point of view of the authors, it is more likely that the in vitro conditions of oocyte maturation might explain these meiotic abnormalities rather than the PCOS itself.

4.2.2 Genetic Approach

The decrease of fertilization rate and the increase in pregnancy loss in certain subgroups of PCOS patients have led to the hypothesis that oocyte and embryos could be of poorer quality due to a higher aneuploidy rate. Morphological

examination is insufficient to detect genetic abnormalities such as aneuploidy. Currently, the only method yielding a definitive evaluation of oocytes/embryos is aneuploidy diagnosis, which provides information of normal or abnormal chromosomal constitution. Moreover, aneuploidy detection requires breach of the zona pellucida and biopsy of the polar bodies from the oocyte that is obviously invasive. Because of the difficulty to directly evaluate the ploidy of the oocytes, some studies investigated indirectly the genetic potential of the PCOM gamete. Sengoku et al. [29] performed cytogenetic analysis on the unfertilized oocytes with normal morphology from PCOS and control patients but didn't find any difference in the incidence of aneuploidy or diploidy [29]. Weghofer et al. [30] examined the association between PCOS and embryonic aneuploidy. They compared the results of preimplantation genetic diagnosis (PGD) between properly documented PCOS patients and controls. Despite a statistically higher absolute number of euploid embryos in PCOS group due to the higher number of metaphase II oocytes retrieved, there was no difference regarding the rate of aneuploidy [30]. Wang et al. [31] conducted a prospective cohort study by performing genetic analysis on abortuses from PCOS and non-PCOS patients who conceived after IVF. The aneuploidy rate was not significantly higher in the PCOS group but significantly lower in comparison with the non-PCOS patients [31].

4.2.3 OMICS Approach

The emergence of the—OMICS technologies, i.e. epigenomics, genomics, transcriptomics, proteomics and metabolomics, provides a huge amount of new information regarding the biological processes involved in the reproductive field. Although using an invasive technology, microarray-based transcription profiles of oocytes at various stage of growth and maturation have led to a better understanding of the genes expressed during oocyte development: disruption of transcription within an oocyte or any modification of their adequate transcriptomes could compromise its growth and development, as well as the resulting embryo, since oocyte mRNAs pool is strongly correlated with the ability to develop into the blastocyst stage [32].

Recent cluster of analysis revealed differences in global gene expression profile between normal and PCOS tissues and oocytes [33]. Wood et al. [34] identified 374 genes with different mRNA transcript levels when analyzing metaphase II oocytes from normal responders and PCOS patients. A subset of these genes found to be differentially expressed in PCOS is involved in spindle dynamics, homologous recombination/chromosome alignment, cell cycle checkpoint and centrosome function during mitosis and/or meiosis [34]. Furthermore, some of the other differentially expressed genes contain putative androgen receptors and/or peroxisome proliferating Y binding sites [34]. The authors make the assumption that these observations could be related to a lower oocyte quality.

Cai et al. [35] interested in the in vitro effects of overexpression of Hsp27 on oocyte maturation and development derived from PCOS patients as emerging

evidence indicates this heat-shock protein has strong antiapoptotic properties and has been shown to be mainly expressed in human oocytes. Interestingly, the same team has previously shown that Hsp27 was downregulated in ovarian tissue and in oocytes isolated from women with PCOS [36]. The results of upregulation of Hsp27 expression were a lowered oocyte maturation rate, similar fertilization but high-quality embryo blastocyst formation rates leading the authors to postulate that Hsp27 could be involved in the apoptotic imbalance of the oocytes via growth and differentiation factor 9 (GDF9) and bone morphogenic protein (BMP15) [36].

It is now recognized that there is a continuous bidirectional cross-talk between oocytes and somatic cells during folliculogenesis through gap junctions and paracrine signalling [19]. If it is sure that oocyte is nurtured and supported by the closely associated somatic cells, especially those of the cumulus, the fact remains that oocyte itself plays an active role via secretion of paracrine signalling factors, such as GDF9 and BMP15, which maintain an appropriate microenvironment for a proper follicular growth [19, 33]. It is now possible to identify the transcriptome of GCs with the microarray technology, and evidence supporting GCs genes markers as valuable and non-invasive predictors of oocyte competence is rapidly emerging [37].

As folliculogenesis disturbances frequently occur in PCO patients, it seemed logical to investigate this oocyte/granulosa cells dialogue. Ouandaogo et al. [38] compared the transcriptome profiles of cumulus cells (CCs) isolated from in vivo and IVM cumulus-oocyte complexes (COCs) at different nuclear maturation stages from normal responders and PCOS patients undergoing ICSI following in vivo or IVM. In the PCOS subgroup, the authors found a strong alteration of the expression profile of the CCs derived from IVM metaphase II oocytes in comparison to in vivo metaphase II oocytes [37]. The expression profile also differed significantly between normal and PCOS patients, but the authors conclude that these significant differences were related to the culture condition, not to the PCOS per se [37]. The same team focused few months later on the gene expression profile in CCs of in vivo metaphase II oocytes from PCOS and non-PCOS patients using the DNA microarray technology [39]. There were significant differences between groups in the gene expression profile. In addition, CCs from PCOS patients were characterized by abnormal expression of many growth factors, including members of the epidermal growth factor-like and IGF-like families that are known to play a role in oocyte competence [39]. Likewise, mRNA transcripts of factors involved in steroid metabolism seem to be deregulated in PCOS CCs [39].

miRNAs are small, non-coding RNAs detected in biological fluids that are able to regulate gene expression at the post-transcriptional level and which may be involved in reproductive function [40]. A limited number of studies have aimed to extensively profile circulating microRNAs (miRNAs) expression and function in the follicular fluid within a PCOS study population, and the results are at times not yet conclusive [40, 41]. Both highlight different miRNA expression between PCOS and non-PCOS patients but with no clear correlation with oocyte maturation or fertilization competence [40, 41].

4.3 Clinical Data

Research on oocyte is extremely complex, especially in PCOS patients undergoing IVF. Firstly, the fact that PCOS patients need IVF, independently of tubal or sperm alterations, introduces some recruitment bias. Secondly, the high ovarian response under COH in these patients may have detrimental effects on the oocyte quality through vascular and inflammatory factors. Likewise, the in vitro/in vivo culture conditions may play a major role in the oocyte quality. PCOS underlies endocrine, ovulatory and/or metabolic dysfunction. These three components are solely or synergistically strong confounders regarding the interpretation of the studies on oocyte quality. Indeed, the source of oocytes is highly variable according to the different PCOS phenotypes, from the lean PCOM-only patient to the obese PCOS patient. In addition, the question as to whether asymptomatic women with PCOM constitute a heterogeneous population in terms of ovarian dysfunction ranging from entirely healthy ovulatory women to women with mild occult PCOS [2] or, alternatively, constitute a homogenous population representing the milder end of the PCOS spectrum remains debatable. More data about PCOS phenotypes are discussed in Chaps. 2 and 7.

Undoubtedly, oocytes from PCOM in IVF condition exhibit molecular specificities in comparison with oocytes from non-PCOM patients. But surely, the key point is to have sufficient oocytes of quality to give substantial chances of pregnancy. One has to recognize that the larger prospective studies regarding the IVF results in PCOS failed to highlight poor results in terms of pregnancy chances, at least in nonobese patients.

Heijnen et al. [20] reported in a meta-analysis the comparative IVF outcome of PCOS patients defined by the Rotterdam criteria to the one of matched non-PCOS controls. Except the higher number of oocytes in PCOS patients, they failed to find any difference between fertilization, pregnancy and take baby home rates between groups [20]. Likewise, Sigala et al. [21] in a large prospective comparative study have shown the same rate of metaphase II and morphologically normal oocytes in nonobese PCOM versus nonobese non-PCOM patients. The rate of top-quality embryo was equivalent in the two groups while the implantation and clinical pregnancy rates were even better in the PCOM group [21]. These results were also confirmed after having divided the PCOM group in PCOS and "sonographic-only" PCOM. Engmann et al. [15] previously reported same results in PCOM patients. Hence, the bad prognosis frequently argued regarding pregnancy rates and/or abortion risk could be more related to the metabolic profile than to PCOM per se. It is well known that high body mass index (BMI) and hyperinsulinaemia are main contributors to the follicular microenvironment disturbances [9]. Indeed, studies in follicular fluids from PCOS patients highlight high levels of interrelated endocrino-metabolic factors such as androgens, VEGF, AMH, insulin and IGF, all of them playing an active role in the oocyte-CCs dialogue [9]. Moreover, numerous studies highlighted benefits from taking insulin-sensitizing agents like metformin as a co-treatment before and during the IVF process [42, 43]. The use of insulin-sensitizing drugs in PCOS is reviewed in Chap. 11.

From another point of view, as mentioned above, it seems that the meiotic/mitotic cell cycle pathway is altered in PCOS oocytes [34], but no more aneuploid oocytes were detected in PCOS patients [29] as well as no more aneuploidy embryos were observed [30]. It is therefore difficult to make the connection between such molecular specificities and the reality of the clinical experience.

Conclusion

It remains difficult to conclude about the oocyte competence in PCOS. Molecular specificities have been properly highlighted in PCOS oocytes, but it doesn't mean that there are abnormalities in their reproductive competence. Too few studies have taken into account the very wide spectrum of PCOS phenotypes and the potential influence of comorbidity factors such as obesity and insulin resistance in their analysis. However, the largest prospective studies performed in nonobese PCOM patients so far are in favour to good pregnancy chances due to a high number of good-quality oocytes. The coexistence of a metabolic syndrome to PCOM seems to impair the oocyte competence and the pregnancy rates. Prospective studies with consensual international diagnosis criteria are needed to allow in better understanding of the exact molecular mechanisms involved in the oocyte competence according to each phenotype of PCOS and would elucidate if the PCOS oocyte deserve its bad reputation.

References

1. Hull MG, Glazener CM, Kelly NJ, Conway DI, Foster PA, Hinton RA, et al. Population study of causes, treatment, and outcome of infertility. Br Med J. 1985;291:1693–7.
2. Mortensen M, Ehrmann DA, Littlejohn E, Rosenfield RL. Asymptomatic volunteers with a polycystic ovary are a functionally distinct but heterogeneous population. J Clin Endocrinol Metab. 2009;94:1579–86.
3. Kim YJ, Ku S-Y, Jee BC, Suh CS, Kim SH, Choi YM, et al. A comparative study on the outcomes of in vitro fertilization between women with polycystic ovary syndrome and those with sonographic polycystic ovary-only in GnRH antagonist cycles. Arch Gynecol Obstet. 2010;282:199–205.
4. March WA, Moore VM, Willson KJ, Phillips DIW, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod. 2010;25:544–51.
5. Jonard S. The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, may be the main culprit for the follicular arrest. Hum Reprod Update. 2004;10:107–17.
6. Franks S, Stark J, Hardy K. Follicle dynamics and anovulation in polycystic ovary syndrome. Hum Reprod Update. 2008;14:367–78.
7. Homburg R. Androgen circle of polycystic ovary syndrome. Hum Reprod. 2009;24:1548–55.
8. Dumesic D, Abbott D. Implications of polycystic ovary syndrome on oocyte development. Semin Reprod Med. 2008;26:53–61.
9. Qiao J, Feng HL. Extra- and intra-ovarian factors in polycystic ovary syndrome: impact on oocyte maturation and embryo developmental competence. Hum Reprod Update. 2010;17:17–33.
10. Kenigsberg S, Bentov Y, Chalifa-Caspi V, Potashnik G, Ofir R, Birk OS. Gene expression microarray profiles of cumulus cells in lean and overweight-obese polycystic ovary syndrome patients. Mol Hum Reprod. 2008;15:89–103.

11. Kwon H, Choi D-H, Bae J-H, Kim J-H, Kim Y-S. mRNA expression pattern of insulin-like growth factor components of granulosa cells and cumulus cells in women with and without polycystic ovary syndrome according to oocyte maturity. Fertil Steril. 2010;94:2417–20.
12. Ludwig M, Finas DF, Al-Hasani S, Diedrich K, Ortmann O. Oocyte quality and treatment outcome in intracytoplasmic sperm injection cycles of polycystic ovarian syndrome patients. Hum Reprod. 1999;14:354–8.
13. Sahu B, Ozturk O, Ranierri M, Serhal P. Comparison of oocyte quality and intracytoplasmic sperm injection outcome in women with isolated polycystic ovaries or polycystic ovarian syndrome. Arch Gynecol Obstet. 2008;277:239–44.
14. Esinler I, Bayar U, Bozdag G, Yarali H. Outcome of intracytoplasmic sperm injection in patients with polycystic ovary syndrome or isolated polycystic ovaries. Fertil Steril. 2005;84:932–7.
15. Engmann L, Maconochie N, Sladkevicius P, Bekir J, Campbell S, Tan SL. The outcome of in-vitro fertilization treatment in women with sonographic evidence of polycystic ovarian morphology. Hum Reprod. 1999;14:167–71.
16. Esmailzadeh S, Faramarzi M, Jorsarai G. Comparison of in vitro fertilization outcome in women with and without sonographic evidence of polycystic ovarian morphology. Eur J Obstet Gynecol Reprod Biol. 2005;121:67–70.
17. Zhong Y-P, Ying Y, Wu H-T, Zhou C-Q, Xu Y-W, Wang Q, et al. Comparison of endocrine profile and in vitro fertilization outcome in patients with PCOS, ovulatory PCO, or normal ovaries. Int J Endocrinol. 2012;2012:1–6.
18. Rienzi L, Balaban B, Ebner T, Mandelbaum J. The oocyte. Hum Reprod. 2012;27:2–21.
19. Li Q, McKenzie LJ, Matzuk MM. Revisiting oocyte-somatic cell interactions: in search of novel intrafollicular predictors and regulators of oocyte developmental competence. Mol Hum Reprod. 2008;14:673–8.
20. Heijnen EM, Eijkemans MJ, Hughes EG, Laven JS, Macklon NS, Fauser BC. A meta-analysis of outcomes of conventional IVF in women with polycystic ovary syndrome. Hum Reprod Update. 2006;12:13–21.
21. Sigala J, Sifer C, Dewailly D, Robin G, Bruyneel A, Ramdane N, Lefebvre-Khalil V, Mitchell V, Decanter C. Is polycystic ovarian morphology related to a poor oocyte quality after controlled ovarian hyperstimulation for intracytoplasmic sperm injection? Results from a prospective, comparative study. Fertil Steril. 2015;103:112–8.
22. Rienzi L, Vajta G, Ubaldi F. Predictive value of oocyte morphology in human IVF: a systematic review of the literature. Hum Reprod Update. 2011;17:34–45.
23. Setti AS, Figueira RCS, Braga DPAF, Colturato SS, Iaconelli A, Borges E. Relationship between oocyte abnormal morphology and intracytoplasmic sperm injection outcomes: a meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2011;159:364–70.
24. Piomboni P, Focarelli R, Capaldo A, Stendardi A, Cappelli V, Cianci A, La Marca A, Luddi A, De Leo V. Protein modification as oxidative stress marker in follicular fluid from women with polycystic ovary syndrome: the effect of inositol and metformin. J Assist Reprod Genet. 2014;31:1269–76.
25. Rienzi L, Ubaldi F, Martinez F, Iacobelli M, Minasi MG, Ferrero S, Tesarik J, Greco E. Relationship between meiotic spindle location with regard to the polar body position and oocyte developmental potential after ICSI. Hum Reprod. 2003;18:1289–93.
26. Tilia L, Venetis C, Kilani S, Cooke S, Chapman M. Is oocyte meiotic spindle morphology associated with embryo ploidy? A prospective cohort study. Fertil Steril. 2016;105:1085–92.
27. Li Y, Feng HL, Cao YJ, Zheng GJ, Yang Y, Mullen S, Critser JK, Chen ZJ. Confocal microscopic analysis of the spindle and chromosome configurations of human oocytes matured in vitro. Fertil Steril. 2006;85:827–32.
28. Vieira RC, Barcelos ID, Ferreira EM, Martins WP, Ferriani RA, Navarro PA. Spindle and chromosome configurations of in vitro-matured oocytes from polycystic ovary syndrome and ovulatory infertile women: a pilot study. J Assist Reprod Genet. 2011;28:15–21.
29. Sengoku K, Tamate K, Takuma N, Yoshida T, Goishi K, Ishikawa M. The chromosomal normality of unfertilized oocytes from patients with polycystic ovarian syndrome. Hum Reprod. 1997;12:474–7.

30. Weghofer A, Munne S, Chen S, Barad D, Gleicher N. Lack of association between polycystic ovary syndrome and embryonic aneuploidy. Fertil Steril. 2007;88:900–5.
31. Wang Q, Luo L, Lei Q, Lin MM, Huang X, Chen MH, Zeng YH, Zhou CQ. Low aneuploidy rate in early pregnancy loss abortuses from patients with polycystic ovary syndrome. Reprod Biomed Online. 2016;33:85–92.
32. Egea RR, Puchalt NG, Escrivá MM, Varghese AC. OMICS: current and future perspectives in reproductive medicine and technology. J Hum Reprod Sci. 2014;7:73–92.
33. Assou S, Boumela I, Haouzi D, Anahory T, Dechaud H, De Vos J, Hamamah S. Dynamic changes in gene expression during human early embryo development: from fundamental aspects to clinical applications. Hum Reprod Update. 2011;17:272–90.
34. Wood JR, Dumesic DA, Abbott DH, Strauss JF 3rd. Molecular abnormalities in oocytes from women with polycystic ovary syndrome revealed by microarray analysis. J Clin Endocrinol Metab. 2007;92:705–13.
35. Cai L, Ma X, Liu S, Liu J, Wang W, Cui Y, Ding W, Mao Y, Chen H, Huang J, Zhou Z, Liu J. Effects of upregulation of Hsp27 expression on oocyte development and maturation derived from polycystic ovary syndrome. PLoS One. 2013;8:e83402.
36. Ma X, Fan L, Meng Y, Hou Z, Mao YD, Wang W, Ding W, Liu JY. Proteomic analysis of human ovaries from normal and polycystic ovarian syndrome. Mol Hum Reprod. 2007;13:527–35.
37. Ouandaogo ZG, Frydman N, Hesters L, Assou S, Haouzi D, Dechaud H, Frydman R, Hamamah S. Differences in transcriptomic profiles of human cumulus cells isolated from oocytes at GV, MI and MII stages after in vivo and in vitro oocyte maturation. Hum Reprod. 2012;27:2438–47.
38. Haouzi D, Assou S, Monzo C, Vincens C, Dechaud H, Hamamah S. Altered gene expression profile in cumulus cells of mature MII oocytes from patients with polycystic ovary syndrome. Hum Reprod. 2012;27:3523–30.
39. Fragouli E, Lalioti MD, Wells D. The transcriptome of follicular cells: biological insights and clinical implications for the treatment of infertility. Hum Reprod Update. 2014;20:1–11.
40. Sørensen AE, Wissing ML, Salö S, Englund AL, Dalgaard LT. MicroRNAs related to polycystic ovary syndrome (PCOS). Genes (Basel). 2014;5:684–708.
41. Scalici E, Traver S, Mullet T, Molinari N, Ferrières A, Brunet C, Belloc S, Hamamah S. Circulating microRNAs in follicular fluid, powerful tools to explore in vitro fertilization process. Sci Rep. 2016;6:24976.
42. Palomba S, Falbo A, La Sala GB. Effects of metformin in women with polycystic ovary syndrome treated with gonadotrophins for in vitro fertilisation and intracytoplasmic sperm injection cycles: a systematic review and meta-analysis of randomised controlled trials. BJOG. 2013;120:267–76.
43. Tso LO, Costello MF, Albuquerque LE, Andriolo RB, Macedo CR. Metformin treatment before and during IVF or ICSI in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2014;11:CD006105.

Endometrial Receptivity in PCOS

5

Giuseppe Benagiano, Paola Bianchi, and Ivo Brosens

5.1 Introduction

The presence of the polycystic ovary syndrome (PCOS) has profound medical implications for the health of women, well beyond reproduction. This has now been documented in a comparative longitudinal study that followed women with the disease and control subjects and found that the presence of PCOS was associated with an increase in hospitalization for reasons unrelated to reproduction, including diabetes, obesity, hypertensive disorders, ischemic heart disease, etc. Interestingly, affected subjects had a higher admission rate for treatment of infertility (40.9 vs. 4.6%), and miscarriage (11.1 vs. 6.1%), and were more likely to require in vitro fertilization (IVF) treatments (17.2 vs. 2.0%) [1]. The number and complexity of abnormalities observed in women with PCOS suggests that other factors behind anovulation may play a role in the infertility or subfertility state of these women, although the heterogeneity of the syndrome, including altered expression of genes affecting signal transduction pathways controlling, among others, steroidogenesis, steroid hormone and gonadotrophin action and regulation, insulin secretion and sensitivity, energy homeostasis and chronic inflammation, makes a systematic approach very difficult [2].

It is generally believed that in most cases, PCOS-related infertility results from the absence of ovulation. At the same time, expert opinions and international guidelines underline that there is evidence that anovulation is not the only reason for these women's failure to conceive [3–5].

G. Benagiano (✉)
Department of Obstetrics, Gynaecology and Urology, Sapienza, University of Rome "La Sapienza", Rome, Italy
e-mail: pinoingeneva@bluewin.ch

P. Bianchi
Department of Medico-Surgical Sciences and Translational Medicine, Sant'Andrea Hospital, Faculty of Medicine and Psychology, University of Rome "La Sapienza", Rome, Italy

I. Brosens
Faculty of Medicine, Catholic University of Leuven, Leuven, Belgium

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_5

Irrespective of its origin, PCOS-related infertility can be successfully treated as documented in a very recent review of 9068 women with PCOS showing that standardized fertility ratios before and after treatment went from 0.80 [95% confidence interval (CI) 0.77 to 0.83] to 1.16 (95%CI 1.12 to 1.20), similar to that of the background population [6]. A recent meta-analysis of randomized controlled trials (RCTs) comparing outcomes of IVF in infertile women with and without PCOS confirmed the success of available treatments, since no difference could be found between groups in clinical pregnancy and live birth rates per cycle [7].

Going back to the origin of PCOS-related infertility, as mentioned available evidence indicates that additional factors may contribute to subfertility in these subjects. A factor that can obviously influence conception rates is oocyte quality, which will be discussed in the Chap. 4. Among other specific factors influencing pregnancy rates in PCOS, a special role can be attributed to endometrial dysfunction, a phenomenon also characterized by a variety of changes in endometrial histomorphology and receptivity markers that apparently cannot be corrected by conventional doses of progesterone [8]. Indeed, notwithstanding the availability of diverse protocols capable of correcting ovulatory disorders, spontaneous pregnancy rates in PCOS have remained comparatively low [9, 10], although they seem to have improved with the use of metformin [11].

Intriguingly, complications do not seem to stop once a woman with PCOS conceives, since she will be at a higher risk of miscarriage, both after spontaneous or assisted conception (ART) [12–14]. This situation can be improved through the use of metformin, an indirect proof that—at least in part—hyperinsulinaemic insulin resistance (IR) contributes to early pregnancy losses (EPL) [15]. Indeed, IR independently increases eightfold the risk of spontaneous abortion after ART, suggesting to be one of the main risk factors for EPL [16]. Also, a meta-analysis of eight studies including 1106 patients with PCOS calculated that risk of EPL could be reduced by about 70% when using metformin [17]. In addition, meta-analyses and literature reviews have documented the existence of an association between the presence of PCOS and major obstetric complications [18–20] and in the already mentioned review of almost 10,000 cases, adjusted odds for preeclampsia, gestational diabetes and premature delivery were all increased compared with controls [6]. This topic will be extensively discussed in Chap. 22, and the reader is referred to it.

Based on these considerations, in the present chapter we will focus on abnormalities in endometrial receptivity and persistence in these subjects of ontogenetic progesterone resistance observed at birth in a majority of neonates. These two important and somewhat neglected features will be illustrated, and suggestions will be offered on how to improve the clinical situation.

5.2 Endometrial Functions and Receptivity in Women with PCOS

First of all, it should be stressed that different information is obtained when reviewing experimental data on the effect of PCOS on the endometrium and therefore on the fertile window and implantation, then when analysing clinical information. In

the latter case, a number of potential confounders exist. Indeed, the effect on the endometrium of factors, such as hyperandrogenism, insulin resistance and obesity, must be taken into consideration before a firm conclusion can be drawn. As we will see when discussing implantation (see Sect. 5.3), endometrial competence is of paramount importance for the establishment of a successful pregnancy, and factors like obesity, hyperinsulinaemia and more generally metabolic alterations can impair endometrial receptivity.

On the other hand, impaired endometrial function in subfertile women can exist even in the absence of PCOS. Finally, the possibility exists that different PCOS phenotypes may contribute differently to endometrial abnormalities, representing yet another possible variable. In this regard, a very recent review of modern knowledge of markers of endometrial abnormalities in PCOS women reported that they relate to steroid hormone action [21]. Among the modifications observed there were alterations of:

1. Oestrogen, progesterone and androgen receptors and their coactivators
2. Endometrial receptivity/decidualization markers, such as the homeobox protein HOXA10, αvβ3 integrin and insulin-like growth factor-binding protein 1 (IGFBP-1)
3. Insulin receptors and growth factors, glucose transporters
4. Markers of inflammation/immune cell migration, such as interleukin 6, CC-motif ligand and uterine natural killer cells

As a consequence, sequential changes in gene expression are perturbed.

On the other hand, if there is persistent anovulation (often associated to hyperinsulinaemia and hyperandrogenaemia), circulating levels of oestradiol (E_2) are relatively constant in the range of those observed in the early follicular phase. These levels, however, may be enhanced through an increased peripheral conversion of androstenedione to oestrone in adipose tissue. In this connection, the expression of oestrogen receptors alpha and beta (ERα, ERβ) and of the G-protein-coupled ER (GPR30) (a transmembrane receptor that promotes specific binding of naturally occurring and synthetic oestrogens) has been measured in the endometrium of PCOS patients and controls during the window of implantation and resulted significantly lower in the PCOS group [22]. Also found was a significant difference in the endometrial pattern, as measured by ultrasound [22].

In spite of these uncertainties, a careful analysis of the specific features of the endometrium in women with PCOS is of great value for a better understanding of the role of factors other than anovulation in the pathogenesis of subfertility in these subjects (Table 5.1).

It seems that when PCOS subjects are oligo- or anovulatory, the regulatory role of progesterone is suboptimal or absent, causing a relatively enhanced response to E_2 [23]. Under these conditions, if folliculogenesis is altered (see Chap. 4), it stands to reason that also luteogenesis will be altered; therefore, in subjects with PCOS, two different anomalies may exist with regard to progesterone and its effects on the endometrium: altered production and altered utilization. The latter phenomenon, known as ‘progesterone resistance’, will be discussed in the subsequent section.

Table 5.1 Different endometrial markers in proliferative phase, secretory phase and hyperplasia in women with PCOS

Markers	PE	SE	HP
Glucose metabolism			
IGFBP-1	▼	▼	
GLUT 4	▼		▼
IRS-1	▼		
Glucose action	▼		
Inflammation			
IL-6	Δ	Δ	
CCL2 (MCP-1)	Δ		
IL-8	Δ	Δ	
RANTES		Δ	
uNK cells		▼	
MMPs			
MMP2		Δ	
MMP3		Δ	
Steroid hormone action			
HOXA10		▼	
AR	Δ	Δ	Δ
PR		Δ	
ERα	Δ(−)	Δ	Δ
ERβ	Δ	(−)	Δ
(avb3) integrin	▼	▼	
MUC1		Δ▼	
Steroid hormone coactivators			
AIBI	Δ	Δ	
TIF2		Δ	
NCoR	(−)		

PE proliferative phase, *SE* secretory phase, *HP* hyperplasia, *IGFBP1* insulin-like growth factor-binding protein 1, *GLUT 4* glucose transporter type 4, *IRS-1* insulin receptor substrate 1, *IL-6* interleukin 6, *CCL2(MCP-1)* chemokine CC ligand-2 Monocyte chemoattractant protein-1, *IL-8* interleukin 8, *RANTES* a cytokine that is a member of the interleukin-8 superfamily of cytokines. RANTES is a protein, *uNK cells* uterine natural killer, *MMPs* matrix metalloproteinases, *MMP2* matrix metalloproteinase 2, *MMP3* matrix metalloproteinase 3, *HOXA 10* homeobox protein Hox-A10 is a protein that in humans is encoded by the HOXA10 gene, *(avb3) integrin* the alpha v beta 3 "vitronectin receptor" is a member of the integrin superfamily of adhesion molecules, *MUC1 Mucin 1* cell surface associated, *AIB1* amplified in breast cancer 1 (AIBI), *TIF 2* transcriptional mediators/intermediary factor 2, *NCoR* nuclear receptor co-repressor, ▼ decreased, Δ increased, (−) no different

Given all these alterations, it is reasonable to assume that the receptivity of the endometrium is perturbed in the presence of PCOS and that such an alteration may contribute to infertility. A comprehensive review of endometrial aspects of the so-called 'window of implantation' stressed that nidation is a highly coordinated event involving both embryonic and endometrial participation [24]. Many of the proteins involved are temporally aligned within the 'window' and act as chemical messengers recognizable by the embryo; they act by facilitating embryonic growth and differentiation. These proteins have been progressively utilized as biomarkers capable of identifying infertile women with implantation failure. An analysis of these biomarkers in women with PCOS suggests that endometrial receptivity may

represent the major limiting factor for the establishment of pregnancy in these subjects.

In addition, some 15 years ago, overexpression of p160 steroid receptor coactivators was observed in the endometrium of women with PCOS [25]. These proteins serve as transcriptional coactivators for a number of nuclear and non-nuclear receptors, and the observation seems consistent with an altered response to progesterone. Therefore, it is likely that two mechanisms are at work in women with PCOS: an increased sensitivity to oestrogens and a relative resistance to progesterone. In theory, both may contribute to disrupting implantation.

A number of other investigations have documented the existence in the endometrium of women with PCOS of an impaired endometrial receptivity during the implantation window. A 2011 review of available data on the endometrium in women with PCOS at the time of the implantation window analysed 105 published articles and concluded that endometrial receptivity is the major limiting factor for the establishment of pregnancy in women with PCOS (as well as of in a large number of other gynaecological diseases) [26].

The upregulation of the homeobox gene *HOXA10* (necessary for the receptivity to embryo implantation) has been also investigated [27]. In vitro findings, as well as endometrial biopsies obtained from women with PCOS, show that testosterone decreases HOXA10-mRNA, leading to the conclusion that diminished uterine HOXA10 expression may contribute to the diminished reproduction potential of women with PCOS. A subsequent evaluation of the gene and protein expression of steroid and nuclear receptor co-regulators, as well as of markers of uterine receptivity in the endometrium of women with PCOS during the mid-secretory phase, found that the endometria exhibited higher levels of mRNA and protein for ERα and coactivators. A greater progesterone receptor (PR) and lower β3-integrin expression were also observed, leading to the conclusion that these alterations may contribute to an impaired implantation [28]. In a comparative study using microarray techniques, 21,571 genes were screened in the endometrium of PCOS and normal subjects [29]. In PCOS, many genes, including those regulating membrane function, adhesion, invasive growth and the cytoskeleton functioning, resulted downregulated during the implantation window. Specifically, the expression of transmembrane superfamily member 4 (associated with adhesion mechanisms) and matrix metalloproteinase 26 (shown to be related to degradation of extracellular matrix) resulted significantly downregulated in women with PCOS [29].

The effect of androgens on the expression of genes involved in oxidative stress resistance in decidualized human endometrial stromal cells has also been investigated [30]. These cells, isolated from hysterectomy specimens, were decidualized with 8-bromo cyclic adenosine monophosphate (8-br-cAMP) and progesterone in the presence or absence of dihydrotestosterone at various concentrations. It was concluded that androgens might play a critical role in the decidualization process at the time of embryo implantation and trophoblast invasion, by promoting resistance to oxidative stress. In the endometrium of PCOS patients, there are also differences in *FADD* (a gene that plays a role in cell proliferation, cycle regulation and development) and *BCL-2* (a gene encoding a protein that blocks the apoptotic death of certain cells, such as lymphocytes) expression during the window of implantation [31]. This suggests that the decrease in cell apoptosis during the implantation

window in PCOS patients may be one of the causes of reduced endometrial receptivity.

A recent systematic review documented the existence of a differential gene regulation in the endometrium of women with PCOS [32] and its critical effect not only on insulin resistance and hyperandrogenism but also on endometrial receptivity, implantation failure, early pregnancy loss and preterm birth.

The effect of micronized progesterone was comparatively evaluated in the endometrium of women with PCOS and in normal controls during both the proliferative and secretory phases of the cycle [8]. Following treatment, during the secretory phase, the endometrium of women with PCOS exhibited a lower number of glands and thicker luminal epithelium, together with a reduced integrin and MECA-79 immunoexpression. The latter is a marker for the so-called high endothelial venules (specialized postcapillary venous swellings characterized by plump endothelial cells enabling circulating lymphocytes to enter a lymph node). In addition, during both phases the expression of E-cadherin was higher and that of intercellular adhesion molecule-1 was lower during both the secretory and proliferative phases. This led to the conclusion that conventional doses of progesterone may not be enough to correct changes in endometrial histomorphology, as well as the expression of receptivity markers in PCOS women. The obesity may be a factor that interferes with this response.

Recently, the endometrial apolipoprotein A1 expression, another marker of endometrial competence, resulted to be upregulated in PCOS patients, especially during the proliferative phase [33]. It seems therefore that also the abnormal expression of this protein can affect negatively endometrial receptivity. An examination of the expression of epithelial Na^+ channels in the endometrium of overweight/obese women with PCOS during the window of implantation found a decreased expression of the gamma form (γ-ENaC) during the secretory phase in patients with increased serum leptin levels [34]. Clinically, these patients showed a significantly increased biochemical pregnancy rate, suggesting that high serum leptin may reduce endometrial receptivity by activating the signal transducer and activator of transcription protein STAT3 and downregulating γ-ENaC expression in the endometrium.

A recent, systematic review of cell adhesion molecules and ER expression in the endometrium of women with PCOS found conflicting results with respect to MUC1 and αVβ3-integrin expression, with significantly higher and lower levels, respectively [35]. ER expression is enhanced among patients with PCOS as compared to healthy women. This means that endometrial factors influence embryo receptivity, modifying the profile of molecular mediators, including cell adhesion molecules and ERs.

Finally, the presence and variations in the endometrium of glucose transporter 4 (GLUT4), a protein involved in the mechanism of insulin resistance in PCOS patients, have also been studied [36]. It was found that GLUT4 mRNA and its positive immunostaining reaction were present in endometrial epithelial cells of both normal and PCOS subjects. However, significantly higher levels of GLUT4 were observed in normal and lean normoinsulinaemic PCOS subjects whereas in

normo- and hyperinsulinaemic obese PCOS women, GLUT4 was significantly lower than in lean subjects. Interestingly, normoinsulinaemic obese and lean hyperinsulinaemic PCOS patients showed a similar low GLUT4 expression. They concluded that hyperinsulinaemia and obesity probably have a negative effect on endometrial GLUT4 expression.

5.3 Progesterone Resistance in Women with PCOS

As mentioned, the existence of a degree of resistance to the action of progesterone seems to be the other factor responsible for a decrease in fertility in women with PCOS. The concept of 'progesterone resistance' is not new, and the condition has been found in a number of situations. Almost 40 years ago, the case was presented of a young infertile woman who, in repeated late luteal phase endometrial biopsies, showed glandular stromal dissociation with failure to undergo decidualization, in spite of a normal progesterone serum profile. This abnormality could not be corrected by progesterone administration because approximately one half the number of high affinity progesterone-binding sites were present in the cytosol fraction of this subject compared to normal controls. Thus the phenomenon might have been due either to this decrease in the number of stromal cytosol receptors, or to a resistance to specific hormone action, or both [37].

The new concept of 'progesterone resistance' was defined in 1986 [38]; it implies a decreased responsiveness of target tissues to bioavailable progesterone and can be observed in cancer patients [39–41], in women with adenomyosis [42] and endometriosis [43] and in a majority of neonates [44]. Moreover, it has been argued that the term 'progesterone resistance' represents a misnomer, because the phenomenon involves a modification of a series of key endometrial signal transduction pathways [45]. Using these abnormalities as a marker, the phenomenon also occurs in women with recurrent pregnancy loss. It has been conjectured that steroid hormone responses in the endometrium are likely to be much more dynamic and complex than previously appreciated. Progesterone resistance, as manifested in conditions like endometriosis, is not just a consequence of perturbed progesterone signal transduction caused by chronic inflammation but is associated with long-lasting epigenetic reprogramming of steroid hormone responses in the endometrium and beyond. In this context, it is assumed that cyclic endometrial decidualization followed by menstrual shedding is an example of the physiological preconditioning that prepares uterine tissue for the dramatic vascular remodelling associated with deep placentation. Indeed, deep placentation involves the remodelling of the spiral arteries in the placental bed, including the endometrial and, most critically, the myometrial segments. However, both molecular aspects and clinical relevance of this phenomenon are far from having been established, with several mechanisms being proposed, all converging on nuclear PR.

Interestingly, evidence is accumulating that in the endometrium of women with PCOS, there is an impaired response to progesterone. An abnormal response in women with PCOS was first observed in 2008 [46] in two cases of atypical endometrial hyperplasia in subjects with PCOS; in these patients high-dose progestin therapy failed to reverse the hyperplasia, and the two subjects were therefore labelled as 'progesterone resistant'. The administration of metformin and of oral contraceptives caused the endometrium to become proliferative without any further evidence of pathology [46].

The possibility that in PCOS patients there may be a degree of progesterone resistance prompted an investigation to analyse total RNA from normal fertile controls and from women with PCOS, either treated with clomiphene citrate (CC) or with daily administration of progesterone [47]. It was found that among the three groups, there were 5160 significantly different genes, 466 of which were differentially regulated in fertile controls and in PCOS patients. A significantly lower expression of a number of progesterone-regulated genes was found in the endometrium of PCOS patients. Among them are the hypoxia-related mitogen-inducible gene 6; the leukaemia inhibitory factor, an interleukin-6 type of cytokine affecting cell growth by inhibiting differentiation; the GRB2-associated binding protein-1 that plays a central role in cellular growth response, transformation and apoptosis; the S100 calcium-binding protein P; and claudin-4, a membrane protein present in epithelial cell tight junctions. In contrast to this, cell proliferation genes such as anillin and cyclin B1 were upregulated. These anomalies lead to the conclusion that differences in gene expression provide evidence of progesterone resistance in mid-secretory PCOS endometrium. As a matter of fact, dysregulated signalling pathways in the endometrium of patients with PCOS compared to that of normal subjects had already been observed in 2009 [48], when it was found that several biological pathways including cell cycle, apoptosis, glycolysis and integrin-Rho-cytoskeleton network were aberrantly downregulated in the endometrium of women with PCOS.

Further evidence of a resistance to progesterone action in subjects with PCOS has now been produced, and it has been suggested that over-binding of progesterone in stromal cells could lead to E_2-induced epithelial cell proliferation [49]. This hypothesis is based on experimental animal studies showing that functional stromal cells are necessary for proper epithelial cell proliferation and differentiation in the endometrium [50]. It has been speculated that failure of progesterone-induced stromal cell proliferation mediated by PR could be at the origin of progesterone resistance in PCOS patients. Also ERs may play a role; since ERβ is necessary to inhibit E_2-induced epithelial cell proliferation, it is believed that stromal PR and ERβ produce the same inhibitory action on epithelial cell proliferation [49].

In conclusion, modification of endometrium gene expression is one of the conditions present at the onset of PCOS in women with progesterone resistance [50–53]. A similar situation exists in women with endometriosis, both in terms of progesterone resistance and alteration of PR-related genes [54], although differences also exist. For instance, on the one hand, downregulation of PR-related mitogen-inducible gene 6 (active in limiting malignant transformation) is found both in endometriosis and in PCOS [47, 52]; on the other, differences exist in the

regulation of PR-related transforming growth factor β-1 (a potent cell regulator and a multifunctional signalling molecule) between women with PCOS [45] and those with endometriosis [51]. The expression of progesterone-regulated mucin 1 (MUC1) (lining the apical surface of epithelial cells and belonging to a family of glycoproteins protecting the body from infection) has been investigated in women with endometriosis and with PCOS, since this protein is expressed in the endometrium of fertile women and carries selectin ligands recognized by the human blastocyst. As such, an altered MUC1 expression during the window of implantation may contribute to infertility of endometrial origin [52]. The expression of the terminal domain of MUC1-N (MUC1-ND) is significantly higher in ovulatory PCOS than in fertile and anovulatory PCOS patients, even after progesterone stimulation. In contradistinction to this, only MUC1-C-subunit cytoplasmic domain expression was lower in endometriosis patients [52]. Endometrial ER expression was significantly higher in PCOS and endometriosis patients, whereas PR expression was significantly higher in PCOS than in fertile patients. This led to the hypothesis that the different PR-related gene expression profiles between women with PCOS and with endometriosis could be related to differences in PR isoform expression [49].

5.4 Potential Consequences of Progesterone Resistance in PCOS

The possibility of a luteal dysfunction due to progesterone resistance in PCOS patients is not without consequences, as it can be at the origin of an altered endometrial receptivity and, even if implantation occurs, of an abnormal decidualization and placentation.

5.4.1 Abnormal Endometrial Competence

Following fertilization and blastocyst formation, the critical and step-limiting factor for the establishment of a successful pregnancy is the complex phenomenon of implantation. Obviously the condition sine qua non for successful implantation is a good-quality embryo; at the same time, an equally vital role is played by two other phenomena: a temporally coordinated differentiation of endometrial cells to optimize their ability to receive the embryo, and a synchronized dialogue between maternal and embryonic tissues [55]. In this connection, it has been shown through third-party parenting in IVF (where the source of oocytes is separated from the endometrium, making possible to assess separately embryo and endometrial development) that E_2 and progesterone are the only hormones necessary to prepare the endometrium for implantation [56].

There are several conditions in which experimental investigations have shown that the absence or suppression of molecules essential for endometrial receptivity results in decreased implantation rates. Among them is PCOS, where the possibility

of a luteal dysfunction due to progesterone resistance becomes important. Unfortunately, as it has been recently pointed out, in spite of a wide availability of clinical and instrumental methods for assessing endometrial competence, reproducible and reliable diagnostic tests for luteal phase inadequacy are yet to be developed [57].

Physiologically, successful embryonic implantation is the result of three equally important factors: a receptive endometrium, a functional embryo at the blastocyst stage and a synchronized dialogue between maternal and embryonic tissues [58]. As already detailed above (Sect. 5.2), during each menstrual cycle there is a short period of embryonic receptivity known as the 'window of implantation', an expression that refers to the temporally limited ability of the endometrium to allow the blastocyst to attach, penetrate and induce localized changes in its stroma. Such a delicate and well-timed chain of events can be easily deranged, and there is now evidence that gene expression in the endometrium at the time of the implantation window is altered in obese women in general and more specifically in obese PCOS subjects. In fact, it has been observed that the luteal phase endometrial transcriptome is altered in obese women during the window of implantation, with 151 genes dysregulated compared with controls [59]. In addition, it has been observed in an in vitro model [54] that, following hormonal challenge with E_2 and progesterone, in some cases endometrial stromal fibroblasts from women with PCOS showed aberrant decidualization though they all exhibited normal oestrogen-mediated increase in PR-G expression.

Although translation of experimental findings in the clinical setting requires caution, there is today evidence that in vivo these aberrations may result in suboptimal implantation.

A good, yet totally unexplored human model to investigate endometrial resistance to the action of progesterone is the neonatal endometrium, where responses indicative of progesterone resistance were documented over 60 years ago [44]. We know today that both male and female foetuses during pregnancy are exposed to progressively increasingly higher plasma concentrations of unbound oestrogens and progesterone. In particular, in the foetal circulation progesterone rises to values much higher than in the maternal circulation [60]. Yet, in an autopsy study of 169 neonates or term foetuses, it was found that in a majority of them (68%) the endometrium failed to respond to these high circulating levels of progesterone and remained either proliferative or inactive [44]. A partial or early response (presence of subnuclear vacuolization) was found in 27% and full reaction (decidualization or menstrual-like shedding) in only 5%. In addition, an examination of ovaries evidenced that they were frequently polycystic, although they failed to show any sign of ovulation or corpus luteum formation [44]. Thus, remarkably at birth the majority of neonates satisfy the current criteria for the diagnosis of PCOS by the presence of polycystic ovaries, anovulation and progesterone-resistant endometrium [61].

Under the circumstances, the hypothesis can be made that the spectrum of progesterone resistance described above is likely to persist till the onset of puberty when endogenous oestrogens begin stimulating endometrial cells. A persisting degree of

endometrial progesterone resistance after menarche may be linked to defective deep placentation and major obstetrical disorders, including preeclampsia, foetal growth restriction and preterm birth [62, 63]. These complications therefore would be both a consequence of the 'ontogenetic progesterone resistance' and of the need for 'menstrual preconditioning', a concept implying that menstruations (i.e. progesterone withdrawal bleedings) evolved in the human because of the need to initiate decidualization in the absence of pregnancy and protect uterine tissues from the profound hyperinflammation and oxidative stress associated with deep placentation.

According to this theory, the human uterus acquires the competence for deep placentation in response to dynamic remodelling events triggered by true menstruations, miscarriage or parturition [64]. If ontogenetic progesterone resistance persists in some young girls until menarche and if full progesterone responsiveness is achieved gradually after the onset of cyclic menstruations, then the presence of anovulatory cycles early in reproductive life (as may be the case in PCOS) can become the source of complications. In this respect, it is well accepted that the pathogenesis of late-onset preeclampsia in the primigravida is linked to defective deep placentation, defined by a restricted remodelling of the myometrial segments of the spiral arteries in the placental bed [65]. Recently, data were collected from a large cohort of nulliparous teenagers with singleton deliveries aged 13–15, 16–17 and 18–19 years and outcomes compared with those in a very large control group of 25–29-year-old women. Results indicate that teenage mothers face increased risks of complications, such as anaemia, proteinuria, urinary tract infection, pyelonephritis and preeclampsia [66]. Such a risk however decreased with age. Of importance is the finding regarding the risk of preeclampsia and preterm delivery, respectively, about four and threefold higher among 13–15-year-olds [66]. This may be considered as an indirect sign of incomplete endometrial maturation in very young mothers. On the other hand, a complete endometrial maturation in these adolescents is probably achieved only after a series of ovulatory cycles [67].

5.4.2 Decidualization and Placentation

The process of fertilization in women with PCOS and its outcome has been extensively investigated and is reported in the Chap. 4. Therefore, here only issues relating to decidualization and placentation will be discussed.

The rationale for investigating the uteroplacental vessels in women with PCOS has been its association with the so-called great obstetrical syndromes (preeclampsia, intrauterine growth restriction, preterm labour, preterm/premature rupture of membranes, late spontaneous abortion and abruptio placentae). Indeed, these women are at increased risk of pregnancy and neonatal complications [14, 68] and therefore it is important to examine whether this situation is, at least in part, due to defective decidualization and placentation.

In humans decidualization is a progesterone-driven differentiation essential to prepare the uterus for successful embryo implantation and deep placentation. This

process starts in the mid-luteal phase of the menstrual cycle and takes place whether or not fertilization has occurred. In case of pregnancy, full remodelling of some 60 spiral arteries is required for supporting the increase of endometrial blood flow from less than 1% up to 25% of the cardiac output. Stromal cells surrounding the spiral arteries and abundant uterine natural killer (uNK) cells in the endometrium are mounting the early decidual vascular response that results in distension of the vessel [69]. The progressive decidualization of the spiral arteries is reflected by loss of the musculo-elastic structure to be followed by endovascular and interstitial trophoblast invasion from the decidua till the inner myometrium. This process transforms these spiral arteries into large fibro-fibrinoid vessels connecting the larger uterine arteries with the intervillous space of the placenta [70]. Different types of defective deep placentation have been described in association with the 'great obstetrical syndromes' (Figs 5.1 and 5.2). Preeclampsia is characterized by persistence of the musculo-elastic structure in the majority of myometrial spiral arteries, except in the centre of the placental bed [64, 71]. A similar, although milder, defective remodelling occurs in preterm birth [72, 73]. On the other hand, preexisting hypertensive conditions affect the proximal myometrial segments by obstructive atherosclerotic lesions. Several authors have investigated the vascular pathology of the placenta and placental bed in women with PCOS. Unfortunately, the investigations of defective deep placentation in PCOS have—up to now—not included histopathology of placental biopsies in patients with PCOS and adverse pregnancy outcome (Table 5.2).

To investigate whether decidual endovascular trophoblast invasion in pregnant women with PCOS is impaired, a case-control study of pregnant subjects waiting for legal pregnancy termination has been conducted [74] in PCOS patients, and healthy non-PCOS pregnant controls matched for age and body mass index (BMI) without any feature of PCOS. All pregnancies were terminated at the week 12 of gestation, and fragments of placental and decidual tissue were obtained by an aspiration technique. The rate of implantation vessels with endovascular trophoblast was significantly lower in patients with PCOS compared with healthy non-PCOS controls. However, the question arises whether in the absence of endovascular trophoblast any structureless vessel opening in the basal plate can be identified as arterial rather than venous. It is precisely because of this difficulty that the large vessels in the basal plate of the placenta were coined 'sinusoids' till serial sections of placental bed biopsies identified them as arterial by their continuity with myometrial spiral arteries [75]. It has been speculated that pregnancies with a high-resistance uterine artery flow pattern in the first trimester of pregnancy are associated with a less extensive trophoblastic invasion pattern of the decidual vessels [76]. However, it is indeed unlikely that the loose endovascular trophoblast 'plugging' can be expected to result in any vascular resistance prior to the establishment of an effective intervillous blood flow.

A quantitative morphological study of intact hysterectomy specimens with placenta in situ ranging from 8 to 18 weeks' gestation obtained at the time of sterilization by hysterectomy revealed a tendency for maximal invasive activity to occur at the centre and, subsequently, to extend centrifugally to produce an annular pattern

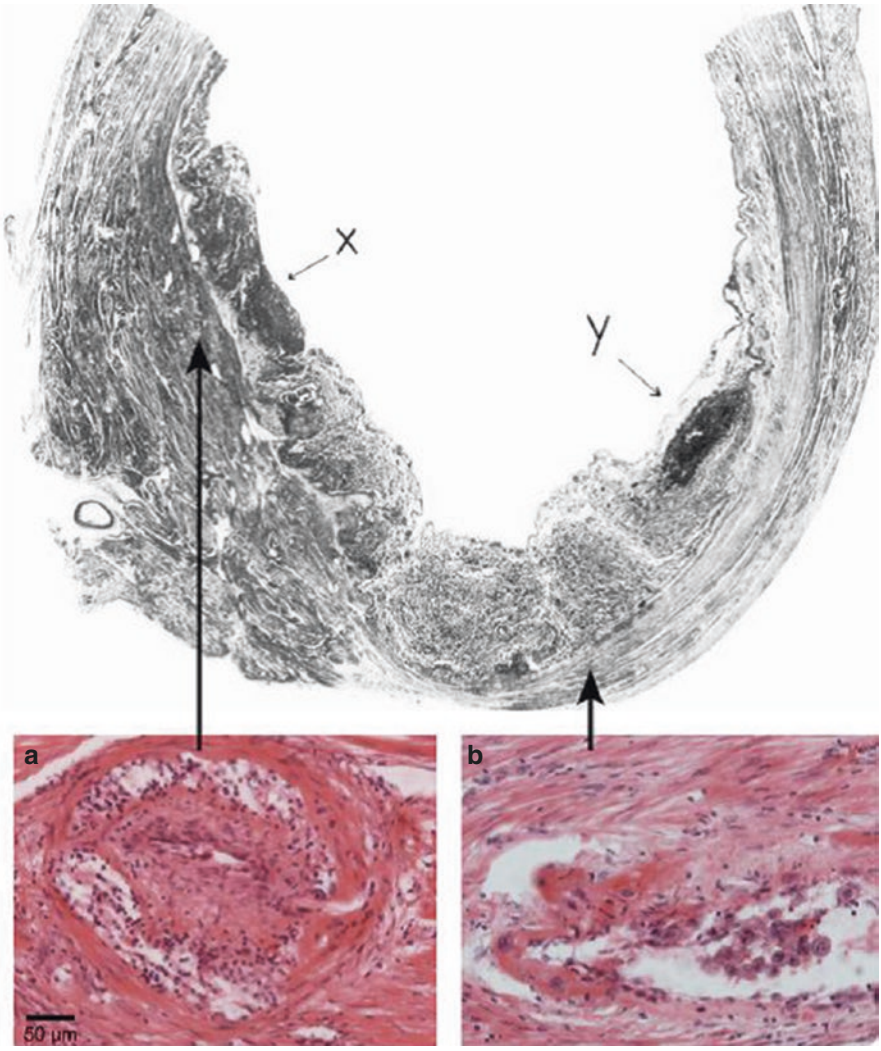


Fig. 5.1 Classification of defective deep placentation. (**a**) Spiral arteries in the centre of the placental bed show full transformation of the arterial wall including the myometrial segment. (**b**) Spiral arteries in the paracentral zone show the absence of transformation of the wall and as seen in this case, the obstructive atherosis in the myometrial spiral artery is underlying a placental infarction. From: Brosens et al. [71]

[76]. This investigation indicates that probably uterine artery resistance and endovascular trophoblast invasion of the decidual arteries are not directly related events. Rather, it is likely that progressive expansion of the remodelling process with decidualization and interstitial and endovascular trophoblast invasion resulting in fibrofibrinoid changes of the spiral arteries in depth as well as centrifugally plays a critical role in the relief of the uterine flow resistance. Indeed, a pattern of full

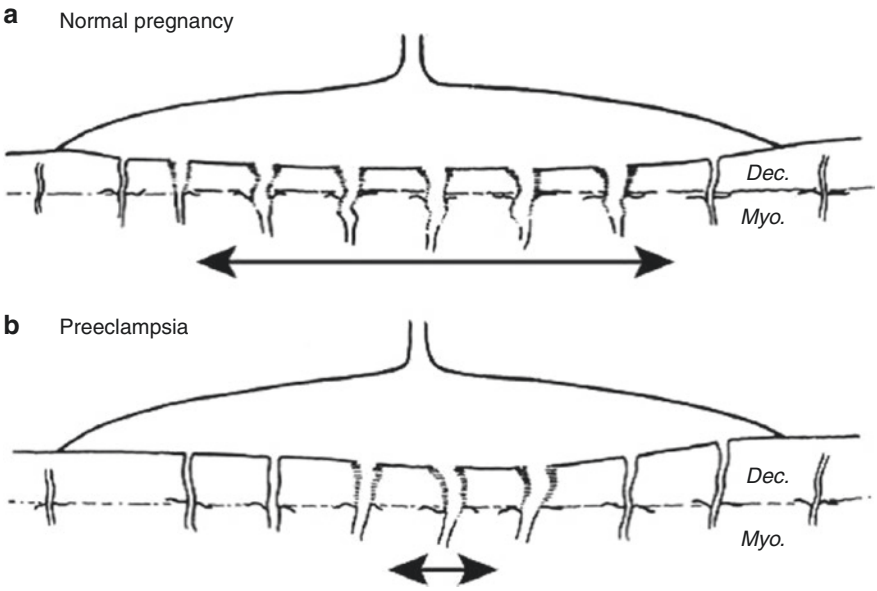


Fig. 5.2 Differences in placental bed in normal and defective deep placentation. (**a**) Normal placental bed with full transformation of the myometrial (*Myo*) spiral arteries, except at the periphery of the placental bed. (**b**) Defective deep placentation is characterized by non-transformation of the myometrial spiral arteries with a reduction of the central area with deep placentation. From Brosens et al. [71]

Table 5.2 Types of defective deep placentation and subsequent obstetrical complication

Remodelling of myometrial spiral artery	Obstetrical complication
Absent (except for centre)	Preeclampsia
Partial	Preterm labour
	Preterm premature rupture of membranes
	Small for gestation age
Absent and obstructive lesions	Small for gestation age with preeclampsia
	Abruptio placentae

remodelling of spiral arteries in the centre, but defective vascular remodelling of the myometrial segment in the paracentral and peripheral zones with placental thrombosis and infarction, has been described in pregnancies complicated by preeclampsia and small for gestational age babies [71]. This raises questions of how representative of the true situation are histological sections from the central region of the placenta.

Finally, on the basis of the variable incidence of microscopic placental lesions, it is believed that early trophoblast invasion and placentation observed in PCOS vary widely according to the phenotype [77, 78].

5.5 Treatment Options to Improved Endometrial Competence

A number of medical, as well as non-medical, interventions have been attempted in order to improve endometrial receptivity and implantation rates in women with PCOS. Unfortunately, no real breakthrough has been obtained in this area, and no systematic evaluation has been carried out.

In any event, the first and most important interventions are those aimed at eliminating abnormalities present in the majority of these patients: obesity, metabolic disorders (dyslipidemia, diabetes), insulin resistance and anovulation.

In this respect a number of promising leads exist.

First, the relationship between regular physical activity and reproductive performance has been assessed in obese infertile patients with stable bodyweight, undergoing IVF [79]. It was found that the percentage of pregnancies was significantly higher in obese patients who did physical activity regularly compared with those who did not, concluding that regular physical activity before IVF is significantly related with improved reproductive performance in obese infertile patients, irrespective of bodyweight loss [79].

Starting from the assumption that PCOS negatively affects the endometrium, in a way that may lead to implantation failure and proliferative aberrations, an attempt has also been made to correct endometrial aberrations through dietary management and physical exercise [80]. The study involved overweight/obese and normal-weight women with PCOS and BMI-matched regularly menstruating controls. Before starting their intervention levels of mRNA ERα, its variant ERα36 (which mediates rapid oestrogen signalling and inhibits genomic oestrogen signalling) and the ERα/ERβ mRNA ratio were lower in proliferative endometrium of overweight/obese PCOS women compared with controls but increased significantly after intervention in proliferative endometrium resulting higher in PCOS women with improved menstrual function than in those without improvement [80]. Thus, although lifestyle intervention improves the clinical features, this per se cannot fully restore normality. However, it has been speculated that manipulating the expression of key endometrial genes with gene or stem cell-based therapies may someday be used to normalize implantation rates [55].

An obvious objective in any effort to improve the endometrial competence in PCOS patients is the elimination/improvement of progesterone resistance. Unfortunately, since the very first description of the condition, it appeared that high doses of progesterone fail to work [37]. As documented by neonatal progesterone resistance, the issue here is not a lack of progesterone; rather it is the inability of the endometrium to respond to it [44].

Changes in the endometrium capable of optimizing endometrial receptivity are metabolically demanding, and glucose metabolism is important for the preparation of the endometrium for embryo implantation [58]. Specifically, decidualization of endometrial stromal cells is dependent on increasing expression of glucose and its transporter. Since one of the symptoms of PCOS is obesity, this is one more reason why it may influence the rate of implantation. Finally, in an attempt to rectify the endometrial insulin signalling in overweight/obese women with PCOS, the effectiveness of lifestyle interventions aiming at weight loss has been formally

tested [81]. It was found that such an intervention can upregulate, both at the mRNA and protein levels, components of insulin signalling in the endometrium of obese or overweight patients, as clinically shown by an improved menstrual pattern. Indeed, in this study, following weight loss menstrual patterns improved in 65% of the subjects, and levels of insulin receptor substrate-1 (IRS1) and GLUT were significantly upregulated in their endometrium. The study concluded that upregulation of these two factors can help improving glucose homeostasis and restoring the functioning of the endometrium in women with PCOS [81].

On the pharmacological side, a molecular analysis of the endometrium of women with PCOS has been carried out following administration of CC or letrozole [82]. It was found that only the latter was able to influence positively several markers of endometrial receptivity, including endometrial thickness, resistance and pulsatility indices of sub-endometrial and endometrial blood flow [83]. Finally, in a large double-blind, multicentre RCT, women who received letrozole had more cumulative ovulation and live births than those who received CC [84].

A technique recently suggested to improve the probability of embryo implantation in women undergoing IVF involves causing endometrial injury by scratching. This technique has been applied also to women with PCOS. As to the mechanism of action, endometrial injury may trigger a series of biological responses, although no particular pathway seems responsible. Rather, there seems to be a cluster of events in response to trauma which benefits embryo implantation [85, 86]. The ensuing inflammatory response is documented by a statistically significant increase in macrophages/dendritic cells and of pro-inflammatory cytokines, tumour necrosis factor-α, growth-regulated oncogene-α, interleukin-15 and macrophage inflammatory protein 1B [87].

The validity of this technique has been tested in 2014 in a large RCT involving 300 subfertile women scheduled for IVF [88]. Although the study concluded that the technique does not improve ongoing pregnancy rates, the cases included in the study were totally unselected [89]. Therefore, because of potential biases, the possibility of a therapeutic effect remains uncertain. To resolve the issue, three clinical trials have been designed and are currently in progress [90]. Named ‘Pipelle for Pregnancy’ (PIP), these trials will evaluate endometrial scratching in three different populations, one being women with PCOS undergoing IVF.

Conclusion

The clinical significance of endometrial abnormalities found in women with PCOS is still unclear and controversial. The reason is simple: the majority of data available come from molecular biology studies, and they have not been clinically validated. In addition, no single marker exists to predict the clinical outcome of an attempt to establish a successful pregnancy, and, to this day, no agreed screening protocols/recommendations for women with PCOS have been established. Also, no standardized and agreed clinical protocol exists for the treatment of endometrial abnormalities in women with PCOS. Probably this is due to the fact that meta-analytic clinical data found no difference in the chance of embryo transfer per oocyte retrieval and no significant difference in clinical pregnancy rates per cycle in infertile IVF patients with PCOS [7]. This means that, at least in patients subjected to IVF cycles, there is little impairment in endometrial receptivity.

In conclusion, what are urgently needed are studies correlating the experimentally observed endometrial abnormalities to pregnancy outcome.

References

1. Hart R, Doherty DA. The potential implications of a PCOS diagnosis on a woman's long-term health using data linkage. J Clin Endocrinol Metab. 2015;100:911–9.
2. Prapas N, Karkanaki A, Prapas I, Kalogiannidis I, Katsikis I, Panidis D. Genetics of polycystic ovary syndrome. Hippokratia. 2009;13:216–23.
3. Legro RS, Zaino RJ, Demers LM, Kunselman AR, Gnatuk CL, Williams NI, Dodson WC. The effects of metformin and rosiglitazone, alone and in combination, on the ovary and endometrium in polycystic ovary syndrome. Am J Obstet Gynecol. 2007;196:402.e1–402.e11.
4. Fauser BCJM, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, Carmina E, Chang J, Yildiz BO, Laven JSE, Boivin J, Petraglia F, Wijeyeratne CN, Norman RJ, Dunaif A, Franks S, Wild RA, Dumesic D, Barnhart K. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril. 2012;97:28–38.
5. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, Welt CK. Diagnosis and treatment of polycystic ovary syndrome: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2013;98:4565–92.
6. Rees DA, Jenkins-Jones S, Morgan CL. Contemporary reproductive outcomes for patients with polycystic ovary syndrome: a retrospective observational study. J Clin Endocrinol Metab. 2016;101:1664–72.
7. Heijnen EM, Eijkemans MJ, Hughes EG, Laven JS, Macklon NS, Fauser BC. A meta-analysis of outcomes of conventional IVF in women with polycystic ovary syndrome. Hum Reprod Update. 2006;12:13–21.
8. Lopes IM, Maganhin CC, Oliveira-Filho RM, Simões RS, Simões MJ, Iwata MC, Baracat EC, Soares JM. Histomorphometric analysis and markers of endometrial receptivity embryonic implantation in women with polycystic ovary syndrome during the treatment with progesterone. Reprod Scie. 2014;21:930–8.
9. López E, Joanne G, Daya S, Parrilla JJ, Abad L, Balash J. Ovulation induction in women with polycystic ovary syndrome: randomized trial of clomiphene citrate versus low-dose recombinant FSH as first line therapy. Reprod Biomed Online. 2004;9:382–90.
10. Leader A, The Monofollicular Ovulation Induction Study Group. Improved monofollicular ovulation in anovulatory or oligo-ovulatory women after a low-dose step-up protocol with weekly increments of 25 international units of follicle-stimulating hormone. Fertil Steril. 2006;85:1766–73.
11. Tso LO, Costello MF, Albuquerque LE, Andriolo RB, Macedo CR. Metformin treatment before and during IVF or ICSI in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2014;11:CD006105.
12. Sagle M, Bishop K, Ridley N, Alexander FM, Michel M, Bonney RC, Beard DW, Franks S. Recurrent early miscarriage and polycystic ovaries. Brit Med J. 1988;297:1027–8.
13. Balen AH, Tan SL, MacDougall J, Jacobs HS. Miscarriage rates following in-vitro fertilization are increased in women with polycystic ovaries and reduced by pituitary desensitization with buserelin. Hum Reprod. 1993;8:959–64.
14. Boomsma CM, Fauser BCJM, Macklon NS. Pregnancy complications in women with polycystic ovary syndrome. Sem Reprod Med. 2008;26:72–84.
15. Jakubowicz DJ, Iuorno MJ, Jakubowicz S, Roberts KA, Nestler JE. Effects of metformin on early pregnancy loss in the polycystic ovary syndrome. J Clin Endocrinol Metab. 2002;87:524–9.
16. Tian L, Shen H, Lu Q, Norman RJ, Wang J. Insulin resistance increases the risk of spontaneous abortion after assisted reproduction technology treatment. J Clin Endocrinol Metab. 2007;92:1430–3.

17. Zheng J, Shan PF, Gu W. The efficacy of metformin in pregnant women with polycystic ovary syndrome: a meta-analysis of clinical trials. J Endocrinol Investig. 2013;36:797–802.
18. Boomsma CM, Eijkemans MJ, Hughes EG, Visser GH, Fauser BC, Macklon NS. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. Hum Reprod Update. 2006;12:673–83.
19. Kjerulff LE, Sanchez-Ramos L, Duffy D. Pregnancy outcomes in women with polycystic ovary syndrome: a metaanalysis. Am J Obstet Gynecol. 2011;204:558.e1–6.
20. Qin JZ, Pang LH, Li MJ, Fan XJ, Huang RD, Chen HY. Obstetric complications in women with polycystic ovary syndrome: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2013;26:11–56.
21. Piltonen TT. Polycystic ovary syndrome: endometrial markers. Best Pract Res Clin Obstet Gynaecol. 2016;37:66–79.
22. Wang A, Ji L, Shang W, Li M, Chen L, White RE, HAN G. Expression of GPR30, ERac and ERfl in endometrium during window of implantation in patients with polycystic ovary syndrome: a pilot study. Gynecol Endocrinol. 2011;27:251–5.
23. Giudice LC. Endometrium in PCOS: implantation and predisposition to endocrine CA. Best Pract Res Clin Endocrinol Metab. 2006;20:235–44.
24. Lessey BA. Endometrial receptivity and the window of implantation. Baillières Best Pract Res Clin Obstet Gynaecol. 2000;14:775–88.
25. Gregory CW, Wilson EM, Apparao KBC, Lininger RA, Meyer WR, Kowalik A, Fritz MA, Lessey BA. Steroid receptor coactivator expression throughout the menstrual cycle in normal and abnormal endometrium. J Clin Endocrinol Metab. 2002;87:2960–6.
26. Ribeiro Soares Lopes IM, Pinheir Baracat MC, De Jesus Simoes M, Santos Simoes R, Chada Baracat E, Jose Maria Soares JM Jr. Endometriurn in women with polycystic ovary syndrome during window of implantation. Rev Assoc Med Bras. 2011;57:688–95.
27. Cermik D, Selam B, Taylor HS. Regulation of HOXA-10 expression by testosterone in vitro and in the endometrium of patients with polycystic ovary syndrome. J Clin Endocrinol Metab. 2003;88:238–43.
28. Quezada S, Avellaira C, Johnson MC, Gabler F, Fuentes A, Vega M. Evaluation of steroid receptors, coregulators, and molecules associated with uterine receptivity in secretory endometria from untreated women with polycystic ovary syndrome. Fertil Steril. 2006;85:1017–26.
29. Qiao J, Wang L, Li R, Zhang X. Microarray evaluation of endometrial receptivity in Chinese women with polycystic ovary syndrome. Reprod Biomed Online. 2008;17:425–35.
30. Kajihara T, Tochigi H, Prechapanich J, Uchino S, Itakura A, Brosens JJ, Ishihara O. Androgen signaling in decidualizing human endometrial stromal cells enhances resistance to oxidative stress. Fertil Steril. 2012;97:185–91.
31. Yan L, Wang A, Chen L, Shang W, Li M, Zhao Y. Expression of apoptosis-related genes in the endometrium of polycystic ovary syndrome patients during the window of implantation. Gene. 2012;506:350–4.
32. Jesintha M, Deecaraman M, Vijayalakshmi M, Umashankar V. A systemic review on differential regulation of genes in polycystic ovarian syndrome disease. Int J Pharma Bio Scie. 2015;6:B893–900.
33. Amjadi F, Aflatoonian R, Javanmard SH, Saifi B, Ashrafi M, Mehdizadeh M. Apolipoprotein A1 as a novel anti-implantation biomarker in polycystic ovary syndrome: a case-control study. J Res Med Sci. 2015;20:1039–45.
34. Lin X-H, Liu M-E, H-Y X, Chen X-J, Wang H, Tian S, Sheng J-Z, Huang H-F. Leptin down-regulates γ-ENaC expression: a novel mechanism involved in low endometrial receptivity. Fertil Steril. 2015;103:228–35.
35. Baracat MC, Serafini PC, Simões Rodos S, Maciel GA, Soares JM Jr, Baracat EC. Systematic review of cell adhesion molecules and estrogen receptor expression in the endometrium of patients with polycystic ovary syndrome. Int J Gynaecol Obstet. 2015;129:1–4.
36. Mioni R, Chiarelli S, Xamin N, Zuliani P, Granzotto M, Mozzanega B, Maffei P, Martini C, Blandamura S, Sicolo N, Vettormioni R. Evidence for the presence of glucose transporter 4 in

the endometrium and its regulation in polycystic ovary syndrome patients. J Clin Endocrinol Metab. 2004;89:4089–96.
37. Keller DW, Wiest WG, Askin FB, Johnson LW, Strickler RC. Pseudocorpus luteum insufficiency: a local defect of progesterone action on endometrial stroma. J Clin Endocrinol Metab. 1979;48:127–32.
38. Chrousos GP, MacLusky NJ, Brandon DD, Tomita M, Renquist DM, Loriaux DL, Lipsett MB. Progesterone resistance. Adv Exp Med Biol. 1986;196:317–28.
39. Simpson HW, McArdle CS, Griffiths K, Turkes A, Beastall GH. Progesterone resistance in women who have had breast cancer. Br J Obstet Gynaecol. 1998;105:345–51.
40. Xu Y, Tong J, Ai Z, Wang J, Teng Y. Epidermal growth factor receptor signaling pathway involved in progestin-resistance of human endometrial carcinoma: in a mouse model. J Obstet Gynaecol Res. 2012;38:1358–66.
41. Xu W, Zhu S, Zhou Y, Jin Y, Dai H, Wang X. Upregulation of mitogen-inducible gene 6 triggers antitumor effect and attenuates progesterone resistance in endometrial carcinoma cells. Cancer Gene Ther. 2014;22:536–41.
42. Benagiano G, Brosens I. The endometrium in adenomyosis. Women's Health (Lond Engl). 2012;8:301–12.
43. Taylor HS, Bagot C, Kardana A, Olive D, Arici A. HOX gene expression is altered in the endometrium of women with endometriosis. Hum Reprod. 1999;14:1328–31.
44. Ober WB, Bernstein J. Observations on the endometrium and ovary in the newborn. Pediatrics. 1955;16:445–60.
45. Al-Sabbagh M, Lam EW, Brosens JJ. Mechanisms of endometrial progesterone resistance. Mol Cell Endocrinol. 2012;358:208–15.
46. Shen Z-Q, Zhu H-T, Lin J-F. Reverse of progestin-resistant atypical endometrial hyperplasia by metformin and oral contraceptives. Obstet Gynecol. 2008;112:465–7.
47. Savaris RF, Groll JM, Young SL, DeMayo FJ, Jeong JW, Hamilton AE, Giudice LC, Lessey BA. Progesterone resistance in PCOS endometrium: a microarray analysis in clomiphene citrate-treated and artificial menstrual cycles. J Clin Endocrinol Metab. 2011;96:1737–46.
48. Kim JY, Song H, Kim H, Kang HJ, Jun JH, Hong SR, Koong MK, Kim IS. Transcriptional profiling with a pathway-oriented analysis identifies dysregulated molecular phenotypes in the endometrium of patients with polycystic ovary syndrome. J Clin Endocrinol Metab. 2009;94:1416–26.
49. Li X, Feng Y, Lin JF, Billing H, Shao R. Endometrial progesterone resistance and PCOS. J Biomed Sci. 2014;21:2.
50. Kim JJ, Kurita T, Bulun SE. Progesterone action in endometrial cancer, endometriosis, uterine fibroids, and breast cancer. Endocr Rev. 2013;34:130–62.
51. Burney RO, Talbi S, Hamilton AE, Vo KC, Nyegaard M, Nezhat CR, Lessey BA, Giudice LC. Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. Endocrinology. 2007;148:3814–26.
52. Margarit L, Taylor A, Roberts MH, Hopkins L, Davies C, Brenton AG, Conlan RS, Bunkheila A, Joels L, White JO, Gonzalez D. MUC1 as a discriminator between endometrium from fertile and infertile patients with PCOS and endometriosis. J Clin Endocrinol Metab. 2010;95:5320–9.
53. Bulun SE, Cheng YH, Pavone ME, Xue Q, Attar E, Trukhacheva E, Tokunaga H, Utsunomiya H, Yin P, Luo X, et al. Estrogen receptor-beta, estrogen receptor-alpha, and progesterone resistance in endometriosis. Semin Reprod Med. 2010;28:36–43.
54. Piltonen TT, Chen JC, Khatun M, Kangasniemi M, Liakka A, Spitzer T, Tran N, Huddleston H, Irwin JC, Giudice LC, Endometrial LC. Stromal fibroblast s from women with polycystic ovary syndrome have impaired progesterone-mediated decidualization, aberrant cytokine profiles and promote enhanced immune cell migration in vitro. Hum Reprod. 2015;30:1203–15.
55. Cakmak H, Taylor HS. Implantation failure: molecular mechanisms and clinical treatment. Hum Reprod Update. 2011;17:242–53.
56. Paulson RJ. Hormonal induction of endometrial receptivity. Fertil Steril. 2011;96:530–5.

57. Palomba S, Santagni S, La Sala GB. Progesterone administration for luteal phase deficiency in human reproduction: an old or new issue? J Ovarian Res. 2015;8:77–92.
58. Schulte MMB, Tsai J-H, Moley KH. Obesity and PCOS: the effect of metabolic derangements on endometrial receptivity at the time of implantation. Reprod Sci. 2015;22:6–14.
59. Bellver J, Martínez-Conejero JA, Labarta E, Alamá P, Barreto Melo MA, Remohí J, Pellicer A, Horcajadas JA. Endometrial gene expression in the window of implantation is altered in obese women especially in association with polycystic ovary syndrome. Fertil Steril. 2011;95:2335–41.
60. Tulchinsky D, Hobel CJ, Yeager E, Marshall JR. Plasma estrone, estradiol, estriol, progesterone, and 17-hydroxyprogesterone in human pregnancy: I, normal pregnancy. Am J Obstet Gynecol. 1972;112:1095–100.
61. Carmina E, Azziz R. Diagnosis, phenotype, and prevalence of polycystic ovary syndrome. Fertil Steril. 2006;86:S7–8.
62. Brosens I, Brosens J, Benagiano G. Neonatal uterine bleeding as antecedent of pelvic endometriosis. Hum Reprod. 2013;28:2893–7.
63. Gargett E, Schwab KE, Brosens JJ, Puttemans P, Benagiano G, Brosens I. Potential role of endometrial stem/progenitor cells in the pathogenesis of early-onset endometriosis. Mol Hum Reprod. 2014;20:591–8.
64. Brosens JJ, Parker MG, McIndoe A, Pijnenborg R, Brosens IA. A role for menstruation in preconditioning the uterus for successful pregnancy. Am J Obstet Gynecol. 2009;200:615.e1–6.
65. Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of preeclampsia. Obstet Gynecol Ann. 1972;1:177–91.
66. Leppälahti S, Gissler M, Mentula M, Heikinheimo O. Is teenage pregnancy an obstetric risk in a welfare society? A population-based study in Finland, from 2006 to 2011. BMJ Open. 2013;3:e003225.
67. Brosens I, Benagiano G. Menstrual preconditioning for the prevention of major obstetrical syndromes in polycystic ovary syndrome. Am J Obstet Gynecol. 2015;213:488–93.
68. Doherty DA, Newnham JP, Bower C, Hart R. Implications of polycystic ovary syndrome for pregnancy and for the health of offspring. Obstet Gynecol. 2015;125:1397–406.
69. Fraser R, Whitley GSJ, Thilaganathan B, Judith E, Cartwright JE. Decidual natural killer cells regulate vessel stability: implications for impaired spiral artery remodelling. J Reprod Immunol. 2015;110:54–60.
70. Brosens I, Robertson WB, Dixon HG. The physiological response of the vessels of the placental bed to normal pregnancy. J Pathol Bacteriol. 1967;93:569–79.
71. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "great obstetrical syndromes" are associated with disorders of deep placentation. Am J Obstet Gynecol. 2011;204:193–201.
72. Kim YM, Chaiworapongsa T, Gomez R, Bujold E, Yoon BH, Rotmensch S, Thaler HT, Romero R. Failure of physiologic transformation of the spiral arteries in the placental bed in preterm premature rupture of membranes. Am J Obstet Gynecol. 2002;187:1137–42.
73. Kim YM, Bujold E, Chaiworapongsa T, Gomez R, Yoon BH, Thaler HT, Rotmensch S, Romero R. Failure of physiologic transformation of the spiral arteries in patients with preterm labor and intact membranes. Am J Obstet Gynecol. 2003;189:1063–9.
74. Palomba S, Russo T, Falbo A, Di Cello A, Amendola G, Mazza R, Tolino A, Zullo F, Tucci L, La Sala GB. Decidual endovascular trophoblast invasion in women with polycystic ovary syndrome: an experimental case-control study. J Clin Endocrinol Metab. 2012;97:2441–9.
75. Prefumo F, Sebire NJ, Thilaganathan B. Decreased endovascular trophoblast invasion in first trimester pregnancies with high-resistance uterine artery Doppler indices. Hum Reprod. 2004;19:206–9.
76. Pijnenborg R, Bland JM, Robertson WB, Dixon G, Brosens I. The pattern of interstitial trophoblastic invasion of the myometrium in early human pregnancy. Placenta. 1981;2:303–16.
77. Palomba S, Russo T, Falbo A, Di Cello A, Tolino A, Tucci L, La Sala GB, Zullo F. Macroscopic and microscopic findings of the placenta in women with polycystic ovary syndrome. Hum Reprod. 2013;28:2838–47.

78. Palomba S, Falbo A, Chiossi G, Tolino A, Tucci L, La Sala G, Zullo F. Early trophoblast invasion and placentation in women with different PCOS phenotypes. Reprod Biomed Online. 2014;29:370–81.
79. Palomba P, Falbo A, Valli B, Morini D, Villani MT, Nicoli A, La Sala GB. Physical activity before IVF and ICSI cycles in infertile obese women: an observational cohort study. Reprod Biomed Online. 2014;29:72–9.
80. Hulchiy M, Nybacka Å, Sahlin L, Lindén Hirschberg A. Endometrial expression of estrogen receptors and the androgen receptor in women with polycystic ovary syndrome: a lifestyle intervention study. J Clin Endocrinol Metab. 2016;101:561–71.
81. Ujvaril D, Huichiyi M, Calabyl A, Nybackal A, Byström B, Hirschberg AL. Lifestyle intervention up-regulates gene nd protein levels of molecules involved in insulin signaling in the endometrium of overweight/obese women with polycystic ovary syndrome. Hum Reprod. 2014;29:1526–35.
82. Wallace KL, Johnson V, Sopelak V, Hines R. Lomiphene citrate versus letrozole: molecular analysis of the endometrium in women with polycystic ovary syndrome. Fertl Steril. 2011;96:1051–6.
83. Selim MF, Borg TF. Letrozole and clomiphene citrate effect on endometrial and subendometrial vascularity in treating infertility in women with polycystic ovary syndrome. J Gynecol Surg. 2011;28:405–10.
84. Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P, Christman GM, Huang H, Yan Q-H, Alvero R, Haisenleder DJ, Barnhart KT, Wright Bates G, Usadi R, Lucidi S, Baker V, Trussell JC, Krawetz SA, Snyder P, Ohl D, Santoro N, Eisenberg E, Zhang H-P. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. N Engl J Med. 2014;371:119–29.
85. Almog B, Shalom-Paz E, Dufort D, Tulandi T. Promoting implantation by local injury to the endometrium. Fertil Steril. 2010;94:2026–9.
86. Siristatidis C, Vrachnis N, Vogiatzi P, Chrelias C, Retamar AQ, Bettocchi S, Glujovsky D. Potential pathophysiological mechanisms of the beneficial role of endometrial injury in in vitro fertilization outcome. Reprod Sci. 2014;21:955–65.
87. Gnainsky Y, Granot I, Aldo PB, Barash A, Or Y, Schechtman E, Mor G, Dekel N. Local injury of the endometrium induces an inflammatory response that promotes successful implantation. Fertil Steril. 2010;94:2030.
88. Yeung TW, Chai J, Li RH, Lee VC, Ho PC, Ng EH. The effect of endometrial injury on ongoing pregnancy rate in unselected subfertile women undergoing in vitro fertilization: a randomized controlled trial. Hum Reprod. 2014;29:2474–81.
89. Nastri CO, Lensen S, Polanski L, Raine-Fenning N, Farquhar CM, Martins WP. Endometrial injury and reproductive outcomes: there's more to this story than meets the horse's blind eye. Hum Reprod. 2015;20:749.
90. Lensen S, Martins W, Nastri C, Sadler L, Farquhar C. Pipelle for pregnancy (PIP): study protocols for three randomised controlled trials. Trials. 2016;17:216–29.

Infertility and Subfertility Cofactors in Women with PCOS

6

Tal Shavit and Togas Tulandi

6.1 Introduction

Infertility is defined as a failure to conceive after 12 months of unprotected intercourse [1]. Some prefer the term subfertility since many couples are not sterile but exhibit decrease reproductive potential or will have a child after fertility intervention. Due to the declining fertility with increasing age, couples in which the female partner age is older than 35 years may be considered as infertile after 6 months of unprotected intercourse [1]. Recent data show that human fertility is probably higher than has previously been estimated. It is estimated that monthly fecundability is 30–38%. Conception usually occurs within 6 cycles of timed intercourse, 85–92% in 12 months [2, 3]. Infertility affects 10–15% of couples and has important psychological, economic, demographic, and medical implications [4, 5]. Contrary to popular belief, the overall incidence of infertility has remained relatively stable during the last four decades. However, the evaluation and treatment of infertility has improved dramatically.

Evaluation of infertility should focus on the couple and not solely on the female partner. The World Health Organization (WHO) Task Force on Diagnosis and Treatment of Infertility evaluated 8500 infertile couples and utilized standard diagnostic criteria to determine the medical conditions contributing to infertility. In developed countries, female factor infertility accounts for 37% of infertile couples, male factor infertility for 8%, and both male and female factors for 35% [6]. The main causes of infertility include ovulatory dysfunction (20–40%), tubal and uterine factors (30–40%), endometriosis (6%), and male factor (30–40%). In about 15% of cases, no clear cause of infertility could be found (unexplained infertility) [7–9]. The prevalence of each cause of infertility varies with age [10]. Couples in which

T. Shavit • T. Tulandi (✉)
Department of Obstetrics and Gynecology, McGill University,
Montreal, QC H3A 1A1, Canada
e-mail: togas.tulandi@mcgill.ca

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_6

the female partner has PCOS may have additional factors contributing to infertility like those in the general population such as tubal or male factor. It is important to perform complete fertility evaluation for the infertile couple and not to focus only on the polycystic ovary syndrome (PCOS) [11].

Infertility investigation is usually performed after a year of infertility. Earlier evaluation should be offered to those with conditions contributing to infertility such as irregular menses, history of pelvic inflammatory disease or endometriosis, possible male factor, and women over 35 years old. One of the common causes of infertility is ovulatory disorder usually due to PCOS. It affects 5–7% of reproductive-aged women [12]. Other possible causes of anovulation include hyper or hypothyroidism, hyperprolactinemia, congenital adrenal hyperplasia, Cushing syndrome, and androgen-secreting tumor.

In infertile patients with PCOS, the infertility can be due to oligo-anovulation, usually related to hyperandrogenism (see Chap. 3), although several additional factors may contribute to infertility (Fig. 6.1). In fact, the increasing prevalence of the PCOS may be related to environmental factors, including dietary habits, behavior, or other undefined factors. Several factors are also responsible for the increase in obesity, insulin resistance, and metabolic syndrome which may contribute directly or indirectly to PCOS and its comorbidities. Those environmental and behavioral changes may also be cofactors leading to impaired fertility [13]. Abnormalities of endometrial and oocytes competences cannot be excluded, as discussed in Chaps. 4 and 5. Finally, other concomitant factors of subfertility affecting the couple can play a clinical and pathogenic role.

This chapter discusses factors leading to anovulation in women with PCOS and additional characteristics of PCOS women that may affect their fertility potential.

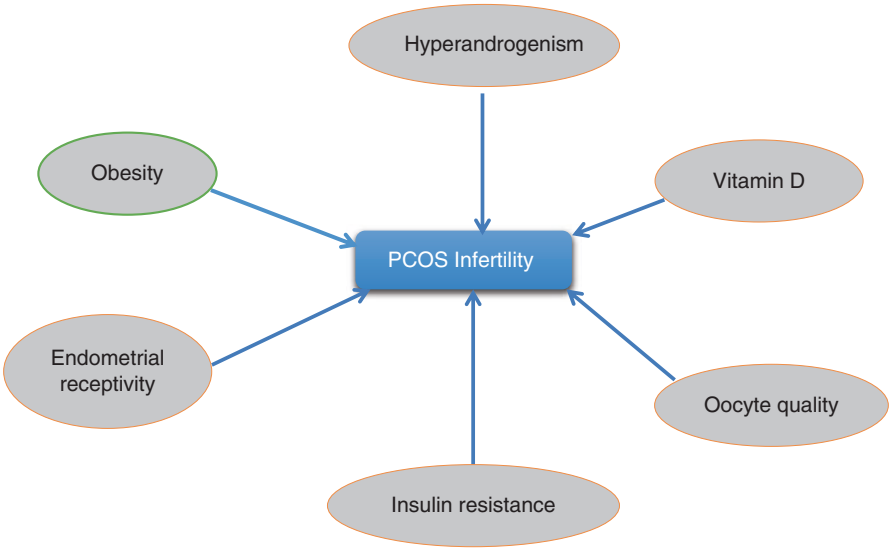


Fig. 6.1 Main PCOS-related factors that may contribute to infertility in women with the syndrome

6.2 PCOS-Related Factors of Infertility

6.2.1 Hyperandrogenism

Hyperandrogenism is the main feature of PCOS. According to the Rotterdam criteria, PCOS can be defined in the absence of hyperandrogenism. However, many believe that the occurrence of hyperandrogenism is a must criterion for PCOS that plays an important role in the pathophysiology of PCOS.

Hyperandrogenism evaluation should include clinical features (hirsutism, acne, or male-pattern alopecia) and hormonal measurements (see also Chap. 2). Serum androgen profiles in PCOS are characterized by elevated total testosterone, increased levels of bioavailable testosterone, and decreased levels of sex hormone binding globulin (SHBG). Hyperandrogenism is due to overproduction of those hormones from the ovary [14–16] and to a less extent from the adrenal gland [17]. The first step in androgen synthesis takes place in the LH-stimulated theca cells mediated by microsomal P450c17 [18]. In fact, alterations of P450c17 activity at transcriptional and posttranscriptional levels have been implicated in PCOS etiology. Exaggerated ovarian response to LH is further amplified by increased LH levels in amplitude and frequency [19].

Androgens play a critical role in the local ovarian environment. Androgens are converted to estrogen by aromatase enzyme. Early follicles acquire androgen receptors, and androgen affects folliculogenesis at the early follicle-stimulating hormone (FSH)-independent phase contributing to early follicular growth [20]. At a more advanced stage, androgens play a synergistic role with insulin and luteinizing hormone (LH) hindering follicular development.

Local ovarian androgens as commonly seen in PCOS are converted to more potent 5α-reduced androgens which cannot be converted to estrogen. Those androgens inhibit aromatase activity and FSH induction of LH receptors on granulosa cells preventing follicular development. Follicles continue to grow but are arrested at early stage before maturation. It leads to the classic polycystic ovarian morphology (PCOM), multiple small follicular cysts surrounding hypertrophic theca cells (see also Chap. 8). The exact mechanism leading to the arrest of follicular growth is yet to be established. Perhaps, it is related to premature activation of the follicles by LH. Willis et al. [21] found LH-induced secretion of estradiol and progesterone from follicles as small as 4 mm from anovulatory PCOS women compared to 9–10 mm from ovulatory women. This premature response to LH is associated with accumulation of cAMP which is responsible for follicular developmental arrest [22]. Consequently, high level of ovarian androgen impedes follicular maturation, promotes follicular atresia, and prevents the development of a dominant follicle.

Hyperinsulinemia and obesity potentiate LH activity even more and contribute to the development of hyperandrogenic state [23, 24]. Circulating androgen is increased due to direct ovarian stimulation by hyperinsulinemia and by decreased SHBG production in the liver. Abnormal genetic expression leading to impaired regulation of several steroidogenesis enzymes is also another possible mechanism [25, 26]. In short, hyperandrogenism is the leading cause of anovulation and infertility among women with PCOS.

6.2.2 Obesity

In the last four decades, the incidence of obesity has been increasing in the United States (US) and Europe [27–29], and in 2008 64% of women in the USA were overweight or obese [30] (see also Chap. 13). Obese women are prone to develop comorbidities, particularly type II diabetes mellitus, metabolic syndrome, a variety of cancer, and cardiovascular diseases [31, 32]. They may also have hormone-dependent comorbidities and infertility mostly related to PCOS-related anovulation [33]. In fact, the risk of PCOS rises with increasing obesity [34, 35]. The prevalence of overweight or obesity among PCOS women is 30% to 75% [13]. Abdominal and visceral adipose tissue plays a key role in the development of this disorder, as increased abdominal fat is observed in normal weight PCOS women as well.

Whereas hyperandrogenism and menstrual irregularities represent major complaints in adolescence with the PCOS, symptoms related to androgen excess, oligomenorrhea or amenorrhea, and infertility are the main complaints of reproductive-aged women. Obesity has an important impact on the severity PCOS particularly in the presence of increased abdominal fat [36]. The chance to conceive among obese women with PCOS is lower than that in those with normal weight [36]. Furthermore, obese PCOS women require increase doses of ovulation-inducing drugs to achieve ovulation [37–39].

The followings are specific characteristics of obese PCOS women that may be cofactors contributing to infertility.

6.2.2.1 Central Obesity Leading to Hyperandrogenism

Direct association between body fat and SHBG is well established [40–42]. Additional factors such as insulin, estrogen, and androgen levels that are altered in women with PCOS are also responsible for regulating SHBG levels. Women with central obesity have lower SHBG than those with peripheral obesity [43]. They also have increased testosterone and dihydrotestosterone production rates [44].

The decrease in SHBG which is characteristic for women with central obesity leads to increased circulating free androgen leading to hyperandrogenism and subfertility [45]. This fact is pertinent for women with PCOS; even those with normal BMI may have enhanced abdominal fatness [46, 47].

6.2.2.2 Leptin

Leptin is a 167-amino acid peptide secreted in adipose tissue. In circulation, it is bound to a family of proteins. It acts on the central nervous system (CNS) neurons that regulate eating behavior and energy balance. Some authors reported that PCOS patients have elevated leptin levels [48, 49]. However, others reported that leptin levels in women with PCOS are comparable to weight and age-matched controls [50, 51].

The role of leptin in reproduction and in the regulation of gonadotrophin concentrations has been demonstrated [52–54]. Leptin acts not only at central levels

to modulate the hypothalamo-pituitary axis [55] but also directly at the ovarian level. It is expressed in the granulosa cells and in the follicular fluid from PCOS women [56]. Increased leptin concentration in the ovary may impair the formation of the dominant follicle and the maturation of the oocyte [57]. Leptin inhibits FSH stimulation of insulin-like growth factor I (IGF-I) as well as the segregation of IGF-I on FSH stimulation of estradiol production [58]. It also contributes to the state of insulin resistance and hyperandrogenism in most women with PCOS. In animal model, leptin infusion decreased ovulation rate [57]. High leptin levels decrease epithelial Na (+) channel (ENaC) expression in the endometrium of overweight/obese women with PCOS during the window of implantation leading to reduce endometrial receptivity and implantation rate [59]. The endometrial competence in women with PCOS is widely discussed in Chap. 5.

In short, obesity-induced hyperleptinemia in PCOS may cause insulin resistance as well as impaired ovarian function.

6.2.2.3 Adiponectin

Adiponectin is an adipocytokine expressed mainly in adipose tissue and is the most abundant circulating adipose-specific protein in humans [60]. The production of adiponectin is decreased in obesity, and its serum level correlates negatively with waist circumference and body mass index (BMI) [61, 62]. As adiponectin possesses insulin-sensitizing, antidiabetic, and antiatherogenic properties, and because its circulating levels are reduced with obesity and type 2 diabetes mellitus, it may also play a role in the pathogenesis of PCOS. Yet, plasma levels of adiponectin in women with PCOS are lower than or comparable to those in control women [63, 64].

Low adiponectin levels may increase insulin resistance and androgen production through a decrease in its inhibitory effects on LH and insulin/IGF-I-stimulated androstenedione production by the ovary [65]. Whether circulating androgens are important modulators of adiponectin serum levels in PCOS or if changes in adiponectin levels precede variations in androgen levels remains unclear. However, treating PCOS women with metformin enhances both adiponectin activity and insulin sensitivity, resulting in a less hyperandrogenic state [66] (see also Chap. 11).

In conclusion, obesity is a prominent feature of several phenotypes of PCOS. The evaluation of BMI in all infertile women with PCOS should be considered crucial because the presence of obesity is related to anovulation; decreasing the potential treatment success and increasing maternal and perinatal complications during pregnancy (see Chap. 22). Weight reduction and other lifestyle modifications are the first-line treatments for infertile PCOS women, as discussed in Chap. 13.

6.2.3 Insulin Resistance

Insulin resistance is defined as the inability of exogenous or endogenous insulin to increase glucose uptake and its utilization [67]. Insulin resistance and hyperandrogenism play a key role in the pathophysiology of PCOS. In fact, insulin resistance

is found in 85% of PCOS women (75% and 95% for nonobese and obese subjects, respectively) [68]. Insulin resistance occurs when insulin-responsive tissues such as the liver becomes less sensitive to insulin, leading the pancreas to produce increased compensatory insulin and eventually leads to hyperinsulinemia [67]. Recent data show that hyperinsulinemia secondary to insulin resistance is the primary factor of increased androgen production [69–71].

Several hypotheses on the mechanism of insulin resistance contribution to hyperandrogenism have been proposed. In human and animal model, insulin stimulates ovarian androgen secretion directly or enhances LH prompt androgen secretion [72, 73], acts indirectly to enhance the amplitude of GnRH-stimulated LH pulses [74], decreases hepatic production of serum SHBG [75, 76], decreases IGF-binding protein-1 (IGFBP-1), and increases the availability of free IGF-1 stimulating the androgen production [77–79]. Finally, hyperinsulinemia may contribute to mid-antral follicular arrest in PCOS [80].

One of the indirect clinical evidence of the role of insulin resistance as a cofactor for PCOS-related infertility is the efficacy of metformin in those women (see also Chap. 11). In theory, metformin may act indirectly by reducing serum insulin levels and directly within the ovary by reducing P450c17a enzyme activity and subsequent androgen production. Further, it increases IGFBP-1 and reduces the availability of IGF-1 [79, 81, 82]. However, the efficacy of metformin alone in enhancing fertility in women with PCOS remains unclear [83–85].

6.3 Endometrial Receptivity

While anovulation is an obvious cause of infertility in women with PCOS, impaired endometrial receptivity may also play a role [86]. The alterations and peculiarities of the endometrium and the role played by endometrium receptivity in infertile PCOS patients are discussed in Chap. 5.

Briefly, due to ovulatory disorder, the endometrium is exposed to unopposed estrogen stimulation leading to altered endometrial milieu. Indeed, women with PCOS tend to have decreased implantation rate and increased miscarriage rate that has been attributed to decreased endometrial receptivity. Several publications demonstrated an increased risk (up to 50%) for miscarriage among women with PCOS [32, 87–89]. However, others found similar miscarriage rates among women with PCOS, and fertile women and women with other infertility diagnosis [90, 91].

Endometrium preparation prior to implantation is regulated by steroids and by several gene expressions especially HOXA10 and HOXA11. These genes are essential for endometrial growth, differentiation, and receptivity by mediating steroid hormone effects [92]. Other endometrial receptivity-related mediators including avb3 integrin, IGFBP-1, and leukemia inhibitory factor (LIF) are also regulated by HOX genes [83]. In recent years, a number of publications demonstrated several endometrial characteristics/markers important for implantation that may explain lower implantation rate in women with PCOS. They include increased expression of

the estrogen receptor in the glandular epithelium [84] and in androgen receptors [85]. Further, women with PCOS have decreased HOXA10 expression during the secretory phase and decreased integrin [93].

Another marker is the WT1. WT1 expression is downregulated in the endometrium of PCOS women during the window of implantation [94]. Changes in this gene expression may lead to abnormal implantation and lower birth rates. Modifications in the implantation window and possibly the endometrial receptivity are also mediated by abnormal steroid environment [95, 96]. In vitro study demonstrated impaired decidualization response with local altered endometrial inflammatory profile in women with PCOS. The endometrial decidualized stromal cells seem to play a role in active embryo selection.

6.4 Oocytes and Embryo Quality

The potential alterations of the folliculogenesis and of the oocyte competence in PCOS patients are specifically discussed in Chap. 4. Briefly, PCOS women treated with control ovarian stimulation tends to produce a high number of follicles. However, the oocytes have been reported poor in quality leading to low fertilization and implantation rate and higher miscarriage rate [97–102]. This could be due to impaired oocyte competence and embryonic development that may be related to alterations in the intrafollicular microenvironment during folliculogenesis and in follicle maturation. Perhaps, it is related to inadequate dialogue between the cumulus cells and the oocytes or to abnormal paracrine/endocrine factors and metabolic dysfunction [103–108]. In a mouse PCOS model induced with DHEA, the number of MII oocyte is reduced. They had decrease mtDNA copy number, ATP content, excessive oxidative stress, and impaired embryo development competence compared to control mice. The authors concluded that excessive androgen may be detrimental to oocyte quality [109].

Yet, compared to normo-ovulatory women, PCOS women undergoing controlled ovarian hyperstimulation had comparable or even better oocyte and embryo quality [97, 110, 111], suggesting that PCOS is not related to adverse oocyte quality, at least in the context of nuclear maturation [112].

6.5 Vitamin D

Vitamin D or calcitriol is a steroid hormone, synthesized mainly by the skin on exposure to ultraviolet light. Additional 10–20% of vitamin D comes from diet. Vitamin D is converted to 25-hydroxyvitamin D (25OH-D) by hepatic 25-hydroxylase. Subsequently, it is converted by renal 1a–hydroxylase to the active form 1,25-dihydroxyvitamin D3 [113, 114].

High prevalence of vitamin D deficiency was reported in PCOS women (67–85% compared to 20–48% in the general population) [115, 116] (see also Chap. 14). In women with PCOS, vitamin D levels are related to hormonal dysfunction and

metabolic status. Vitamin D deficiency might also be a contributing factor to insulin resistance, and metabolic syndrome [117–122]. Note that obesity may decrease the circulating 25OH-D levels by trapping the lipophilic vitamin in the adipose tissue [123].

Asadi et al. reported that genetic variant of the vitamin D receptor was associated with the severity of PCOS clinically [124]. A correlation between vitamin D deficiency and infertility has also been reported. Recent studies showed that low levels of vitamin D in the follicular fluid were associated with lower implantation and live birth rates [125–128]. However, the effect of vitamin D supplementation to vitamin D-depleted women undergoing IVF treatment is still unclear [129].

Using data collected in the Pregnancy in PCOS I (PPCOS-I) study, the authors found direct correlation between vitamin D levels and ovulatory rates in infertile PCOS women treated with clomiphene, metformin, or both. Of interest, each 1 ng/mL (2.5 nmol/L) increase in serum 25OH-D enhanced the likelihood of live birth by 2% [130]. Another study found that vitamin D supplementation increased endometrial thickness in PCOS women undergoing intrauterine insemination (IUI) treatment. However, the pregnancy rates in women treated and not treated with vitamin D were comparable [131]. Results of studies evaluating the effect of vitamin D supplementation and insulin resistance have been mixed. While some reported an improvement in insulin resistance in obese PCOS women [132], others did not demonstrate the effect of vitamin D [133]. It appears that vitamin D deficiency is a common finding in PCOS women. It might be related to insulin resistance, ovulatory dysfunction, and infertility. Currently there is a paucity of data supporting routine use of vitamin D in women with PCOS.

6.6 Fertility Evaluation in PCOS Women

Fertility evaluation is usually recommended after 12 months of failure to conceive with reasonable frequency of unprotected intercourse. For women with suspected PCOS, infertility investigations should be started after 6 months of trying. For most PCOS women, the most likely diagnosis will be anovulation. However, other causes of infertility should be eliminated before starting treatment. Note that 40% of infertile couples have multifactorial causes of infertility.

According to the National Institute of Excellence (NICE) [https://www.nice.org.uk/guidance/cg156] and American Society of Reproductive Medicine (ASRM) guidelines [134], the basic infertility evaluation should include the following.

6.6.1 Medical and Reproductive History

Women with PCOS and their partner should undergo general routine medical history to exclude other possible causes of infertility. History should include questions regarding possible diabetes or insulin resistance status and family history of

diabetes. The reproductive history should focus on the menstrual cycle characteristics. Women with oligomenorrhea or amenorrhea generally do not require specific diagnostic tests to establish anovulation. The history should include questions about symptoms related to adrenal disease, thyroid disease or hyperprolactinemia, or other causes of oligomenorrhea such as weight gain or loss and excessive exercise. Assessment of lifestyle habits is important particularly in this population. They are at risk to develop metabolic syndrome.

6.6.2 Physical Examination

In addition to the routine physical examination, women with PCOS should be evaluated for signs of androgen access. Hirsutism can be evaluated using the Ferriman-Gallwey score (see Chap. 2). BMI have to be calculated for each patient with suspected PCOS. Waist circumference or waist-to-hip ratio is also useful to suspect a visceral obesity. The thyroid should be palpated to rule out goiter and the breast to identify galactorrhea. The skin should be examined for hyperkeratosis and acanthosis nigricans.

6.6.3 Basal Body Temperature (BBT)

BBT measurements provide a simple and inexpensive method for evaluating ovulatory function. In cycles monitored with BBT, the period of highest fertility spans the 7 days prior to the mid-cycle rise in BBT. Anovulatory cycles typically result in monophasic patterns. However, BBT measurement is not always reliable.

6.6.4 Laboratory Test

In addition to the routine blood test performed as part of the initial work-up infertility evaluation, several additional tests are recommended for women with suspected PCOS (see also Chap. 2).

- LH/FSH ratio may serve as an additional indicator (in case of ratio equal or higher than 2) favoring PCOS.
- Serum levels of androgens and especially testosterone are usually measured. Yet, its contribution to establish the diagnosis of PCOS is controversial.
- Mid-luteal phase serum progesterone greater than 15 ng/mL provides presumptive but reliable evidence of recent ovulation. Endometrial biopsy (EB) and histology can demonstrate secretory endometrial development. Due to its invasive nature, most fertility specialists have abandoned the routine practice of EB.
- AMH levels are two to threefold higher in women with PCOS compared to unaffected women and relatively higher in relation to antral follicles.

– Serum levels of 17-hydroxyprogesterone should be measured to rule out the presence of late-onset congenital adrenal hyperplasia.
– Oral glucose tolerance test (OGTT). In women with risk factors for diabetes mellitus including dyslipidemia, additional tests including fasting glucose and HbA1c measurement should be done. The time when the patients are undergoing infertility investigations is also a good opportunity to evaluate the general health including comorbidities. Treatment of those comorbidities could help the patients to conceive and to decrease pregnancy complications (see Chap. 22).

6.6.5 Transvaginal Ultrasound (TVS)

TVS is mandatory to evaluate the ovarian appearance and AFC as part of the PCOS investigation. In addition, it also provides information about the endometrium that may be decidualized in PCOS, endometrial hyperplasia, or possibly endometrial carcinoma. Evaluation of the baseline endometrial thickness is important in the initial work-up of those women. TVS is also important to evaluate the uterine cavity and to rule out the presence of intrauterine polyp, myoma, or uterine septum.

6.6.6 Tubal Patency

Tubal disease is an important cause of infertility and should be specifically excluded. The conventional method to evaluate tubal patency is hysterosalpingography (HSG) examination. A newer method is hysterosonography (or saline infusion sonohysterography, SIS). In this era of IVF, diagnostic laparoscopy is rarely performed. However, laparoscopy plays a role in cases with pelvic pain or with suspected endometriosis. In addition, although controversial ovarian drilling can be performed at the time of laparoscopy (see Chap. 15).

6.6.7 Semen Analysis

Semen analysis should be performed routinely. About 40% of infertile couples have multifactorial infertility mostly combined male and female abnormalities. The sperm analysis should be performed according to the WHO guidelines. In Fig. 6.2 the lower reference limits (5th centiles and their 95% confidence intervals) for semen characteristics are reported.

Parameter	Lower reference limit
Semen volume (ml)	1.5 (1.4–1.7)
Total sperm number (10^6 per ejaculate)	39 (33–46)
Sperm concentration (10^6 per ml)	15 (12–16)
Total motility (PR+ NP, %)	40 (38–42)
Progressive motility (PR, %)	32 (31–34)
Vitality (live spermatozoa,%)	58 (55–63)
Sperm morphology (normal forms, %)	4 (3.0–4.0)

Fig. 6.2 Lower reference limits (5th centiles and their 95% confidence intervals) for semen characteristics according to the WHO guidelines

Conclusion

Infertility in PCOS women is usually related to anovulation. However, several investigators suggested association between PCOS and other infertility factor such as endometriosis [135] or possibly with leiomyomas [136]. Because many infertile couples have more than one infertility diagnosis, other causes of infertility should be evaluated. The contribution of cofactors leading to anovulation may be different among the diverse PCOS phenotypes. Fertility varies according to the specific PCOS phenotype and related comorbidities. Insulin resistance, obesity, hyperandrogenism, and infertility are clearly related to PCOS. Others factors including endometrial and oocyte competence, as well as the role of vitamin D, need further investigation. Treatment should be tailored accordingly.

References

1. Practice Committee of American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertil Steril. 2013;99:63.
2. Wang X, et al. Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study. Fertil Steril. 2003;79:577–84.
3. Gnoth C, et al. Time to pregnancy: results of the German prospective study and impact on the management of infertility. Hum Reprod. 2003;18:1959–66.
4. Gnoth C, et al. Definition and prevalence of subfertility and infertility. Hum Reprod. 2005;20:1144–7.
5. Evers JL. Female subfertility. Lancet. 2002;360:151–9.
6. WHO. Recent advances in medically assisted conception. Report of a WHO Scientific Group. World Health Organ Tech Rep Ser. 1992;820:1–111.
7. Hull MG, et al. Population study of causes, treatment, and outcome of infertility. Br Med J (Clin Res Ed). 1985;291:1693–7.

8. Templeton A, Fraser C, Thompson B. The epidemiology of infertility in Aberdeen. BMJ. 1990;301:148–52.
9. Bhattacharya S, et al. The epidemiology of infertility in the North East of Scotland. Hum Reprod. 2009;24:3096–107.
10. Miller JH, et al. The pattern of infertility diagnoses in women of advanced reproductive age. Am J Obstet Gynecol. 1999;181:952–7.
11. McGovern PG, et al. Utility of screening for other causes of infertility in women with "known" polycystic ovary syndrome. Fertil Steril. 2007;87:442–4.
12. Azziz R, et al. The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab. 2004;89:2745–9.
13. Ehrmann DA. Polycystic ovary syndrome. N Engl J Med. 2005;352:1223–36.
14. Cedars MI, et al. Long-term administration of gonadotropin-releasing hormone agonist and dexamethasone: assessment of the adrenal role in ovarian dysfunction. Fertil Steril. 1992;57:495–500.
15. Kumar A, et al. Prevalence of adrenal androgen excess in patients with the polycystic ovary syndrome (PCOS). Clin Endocrinol (Oxf). 2005;62:644–9.
16. Baskind NE, Balen AH. Hypothalamic-pituitary, ovarian and adrenal contributions to polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol. 2016;37:80–97.
17. Yildiz BO, Azziz R. The adrenal and polycystic ovary syndrome. Rev Endocr Metab Disord. 2007;8:331–42.
18. Baptiste CG, et al. Insulin and hyperandrogenism in women with polycystic ovary syndrome. J Steroid Biochem Mol Biol. 2010;122:42–52.
19. Hendriks ML, et al. LH as a diagnostic criterion for polycystic ovary syndrome in patients with WHO II oligo/amenorrhoea. Reprod Biomed Online. 2008;16:765–71.
20. Rice S, et al. Stage-specific expression of androgen receptor, follicle-stimulating hormone receptor, and anti-Mullerian hormone type II receptor in single, isolated, human preantral follicles: relevance to polycystic ovaries. J Clin Endocrinol Metab. 2007;92:1034–40.
21. Willis DS, et al. Premature response to luteinizing hormone of granulosa cells from anovulatory women with polycystic ovary syndrome: relevance to mechanism of anovulation. J Clin Endocrinol Metab. 1998;83:3984–91.
22. Hugues JN, Durnerin IC. Impact of androgens on fertility–physiological, clinical and therapeutic aspects. Reprod Biomed Online. 2005;11:570–80.
23. Qu J, et al. Insulin resistance directly contributes to androgenic potential within ovarian theca cells. Fertil Steril. 2009;91(5 Suppl):1990–7.
24. Cupisti S, et al. Body mass index and ovarian function are associated with endocrine and metabolic abnormalities in women with hyperandrogenic syndrome. Eur J Endocrinol. 2008;158:711–9.
25. Doi SA. Neuroendocrine dysfunction in PCOS: a critique of recent reviews. Clin Med Res. 2008;6:47–53.
26. Strauss 3rd JF. Some new thoughts on the pathophysiology and genetics of polycystic ovary syndrome. Ann N Y Acad Sci. 2003;997:42–8.
27. Harlan WR, et al. Secular trends in body mass in the United States, 1960–1980. Am J Epidemiol. 1988;128:1065–74.
28. Kuczmarski RJ, et al. Increasing prevalence of overweight among US adults. The National Health and Nutrition Examination Surveys, 1960 to 1991. JAMA. 1994;272:205–11.
29. Flegal KM, et al. Prevalence and trends in obesity among US adults, 1999–2000. JAMA. 2002;288:1723–7.
30. Flegal KM, et al. Prevalence and trends in obesity among US adults, 1999–2008. JAMA. 2010;303:235–41.
31. Ford ES. Prevalence of the metabolic syndrome in US populations. Endocrinol Metab Clin North Am. 2004;33:333–50.

32. Hart R, Doherty DA. The potential implications of a PCOS diagnosis on a woman's long-term health using data linkage. J Clin Endocrinol Metab. 2015;100:911–9.
33. Pasquali R, et al. Obesity and reproductive disorders in women. Hum Reprod Update. 2003;9:359–72.
34. Yildiz BO, Knochenhauer ES, Azziz R. Impact of obesity on the risk for polycystic ovary syndrome. J Clin Endocrinol Metab. 2008;93:162–8.
35. Alvarez-Blasco F, et al. Prevalence and characteristics of the polycystic ovary syndrome in overweight and obese women. Arch Intern Med. 2006;166:2081–6.
36. Gambineri A, et al. Obesity and the polycystic ovary syndrome. Int J Obes Relat Metab Disord. 2002;26:883–96.
37. Galtier-Dereure F, et al. Choice of stimulation in polycystic ovarian syndrome: the influence of obesity. Hum Reprod. 1997;12:88–96.
38. White DM, et al. Induction of ovulation with low-dose gonadotropins in polycystic ovary syndrome: an analysis of 109 pregnancies in 225 women. J Clin Endocrinol Metab. 1996;81:3821–4.
39. Fedorcsak P, et al. The impact of obesity and insulin resistance on the outcome of IVF or ICSI in women with polycystic ovarian syndrome. Hum Reprod. 2001;16:1086–91.
40. Glass AR, et al. Low serum testosterone and sex-hormone-binding-globulin in massively obese men. J Clin Endocrinol Metab. 1977;45:1211–9.
41. Stefan N, Schick F, Haring HU. Sex hormone-binding globulin and risk of type 2 diabetes. N Engl J Med. 2009;361:2675–6. author reply 2677–8
42. Peter A, et al. Relationships of circulating sex hormone-binding globulin with metabolic traits in humans. Diabetes. 2010;59:3167–73.
43. Pasquali R, Vicennati V, and U Pagotto, Endocrine determinants of fat distribution, in Handbook of obesity, Bouchard C. Bray GA, Editor. 2003, . Marcel Dekker: New York. p. 671–692.
44. Kirschner MA, et al. Androgen-estrogen metabolism in women with upper body versus lower body obesity. J Clin Endocrinol Metab. 1990;70:473–9.
45. Simo R, et al. Novel insights in SHBG regulation and clinical implications. Trends Endocrinol Metab. 2015;26:376–83.
46. de Mendonca-Louzeiro MR, Annichino-Bizzacchi JM, Benetti-Pinto CL. Android fat distribution affects some hemostatic parameters in women with polycystic ovary syndrome compared with healthy control subjects matched for age and body mass index. Fertil Steril. 2015;104:467–73.
47. Barber TM, et al. Obesity and polycystic ovary syndrome. Clin Endocrinol (Oxf). 2006;65:137–45.
48. Houjeghani S, Pourghassem Gargari B, Farzadi L. Serum leptin and ghrelin levels in women with polycystic ovary syndrome: correlation with anthropometric, metabolic, and endocrine parameters. Int J Fertil Steril. 2012;6:117–26.
49. Pehlivanov B, Mitkov M. Serum leptin levels correlate with clinical and biochemical indices of insulin resistance in women with polycystic ovary syndrome. Eur J Contracept Reprod Health Care. 2009;14:153–9.
50. Chen X, et al. Adipokines in reproductive function: a link between obesity and polycystic ovary syndrome. J Mol Endocrinol. 2013;50:R21–37.
51. Mantzoros CS, Dunaif A, Flier JS. Leptin concentrations in the polycystic ovary syndrome. J Clin Endocrinol Metab. 1997;82:1687–91.
52. Budak E, et al. Interactions of the hormones leptin, ghrelin, adiponectin, resistin, and PYY3–36 with the reproductive system. Fertil Steril. 2006;85:1563–81.
53. Barash IA, et al. Leptin is a metabolic signal to the reproductive system. Endocrinology. 1996;137:3144–7.
54. Cunningham MJ, Clifton DK, Steiner RA. Leptin's actions on the reproductive axis: perspectives and mechanisms. Biol Reprod. 1999;60:216–22.

55. Mitchell M, et al. Adipokines: implications for female fertility and obesity. Reproduction. 2005;130:583–97.
56. Loffler S, et al. Evidence of leptin expression in normal and polycystic human ovaries. Mol Hum Reprod. 2001;7:1143–9.
57. Duggal PS, et al. The in vivo and in vitro effects of exogenous leptin on ovulation in the rat. Endocrinology. 2000;141:1971–6.
58. Cioffi JA, et al. Novel B219/OB receptor isoforms: possible role of leptin in hematopoiesis and reproduction. Nat Med. 1996;2:585–9.
59. Lin XH, et al. Leptin down-regulates gamma-ENaC expression: a novel mechanism involved in low endometrial receptivity. Fertil Steril. 2015;103:228–35. e3
60. Arita Y, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 1999;257:79–83.
61. Weyer C, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab. 2001;86:1930–5.
62. Vilarrasa N, et al. Distribution and determinants of adiponectin, resistin and ghrelin in a randomly selected healthy population. Clin Endocrinol (Oxf). 2005;63:329–35.
63. Ardawi MS, Rouzi AA. Plasma adiponectin and insulin resistance in women with polycystic ovary syndrome. Fertil Steril. 2005;83:1708–16.
64. Aroda V, et al. Circulating and cellular adiponectin in polycystic ovary syndrome: relationship to glucose tolerance and insulin action. Fertil Steril. 2008;89:1200–8.
65. Lagaly DV, et al. Role of adiponectin in regulating ovarian theca and granulosa cell function. Mol Cell Endocrinol. 2008;284:38–45.
66. Hamed HO. Role of adiponectin and its receptor in prediction of reproductive outcome of metformin treatment in patients with polycystic ovarian syndrome. J Obstet Gynaecol Res. 2013;39:1596–603.
67. Lebovitz HE. Insulin resistance: definition and consequences. Exp Clin Endocrinol Diabetes. 2001;109:S135–48.
68. Stepto NK, et al. Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic-hyperinsulaemic clamp. Hum Reprod. 2013;28:777–84.
69. Cussons AJ, et al. Cardiometabolic risk in polycystic ovary syndrome: a comparison of different approaches to defining the metabolic syndrome. Hum Reprod. 2008;23:2352–8.
70. Geffner ME, et al. Persistence of insulin resistance in polycystic ovarian disease after inhibition of ovarian steroid secretion. Fertil Steril. 1986;45:327–33.
71. Diamanti-Kandarakis E, et al. Insulin sensitivity and antiandrogenic therapy in women with polycystic ovary syndrome. Metabolism. 1995;44:525–31.
72. Barbieri RL, Makris A, Ryan KJ. Insulin stimulates androgen accumulation in incubations of human ovarian stroma and theca. Obstet Gynecol. 1984;64(3 Suppl):73S–80S.
73. Hernandez ER, et al. Insulin as a regulator of androgen biosynthesis by cultured rat ovarian cells: cellular mechanism(s) underlying physiological and pharmacological hormonal actions. Endocrinology. 1988;122:2034–43.
74. Adashi EY, Hsueh AJ, Yen SS. Insulin enhancement of luteinizing hormone and follicle-stimulating hormone release by cultured pituitary cells. Endocrinology. 1981;108:1441–9.
75. Teede HJ, Stuckey BG. Polycystic ovary syndrome and abnormal glucose tolerance. Med J Aust. 2007;187:324–5.
76. Nestler JE, et al. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. J Clin Endocrinol Metab. 1991;72:83–9.
77. Lee PD, Conover CA, Powell DR. Regulation and function of insulin-like growth factor-binding protein-1. Proc Soc Exp Biol Med. 1993;204:4–29.
78. Ibanez L, et al. Hyperinsulinemia and decreased insulin-like growth factor-binding protein-1 are common features in prepubertal and pubertal girls with a history of premature pubarche. J Clin Endocrinol Metab. 1997;82:2283–8.

79. De Leo V, et al. Effect of metformin on insulin-like growth factor (IGF) I and IGF-binding protein I in polycystic ovary syndrome. J Clin Endocrinol Metab. 2000;85: 1598–600.
80. Franks S, et al. Insulin action in the normal and polycystic ovary. Endocrinol Metab Clin North Am. 1999;28:361–78.
81. Diamanti-Kandarakis E, et al. Metformin: an old medication of new fashion: evolving new molecular mechanisms and clinical implications in polycystic ovary syndrome. Eur J Endocrinol. 2010;162:193–212.
82. Nestler JE, Jakubowicz DJ. Decreases in ovarian cytochrome P450c17 alpha activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. N Engl J Med. 1996;335:617–23.
83. Xu B, et al. Regulation of endometrial receptivity by the highly expressed HOXA9, HOXA11 and HOXD10 HOX-class homeobox genes. Hum Reprod. 2014;29:781–90.
84. Quezada S, et al. Evaluation of steroid receptors, coregulators, and molecules associated with uterine receptivity in secretory endometria from untreated women with polycystic ovary syndrome. Fertil Steril. 2006;85:1017–26.
85. Apparao KB, et al. Elevated endometrial androgen receptor expression in women with polycystic ovarian syndrome. Biol Reprod. 2002;66:297–304.
86. Giudice LC. Endometrium in PCOS: implantation and predisposition to endocrine CA. Best Pract Res Clin Endocrinol Metab. 2006;20:235–44.
87. Balen AH, et al. Miscarriage rates following in-vitro fertilization are increased in women with polycystic ovaries and reduced by pituitary desensitization with buserelin. Hum Reprod. 1993;8:959–64.
88. Sagle M, et al. Recurrent early miscarriage and polycystic ovaries. BMJ. 1988;297:1027–8.
89. Rees DA, Jenkins-Jones S, Morgan CL. Contemporary reproductive outcomes for patients with polycystic ovary syndrome: a retrospective observational study. J Clin Endocrinol Metab. 2016;101:1664–72.
90. West S, et al. Irregular menstruation and hyperandrogenaemia in adolescence are associated with polycystic ovary syndrome and infertility in later life: northern Finland birth cohort 1986 study. Hum Reprod. 2014;29:2339–51.
91. Palomba S, et al. Pregnancy complications in women with polycystic ovary syndrome. Hum Reprod Update. 2015;21:575–92.
92. Cakmak H, Taylor HS. Implantation failure: molecular mechanisms and clinical treatment. Hum Reprod Update. 2011;17:242–53.
93. Piltonen TT. Polycystic ovary syndrome: endometrial markers. Best Pract Res Clin Obstet Gynaecol. 2016;37:66–79.
94. Gonzalez D, et al. Loss of WT1 expression in the endometrium of infertile PCOS patients: a hyperandrogenic effect? J Clin Endocrinol Metab. 2012;97:957–66.
95. Baracat MC, et al. Systematic review of cell adhesion molecules and estrogen receptor expression in the endometrium of patients with polycystic ovary syndrome. Int J Gynaecol Obstet. 2015;129:1–4.
96. Lopes IM, et al. Endometrium in women with polycystic ovary syndrome during the window of implantation. Rev Assoc Med Bras. 2011;57:702–9.
97. Ludwig M, et al. Oocyte quality and treatment outcome in intracytoplasmic sperm injection cycles of polycystic ovarian syndrome patients. Hum Reprod. 1999;14:354–8.
98. Boomsma CM, Fauser BC, Macklon NS. Pregnancy complications in women with polycystic ovary syndrome. Semin Reprod Med. 2008;26:72–84.
99. Heijnen EM, et al. A meta-analysis of outcomes of conventional IVF in women with polycystic ovary syndrome. Hum Reprod Update. 2006;12:13–21.
100. Sahu B, et al. Comparison of oocyte quality and intracytoplasmic sperm injection outcome in women with isolated polycystic ovaries or polycystic ovarian syndrome. Arch Gynecol Obstet. 2008;277:239–44.

101. Mulders AG, et al. IVF outcome in anovulatory infertility (WHO group 2)–including polycystic ovary syndrome–following previous unsuccessful ovulation induction. Reprod Biomed Online. 2003;7:50–8.
102. Sengoku K, et al. The chromosomal normality of unfertilized oocytes from patients with polycystic ovarian syndrome. Hum Reprod. 1997;12:474–7.
103. Kenigsberg S, et al. Gene expression microarray profiles of cumulus cells in lean and overweight-obese polycystic ovary syndrome patients. Mol Hum Reprod. 2009;15:89–103.
104. Kwon H, et al. mRNA expression pattern of insulin-like growth factor components of granulosa cells and cumulus cells in women with and without polycystic ovary syndrome according to oocyte maturity. Fertil Steril. 2010;94:2417–20.
105. Dumesic DA, Abbott DH. Implications of polycystic ovary syndrome on oocyte development. Semin Reprod Med. 2008;26:53–61.
106. Dumesic DA, Abbott DH, Padmanabhan V. Polycystic ovary syndrome and its developmental origins. Rev Endocr Metab Disord. 2007;8:127–41.
107. Wood JR, et al. Molecular abnormalities in oocytes from women with polycystic ovary syndrome revealed by microarray analysis. J Clin Endocrinol Metab. 2007;92:705–13.
108. Franks S, Roberts R, Hardy K. Gonadotrophin regimens and oocyte quality in women with polycystic ovaries. Reprod Biomed Online. 2003;6:181–4.
109. Huang Y, et al. Impaired oocyte quality induced by dehydroepiandrosterone is partially rescued by metformin treatment. PLoS One. 2015;10:e0122370.
110. Kdous M, et al. Oocyte and embryo quality and outcome of ICSI cycles in patients with polycystic ovary syndrome (PCOS) versus normo-ovulatory. J Gynecol Obstet Biol Reprod (Paris). 2009;38:133–43.
111. Sermondade N, et al. Impact of polycystic ovary syndrome on oocyte and embryo quality. Gynecol Obstet Fertil. 2013;41:27–30.
112. Sigala J, et al. Is polycystic ovarian morphology related to a poor oocyte quality after controlled ovarian hyperstimulation for intracytoplasmic sperm injection? Results from a prospective, comparative study. Fertil Steril. 2015;103:112–8.
113. Bouillon R, et al. Vitamin D metabolism and action. Osteoporos Int. 1998;8:S13–9.
114. Wagner CL, et al. Vitamin D and its role during pregnancy in attaining optimal health of mother and fetus. Nutrients. 2012;4:208–30.
115. Thomson RL, Spedding S, Buckley JD. Vitamin D in the aetiology and management of polycystic ovary syndrome. Clin Endocrinol (Oxf). 2012;77:343–50.
116. Forrest KY, Stuhldreher WL. Prevalence and correlates of vitamin D deficiency in US adults. Nutr Res. 2011;31:48–54.
117. de Groot PC, et al. PCOS, coronary heart disease, stroke and the influence of obesity: a systematic review and meta-analysis. Hum Reprod Update. 2011;17:495–500.
118. Khanna R, Wu X, Shen B. Low levels of vitamin D are common in patients with ileal pouches irrespective of pouch inflammation. J Crohns Colitis. 2013;7:525–33.
119. Gallea M, et al. Insulin and body weight but not hyperandrogenism seem involved in seasonal serum 25-OH-vitamin D3 levels in subjects affected by PCOS. Gynecol Endocrinol. 2014;30:739–45.
120. Wehr E, et al. Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. Eur J Endocrinol. 2009;161:575–82.
121. Hahn S, et al. Low serum 25-hydroxyvitamin D concentrations are associated with insulin resistance and obesity in women with polycystic ovary syndrome. Exp Clin Endocrinol Diabetes. 2006;114:577–83.
122. Ngo DT, et al. Determinants of insulin responsiveness in young women: impact of polycystic ovarian syndrome, nitric oxide, and vitamin D. Nitric Oxide. 2011;25:326–30.
123. He C, et al. Serum vitamin D levels and polycystic ovary syndrome: a systematic review and meta-analysis. Nutrients. 2015;7:4555–77.

124. Zadeh-Vakili A, et al. Genetic polymorphism of vitamin D receptor gene affects the phenotype of PCOS. Gene. 2013;515:193–6.
125. Rudick B, et al. Characterizing the influence of vitamin D levels on IVF outcomes. Hum Reprod. 2012;27:3321–7.
126. Ozkan S, et al. Replete vitamin D stores predict reproductive success following in vitro fertilization. Fertil Steril. 2010;94:1314–9.
127. Rudick BJ, et al. Influence of vitamin D levels on in vitro fertilization outcomes in donor-recipient cycles. Fertil Steril. 2014;101:447–52.
128. Estes SJ, et al. A proteomic analysis of IVF follicular fluid in women ≤32 years old. Fertil Steril. 2009;92:1569–78.
129. Irani M, Merhi Z. Role of vitamin D in ovarian physiology and its implication in reproduction: a systematic review. Fertil Steril. 2014;102:460–8. e3
130. Pal L, et al. Vitamin D status relates to reproductive outcome in women with polycystic ovary syndrome: secondary analysis of a multicenter randomized controlled trial. J Clin Endocrinol Metab. 2016;101:3027–35.
131. Asadi M, et al. Vitamin D improves endometrial thickness in PCOS women who need intrauterine insemination: a randomized double-blind placebo-controlled trial. Arch Gynecol Obstet. 2014;289:865–70.
132. Selimoglu H, et al. The effect of vitamin D replacement therapy on insulin resistance and androgen levels in women with polycystic ovary syndrome. J Endocrinol Invest. 2010;33:234–8.
133. Ardabili HR, Gargari BP, Farzadi L. Vitamin D supplementation has no effect on insulin resistance assessment in women with polycystic ovary syndrome and vitamin D deficiency. Nutr Res. 2012;32:195–201.
134. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile female: a committee opinion. Fertil Steril. 2015;103:e44–50.
135. Singh KB, Patel YC, Wortsman J. Coexistence of polycystic ovary syndrome and pelvic endometriosis. Obstet Gynecol. 1989;74:650–2.
136. Wise LA, et al. Polycystic ovary syndrome and risk of uterine leiomyomata. Fertil Steril. 2007;87:1108–15.

PCOS Phenotypes: Impact on Fertility

7

Enrico Carmina

7.1 Introduction

One of the main characters of polycystic ovary syndrome (PCOS) is its heterogeneity [1, 2]. While the classic image of the PCOS patient is a hirsute, hyperandrogenic woman with menstrual irregularities and metabolic problems, the clinical picture may be very different. Many patients have normal body weight, others may not be hirsute, androgen levels may be normal and metabolic problems may not be important.

For several years, most experts used a mainly clinical diagnostic approach. Patients presenting hyperandrogenism (clinical or biochemically demonstrated) and chronic anovulation were considered affected by PCOS, while all other patients were excluded from the disorder [3]. However, these criteria were too much restrictive. Important similarities between ovulatory and anovulatory hyperandrogenic women with polycystic ovarian morphology (PCOM) were observed, and we suggested that both groups were part of the same disorder [4, 5]. The same patient during her life, just changing body weight, could move from a condition of chronic anovulation to ovulatory cycles and back [6]. In many countries, National Institutes of Health (NIH) criteria were never used and diagnosis was based on PCOM [7]. Finally, an agreement was found to include in the disorder patients presenting at least two out of three criteria (hyperandrogenism, anovulation, PCOM) [8]. While Androgen Excess and PCOS (AE-PCOS) Society expressed concern about the possibility of including also non-hyperandrogenic patients in the syndrome [9], the so-called Rotterdam criteria have been accepted by most experts and finally endorsed by most scientific societies and by NIH [10]. For a deeper discussion about diagnostic criteria, see also Chap. 2.

E. Carmina
Department of Health Sciences and Mother and Child Care, University of Palermo, Palermo 90139, Italy
e-mail: enrico.carmina@ae-society.org

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_7

7.2 PCOS Phenotypes

Using Rotterdam criteria, four main phenotypes may be distinguished:

1. Hyperandrogenism, chronic anovulation and PCOM
2. Hyperandrogenism, chronic anovulation and normal ovaries
3. Hyperandrogenism, PCOM and ovulatory cycles
4. Chronic anovulation, PCOM and no clinical and/or biochemical signs of androgen excess

There is no agreement on the way to denominate these phenotypes, and many societies (and NIH) [10] use the names phenotypes A, B, C and D. We have preferred giving names that are more related to their main characters. According to our definition [11], the following phenotypes may be distinguished:

1. Classic PCOS
   (a) With PCOM (phenotype A)
   (b) With normal ovaries (phenotype B)
2. Ovulatory PCOS (phenotype C)
3. Normoandrogenic PCOS (phenotype D)

While the understanding that PCOS is a very heterogeneous disorder has been important, it is also necessary to realise that the different PCOS phenotypes, having very different clinical problems, need different treatments. In this review, we will focus on the characters of main PCOS phenotypes and then we will discuss their impact on fertility.

7.3 Relative Prevalence of Different PCOS Phenotypes

In clinical setting, the classic PCOS phenotype is the most common [11–14]. In some studies, 90% of PCOS patients present with the classic phenotype. However, the relative prevalence of the different PCOS phenotypes varies according to many factors, the most important being the mean body weight of the population [15]. In our experience 60–65% of PCOS patients have the NIH classic phenotype. Of these, the large majority have the phenotype A, while a few patients (<10%) have the phenotype B.

In our clinic [11], the ovulatory phenotype (phenotype C) is also common with almost 30% of the PCOS patients presenting this phenotype, while the normoandrogenic phenotype is relatively uncommon being observed in <10% of the patients. In other clinical settings (mainly in studies reporting patients referred to obstetrics and gynaecology clinics), the ovulatory phenotype is uncommon and normoandrogenic phenotype may be present in about 20–30% of the patients [12, 16, 17].

The relative prevalence of the PCOS phenotypes C varies also depending on ethnic groups with normoandrogenic phenotype being observed in more than 30% of PCOS patients of some countries of East Asia [18, 19].

Studies in general population are few, but ovulatory phenotype (phenotype C) seems to be the most represented phenotype [17, 20]. Probably, many of these subjects never go to a clinic because they do not suffer of problems like infertility and menstrual disorders.

7.4 Main Characters of Different PCOS Phenotypes

7.4.1 Classic PCOS (Phenotypes A and B)

Classic PCOS phenotype corresponds to the original NIH phenotype (see also Chap. 2) and is generally characterised by menstrual irregularities linked to a condition of chronic anovulation and hyperandrogenism (Table 7.1). Inside this group, there is also a significant heterogeneity with patients presenting both clinical and biochemical signs of hyperandrogenism and others presenting only clinical or biochemical hyperandrogenism. Obesity is common but may be absent with a variable percent of patients presenting normal body weight. Luteinising hormone (LH)/follicle-stimulating hormone (FSH) ratio are generally increased but may be normal in a significant number of patients. Insulin resistance and hyperinsulinemia are common but some patients present normal insulin levels and a normal insulin sensitivity. Anti-Mullerian hormone (AMH) is generally increased but may be normal [21] (see also Chaps. 3 and 8). PCOM is very common but a subgroup of patients (phenotype B) present normal ovarian morphology. Finally, increased ovarian size is common but a significant number of patients present normal ovarian size [21].

Very few studies have tried to assess differences between PCOS patients with phenotypes A and B. Some years ago, we found that these phenotypes are very similar in body weight, androgen levels, insulin levels and insulin sensitivity but that patients with phenotype A present much higher LH levels than patients with phenotype B [11]. The relationship between these findings and the ovarian

Table 7.1 Characters of main PCOS phenotypes

	Hyperandrogenemia	Anovulation	PCOM	AMH	Metabolic issues
Classic PCOS (phenotype A)	Yes	Yes	Yes	Increased	Yes
Classic PCOS (phenotype B)	Yes	Yes	Not	Unknown	Yes
Ovulatory PCOS (phenotype C)	Yes	Not	Yes	Mild increase	Mild
Normoandrogenic PCOS (phenotype D)	Not	Yes	Yes	Mild increase	Not

AMH anti-Mullerian hormone, *PCOM* polycystic ovarian morphology

morphology is unclear. No data on AMH secretion in patients of phenotype B have been reported.

Patients with phenotype B have LH values that are similar to the controls and patients with ovulatory PCOS but higher LH/FSH ratio than those two groups [11].

7.4.2 Ovulatory PCOS (Phenotype C)

These PCOS patients present normal ovulatory cycles but PCOM and clinical or biochemical signs of hyperandrogenism. In many ways these patients represent a mild form of PCOS: they have normal LH and LH/FSH ratio and their hyperandrogenism is less severe than patients with classic PCOS. Their insulin levels are normal or only slightly elevated and insulin resistance is less common and less severe. Obesity may be present but most patients with ovulatory PCOS present normal body weight or are just overweight. Increased AMH is present in only about 50% of patients with phenotype C and enlarged ovarian size has a similar prevalence [21]. In these patients, ovulatory cycles correspond to normal fertility. Initial data suggesting impairment of fertility in PCOS women of phenotype C (because of short or insufficient luteal function) [22] have been not confirmed by successive studies.

Interestingly, prevalence of phenotype C is higher in countries where mean body weight is lower, and it has been shown that patients may move from phenotype A to phenotype C and back with changes of body weight [6]. All of it suggests that phenotype C represents a milder form of phenotype A with lower metabolic problems, and it is in some way linked to lower androgen excess and to lower fertility problems.

7.4.3 Normoandrogenic PCOS (Phenotype D)

Patients with normoandrogenic PCOS have chronic anovulation and PCOM but no clinical or biochemical signs of hyperandrogenism. Although their androgen values are in the normal range, the mean testosterone levels are significantly higher compared with controls. They present increased LH and LH/FSH ratio than the controls but low prevalence of obesity and normal mean body weight, normal levels of insulin and no signs of insulin resistance. Ovarian size and AMH are increased in only 50% of these patients [21].

These patients represent a unique group because they represent some characters of classic PCOS (menstrual irregularities, chronic anovulation, increased LH) but at the same time present few metabolic problems and on this respect should be considered a form of mild PCOS.

7.5 Pathogenesis of the Different PCOS Phenotypes

It is unclear why some patients present a classic PCOS and others may be ovulatory or normoandrogenic. Of course, it may be just the result of different genetic and/or environmental influences. However, genetic studies do not give evidence of it.

Genome-wide association studies (GWAS) were unable to differentiate between the different PCOS phenotypes [23] suggesting that environmental factors play a main role on heterogeneity of the syndrome. In some way, the main difference between classic and ovulatory PCOS patients is the body weight [11], and it suggests that obesity, partially related to environmental components, may be the main factor determining the appearance of a classic phenotype, while patients maintaining a normal body weight will develop an ovulatory PCOS.

However, this explanation, while probable in many patients, cannot explain the occurrence of classic PCOS in normal-weight or overweight PCOS. More accurate genetic studies involving different groups of patients and a better understanding of PCOS pathophysiology are needed to establish the cause(s) of the heterogeneity of PCOS.

7.6 PCOS Phenotypes and Infertility

It is well known that, with the exception of phenotype C, patients with PCOS present a chronic anovulation. In turn, chronic anovulation determines infertility, and many PCOS patients are referred to specialised clinical settings because of infertility.

The mechanisms of chronic anovulation in PCOS are not perfectly understood [24]. While this issue will be treated more in depth in another chapter of this book (Chap. 3), available data suggest that both main endocrine alterations of PCOS, increased androgen production and hyperinsulinemia, participate to the mechanism of chronic anovulation. In fact, reduction in one of these endocrine alterations may transform a classic anovulatory phenotype in an ovulatory phenotype. About 50% of anovulatory women with PCOS become ovulatory when they lose weight or are treated by a product that reduces insulin secretion (generally an insulin-sensitising drug-like metformin) [24]. The role of androgens is less clear, but reduction in ovarian (and adrenal) androgen secretion during late reproductive age may be an important mechanism in improving the PCOS presentation and transforming a classic phenotype in ovulatory phenotype [25].

While anovulation is the main factor determining infertility in PCOS, women with PCOS have also risk factors that may cause reduced fertility. Some studies have shown alterations of the oocyte and endometrial competence (see Chaps. 4 and 5) but contrasting data have been presented and evidence-based data are missing [26]. Women with PCOS have been reported to have also three- to fourfold risk for pregnancy-induced hypertension, pre-eclampsia and gestational diabetes and twofold risk for prematurity [27]. All these risks are associated to obesity and insulin resistance and are much higher in patients with phenotypes A and B than in patients with phenotype C or D. Some studies have suggested an additional role of hyperandrogenism on these events. It may suggest that patients with phenotype D (normoandrogenic and normal weight) have a lower or normal risk of pregnancy complications. However, data on pregnancy complications in selected phenotypes of PCOS are missing.

Conclusion

PCOS phenotypes should not be considered a fixed clinical and endocrine presentation because changes in lifestyle, administration of drugs and modifications in hormonal (mainly in androgen production) linked to physiologic processes of ageing may move the same patient from a phenotype to another.

Because of it, the prognosis of infertility in women with PCOS is often better than that generally anticipated during young age [24]. Many patients get spontaneous fertility and children without any treatment, and this phenomenon is particularly relevant after their forties because of the physiologic reduction of ovarian androgen secretion that presents at that age. Probably, during late reproductive age, at least 50% of PCOS patients with chronic anovulation (phenotypes A and B) become ovulatory (phenotype C) and regain spontaneous fertility. While treatment of infertility cannot wait for possible spontaneous improvement during late reproductive age, our counselling of PCOS women should take in account these changes and tell the patients that their disorder and their anovulatory status may improve with lifestyle changes but also because of spontaneous modifications of hormonal function with ageing.

References

1. Carmina E. The spectrum of androgen excess disorders. Fertil Steril. 2006;85:1582–5.
2. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS, Lizneva D, Natterson-Horowtiz B, Teede HJ, Yildiz BO. Polycystic ovary syndrome. Nat Rev Dis Primers. 2016;2:16057.
3. Zawadzki JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens JR, Haseltine F, Merriam GR, editors. Polycystic ovary syndrome. Boston, MA: Mass Blackwell Scientific; 1992. p. 377–84.
4. Carmina E, Lobo RA. Do hyperandrogenic women with normal menses have PCOS? Fertil Steril. 1999;71:319–22.
5. Carmina E, Lobo RA. Polycystic ovaries in women with normal menses. Am J Med. 2001;111:602–6.
6. Carmina E. Mild androgen disorders. Best Pract Res Clin Endocrinol. 2006;20:207–20.
7. Balen A. What is the polycystic ovary syndrome? Are national views important? Hum Reprod. 2002;17:2219–27.
8. Rotterdam ESHRE-ASRM Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Hum Reprod. 2004;19:41–7.
9. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE, Witchel SF. The androgen excess and PCOS society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril. 2009;91:456–88.
10. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, Welt CK, Endocrine Society. Endocrine Society diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2013;98:4565–92.
11. Guastella E, Longo RA, Carmina E. Clinical and endocrine characteristics of the main PCOS phenotypes. Fertil Steril. 2010;94:2197–201.
12. Azziz R, Sanchez LA, Knochenhauer ES, Moran C, Lazenby J, Stephens KC, Taylor K, Boots LR. Androgen excess in women: experience with over 1000 consecutive patients. J Clin Endocrinol Metab. 2004;89:453–62.

13. Carmina E, Rosato F, Jannì A, Rizzo M, Longo RA. Relative prevalence of different androgen excess disorders in 950 women referred because of clinical hyperandrogenism. J Clin Endocrinol Metab. 2006;91:2–6.
14. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod. 2016;31:2841–55.
15. Carmina E, Legro RS, Stamets K, Lowell J, Lobo RA. Difference in body weight between American and Italian women with polycystic ovary syndrome: influence of the diet. Hum Reprod. 2003;11:2289–93.
16. Clark NM, Podolski AJ, Brooks ED, Chizen DR, Pierson RA, Lehotay DC, Lujan ME. Prevalence of polycystic ovary syndrome phenotypes using updated criteria for polycystic ovarian morphology: an assessment of over 100 consecutive women self-reporting features of polycystic ovary syndrome. Reprod Sci. 2014;21:1034–43.
17. Lizneva D, Kirubakaran R, Mykhalchenko K, Suturina L, Galina C, Diamond MP, Azziz R. Phenotypes and body mass in women with polycystic ovary syndrome identified in referral versus unselected populations: systematic review and meta-analysis. Fertil Steril. 2016;106:1510.e2–20.e2.
18. Carmina E, Koyama T, Chang L, Stanczyk FZ, Lobo RA. Does ethnicity influence the prevalence of adrenal hyperandrogenism and insulin resistance in polycystic ovary syndrome? Am J Obstet Gynecol. 1992;167:1807–12.
19. Zhang HY, Guo CX, Zhu FF, PP Q, Lin WJ, Xiong J. Clinical characteristics, metabolic features, and phenotype of Chinese women with polycystic ovary syndrome: a large-scale case-control study. Arch Gynecol Obstet. 2013;287:525–31.
20. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod. 2010;25:544–51.
21. Carmina E, Campagna AM, Fruzzetti F, Lobo RA. AMH measurement versus ovarian ultrasound in the diagnosis of polycystic ovary syndrome (PCOS) in different phenotypes. Endocr Pract. 2016;22:287–93.
22. Joseph-Home R, Mason H, Batty S, White D, Hillier S, Urquhart M, Franks S. Luteal phase progesterone excretion in ovulatory women with polycystic ovaries. Hum Reprod. 2002;17:1459–63.
23. Shi Y, Zhao H, Shi Y, Cao Y, Yang D, Li Z, Zhang B, Liang X, Li T, Chen J, Shen J, Zhao J, You L, Gao X, Zhu D, Zhao X, Yan Y, Qin Y, Li W, Yan J, Wang Q, Zhao J, Geng L, Ma J, Zhao Y, He G, Zhang A, Zou S, Yang A, Liu J, Li W, Li B, Wan C, Qin Y, Shi J, Yang J, Jiang H, JE X, Qi X, Sun Y, Zhang Y, Hao C, Ju X, Zhao D, Ren CE, Li X, Zhang W, Zhang Y, Zhang J, Wu D, Zhang C, He L, Chen ZJ. Genome-wide association study identifies eight new risk loci for polycystic ovary syndrome. Nat Genet. 2012;44:1020–5.
24. Carmina E. Reproductive system outcome among patients with polycystic ovarian syndrome. Endocrinol Metab Clin N Am. 2015;44:787–97.
25. Carmina E, Campagna AM, Lobo RA. A 20-year follow-up of young women with polycystic ovary syndrome. Am J Obstet Gynecol. 2012;119:263–9.
26. Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, Carmina E, Chang J, Yildiz BO, Laven JS, Boivin J, Petraglia F, Wijeyeratne CN, Norman RJ, Dunaif A, Franks S, Wild RA, Dumesic D, Barnhart K. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril. 2012;97:28.e25–38.e25.
27. Piltonen TT. Polycystic ovary syndrome: endometrial markers. Best Pract Res Clin Obstet Gynaecol. 2016;37:66–79.

Follicle Excess and Abnormalities in Women with PCOS: Pathophysiology, Assessment and Clinical Role

8

Agathe Dumont, Pauline Plouvier, and Didier Dewailly

8.1 Introduction

Follicle excess is the cornerstone of polycystic ovarian morphology (PCOM). Besides its paramount importance for the diagnosis of polycystic ovary syndrome (PCOS) (see also Chap. 2), it has a major role in the pathophysiology of the ovulation disorder that is observed in most of the PCOS phenotypes. However, as regards in vitro fertilisation (IVF) outcomes, this ovarian richness turns out to be an advantage as PCOM [or high antral follicular count (AFC) in non-PCOS women] is now considered as a good prognosis factor for oocyte yield and thus for pregnancy [1]. This chapter will discuss first the pathophysiology of the follicle excess in PCOS, then its diagnosis and, finally, its clinical role.

8.2 Pathophysiology of the Follicle Excess

The ovarian reserve refers to the number of primordial follicles, defined at birth (around one million). This follicular capital decreases gradually throughout reproductive life, with the continuous initiation of growth of some follicles and then mostly their apoptosis. There are about 400,000 follicles in adolescents' ovaries (leading roughly to 400 ovulations), whereas only a thousand remains at the time of menopause [2].

PCOS is characterised by an increased number of follicles at all growing stages [3, 4], especially preantral and small antral follicles. Moreover, in PCOS, the follicular growth is slowed down, aggravating the accumulation of growing follicles in

A. Dumont • P. Plouvier • D. Dewailly (✉)
Service de Gynécologie Endocrinienne et de Médecine de la Reproduction, Hôpital Jeanne de Flandre, CHRU Lille, Lille, France
e-mail: agathe.dumont@hotmail.fr; pauline.plouvier@chru-lille.fr; didier.dewailly@chru-lille.fr

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_8

the ovarian cortex [4, 5]. In PCOS, there is also a follicular apoptosis defect, which worsens the follicle excess [6, 7]. All these three phenomenons are mainly explained by an excessive intraovarian androgen secretion (see also Chap. 3). This overproduction of androgens could be an intrinsic defect of theca cells [8–11]. Undeniably, there is a positive correlation between intraovarian hyperandrogenism and excessive early follicular growth (up to the 2–5 mm follicular stage), independently from luteinising hormone (LH) and insulin [12]. Indeed, some experiments showed higher number of antral follicles after injection of androgens in female animals and in female to male transsexuals [13, 14]. Despite the follicle excess, there is also an inhibition of the terminal follicular growth, the so-called follicular arrest [15]. According to previous theories, the selection of the dominant follicle would be impaired by a premature acquisition of LH receptors in the granulosa cells [16, 17], leading to their early luteinisation and the premature arrest of their growth [18]. High insulin and androgen levels have also been incriminated in this phenomenon.

Elevated serum anti-Müllerian hormone (AMH) level is strongly related to the follicle excess [9, 19], as it is a reflection of the preantral and small antral follicle pool. AMH is synthesised at its highest level in small antral follicles, which are precisely the ones seen on ultrasound.

Therefore, high serum AMH level in PCOS is not only due to the higher number of preantral and small antral follicles but also to an intrinsic dysregulation of the granulosa cells, producing more AMH [20–23]. The cause of this dysregulation is currently unknown, but there is evidence to support a role played by androgens. For more details on the pathophysiology of the anovulation in women with PCOS, see Chap. 3.

In PCOS, there is also a specific relationship between follicle-stimulating hormone (FSH) and AMH [24]: FSH would directly stimulate AMH in small antral follicles, as long as they do not express aromatase. Conversely, in larger follicles, the FSH-induced increasing E_2 production would have a direct inhibitory effect on AMH expression (Fig. 8.1) [24]. Moreover, it has been demonstrated that AMH significantly decreases not only the FSH receptor expression but also ovarian aromatase expression [25], allowing protection of the small follicles from premature aromatase expression. However, this protective effect exceeds its physiological role when AMH is in excess, thus resulting in a defect in the selection of the dominant follicle. The fact that AMH is inhibitory to FSH-dependent factors required for follicle dominance adds considerable significance to the high serum AMH expression found in PCOS and makes AMH a putative central actor of the ‘follicular arrest’. In good agreement, clinical studies have shown a relationship between high AMH and ovulatory disorder [23]. In addition, LH seems to stimulate AMH production by the granulosa cells in women with PCOS but not in controls [23]. Conversely, some authors demonstrated that LH reduces AMH receptor II (RII) expression in granulosa luteal cells from women with normal ovaries and from women with normoovulatory PCOS, whereas it cannot do so in women with anovulatory PCOS [26, 27]. Besides the LH-stimulating effect on AMH expression, this lack of LH-induced downregulation of AMHRII expression in women with anovulatory PCOS could contribute to anovulation. Therefore, the premature action of LH involved in the ‘follicular arrest’ could be partially explained through the AMH system [16, 17].

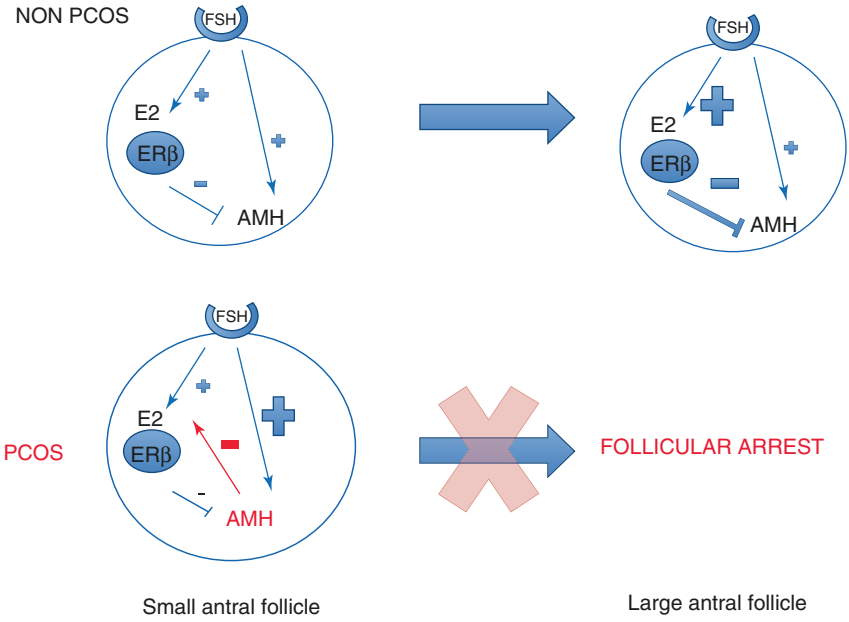


Fig. 8.1 Schematic diagram of AMH regulation by FSH and E2 in GC of small and large antral follicles**.** Adapted from Grynberg et al. [24]. Until the small antral stage, AMH secretion is stimulated by different factors like FSH. Oestradiol (E2) production under the influence of FSH is impaired by the inhibiting effect of AMH on aromatase. When oestradiol concentration reaches a certain threshold in large antral follicles, it is capable of completely inhibiting AMH expression through ERβ, which predominates in growing follicles, thus overcoming the stimulation by FSH. In large follicles from PCO, the lack of FSH-induced E2 production and the high level of AMH impair the shift from the AMH to the E2 tone, thus leading to the follicular arrest

In conclusion, the abnormalities of folliculogenesis in PCOS are multiple: (1) increased number of small growing follicles; (2) inhibition of the terminal follicular growth, resulting in a lack of selection of the dominant follicle, the so-called the follicle arrest; and (3) a follicular apoptosis defect aggravating the excess of growing follicles. Intraovarian hyperandrogenism seems to be the greater culprit of all those abnormalities, via pathways involving FSH, LH and AMH secretion.

8.3 Assessment of the Follicle Excess

8.3.1 Ultrasounds

The Rotterdam consensus of 2003 defined the PCOM as a follicle number per ovary (FNPO) ≥ 12 and/or an ovarian volume ≥ 10 mL [28, 29]. Theoretically, the word ‘polycystic’ is not appropriated, since there is no cyst in PCOS but antral follicle excess, and should be replaced by ‘multifollicular’.

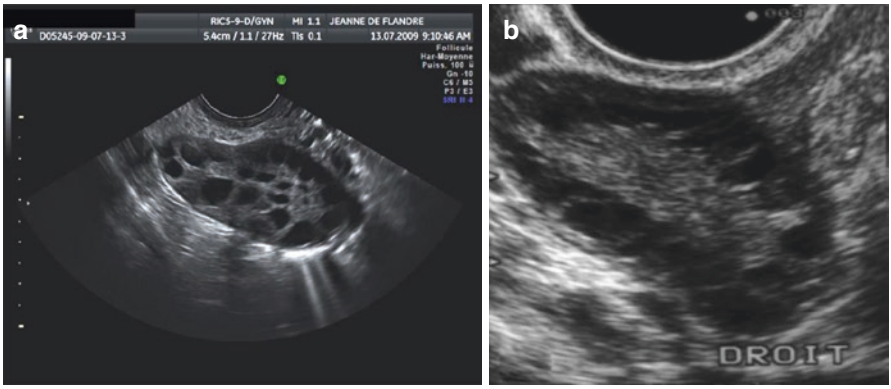


Fig. 8.2 Ovarian polycystic aspect with ultrasound equipment from 2009 (**a**) and 2001 (**b**)

8.3.1.1 B-Mode Ultrasonography

Abdominal pelvic ultrasound is first performed to see the position of the uterus and ovaries and to exclude any abdominal mass. Then, to visualise at best the ovaries and to precisely count the number of follicles, the ultrasound should be done vaginally (Fig. 8.2). Abdominal pelvic ultrasound can be an alternative if the patient is virgin or refuses the vaginal probe, but only the ovarian volume can be estimated this way [30]. This exam must be performed between day 2 and day 5 of the cycle, to prevent any growing follicle from hiding small antral follicles and/or modifying the ovarian volume.

The best ultrasound criterion for PCOM is the high number of follicles [31]. International experts have submitted practical recommendations for a better standardisation of the way to count the follicles [32]. It is recommended to first have a global look of the ovary, in both plans, until the limits of the ovary are clear. First, all follicles >10 mm should be identified and measured, as they are not included in the FNPO. Then, the evaluation of the FNPO is finally done (follicles between 2 and 9 mm), slowly from one border of the ovary to the other. Thus, assessment of FNPO to define PCOM should follow the same procedure as assessment of AFC to evaluate the ovarian reserve prior to IVF. The only difference is that AFC corresponds to the sum of follicles in both ovaries, while FNPO to define PCOM is for one ovary. Unilateral excess in FNPO allows retaining the diagnosis of PCOM, as stated by the Rotterdam recommendations [29].

The ovarian volume is also a criterion for the diagnosis of PCOM. It can be automatically measured by the ultrasound unit or calculated with the formula: length × width × thickness × 0.523 [33]. Those three diameters must be measured in strict orthogonal plans.

The ovarian area can also be used for appreciation of PCOM. It is measured using an automatic ellipse or by delimiting manually the contours of the ovary, from a picture where it seems the biggest. It can also be calculated with the formula: length × width × 0.8. Even if the Rotterdam consensus does not recommend the

ovarian area as a diagnostic criterion of PCOS, it is still a good assessment of PCOM, when greater than 5 cm^2 (sensitivity 77.6%, specificity 94.7%) [34].

Stromal hypertrophy (defined by increased volume and echogenicity of the central part of the ovary) and peripheral distribution of the follicles are very subjective and variable assessment, depending on the ultrasound unit. Thus, at the Rotterdam consensus conference, those two criteria were not retained to define PCOM.

8.3.1.2 Three-Dimensional Ultrasonography

Using the three octagonal plans, three-dimensional (3D) ultrasonography is theoretically an easier way to measure the ovarian volume and to assess the number of follicles (Fig. 8.3) [35]. Actually, the automatic count is not reliable enough for small follicles, and a post hoc analysis on stored scans is required to get a reliable FNPO, which is time-consuming. With the sono-automatic volume calculation (AVC) mode, the assessment of ovarian volume is reliable, but in common use, 3D ultrasonography doesn't seem to be superior to the B-mode and requires expensive equipment and regular training [36].

8.3.1.3 Controversy of Ultrasonography

Since 2003, many studies have questioned the threshold of 12 follicles defined by the Rotterdam consensus, as evidence of PCOM [37–39]. Indeed, this cut-off is highly dependent on ultrasound equipment and operator skill. Therefore, with the latest ultrasound generation, it is now possible to distinguish very small follicles (<2 mm). In 2011, Dewailly et al. [37] proposed a new threshold for FNPO of 19 (sensitivity 81%, specificity 92%). Similarly, a panel of international experts has recently suggested a threshold of 25, when the maximum frequency of the probe is greater than 8 MHz [40]. However, this threshold is highly dependent on ultrasound equipment and operator skill, as demonstrated by Dewailly et al. [41]; thus, each centre has to establish its own threshold. Concerning the ovarian volume, some

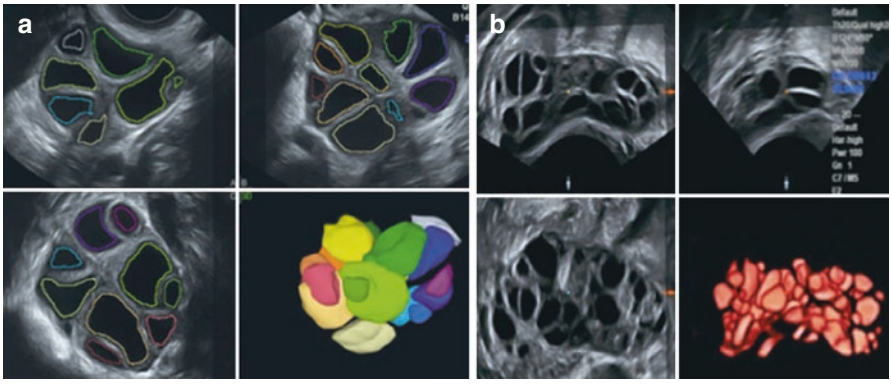


Fig. 8.3 3D ultrasonography: Sono-AVC mode (**a**) and surface mode (**b**)**.** Pictures from Levaillant et al. [35]. Authorisation from Dr. Yves Ardaens

authors proposed to reassess the threshold of 10 cm^3 as diagnostic criterion of PCOM [34, 42], but no recommendation has yet been made [30].

8.3.1.4 Magnetic Resonance Imaging (MRI)

MRI has been proposed as an alternative to ultrasound to count the FNPO, and to evaluate the ovarian areas and volumes, the stromal hypertrophy and vascularisation (with gadolinium injection). However, the spatial resolution is not as good as in vaginal ultrasound, and the cost is much higher. MRI is therefore not a first-line tool in the diagnosis of PCOS, but it is still very helpful in cases of severe hyperandrogenism, when it is important to exclude an ovarian tumour.

8.3.2 Anti-Müllerian Hormone

AMH was isolated and purified in 1984 [43] and has been predominantly known for its role in male sexual differentiation [44]. In women, its expression is restricted to one cell type: the granulosa cells of the ovary. It starts around the 25th week of gestation, continuing until menopause [43, 45].

The functional role of AMH in early follicular growth has been characterised by the study of "knocked out" models for the AMH gene (AMHKO) [46–48]. In the absence of AMH ("knocked out" mice), there is an increased initiation of primordial follicles into the growing pool (they are recruited faster), resulting in an exhausted primordial follicle pool at a younger age than in wild-type animals [46]. AMH therefore has an inhibitory effect on early follicular recruitment preventing the entry of primordial follicles into the growing pool and thus premature exhaustion of follicles/oocytes [49].

AMH is expressed as soon as primordial follicles are recruited to grow into small preantral follicles. Its highest expression is observed in preantral and small antral follicles; then it decreases with the selection of follicles for dominance and in atretic follicles (Fig. 8.4) [40, 48, 50, 51]. Thus, serum AMH concentration is strongly correlated with the number of growing follicles [8, 9]. Considering that the rate of initiation of follicle growth is deeply related to the initial follicular pool, we can assume that serum AMH is an indirect reflection of ovarian reserve. There is actually a very good correlation between serum AMH levels and FNPO [52], since circulating AMH is mostly produced by granulosa cells of follicles from 2 to 9 mm (60%), which are precisely the ones counted on the ultrasound for FNPO assessment [53]. Serum AMH could therefore be used as a surrogate for the FNPO in the diagnosis of PCOS.

8.3.2.1 Serum AMH Assessment for PCOS/PCOM Patients

In most patients with PCOS, serum AMH concentration is greatly increased and was found to be twofold to fourfold higher in women with PCOS than in healthy women [9, 54]. This increase is not only due to the higher number of antral follicles secreting AMH but also to a greater production of AMH from the granulosa cells [23].

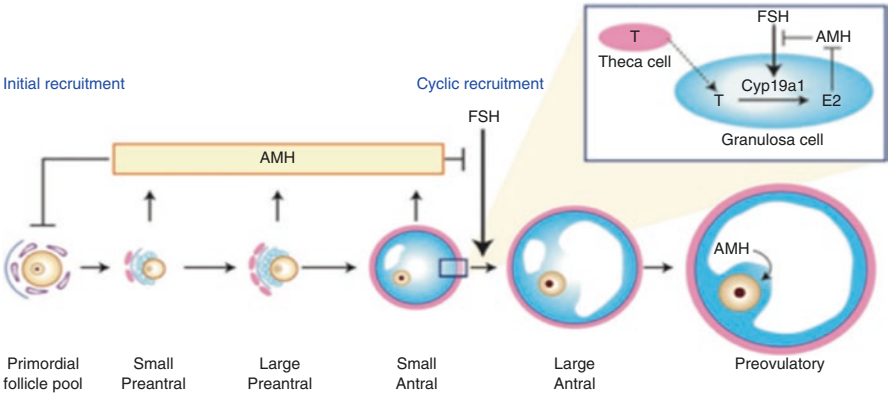


Fig. 8.4 Schematic model of AMH actions in the ovary. From Dewailly et al. [40]. AMH, produced by the granulosa cells of small growing follicles, inhibits initial follicle recruitment and FSH-dependent growth and selection of preantral and small antral follicles. In addition, AMH remains highly expressed in cumulus cells of mature follicles. The inset shows in more detail the inhibitory effect of AMH on FSH-induced CYP19a1 expression, leading to reduced oestradiol (E2) levels, and the inhibitory effect of E2 itself on AMH expression. *T* testosterone, *Cyp19a1* aromatase. Figure modified from van Houten et al. [51]

Due to the great number of assay kits available for serum AMH, there is currently no international threshold to define follicle excess (cf. Sect. 8.3.2.2). In our centre, we have defined our own cut-off at 35 pmol/L (4.9 ng/mL) for the diagnosis of follicle excess and prediction of PCOS, using the enzyme immunoassay AMH-EIA (EIA AMH/MIS kit) ('Immunotech', Ref. A16507) provided by Beckman Coulter (France) [37]. This result was obtained after exclusion of women with asymptomatic PCOM from the control group through cluster analysis, a mathematical procedure that avoids using predefined thresholds for AMH and FNPO. This mathematical approach has already been replicated in another setting [30]. In our series, the threshold of 35 pmol/L had a good specificity (97%) and a better sensitivity than the FNPO (92%) to distinguish women with PCOS from normal women [37]. However, this threshold cannot be extrapolated to other centres using different control populations and AMH assays. In addition, the Immunotech assay is no longer marketed.

Recently, Pigny et al. [55] have compared the five currently available serum AMH assays in the diagnosis of PCOS (cf. Sect. 8.3.2.2). With manual ELISA assays, they proposed a higher cut-off at 5.6 ng/mL (40 pmol/L), for the prediction of PCOM (corresponding to the 95th percentile of 'pure' controls). They also proposed a threshold at 4.2 ng/mL (30 pmol/L) for the automatic assays. If confirmed with the new automatised serum AMH assays or the ultrasensitive assay, a high serum AMH level could then become a reliable and accurate marker for PCOM.

8.3.2.2 Controversy of AMH

In clinical application, AMH presents many opportunities but unfortunately there are difficulties due to several biological features of this molecule [56]. First, there is a molecular heterogeneity of the circulating AMH level with a non-cleaved

biologically inactive form and a cleaved biologically active form [57, 58]. Also, there is variable sensitivity of the immunoassays to interference by complement C1q and C3 [59]. Measurement thus involves different assays with different sensitivities. The last technical issue is the inter-laboratory variability, mainly for low values of serum AMH. The difficulty lies in the fact there are currently different ELISA immunoassays used worldwide. There are still two 'manual' assays (Gen II of Beckman Coulter and AL-105-i of Anshlabs), which use different monoclonal antibodies and different standards [25]. For some time, there has been lack of agreement between these assays which explains the absence of consensual reference values and decision thresholds between teams in the literature [49]. But progresses have been made and the two assays now seem superimposable [55]. Likewise, automation on immuno-analysers (Access Dxi of Beckman Coulter and Elecsys of Roche Diagnostics) yields nearly identical values [60, 61]. The development of an ultrasensitive assay ('pico AMH' kit, Anshlabs) is also a progress but this is not relevant for PCOM. Hopefully, an international standard for serum AMH assay will be established soon in order to maximise its clinical utility.

8.4 Clinical Role of the Assessment of Follicle Excess

8.4.1 Diagnostic Performance

The robust association between AMH and FNPO has led some authors to compare their performance in the diagnosis of PCOS [62, 63]. If results from the current literature are not quite homogeneous [49], serum AMH assay is definitively more sensitive and specific than the FNPO as it also reflects preantral and small antral follicles (<2 mm), which are hardly seen on ultrasound. Serum AMH is therefore a deeper 'probe' for the growing follicular pool than the FNPO (Fig. 8.5) [37, 40, 53].

Serum AMH assay has other benefits over the FNPO, as its serum level is quite stable from one cycle to another and throughout the same cycle (since the dominant follicle and corpus luteum do not secrete AMH) [64, 65]. Conversely, the FNPO has to be measured on the first 5 days of the cycle [66], to prevent any developing follicle from miscalculating the number of follicles or the ovarian volume.

Serum AMH level is rather independent from the hypothalamic pituitary axis and as such is not modified in pathologies such as hyperprolactinemia and functional hypothalamic amenorrhea or in incomplete and recent hypogonadotropic hypogonadism, providing serum FSH level which remains normal or subnormal [67]. However, serum AMH might be influenced by some factors, and controversies persist, in particular for obesity [68–70] and hormonal contraception [71–73].

Serum AMH assay is also interesting in adolescent, where it can be difficult to evaluate the ovaries with abdominal ultrasonography, and where it is sometimes hard to estimate the share of the physiological and pathological (PCOS), concerning acne and/or cycle disorders [74].

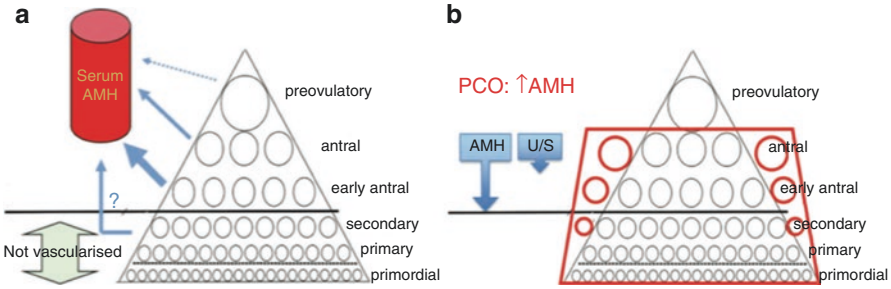


Fig. 8.5 Rationale for the use of serum AMH assay as a probe for PCOM. From Dewailly et al. [40]. (**a**) All growing follicles secrete AMH, but serum AMH reflects only the secretion from bigger follicles that are in contact with the vascular bed. As the numbers of follicles in all growth stages are strongly related to each other, serum AMH is considered to reflect the sum of growing follicles but not the number of primordial follicles that do not secrete AMH. (**b**) In PCO, the numbers of all growing follicles are increased, resulting in a marked increase in serum AMH level. AMH may be considered as a deeper and more sensitive probe to define follicle excess than the follicle count by ultrasound (U/S) since it appraises more follicle classes (*blue arrows*)

8.4.2 PCOS Phenotypes

Serum AMH level is correlated to the severity of PCOS symptoms [27] and is higher when hyperandrogenism [75] or oligo-anovulation is present [8, 76]. Indeed, AMH production in vitro was found much higher in granulosa cells from anovulatory PCOS than in normal ovaries or normo-ovulatory PCOS (75- and 20-fold, respectively) [23] (see also Chap. 7).

By principal component analysis, it has been shown that a high serum AMH level and an excessive FNPO can be considered as a marker of hyperandrogenism and could equally be used as a substitute for the classical markers of ovarian hyperandrogenism [77]. This would reconcile the different classifications currently available for PCOS because some require hyperandrogenism as a necessary criterion [78]. In 2011, the following strategy was proposed [37]: for the diagnosis of PCOS, hyperandrogenism and oligo-anovulation should be first sought, after excluding all alternative diagnoses. If one is missing, PCOM (i.e., high FNPO and/or high serum AMH level) can be used instead (Table 8.1) [37]. Thus, there are four PCOS phenotypes, and it is important to differentiate them, as they do not involve the same reproductive concerns and/or metabolic consequences (see also Chap. 2).

Many studies have tried to identify a predictive level of serum AMH for the different phenotypes but results remain mixed [79, 80]. Although it is clear that complete phenotypes of PCOS (phenotype A) have the highest serum AMH levels, it was observed that non-hyperandrogenic oligo-anovulatory phenotypes (phenotype D) had higher median serum AMH levels than hyperandrogenic normo-ovulatory phenotypes (phenotype C) [41]. Alebic et al. [21] described a steadily increase in median serum AMH levels across the phenotypes (PCOM < phenotype C < phenotype D < phenotype A). They also used the ratio AMH/AFC as a marker of per-follicle AMH production and found it to be significantly increased in a stepwise

Table 8.1 Adaptation of the previous classifications for the diagnosis of PCOS, proposing an excessive follicle number (FN) of >19 or serum AMH concentration > 35 pmol/L or >5 ng/mL as a surrogate when either oligo-anovulation or hyperandrogenism (HA) is missing

Oligo-anovulation	Clinical and/or biological HA	FN > 19 and/or AMH > 35 pmol/l[a]	Diagnosis
+	+	(±)[b]	PCOS
+	–	+	PCOS
–	+	+	PCOS
–	–	+	Normal woman with PCOM
+	–	–	Idiopathic anovulation
–	+	–	Idiopathic hyperandrogenism

[a](5 ng/ml)
[b]Not necessary for the diagnosis
AMH anti-Müllerian hormone, *FN* follicle number, *PCOM* polycystic ovarian morphology, *PCOS* polycystic ovarian syndrome
From Dewailly et al. [37]

manner: low in controls, intermediate in eumenorrheic women (PCOM/phenotype C) and high in oligo-amenorrheic women, regardless of androgen status (phenotype A/phenotype D) [21]. Recently, Carmina et al. [81] reported that FNPO was significantly more sensitive than serum AMH in the overall diagnosis of PCOS (93% versus 79%) and especially in non-hyperandrogenic phenotypes (93% versus 53%) or ovulatory phenotypes (95% versus 50%). AMH appeared to be helpful in anovulatory phenotypes (sensitivity 91% versus 92%) [81].

8.4.3 Utility in Infertility Treatment

PCOS is a frequent cause of infertility because of the dysovulation often associated. Therefore, induction ovulation treatments are frequently used, but the follicle excess present in this pathology can be responsible for an ovarian overresponse with an increased risk of ovarian hyperstimulation syndrome (OHSS). This is a challenging situation as the minimal effective dose is often very close to the overdose leading to hyperstimulation. Thus, serum AMH level and FNPO can be useful to establish treatment protocols and to define the best strategy for ovulation induction in infertile women with PCOS.

8.4.3.1 Clomiphene Citrate

So far, very few studies have examined the predictive power of the follicle excess in the response to clomiphene citrate (CC) (see Chap. 10). Only Mahran et al. [82] have proposed a threshold for serum AMH at 3.4 ng/mL, above which a resistance to CC is highly expected, suggesting a higher starting dose should be used.

8.4.3.2 In Vitro Stimulation

The value of serum AMH for pregnancy prediction in PCOS women undergoing IVF treatment was evaluated in very few studies and results were conflicting [83, 84].

Nevertheless, it seems that neither serum AMH nor AFC have proven to be predictive for clinical pregnancy rates in women with PCOS undertaking IVF treatment [80]. However, serum AMH level and FNPO appear to be good predictive markers for the risk of ovarian hyperstimulation syndrome (OHSS) in IVF cycle [85], mostly occurring in PCOS [86]. A recent study proposed a threshold of serum AMH level at 6.95 ng/mL, above which the risk of OHSS was high (75% sensitivity, 84% specificity) [87]. Ocal et al. [88] used a threshold of serum AMH > 3.3 ng/mL to determine the risk of OHSS (90% sensitivity, 71% specificity) and found that serum AMH was a better predictor than AFC, LH or FSH. However, the establishment of an accurate threshold remains difficult because of the heterogeneity of the OHSS definition (see Chaps. 12, 18 and 20).

8.4.3.3 Laparoscopic Ovarian Drilling

Laparoscopic ovarian drilling (LOD) is currently recommended as a successful second-line treatment for ovulation induction in women with PCOS (see Chap. 15). It is considered to be an alternative to gonadotrophin stimulation in the case of CC resistance [89].

The aim is to trigger spontaneous ovulation by destroying small amounts of ovarian cortex. The utility of the AMH assay as a predictor for LOD outcome has been recently questioned [90–92], and one study showed that women who ovulated after LOD had lower preoperative AMH levels [91]. They identified a pre-LOD serum AMH level threshold of 7.7 ng/mL, which could predict failure of LOD (sensitivity 78%, specificity 76%). However, these data need confirmation, as the statistical power of those studies was small.

Conclusion

The follicle excess is an important, but not exclusive, criterion for the diagnosis of PCOS. In the Rotterdam classification, it could be used as a surrogate for either oligo-anovulation or hyperandrogenism. Despite considerable efforts to determine the cause of PCOM, its pathophysiology is not fully understood as it involves multiple mechanisms (hyperandrogenism, FSH, LH and AMH secretions). The follicle excess can be evaluated morphologically by ultrasound or biologically by serum AMH level. It has been demonstrated that FNPO suffers a great variability from an ultrasound machine to another and from an operator to another. Serum AMH appears therefore to be a good substitute, but the lack of international standard, due to different assays used worldwide, makes it difficult nowadays to consider serum AMH as the ‘gold standard’ in the recognition of PCOM.

As regards the use of AMH and/or FNPO for predicting ovarian response to controlled ovarian stimulation, the situation might be different between non-PCOS ovulatory women with high FNPO/AMH and women with genuine PCOS. In the latter, other cofactors such as obesity and/or insulin resistance, hyperandrogenism or severity of menstrual disorder have been shown to be predictive, independently from PCOM markers [93, 94]. This should be borne in mind, especially when deciding the starting dose of gonadotrophins in IVF cycles.

References

1. Sigala J, Sifer C, Dewailly D, Robin G, Bruyneel A, Ramdane N, Lefebvre-Khalil V, Mitchell V, Decanter C. Is polycystic ovarian morphology related to a poor oocyte quality after controlled ovarian hyperstimulation for intracytoplasmic sperm injection? Results from a prospective, comparative study. Fertil Steril. 2015;103:112–8.
2. Block E. Quantitative morphological investigations of the follicular system in women; variations at different ages. Acta Anat (Basel). 1952;14:108–23.
3. Webber LJ, Stubbs S, Stark J, Trew GH, Margara R, Hardy K, Franks S. Formation and early development of follicles in the polycystic ovary. Lancet. 2003;362:1017–21.
4. Maciel GA, Baracat EC, Benda JA, Markham SM, Hensinger K, Chang RJ, Erickson GF. Stockpiling of transitional and classic primary follicles in ovaries of women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2004;89:5321–7.
5. Catteau-Jonard S, Dewailly D. Physiopathologie des perturbations de la folliculogenèse dans le SOPK. Méd Reprod Gynécol Endocrinol. 2009;11:191–7.
6. Das M, Djahanbakhch O, Hacihanefioglu B, Saridogan E, Ikram M, Ghali L, Raveendran M, Storey A. Granulosa cell survival and proliferation are altered in polycystic ovary syndrome. J Clin Endocrinol Metab. 2008;93:881–7.
7. Webber LJ, Stubbs SA, Stark J, Margara RA, Trew GH, Lavery SA, Hardy K, Franks S. Prolonged survival in culture of preantral follicles from polycystic ovaries. J Clin Endocrinol Metab. 2007;92:1975–8.
8. Laven JS, Mulders AG, Visser JA, Themmen AP, De Jong FH, Fauser BC. Anti-Mullerian hormone serum concentrations in normoovulatory and anovulatory women of reproductive age. J Clin Endocrinol Metab. 2004;89:318–23.
9. Pigny P, Merlen E, Robert Y, Cortet-Rudelli C, Decanter C, Jonard S, Dewailly D. Elevated serum level of anti-Mullerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest. J Clin Endocrinol Metab. 2003;88:5957–62.
10. Gilling-Smith C, Willis DS, Beard RW, Franks S. Hypersecretion of androstenedione by isolated thecal cells from polycystic ovaries. J Clin Endocrinol Metab. 1994;79:1158–65.
11. Carlsen SM, Vanky E, Fleming R. Anti-Mullerian hormone concentrations in androgen-suppressed women with polycystic ovary syndrome. Hum Reprod. 2009;24:1732–8.
12. Jonard S, Robert Y, Cortet-Rudelli C, Pigny P, Decanter C, Dewailly D. Ultrasound examination of polycystic ovaries: is it worth counting the follicles? Hum Reprod. 2003;18:598–603.
13. Pache TD, Hop WC, de Jong FH, Leerentveld RA, van Geldorp H, Van de Kamp TM, Gooren LJ, Fauser BC. 17 beta-Oestradiol, androstenedione and inhibin levels in fluid from individual follicles of normal and polycystic ovaries, and in ovaries from androgen treated female to male transsexuals. Clin Endocrinol. 1992;36:565–71.
14. Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. J Clin Invest. 1998;101:2622–9.
15. Jonard S, Dewailly D. The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, may be the main culprit for the follicular arrest. Hum Reprod Update. 2004;10:107–17.
16. Willis DS, Watson H, Mason HD, Galea R, Brincat M, Franks S. Premature response to luteinizing hormone of granulosa cells from anovulatory women with polycystic ovary syndrome: relevance to mechanism of anovulation. J Clin Endocrinol Metab. 1998;83:3984–91.
17. Jakimiuk AJ, Weitsman SR, Navab A, Magoffin DA. Luteinizing hormone receptor, steroidogenesis acute regulatory protein, and steroidogenic enzyme messenger ribonucleic acids are overexpressed in thecal and granulosa cells from polycystic ovaries. J Clin Endocrinol Metab. 2001;86:1318–23.
18. Hillier SG. Current concepts of the roles of follicle stimulating hormone and luteinizing hormone in folliculogenesis. Hum Reprod. 1994;9:188–91.
19. Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, Kramer P, Fauser BC, Themmen AP. Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod. 2004;10:77–83.

20. Catteau-Jonard S, Jamin SP, Leclerc A, Gonzales J, Dewailly D, di Clemente N. Anti-Mullerian hormone, its receptor, FSH receptor, and androgen receptor genes are overexpressed by granulosa cells from stimulated follicles in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2008;93:4456–61.
21. Alebic MS, Stojanovic N, Duhamel A, Dewailly D. The phenotypic diversity in per-follicle anti-Mullerian hormone production in polycystic ovary syndrome. Hum Reprod. 2015;30:1927–33.
22. Bhide P, Dilgil M, Gudi A, Shah A, Akwaa C, Homburg R. Each small antral follicle in ovaries of women with polycystic ovary syndrome produces more antimullerian hormone than its counterpart in a normal ovary: an observational cross-sectional study. Fertil Steril. 2014;103:537–41.
23. Pellatt L, Hanna L, Brincat M, Galea R, Brain H, Whitehead S, Mason H. Granulosa cell production of anti-Mullerian hormone is increased in polycystic ovaries. J Clin Endocrinol Metab. 2007;92:240–5.
24. Grynberg M, Pierre A, Rey R, Leclerc A, Arouche N, Hesters L, Catteau-Jonard S, Frydman R, Picard JY, Fanchin R, Veitia R, di Clemente N, Taieb J. Differential regulation of ovarian anti-mullerian hormone (AMH) by estradiol through alpha- and beta-estrogen receptors. J Clin Endocrinol Metab. 2012;97:E1649–57.
25. Pellatt L, Rice S, Dilaver N, Heshri A, Galea R, Brincat M, Brown K, Simpson ER, Mason HD. Anti-Mullerian hormone reduces follicle sensitivity to follicle-stimulating hormone in human granulosa cells. Fertil Steril. 2011;96:1246–51.e1.
26. Pierre A, Peigne M, Grynberg M, Arouche N, Taieb J, Hesters L, Gonzales J, Picard JY, Dewailly D, Fanchin R, Catteau-Jonard S, di Clemente N. Loss of LH-induced down-regulation of anti-Mullerian hormone receptor expression may contribute to anovulation in women with polycystic ovary syndrome. Hum Reprod. 2013;28:762–9.
27. Pellatt L, Rice S, Mason HD. Anti-Mullerian hormone and polycystic ovary syndrome: a mountain too high? Reproduction. 2010;139:825–33.
28. Balen AH, Laven JS, Tan SL, Dewailly D. Ultrasound assessment of the polycystic ovary: international consensus definitions. Hum Reprod Update. 2003;9:505–14.
29. Rotterdam ESHRE. ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19:41–7.
30. Dewailly D, Lujan ME, Carmina E, Cedars MI, Laven J, Norman RJ, Escobar-Morreale HF. Definition and significance of polycystic ovarian morphology: a task force report from the Androgen Excess and Polycystic Ovary Syndrome Society. Hum Reprod Update. 2014;20:334–52.
31. Christ JP, Willis AD, Brooks ED, Vanden Brink H, Jarrett BY, Pierson RA, Chizen DR, Lujan ME. Follicle number, not assessments of the ovarian stroma, represents the best ultrasonographic marker of polycystic ovary syndrome. Fertil Steril. 2014;101:280–87.e1.
32. Broekmans FJ, de Ziegler D, Howles CM, Gougeon A, Trew G, Olivennes F. The antral follicle count: practical recommendations for better standardization. Fertil Steril. 2010;94:1044–51.
33. Sample WF, Lippe BM, Gyepes MT. Gray-scale ultrasonography of the normal female pelvis. Radiology. 1977;125:477–83.
34. Jonard S, Robert Y, Dewailly D. Revisiting the ovarian volume as a diagnostic criterion for polycystic ovaries. Hum Reprod. 2005;20:2893–8.
35. Ardaens Y, Guérin du Masgenêt B, Coquel P, Levaillant JM, Poncelet E. Échographie et imagerie pelvienne en pratique gynécologique. Issy-les-Moulineaux: Elsevier Masson; 2012.
36. Lam PM, Raine-Fenning N. The role of three-dimensional ultrasonography in polycystic ovary syndrome. Hum Reprod. 2006;21:2209–15.
37. Dewailly D, Gronier H, Poncelet E, Robin G, Leroy M, Pigny P, Duhamel A, Catteau-Jonard S. Diagnosis of polycystic ovary syndrome (PCOS): revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. Hum Reprod. 2011;26:3123–9.

38. Lujan ME, Jarrett BY, Brooks ED, Reines JK, Peppin AK, Muhn N, Haider E, Pierson RA, Chizen DR. Updated ultrasound criteria for polycystic ovary syndrome: reliable thresholds for elevated follicle population and ovarian volume. Hum Reprod. 2013;28:1361–8.
39. Kristensen SL, Ramlau-Hansen CH, Ernst E, Olsen SF, Bonde JP, Vested A, Toft G. A very large proportion of young Danish women have polycystic ovaries: is a revision of the Rotterdam criteria needed? Hum Reprod. 2010;25:3117–22.
40. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, Griesinger G, Kelsey TW, La Marca A, Lambalk C, Mason H, Nelson SM, Visser JA, Wallace WH, Anderson RA. The physiology and clinical utility of anti-Mullerian hormone in women. Hum Reprod Update. 2014;20:370–85.
41. Dewailly D, Alebic MS, Duhamel A, Stojanovic N. Using cluster analysis to identify a homogeneous subpopulation of women with polycystic ovarian morphology in a population of non-hyperandrogenic women with regular menstrual cycles. Hum Reprod. 2014;29:2536–43.
42. Carmina E, Orio F, Palomba S, Longo RA, Lombardi G, Lobo RA. Ovarian size and blood flow in women with polycystic ovary syndrome and their correlations with endocrine parameters. Fertil Steril. 2005;84:413–9.
43. Rajpert-De Meyts E, Jorgensen N, Graem N, Muller J, Cate RL, Skakkebaek NE. Expression of anti-Mullerian hormone during normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. J Clin Endocrinol Metab. 1999;84:3836–44.
44. Jost A. The age factor in the castration of male rabbit fetuses. Proc Soc Exp Biol Med. 1947;66:302.
45. Kuiri-Hanninen T, Kallio S, Seuri R, Tyrvainen E, Liakka A, Tapanainen J, Sankilampi U, Dunkel L. Postnatal developmental changes in the pituitary-ovarian axis in preterm and term infant girls. J Clin Endocrinol Metab. 2011;96:3432–9.
46. Durlinger AL, Kramer P, Karels B, de Jong FH, Uilenbroek JT, Grootegoed JA, Themmen AP. Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary. Endocrinology. 1999;140:5789–96.
47. Durlinger AL, Gruijters MJ, Kramer P, Karels B, Kumar TR, Matzuk MM, Rose UM, de Jong FH, Uilenbroek JT, Grootegoed JA, Themmen AP. Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology. 2001;142:4891–9.
48. Durlinger AL, Gruijters MJ, Kramer P, Karels B, Ingraham HA, Nachtigal MW, Uilenbroek JT, Grootegoed JA, Themmen AP. Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology. 2002;143:1076–84.
49. Iliodromiti S, Kelsey TW, Anderson RA, Nelson SM. Can anti-Mullerian hormone predict the diagnosis of polycystic ovary syndrome? A systematic review and meta-analysis of extracted data. J Clin Endocrinol Metab. 2013;98:3332–40.
50. Salmon NA, Handyside AH, Joyce IM. Oocyte regulation of anti-Mullerian hormone expression in granulosa cells during ovarian follicle development in mice. Dev Biol. 2004;266:201–8.
51. van Houten EL, Themmen AP, Visser JA. Anti-Mullerian hormone (AMH): regulator and marker of ovarian function. Ann Endocrinol (Paris). 2010;71:191–7.
52. Fanchin R, Schonauer LM, Righini C, Guibourdenche J, Frydman R, Taieb J. Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. Hum Reprod. 2003;18:323–7.
53. Jeppesen JV, Anderson RA, Kelsey TW, Christiansen SL, Kristensen SG, Jayaprakasan K, Raine-Fenning N, Campbell BK, Yding Andersen C. Which follicles make the most anti-Mullerian hormone in humans? Evidence for an abrupt decline in AMH production at the time of follicle selection. Mol Hum Reprod. 2013;19:519–27.
54. Li HW, Anderson RA, Yeung WS, Ho PC, Ng EH. Evaluation of serum antimullerian hormone and inhibin B concentrations in the differential diagnosis of secondary oligoamenorrhea. Fertil Steril. 2011;96:774–9.

55. Pigny P, Gorisse E, Ghulam A, Robin G, Catteau-Jonard S, Duhamel A, Dewailly D. Comparative assessment of five serum antimullerian hormone assays for the diagnosis of polycystic ovary syndrome. Fertil Steril. 2016;105:1063–1069.e3.
56. Pigny P. Anti-Mullerian hormone assay: what's up in 2013? Médecine de la reproduction. Gynecol Endocrinol. 2014;16:16–20.
57. Pankhurst MW, McLennan IS. Human blood contains both the uncleaved precursor of anti-Mullerian hormone and a complex of the NH2- and COOH-terminal peptides. Am J Physiol Endocrinol Metab. 2013;305:E1241–7.
58. Nachtigal MW, Ingraham HA. Bioactivation of Mullerian inhibiting substance during gonadal development by a kex2/subtilisin-like endoprotease. Proc Natl Acad Sci U S A. 1996;93:7711–6.
59. Han X, McShane M, Sahertian R, White C, Ledger W. Pre-mixing serum samples with assay buffer is a prerequisite for reproducible anti-Mullerian hormone measurement using the Beckman Coulter gen II assay. Hum Reprod. 2014;29:1042–8.
60. van Helden J, Weiskirchen R. Performance of the two new fully automated anti-Mullerian hormone immunoassays compared with the clinical standard assay. Hum Reprod. 2015;30:1918–26.
61. Nelson SM, Pastuszek E, Kloss G, Malinowska I, Liss J, Lukaszuk A, Plociennik L, Lukaszuk K. Two new automated, compared with two enzyme-linked immunosorbent, antimullerian hormone assays. Fertil Steril. 2015;104:1016–21.e6.
62. Eilertsen TB, Vanky E, Carlsen SM. Anti-Mullerian hormone in the diagnosis of polycystic ovary syndrome: can morphologic description be replaced? Hum Reprod. 2012;27:2494–502.
63. Singh AK, Singh R. Can anti-Mullerian hormone replace ultrasonographic evaluation in polycystic ovary syndrome? A review of current progress. Indian J Endocrinol Metab. 2015;19:731–43.
64. La Marca A, Stabile G, Artenisio AC, Volpe A. Serum anti-Mullerian hormone throughout the human menstrual cycle. Hum Reprod. 2006;21:3103–7.
65. Tsepelidis S, Devreker F, Demeestere I, Flahaut A, Gervy C, Englert Y. Stable serum levels of anti-Mullerian hormone during the menstrual cycle: a prospective study in normo-ovulatory women. Hum Reprod. 2007;22:1837–40.
66. van Disseldorp J, Lambalk CB, Kwee J, Looman CW, Eijkemans MJ, Fauser BC, Broekmans FJ. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. Hum Reprod. 2010;25:221–7.
67. Tran ND, Cedars MI, Rosen MP. The role of anti-mullerian hormone (AMH) in assessing ovarian reserve. J Clin Endocrinol Metab. 2011;96:3609–14.
68. Freeman EW, Gracia CR, Sammel MD, Lin H, Lim LC, Strauss 3rd JF. Association of anti-mullerian hormone levels with obesity in late reproductive-age women. Fertil Steril. 2007;87:101–6.
69. Moy V, Jindal S, Lieman H, Buyuk E. Obesity adversely affects serum anti-mullerian hormone (AMH) levels in Caucasian women. J Assist Reprod Genet. 2015;32:1305–11.
70. Kriseman M, Mills C, Kovanci E, Sangi-Haghpeykar H, Gibbons W. Antimullerian hormone levels are inversely associated with body mass index (BMI) in women with polycystic ovary syndrome. J Assist Reprod Genet. 2015;32:1313–6.
71. Somunkiran A, Yavuz T, Yucel O, Ozdemir I. Anti-Mullerian hormone levels during hormonal contraception in women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol. 2007;134:196–201.
72. Dolleman M, Verschuren WM, Eijkemans MJ, Dolle ME, Jansen EH, Broekmans FJ, van der Schouw YT. Reproductive and lifestyle determinants of anti-Mullerian hormone in a large population-based study. J Clin Endocrinol Metab. 2013;98:2106–15.
73. Kallio S, Puurunen J, Ruokonen A, Vaskivuo T, Piltonen T, Tapanainen JS. Antimullerian hormone levels decrease in women using combined contraception independently of administration route. Fertil Steril. 2013;99:1305–10.

74. Goodman NF, Cobin RH, Futterweit W, Glueck JS, Legro RS, Carmina E. American association of clinical endocrinologists, american college of endocrinology, and androgen excess and pcos society disease state clinical review: guide to the best practices in the evaluation and treatment of polycystic ovary syndrome - part 1. Endocr Pract. 2015;21:1291–300.
75. Eldar-Geva T, Margalioth EJ, Gal M, Ben-Chetrit A, Algur N, Zylber-Haran E, Brooks B, Huerta M, Spitz IM. Serum anti-Mullerian hormone levels during controlled ovarian hyperstimulation in women with polycystic ovaries with and without hyperandrogenism. Hum Reprod. 2005;20:1814–9.
76. Catteau-Jonard S, Bancquart J, Poncelet E, Lefebvre-Maunoury C, Robin G, Dewailly D. Polycystic ovaries at ultrasound: normal variant or silent polycystic ovary syndrome? Ultrasound Obstet Gynecol. 2012;40:223–9.
77. Dewailly D, Pigny P, Soudan B, Catteau-Jonard S, Decanter C, Poncelet E, Duhamel A. Reconciling the definitions of polycystic ovary syndrome: the ovarian follicle number and serum anti-Mullerian hormone concentrations aggregate with the markers of hyperandrogenism. J Clin Endocrinol Metab. 2010;95:4399–405.
78. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE, Witchel SF. The androgen excess and PCOS society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril. 2009;91:456–88.
79. Hwang YI, Sung NY, Koo HS, Cha SH, Park CW, Kim JY, Yang KM, Song IO, Koong MK, Kang IS, Kim HO. Can high serum anti-Mullerian hormone levels predict the phenotypes of polycystic ovary syndrome (PCOS) and metabolic disturbances in PCOS patients? Clin Exp Reprod Med. 2013;40:135–40.
80. Sahmay S, Atakul N, Oncul M, Tuten A, Aydogan B, Seyisoglu H. Serum anti-Mullerian hormone levels in the main phenotypes of polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol. 2013;170:157–61.
81. Carmina E, Campagna AM, Fruzzetti F, Lobo RA. AMH measurement versus ovarian ultrasound in the diagnosis of polycystic ovary sundrome in different phenotypes. Endocr Pract. 2016;22:287–93.
82. Mahran A, Abdelmeged A, El-Adawy AR, Eissa MK, Shaw RW, Amer SA. The predictive value of circulating anti-Mullerian hormone in women with polycystic ovarian syndrome receiving clomiphene citrate: a prospective observational study. J Clin Endocrinol Metab. 2013;98:4170–5.
83. Kaya C, Pabuccu R, Satiroglu H. Serum antimullerian hormone concentrations on day 3 of the in vitro fertilization stimulation cycle are predictive of the fertilization, implantation, and pregnancy in polycystic ovary syndrome patients undergoing assisted reproduction. Fertil Steril. 2010;94:2202–7.
84. Xi W, Gong F, Lu G. Correlation of serum anti-Mullerian hormone concentrations on day 3 of the in vitro fertilization stimulation cycle with assisted reproduction outcome in polycystic ovary syndrome patients. J Assist Reprod Genet. 2012;29:397–402.
85. Knez J, Kovacic B, Medved M, Vlaisavljevic V. What is the value of anti-Mullerian hormone in predicting the response to ovarian stimulation with GnRH agonist and antagonist protocols? Reprod Biol Endocrinol. 2015;13:58.
86. Broer SL, Dolleman M, van Disseldorp J, Broeze KA, Opmeer BC, Bossuyt PM, Eijkemans MJ, Mol BW, Broekmans FJ, Group I-ES. Prediction of an excessive response in in vitro fertilization from patient characteristics and ovarian reserve tests and comparison in subgroups: an individual patient data meta-analysis. Fertil Steril. 2013;100:420–9. e7
87. Aghssa MM, Tarafdari AM, Tehraninejad ES, Ezzati M, Bagheri M, Panahi Z, Mahdavi S, Abbasi M. Optimal cutoff value of basal anti-mullerian hormone in iranian infertile women for prediction of ovarian hyper-stimulation syndrome and poor response to stimulation. Reprod Health. 2015;12:85.
88. Ocal P, Sahmay S, Cetin M, Irez T, Guralp O, Cepni I. Serum anti-Mullerian hormone and antral follicle count as predictive markers of OHSS in ART cycles. J Assist Reprod Genet. 2011;28:1197–203.

89. Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Hum Reprod. 2008;23:462–77.
90. Elmashad AI. Impact of laparoscopic ovarian drilling on anti-Mullerian hormone levels and ovarian stromal blood flow using three-dimensional power Doppler in women with anovulatory polycystic ovary syndrome. Fertil Steril. 2011;95:2342–6.
91. Amer SA, Li TC, Ledger WL. The value of measuring anti-Mullerian hormone in women with anovulatory polycystic ovary syndrome undergoing laparoscopic ovarian diathermy. Hum Reprod. 2009;24:2760–6.
92. Abu Hashim H. Predictors of success of laparoscopic ovarian drilling in women with polycystic ovary syndrome: an evidence-based approach. Arch Gynecol Obstet. 2015;291:11–8.
93. Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC. A nomogram to predict the probability of live birth after clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. Fertil Steril. 2002;77:91–7.
94. Imani B, Eijkemans MJ, Faessen GH, Bouchard P, Giudice LC, Fauser BC. Prediction of the individual follicle-stimulating hormone threshold for gonadotropin induction of ovulation in normogonadotropic anovulatory infertility: an approach to increase safety and efficiency. Fertil Steril. 2002;77:83–90.

Part II

Medical Treatments

Antiestrogens

9

Richard S. Legro

9.1 Introduction

This chapter will review current strategies for ovulation induction in women with polycystic ovary syndrome (PCOS) focusing primarily on clinical aspects of treating infertility with antiestrogens in women with PCOS. Antiestrogens are a broad category; thus, only most common forms of antiestrogens, selective estrogen receptor modulators (SERMs), which include clomiphene citrate (CC) and tamoxifen and raloxifene, will be discussed. However, given the overwhelming number of studies with CC, as well as its accepted clinical role over other SERMs in the treatment of PCOS, this chapter will largely focus on CC.

9.2 Clomiphene Citrate (CC)

9.2.1 Overview

CC is the most commonly used SERM in ovulation induction in women with PCOS, although tamoxifen has also been studied for this indication. These treatments were originally studied for the treatment of hormone-dependent breast cancer as were also the estrogen receptor antagonists and also the aromatase inhibitors. When you consider that many of the chronic treatments for hirsutism were adapted from antiandrogens developed for the treatment of hormone-dependent prostate cancer, a large debt is owed to hormone-dependent cancers and drug repurposing in the treatment of women with PCOS.

R.S. Legro
Department of Obstetrics and Gynecology and Public Health Sciences, Penn State College of Medicine, Hershey, PA, USA
e-mail: rsl1@psu.edu

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_9

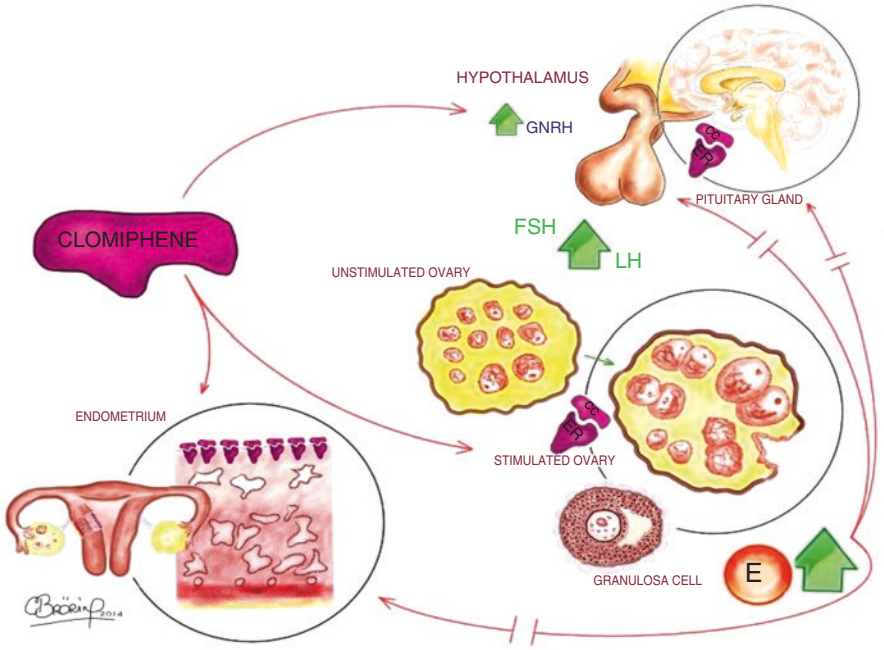


Fig. 9.1 Mechanism of action of CC (From Palomba [1])

Although SERMs, especially CC, are among the longest utilized drugs to treat anovulatory infertility in women with PCOS, their mechanism of action is still incompletely understood. Figure 9.1 summarizes the main and known mechanism of action. Specifically, they are thought to function as estrogen receptor antagonists in the hypothalamus and stimulate gonadotrophin-releasing hormone (GnRH) and subsequent follicle-stimulating hormone (FSH) secretion. This increase is FSH, especially relative to the excess of luteinizing hormone (LH) secretion that characterizes women with PCOS and restores follicular development, in many cases multi-follicular development in women with PCOS, who suffer from an excess of arrested antral follicles. They may also have similar effects elsewhere in the body; for instance, they may antagonize estrogen-stimulated endometrial development leading to a thinner luteal endometrial thickness and contributing to a lower chance of embryo implantation despite the increase in ovulatory rate. This theory, however, has been challenged by clinical trials of letrozole vs. CC in women with PCOS which have noted that midluteal increase in thickness is significantly higher with CC than with letrozole [2]. Such findings may dispel myths about the meaning of easily obtainable but clinically meaningless surrogate endpoints in predicting pregnancy outcomes.

Overall, CC has an estrogenic effect as indicated by the significant increases in circulating sex hormone-binding globulin (SHBG) levels after even short exposures (i.e., 5 days). The metabolism of CC is complex as it is a racemic mixture of two

isomers (zu- and en-CC which may have varying effects) and has a long half-life (5–7 days) such that metabolites (especially zu-CC) may accumulate over time with carryover effects in consecutive cycles [3].

9.2.2 Efficacy

The cumulative success rate of CC will depend on the population studied, such that cumulative 3–6-month live birth rates vary greatly from 20–80% in the literature [4, 5]. Table 9.1 summarizes a variety of predictive factors that have been identified from studies [5–8]. A caveat is that although known predictive factors have been reported from multiple groups, rarely is a predictive model for pregnancy validated in a subsequent prospective clinical trial [9]. Most infertility studies will screen out other causes of infertility prior to ovulation induction, and that process makes good clinical sense before commencing with this infertility therapy. The reason is that in an unselected population of women with PCOS seeking infertility, there is a high proportion of other infertility factors (see also Chap. 6), e.g., 10% of males have oligospermia requiring further evaluation or alternate treatments, and ~5% of women have bilateral tubal occlusion on tubal testing [by hysterosalpingography (HSG), sonohysterography (SHG), or laparoscopy], and a larger percentage have endometrial filling defects or unilateral tubal occlusion which may impair response to treatment [10].

There is emerging evidence that excessively high anti-Mullerian hormone (AMH) levels are associated with both poor responses to CC, i.e., increased resistance to ovulation as well as lower pregnancy rates [9]. This may be related to the general concept that the more severe cases of PCOS are less responsive to first-line therapy than the milder cases. It is often difficult to separate the relative contributions of reproductive abnormalities such as hyperandrogenism from metabolic abnormalities such as insulin resistance (see Chap. 6). A good example for this is the examination of SHBG which increases both in response to lower circulating androgens and higher circulating estrogens but also to lower insulin levels and improved insulin sensitivity [11, 12]. Both higher baseline SHBG and increases in response to treatment have been associated with improved pregnancy rates in PCOS [9, 13].

Table 9.1 Predictive factors for pregnancy with ovulation induction with CC

Known predictive factors	Suspected predictive factors
Younger age	Exclusion of other infertility factors
Shorter duration of attempting pregnancy	Prior nonresponse to SERMs
Lower BMI	Lower levels of AMH (above the normal lower limit cutoff)
Less hyperandrogenism	
Less insulin resistance	
Recent pregnancy loss	

9.2.3 Adverse Events

Hot flashes are noted as a particularly annoying side effect by patients. Further there is a theoretical concern about a sudden development of visual symptoms due to potential pituitary enlargement and pressure on the optic chiasm. This can be a reason for treatment discontinuation though clinically it is often to determine the source of such symptoms without brain imaging, which is typically not performed in such cases. The most common side effects to CC are related to successful response, i.e., abdominal pain and cramps, dysmenorrhea, breast tenderness, etc., related to follicular development, ovulation, and menses, if the patient does not conceive. Patients, who, due to their anovulation, are not used to such symptoms, should be counseled about the normalcy of such symptoms in the context of ovulatory response. CC can induce the formation of follicular cysts requiring treatment cessation until cysts resolve.

Multiple pregnancy rates are in the range of 4–8% and most are twins, though case reports have also documented high-order multiple pregnancies after CC use. From our experience with the use of CC in both PCOS and unexplained infertility, multiple pregnancy rates (among clinically recognized pregnancies) trend higher in the population with unexplained infertility (9.4%) compared to PCOS (4.0%) [2, 14].

There are no known patterns of congenital birth defects related to CC, though greater attention has focused on this serious adverse event with the increased scrutiny of birth defects in patients who conceived with letrozole (see also Chap. 10). One retrospective chart review noted an increased prevalence of cardiac-related malformations with CC compared to letrozole in women with infertility [15]. Another registry-based study in Australia also noted a high prevalence of birth defects after CC use compared to other forms of infertility therapy, but this may be a type 1 error due to the low number of patients in this category [16]. Prospective trials of letrozole and CC in both unexplained infertility and PCOS have noted comparable congenital malformation rates below 5% with both drugs. Thus, currently there are not supporting data to counsel patients of an increased congenital malformation rate with the use of CC compared to other therapies.

9.2.4 Protocols

CC is given in the early follicular phase or, more correctly stated, the constant follicular phase of anovulatory women with PCOS. Thus, it is a moot point whether to begin on day 3 or day 5 of the cycle, since the women are acyclic. If a woman with PCOS ovulates, there is no reason to recommend a day 3 or day 5 cycle start in a subsequent cycle. The starting dose for clomiphene is 50 mg a day for 5 days. Many groups will perform a baseline ultrasound with serum progesterone screening to rule out periodic and unexpected ovulation. Further baseline ultrasounds (or midluteal ultrasounds) will be obtained to rule out the presence of large residual cysts that may lead to symptoms, ovarian torsion, or unilateral ovarian suppression of follicle development, due to a mass effect. At a minimum, it is prudent to perform a urine

pregnancy test to rule out potential exposure of an early pregnancy to the medication before any dose is given. This advice is practical for all ovulation induction methods in women with PCOS.

In terms of monitoring, verifying follicular development or ovulation with a serum progesterone, ultrasound, or both may allow for more rapid advancement to therapeutic doses. If there is no ovulation or follicular development, the so-called stairstep protocol may be utilized, which recommends dose increases every 2–3 weeks based on follicular response [17]. Other regimens proposed in the literature but less used in the clinical practice are the "extended regimen," i.e., CC administration at doses of 100 mg daily starting on day 2 of menses for 9 days; the "luteal phase regimen," i.e., CC administration at doses of 100 mg daily starting the next day after finishing medroxyprogesterone acetate given for 5 days; and the "repeated intra-cycle regimen," i.e., CC administration at the same dose per 5 days at regular intervals.

There are limited data to suggest that follicular phase monitoring and triggering of ovulation with hCG is superior to no monitoring and timed intercourse in anovulatory patients. The dose of CC is increased by 50 mg a day up to a maximum daily dose of 150 mg a day or 750 mg per cycle as per the US Food and Drug Administration package insert recommendations. Higher daily doses of CC have been given with reported success or longer duration of dosing beyond 5 days [18].

It is debatable whether an induced withdrawal bleed is necessary prior to ovulation induction or between anovulatory cycles if a patient is nonresponsive to medication (i.e., CC resistant) [19]. The rationale for this likely extends back to the concept that a "fresh" endometrium has a better prognosis than one that has seen a prolonged follicular phase; however, there is also the potential for harm through excessive shedding of the endometrium or through prolonged suppression of the hypothalamic pituitary axis through progestin challenge. Further prospective studies are needed supporting lower pregnancy rates with this practice before a definitive recommendation to discontinue progestin challenge can be made [20].

9.2.5 Adjuvant Infertility Therapies with CC

CC may see its fullest range of use in combination with other medications. A summary table (Table 9.2) of select adjuvant agents in which some benefit from at least one prospective trial is noted is included in this chapter. Of note, the only adjuvant therapy to clomiphene which in the Cochrane systematic reviews of antiestrogen use in PCOS was found to significantly improve pregnancy rates was dexamethasone [21]. CC plus dexamethasone treatment was effective in increasing pregnancy rate of about tenfold compared to CC alone [odds ratio (OR) 9.46, 95% confidence interval (CI) 5.1 to 17.7]. Metformin has been studied most extensively as an adjuvant therapy to CC, and generally in large studies, the combination has not been statistically superior to CC alone in improving pregnancy rates [4, 22, 23]. However there may be a benefit in certain subsets of patients, particularly obese patients [4, 24]. Further

Table 9.2 Concurrent adjuvant infertility therapies with CC

Ovarian stimulation agents
Gonadotrophins [25]
Letrozole [32]
Adrenal agents
Dexamethasone [33]
Metabolic agents
Metformin [34]
Rosiglitazone [35]
Supplements
L-Carnitine [36]
N-Acetyl Cysteine [37]
Inositols [20]

such studies were useful for showing the comparative difference in quality of ovulation with CC versus metformin. CC may also be useful as an adjuvant therapy to reduce the utilization of more expensive medications such as gonadotrophins [25], reducing the rate of complications rates such as ovarian hyperstimulation syndrome (OHSS) when used as an adjuvant.

9.2.6 Areas of Uncertainty

Many issues with CC still need to be addressed. The ideal number of cycles for first-line therapy has not been established, but longer studies have shown that time does not diminish the per cycle pregnancy rates with CC over five or six cycles [2, 4, 22]. Thus, if a patient is ovulating, a longer course of ovulation induction with CC may be indicated if other factors do not lead to choosing alternate and more successful therapies (i.e., gonadotrophins or IVF). CC resistance remains an issue as up to 25% of patients will not ovulate even when challenged with the highest dose of 150 mg/day. Adjuvant therapies or preconception weight loss in obese patients may improve the ovulation rate with CC [26, 27]. Such studies have formed the basis for the recommendation by experts for obese women with PCOS to lose weight prior to ovulation induction. However, more recent studies have noted a relative harm in obese women who delay infertility treatment to pursue lifestyle modification, with significantly lower cumulative live birth rates over a 2-year period of follow-up [28]. Further studies in this area are needed.

9.3 Other SERMs: Tamoxifen and Raloxifene

Tamoxifen and CC have very similar structures with only a difference in a single side chain between them (Fig. 9.2). This may explain the fact that there appears to be little difference between them in terms of ovulation rates and pregnancy rates in comparative trials between CC and tamoxifen [21, 29, 30].

At the moment, there has been one small trial comparing CC to raloxifene, a newer SERM for ovulation induction in women with PCOS, which noted

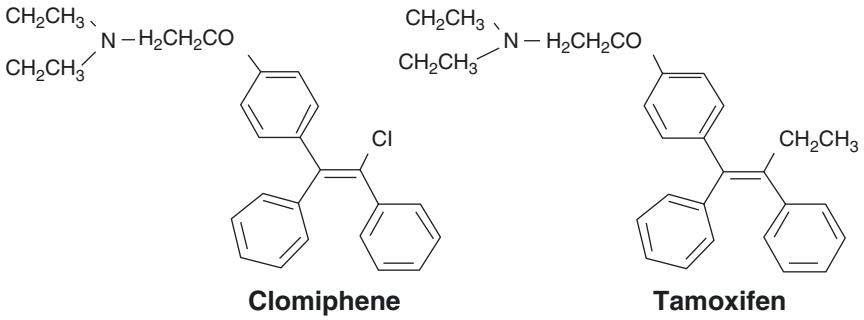


Fig. 9.2 Chemical structures of CC and tamoxifen

comparable ovulation rates [31]. Thus, currently there appears to be little rationale for choosing tamoxifen or raloxifene over CC or using tamoxifen or raloxifene in the face of CC resistance or CC failure.

Conclusion

Despite the emergence of aromatase inhibitors as first-line infertility therapy in women with PCOS, CC remains a time-honored relatively safe and effective means for ovulation induction in women with PCOS. Many opportunities exist to improve our utilization of CC including pharmacogenetic studies to identify responders, further studies of combination therapy with other safe and relatively effective adjuvants, and better long-term studies of prolonged treatment courses with CC in ovulatory patients. Such studies could help us to clarify the role of clomiphene in the future treatment of women with PCOS-related infertility.

Conflict of Interest Dr. Legro reports consulting fees from Euroscreen, Kindex, Bayer, and Millendo Pharmaceuticals and research funding from Ferring.

Funding This work was supported by the Eunice Kennedy Shriver National Institutes of Child Health and Human Development (NICHD) Grants R01 HD056510 and U10 HD38992 (to R.S.L.)

References

1. Palomba S. Aromatase inhibitors for ovulation induction. J Clin Endocrinol Metab. 2015;100:1742–7.
2. Legro RS, Brzyski RG, Diamond MP, et al. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. N Engl J Med. 2014;371:119–29.
3. Adashi EY. Clomiphene citrate: mechanism(s) and site(s) of action–a hypothesis revisited. Fertil Steril. 1984;42:331–44.

4. Legro RS, Barnhart HX, Schlaff WD, et al. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. N Engl J Med. 2007;356:551–66.
5. Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC. Predictors of chances to conceive in ovulatory patients during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. J Clin Endocrinol Metab. 1999;84:1617–22.
6. Rausch ME, Legro RS, Barnhart HX, et al. Predictors of pregnancy in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2009;94:3458–66.
7. Imani B, Eijkemans MJ, de Jong FH, et al. Free androgen index and leptin are the most prominent endocrine predictors of ovarian response during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. J Clin Endocrinol Metab. 2000;85:676–82.
8. Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC. Predictors of patients remaining anovulatory during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. J Clin Endocrinol Metab. 1998;83:2361–5.
9. Mumford SL, Legro RS, Diamond MP, et al. Baseline AMH level associated with ovulation following ovulation induction in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2016;101:3288–96.
10. McGovern PG, Legro RS, Myers ER, et al. Utility of screening for other causes of infertility in women with "known" polycystic ovary syndrome. Fertil Steril. 2007;87:442–4.
11. Nestler JE, Powers LP, Matt DW, et al. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. J Clin Endocrinol Metab. 1991;72:83–9.
12. Stener-Victorin E, Holm G, Labrie F, Nilsson L, Janson PO, Ohlsson C. Are there any sensitive and specific sex steroid markers for polycystic ovary syndrome? J Clin Endocrinol Metab. 2010;95:810–9.
13. Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC. A nomogram to predict the probability of live birth after clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. Fertil Steril. 2002;77:91–7.
14. Diamond MP, Legro RS, Coutifaris C, et al. Letrozole, gonadotropin, or clomiphene for unexplained infertility. N Engl J Med. 2015;373:1230–40.
15. Tulandi T, Martin J, Al-Fadhli R, et al. Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. Fertil Steril. 2006;85:1761–5.
16. Davies MJ, Moore VM, Willson KJ, et al. Reproductive technologies and the risk of birth defects. N Engl J Med. 2012;366:1803–13.
17. Hurst BS, Hickman JM, Matthews ML, Usadi RS, Marshburn PB. Novel clomiphene "stairstep" protocol reduces time to ovulation in women with polycystic ovarian syndrome. Am J Obst Gynecol. 2009;200:510.e1–4.
18. Lobo RA, Granger LR, Davajan V, Mishell Jr DR. An extended regimen of clomiphene citrate in women unresponsive to standard therapy. Fertil Steril. 1982;37:762.
19. Diamond MP, Kruger M, Santoro N, et al. Endometrial shedding effect on conception and live birth in women with polycystic ovary syndrome. Obstet Gynecol. 2012;119:902–8.
20. Dong X, Zheng Y, Liao X, Xiong T, Zhang H. Does progesterone-induced endometrial withdrawal bleed before ovulation induction have negative effects on IUI outcomes in patients with polycystic ovary syndrome? Int J Clin Exp Pathol. 2013;6:1157–63.
21. Brown J, Farquhar C. Clomiphene and other antioestrogens for ovulation induction in polycystic ovarian syndrome. Cochrane Database Syst Rev. 2016;12:CD002249. doi: 10.1002/14651858.CD002249.pub5. PMID: 27976369.
22. Moll E, Bossuyt PM, Korevaar JC, Lambalk CB, van der Veen F. Effect of clomiphene citrate plus metformin and clomiphene citrate plus placebo on induction of ovulation in women with newly diagnosed polycystic ovary syndrome: randomised double blind clinical trial. BMJ. 2006;332:1485.
23. Zain MM, Jamaluddin R, Ibrahim A, Norman RJ. Comparison of clomiphene citrate, metformin, or the combination of both for first-line ovulation induction, achievement of pregnancy,

and live birth in Asian women with polycystic ovary syndrome: a randomized controlled trial. Fertil Steril. 2009;91:514–21.
24. Morin-Papunen L, Rantala AS, Unkila-Kallio L, et al. Metformin improves pregnancy and live-birth rates in women with polycystic ovary syndrome (PCOS): a multicenter, double-blind, placebo-controlled randomized trial. J Clin Endocrinol Metab. 2012;97:1492–500.
25. Ghanem ME, Elboghdady LA, Hassan M, et al. Clomiphene citrate co-treatment with low dose urinary FSH versus urinary FSH for clomiphene resistant PCOS: randomized controlled trial. J Assist Reprod Genet. 2013;30:1477–85.
26. Legro RS, Dodson WC, Kris-Etherton PM, et al. Randomized controlled trial of preconception interventions in infertile women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2015;100:4048–58.
27. Clark AM, Ledger W, Galletly C, et al. Weight loss results in significant improvement in pregnancy and ovulation rates in anovulatory obese women. Hum Reprod. 1995;10:2705–12.
28. Mutsaerts MA, van Oers AM, Groen H, et al. Randomized trial of a lifestyle program in obese infertile women. N Engl J Med. 2016;374:1942–53.
29. Dhaliwal LK, Suri V, Gupta KR, Sahdev S. Tamoxifen: an alternative to clomiphene in women with polycystic ovary syndrome. J Human Reprod Sci. 2011;4:76–9.
30. Nardo LG. Management of anovulatory infertility associated with polycystic ovary syndrome: tamoxifen citrate an effective alternative compound to clomiphene citrate. Gynecol Endocrinol. 2004;19:235–8.
31. de Paula Guedes Neto E, Savaris RF, von Eye Corleta H, de Moraes GS, do Amaral Cristovam R, Lessey BA. Prospective, randomized comparison between raloxifene and clomiphene citrate for ovulation induction in polycystic ovary syndrome. Fertil Steril. 2011;96:769–73.
32. Hajishafiha M, Dehghan M, Kiarang N, Sadegh-Asadi N, Shayegh SN, Ghasemi-Rad M. Combined letrozole and clomiphene versus letrozole and clomiphene alone in infertile patients with polycystic ovary syndrome. Drug Des Devel Ther. 2013;7:1427–31.
33. Parsanezhad ME, Alborzi S, Motazedian S, Omrani G. Use of dexamethasone and clomiphene citrate in the treatment of clomiphene citrate-resistant patients with polycystic ovary syndrome and normal dehydroepiandrosterone sulfate levels: a prospective, double-blind, placebo-controlled trial. Fertil Steril. 2002;78:1001–4.
34. Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. N Engl J Med. 1998;338:1876–80.
35. Ghazeeri G, Kutteh WH, Bryer-Ash M, Haas D, Ke RW. Effect of rosiglitazone on spontaneous and clomiphene citrate-induced ovulation in women with polycystic ovary syndrome. Fertil Steril. 2003;79:562–6.
36. Ismail AM, Hamed AH, Saso S, Thabet HH. Adding L-carnitine to clomiphene resistant PCOS women improves the quality of ovulation and the pregnancy rate. A randomized clinical trial. Eur J Obstet Gynecol Reprod Biol. 2014;180:148–52.
37. Rizk AY, Bedaiwy MA, Al-Inany HG. N-acetyl-cysteine is a novel adjuvant to clomiphene citrate in clomiphene citrate-resistant patients with polycystic ovary syndrome. Fertil Steril. 2005;83:367–70.

Aromatase Inhibitors

10

Nivin Samara and Robert F. Casper

10.1 Introduction

Aromatase (AROM) is the rate-limiting enzyme in oestrogen biosynthesis, and inhibition of its activity reduces oestrogen blood levels and negative feedback on gonadotrophin secretion. More than 15 years have passed since the first use of an aromatase inhibitor (AI) as a treatment for induction of ovulation in patients with polycystic ovary syndrome (PCOS) [1]. Letrozole (LTZ) is the dominant AI in use in infertility treatment. It is now the first treatment option for different subfertility/infertility indications. Induction of ovulation in both naïve and clomiphene citrate (CC) resistant infertility patients with PCOS is the main indication. LTZ is also widely administered in treatment of unexplained infertility and mild male factor infertility either as a sole treatment or in combination with gonadotrophins and intrauterine insemination (IUI) [2]. Recently more and more studies demonstrate the potential beneficial use of LTZ in in vitro fertilisation (IVF) cycles especially in breast cancer patients going through fertility preservation treatment [3–5]. LTZ is used "off label" in North America and many other Countries around the world. The warning letter published by the original manufacturer is still the main obstacle to wider acceptance [6]. In the last 15 years, many studies have been published describing favourable results with LTZ use in reproductive technologies with no significant short or long-term side effects [7]. We believe, in the light of the accumulating clinical research evidence, that LTZ is safe for use in assisted reproduction. Meanwhile, the use of AIs for induction of ovulation in PCOS patients is well documented. LTZ has

N. Samara • R.F. Casper (✉)
Division of Reproductive Sciences, University of Toronto, Toronto, ON M5S 1A1, Canada

Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital,
Toronto, ON M5G 1X5, Canada

TRIO Fertility, 655 Bay St, Suite 1101,
Toronto, ON M5G2K4, Canada
e-mail: casper@lunenfeld.ca

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_10

several benefits over the traditional treatment with CC [8] including the development of normal endometrial thickness and normal cervical mucus, predominantly monofollicular ovulation with reduced risk of multiple pregnancy and a short half-life that prevents accumulation with serial use. In the following chapter we will review use of AIs in ovulation induction, focusing primarily on World Health Organization (WHO) type 2 classification, mainly PCOS.

10.2 History

For decades, the cytochrome P450 AROM) has been under intensive investigation to understand its function in conversion of the androgens, androstendione, testosterone and 16 α-hydroxytestosterone to the oestrogens, oestrone (E1), 17β-oestradiol (E2) and 17β, 16α-oestriol (E3), respectively. AROM functions in various tissues and organs including ovaries, adipose tissue, brain, liver and breast [9]. The main interest in this field has been in the development of an improved therapy for postmenopausal oestrogen-dependent breast cancer [10]. Aminoglutethimide (AG) was discovered in 1961 and first used as an antiepileptic treatment. It represents the first generation of AIs and was used as early as 1974 [11]. A reduction in E1 levels was described in postmenopausal breast cancer patients treated with AG [12, 13]. Its main side effects were a result of its low selectivity; AG inhibited AROM but also inhibited the activity of other P450s, e.g. P450scc and some enzymes involved in thyroid biosynthesis [14].

Formestane is a steroidal AI, synthesised in 1973 [15], and first reported as an AI in 1984 [16]. Formestane belongs to the second generation AIs, has fewer side effects compared with AG and underwent several clinical trials in breast cancer treatment [16]. Formestane was never approved for clinical use, mostly, because of low oral bioavailability [17] and the discovery of the third generation AIs with more selective and more potent AROM inhibition [10]. The other second generation AI is fadrozole, it was a non-steroidal reversible AI like AG but more potent and specific [18, 19]. Fadrozole was used as early as 1987, and was part of clinical trials of breast cancer treatment in 1989. Fadrozole was approved for clinical treatment in Japan but never by the Food Drug Administrations (FDA) in the United States (US). Despite relatively high selectivity, fadrozole had some side effects including nausea, vomiting, fatigue and suppression of aldosterone [16].

Third generation non-steroidal AIs include anastrozole (ANZ), LTZ and exemstane (EXM), all approved by the FDA in the late nineties for clinical use for postmenopausal oestrogen-dependent breast cancer [20]. The prominent feature of the third generation drugs is high selectivity for AROM and as a result fewer side effects [21, 22]. It is noteworthy that all three AIs are equally effective in breast cancer therapy [23, 24]. All three AIs have shown superior efficacy and greater disease free survival when compared to tamoxifen, a selective oestrogen receptor modulator (SERM) in treating breast cancer [21, 22], more recently employed for ovulation induction too (see Chap. 9). Among third generation AIs, the most common adverse effects are fatigue and arthralgia, in addition to hypoestrogenism-related effects including hot flashes and osteoporosis [21, 22, 25, 26].

10.3 Pharmacokinetics and Pharmacodynamics

Third generation AIs have a relatively short half-life. This feature is especially important for fertility indications. The faster the elimination from the circulation, the less chance exists of exposure of the fertilised oocyte or developing embryo through implantation and beyond, thereby reducing the possibility of any teratogenic effect. LTZ has a half-life of about 45 h. It is completely cleared from blood within approximately 10 days or 5 times the half-life length. Oral LTZ bioavailability is 100%, and it reaches a blood steady state level in 4–8 h. LTZ at the 2.5 mg dose suppresses oestrone and oestradiol by ~78% in postmenopausal women, with maximum suppression achieved after 48–72 h. Contrast that pharmacokinetic with those of CC below.

CC was considered for six decades as the sole oral treatment for ovulation induction in PCOS patients, despite accumulating evidence of undesirable side effects that impaired pregnancy success compared to ovulation efficacy (see also Chap. 9) [7]. CC is a SERM that blocks oestrogen negative feedback in the pituitary and hypothalamus by depleting oestrogen receptors (ER), resulting in the increase in secretion of luteinising hormone (LH) and follicle stimulating hormone (FSH). This anti-ER effect is non-specific and is the explanation for the detrimental impact of CC on cervical mucus and the endometrium [27, 28]. A proportion of patients with PCOS demonstrates resistance to CC and do not ovulate. Following resistance to CC for ovulation induction, historically most physicians would switch to injectable gonadotrophins with potentially unfavourable results such as multiple pregnancy and a risk of ovarian hyperstimulation syndrome (OHSS) (see also Chap. 21). The optimal alternative to CC, therefore, would be a similar oral, inexpensive medication with a short half- life [29, 30], and no anti-estrogenic effects.

LTZ – 4,4′-[1H-1,2,4-triazol-1-ylmethylene]-bis-ben is highly selective for AROM. Originally suggested as a possible contraceptive agent, multiple follicle formation was observed following administration of LTZ in Bonnet monkeys [31]. Several years later, in 2001, Mitwally and Casper [1] published their work on a group of patients who failed to ovulate or who demonstrated thin endometrium and failure to conceive with CC treatment. The patients had mixed anovulatory and ovulatory infertility disorders. They were treated with LTZ, 2.5 mg a day, from day 3–7 of the cycle. Seventy-five percent of PCOS patients ovulated and 90% of ovulatory patients had one or more follicles with adequate endometrial thickness. Seventeen percent of the patients conceived following LTZ treatment.

This was the first study suggesting LTZ as an alternative to CC, and proposing a solution for the adverse anti-oestrogenic effects of CC on extra-ovarian tissues. Gradually, since the first report described above, LTZ has become a popular, off label, treatment for ovulation induction and stimulation. Numerous studies have been and still are conducted on LTZ. This active research and clinical use contributes to continuous improvement in efficacy and safety of LTZ treatment protocols. The selection of LTZ for fertility treatment, rather than other third generation AI's, was serendipitous, after a patient with breast cancer was prescribed LTZ by

her oncologist and she asked us about the medication. It is noteworthy that LTZ has become the predominant AI in use in assisted reproductive technology (ART) and the rest of this review will focus on data related to LTZ.

10.4 Mechanism of Action

Both peripheral oestrogen production in ovaries and fat tissue, and central estrogenic secretion in the brain result in negative feedback on gonadotrophin secretion from the hypothalamus-pituitary axis [32–34]. By eliminating oestrogen production in the early follicular phase, LTZ will cause secretion of more FSH and recruitment of one or more follicles. A smaller fraction of the FSH increase is attributed to enhanced activin discharge from the pituitary following reduced oestrogen levels. Activin locally, stimulates FSH secretion from gonadotrophes [35].

In 2009, cytochrome P450 AROM was purified in its crystal form for the first time enabling a better understanding of its function in conversion of androgens to oestrogens. [10]. Androstendione is the natural substrate of AROM, binding to the active site of AROM, a heme-distal group of the P450 subunit. LTZ binds reversibly to this active group of AROM, inhibiting androgen transformation to oestrogens [10]. As already before reported, LTZ has relatively short half-life about 45 h [7, 36–39] and LTZ and the other AIs do not down-regulate oestrogen receptors, nor have any direct estrogenic or anti-estrogenic activity.

Some studies reported that testosterone enhances FSH receptor expression in the ovaries promoting follicular development in the follicular phase [40–42]. The LTZ inhibition of androgen aromatisation may result in accumulation of androstenedione and testosterone in the ovaries. Androgens may also increase local secretion of endocrine and paracrine factors involved in follicular development such as insulin-like growth factor (IGF) [43, 44].

PCOS patients have typically relatively low FSH levels. One possible explanation is that central oestrogen levels are high as a result of aromatisation of the abundant androgens in these patients. It is expected that this effect would be counteracted by the inhibition of aromatisation by LTZ resulting in an increase in FSH levels and recruitment of multiple follicles in the early follicular phase. However, the FSH increase remains modest because once the AROM inhibition effect wears off; oestrogen negative feedback is resumed since unlike CC, LTZ results in no ER depletion. In addition, high levels of inhibin in PCOS patients may modulate the rise in FSH [45].

Anti-Mullerian hormone (AMH) has been suggested as an active factor in the pathogenesis of PCOS (see also Chap. 8). AMH is usually elevated in PCOS as a result of abundant secretion from granulosa cells of small follicles in the PCOS ovary [46]. Some evidence supports the assumption that AMH is not merely an indicator of antral follicle number but is also active in inhibiting early follicle growth. AMH is secreted from the primary follicle stage onwards and its secretion is highest during in the pre-antral and small antral follicle stages and declines after the follicle reaches a diameter of about 8 mm [47, 48]. AMH inhibits follicular growth [49]. In in vitro experiments, AMH treatment reduced FSH-induced [50]

and cAMP-stimulated AROM activity, and AROM mRNA expression and E2 production [49–51]. PCOS patients have higher serum levels of AMH compared to normal cycling women [49]. The increase in AMH was also demonstrated in follicular fluid [52] and granulosa cells [53] of anovulatory PCOS patients compared with ovulatory women. A higher total AMH and higher AMH per follicle in PCOS patients who did not respond to ovulation induction with CC were demonstrated [54]. In a study comparing LTZ to CC for ovulation induction in PCOS patients, a greater reduction in AMH levels in the LTZ group compared with CC group was found [8]. These data suggest another possible route of a potential positive effect of LTZ on ovarian response in PCOS patients.

This complex system of hormonal balance and interactions combine to form a favourable treatment result with LTZ in PCOS patients. AIs achieve induction of ovulation without interfering with the negative feedback system in the hypothalamus and pituitary and with no adverse effect on endometrial development. Monofollicular response in anovulatory PCOS patients is a reasonable goal that can be obtained by LTZ since the short half-life allows rapid resumption of follicular oestrogen production and normal negative feedback of oestrogen on FSH to prevent further follicular recruitment. Nonetheless, dose management and monitoring with ultrasound must be individualised to be optimal.

10.5 Clinical Data

Induction of ovulation is the main target of fertility treatment for PCOS patients. It is widely accepted that lifestyle change is a first line treatment in overweight or obese patients (see also Chap. 13). However, a Cochrane meta-analysis concluded that there is no data regarding pregnancy, live birth and miscarriage rates in PCOS women with lifestyle modification, even though restoration of ovulation and cycle regularity has been reported following weight loss [55]. A recent large study from the Netherlands compared lifestyle intervention for weight loss for 6 months before fertility treatment in obese PCOS patients compared to immediate ovulation induction. The results of this study showed no benefit of lifestyle intervention. Significantly more women had a healthy live birth by 24 months in the immediate ovulation induction group compared to the lifestyle intervention group [56]. The timing of interventional treatment is usually matched to the patient's desire for pregnancy, age, and concurrent infertility factors. Because of variability in response to weight loss, CC has been widely prescribed for ovulation induction. For the last 60 years, CC has been the first line pharmacological treatment for PCOS patients (see Chap. 9). CC has the advantages of easy oral use and low cost. However, the cumulative birth rate is relatively low (22% in up to 6 cycles [56]). As mentioned above CC has unwanted anti-estrogenic side effects, and the potential for multiple pregnancy (3–8%) [8] compared to 1% in naturally conceived pregnancies. Failure of ovulation or resistance to CC leads usually to a progression to gonadotrophin therapy. The recommended gonadotrophin protocol in PCOS patients is "low and slow" meaning low dose of gonadotrophins for several days before a gradual increase in

dose and continuous intensive monitoring to reduce the risk of multiple ovulation and multiple pregnancy or OHSS [57, 58]. Gonadotrophins are administrated as daily injections and they are expensive. More details on gonadotrophin ovarian stimulation are given in the Chap. 12).

As described above, the preliminary study by Mitwally and Casper reported 75% ovulation and 17% pregnancy rate with one cycle of treatment in PCOS patients who did not response to CC [1]. Similar ovulation rates were described in other studies. Many clinical studies have been conducted since 2001 comparing LTZ with other ovulation induction treatments. Various protocols have been tried, and gradually a diversity of indications has resulted.

The early studies described patients treated with LTZ as second line therapy after failure to induce ovulation with CC or failure to achieve pregnancy in PCOS patients. As more and more comparative studies accumulated, however, more evidence has been collected supporting the use of LTZ as a substitute for CC and a primary treatment for PCOS. A Cochrane meta-analysis examined the outcomes of 26 randomised controlled trials (RCTs) and confirmed that LTZ improved live birth and pregnancy rates compared to CC [59, 60]. Odds ratio (OR) of live birth was 1.64 and OR of clinical pregnancy rate was 1.4 for LTZ compared to CC. No evidence of difference in miscarriage rates was demonstrated between pregnancies achieved after LTZ vs. CC. LTZ was associated with reduced risk of multiple pregnancy, estimated OR 0.38. Recently, Legro et al. [8] have published what has been considered to be the definitive comparison of LTZ and CC for ovulation induction in PCOS. That study showed improved ovulation rate (88.5% vs. 76.6%), improved conception rate (41.2% vs. 27.4%), improved clinical pregnancy rate (31.3% vs. 21.5%) and live birth rate (27.5% vs. 19.1%) in LTZ treated patients compared to CC (see also Chap. 9).

10.5.1 CC Resistance

Fifteen to 40% of PCOS patients fail ovulate following multiple treatment cycles with CC and are considered to be CC resistant [61]. Moreover, the definition of CC resistant is very variable among available studies. One study defined CC resistance as failure to ovulate after 5 cycles of CC up to 250 mg [62]. LTZ has been proposed as an alternative treatment in these women. In fact, the first study conducted by Mitwally and Casper [1] was conducted in CC resistant PCOS patients. Since then many studies have been published comparing LTZ with other treatments in management of CC resistant patients with a success rate of LTZ to induce ovulation of around 33% [63]. In a comparative study of LTZ and tamoxifen in CC resistant patients, ovulation rates were 23.3% vs. 8.89% with LTZ and tamoxifen, respectively and pregnancy rates were more than doubled in the LTZ group [64]. Another study compared LTZ with CC combined with metformin in treatment of CC resistant patients, and the ovulation rates were equivalent between these two groups (64.9% vs. 69.6%, respectively) [65].

Another treatment that has been proposed for CC resistant patients is laparoscopic ovarian drilling (LOD). In our opinion, LOD should be an obsolete treatment for induction of ovulation in PCOS (see also Chap. 15). Conventionally, it is reserved as an alternative to gonadotrophins in CC resistant PCOS patients. Even though LOD is a surgical procedure, it appeals to patients who cannot afford expensive gonadotrophin treatments and reduces the risk of multiple pregnancy associated with gonadotrophins. However, we have demonstrated by second-look laparoscopy, that LOD is associated with consistent formation of ovarian adhesions [66] and should probably be abandoned, especially since the ovulation and pregnancy rate with LTZ treatment was found to be equally as, or more effective than, LOD. No difference was observed in miscarriage rates and live birth rates were also increased with LTZ [67, 68].

10.6 Protocols and Doses

In their initial study, Mitwally and Casper used LTZ 2.5 mg daily for 5 days from cycle days 3 to 7 with good results. Since then, LTZ 2.5 mg has been the usual treatment dose that is successful in inducing ovulation in most patients with PCOS. Al-Fadhli et al. prospectively compared 2.5 mg and 5 mg of LTZ for 5 days and observed a higher number of dominant follicles and significantly higher pregnancy rates in the 5 mg per day arm [69]. Badawy et al. compared 2.5, 5 and 7.5 mg of LTZ per day in unexplained infertility patients. They found a higher number of follicles in the 7.5 mg group but no significant difference in pregnancy rates between the three doses [70]. Additional studies looked at 2.5 mg vs 5 mg of LTZ daily together with recombinant FSH (rFSH) in women with unexplained infertility. No difference in pregnancy rates was detected but lower amounts of rFSH were needed with the 5 mg LTZ arm making it more cost-effective [71].

Three other protocols of LTZ administration that may be interesting have been reported. In a nonrandomised study, a single dose of 20 mg LTZ given on cycle day 3 was compared with daily dose of 2.5 mg for 5 days [72]. The single dose administration was comparable to the 5-day protocol and may have several benefits including ease of use, safety due to more rapid clearance of LTZ than the 5-day treatment regimen, and improved patient compliance. Another study reported by Mitwally et al. suggested that a step-up protocol could be used if multiple follicle ovulation is desired [73]. Finally, in some women who fail to ovulate with the standard dose of LTZ given for 5 days, a 10-day course has been reported to be successful [74].

Nevertheless, with young PCOS patients the risk of overstimulation must be kept in mind regardless of the protocol used, and the risk of multiple and even higher order multiple pregnancies is always present [8, 75]. For example, even though monofollicular ovulation is the rule with LTZ, a sextuplet pregnancy has been reported after un-monitored LTZ use for ovulation induction in a PCOS patient with more than 50 basal antral follicles [85]. Therefore, we recommend cycle monitoring to follow response to treatment and to prevent high order multiple pregnancies with any type of stimulation protocol.

10.7 Side Effects

The main side effects of LTZ use in fertility patients include headaches in 1% and leg cramps in 1% of women and these seem to be idiosyncratic, i.e. unrelated to dose but possibly impacted by vitamin D deficiency [76]. Most of the reported side effects were inferred from studies and observations in breast cancer patients. This population is totally different from the infertility treatment population. The former are usually postmenopausal women while the latter are young healthy patients. The duration of LTZ treatment is very short in ART. Therefore, the long-term side effects are not expected to occur.

10.8 Foetal Safety and Teratogenic Effects

Concerns about safety of LTZ for ovulation induction emerged based on early studies of LTZ exposure during pregnancy in animal models. Exposure during organogenesis in pregnancy resulted in congenital anomalies in rats and rabbits and foetal mortality at increased doses [77, 78]. These studies examined the effect of LTZ during a time period which is not consistent with the use of LTZ in fertility treatment for induction of ovulation. In a more relevant animal model, mice treated for 6 weeks with LTZ and then allowed to conceive 2 weeks after last dose, demonstrated no foetal anomalies [79].

Clinical concern about the safety of LTZ arose during the Annual Meeting of the American Society for Reproductive Medicine (ASRM) 2005 where an abstract presentation described a possible link between LTZ treatment for ovulation induction and new-born congenital cardiac and bone anomalies [80]. The comparison in this study was between a small group of 150 babies delivered after LTZ treatment and a group of 36,050 low risk deliveries conceived spontaneously. The methodological design of this retrospective study was criticised from several aspects. The study did not take into consideration that infertility patients have a higher risk of anomalies regardless of treatment modality. The two groups were not comparable in age (mean age 35 years in the LTZ group compared with 30 years in the controls) nor in other potential risk factors like diabetes and twins, which were more common in the LTZ group. Additionally, many spontaneous pregnancies with foetal anomalies or complications were referred to a high-risk hospital, and not included in the low risk hospital registration. A peer-reviewed manuscript of the abstract presentation was not accepted for publication. Nevertheless, Novartis Pharmaceuticals, the manufacturer of LTZ, published a warning notice to physicians declaring that the use of LTZ was contraindicated in reproductive age women or for fertility treatment.

In the year following the aforementioned presentation, Tulandi et al. [81] published a multicentre Canadian study comparing the neonatal congenital malformation rate in 504 babies conceived after LTZ treatment and in 397 babies conceived after CC use. Major malformations (VSD, oesophageal atresia and cleft palate) were 1.2% in the LTZ group and 3% in the CC group, resulting not significantly different. VSD was more prevalent in new-borns in the CC group 1% vs. the LTZ

group 0.2%. Moreover, the rate of overall cardiac anomalies was significantly higher in the CC group than in LTZ group (1.8% vs. 0.2%, respectively). No difference was observed in regard to minor malformation (e.g. pre-auricular skin tag, congenital ptosis, plagiocephaly) in the CC group (4.8%) vs. the LTZ group (2.4%).

This study by Tulandi et al. [81] was the first to uncover a possible teratogenic effect of CC. This association has some biologic plausibility since the half-life of the zu-clomiphene isomer is about 2 weeks [82] suggesting that the complete clearance of CC from the body may take around 10 weeks (5 half-lives) and does include part of the period of organogenesis in the foetus. Since then, several publications have focused on the safety of CC. One recent publication concerning CC and birth defects was published by Reefhius et al. [83] using data from the Centers for Disease Control National Birth Defects Prevention Study (NBDPS). The authors observed a significant association between CC exposure and the occurrence of anencephaly, Dandy Walker malformation, coarctation of the aorta, esophageal atresia, cloacal exstrophy, craniosynostosis and omphalocele. In addition, this study confirmed the previous findings of Tulandi et al. [81] regarding a significant increased risk of septal heart defects including muscular ventricular septal defect (VSD). Another recent study found an increased association of neural tube defects with CC use that was independent of ART use [84]. However, the latter two findings need to be viewed with caution since the babies in the CC patients were compared to babies from spontaneous pregnancies. Patients with PCOS in whom ovulation induction is indicated may be at increased risk of birth defects because of underlying associated obesity or defects in glucose metabolism.

Our understanding of the pharmacokinetics of LTZ strengthens the safety of this medication for induction of ovulation. Its short half-life (~45 h) ensures that early follicular use (day 3–7 of the cycle) of LTZ eliminates oocyte or embryo exposure to LTZ thereby reducing any theoretical teratogenic effect. To further increase safety, we recommend a pregnancy test prior to LTZ start to reduce the chance of undiagnosed early pregnancy exposure.

10.9 Other Infertility Indications for Aromatase Inhibitors Treatment

10.9.1 Unexplained Infertility

By definition, unexplained infertility is diagnosed when no cause of infertility or subfertility has been determined. Treatment is not targeting a specific problem and one strategy is to increase follicular numbers or to bypass undefined obstacles. LTZ, with its usually monofollicular ovulation, is less effective in treatment of unexplained infertility patients compared to patients with PCOS [85]. Comparative studies between LTZ, CC and gonadotrophin stimulation in unexplained infertility patients showed lower cumulative pregnancy rates after 4 cycles of LTZ or CC compared with gonadotrophins, but at the expense of a 30% multiple pregnancy rate in the gonadotrophin group including high order multiples. In contrast, no high order multiple pregnancy was reported in either the LTZ or CC groups [86].

10.9.2 Breast Cancer and Fertility Preservation

Increasing numbers of breast cancer patients survive the disease and are cured due to effective but nonetheless gonadotoxic chemotherapy [87]. Therefore, fertility preservation counselling is important for these patients. Oocyte or embryo cryopreservation is the most effective method of preserving fertility for these young patients [88]. Two main concerns are predominant in addressing this option: time available before chemotherapy start and serum oestrogen levels during ovarian stimulation. In general, during controlled ovarian stimulation for IVF, oestrogen levels may rise to be 10–20 times the physiologic levels and there is concern that this increase might accelerate the breast cancer growth if the tumour is positive for ER. Combined ovarian stimulation with gonadotrophins and the addition of LTZ to lower serum oestrogen concentrations was initially described for oocyte cryopreservation in breast cancer patients with positive ER [89, 90]. Oktay et al. [91] studied a protocol in which LTZ was initiated at day 2–3 of cycle together with gonadotrophins and both medications were continued until the trigger for follicular maturation. Oestradiol levels throughout stimulation and after ovulation were significantly lower in the LTZ-gonadotrophin protocol compared with the conventional long protocol of stimulation in infertility patients. The number of retrieved oocytes and fertilisation rates were comparable in the two groups. Significantly lower amounts of FSH were needed in the LTZ-gonadotrophin stimulation protocol [70, [91] A more recent publication [92] from the Cornell group compared 220 patients with breast cancer undergoing oocyte retrieval with gonadotrophins and letrozole for oocyte preservation with 451 age matched patients undergoing elective oocyte cryopreservation using gonadotrophins alone. This study observed significantly more total and mature oocytes retrieved in the breast cancer patients with lower oestradiol concentrations compared to the control group. Fifty-six of the breast cancer patients subsequently had frozen embryo transfer with a 32% live birth rate [92].

Multiple other protocols have been assessed in breast cancer patients including a comparison of tamoxifen and LTZ supplementation during gonadotrophin stimulation [93]. LTZ appears to provide a favourable response by achieving multiple desired effects. First, LTZ causes a decrease of oestrogen negative feedback centrally thereby releasing more endogenous FSH and recruitment of more follicles. Second, LTZ provides direct protection of the breast tissue by reduction of oestrogen levels in the breast tissue itself. Third, as mentioned above, LTZ reduces circulating oestrogen during ovarian stimulation [91, 94]. Ovarian stimulation with LTZ-gonadotrophin for IVF has not been shown to increase breast cancer recurrence rate [95].

Random start of follicular stimulation has become more widely used after the demonstration that ovarian follicles develop in a wave pattern, and that these waves of development occur throughout the cycle [96]. Therefore, follicular stimulation can be initiated in any phase of the menstrual cycle. This observation enables physician to shorten the critical period to ovum pick-up, preventing a delay in chemotherapy start time.

10.9.3 Prevention of OHSS

The findings observed in breast cancer patients undergoing ovarian stimulation may contribute to development of new management strategies to prevent OHSS. Whether LTZ might reduce the risk of OHSS by reducing oestrogen levels is controversial [76]. A study in a rat model of OHSS demonstrated that LTZ reduced vascular endothelial growth factor (VEGF) and increased pigment epithelium derived factor (PEDF) with reduced vascular permeability [97]. A recent clinical study demonstrated a dose dependent decrease in the levels of VEGF with increasing doses of LTZ administered in the luteal phase [78]. These findings suggest that LTZ could decrease the risk of OHSS although it is not clear if LTZ has a direct effect on VEGF and PEDF secretion or an indirect effect through a reduction in oestradiol.

Conclusion

Recent level one evidence now points to LTZ as the first line treatment for ovulation induction in women with PCOS. The absence of anti-oestrogenic effects on the endometrium and cervix and the maintenance of normal oestrogen feedback on FSH release resulting in mainly monofollicular ovulation suggest that LTZ may be safer than CC for use by community gynaecologists without ready access to ultrasound monitoring. However, a case report of a sextuplet pregnancy after the use of LTZ alone in a PCOS patient [75] points to the need for at least minimal monitoring when any method of ovulation induction is considered. In unexplained infertility, CC and LTZ appear to have similar pregnancy rates, although the long half-life of CC raises concerns about persistence of the SERM during early pregnancy and the possible association with foetal teratogenicity, specifically cardiac anomalies. From that viewpoint alone, LTZ may be a preferred choice for management of unexplained infertility as well.

References

1. Mitwally MF, Casper RF. Use of an aromatase inhibitor for induction of ovulation in patients with an inadequate response to clomiphene citrate. Fertil Steril. 2001;75:305–9.
2. Kar S. Current evidence supporting "letrozole" for ovulation induction. J Hum Reprod Sci. 2013;6:93–8.
3. Beckmann MW, Findeklee S. Fertility preservation in breast cancer patients by embryo cryopreservation after ovarian stimulation with letrozole and FSH. Strahlenther Onkol. 2015;191:895–6.
4. Goldrat O, et al. Progesterone levels in letrozole associated controlled ovarian stimulation for fertility preservation in breast cancer patients. Hum Reprod. 2015;30:2184–9.
5. Turan V, et al. Safety and feasibility of performing two consecutive ovarian stimulation cycles with the use of letrozole-gonadotropin protocol for fertility preservation in breast cancer patients. Fertil Steril. 2013;100:1681–5.e1.
6. Klement AH, Casper RF. The use of aromatase inhibitors for ovulation induction. Curr Opin Obstet Gynecol. 2015;27:206–9.
7. Palomba S. Aromatase inhibitors for ovulation induction. J Clin Endocrinol Metab. 2015;100:1742–7.

8. Legro RS, et al. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. N Engl J Med. 2014;371:119–29.
9. Haynes BP, et al. The pharmacology of letrozole. J Steroid Biochem Mol Biol. 2003;87:35–45.
10. Ghosh D, Lo J, Egbuta C. Recent progress in the discovery of next generation inhibitors of aromatase from the structure-function perspective. J Med Chem. 2016;59:5131–48.
11. Lipton A, Santen RJ. Proceedings: medical adrenalectomy using aminoglutethimide and dexamethasone in advanced breast cancer. Cancer. 1974;33:503–12.
12. Dowsett M, et al. Effective inhibition by low dose aminoglutethimide of peripheral aromatization in postmenopausal breast cancer patients. Br J Cancer. 1985;52:31–5.
13. Santen RJ, et al. Aminoglutethimide inhibits extraglandular estrogen production in postmenopausal women with breast carcinoma. J Clin Endocrinol Metab. 1978;47:1257–65.
14. Pittman JA, Brown RW. Antithyroid and antiadrenocortical activity of aminoglutethimide. J Clin Endocrinol Metab. 1966;26:1014–6.
15. Burnett RD, Kirk DN. Some observations on the preparation of 2-hydroxy-steroid 4-en-3 ones. J Chem Soc Perkin 1. 1973;17:1830–6.
16. Coombes RC, et al. 4-Hydroxyandrostenedione in treatment of postmenopausal patients with advanced breast cancer. Lancet. 1984;2:1237–9.
17. Dowsett M, et al. Dose-related endocrine effects and pharmacokinetics of oral and intramuscular 4-hydroxyandrostenedione in postmenopausal breast cancer patients. Cancer Res. 1989;49:1306–12.
18. Santen RJ, et al. Potency and specificity of CGS-16949A as an aromatase inhibitor. Endocr Res. 1990;16:77–91.
19. Browne LJ, et al. Fadrozole hydrochloride: a potent, selective, nonsteroidal inhibitor of aromatase for the treatment of estrogen-dependent disease. J Med Chem. 1991;34:725–36.
20. Lo J, et al. Structural basis for the functional roles of critical residues in human cytochrome p450 aromatase. Biochemistry. 2013;52:5821–9.
21. Smith IE, Dowsett M. Aromatase inhibitors in breast cancer. N Engl J Med. 2003;348:2431–42.
22. Brodie AM, Njar VC. Aromatase inhibitors in advanced breast cancer: mechanism of action and clinical implications. J Steroid Biochem Mol Biol. 1998;66:1–10.
23. Goss PE, et al. Exemestane versus anastrozole in postmenopausal women with early breast cancer: NCIC CTG MA.27–a randomized controlled phase III trial. J Clin Oncol. 2013;31:1398–404.
24. Murray J, et al. A randomised study of the effects of letrozole and anastrozole on oestrogen receptor positive breast cancers in postmenopausal women. Breast Cancer Res Treat. 2009;114:495–501.
25. Miller WR. Biology of aromatase inhibitors: pharmacology/endocrinology within the breast. Endocr Relat Cancer. 1999;6:187–95.
26. Gibson LJ, et al. Aromatase inhibitors for treatment of advanced breast cancer in postmenopausal women. Cochrane Database Syst Rev. 2007;1:CD003370.
27. Randall JM, Templeton A. Cervical mucus score and in vitro sperm mucus interaction in spontaneous and clomiphene citrate cycles. Fertil Steril. 1991;56:465–8.
28. Gonen Y, Casper RF. Sonographic determination of a possible adverse effect of clomiphene citrate on endometrial growth. Hum Reprod. 1990;5:670–4.
29. Lipton A, et al. Letrozole (CGS 20267). A phase I study of a new potent oral aromatase inhibitor of breast cancer. Cancer. 1995;75:2132–8.
30. Iveson TJ, et al. Phase I study of the oral nonsteroidal aromatase inhibitor CGS 20267 in postmenopausal patients with advanced breast cancer. Cancer Res. 1993;53:266–70.
31. Shetty G, et al. Effect of estrogen deprivation on the reproductive physiology of male and female primates. J Steroid Biochem Mol Biol. 1997;61:157–66.
32. Kamat A, et al. Mechanisms in tissue-specific regulation of estrogen biosynthesis in humans. Trends Endocrinol Metab. 2002;13:122–8.

33. Naftolin F, et al. The cellular effects of estrogens on neuroendocrine tissues. J Steroid Biochem. 1988;30:195–207.
34. Naftolin F, Romero R. H2-receptor antagonists and sexual differentiation. Gastroenterology. 1984;87:248–9.
35. Mason AJ, et al. Activin B: precursor sequences, genomic structure and in vitro activities. Mol Endocrinol. 1989;3:1352–8.
36. Bao SH, et al. Effects of letrozole and clomiphene citrate on the expression of HOXA10 and integrin alpha v beta 3 in uterine epithelium of rats. Fertil Steril. 2009;91:244–8.
37. Casper RF, Mitwally MF. A historical perspective of aromatase inhibitors for ovulation induction. Fertil Steril. 2012;98:1352–5.
38. Sioufi A, et al. Absolute bioavailability of letrozole in healthy postmenopausal women. Biopharm Drug Dispos. 1997;18:779–89.
39. Sioufi A, et al. Comparative bioavailability of letrozole under fed and fasting conditions in 12 healthy subjects after a 2.5 mg single oral administration. Biopharm Drug Dispos. 1997;18:489–97.
40. Weil S, et al. Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. J Clin Endocrinol Metab. 1999;84:2951–6.
41. Vendola K, et al. Androgens promote oocyte insulin-like growth factor I expression and initiation of follicle development in the primate ovary. Biol Reprod. 1999;61:353–7.
42. Vendola KA, et al. Androgens stimulate early stages of follicular growth in the primate ovary. J Clin Invest. 1998;101:2622–9.
43. Giudice LC. Insulin-like growth factors and ovarian follicular development. Endocr Rev. 1992;13:641–69.
44. Yen SS, Laughlin GA, Morales AJ. Interface between extra- and intraovarian factors in polycystic ovarian syndrome. Ann N Y Acad Sci. 1993;687:98–111.
45. Casper RF. Aromatase inhibitors in ovarian stimulation. J Steroid Biochem Mol Biol. 2007;106:71–5.
46. Laven JS, et al. Anti-Mullerian hormone serum concentrations in normoovulatory and anovulatory women of reproductive age. J Clin Endocrinol Metab. 2004;89:318–23.
47. Stubbs SA, et al. Anti-mullerian hormone protein expression is reduced during the initial stages of follicle development in human polycystic ovaries. J Clin Endocrinol Metab. 2005;90:5536–43.
48. Weenen C, et al. Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod. 2004;10:77–83.
49. Garg D, Tal R. The role of AMH in the pathophysiology of polycystic ovarian syndrome. Reprod Biomed Online. 2016;33:15–28.
50. Pellatt L, et al. Granulosa cell production of anti-Mullerian hormone is increased in polycystic ovaries. J Clin Endocrinol Metab. 2007;92:240–5.
51. Grossman MP, et al. Mullerian-inhibiting substance inhibits cytochrome P450 aromatase activity in human granulosa lutein cell culture. Fertil Steril. 2008;89(5 Suppl):1364–70.
52. Das M, et al. Anti-Mullerian hormone is increased in follicular fluid from unstimulated ovaries in women with polycystic ovary syndrome. Hum Reprod. 2008;23:2122–6.
53. Catteau-Jonard S, et al. Anti-Mullerian hormone, its receptor, FSH receptor, and androgen receptor genes are overexpressed by granulosa cells from stimulated follicles in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2008;93:4456–61.
54. Mumford SL, et al. Baseline AMH level associated with ovulation following ovulation induction in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2016;101:3288–96.
55. Moran LJ, et al. Lifestyle changes in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2011;7:CD007506.
56. Legro RS, et al. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. N Engl J Med. 2007;356:551–66.

57. Nugent D, et al. Gonadotrophin therapy for ovulation induction in subfertility associated with polycystic ovary syndrome. Cochrane Database Syst Rev. 2000;4:CD000410.
58. Homburg R, Levy T, Ben-Rafael Z. A comparative prospective study of conventional regimen with chronic low-dose administration of follicle-stimulating hormone for anovulation associated with polycystic ovary syndrome. Fertil Steril. 1995;63:729–33.
59. Franik S, et al. Aromatase inhibitors for subfertile women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2014:CD010287.
60. Franik S, et al. Aromatase inhibitors for subfertile women with polycystic ovary syndrome: summary of a Cochrane review. Fertil Steril. 2015;103:353–5.
61. Palomba S, Falbo A, Zullo F. Management strategies for ovulation induction in women with polycystic ovary syndrome and known clomifene citrate resistance. Curr Opin Obstet Gynecol. 2009;21:465–73.
62. Parsanezhad ME, et al. Use of dexamethasone and clomiphene citrate in the treatment of clomiphene citrate-resistant patients with polycystic ovary syndrome and normal dehydroepiandrosterone sulfate levels: a prospective, double-blind, placebo-controlled trial. Fertil Steril. 2002;78:1001–4.
63. Kamath MS, et al. Aromatase inhibitors in women with clomiphene citrate resistance: a randomized, double-blind, placebo-controlled trial. Fertil Steril. 2010;94:2857–9.
64. El-Gharib MN, Mahfouz AE, Farahat MA. Comparison of letrozole versus tamoxifen effects in clomiphen citrate resistant women with polycystic ovarian syndrome. J Reprod Infertil. 2015;16:30–5.
65. Abu Hashim H, Shokeir T, Badawy A. Letrozole versus combined metformin and clomiphene citrate for ovulation induction in clomiphene-resistant women with polycystic ovary syndrome: a randomized controlled trial. Fertil Steril. 2010;94:1405–9.
66. Greenblatt EM, Casper RF. Adhesion formation after laparoscopic ovarian cautery for polycystic ovarian syndrome: lack of correlation with pregnancy rate. Fertil Steril. 1993;60:766–70.
67. Abdellah MS. Reproductive outcome after letrozole versus laparoscopic ovarian drilling for clomiphene-resistant polycystic ovary syndrome. Int J Gynaecol Obstet. 2011;113:218–21.
68. Liu W, et al. Randomized controlled trial comparing letrozole with laparoscopic ovarian drilling in women with clomiphene citrate-resistant polycystic ovary syndrome. Exp Ther Med. 2015;10:1297–302.
69. Al-Fadhli R, et al. A randomized trial of superovulation with two different doses of letrozole. Fertil Steril. 2006;85:161–4.
70. Badawy A, Metwally M, Fawzy M. Randomized controlled trial of three doses of letrozole for ovulation induction in patients with unexplained infertility. Reprod Biomed Online. 2007;14:559–62.
71. Noriega-Portella L, et al. Effect of letrozole at 2.5 mg or 5.0 mg/day on ovarian stimulation with gonadotropins in women undergoing intrauterine insemination. Fertil Steril. 2008;90:1818–25.
72. Mitwally MF, Casper RF. Single-dose administration of an aromatase inhibitor for ovarian stimulation. Fertil Steril. 2005;83:229–31.
73. Mitwally MF, et al. Letrozole step-up protocol: a successful superovulation protocol. Fertil Steril. 2008;89:S23–4.
74. Badawy A, et al. Extended letrozole therapy for ovulation induction in clomiphene-resistant women with polycystic ovary syndrome: a novel protocol. Fertil Steril. 2009;92:236–9.
75. Warraich G, Vause TD. First reported case of sextuplets conceived via letrozole for ovulation induction. Fertil Steril. 2015;103:535–6.
76. Arul Vijaya Vani S, et al. Effects of vitamin D and calcium supplementation on side effects profile in patients of breast cancer treated with letrozole. Clin Chim Acta. 2016;459:53–6.
77. Tiboni GM, et al. Effects of the aromatase inhibitor letrozole on in utero development in rats. Hum Reprod. 2008;23:1719–23.

78. Tiboni GM, et al. Impact of estrogen replacement on letrozole-induced embryopathic effects. Hum Reprod. 2009;24:2688–92.
79. Luthra R, et al. Use of letrozole as a chemopreventive agent in aromatase overexpressing transgenic mice. J Steroid Biochem Mol Biol. 2003;86:461–7.
80. Biljan MM, Hemmings R, Brassard N. The outcome of 150 babies following the treatment with letrozole or letrozole and gonadotropins. Fertil Steril. 2005;84:S95.
81. Tulandi T, et al. Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. Fertil Steril. 2006;85:1761–5.
82. Young SL, Opsahl MS, Fritz MA. Serum concentrations of enclomiphene and zuclomiphene across consecutive cycles of clomiphene citrate therapy in anovulatory infertile women. Fertil Steril. 1999;71:639–44.
83. Reefhuis J, et al. Use of clomiphene citrate and birth defects, National Birth Defects Prevention Study, 1997–2005. Hum Reprod. 2011;26:451–7.
84. Benedum CM, et al. Association of Clomiphene and Assisted Reproductive Technologies with the risk of neural tube defects. Am J Epidemiol. 2016;183:977–87.
85. Polyzos NP, et al. Aromatase inhibitors for female infertility: a systematic review of the literature. Reprod Biomed Online. 2009;19:456–71.
86. Diamond MP, et al. Letrozole, gonadotropin, or clomiphene for unexplained infertility. N Engl J Med. 2015;373:1230–40.
87. Oktay K, et al. Fertility preservation success subsequent to concurrent aromatase inhibitor treatment and ovarian stimulation in women with breast cancer. J Clin Oncol. 2015;33:2424–9.
88. Loren AW, et al. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2013;31:2500–10.
89. Siegel R, et al. Cancer statistics, 2014. CA Cancer J Clin. 2014;64:9–29.
90. Anders CK, et al. Breast cancer before age 40 years. Semin Oncol. 2009;36:237–49.
91. Oktay K, et al. Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. J Clin Endocrinol Metab. 2006;91:3885–90.
92. Pereira N, et al. Comparison of ovarian stimulation response in patients with breast cancer undergoing ovarian stimulation with letrozole and gonadotropins to patients undergoing ovarian stimulation with gonadotropins alone for elective cryopreservation of oocytesdagger. Gynecol Endocrinol. 2016;32:823–6.
93. Oktay K, et al. Fertility preservation in breast cancer patients: a prospective controlled comparison of ovarian stimulation with tamoxifen and letrozole for embryo cryopreservation. J Clin Oncol. 2005;23:4347–53.
94. Checa Vizcaino MA, et al. The effects of letrozole on ovarian stimulation for fertility preservation in cancer-affected women. Reprod Biomed Online. 2012;24:606–10.
95. Azim AA, Costantini-Ferrando M, Oktay K. Safety of fertility preservation by ovarian stimulation with letrozole and gonadotropins in patients with breast cancer: a prospective controlled study. J Clin Oncol. 2008;26:2630–5.
96. Baerwald AR, Pierson RA. Endometrial development in association with ovarian follicular waves during the menstrual cycle. Ultrasound Obstet Gynecol. 2004;24:453–60.
97. Sahin N, et al. Comparison of the effects of letrozole and cabergoline on vascular permeability, ovarian diameter, ovarian tissue VEGF levels, and blood PEDF levels, in a rat model of ovarian hyperstimulation syndrome. Arch Gynecol Obstet. 2016;293:1101–6.

Insulin-Sensitising Drugs

11

Stefano Palomba, Angela Falbo,
and Giovanni Battista La Sala

11.1 Introduction

Metformin (1,1-dimethylbuguanidehydrochloride) is a biguanide currently used as an oral antihyperglycemic agent approved by the US Food and Drug Administration (USFDA) to treat type 2 diabetes mellitus (DM) and the insulin-sensitising drug (ISD) most extensively studied in the treatment of infertility in women with polycystic ovary syndrome (PCOS). In fact, about 90% of the papers on ISDs used in PCOS management concern the metformin. To this regard, given the varied risk-benefit ratio of other ISDs, there is insufficient evidence to recommend the use of other ISDs such as thiazolidinediones, d-chiro-inositol and myo-inositol in the treatment of anovulatory PCOS (see also Chap. 16), and metformin remains the main ISD in the management of infertility in PCOS. Thus, our focus will be on metformin in the treatment of infertility associated with PCOS.

In 1994, Velasquez et al. [1] first evaluated the effects of metformin administration in 26 obese anovulatory PCOS patients, relying on the role of insulin resistance in the pathogenesis of PCOS. After 6 months of 1500 mg/d of metformin, they reported a reduction in androgen levels and body weight, and a restoration of regular ovulatory cycles in these patients [1]. During the years, the drug has been extensively studied, and, to date, it is widely used by gynaecologists and endocrinologists for the treatment of ovulatory disorders in women with PCOS as off-label drug [2] because neither in Europe nor in the United States (US) metformin is approved for the treatment of PCOS patients.

S. Palomba (✉) • A. Falbo
Unit of Gynaecology and Obstetrics, IRCCS–Arcispedale Santa Maria Nuova,
Viale Risorgimento 80, 42123 Reggio Emilia, Italy
e-mail: stefanopalomba@tin.it

G.B. La Sala
Unit of Gynaecology and Obstetrics, IRCCS–Arcispedale Santa Maria Nuova,
Viale Risorgimento 80, 42123 Reggio Emilia, Italy

University of Modena and Reggio Emilia, Via Università, 4, 41121 Modena, Italy

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_11

This chapter will describe the general pharmacology, regimes and side effects of the administration of metformin and its specific uses for managing infertile women with PCOS.

11.2 Pharmacology

11.2.1 Pharmacokinetics

Pharmacokinetics pathway of metformin is attributable to a two-compartment open model with first-order absorption [3, 4]. Specifically, metformin has an incomplete gastrointestinal absorption ranging from 20% to 30% [3, 4]. Absorption is dose dependent, it is complete within 6 h from administration and is slower than the elimination, determining the drug's disposal rate [3, 4]. The bioavailability of metformin is limited to 50–60% because the amount available may result from presystemic clearance or binding to the intestinal wall [3, 4].

After the absorption of 1.5 g of metformin, a linear pharmacokinetics of metformin was reported in both diabetic and nondiabetic subjects [3, 4]. Once absorbed, it is rapidly accumulated in the oesophagus, stomach, duodenum, salivary glands and kidneys. It is transported by at least two organic cation transporters (OCTs), OCT1 and OCT2, which are saturable and genetically influenced by polymorphisms [3, 4], and is not metabolised but is excreted by the kidney with a mean 4- to 8-h half-life in healthy volunteers [3, 4]. Ranges of values for kidney and total clearance are reported to be 20.1–36.9 and 26.5–42.4 L/h, respectively [3, 4].

Metformin freely passes the placenta by an OCT bidirectionally, with a higher transfer rate from the foetal to the maternal compartment [3, 4], resulting in the exposure of the foetus to metformin therapeutic concentrations [3, 4]. Furthermore, no effect on human placental glucose uptake or transport has been demonstrated [5]. The concentrations of metformin in breast milk are generally low.

Type 2 DM and different oral preparations have no effect on metformin disposition [3, 4]. On the contrary, the food and several compounds, such as guar gum, glucosidase inhibitor acarbose and histamine H2-receptor antagonist cimetidine, significantly influence metformin pharmacokinetics. Excretion is prolonged in patients with renal impairment and correlates with creatinine clearance [3, 4].

11.2.2 Pharmacodynamics

Several data regarding metformin's pharmacodynamics were obtained in patients with type 2 DM, and they could be translated in PCOS patients. Metformin is an antihyperglycemic agent which improves glucose tolerance in patients with type 2 DM, lowering both basal and postprandial plasma glucose. Its pharmacologic mechanism of action is different from other classes of oral antihyperglycemic agents.

Metformin decreases hepatic glucose production by 9–30% in patients with type 2 DM [4, 6]. Experimental studies [7] evaluating glucose production from collagenase-isolated hepatocytes of starved rats demonstrated that metformin potentiates the antigluconeogenic effect of insulin by enhancing the suppression of gluconeogenesis and by reducing the glucagon-stimulating gluconeogenesis. However, the nature of the mechanism of metformin action on hepatic glucose production remains unclear. Data from in vitro studies suggest several effects of metformin on the reduction of hepatic gluconeogenesis through short- (metabolic) and long-term (gene expression) effects [4]. In addition, metformin decreases intestinal absorption of glucose and decreases free fatty acid (FFA) oxidation, contributing in reducing gluconeogenesis [4, 8]. An accelerated FFA oxidation promotes hepatic gluconeogenesis by providing acetyl-coenzyme A, adenosine-5-triphosphate (ATP) and a reduction in equivalents [4, 8] and reduces glucose utilisation in peripheral tissues secondarily to an inhibition of pyruvate dehydrogenase activity [4, 8]. On the other hand, the decreased FFA oxidation due to metformin treatment [4, 8] decreases hepatic gluconeogenesis and increases glucose uptake and oxidation in skeletal muscle [4, 8], improving insulin sensitivity.

Metformin improves insulin sensitivity by increasing peripheral glucose uptake and utilisation. Studies on human muscle cell cultures [9] and rat adipocytes [10] demonstrated that metformin increases glucose uptake through the glucose transport system. In fact, it facilitates the translocation of glucose transporters (GLUTs) from intracellular sites to the plasma membrane. This mechanism has been demonstrated also in PCOS women [4, 9]. Experimental data demonstrate also that metformin activate the 5-AMP-activated protein kinase (AMPK) pathway [11–13]. AMPK is a pleiotropic serine/threonine kinase that acts as a fuel gauge in regulating energy metabolism, especially under stress conditions where biosynthetic pathways are blocked by phosphorylation of downstream AMPK substrates. In particular, AMPK activation restores cellular adenosine-5-triphosphate (ATP) level by switching on the catabolic pathway and switching off catabolic pathways [11–13]. Finally, to support the hypothesis on long-term clinical effects of metformin, in vitro experiments on cultured starved rat hepatocytes indicated that metformin can regulate the expression of specific genes encoding regulatory proteins of the phosphoenolpyruvate/pyruvate cycle in an insulin-independent manner [4].

In obese women with PCOS, metformin reduces the fasting and glucose-stimulated insulin levels and decreases ovarian cytochrome P450c17 activity, reducing the serum-free testosterone concentration [14]. These results were also confirmed in lean PCOS patients [15]. The reduction in insulin levels after metformin treatment in PCOS patients is associated with an increase in insulin growth factor (IGF)-binding protein (BP)-1 and a decrease in the IGF-I/IGFBP-1 ratio. IGF-I stimulates oestrogen production by granulosa cells [16] and acts synergistically with follicle-stimulating hormone (FSH) and luteinising hormone (LH) in controlling granulosa cell aromatase levels. Thus, by reducing plasma insulin levels and IGF-I availability to the ovaries, metformin may modify the hyperandrogenic intrafollicular milieu recognised in PCOS.

Moreover, metformin may inhibit ovarian gluconeogenesis through a direct effect, thus reducing ovarian steroidogenesis, specifically, androgen production [14]. Similarly, metformin has a possible inhibiting effect on adrenal androgen production, too. On the other hand, the effect of metformin on hyperandrogenism could also be due to a direct effect of metformin on LH secretion. In particular, metformin modulates LH secretion, decreasing LH pulse amplitude but not pulse frequency [4]. This effect seems to be mediated by AMPK pathway at the hypothalamic level. In fact, in rat model, metformin increases the AMPK activation by phosphorylation at Thr172 in GnRH neurones, and, thus, the modulation of gonadotrophin-releasing hormone (GnRH) releases [4]. Lastly, some metabolic hormones, such as adipokines (leptin, resistin, adiponectin) and ghrelin, which are involved in the control of the reproductive functions at the hypothalamus-pituitary-gonadal axis level and of several processes of tumour genesis, might act through AMPK signalling [4].

11.3 Regimens

Therapeutic regimens of metformin administration are not well standardised in clinical practice, and heterogeneous protocols were used in the various studies available in literature.

Metformin is available as oral caps, in two formulations, i.e. immediate release and extended release. Metformin at immediate release is available as 500-, 850- and 1000-mg tablets, while metformin at extended release is available as 1000- and 2000-mg tablets.

The dose of metformin used in clinical practice ranges from 1500 to 2550 mg/d. Although a dose-finding study [17] showed that 2000 mg/d of metformin had maximal benefit in lowering plasma glucose and glycated haemoglobin in patients with DM, no well-done dose-finding study is to date available for PCOS women, probably because of the complexity of clinical endpoints.

To minimise the gastrointestinal drug-related adverse effects, it has been suggested to assume metformin on an empty stomach, starting with a low dosage and gradually increasing over a period of 4–6 weeks. Classically, it was suggested to take immediate-release metformin initially at a low dose at meals, beginning with 500 mg at dinner for 3–4 days, and then increasing by 500 mg every 3–4 days up to a maximal dosage of 1000 mg twice daily [18, 19]. Extended-release metformin is usually taken with the evening meal, and the only suggestion to minimise potential adverse effects is to divide the tablets into two administrations.

Almost all published studies including PCOS patients used metformin in immediate-release preparation; however, no clear data regarding the correct formulation of metformin in infertile PCOS patients is to date available. Moreover, several studies regarding metformin for ovulation induction in PCOS infertile patients used 1500–1700 mg/d, which could be a suboptimal dosage. Furthermore, several variables should be studied to set the optimal dose of metformin. For example, recent data suggest that metformin is more effective in insulin-resistant PCOS patients with low body mass index (BMI), thus metformin dose should probably be adjusted

according to the patient's BMI and insulin resistance [20]. Unfortunately, no model is currently validated to calculate the right dose according to these characteristics.

Finally, also the length of metformin treatment in PCOS patients is not standardised. In fact, it is still unknown whether metformin administration should be considered as a symptomatic treatment or as a curative therapy, consecutively; it is not clear for how long metformin should be administrated and how long metformin effects are maintained after its suspension. At a metabolic level, our previous data [21] on a non-insulin-resistant PCOS population showed that treatment suspension is related to a quick reversion of its beneficial effect on peripheral insulin sensitivity. Conversely, a slight but significant worsening of the insulin resistance, hyperandrogenism, and menstrual cyclicity can be observed after metformin suspension.

From a clinical point of view and based on our clinical experience, we suggest that metformin should be administered in a slow and increasing manner up to a maximal tolerated dosage [20].

11.4 Drug Safety

The safety profile of metformin is well known. In fact, metformin is used worldwide to treat type 2 DM, and, during the last years, it has been used on women with PCOS.

Metformin is generally a well-tolerated drug. A meta-analysis comparing metformin, clomiphene citrate (CC) or both for anovulatory infertility in therapy-naïve PCOS women [22] showed a similar effect on the discontinuation rate for adverse events [odds ratio (OR) 0.71, 95% confidence interval (CI) 0.22–2.25] with homogeneous data [22]. On the other hand, no significant effect of metformin on discontinuation rate for adverse events (OR 0.23, 95%CI 0.04–1.24) was observed when studies comparing the combination of metformin plus CC vs. CC alone were pooled, even if a significant heterogeneity was detected [22].

Gastrointestinal symptoms are the most frequent drug-related adverse events occurring in about 30% of patients taking metformin, limiting the compliance to treatment. A significant increase of nausea, vomiting and gastrointestinal distress was reported in women with PCOS under metformin.

Meta-analytic data [22] regarding metformin side effects from studies using metformin at immediate-release formulation with a treatment duration ranging from 6 weeks [23] to 12 week or more [24–26] reported a significantly higher incidence of nausea or vomiting (OR 3.84, 95%CI 1.07–13.81) and other gastrointestinal disturbances (OR 4.40, 95%CI 1.82–10.66). Further randomised controlled studies (RCTs) reported a rate of adverse events ranging from 7.9% [27] to 22.2% [28] by using metformin at immediate release.

The rate of gastrointestinal side effects seems to be lower with the use of the extended-release formulation [29], but clear data are still unknown because the studies in which this kind of formulation is used are very few.

Serious adverse events of metformin treatment are rare. Metformin toxicity is manifested at a concentration of 100 g/mL or higher, but in vivo data showed plasma

levels of metformin less than 5 g/mL, even at maximum dosage. Lactic acidosis is a rare but severe complication of metformin administration reported in 5.1 cases per 100,000 patient-years, with a mortality of 50% [30]. In almost all cases, lactic acidosis was reported in patients who received metformin due to type 2 DM, whereas no case of lactic acidosis in women receiving metformin for PCOS was currently described. This risk increases in patients with hepatic or renal impairment, cardiac or respiratory insufficiency, severe infection or alcoholism, conditions that are, in themselves, associated with hypoxia and lactic acidosis [31, 32]. Further very rare serious side effects were described under metformin administration, never in PCOS patients.

Pregnancy outcomes in PCOS patients receiving metformin during pregnancy were studied [33, 34]. To date, metformin is still found in the B classification for US Food Drug Administration (FDA) pregnancy category, i.e. no teratogenic effect was demonstrated in animal models, but human safety studies are not adequate. However, a wide clinical trial confirmed that metformin alone or with insulin addition was as safe as insulin alone in patients with gestational DM [35]. No teratogenic effects or adverse foetal outcomes were actually reported from metformin in pregnant women with type 2 DM or gestational DM [36], even if treatment started after pregnancy had begun. Two meta-analyses on metformin safety did not find any evidence for adverse pregnancy outcome in women undergoing treatment with metformin [37, 38].

Preliminary data on the use of metformin during pregnancy in patients with PCOS confirmed the safety profile of metformin [39]. Despite these safety data, in clinical practice, metformin is usually discontinued during pregnancy in women with PCOS who conceived while receiving the drug.

11.5 Patients Selection

Several studies [4] demonstrate that metformin beneficial effects vary according to the clinical characteristics of the patients. Thus, the personalisation of therapy with a right selection of the patients could be a key point to optimise the metformin's therapy, improving its efficacy and its safety profile [40]. However, this principle becomes much more complex when applied to women with PCOS due to the heterogeneity of the clinical presentation of the syndrome.

To date, even if the use of metformin to treat ovulatory disorders in PCOS women is widespread, few guidelines regarding the selection of patients are available. In particular, the Androgen Excess and PCOS Society (AEPS) [41] suggests that metformin could be used to treat and to prevent progression to impaired glucose tolerance (IGT) in PCOS patients, and the American Association of Clinical Endocrinologists [42] recommends metformin as an initial intervention in overweight and obese patients with PCOS.

At the moment, the European Society of Human Reproduction and Embryology (ESHRE)/American Society of Reproductive Medicine (ASRM)-sponsored PCOS Consensus Workshop Group [43] concluded that ISDs should not be used as first choice agents in ovulation induction of women with PCOS, but they should be restricted to those patients with IGT.

11.6 Efficacy Data

11.6.1 Metformin Administration to Prevent an Infertility Diagnosis

From the 1990s until now, several studies that evaluate the efficacy of metformin on menstrual/ovulatory disorders in women with PCOS, before a definite diagnosis of infertility, have been published.

Several systematic reviews with meta-analysis [44–49] are available about this issue, and unanimous conclusions have been drawn. In particular, metformin monotherapy represents a safe and valid therapeutic option for the improvement of ovulation in PCOS patients [44–49]. Metformin is more effective than placebo or no treatment in the restoration of normal menstrual cycles and in inducing ovulatory cycles in oligomenorrheic PCOS patients (OR 3.88, 95%CI 2.25–6.69; [45], and OR 1.50, 95%CI 1.13–1.99 [45]). However, no benefit from metformin administration was found in terms of pregnancies (OR 2.76, 95%CI 0.85–8.98 [44], and OR 1.07, 95%CI 0.20–5.74 [45]), clinical pregnancies (OR 3.3, 95%CI 0.92–11) [48] and live births (OR 1.00, 95%CI 0.13–7.79 [45]). A more recent meta-analysis of RCTs [49] confirmed the beneficial effects of metformin on metformin over placebo on ovulation rate (OR 2.94, 95%CI 1.43–6.02), whereas any effect on pregnancy (OR 1.56, 95%CI 0.74–3.33) or live births (OR 0.44, 95%CI, 0.03–5.88) was reported.

11.6.2 Metformin Administration as First-Line Treatment in Anovulatory Infertility

Several studies aimed to define the role of metformin as first-line drug for anovulatory infertility in PCOS patients were published. Most of them compared metformin, alone or in combination to CC (see also Chap. 9).

Meta-analyses [22, 48, 50–52] available on this issue show contrasting data probably due to the great heterogeneity in the protocols used and in the populations studied. First meta-analytic data [48] evaluating the efficacy of metformin, CC or both drugs in therapy-naïve PCOS patients demonstrated no significant benefit from metformin administration over CC as first-line therapy in PCOS patients in terms of clinical pregnancy (OR 0.88, 95%CI 0.19–4.1) and live birth (OR 0.96, 95%CI 0.11–8.2) rates. Furthermore, these results were biased from many factors, i.e. the metformin exposure as pretreatment and the use of a fixed model for analysing heterogeneous data [53]. Successively, a systematic review with meta-analysis [22], including four well-selected head-to-head randomised controlled studies (RCTs) [27, 28, 54, 55], was published to clarify the efficacy of CC and metformin, alone or in combination, as a first-step approach in treating anovulatory infertility in PCOS patients [22]. Interestingly, the heterogeneity among the studies included in the analysis was confirmed. Thus, the use of random model demonstrated no significant difference between metformin and CC in terms of live birth (OR 1.17, 95%CI 0.16–8.61), pregnancy (OR 1.22, 95%CI 0.23–6.55) and ovulation (OR 1.55, 95%CI 0.77–5.99) rates

[22]. The lack of difference in live birth (OR 0.83, 95%CI 0.22–3.24) and pregnancy (OR 0.91, 95%CI 0.35–2.35) rates was also detected by Johnson [50]. In that meta-analysis were included only data from nonobese patients with PCOS, considering that in New Zealand, as well as in other states, obese patients are unable to access assisted conception [50].

In order to avoid heterogeneous results, Tang et al. [56] evaluated the effect of metformin administration in therapy-naïve infertile patients with PCOS with a meta-analysis subgrouping studies according to obesity/nonobesity. Data from two, five and four RCTs for the evaluation of the pooled live birth, pregnancy and ovulation rates, respectively, were analysed. In nonobese patients, still a high heterogeneity was detected regarding the effect of metformin (compared with CC) on live birth with a risk between two RCTs ranging from an OR of 4.94 (95%CI 1.99–12.26) to an OR of 0.34 (95%CI 0.13–0.91) [56]. In obese patients, a significant benefit of CC over metformin was observed in terms of live birth rate (OR 0.30, 95%CI 0.17–0.52). Data on the clinical pregnancy rates under metformin resulted higher (OR 1.94, 95%CI 1.19–3.16) and lower (OR 0.34, 95%CI 0.21–0.55) than CC, respectively, in nonobese and obese patients [56]. The ovulation rate resulted not different between metformin and CC in nonobese patients (OR 0.87, 95%CI 0.60–1.26), whereas it was better under CC in obese subjects (OR 0.43, 95%CI 0.36–0.51) [56].

Even if Siebert et al. [51] confirmed that metformin is related to a significantly lower live birth rate when compared with CC (OR 0.48, 95%CI 0.31–0.73) after the data synthesis of 14 prospective studies, a further systematic review and meta-analysis [52] demonstrated no difference between metformin and CC in terms of ovulation, pregnancy, live birth, miscarriage and multiple pregnancy rates when the analysis was restricted to women with PCOS and a BMI lower than 32 kg/m^2 [52]. Moreover, the authors concluded that a lack of superiority of one treatment should not be considered as evidence for equivalence, thus caution should be exercised when prescribing metformin as first-line pharmacological therapy in nonobese PCOS women.

In conclusion, owing to the lack of evidence, metformin should not be considered as primary treatment for PCOS-related infertility, and methodologically rigorous trials are required to determine whether there is a difference in effectiveness between metformin and placebo (or no treatment) or between metformin and CC [57].

Considering the insulin-sensitising action of metformin, several authors hypothesised that the addition of metformin to CC could improve the efficacy of CC alone in therapy-naïve infertile PCOS patients. In the meta-analysis by Moll et al. [48] on seven RCTs comparing the combination CC and metformin with CC, a significantly higher clinical pregnancy rate (OR 1.9, 95% CI 1.2–3.3) was observed in patients treated with metformin plus CC compared with those treated with CC alone, although a significant heterogeneity in treatment effect across the trials included in the meta-analysis was reported. Furthermore, no significant benefit on the live birth rate of the combined therapy (OR 1.0, 95%CI 0.82–1.3), and no significant heterogeneity in treatment effect were reported [48].

By using stricter criteria and updating of data found in literature, three head-to-head RCTs [28, 54, 55] comparing reproductive efficacy of metformin plus CC

combination vs. CC monotherapy in therapy-naïve PCOS patients were available. After meta-analysis [22] of these three studies, metformin plus CC combination was shown to be no more effective than CC in terms of ovulation (OR 0.84, 95%CI 0.60–1.18), pregnancy (OR 0.85, 95%CI 0.62–1.15) and live birth (OR 0.99, 95%CI 0.70–1.40) rates. Of note, no significant heterogeneity was detected for all three parameters. However, a further meta-analysis [49] showed a significant benefit of metformin when added to CC in CC-naïve PCOS patients on clinical pregnancy (RR 1.70, 95%CI 0.99–2.94) and ovulation (RR 3.84, 95%CI 1.38–10.68) rates, whereas no data on live birth rate was provided. More recently, Siebert et al. [51] published a further meta-analysis aimed to evaluate the efficacy of metformin plus CC treatment in CC-naïve patients including 14 prospective clinical trials. Metformin plus CC increased ovulation (OR 1.6, 95%CI 1.2–2.1) and pregnancy (OR 1.3, 95%CI 1.0–1.6) rates, but no effect on live birth rate (OR 1.1, 95%CI 0.8–1.5) was detected [51].

An interesting multicentre double-blind placebo-controlled RCT [58] was recently published in order to assess the efficacy of several strategies for PCOS-related anovulatory infertility [58]. In particular, 320 women with PCOS and anovulatory infertility were randomised to metformin or placebo; after 3 months of treatment, another appropriate infertility drug was combined if necessary. Metformin improved pregnancy (53.6 vs. 40.4%) and live birth rates (41.9 vs. 28.8%) rates. Moreover, cox regression analysis showed that metformin plus standard infertility treatment increased the chance of pregnancy 1.6 times (hazard rate 1.6, 95%CI 1.13–2.27) [58].

In conclusion, on the basis of actual evidences from the literature, in anovulatory infertile therapy-naïve PCOS patients, the combined approach of metformin plus CC is not better than CC alone, and the quality of life in women with PCOS treated with CC plus metformin was even significantly lower than in women treated with CC plus placebo [59]. Moreover, there are insufficient data to determine the optimal duration of pretreatment with metformin before the initiation of CC for ovulation induction in infertile women with PCOS [60]. On the other hand, the choice between CC and metformin as first-step treatment should be drawn considering also contingent circumstances because of the lack of clear evidence.

11.6.3 Metformin Administration as Second-Line Treatment and/or in CC-Resistant Patients

Several studies evaluated the efficacy of metformin as second-line approach in the treatment of anovulatory infertility in PCOS patients. In particular, metformin was used as single agent (treatment), combined agent (co-treatment) and/or before other treatments (pretreatment).

11.6.3.1 Metformin Treatment

Few studies [24, 61, 62] addressed the potential role of metformin as a single agent in CC-resistant patients. The first study [24] on 20 infertile CC-resistant patients showed no benefit of metformin over placebo in terms of ovulation, pregnancy and

live birth rates. A successive RCT [61] compared metformin as single treatment with laparoscopic ovarian diathermy (LOD) in 120 CC-resistant PCOS patients. No difference between metformin and LOD was found in the ovulation rate (54.8 vs. 53.2%, respectively), whereas metformin was more effective than LOD in terms of pregnancy (21.8 vs. 13.4%, respectively) and live birth (86.0 vs. 64.5%) rates. Finally, metformin was about 20-fold less expensive than LOD [61]. A successive meta-analysis [48] confirmed the beneficial effects of metformin over LOD in live birth rate (OR 1.6, 95%CI 1.1–2.5), whereas no evidence of difference in clinical pregnancy rate (OR 1.3, 95%CI 0.96–1.7) was obtained.

11.6.3.2 Metformin Co-treatment

The potential effects of metformin as second-line treatment and/or in CC-resistant patients were assessed in PCOS patients receiving other treatments, i.e. CC, aromatase inhibitors and surgical ovulation induction. For more details, please see also Chaps. 9, 10, and 15, respectively.

With regard to metformin-CC co-treatment, two meta-analyses [45, 46] agreed in demonstrating a significant benefit of metformin co-administration in comparison with CC alone, even if a significant heterogeneity was observed between studies. In particular, in CC-resistant PCOS women, metformin-CC co-treatment was more effective than CC alone in terms of ovulation (OR 4.41, 95%CI 2.37–8.22 [44], and OR 3.04, 95%CI 1.77–5.24 [45]) and pregnancy (OR 4.40, 95%CI 1.96–9.85 [44], and OR 3.65, 95%CI 1.11–11.99 [45]) rates.

A successive meta-analysis [47], designed to assess metformin co-administration as a second-step approach for CC-resistant PCOS patients, confirmed the beneficial effect of metformin addition to CC than CC alone in inducing ovulation (OR 6.82, 95%CI 3.59–12.96), even if a significant heterogeneity was demonstrated across studies, whereas no data were provided regarding its effect on pregnancy and live birth rates. Successively, data on the effect of metformin-CC co-administration were provided by Moll et al. [48] in a meta-analysis of RCTs, demonstrating the superiority of the combined therapy than CC alone in terms of clinical pregnancy (OR 5.6, 95%CI 2.3–13) and live birth (OR 6.4, 95%CI, 1.2–34; $P = 0.03$) rates without significant heterogeneity in treatment effect across trials.

More recently, a meta-analysis of selected placebo-controlled RCTs [49] showed higher ovulation (OR, 4.39; 95%CI, 1.94–9.96) and pregnancy (OR, 2.67; 95%CI, 1.45–4.94) rates in PCOS patients receiving CC, even if heterogeneity across studies were detected. On the contrary, metformin did not have any effect on live births (OR, 1.74; 95%CI, 0.79–3.86), and no significant heterogeneity was observed. Moreover, the sub-analysis of data according to CC resistance, obesity and duration of treatment showed that metformin is more effective than placebo in PCOS patients treated for short periods and not CC resistant, whereas the benefits of metformin-CC combination vs. CC were significantly higher in CC-resistant and obese PCOS patients [49]. Finally, new data [63, 64] confirm that the clinical pregnancy rate is improved when adding metformin to CC in women with CC resistance, in both obese and nonobese patients (OR 1.59, 95%CI 1.25–2.02), although the addition of metformin to CC did not improve live birth rates (OR 1.21, 95%CI 0.91–1.61).

The crucial point to be considered in data regarding metformin-CC combination is the length of metformin administration. In fact, by a physiological point of view, the effectiveness of metformin should be optimal after at least 3 months of its administration, whereas short-course metformin could be a suboptimal pretreatment period before beginning CC [65]. Unfortunately, to the present, there are insufficient data to determine whether long-course metformin pretreatment, before the initiation of CC for ovulatory infertility treatment, is more effective than short-course pretreatment [66].

Metformin-CC co-treatment was demonstrated to be more effective also than surgical ovulation induction by LOD in CC-resistant PCOS patients with anovulatory infertility [67]. In particular, metformin plus CC association was related to higher ovulation rates than LOD, even if no difference in the rates of pregnancies, live births and miscarriages were detected between two approaches [67]. In one trial [62], 42 CC-resistant PCOS patients were randomised to LOD followed by metformin or LOD alone. Metformin addition to LOD resulted more effective in terms of ovulations (86.1 vs. 44.6%) and pregnancies (47.6 vs. 19.1%). Furthermore, a successive meta-analysis [48] demonstrated no significant benefit in clinical pregnancy rate (OR 2.3, 95%CI 0.82–6.2) or live birth rate (OR 1.3, 95%CI 0.39–4.0) for the metformin administration after LOD.

The combination of metformin plus letrozole, the most studied aromatase inhibitor for ovulation induction in PCOS patients, vs. metformin plus CC in CC-resistant PCOS patients was evaluated in a RCT [68]. Serum oestradiol (E_2) levels and E_2 levels per mature follicle were significantly higher in CC patients without differences in mature follicles, ovulation and pregnancy rates [68]. However, endometrial thickness and full-term pregnancies were significantly higher in patients treated with metformin plus letrozole [68].

11.6.3.3 Metformin Pretreatment

Several RCTs [69–74] evaluated the efficacy of metformin pretreatment before CC in CC-resistant PCOS patients. Studies were very heterogeneous and were performed on small populations. Data obtained are contrasting; however, most of them [69, 71, 72] seemed to suggest that metformin pretreatment improves the efficacy of CC in PCOS patients with CC resistance. These findings could be explained with the insulin-sensitising action of metformin that hypothetically facilitates the induction of ovulation by using CC in PCOS patients previously resistant to CC ovulation induction [75].

11.6.3.4 Metformin in Patients Who Receive Gonadotrophins

Metformin was also proposed in PCOS patients who received gonadotrophins for inducing mono-ovulatory cycles or multiple follicular development in in vitro fertilisation (IVF) cycles (see also Chaps. 12 and 19). Although the exact mechanism by which metformin could exert its beneficial action during gonadotrophin stimulation remains unknown, hypothetically, metformin could act on the regulation of ovarian response to exogenous gonadotrophins improving insulin resistance. In fact, a reduction in serum testosterone and insulin levels in follicular fluid was observed after metformin treatment [76]. Thus, the improvement of the hyperinsulinemic and

hyperandrogenic ovarian environment might be crucial for a normal folliculogenesis, homogeneous development and responsiveness of follicles and atresia of the small cohort of follicles.

Metformin in Patients Who Receive Gonadotrophins for Mono-ovulatory Cycles

The first meta-analysis regarding the effects of metformin in patients who received gonadotrophins for controlled ovarian stimulation (COS) was published in 2006 by Costello et al. [77]. Metformin was not effective in improving clinical reproductive outcomes [77]. In particular, metformin did not improve ovulation (90% vs. 73.3%; OR 3.27, 95%CI 0.31–34.72) and pregnancy (28 vs.10%; OR 3.46, 95%CI 0.98–12.2) rates during COS with gonadotrophins, whereas no RCT reporting live births was identified. On the other hand, by the meta-analysis of secondary endpoints, metformin seemed to improve the ovarian responsiveness to gonadotrophins [77]. In fact, a significant reduction in the ovarian stimulation length [weighted mean duration (WMD) -4.14 days, 95%CI −6.36 to −1.93] and in the total dose of gonadotrophins used (WMD -425.05 IU, 95%CI -507.08 to −343.03) was reported by using metformin, even if a significant heterogeneity was found between pooled studies. Finally, no RCT reporting ovarian hyperstimulation syndrome (OHSS) as an outcome measure was identified [77].

Successively, two RCTs [78, 79] evaluating whether metformin changes ovarian responsiveness in COS cycles were published. The first RCT [78] on 70 non-obese insulin-resistant PCOS patients who received a low-dose step-up gonadotrophin stimulation protocol followed by timed intercourse or intrauterine insemination demonstrated a significant effect of metformin in increasing the rate of mono-ovulatory cycles and in reducing those of cancelled cycles. Furthermore, no effect of metformin pretreatment and co-administration was confirmed in ovulation, cycle cancellation, pregnancy, abortion, live births, multiple pregnancies or OHSS. The second study [79] showed that metformin improved the endocrine profile in insulin-resistant PCOS patients receiving gonadotrophins in a step-up protocol and confirmed that it promoted the mono-follicular development during COS cycles.

Finally, a more recent meta-analysis [48] on four RCTs demonstrated a significantly higher clinical pregnancy rate when metformin was added to gonadotrophins than with gonadotrophins alone (OR 1.7, 95%CI 1.1–2.8), whereas no significant benefit on live birth rate was reported (OR 1.6, 95%CI 1.0–2.9). No heterogeneity in treatment effect across trials was reported for either pregnancy or live birth rate. In addition, metformin was demonstrated to be effective in reducing multiple pregnancies (OR 0.26, 95%CI 0.07–0.96) but not OHSS (OR 0.59, 95%CI 0.17–2.1). The lack of an effect of metformin on this endpoint was probably due to the low incidence of OHSS during ovarian stimulation with low-dose step-up gonadotrophin protocols [80].

In conclusion, in patients who received gonadotrophins as treatment for anovulation, metformin addition reduces the duration of gonadotrophins administration and

the doses of gonadotrophins required and increases the rate of mono-ovulations, reducing the risk of cancelled cycles.

Metformin in Patients Who Receive Gonadotrophins in Multiple Ovulatory Cycles for IVF Procedures

On the basis of a retrospective evaluation utilising the results of a web-based survey [81], in clinical practice, metformin is used worldwide as an adjunct to standard IVF protocols, even if there is much variation in its use and the majority of centres report the lack of evidence supporting its use. On the other hand, data from the literature seem to be still inconclusive, suggesting that metformin do not improve the efficacy of gonadotrophins in IVF cycles. In particular, a meta-analysis [82] on ten RCTs assessed the effects of metformin administration in infertile patients with PCOS who receive gonadotrophins for IVF and intracytoplasmic sperm injection (ICSI) cycles. Metformin had no clinical effect on rates of pregnancy (OR 1.20, 95%CI 0.90–1.61) or live birth (OR 1.69, 95%CI 0.85–3.34). However, it reduced the risk of OHSS (OR 0.27, 95%CI 0.16–0.46) and improved the rates of miscarriage (OR 0.50, 95%CI 0.30–0.83) and implantation (OR 1.42, 95%CI 1.24–2.75) [82]. Finally, a more recent meta-analysis [82] confirmed that metformin did not affect live birth rate (OR 1.39, 95%CI 0.81–2.40) but significantly reduced the OHSS incidence (OR 0.29, 95%CI 0.18–0.49). On the other hand, metformin significantly increased pregnancy rate (OR 1.52, 95%CI 1.07–2.15) [82].

In conclusion, evidence from the literature agreed that in infertile patients with PCOS treated with gonadotrophins for IVF/ICSI cycles, metformin reduces the risk of OHSS, whereas data on live birth and pregnancy are still inconclusive. Further RCTs are needed to assess the reproductive effect of metformin in young well-selected patients with PCOS and specific phenotypes and features.

Conclusion

Metformin is the most studied ISD in the treatment of infertility in PCOS women. However, its use is off-label. The knowledge regarding its effects and regimens of administration in PCOS patients is still incomplete, thus only weak recommendations can be made.

Metformin could be used alone to improve ovulation rate and pregnancy rate in young women with PCOS who are anovulatory and are infertile with no other infertility factors. However, its efficacy in therapy-naïve infertile PCOS patients is lower than CC. Metformin-CC co-treatment could improve fertility outcomes in infertile CC-resistant patients with PCOS with no other infertility factors.

In patients who received gonadotrophins as treatment for anovulation, metformin addition reduces the duration of the treatment and the doses required and increases the rate of mono-ovulations, reducing the risk of cancelled cycles. On the other hand, in infertile patients with PCOS treated with gonadotrophins for IVF/ICSI cycles, metformin reduces the risk of OHSS.

References

1. Velazquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ. Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism. 1994;43:647–54.
2. Vitek W, Alur S, Hoeger KM. Off-label drug use in the treatment of polycystic ovary syndrome. Fertil Steril. 2015;103:605–11.
3. Dunn CJ, Peters DH. Metformin: a review of its pharmacological properties and therapeutic use in non-insulin-dependent diabetes mellitus. Drugs. 1995;49:721–49.
4. Palomba S, Falbo A, Zullo F, Orio Jr F. Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. Endocr Rev. 2009;30:1–50.
5. Elliott BD, Langer O, Schuessling F. Human placental glucose uptake and transport are not altered by the oral antihyperglycemic agent metformin. Am J Obstet Gynecol. 1997;176:527–30.
6. Stumvoll M, Nurjhan N, Perriello G, Dailey G, Gerich JE. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. N Engl J Med. 1995;333:550–4.
7. Wollen N, Bailey CJ. Inhibition of hepatic gluconeogenesis by metformin. Synergism with insulin. Biochem Pharmacol. 1988;37:4353–8.
8. Bailey CJ, Turner RC. Metformin. N Engl J Med. 1996;334:574.
9. Sarabia V, Lam L, Burdett E. Glucose transport in human skeletal muscle cells in culture. Stimulation by insulin and metformin. J Clin Invest. 1992;90:1386–95.
10. Matthaei S, Hamann A, Klein HH. Evidence that metformin increases insulin-stimulated glucose transport by potentiating insulin-induced translocation of glucose transporters from an intracellular pool to the cell surface in rat adipocytes. Horm Metab Res. 1992;26:S34–41.
11. Coyral-Castel S, Tosca L, Ferreira G, Jeanpierre E, Rame C, Lomet D, Caraty A, Monget P, Chabrolle C, Dupont J. The effect of AMP-activated kinase activation on gonadotrophin-releasing hormone secretion in GT1-7cells and its potential role in hypothalamic regulation of the oestrous cyclicity in rats. J Neuroendocrinol. 2008;20:335–46.
12. Tosca L, Chabrolle C, Uzbekova S, Dupont J. Effects of metformin on bovine granulose cells steroidogenesis: possible involvement of adenosine 5′monophosphate-activated protein kinase (AMPK). Biol Reprod. 2007;76:368–78.
13. Tosca L, Solnais P, Ferrè P, Foufelle F, Dupont J. Metformin-induced stimulation of adenosine 5′monophosphate-activated protein kinase (PRKA) impairs progesterone secretion in rat granulosa cells. Biol Reprod. 2006;75:342–51.
14. Nestler JE, Jakubowicz DJ. Decreases in ovarian cytochrome P450c17 activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. N Engl J Med. 1996;335:617–23.
15. Nestler JE, Jakubowicz DJ. Lean women with polycystic ovary syndrome respond to insulin reduction with decreases in ovarian P450c17 activity and serum androgens. J Clin Endocrinol Metab. 1997;82:4075–9.
16. Erickson GF, Magoffin DA, Cragun JR, Chang RJ. The effects of insulin and insulin-like growth factors-I and -II on estradiol production by granulosa cells of polycystic ovaries. J Clin Endocrinol Metab. 1990;70:894–902.
17. Garber AJ, Duncan TG, Goodman AM, Mills DJ, Rohlf JL. Efficacy of metformin in type II diabetes: results of a double-blind, placebo-controlled, dose-response trial. Am J Med. 1997;103:491–7.
18. Nestler JE, Stovall D, Akhter N, Iuorno MJ, Jakubowicz DJ. Strategies for the use of insulin-sensitizing drugs to treat infertility in women with polycystic ovary syndrome. Fertil Steril. 2002;77:209–15.
19. Nestler JE. Metformin for the treatment of the polycystic ovary syndrome. N Engl J Med. 2008;358:47–54.
20. Palomba S, Falbo A, Orio F, Tolino A, Zullo F. Efficacy predictors for metformin and clomiphene citrate treatment in anovulatory infertile patients with polycystic ovary syndrome. Fertil Steril. 2008;91:2557–67.

21. Palomba S, Falbo A, Russo T, Manguso F, Tolino A, Zullo F, De Feo P, Orio F. Insulin sensitivity after metformin suspension in normal-weight women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2007;92:3128–35.
22. Palomba S, Pasquali R, Orio Jr F, Nestler JE. Clomiphene citrate, metformin or both as first-step approach in treating anovulatory infertility in patients with polycystic ovary syndrome (PCOS): a systematic review of head-to-head randomized controlled studies and meta-analysis. Clin Endocrinol. 2009;70:311–21.
23. Yarali H, Yildiz BO, Demirol A, Zeyneloglu HB, Yigit N, Bukulmez O, Koray Z. Co-administration of metformin during rFSH treatment in patients with clomiphene citrate-resistant polycystic ovarian syndrome: a prospective randomized trial. Hum Reprod. 2002;17:289–94.
24. Ng EH, Wat NM, Ho PC. Effects of metformin on ovulation rate, hormonal and metabolic profiles in women with clomiphene resistant polycystic ovaries: a randomized, double-blinded placebo-controlled trial. Hum Reprod. 2001;16:1625–31.
25. Fleming R, Hopkinson ZE, Wallace AM, Greer IA, Sattar N. Ovarian function and metabolic factors in women with oligomenorrhea treated with metformin in a randomized double blind placebo-controlled trial. J Clin Endocrinol Metab. 2002;87:569–74.
26. Moghetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M, Zanolin E, Muggeo M. Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. J Clin Endocrinol Metab. 2000;85:139–46.
27. Zain MM, Jamaluddin R, Ibrahim A, Norman RJ. Comparison of clomiphene citrate, metformin, or the combination of both for first-line ovulation induction, achievement of pregnancy, and live birth in Asian women with polycystic ovary syndrome: a randomized controlled trial. Fertil Steril. 2009;91:514–21.
28. Palomba S, Orio Jr F, Falbo A, Manguso F, Russo T, Cascella T, Tolino A, Carmina E, Colao A, Zullo F. Prospective parallel randomized, double-blind, double-dummy controlled clinical trial comparing clomiphene citrate and metformin as the first-line treatment for ovulation induction in nonobese anovulatory women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2005;90:4068–74.
29. Stepensky D, Friedman M, Srour W, Raz I, Hoffman A. Preclinical evaluation of pharmacokinetic-pharmacodynamic rationale for oral CR metformin formulation. J Control Release. 2001;71:107–15.
30. Salpeter SR, Greyber E, Pasternak GA, Salpeter EE. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus: systematic review and meta-analysis. Arch Intern Med. 2003;163:2594–602.
31. Brown JB, Pedula K, Barzilay J, Herson MK, Latare P. Lactic acidosis rates in type 2 diabetes. Diabetes Care. 1998;21:1659–63.
32. Jones GC, Macklin JP, Alexander WD. Contraindications to the use of metformin. BMJ. 2003;326:4–5.
33. Checa MA, Requena A, Salvador C, Tur R, Callejo J, Espinos JJ, Fabregues F, Herrero J. Reproductive Endocrinology Interest Group of the Spanish Society of Fertility. Insulin-sensitizing agents: use in pregnancy and as therapy in polycystic ovary syndrome. Hum Reprod Update. 2005;11:375–90.
34. Rowan JA, Hague WM, Gao W, Battin MR, Moore MP, Investigators MGT. Metformin versus insulin for the treatment of gestational diabetes. N Engl J Med. 2008;358:2003–15.
35. Coetzee EJ, Jackson WP. Oral hypoglycaemics in the first trimester and fetal outcome. S Afr Med J. 1984;65:635–7.
36. Koren G, Gilbert C, Valois M. Metformin use during the first trimester of pregnancy. Is it safe? Can Fam Physician. 2006;52:171–2.
37. Gilbert C, Valois M, Koren G. Pregnancy outcome after first trimester exposure to metformin: a meta-analysis. Fertil Steril. 2006;86:658–63.
38. Thatcher SS, Jackson EM. Pregnancy outcome in infertile patients with polycystic ovary syndrome who were treated with metformin. Fertil Steril. 2006;85:1002–9.

39. Fauser BC, Diedrich K, Devroey P, on behalf of the Evian Annual Reproduction (EVAR) Workshop Group 2007. Predictors of ovarian response: progress towards individualized treatment in ovulation induction and ovarian stimulation. Hum Reprod Update. 2008;14:1–14.
40. Salley KE, Wickham EP, Cheang KI, Essah PA, Karjane NW, Nestler JE. Glucose in tolerance in polycystic ovary syndrome: a position statement of the Androgen Excess Society. J Clin Endocrinol Metab. 2007;92:4546–56.
41. Polycystic Ovary Syndrome Writing Committee. American Association of Clinical Endocrinologists position statements on metabolic and cardiovascular consequences of polycystic ovary syndrome. Endocr Pract. 2005;11:126–34.
42. Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Fertil Steril. 2008;89:505–22.
43. Costello MF, Eden JA. A systematic review of the reproductive system effects of metformin in patients with polycystic ovary syndrome. Fertil Steril. 2003;79:1–13.
44. Lord JM, Flight IH, Norman RJ. Metformin in polycystic ovary syndrome: systematic review and meta-analysis. BMJ. 2003;327:951–3.
45. Kashyap S, Wells GA, Rosenwaks Z. Insulin-sensitizing agents as primary therapy for patients with polycystic ovarian syndrome. Hum Reprod. 2004;19:2474–83.
46. Siebert TI, Kruger TF, Steyn DW, Nosarka S. Is the addition of metformin efficacious in the treatment of clomiphene citrate resistant patients with polycystic ovary syndrome? A structured literature review. Fertil Steril. 2006;86:1432–7.
47. Moll E, van der Veen F, van Wely M. The role of metformin in polycystic ovary syndrome: a systematic review. Hum Reprod Update. 2007;13:527–37.
48. Creanga AA, Bradley HM, McCormick C, Witkop CT. Use of metformin in polycystic ovary syndrome: a meta-analysis. Obstet Gynecol. 2008;111:959–68.
49. Palomba S, Falbo A. Metformin in therapy naïve patients with polycystic ovary syndrome. Hum Reprod Update. 2008;14:193.
50. Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. Cochrane Database Syst Rev. 2012;5:CD003053.
51. Misso ML, Costello MF, Garrubba M, Wong J, Hart R, Rombauts L, Melder AM, Norman RJ, Teede HJ. Metformin versus clomiphene citrate for infertility in non-obese women with polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update. 2013;19:2–11.
52. Abu Hashim H. Twenty years of ovulation induction with metformin for PCOS; what is the best available evidence? Reprod Biomed Online. 2016;32:44–53.
53. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, Steinkampf MP, Coutifaris C, McGovern PG, Cataldo NA, Gosman GG, Nestler JE, Giudice LC, Leppert PC, Myers ER, Cooperative Multicenter Reproductive Medicine Network. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. N Engl J Med. 2007;356:551–66.
54. Moll E, Bossuyt PM, Korevaar JC, Lambalk CB, van der Veen F. Effect of clomifene citrate plus metformin and clomifene citrate plus placebo on induction of ovulation in women with newly diagnosed polycystic ovary syndrome: randomised double blind clinical trial. BMJ. 2006;332:1485.
55. Johnson N. Metformin is a reasonable first-line treatment option for non-obese women with infertility related to anovulatory polycystic ovary syndrome-a meta-analysis of randomised trials. Aust N Z J Obstet Gynaecol. 2011;51:125–9.
56. Siebert TI, Viola MI, Steyn DW, Kruger TF. Is metformin indicated as primary ovulation induction agent in women with PCOS? A systematic review and meta-analysis. Gynecol Obstet Investig. 2012;73:304–13.
57. Morin-Papunen L, Rantala AS, Unkila-Kallio L, Tiitinen A, Hippeläinen M, Perheentupa A, Tinkanen H, Bloigu R, Puukka K, Ruokonen A, Tapanainen JS. Metformin improves pregnancy and live-birth rates in women with polycystic ovary syndrome (PCOS): a multicenter, double-blind, placebo-controlled randomized trial. J Clin Endocrinol Metab. 2012;97:1492–500.

58. Moll E, van Wely M, Lambalk CB, Bossuyt PM, van der Veen F. Health-related quality of life in women with newly diagnosed polycystic ovary syndrome randomized between clomifene citrate plus metformin or clomifene citrate plus placebo. Hum Reprod. 2012;27:3273–8.
59. Sinawat S, Buppasiri P, Lumbiganon P, Pattanittum P. Long versus short course treatment with metformin and clomiphene citrate for ovulation induction in women with PCOS. Cochrane Database Syst Rev. 2012;10:CD006226.
60. Palomba S, Orio Jr F, Nardo LG, Falbo A, Russo T, Corea D, Doldo P, Lombardi G, Tolino A, Colao A, Zullo F. Metformin administration versus laparoscopic ovarian diathermy in clomiphene citrate-resistant women with polycystic ovary syndrome: a prospective parallel randomized double-blind placebo-controlled trial. J Clin Endocrinol Metab. 2004;89:4801–9.
61. Kocak I, Ustun C. Effects of metformin on insulin resistance, androgen concentration, ovulation and pregnancy rates in women with polycystic ovary syndrome following laparoscopic ovarian drilling. J Obstet Gynaecol Res. 2006;32:292–9.
62. Balen AH, Morley LC, Misso M, Franks S, Legro RS, Wijeyaratne CN, Stener-Victorin E, Fauser BC, Norman RJ, Teede H. The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. Hum Reprod Update. 2016;22:687–708.
63. Morley LC, Tang T, Yasmin E, Lord JM, Norman RJ, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. Cochrane Database Syst Rev. 2016:CD003053.
64. Palomba S, Orio Jr F, Falbo A, Russo T, Tolino A, Zullo F. Clomiphene citrate versus metformin as first-line approach for the treatment of anovulation in infertile patients with polycystic ovary syndrome. J Clin Endocrinol Metab. 2007;92:3498–503.
65. Sinawat S, Buppasiri P, Lumbiganon P, Pattanittum P. Long versus short course treatment with metformin and clomiphene citrate for ovulation induction in women with PCOS. Cochrane Database Syst Rev. 2008:CD006226.
66. Palomba S, Falbo A, Battista L, Russo T, Venturella R, Tolino A, Orio F, Zullo F. Laparoscopic ovarian diathermy vs clomiphene citrate plus metformin as second-line strategy for infertile anovulatory patients with polycystic ovary syndrome: a randomized controlled trial. Am J Obstet Gynecol. 2010;202:577.e1–8.
67. Sohrabvand F, Ansari SH, Bagheri M. Efficacy of combined metformin-letrozole in comparison with metformin-clomiphene citrate in clomiphene-resistant infertile women with polycystic ovarian disease. Hum Reprod. 2006;21:1432–5.
68. George SS, George K, Irwin C, Job V, Selvakumar R, Jeyaseelan V, Seshadri MS. Sequential treatment of metformin and clomiphene citrate in clomiphene-resistant women with polycystic ovary syndrome: a randomized, controlled trial. Hum Reprod. 2003;18:299–304.
69. Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. N Engl J Med. 1998;338:1876–80.
70. Palomba S, Orio Jr F, Falbo A, Russo T, Caterina G, Manguso F, Tolino A, Colao A, Zullo F. Metformin administration and laparoscopic ovarian drilling improve ovarian response to clomiphene citrate (CC) in oligo-anovulatory CC-resistant women with polycystic ovary syndrome. Clin Endocrinol. 2005;63:631–5.
71. Khorram O, Helliwell JP, Katz S, Bonpane CM, Jaramillo L. Two weeks of metformin improves clomiphene citrate-induced ovulation and metabolic profiles in women with polycystic ovary syndrome. Fertil Steril. 2006;85:1448–51.
72. Sturrock ND, Lannon B, Fay TN. Metformin does not enhance ovulation induction in clomiphene resistant polycystic ovary syndrome in clinical practice. Br J Clin Pharmacol. 2002;53:469–73.
73. Hwu YM, Lin SY, Huang WY, Lin MH, Lee RK. Ultra-short metformin pretreatment for clomiphene citrate-resistant polycystic ovary syndrome. Int J Gynaecol Obstet. 2005;90:39–43.
74. Palomba S, Falbo A, Orio F, Zullo F. Insulin sensitizing agents and reproductive function in polycystic ovary syndrome patients. Curr Opin Obstet Gynecol. 2008;20:364–73.

75. Stadtmauer LA, Toma SK, Riehl RM, Talbert LM. Metformin treatment of patients with polycystic ovary syndrome undergoing in vitro fertilization improves outcomes and is associated with modulation of the insulin-like growth factors. Fertil Steril. 2001;75:505–9.
76. Costello MF, Chapman M, Conway U. A systematic review and meta-analysis of randomized controlled trials on metformin co-administration during gonadotrophin ovulation induction or IVF in women with polycystic ovary syndrome. Hum Reprod. 2006;21:1387–99.
77. Palomba S, Falbo A, Orio Jr F, Manguso F, Russo T, Tolino A, Colao A, Dale B, Zullo F. A randomized controlled trial evaluating metformin pre-treatment and co-administration in nonobese insulin-resistant women with polycystic ovary syndrome treated with controlled ovarian stimulation plus timed intercourse or intrauterine insemination. Hum Reprod. 2005;20:2879–86.
78. van Santbrink EJ, Hohmann FP, Eijkemans MJ, Laven JS, Fauser BC. Does metformin modify ovarian responsiveness during exogenous FSH ovulation induction in normogonadotrophic anovulation? A placebo-controlled double-blind assessment. Eur J Endocrinol. 2005;152:611–7.
79. Gorry A, White DM, Franks S. Infertility in polycystic ovary syndrome: focus on low-dose gonadotropin treatment. Endocrine. 2006;30:27–33.
80. Christianson MS, Wu H, Zhao Y, Yemini M, Leong M, Shoham Z. Metformin use in patients undergoing in vitro fertilization treatment: results of a worldwide web-based survey. J Assist Reprod Genet. 2015;32:401–6.
81. Palomba S, Falbo A, La Sala GB. Effects of metformin in women with polycystic ovary syndrome treated with gonadotrophins for in vitro fertilisation and intracytoplasmic sperm injection cycles: a systematic review and meta-analysis of randomised controlled trials. BJOG. 2013;120:267–76.
82. Tso LO, Costello MF, Albuquerque LE, Andriolo RB, Macedo CR. Metformin treatment before and during IVF or ICSI in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2014;11:CD006105.

Gonadotrophins

12

Sophie Christin-Maitre

12.1 Introduction

In women with polycystic ovary syndrome (PCOS), gonadotrophins are second-line pharmacological therapy of infertility, after clomiphene citrate (CC) or aromatase inhibitors (AIs) (see also Chaps. 9 and 10). Indeed, according to the workshop endorsed by the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM), held in Thessaloniki, in Greece, gonadotrophins should be proposed to CC-resistant patients [1]. Recently, the development of a global World Health Organization (WHO) guidance in the management of anovulatory infertility in women with PCOS has been suggested [2].

Human gonadotrophins have been used in the treatment of infertility, since the late 1960s. The main issue of this treatment is to produce a single ovulation and a healthy term single fetus, avoiding ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies. After a brief presentation of follicle-stimulating hormone (FSH) and luteinising hormone (LH), this chapter will describe their respective roles in human ovarian physiology, the rationale for using gonadotrophins, and the different types of gonadotrophins available nowadays and will then discuss the use of gonadotrophins in mono-ovulation induction in PCOS women.

S. Christin-Maitre
Reproductive Endocrine Unit, Hôpital St. Antoine, AP-HP, University Pierre and Marie Curie, Sorbonne, Paris, France

Unité INSERM UMR_S933, Paris, France
e-mail: sophie.christin-maitre@aphp.fr

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_12

12.2 The Role of Gonadotrophins in Human Physiology

FSH and LH are heterodimeric hormones, synthesised by the pituitary, in a pulsatile manner, under the control of hypothalamic gonadotrophin-releasing hormone (GnRH). Those glycoproteins consist of two different subunits, a common α subunit (92 amino acids) and a β-specific subunit (Fig. 12.1). In humans, βFSH and βLH contain 111 and 115 amino acids, respectively. Both α and β subunits are non-convalently linked. FSH and LH are glycosylated proteins, as α and β subunits each contain two N-linked carbohydrate groups. The type and size of sugars attached may vary, depending mainly on the amount of sialic acid. At least 20 different types of FSH have been identified in human pituitary; they are called isoforms [3]. The rate of acidic isoforms in the serum is the lowest during the preovulatory and ovulatory phase. The basic isoforms are secreted before ovulation. Bioactivity of FSH and LH isoforms has been studied *in vivo* as well as *in vitro*, using bioassays [4]. When FSH activity is measured *in vitro*, acidic isoforms of FSH have a lower activity than the more basic isoforms. In contrast, when the activity is measured *in vivo*, the acidic isoforms of FSH have a higher activity than the basic isoforms. Acidic isoforms have a longer half-life as compared to the basic preparations. The respective role of each isoform has not been totally elucidated.

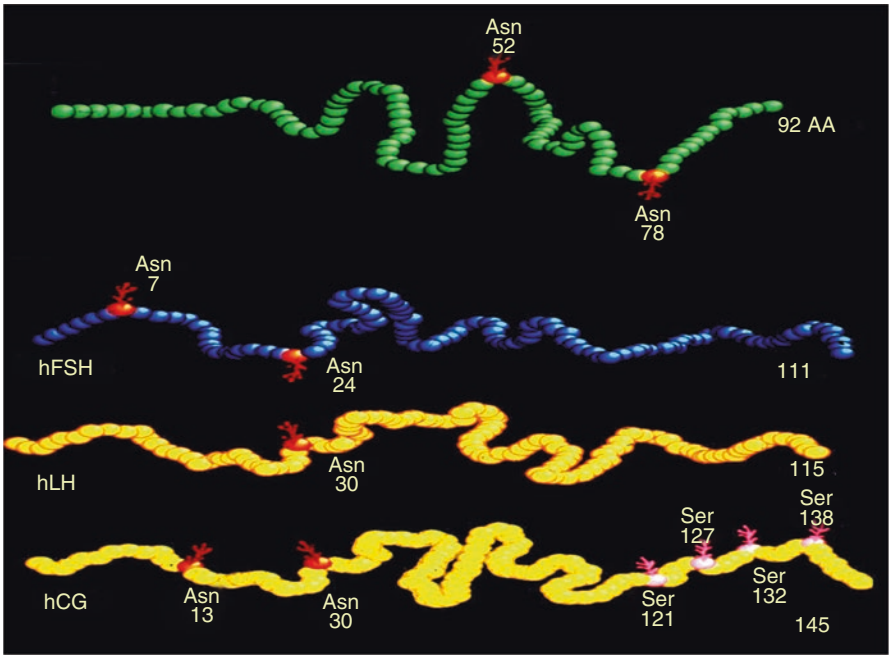


Fig. 12.1 Gonadotrophins, in *green* the common α subunit, in *blue* the specific β subunit of FSH and in *yellow* the specific hLHβ and hCGβ sub units. AA for amino acids; the sites of glycosylation are indicated in *red* and *pink*. Asn for asparagine site of N glycosylation; Ser for serine site of O glycosylation

Free α and β subunits when they are not associated are not recognised by gonadotrophin receptors and therefore are not biologically active. Depending on the amount of sialic acid attached on the carbohydrate groups, the half-lives of each gonadotrophin differ. The half-lives of FSH and LH are 5 h and 1 h, respectively [5].

In physiology, during the menstrual cycle, FSH rises during the luteo-follicular transition and progressively increases during the follicular phase. A major role of FSH is to induce follicular recruitment and follicular maturation [6]. Indeed, defects in folliculogenesis have been described in few patients presenting loss-of-function mutations of FSH or its receptor. Their follicles are blocked at the early preantral stage and are not able to achieve complete follicular maturation up to the preovulatory stage. Those rare patients illustrate the fact that during folliculogenesis, the first stages of follicular maturation are FSH independent and the last steps, up to the preovulatory stage, are FSH dependent.

At the beginning of each menstrual cycle, a growing cohort of 5–8 small follicles is present. However, in humans, only one follicle is selected and reaches the preovulatory stage. The idea of a threshold of FSH has been suggested in 1978 [7]. Above this threshold, a follicle leaves the cohort of growing follicles. A 10–30% increase of FSH above the threshold induces final follicle maturation. As each follicle has a different threshold of FSH, the concept of follicular asynchrony has risen.

Within the ovarian follicle, FSH binds to its receptors, mainly localised on the membrane of granulosa cells. FSH receptors (FSHR) belong to the G protein-coupled family of receptors. LH binds to its receptors localised on the membrane of theca cells. According to the "two cell-two gonadotrophins" theory, LH, after binding to its receptor, in theca cells, increases androgen production, mainly androstenedione [8]. Androgens cross the basal membrane of the follicle and reach granulosa cells (Fig. 12.2). In the dominant follicle, they are aromatised to oestradiol (E_2),

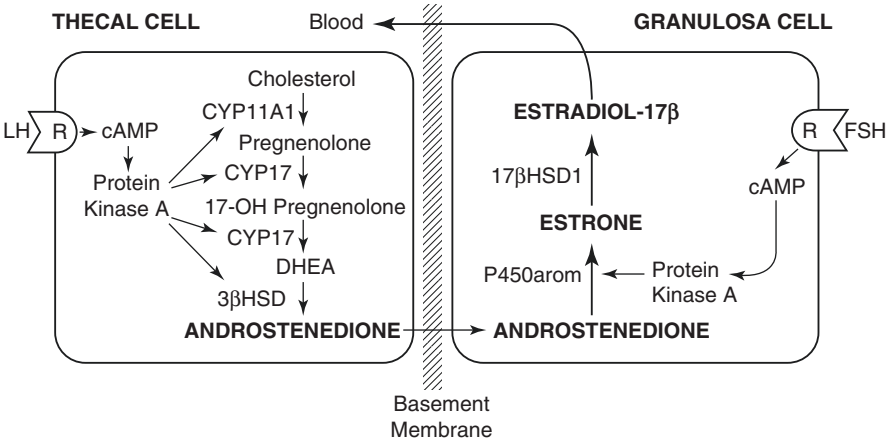


Fig. 12.2 Two cell-two gonadotrophin theory: thecal and granulosa cells; LH and FSH. LH binds to its receptor, located on thecal cell membrane, inducing the production of androstenedione. This androgen is then transferred to granulosa cells. After the binding of FSH on its receptor, located on the membrane of granulosa cells, androstenedione is aromatised to oestradiol

under the control of FSH. The progressive rise of E_2 during the follicular phase in human exerts at first a negative feedback on FSH. Therefore, the most sensitive follicles to FSH continue their maturation up to the preovulatory stage [9]. On the other hand, the 4–5 remaining follicles of the growing cohort that are less sensitive to FSH undergo apoptosis. This process represents the basis of follicular dominance and explains the monofollicular development in human physiology. On the contrary, in other species, such as the rabbit, there is no decrease of FSH at the end of the follicular phase. Therefore, multifollicular development occurs and spontaneous multiple gestations. This progressive rise and decrease of FSH has been named the "FSH window". David Baird first suggested that the rise of FSH during the luteo-follicular phase could represent the opening of the window [10]. The decrease of FSH at the end of the follicular phase, before the preovulatory surge, could represent the shutting of the window [11]. According to this concept, the wider is the window, the larger will be the number of follicles recruited during the cycle.

At the end of the follicular phase, the E_2 feedback is inverted, and the rise of E_2 exerts a positive feedback on the hypothalamus and the pituitary, increasing GnRH pulses and therefore LH and FSH preovulatory peak. During the follicular phase, the negative E_2 feedback becomes positive. In monkeys, the positive or negative feedbacks of E_2 depend on the localisation of hypothalamic Kiss neurons involved. Whether they are located in the arcuate nucleus of the hypothalamus (ARC) or the paraventricular region of the preoptic area (AVPV), they induce, respectively, negative or positive feedbacks [12].

The respective role of FSH and LH in human physiology has been illustrated in the early 1990s, as recombinant gonadotrophins have been available. A small amount of LH is necessary during the follicular phase in order to produce androgens by the theca cells and therefore E_2 by the granulosa cells. A treatment with recombinant FSH in women lacking both gonadotrophins due to pituitary deficiency induces follicular development, but the treated women lack E_2 and their endometrium does not proliferate [13]. At mid-cycle, the LH rise plays three major roles. First of all, it stops granulosa cell proliferation and produces corpus luteum. Secondly, LH rise enables oocyte meiosis and finally it induces ovulation.

In the human pituitary, a small amount of human chorionic gonadotrophin (hCG) is synthesised. Due to its carboxyl-terminal extension of 30 amino acids with four O-linked oligosaccharides, hCG's half-life is longer than other gonadotrophins, reaching 24 h. LH and hCG bind to the same receptor in the follicle; therefore, LH or hCG may be used in order to induce ovulation.

12.3 Rationale for Gonadotrophin Treatment in Women with PCOS

Women with PCOS have an altered folliculogenesis with an increased number of small follicles, a defect in follicular recruitment and in follicular growth, leading to anovulation. Patients with PCOS are considered a subgroup of WHO type II anovulation, and an important role in follicle maturation failure could be related to high

anti-Mullerian hormone (AMH) levels. Indeed, several studies have demonstrated that AMH inhibits FSH effects on granulosa cells, therefore inducing a relative defect in endogenous FSH [14].

12.4 Different Types of Gonadotrophins Available for Fertility Treatment

Initially, gonadotrophins were isolated from pituitaries. However, the use of pituitary gonadotrophic preparations has been abandoned, since the source of human pituitaries is limited and because of potential viral contamination. Indeed, many cases of Creutzfeldt-Jakob disease have been reported after using human growth hormone preparations issued from human pituitaries. Four cases have been reported after gonadotrophin treatment issued from pituitaries [15].

Donini et al. extracted human menopausal gonadotrophins (hMG) from urine of postmenopausal women [16]. Such preparations contain around the same amount of FSH and LH. FSH and LH activities have been standardised at 75 IU for each type of gonadotrophin. If no hCG is added after purification, the ratio of FSH to LH bioactivity is 3:1. Progressively, over the years, as FSH is a major player in follicular recruitment and maturation, purified urinary FSH (p-FSH) preparations have been prepared. The ratio of FSH to LH bioactivity in those preparations is higher than 60:1. This has been obtained by removing LH with polyclonal antibodies. Later on, since 1993, highly purified urinary FSH (hp-FSH) preparations have been developed. They contain less and 0.1 IU of LH and less than 5% of unidentified urinary proteins. Due to a high degree of purity, they may be administered subcutaneously.

Since the mid-1990s, recombinant FSH (r-FSH) preparations have been available. In order to produce r-FSH, mammalian cells, such as Chinese hamster ovary cell line, are transfected by vectors containing the genes coding for the human sequence of α and β FSH subunits. Cells are grown in culture medium. The recombinant gonadotrophins produced are then collected. Preparations of r-FSH are devoid of LH activity [17]. R-FSH was developed in order to assure permanent availability of gonadotrophins, to increase FSH production independent of urine collection and to reduce batch to batch variability. Furthermore, there is a reduced risk of immunological reactions due to impurities. R-FSH, having no protein contamination, may be used subcutaneously. Self-administration by the patient has been developed using prefilled pen devices [18]. Marketed r-FSH available in Europe are follitropin alfa (Gonal-F®) and follitropin beta (Puregon®).

A long-acting FSH has been generated by adding the C-terminal region of hCG, called C-terminal peptide (CTP) to the beta subunit of FSH [19]. This molecule is called FSH-CTP or corifollitropin alfa (Elonva®). Its major indication is during *in vitro* fecondation (IVF) protocols.

The increased cost pressure of many healthcare systems and the patent expiration date of several r-FSH are the source of the development of FSH biosimilars [20]. Biosimilar formulations are products with similarity in physicochemical characteristics, efficacy and safety to an approved product [21]. In 2014, two types of FSH

biosimilars have been approved by the European Medicines Agency (EMA): Bemfola® and Ovaleap®. They are follitropin alfa and have been compared to Gonal-F®. Pharmacokinetic studies have shown bioequivalence of Bemfola® and Ovaleap® with the innovator product Gonal-F®. Both biosimilar preparations have been tested in comparative phase three efficacy as well as tolerance studies, in women using assisted reproduction technologies [22, 23]. The results in IVF protocols are rather similar. However, as mentioned by Orvieto and Seifer, biosimilar may be just siblings and not identical twins [24], as their glycosylation patterns are different.

In order to trigger ovulation, the gold standard is to use hCG. Indeed, it binds to the same receptor as LH, with a higher affinity. It has been initially purified from pregnant women's urine (u-hCG). It is generally administered intramuscularly. Recombinant human LH (r-LH) and recombinant hCG (r-hCG) have been available since 2000 and 2001, respectively. They are administered subcutaneously using prefilled syringes. The most frequent dose of u-hCG, used in PCOS, is a single injection of 5000 IU or 250 μg of r-hCG. The dose of 6500 IU of u-hCG is equivalent to 250 μg of r-hCG [25].

12.5 Different Protocols of Gonadotrophin Treatment in PCOS Women

Initially, FSH starting dose used to treat infertility in PCOS women was 150 IU per day, and the dose was increased by 75 IU/day every 3–5 days [26]. This protocol was named "conventional protocol". As high rates of OHSS and multiple pregnancies have been reported [27, 28], doses of gonadotrophins have been decreased. "Low-dose" protocols using a starting dose of FSH of 37.5–75 IU per day have progressively replaced the conventional protocol [29, 30]. Two main different low-dose protocols have been described, i.e. the step-up and the step-down regimens. In both protocols, FSH is started within the first 7 days of the menstrual cycle, once the cycle has been induced by 10 days of progestins in oligoamenorrheic women.

12.5.1 The Step-Up Protocol

The goal of the step-up protocol is to reproduce physiological follicular selection and single follicular growth (Fig. 12.3). It recreates the progressive rise of FSH during the follicular phase. Follicle development needs to be checked, once a week after initiation of FSH. If follicle growth is not observed, an increase in dose is recommended. On the contrary, once follicular growth is observed, the same FSH dose is maintained. The protocol was then modified, as the duration of the initial dose of FSH was extended from 7 to 14 days and the weekly dose increment was reduced from 100 to 50% of the initial dose. This protocol is also called the "chronic low-dose regimen" [31–35]. According to the Thessaloniki Consensus Workshop Group, the recommended starting daily dose is 37.5–50 IU, and it is increased after

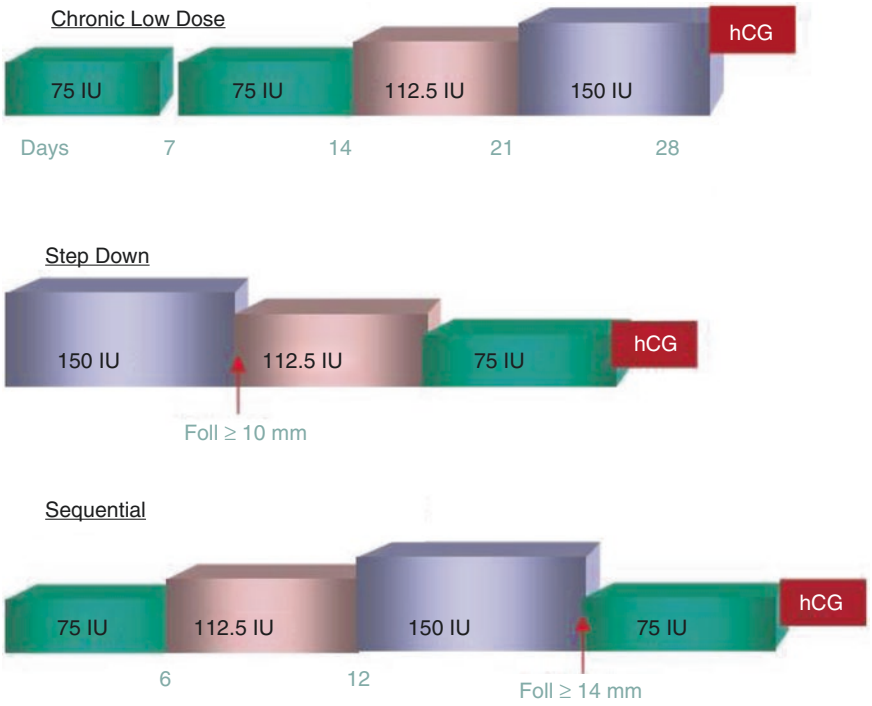


Fig. 12.3 Chronic low-dose step-up protocol, step-down and sequential step-up and step-down protocols

14 days, if there is no response and only by half an ampoule every 7 days [1]. Some protocols have even suggested a starting dose of 25 IU with ultra-low increment of gonadotrophins (8.3–12.5 IU) [36].

12.5.2 The Step-Down Protocol

A loading dose of FSH is given and followed by a subsequent stepwise reduction as soon as follicular development is observed on ultrasound [37, 38]. Follicles which already started to mature have gained increased sensitivity to FSH, but less mature follicles fail to thrive as FSH levels decrease. The starting FSH dose may be 100 or 150 IU daily for 3–4 days, and the dose is then decreased to 50–75 IU daily.

12.5.3 The Combined Step-Up and Step-Down Protocol

This approach of sequential step-up and step-down regimens has been initially described by Hugues et al. [39, 40]. The goal is to reduce the risk of overresponse. The step-up used initially enables to identify the FSH dose susceptible to induce

monofollicular recruitment. The step-down is used in the second cycle; the starting dose is defined just below the individual threshold, therefore recreating the window of FSH.

In all protocols, hCG is administered in order to induce ovulation, when the leading follicle is higher than 17 mm. This size represents the optimal mean diameter, but competence may be achieved at 15 mm diameter. A single dose of 5000 IU of hCG is administered i.m or s.c. In order to avoid hyperstimulation, the number of follicles >14 mm should be lower than 2, with the largest >17 mm. If there are more than three follicles higher than 15 mm, hCG should not be administered and the couple advise to refrain from sexual intercourse or to use condoms.

12.6 Results of Gonadotrophins in Mono-ovulatory Induction

The rates of monofollicular development are rather similar using the step-up and the step-down regimens [30, 41] as well as the pregnancy rate. Using the step-up chronic low-dose protocols, the pregnancy rate per started cycle ranges from 11% [28] to 14% [42]. Using the step-down protocol, it is around 16% [43]. Several studies have shown that the mean duration of treatment in low-dose step-up protocols is longer compared to the step-up protocols, reaching 28–35 days. Interestingly, the risk of multiple pregnancies is lower with low-dose step-up.

Few studies have compared directly, within the same study, step-up and step-down protocols [30, 41, 44]. One of the largest randomised controlled trials (RCT) including 83 women with anovulatory infertility due to PCOS allocated 1:1 step-up or step-down protocols [44]. The starting daily dose of r-FSH was 50 IU in the step-up protocol and 100 IU in the step-down protocol. The mean duration of treatment was significantly longer in the step-up than in the step-down. The total amount of r-FSH was similar in the two protocols. The rate of monofollicular development (one follicle > 16 mm diameter at the time of hCG administration) was higher in the step-up than in the step-down protocols (68.2% versus 32% of treatment cycles, respectively). Similarly, the administration of hCG was performed in 84.6% of the step-up versus 61.8% in the step-down cycles. The rate of hyperstimulation was significantly higher in the step-down group than in the step-up. The fecundity rate was similar. The cumulative rates of gestation during the 3 months from study start were not statistically different (38.6% versus 30.8%, for step-up and step-down protocols, respectively). In this large RCT, chronic low-dose regimen using r-FSH administration resulted as effective as but safer as the step-down regimen.

The cumulative rate of pregnancies after 6 months of CC and FSH has been evaluated. A Dutch study reported rates of 50% and 71% after 12 and 24 months, respectively [45]. A second study reported a singleton live birth of 60% after 1 year and 78% after 2 years of follow-up [46]. The rate of multiple pregnancies was low, below 3%, and no OHSS was observed. The median treatment duration to achieve a pregnancy was 5.1 months (range 0.4–24 months) [46]. According to the Thessaloniki

Consensus, the recommended duration of gonadotrophin treatment should not exceed six ovulatory cycles [1].

Several meta-analyses have compared results of mono-ovulation induction according to the type of FSH preparations used. The rate of OHSS and the pregnancy rate have been evaluated. The meta-analysis published in 2000 showed that administration of FSH is safer than hMG by reducing the risk of OHSS in those patients with high endogenous LH secretion; the rate of pregnancy was similar [47]. More recently, Weiss et al. included in their meta-analysis 14 trials with 1726 women [48]. Ten trials compared r-FSH versus urinary-derived gonadotrophins; four trials compared p-FSH with hMG. There was no evidence of a difference in live birth for r-FSH versus urinary-derived gonadotrophins [odds ratio (OR) 1.26, 95% confidence interval (CI) 0.80–1.99] or clinical pregnancy rate (OR 1.08, 95% CI 0.83–1.39). However, evidence was of low quality. The observed average live birth per woman with urinary-derived FSH was 16%, the chance of live birth following r-FSH between 13% and 26%. Pooling the data, there was no evidence of a difference for r-FSH versus urinary derived on OHSS. However, the long-acting CFT should be avoided for mono-ovulation induction in PCOS patients because the dosage cannot be tailored and the risk of cancelled cycles is high.

12.7 Predictive Markers of Ovarian Response in Women with PCOS

As individual responses to gonadotrophins vary among patients, this is particularly true for patients with PCOS (see Chaps. 7 and 8). Thus, several authors have proposed prediction models. The goals of those models are to evaluate ovarian sensitivity of PCOS patients to gonadotrophins, to identify couples with a poor prognosis and finally to adjust gonadotrophin dose to each patient.

Homburg et al. suggested that fasting insulin level could predict the number of ampoules required for ovulation induction [49]. Several years later, Mulders et al. used androstenedione and the antral follicle count (AFC) [50] in order to predict ovarian sensitivity. Imani et al. found a correlation of ovarian response with body mass index (BMI) and β cell function [51]. More recently, Koninger et al. included 48 infertile PCOS patients, in a prospective cohort. Those patients, aged 18–43 years, received r-FSH, using a step-up protocol [52]. In this study, AMH was the only independent variable for which the effect on FSH dosage was statistically significant in the crude regression model as well as after adjustment for other parameters such as age, BMI, AFC, ovarian volume, androstenedione, testosterone, LH, FSH and LH/FSH ratio. This study reported that an interquartile range increase in AMH was associated with a 51.4% (95%CI 24.7–79%) increase in the mean total r-FSH dosage per cycle. In other terms, a need for a 7.2% increase in the mean total FSH dosage per cycle was necessary per ng/ml of AMH.

Among the factors affecting the outcome of ovulation induction, a single nucleotide polymorphism, in exon 10 of the FSHR gene, has been described. The minor allele encodes an alternative amino acid, serine instead of asparagine at

codon 680 (680^{Ser}) [53]. The Ser 680 FSHR is less sensitive to gonadotrophins, indicating a higher need for exogenous FSH in association with the 680^{Ser} allele. The 680^{Asn} is on the contrary more sensitive [54]. A recent study included 240 anovulatory patients in a prospective cohort and 185 in a replication retrospective cohort. In this study, carriers of the 680^{Ser} allele were as likely to achieve an ongoing pregnancy when treated with exogenous FSH in the retrospective cohort and more likely in the prospective cohort, compared with other FSHR genotypes [55]. However, doses of FSH were not mentioned in this study. Other polymorphisms might be involved such as a polymorphism at position −29 in the promoter of the FSH receptor gene as well as a polymorphism in the promoter region of the FSHB gene, encoding the beta chain of FSH [56]. Pharmacogenetics could be an option in the future.

Conclusion

The major goal using gonadotrophins in PCOS women is to induce a single follicular growth and to avoid OHSS. Gonadotrophin preparations give similar rate of pregnancy; therefore, the most cost-effective should be used. The low-dose step-up protocol and the step-down protocols give similar results in terms of pregnancies, but the step-up seems safer and more convenient to use. However, the duration of treatment is usually longer with the step-up than the step-down protocol. In all cases, it is necessary to tailor every patient's treatment. Therefore, training of physicians to perform mono-ovulation induction is necessary and should be encouraged.

References

1. The Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Hum Reprod. 2008;23:462–77.
2. Balen AH, Morley LC, Misso M, Franks S, Legro RS, Wijeyaratne CN, et al. The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. Hum Reprod Update. 2016;5:1–22.
3. Wide L. Median charge and heterogeneity of human pituitary FSH, LH and TSH. Relationship to sex and age. Acta Endocrinol. 1985;109:190–7.
4. Steelman SL, Pohley FM. Assay of follicle-stimulating hormone based on the augmentation with human chorionic gonadotropin. Endocrinology. 1953;53:604–16.
5. Ulloa-Aguirre A, Espinoza A, Damien-Matsumura P, Chappel SC. Immunological and biological potencies of the different molecular species of gonadotropins. Hum Reprod. 1988;3:491–501.
6. Hsueh AJ, Kawamura K, Cheng Y, Fauser BC. Intraovarian control of early folliculogenesis. Endoc Rev. 2015;36:1–24.
7. Brown JB. Pituitary control of ovarian function-concepts derived from gonadotropin therapy. Aust N Z J Obstet Gynecol. 1978;18:46–54.
8. Ben-Chetrit A, Gotlieb L, Wong PY, Casper RF. Ovarian response to recombinant follicle-stimulating hormone in luteinizing hormone-depleted women: examination of the two cell-two gonadotropin theory. Fertil Steril. 1996;65:711–7.
9. Zeleznik AJ. The physiology of follicle selection. Reprod Biol Endocrinol. 2004;2:31–8.

10. Baird DT. Factors regulating the growth of the preovulatory follicle in the sheep and human. J Reprod Fertil. 1983;69:343–52.
11. Fauser B, Van Heusden AM. Manipulation of human ovarian function: physiological concepts and clinical consequences. Endocr Rev. 1997;18:71–105.
12. Christian CA, Moenter SM. The neurobiology of preovulatory and estradiol-induced gonadotropin releasing hormone surges. Endoc Rev. 2010;31:544–77.
13. Schoot DC, Coelingh Bennink HJ, Mannaerts BM, Lamberts SW, Bouchard P, Fauser BC. Human recombinant follicle-stimulating hormone induces growth of preovulatory follicles without concomitant increase in androgen and estrogen biosynthesis in a woman with isolated gonadotropin deficiency. J Clin Endocrinol Metab. 1992;74:1471–3.
14. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, Griesinger G, Kelsey TW, La Marca A, Lambalk C, Mason H, Nelson SM, Visser JA, Wallace WH, Anderson RA. The physiology and clinical utility of anti-Mullerian hormone in women. Hum Reprod Update. 2014;20:370–85.
15. Barrenetxea G. Iatrogenic prion diseases in humans: an update. Eur J Obstet Gynecol Reprod Biol. 2012;165:165–9.
16. Donini P, Puzzouli D, Montezemolo R. Purification of gonadotropins from human menopause urine. Acta Endocr. 1996;45:321–8.
17. Howles CM. Role of FSH and LH in ovarian function. Mol Cell Endocrinol. 2000;161:25–30.
18. Christen M, Schertz JC, Arriagada P, Keitel J, Uller H. The redesigned follitropin α pen injector for infertility treatment. Expert Opin Drug Deliv. 2011;8:833–9.
19. Duijkers IJ, Klipping C, Boerrigter PJ, Machielsen CS, De Bie JJ, Voortman G. Single dose pharmacokinetics and effects on follicular growth and serum hormones of a long-acting recombinant FSH preparation (FSH-CTP) in healthy pituitary-suppressed females. Hum Reprod. 2002;17:1987–9.
20. Santi D, Simoni M. Biosimilar recombinant follicle stimulating hormones in infertility treatment. Expert Opin Biol Ther. 2014;14:1399–409.
21. Weise M, Bielsky MC, De Smet K, Ehmann F, Ekman N, Narayanan G, Heim HK, Heinonen E, Ho K, Thorpe R, Vleminckx C, Wadhwa M, Schneider CK. Biosimilars-why terminology matters. Nat Biotechnol. 2011;29:690–3.
22. Rettenbacher M, Andersen AN, Garcia-Velasco JA, Sator M, Barri P, Lindenberg S, et al. A multi-centre phase 3 study comparing efficacy and safety of Bemfola (®) versus Gonal-f (®) in women undergoing ovarian stimulation for IVF. Reprod Biomed Online. 2015;30:504–13.
23. Strowitzki T, Kuczynski W, Mueller A, Bias P. Safety and efficacy of Ovaleap® (recombinant human follicle-stimulating hormone) for up to 3 cycles in infertile women using assisted reproductive technology: a phase 3 open-label follow-up to main study. Reprod Biol Endocrinol. 2016;10:14–31.
24. Orvieto R, Seifer DB. Biosimilar FSH preparations- are they identical twins or just siblings. Reprod Biol Endocrinol. 2016;14:32.
25. de Barros F, Leao R, Esteves SC. Gonadotropin therapy in assisted reproduction: an evolutionary perspective from biologics to biotech. Clinics. 2014;69:279–93.
26. Lunenfeld B, Insler V. Classification of amenorrhea states and their treatment by ovulation induction. Clin Endocrinol. 1974;3:223–37.
27. Thompson CR, Hanse LM. Pergonal (menotropins): a summary of clinical experience in the induction of ovulation and pregnancy. Fertil Steril. 1970;21:844–53.
28. Hamilton-Fairley D, Franks S. Common problems in induction of ovulation. Baill Clin Obstet Gynecol. 1990;4:609–25.
29. White D, Polson DW, Kiddy D, Sagle P, Watson H, Gilling-Smith C, Hamilton-Fairley D, Franks S. Induction of ovulation with low-dose gonadotropins in polycystic ovary syndrome: an analysis of 109 pregnancies in 225 women. J Clin Endocrinol Metab. 1996;81:3821–4.
30. Balasch J, Fabregues F, Creus M, Puerto B, Peñarrubia J, Vanrell JA. Follicular development and hormone concentrations following recombinant FSH administration for anovulation associated with polycystic ovarian syndrome: prospective, randomized comparison between low-dose step-up and modified step-down regimens. Hum Reprod. 2001;16:652–6.

31. Kamrava M, Seibel MM, Berger MJ, Thompson I, Taymor ML. Reversal of persistent anovulation in polycystic ovarian disease by administration of chronic low-dose follicle-stimulating hormone. Fertil Steril. 1982;37:520–3.
32. Seibel MM, Kamrava MM, McArdle C, Taymor ML. Treatment of polycystic ovary disease with chronic low-dose follicle stimulating hormone: biochemical changes and ultrasound correlation. Int J Fertil. 1984;29:39–43.
33. Polson DW, Mason HD, Saldahna MB, Franks S. Ovulation of a single dominant follicle during treatment with low-dose pulsatile follicle stimulating hormone in women with polycystic ovary syndrome. Clin Endocrinol. 1987;26:205–12.
34. Sagle MA, Hamilton-Fairley D, Kiddy DS, Franks S. A comparative, randomized study of low-dose human menopausal gonadotropin and follicle-stimulating hormone in women with polycystic ovarian syndrome. Fertil Steril. 1991;55:56–60.
35. Dale O, Tanbo T, Lunde O, Abyholm T. Ovulation induction with low-dose follicle-stimulating hormone in women with the polycystic ovary syndrome. Acta Obstet Gynecol Scand. 1993;72:43–6.
36. Orvieto R, Homburg R. Ultra-low dose follicle-stimulating hormone regimen for patients with polycystic ovary syndrome one click, one follicle, one pregnancy. Fertil Steril. 2009;91:1533–5.
37. Mizunuma H, Takagi T, Yamada K, Andoh K, Ibuki Y, Igarashi M. Ovulation induction by step-down administration of purified urinary follicle-stimulating hormone in patients with polycystic ovarian syndrome. Fertil Steril. 1991;55:1195.
38. Fauser BC, Donderwinkel P, Schoot DC. The step-down principle in gonadotrophin treatment and the role of GnRH analogues. Baillieres Clin Obstet Gynaecol. 1993;7:309–30.
39. Hugues JN, Cédrin-Durnerin I, Avril C, Bulwa S, Hervé F, Uzan M. Sequential step-up and step-down dose regimen: an alternative method for ovulation induction with follicle-stimulating hormone in polycystic ovarian syndrome. Hum Reprod. 1996;11:2581–4.
40. Hugues JN, Cédrin-Durnerin I, Howles CM, FSH OI Study Group. The use of a decremental dose regimen in patients treated with a chronic low-dose step-up protocol for WHO Group II anovulation: a prospective randomized multicentre study. Hum Reprod. 2006;21:2817–22.
41. van Santbrink EJ, Fauser BC. Urinary follicle-stimulating hormone for normogonadotropic clomiphene-resistant anovulatory infertility: prospective, randomized comparison between low dose step-up and step-down dose regimens. J Clin Endocrinol Metab. 1997;82:3597–602.
42. Balen AH, Braat DD, West C, Patel A, Jacobs HS. Cumulative conception and live birth rates after the treatment of anovulatory infertility: safety and efficacy of ovulation induction in 200 patients. Hum Reprod. 1994;9:1563–70.
43. van Santbrink EJ, Donderwinkel PF, van Dessel TJ, Fauser BC. Gonadotrophin induction of ovulation using a step-down dose regimen: single centre clinical experience in 82 patients. Hum Reprod. 1995;10:1048–53.
44. Christin-Maitre S, Hugues JN, Recombinant FSH Study Group. A comparative randomized multicentric study comparing the step-up versus step-down protocol in polycystic ovary syndrome. Hum Reprod. 2003;18:1626–31.
45. Eijkemans MJ, Imani B, Mulders AG, Habbema JD, Fauser BC. High singleton live birth rate following classical ovulation induction in normogonadotrophic anovulatory infertility (WHO 2). Hum Reprod. 2003;18:2357–62.
46. Veltman-Verhuist SM, Fauser BC, Eijkemans MJ. High singleton live birth rate confirmed after ovulation induction in women with anovulatory polycystic ovary syndrome: validation of a prediction model for clinical practice. Fertil Steril. 2012;98:761–8.
47. Nugent D, Vandekerckhove P, Hughes E, Arnot M, Lilford R. Gonadotrophin therapy for ovulation induction in subfertility associated with polycystic ovary syndrome. Cochrane Database Syst Rev. 2000;4:CD000410.
48. Weiss NS, Nahuis M, Bayram N, Mol BW, Van der Veen F, van Wely M. Gonadotrophins for ovulation induction in women with polycystic ovarian syndrome. Cochrane Database Syst Rev. 2015;9:CD010290.

49. Homburg R, Orvieto R, Bar-Hava I, Ben-Rafael Z. Serum levels of insulin-like growth factor-1, IGF binding protein-1 and insulin and the response to human menopausal gonadotrophins in women with polycystic ovary syndrome. Hum Reprod. 1996;11:716–9.
50. Mulders AG, Eijkemans MJ, Imani B, Fauser BC. Prediction of chances for success or complications in gonadotrophin ovulation induction in normogonadotrophic anovulatory infertility. Reprod Biomed Online. 2003;7:170–8.
51. Imani B, Eijkemans MJ, Faessen GH, Bouchard P, Giudice LC, Fauser BC. Prediction of the individual follicle-stimulating hormone threshold for gonadotropin induction of ovulation in normogonadotropic anovulatory infertility: an approach to increase safety and efficiency. Fertil Steril. 2002;77:83–90.
52. Köninger A, Sauter L, Edimiris P, Kasimir-Bauer S, Kimmig R, Strowitzki T, Schmidt B. Predictive markers for the FSH sensitivity of women with polycystic ovarian syndrome. Hum Reprod. 2014;29:518–24.
53. Simoni M, Tempfer CB, Destenaves B, Fauser BC. Functional genetic polymorphisms and female reproductive disorders: part I: polycystic ovary syndrome and ovarian response. Hum Reprod Update. 2008;14:459–84.
54. Perez Mayorga M, Gromoll J, Behre HM, Gassner C, Nieschlag E, Simoni M. Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. J Clin Endocrinol Metab. 2000;85:3365.
55. Valkenburg O, van Santbrink EJ, König TE, Themmen AP, Uitterlinden AG, Fauser BC, Lambalk CB, Laven JS. Follicle-stimulating hormone receptor polymorphism affects the outcome of ovulation induction in normogonadotropic (World Health Organization class 2) anovulatory subfertility. Fertil Steril. 2015;103:1081–8.
56. La Marca A, Papaleo E, Alviggi C, Ruvolo G, De Placido G, Candiani M. The combination of genetic variants of the FSHB and FSHR genes affects serum FSH in women of reproductive age. Hum Reprod. 2013;28:1369–74.

Part III

Lifestyle Management and Other Treatment Approaches

Lifestyle Interventions and Natural and Assisted Reproduction in Patients with PCOS

13

Renato Pasquali

13.1 Introduction

Polycystic ovary syndrome (PCOS) is the most prevalent female hyperandrogenic disorder [1]. It includes signs and symptoms of androgen excess, ovarian dysfunction and anovulatory infertility and several dysmetabolic conditions, including insulin resistance and all features of the metabolic syndrome [1] (see Chap. 2). Approximately 50% of the affected women are overweight or obese, particularly the abdominal-visceral phenotype, with large differences according to geographical areas and ethnicities [2]. Obesity not only greatly affects the severity of PCOS [3] but also plays a specific pathophysiological role in the development and clinical presentation of PCOS [3]. According to this perspective, we have speculated that a "secondary form of PCOS" may develop because of the negative impact of obesity in the pathophysiological mechanism leading to androgen excess and associated whole body insulin resistance, particularly during adolescent years [4, 5].

One of the major consequences of the PCOS status is represented by chronic infertility that is in turn largely affected by the presence of excess weight and obesity [1]. The negative impact of obesity was recognized a long time ago, and, as a consequence, considerable scientific effort has been devoted to this specific issue, in order to improve fertility rates in affected women. In 2008, the Thessaloniki European Society of Human Reproduction and Embryology (ESHRE)/American Society for Reproductive Medicine (ASRM)-sponsored PCOS consensus workshop group [6] provided specific recommendations on how to manage PCOS patients with obesity and PCOS, in order to improve fertility outcomes. The Thessaloniki group emphasized that obesity adversely affects reproduction and is associated with anovulation, pregnancy loss and late-pregnancy complications. Moreover, it was

R. Pasquali
Division of Endocrinology, Department of Medical and Surgical Sciences, University Alma Mater Studiorum, S. Orsola-Malpighi Hospital, Via Massarenti 9, 40138 Bologna, Italy
e-mail: renato.pasquali@unibo.it

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_13

acknowledged that the presence of obesity in these women is associated with failure of infertility treatment and that weight loss prior to infertility treatment may improve ovulation rates, fecundity and pregnancy complications, in spite of the limited scientific evidence. In addition, there was a general agreement that in obese PCOS patients the treatment of choice should be represented, first, by lifestyle modifications (including caloric restriction and physical exercise) as the first-line intervention, as experienced in other areas of medicine, including obesity per se, diabetes type 2 and others [6].

In the same period of time and based on the same scientific evidence, the British Fertility Society decided that women should aim for a normal body mass index (BMI) before starting any form of infertility treatment and that any pharmacological treatment should be deferred until the BMI is less than 35, although in those relatively younger weight reduction to a BMI of less than 30 would be preferable [7]. In any case, these patients should be provided with assistance to lose weight, including psychological support, dietary advice, exercise classes and, where appropriate, weight-reducing agents or even bariatric surgery. Notably, it was considered that even a moderate weight loss of 5–10% of body weight could be sufficient to restore fertility and improve metabolic markers in most of these patients [7].

More recently, the Endocrine Society Guidelines [8] reinforced the concept that menses and ovulation may improve in PCOS women with as little as 5–10% reduction in body weight, although there was no sufficient evidence that these effects may persist in the long term. It was therefore suggested that, although the response to weight loss is variable and not all patients are able to restore ovulation despite similar weight reduction, consistent evidence nonetheless exists for improved pregnancy rates and a decreased requirement for use of ovulation induction techniques or other fertility treatments of infertility.

13.2 Psychological Symptoms and Health-Related Quality of Life (HRQoL) in PCOS

The investigation and management of psychological disorders is an important part of the methodological approach to women with PCOS [1]. Available data show that PCOS status may have significant negative consequences on both the psychological well-being and HRQoL [2]. Psychological disorders are commonly associated with both the development and maintenance of excess body weight, and there is evidence that in this case affected women have a reduced chance of responding to lifestyle intervention strategies even if associated with pharmacological intervention [2].

Prevalence of anxiety and depression is quite common in these women [9]. Anxiety and particularly depressive traits can be related, among other factors, to infertility [10]. In addition, eating disorders can be frequently observed, particularly in younger women with PCOS, and this may represent an additional factor negatively influencing menses regularities and ovulatory performance [9]. Eating disorders per se are not specific of PCOS; in fact, they are often present even in women with excess weight or obesity [11]. Whether in women with PCOS the

psychological distress related to the PCOS phenotype [specifically hirsutism and menses irregularities] may have some responsibility in the development of eating disorders possibly related to the specific hormonal imbalance is relatively underinvestigated [12]. However, there is no doubt that disordered eating and eating disorders may interfere with any lifestyle interventional procedure [13].

The adaptation to chronic stress exposure can also be altered in PCOS patients, and infertility may play a negative role in this context. It is well known that maladaptation to chronic stress exposure may favour the development of chronic diseases, including obesity, metabolic disorder and psychological functions [14]. There are studies demonstrating that chronic stress exposure may influence the features of PCOS, particularly those related to ovarian dysfunction, including infertility [15].

An additional feature of women with PCOS is represented by the frequently observed negative body image that women develop because of their features, including hirsutism [and acne or alopecia] and, eventually, excess body weight [13].

Further well-designed trials in women with PCOS are important to determine the most effective tools and optimal approaches to assess and manage depression and/or anxiety, eating disorders or disordered eating and negative body image and, finally, overall HRQoL in women with PCOS. This may be very important in the approach of infertile women wishing to become pregnant. In any case, the assessment of psychological symptoms, eating disorders or disordered eating, not only by HRQoL can also be performed with appropriate validated questionnaires [2] or structured interviews [13] that are available on the web. Recognition of these psychological disorders may help the clinician in planning lifestyle interventional programs in order to improve patient adherence and compliance and, ultimately, the efficacy of such a treatment, particularly when pregnancy represents the ultimate aim.

13.3 Evaluation of Dietary Habits in Women with PCOS Before Planning a Lifestyle Intervention

Adequate nutritional status is a critical determinant of the onset and maintenance of normal reproductive function. Unfortunately, the association between abnormal dietary habits and history and risk of PCOS has not been examined in depth, and available data are still sparse and often contradictory. Most researchers found that daily energy intake and diet composition did not differ in the majority of PCOS women compared to controls, although minor discrepancies regarding specific nutrients and food categories in subgroups of women were reported [16]. However, whether women with PCOS have different patterns of dietary intake and food preferences with respect to the unaffected population is far from being established. Interestingly, an increased risk of anovulatory infertility has been associated with a higher consumption of animal proteins, total carbohydrates and foods with a high glycaemic index, low-fat dairy foods and cola beverages in different reports from the Nurses' Health Study cohort [17]. In a case–control study in overweight or obese women with PCOS compared with age- and weight-matched non-PCOS

women [18], we found that diet did not differ between the two groups in relation to energy and macronutrient intake. However, compared to controls, we reported that PCOS women had a higher consumption of cheese and high-glycaemic index starchy sweets and a preference for raw oil rather than other cooked fats. This may have some clinical relevance, since there are few studies linking anovulatory infertility [18] or PCOS [19] to high-glycaemic index food intake. Overall, the available data give little support to the hypothesis of a strong dependence of polycystic ovary syndrome status on nutritional factors.

An interesting new advance in this area is represented by the glycosylated end products [AGEs] story. Diet is a major source of AGEs and other oxidants [20]. The origin of AGEs is strongly related to food preparation techniques and their cooking. Foods cooked at high temperatures and under dry conditions have the highest AGE content, especially if the fat content is high [21]. Dietary AGEs contribute to a state of elevated oxidative stress and inflammation [20, 22] and have been shown to play a role in promoting diabetes, insulin resistance and atherosclerosis in mice [20]. Recent data have shown that oxidative stress may also be involved in the pathophysiology of PCOS [23]. Whether AGE intake differs in obese PCOS women compared to normal weight ones or the normal weight reference population is relatively unknown. An observational cross-sectional study aimed at evaluating eating habits was performed in a population of high school students from Emilia Romagna (Italy), aged between 15–19 years (265 females and 227 males) who underwent a personal interview by a dietician regarding their dietary habits, used to assess the macro- and micronutrient composition of the diet, the quality of the diet according to the Mediterranean diet quality index (M-DQI) and the total amounts of AGEs ingested daily [24]. It was found that most (>90%) of the subjects had a relatively poor M-DQI score and that this score was worse in males than females. Interestingly, the total amount of AGEs ingested daily correlated significantly and positively with M-DQI. Interestingly, both AGEs and advanced oxidation protein products have been found to be higher in women with PCOS than in healthy controls [25] and can be reduced by dietary-induced weight loss [26]. At variance, in the previous study cited above, we documented the lack of significant difference in AGE intake in a relatively small number of PCOS women and non-PCOS controls [18]. Therefore, whether AGE intake is higher in women with PCOS requires more investigation. Nonetheless, given the important negative impact of PCOS status on fertility, whether specific dietary changes may provide some significant better benefit on fertility in these women warrants investigation.

13.4 Does a Healthy Diet Positively Influence Fertility Processes?

A healthy diet may improve fertility for women with ovulatory dysfunction, although data regarding the effects of variations in diet on fertility in anovulatory women, particularly those with PCOS, are very few. Apart from lowering the

malformation risk by periconceptional supplementation of folic acid, data on dietary integration with different micronutrients are often anecdotal.

A potential efficacy of the Mediterranean dietary patterns has been emphasized in some studies [27]. Vitamin D levels are often lower than normal in PCOS women, particularly in the presence of obesity [28]. In some studies, supplementation with vitamin D has been shown to improve ovulation in women with PCOS [29]. Whether supplementation with vitamin D may be effective in improving fertility in response to pharmacological ovulation induction or assisted reproductive technologies (ARTs) requires further investigation.

As summarized in a previous paragraph, specific interest has arisen regarding the potential role of AGEs not only in terms of their pathophysiological role in favouring PCOS but also of their potential role in fertility. Reducing AGE intake may have implications in the treatment of infertile women with PCOS, offering specific dietary advice to improve not only their dysmetabolic milieu but also in improving their reproductive potential [21]. The available literature supports the concept that by modifying preparation methods with the aim of containing the lowest amount of AGE could potentially improve ovulation dysfunction associated with PCOS [21]. However, there are no data comparing different ethnic populations with different diets regarding the impact of AGEs [30]. In any case, the environmental source of AGEs can be reduced by dietary modifications.

A new perspective seems to exist to advocate further investigation of nutritional treatments for infertility patients [31]. Since infertility may be favoured by specific dietary deficiencies or imbalance, it may be that the correction or addition of missing components in the diet may help. The US Nurses' Health Study has shown increased risk of ovulatory dysfunction associated with many dietary factors, including protein intake, dietary fats, carbohydrates, alcohol, caffeine and dairy products [32]. For example, the dietary glycaemic index has been positively correlated with ovulatory infertility, whereas intake of vitamins has been inversely correlated with ovulatory infertility [33]. Animal models of obesity have highlighted oocyte dysfunction, including an increase in granulosa cell apoptosis and impaired oocyte maturation, which may imply an impaired mitochondrial function of the oocyte [31]. In a mouse model, a high incidence of spindle abnormalities and increased reactive oxygen species (ROS) generation in oocyte mitochondria have also been demonstrated [34]. Human studies confirm that oocytes from obese women undergoing in vitro fertilization may have abnormal lipid accumulation and oxidative stress, which implies an impaired development [31]. This may suggest some benefit in seeking to improve mitochondrial function for fertility enhancement. Specifically, it has been shown that antioxidants, cofactors and energy enhancer compounds [including nutrients] may reduce the detrimental effects of reactive oxygen. The idea of a "mitochondrial diet" suggests that by supplementing the necessary cofactors, energy enhancers and antioxidants [such as coenzyme Q10, vitamin C, vitamin E, vitamin B6, selenium, catechins, carnitine, proanthocyanidins, alpha-lipoic acid, *N*-acetylcysteine and omega-3 fatty acids] into a diet might positively impact on fertility [31]. Although there are very few available studies in humans, this nonetheless appears to be an exciting area for further research.

13.5 Definition of Lifestyle Intervention

A lifestyle intervention should be recommended to all women planning a pregnancy but above all in patients with PCOS who are overweight or obese [35]. The term "lifestyle" is often misinterpreted and usually refers to a prescription of a specific hypocaloric diet, possibly combined with a standardized physical activity. Unfortunately, this procedure is largely influenced by a list of factors affecting daily life and currently lasts for only a short period of time, rarely exceeding 6 months [35]. A more comprehensive approach, aimed not only at favouring changes in dietary habits and food choice but also in modifying eating behaviour and the increase in self-confidence [which requires a well-defined methodology including psychological models], is rarely used and often is not part of this type of medical act. The recent Australian Guidelines on treatment of PCOS [13] focused attention on the need for well-defined goals to be achieved, shared between doctor and patient, in order to predict good success. The prospect of a pregnancy, regardless of how it is obtained and managed, can be a perfect reason to increase compliance and intervention programs on lifestyle. Unfortunately, the available literature does not help much in this regard, often because the psychopathological aspects coexisting in the same patient are not considered and the organizational aspects in the follow-up are not adequately planned. These aspects should be much more defined and practiced, especially when ovulation induction therapy or ARTs are planned.

13.6 Lifestyle Intervention in PCOS

13.6.1 Clinical Effectiveness

Weight loss per se may improve PCOS to varying extents, and a number of interventional studies with lifestyle modification with or without the association of insulin sensitizers (particularly metformin) have uniformly demonstrated a significant improvement in many key features of PCOS, including androgen blood levels and the dysmetabolic milieu [35]. Unfortunately, most of these studies are short, which represents their major limitation. In addition, a great interindividual variability in the response to weight loss has been reported, and predictive factors are still largely under-evaluated [36]. On the other hand, it has been shown that when the objectives are well defined, patient empowerment can be increased, and the extent of weight loss can therefore be amplified [37].

Lifestyle modification programs including a structured diet and/or physical activity are recommended in overweight or obese PCOS women in order to favour spontaneous pregnancy and also to improve the chances of pregnancy after ovulation therapies or after ARTs [35]. As above described, this should always be associated with a structured intervention to improve psychological disorders, when present. This may favour adherence to the lifestyle program and individual HRQoL.

A large number of uncontrolled intervention studies have been conducted examining the effect of weight loss through dietary restriction alone in overweight or

obese women with PCOS [35]. Unfortunately, no randomized controlled trials (RCTs) comparing dietary intervention to nonstructured dietary intervention are available. Nonetheless, all the studies demonstrate fairly uniform improvements in many key features of PCOS even with a modest but significant weight loss, specifically on menstrual cyclicity and ovulatory rates, other than on metabolic and hormonal parameters [36]. Unfortunately, few studies reported the outcomes on pregnancy or conception rates, and there are few data on the effect of lifestyle modification on pregnancy outcomes [live birth rate] in obese women with PCOS. A very recent large RCT in obese infertile women has been published [38]. The authors randomly assigned a large group of infertile women with a BMI of 29 or higher to a 6-month lifestyle intervention preceding treatment for infertility or to prompt treatment for infertility. A similar group served as a control group, without any lifestyle plan. The primary outcome was the vaginal birth of a healthy singleton at term within 24 months after randomization. The mean weight loss at the intention-to-treat analyses was 4.4 kg in the intervention group and 1.1 kg in the control group. The primary outcome occurred in 27.1% of the women in the intervention group and in 35.2% of those in the control group. These results were fairly discouraging, since it was found that in obese infertile women a lifestyle intervention preceding infertility treatment, as compared with prompt infertility treatment, did not result in higher rates of a physiological delivery. No similar studies have been performed in obese women with PCOS.

There is no evidence that macronutrient composition of the dietary component of a lifestyle program may have some specific benefit, although a low-fat, moderate protein and high carbohydrate intake in conjunction with moderate regular exercise was recommended for the management of obesity and related comorbidities [35]. This has been confirmed by a recent Cochrane review that reported similar weight loss and compliance for a low-fat diet compared with other approaches [36]. Accordingly, there is no evidence that alternative dietary approaches based on changes in the macronutrient proportions may have more favourable hormonal and metabolic effects or may produce different weight loss. Similar findings from small studies have been shown in reproductive outcomes. With regard to a very low carbohydrate approach, it should be considered that these diets may have safety concerns related to the potential nutritional inadequacy; hitch obviously may be particularly important in a pregnancy.

A recent meta-analysis on the role of lifestyle intervention in women with PCOS included RCTs that enrolled woman of any age with PCOS who received lifestyle intervention and compared them against women who received no intervention, minimal intervention or metformin [39]. The results of this study refer to nine trials enrolling 583 women. Unfortunately, most studies recorded a high loss to follow-up rate, lack of blinding and short follow-up. Nonetheless, some metabolic benefits added to weight loss were found, similar to those obtained by metformin. The use of physical exercise and/or hypocaloric dieting seemed to be efficacious in overweight or obese women with PCOS. However, no significant effect of lifestyle intervention on pregnancy rate was found, probably due to the relatively short (6 months) period of treatment.

13.6.2 Controversial Issues

As previously detailed, there is no doubt that a weight loss of 5–10% of the initial value can lead to an improvement of ovulatory rates and a greater chance of a pregnancy and giving birth to a healthy baby. On the other hand, it has still not been clearly defined what the extent of the weight loss should be in each patient in order to obtain the maximum efficiency of the treatment, in particular of a structured lifestyle program, nor in how much time it should be obtained in order to favour as much as possible a spontaneous pregnancy or a better response to drug therapy of ovulation induction or ARTs. On the other hand, there is evidence that a marked weight loss achieved over a relatively long period can successfully improve ovulatory performance and, potentially, pregnancy rates in obese PCOS women. In a long-term retrospective study performed in a relatively large group of obese PCOS patients treated with a hypocaloric diet (1200 kcal/day) followed by mild caloric restriction and programmed physical activity, with careful reinforcement at the periodical check-ups and with a follow-up period of 20.4 ± 12.5 months, we reported that approximately 35 percent of patients completely recovered from all features of PCOS and, in particular, achieved normal ovarian morphology and ovulation rate [40]. A complete improvement in ovulatory performance obviously suggests a greater chance to be fertile. Results from studies on the efficacy of bariatric surgery in obese women on PCOS are extraordinarily positive in this sense.

Available studies on the effects of bariatric surgery in PCOS women with severe obesity represent convincing evidence of the potential recovery from the PCOS phenotype, provided adequate weight loss is achieved. A recent meta-analysis [41], including 13 primary studies and involving more than 2000 female patients, provided definite information on the efficacy of bariatric surgery in obese PCOS women. It showed that the preoperative incidence of PCOS was 45.6%, which significantly decreased to 6.8% at the 12th month follow-up, parallel to the decrease in BMI from 46.3 to 34.2. Focusing on the criteria defining the PCOS phenotypes, the study found that 56.2% of patients reported preoperative menstrual irregularity, which significantly decreased to 7.7% and that of infertility declined from 18.2% to 4.3% [41]. Overall, these data suggest that infertility can be greatly improved in obese women with PCOS, provided an adequate therapeutic procedure is planned and a considerable weight loss is achieved.

13.7 Obesity, Infertility and PCOS: A Personalized Approach?

Since there is consistent evidence that high BMI has adverse effects on ovulation induction treatment outcomes, women undergoing assisted reproduction may offer a unique opportunity to search for associations between preconceptional treatment plans and reproductive outcomes. In addition, although there are clinical recommendations of advising overweight or obese women to lose weight before planning

Table 13.1 Methodological aspects for a personalized approach with lifestyle intervention program

(1) A strategy to select patients potentially responsive to a lifestyle intervention program in either the short or long-term by the doctor
(2) The need to evaluate behavioural and psychological disturbances before planning any lifestyle treatment is mandatory and, based on the clinical approach, a decision on whether a preliminary psychiatric or psychological approach is needed should be considered. For example, the presence of eating disorders needs primary medical intervention to improve adherence to a lifestyle intervention
(3) The evaluation of the HRQoL of the patient at baseline may help the doctor in planning the lifestyle treatment before any pharmacological and technical procedure is planned
(4) Prior evaluation of dietary habits should always be performed, since this may help to plan dietary changes on an individual basis
(5) The inclusion in the therapeutic plan of physical activity may be difficult in some patients, however in selected patients it may be of great help in improving self-esteem
(6) In patients with massive obesity it is likely a medical treatment based on lifestyle intervention may fail; alternatives, including bariatric surgery, should therefore be assessed
(7) It should be considered that planning a pregnancy may involve different therapeutic strategies and that, in any case, these need time to hopefully obtain effective results, regardless of the therapeutic strategy chosen to achieve a pregnancy

any ovulation induction technique, the quality of available studies published on the topic is still unsatisfactory. Overall, this suggests the need for a personalized intervention based on the woman's needs, particularly on how to treat infertility, and including a therapeutic approach able to improve the chance to become pregnant and give birth to a baby. A tailored comprehensive lifestyle program, together with appropriate drugs, may be part of an individualized therapeutic protocol aimed at reducing weight and metabolic alterations and favouring the fertility process. A personalized approach needs a careful evaluation of many methodological aspects (Table 13.1), including a close collaboration between the patient and the doctors.

Methodological aspects that could be used while planning a lifestyle intervention are summarized in Table 13.1.

13.7.1 The Type of Therapeutic Intervention

The available strategies in women with PCOS favour ovulation and therefore a spontaneous natural pregnancy or, conversely, use therapeutic strategies to induce ovulation or the recourse to ARTs. All these therapeutic approaches represent different conditions that may require specific therapeutic protocols, based on individual needs. The decision to apply a lifestyle interventional program in overweight or obese women with PCOS who wish to achieve a physiological pregnancy may require a different strategy with respect to those who are likely to need an ovulation induction therapy or are started on ARTs. In the former, a long-term lifestyle program in

overweight/obese PCOS women who plan a pregnancy in the long term seems to be rational and extremely useful. Although no studies have addressed the use of a long-term lifestyle program before either ovulation induction therapies or the resort to ARTs, this should be planned in order to investigate whether a sustained weight loss may improve the outcomes of these treatments. Intriguingly, in obese or massively obese PCOS women, this obviously requires a long period of time.

The question of whether an unexpected pregnancy occurring in a woman with PCOS may require a soft lifestyle intervention or a healthy diet represents a further challenge in this area of medicine. If the woman is normal weight, a healthy diet can be advised, aimed at controlling weight gain and providing a physiological environment for the foetus. Pregnancy, childbirth and lactation represent the terminal processes of the reproductive potential of a woman, requiring a further important energy adaptation, given the considerable expenditure that these conditions entail. In addition, energy expenditure varies according to initial BMI levels of pregnant women based on the socio-economic condition [42]. In these cases, a healthy diet could be recommended, providing the opportunity to avoid excessive medicalization of the patient.

Conclusion

The use of lifestyle intervention in obese women with PCOS, aimed to improve fertility, still represents a controversial issue. Apart from the Australian Guidelines [13], it was not made any effort in the rest of world to plan the best strategy. It appears of particular importance the opportunity to assess, before starting a lifestyle program, if there are psychological problems that could adversely affect the outcomes. What seems absolutely necessary is a plan for long-term controlled studies that, depending on the type of patient and the therapeutic strategy choice, can lead to effective clinical results. Finally, it should be considered that this type of intervention should be as possible based on individual needs that often require different strategies, with long lead times and clearly defined goals.

References

1. McCartney CR, Marshall JC. Clinical practice. Polycystic ovary syndrome. N Engl J Med. 2016;375:54–64.
2. Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, Gambineri A, Kelestimur F, Macut D, Micic D, Pasquali R, Pfeifer M, Pignatelli D, Pugeat M, Yildiz BO, ESE PCOS Special Interest Group. The polycystic ovary syndrome: a position statement from the European Society of Endocrinology. Eur J Endocrinol. 2014;171:P1–29.
3. Pasquali R, Diamanti-Kandarakis E, Gambineri A. Management of endocrine disease: secondary polycystic ovary syndrome: theoretical and practical aspects. Eur J Endocrinol. 2016;175:R157–69.
4. Pasquali R, Gambineri A. A comprehensive approach in diagnosing the polycystic ovary syndrome. Womens Health (Lond). 2015;11:501–12.
5. Rosenfield RL. Clinical review: adolescent anovulation: maturational mechanisms and implications. J Clin Endocrinol Metab. 2013;98:3572–83.

6. The Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Hum Reprod. 2008;23:462–77.
7. Balen AH, Anderson R. Impact of obesity on female reproductive health: British fertility society, police and practice guidelines. Hum Fertil (Camb). 2007;10:195–206.
8. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, Welt CK, Society E. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2013;98:4565–92.
9. Barry J, Kuczmierczyk A, Hardiman P. Anxiety and depression in PCOS: a systematic review and meta-analysis. Hum Reprod. 2011;26:2442–51.
10. Tan S, Hahn S, Benson S, Janssen O, Dietz T, Kimmig R, Hesse-Huissain J, Mann K, Schedlowsky M, Arck P. Psychological implications of infertility in women with PCOS. Hum Reprod. 2008;23:2064–71.
11. Kerchner A, Lester W, Stuart SP, Dokras A. Risk of depression and other mental health disorders in women with polycystic ovary syndrome: a longitudinal study. Fertil Steril. 2009;91:207–12.
12. Hahn S, Janssen OE, Tan S, Pleger K, Mann K, Schedlowski M, Kimmig R, Benson S, Balamitsa E, Elsenbruch S. Clinical and psychological correlates of quality-of-life in polycystic ovary syndrome. Eur J Endocrinol. 2005;153:853–60.
13. Misso M, Boyle J, Norman R, Teede H. Development of evidenced-based guidelines for PCOS and implications for community health. Semin Reprod Med. 2014;32:230–40.
14. Pasquali R. The hypothalamic-pituitary-adrenal axis and sex hormones in chronic stress and obesity: pathophysiological and clinical aspects. Ann N Y Acad Sci. 2012;1264:20–35.
15. Elsenbruch S, Benson S, Hahn S, Tan S, Mann K, Pleger K, Kimmig R, Jansen O. Determinants of emotional distress in women with PCOS. Hum Reprod. 2006;21:1092–9.
16. Chavarro JE, Rich-Edwards JW, Rosner BA, Willett WC. A prospective study of dairy foods intake and anovulatory infertility. Hum Reprod. 2007;22:1340–7.
17. Chavarro JE, Rich-Edwards JW, Rosner BA, Willett WC. A prospective study of dietary carbohydrate quantity and quality in relation to risk of ovulatory infertility. Eur J Clin Nutr. 2009;63:78–86.
18. Altieri P, Cavazza C, Pasqui F, Morselli AM, Gambineri A, Pasquali R. Dietary habits and their relationship with hormones and metabolism in overweight and obese women with polycystic ovary syndrome. Clin Endocrinol. 2013;78:52–9.
19. Douglas CC, Norris LE, Oster RA, Darnell BE, Azziz R, Gower BA. Difference in dietary intake between women with polycystic ovary syndrome and healthy controls. Fertil Steril. 2006;86:411–7.
20. Merhi Z. Advanced glycation end products and their relevance in female reproduction. Hum Reprod. 2014;29:135–45.
21. Garg D, Merhi Z. Advanced glycation end products: link between diet and ovulatory dysfunction in PCOS? Forum Nutr. 2015;7:10129–44.
22. Piperi C, Adamopoulos C, Dalagiorgou G, Diamanti-Kandarakis E, Papavassiliou AG. Crosstalk between advanced glycation and endoplasmic reticulum stress: emerging therapeutic targeting for metabolic diseases. J Clin Endocrinol Metab. 2012;97:2231–42.
23. Papalou O, Victor VM, Diamanti-Kandarakis E. Oxidative stress in polycystic ovary syndrome. Curr Pharm Des. 2016;22:2709–22.
24. Tarabusi V, Cavazza C, Pasqui F, Gambineri A, Pasquali R. Quality of diet, screened by the Mediterranean diet quality index and the evaluation of the content of advanced glycation end products, in a population of high school students from Emilia Romagna. Mediterr J Nutr Metab. 2010;3:153–7.
25. Diamanti-Kandarakis E, Katsikis I, Piperi C, Kandaraki E, Piouka A, Papavassiliou AG, Panidis D. Increased serum advanced glycation end-products is a distinct finding in lean women with polycystic ovary syndrome (PCOS). Clin Endocrinol. 2008;69:634–41.
26. Tantalaki E, Piperi C, Livadas S, Kollias A, Adamopoulos C, Koulouri A, Christakou C, Diamanti-Kandarakis E. Impact of dietary modification of advanced glycation end products (AGEs) on the hormonal and metabolic profile of women with polycystic ovary syndrome (PCOS). Hormones (Athens). 2014;13:65–73.

27. Vujkovic M, de Vries JH, Lindemans J, Macklon NS, van der Spek PJ, Steegers EA, Steegers-Theunissen RP. The preconception Mediterranean dietary pattern in couples undergoing in vitro fertilization/intracytoplasmic sperm injection treatment increases the chance of pregnancy. Fertil Steril. 2010;94:2096–101.
28. Lerchbaum E, Rabe T. Vitamin D and female fertility. Curr Opin Obstet Gynecol. 2014;26:145–50.
29. Dabrowski FA, Grzechocinska B, Wielgos M. The role of vitamin D in reproductive health–a Trojan horse or the golden fleece? Forum Nutr. 2015;7:4139–53.
30. Pasquali R, Stener-Victorin E, Yildiz BO, Duleba AJ, Hoeger K, Mason H, Homburg R, Hickey T, Franks S, Tapanainen JS, Balen A, Abbott DH, Diamanti-Kandarakis E, Legro RS. PCOS Forum: research in polycystic ovary syndrome today and tomorrow. Clin Endocrinol. 2011;74:424–33.
31. Shaum KM, Polotsky AJ. Nutrition and reproduction: is there evidence to support a "fertility diet" to improve mitochondrial function? Maturitas. 2013;74:309–12.
32. Chavarro JE. Diet and lifestyle in the prevention of ovulatory disorder infertility. Obstet Gynecol. 2007;110:1050–8.
33. Chang AS, Dale AN, Moley KH. Maternal diabetes adversely affects preovulatory oocyte maturation, development, and granulosa cell apoptosis. Endocrinology. 2005;146:2445–53.
34. Wittemer C, Ohl J, Bailly M, Bettahar-Lebugle K, Nisand I. Does body mass index of infertile women have an impact on IVF procedure and outcome? J Assist Reprod Genet. 2000;17:547–52.
35. Moran LJ, Pasquali R, Teede HJ, Hoeger KM, Norman RJ. Treatment of obesity in polycystic ovary syndrome: a position statement of the androgen excess and polycystic ovary syndrome society. Fertil Steril. 2009;92:1966–82.
36. Moran LJ, Hutchison SK, Norman RJ, Teede HJ. Lifestyle changes in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2011;7:CD007506.
37. Crosignani PG, Colombo M, Vegetti W, Somigliana E, Gessati A, Ragni G. Overweight and obese anovulatory patients with polycystic ovaries: parallel improvements in anthropometric indices ovarian physiology and fertility rate induced by diet. Hum Reprod. 2003;18:1928–32.
38. Mutsaerts MAQ, van Oers AM, Groen H, Burggraaff JM, Kuchenbecker WK, Perquin DA, Koks CA, van Golde R, Kaaijk EM, Schierbeek JM, Oosterhuis GJ, Broekmans FJ, Bemelmans WJ, Lambalk CB, Verberg MF, van der Veen F, Klijn NF, Mercelina PE, van Kasteren YM, Nap AW, Brinkhuis EA, Vogel NE, Mulder RJ, Gondrie ET, de Bruin JP, Sikkema JM, de Greef MH, ter Bogt NC, Land JA, Mol BW, Hoek A. Randomized trial of a lifestyle program in obese infertile women. N Engl J Med. 2016;374:1942–53.
39. Domecq JP, Prutsky G, Mullan RJ, Hazem A, Sundaresh V, Elamin MB, Phung OJ, Wang A, Hoeger K, Pasquali R, Erwin P, Bodde A, Montori VM, Murad MH. Lifestyle modification programs in polycystic ovary syndrome: systematic review and meta-analysis. J Clin Endocrinol Metab. 2013;98:4655–63.
40. Pasquali R, Gambineri A, Cavazza C, Ibarra Gasparini D, Ciampaglia W, Cognigni GE, Pagotto U. Heterogeneity in the responsiveness to long-term lifestyle intervention and predictability in obese women with polycystic ovary syndrome. Eur J Endocrinol. 2011;164:53–60.
41. Skubleny D, Switzer NJ, Gill RS, Dykstra M, Shi X, Sagle MA, de Gara C, Birch DW, Karmali S. The impact of bariatric surgery on polycystic ovary syndrome: a systematic review and meta-analysis. Obes Surg. 2016;26:169–76.
42. Butte NF, King JC. Energy requirements during pregnancy and lactation. Public Health Nutr. 2005;8:1010–27.

Dietary Supplements, Phytotherapy and Chinese Herbal Medicine in PCOS

14

Xiao-Ke Wu and Ernest HY Ng

14.1 Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common reproductive endocrinology abnormalities; according to the Rotterdam diagnostic criteria, it affects 5–10% of women of reproductive age in Caucasian population and 5.6% of the Chinese women aged 19–45 years [1]. Western medicines, such as oral contraceptives and insulin sensitisers, have been widely used to improve the symptoms and signs of PCOS. Except the treatment mentioned above, there are some other complementary and alternative medicines (CAMs) including dietary supplements, phytotherapy and Chinese herbal medicine (CHM) for treating PCOS.

14.2 Dietary Supplements

Several dietary supplements may have beneficial effects on women with PCOS. However, most studies in this area are small or uncontrolled. Therefore, well-designed studies are needed to further evaluate the benefits and risks of these supplements in PCOS. In addition, it is important to note that the supplements discussed here are not approved by the Food and Drug Administration (FDA) or other national agencies for the treatment of PCOS.

X.-K. Wu (✉)
Department of Obstetrics and Gynaecology, First Affiliated Hospital, Heilongjiang University of Chinese Medicine, Harbin, China
e-mail: xiaokewu2002@vip.sina.com

E.H. Ng
Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong, China

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_14

14.2.1 Vitamin D

Accumulating evidence suggests that vitamin D deficiency may be a causal factor in the pathogenesis of insulin resistance (IR) and the metabolic syndrome in PCOS [2]. Furthermore, 25-hydroxyvitamin D levels are closely associated with impaired cell function, impaired glucose tolerance (IGT) and the metabolic syndrome in PCOS women [3]. Two small, uncontrolled studies demonstrate that vitamin D may improve IR and lipid profiles in PCOS patients [4, 5]. One of the two studies showed a significant reduction in homeostatic model assessment of insulin resistance (HOMA-IR) 3 weeks after a single oral vitamin D 3 dose of 300,000 IU in 11 obese, insulin-resistant women with PCOS [5, 6]. Moreover, vitamin D supplementation may also improve anovulation in PCOS. A pilot randomised controlled trial (RCT) of 60 infertile PCOS patients showed that the number of dominant follicles (14 mm) during 2–3 months of follow-up was higher in the calcium (1000 mg/day) plus vitamin D (400/day) plus metformin (1500 mg/day) group than in the calcium-vitamin D-only group or the metformin-only group [6]. Besides, a recent study showed that using calcium combined with vitamin D for 8 weeks among overweight and vitamin D-deficient women with PCOS had positive effects on inflammatory factors and biomarkers of oxidative stress compared with using vitamin D or calcium alone [7].

14.2.2 Vitamin B_{12} and Folate

Two recent studies suggest that B vitamins may be important in PCOS. In the first study, IR, obesity and elevated homocysteine were associated with lower serum vitamin B_{12} concentrations in PCOS patients [8]. The second study was a non-randomised, placebo-controlled, double-blind trial that demonstrated that supplementation with folate (400 mg daily) for 6 months increased the beneficial effect of metformin on the vascular endothelium in women with PCOS [9]. However, the mechanisms involved are still unclear.

14.2.3 Green Tea and Spearmint Tea

Tea, next only to water, is the most popularly consumed beverage in the world, with a per capita consumption of 120 ml/day [10]. Green tea has been shown to exert beneficial effects on glucose and lipid metabolism [11, 12] and the hormonal system [13, 14] in rats and humans, which are all very relevant in the management of PCOS patients. In addition, herbal tea reduces body weight and induces ovulation in androgen-sterilised rats [15]. However, there are only two RCTs of herbal tea in PCOS, one using green tea [16] and the other spearmint tea [17]. The principal component of green tea, (−)-epigallocatechin-3-gallate (EGCG), significantly reduced body weight and circulating testosterone, oestradiol, leptin, insulin, insulin growth factor (IGF)-I, luteinizing hormone (LH), glucose, cholesterol and triglyceride in Sprague Dawley rats and lean and obese Zucker rats [15]. In vitro studies

demonstrate that green tea extract and EGCG inhibit basal and stimulated testosterone production in rat Leydig cells. The mechanisms underlying the effects of EGCG involve the in vitro inhibition of the PKA/PKC signalling pathways as well as the inhibition of P-450 side-chain cleavage enzyme and 17-hydroxysteroid dehydrogenase function during testicular steroidogenesis [14].

In an RCT of 34 obese Chinese women with PCOS, the body weight of the green tea capsule group (540 mg EGCG/day) decreased by a non-significant 2.4% after treatment, whereas the body weight, body mass index (BMI) and body fat content of the control group were significantly higher after 3 months [17]. However, there were no significant differences in glucose, lipid metabolism or any of the hormone levels between the two groups. The lack of a positive finding in this study may be due to an inadequate dose of green tea and the small sample size of the study. Furthermore, the response to EGCG may be greater in other ethnic groups, especially those groups who do not already have a strong habit of taking tea in their daily life [18].

With regard to spearmint tea, an RCT of 41 PCOS women showed that spearmint tea twice a day for 1 month significantly decreased free and total testosterone levels, improved patients' subjective assessments of their hirsutism and increased LH and follicle stimulating hormone (FSH) compared with a placebo herbal tea [18]. Further studies are needed to confirm these findings and further elucidate the mechanisms underlying the anti-androgenic effects of spearmint tea.

14.2.4 Cinnamon Extract

Cinnamon extract (a traditional herb) has been shown to potentiate the insulin effect through upregulation of glucose uptake in cultured adipocytes [19–21]. Cinnamon extract also improves insulin action via increasing glucose uptake in vivo, as it has been shown to enhance the insulin signalling pathway in skeletal muscle in rats [22]. An RCT of 15 women with PCOS showed significant reduction in IR in the cinnamon group (333 mg of cinnamon extract, 3 times a day) but not in the placebo group [23].

14.2.5 ω-3 and Other Polyunsaturated Fatty Acids

A small RCT of 25 PCOS women demonstrated that dietary supplementation with ω-3 fatty acid 4 g/day (4 × 1000-mg capsules of 56% docosahexaenoic acid and 27% eicosapentaenoic acid) for 8 weeks has beneficial effects on liver fat content and other cardiovascular risk factors in women with PCOS [24]. Another small study of 17 women with PCOS showed that increased dietary polyunsaturated fatty acid (PUFA) intake from walnuts (48 g walnuts per 800 kcal energy intake) for 3 months increased glucose levels in women with PCOS [25]. Forty-eight grams of walnuts contain 311 kcal (70 kcal from 30 g fat, 28 kcal from 7 g protein, and 36 kcal from 9 g carbohydrates) and provide 19 g of linolenic acid and 3.3 g of

α-linolenic acid. Further studies are needed to determine the risks and benefits of ω-3 fatty acids and other PUFAs in PCOS.

14.2.6 Micronutrients

Several RCTs [26–28] showed that selenium, zinc and chromium supplement reduced serum insulin levels, markers of IR (HOMA-IR and QUICKI) and triglyceride concentrations compared with placebo. Besides, zinc and chromium reduced fasting glucose and VLDL-C concentrations.

14.3 Chinese Herbal Medicine (CHM)

Recently, many studies have been published considering CHM as an alternative treatment for women with PCOS [29]. In both developed and developing countries, there is an increasing public interest in, and use of, a wide range of therapies which lie outside the 'mainstream' or traditional Western medical practice [30]. To be more intuitive, 99% of 648 women responded 'yes' when asked whether they would be interested if their PCOS could be safely and effectively helped by something else besides fertility drugs or birth control pills [30].

14.3.1 Traditional Chinese Medicine (TCM)

TCM is a well-defined healthcare profession with its practice of acupuncture and herbal medicine guided by a coherent and an evolving body of knowledge and underpinned by its unique philosophy, holism and ongoing scientific endeavour [31]. Basic knowledge of TCM includes the philosophy, medical theories, diagnostic system, therapeutic studies including acupuncture or medicinal substances and clinical studies [32]. The major theories of TCM include the Yin-Yang, the Five Elements, Qi and Blood and Zang-fu organ theories. In TCM, the understanding of the human body is based on the holistic understanding of the universe as described in Daoism, and the treatment of illness is based primarily on the diagnosis and differentiation of syndromes.

CHM acts on Zang-fu organs internally, and acupuncture is accomplished by stimulating certain areas of the external body [32]. Chinese medical theory includes traditional physiological concepts, maintenance of health, processes in the development of disease and approaches to therapy. Body and mind are viewed as part of a broader ecological system which includes both environmental and socio-emotional factors [32]. The diagnostic system involves the identification of the disease state and the underlying symptom pattern (zheng). This is often referred to as a dual diagnostic system (bian zheng lun zhi) [32]. Accurate diagnosis of both the disease

and the symptom pattern type is essential for setting treatment priorities and determining the treatment.

CHM is an integral part of TCM [33], which has been practiced for more than 2500 years. It was the only way of healthcare in China before the introduction of modern Western medicine into China. In China today, TCM is often administered as a complement to Western medicine. While TCM traces its roots back thousands of years, it rests, from the view of evidence-based medicine, more on a philosophy than a science. Much of the central philosophy involves maintaining the balanced flow of life energy (qi). TCM views organ systems as contributing to mind-body states and tries to address imbalances of these organ systems [34]. In TCM, all diseases are classified into different syndromes (such as deficiency or excess) [34]. Thus, PCOS can be classified as two diseases: amenorrhoea (failure to menstruate) and infertility according to the presentations (symptoms and signs) of women with PCOS [30]. Even so, it is also aimed to elevate outcomes such as pregnancy rate of PCOS by using CHM.

TCM is based on Chinese medical practice including various forms of herbal medicine, acupuncture, massage (tui na), exercise (qigong) and dietary therapy, but we will just discuss the herbal medicine and the phytotherapy in this chapter. Traditionally, CHMs are combined in varying preparations. Although some preparations are regulated by the government, there remain concern about quality control of individual formulations, given the variation in plant quality from harvest to harvest, and concerns about harmful supplements or by-products of preparation such as heavy metals, herbicides, pesticides, microorganisms, mycotoxins, insects, pharmaceuticals, etc. [35, 36]. CHMs also include many animal by-products that we will not discuss in detail in this chapter too. For example, a common preparation used to induce ovulation in women with PCOS is Di Long (earth dragon), which is made from abdominal extracts of the red earthworm *Lumbricus rubellus* [34].

14.3.2 Physiological Mechanisms of CHMs

The aetiology and clinical characteristics of PCOS still remain controversial but are believed to be related to the disorders of kidneys, liver and spleen, and from TCM perspective, reproductive function is regarded as being governed by kidneys. It is believed that kidney deficiency may be the main problem in PCOS [37, 38].

Currently, the physiological mechanisms for efficacy of most CHMs are unknown in PCOS [34]. Our searches in Chinese databases identified 125 clinical trials using CHM either alone (54 studies) or in combination with conventional drugs (71 studies) for treatment of PCOS (unpublished data). The majority of the studies were RCTs. The formulation of herbal medicine was mainly based on symptom differentiations, and herbal compounds (mixture of herbs) were typically used. Critical appraisals of 15 trials show improvement of the methodological quality in terms of randomisation, blinding and intention to treat in recent trials. Most of the identified

trials reported promising effects for PCOS patients. The potential action of herbal medicine in PCOS may be related to regulation of hormones such as LH, FSH, oestradiol and testosterone. However, publication bias could not be excluded, and further analysis of data in systematic reviews is required [32].

Many may have selective oestrogenic effects and function like clomiphene citrate (CC; see Chap. 9) to induce ovulation. For example, *Rhizoma alismatis* has been found in an in vitro tissue model to inhibit intestinal glucose absorption and stimulate glucose uptake in fibroblasts and adipocytes [39]. Furthermore, in a streptozotocin-induced diabetes mouse model, it lowers plasma glucose and triglycerides and improves insulin levels [40]. Other CHMs, such as *Radix notoginseng*, have been found to have similar antidiabetic effects in mouse models, improving not only glucose tolerance and insulin action in a dose-response fashion but also ameliorating obesity [41]. Similarly, *Salvia miltiorrhiza bunge* has been shown to significantly improve glucose tolerance in a prenatally androgenised rat model of PCOS and to favourably impact insulin signalling in treated animals [42]. Berberine, a component of *Rhizoma coptidis*, has been shown to improve glucose uptake and insulin action in human thecal cells with dexamethasone-induced IR. These favourable changes in glucose metabolism have also been shown to favourably alter sex steroid feedback or production, ameliorating hyperandrogenism in these models [43]. Additionally, antioxidant activity has been noted in vitro for a number of these substances [44, 45].

14.3.3 CHM Formulae

In TCM, there are three different therapeutic strategies to treat PCOS by CHM. Firstly, only one special formula comprising of sovereign medicinal (the ingredient that provides the principal curative action on the main pattern/syndrome or primary symptom) is prescribed to patients throughout the whole menstrual cycle. This formula is occasionally combined with some minister medicinal (the ingredient that helps strengthen the principal curative action) and assistant medicinal (the ingredient that treats the combined pattern/syndrome, relieves secondary symptoms or tempers the action of the sovereign ingredient when the latter is too potent) accordingly to one's individual symptoms and signs [46–53]. Secondly, different formulae are periodically prescribed to patients with PCOS according to individual's menstrual period cycle. This strategy is aimed to resume one's normal reproductive endocrinological function [54, 55]. Last but not least, CHMs are used to combine with the Western medicines in treating PCOS [56–60].

Some of the formula can help treat the PCOS, for example, Bushen Huoxue formula, and its basic formula is as follows: tu si zi 20 g, shu di 10 g, sang ji sheng 20 g, xian ling pi 15 g, bu gu zhi 10 g, huang jing 10 g, zao jiao ci 15 g, tao ren 10 g, shan ci gu 10 g, dan shen 10 g, gan cao 6 g plus huang qi 20 g, shan zha 10 g, fa ban xia 10 g in obese patients plus zhi mu 10 g, huang qin 10 g in hirsutism or acne patients [61].

Tanshinones are a class of bioactive constituents isolated from *S. miltiorrhiza* (danshen), which is a commonly used herb in TCM. Cryptotanshinone is the major bioactive tanshinone in the plant and has several pharmacological effects including anti-inflammatory, anti-oxidative, anticholinesterase, antibacterial and antiplatelet aggregation and anticancer activities [62–64]. CHM has been used for the treatment of PCOS, but the evidence for its efficacy and safety is minimal. Animal experiments showed that cryptotanshinone can induce favourable alterations in androgen excess and IR as well as glucose metabolism [65], but there is still a lack of scientific justification for the use of tanshinone in women with PCOS. In particular, no randomised controlled trials have been performed to evaluate the use of tanshinone on hyperandrogenism, metabolic profiles or the quality of life in women with PCOS who do not wish to conceive.

Cinnamon is obtained from the inner bark of several trees from the genus *Cinnamomum*. In TCM, cinnamon can be used for the treatment of amenorrhoea caused by kidney deficiency. Cinnamon has been found to have insulin-sensitising effects in both animal and human studies [20, 66, 67]. Besides, in a prospective trial, compared with the control group, patients taking cinnamon had a more frequent menstruation [68]. But the specific mechanism is still not clear.

Berberine, the major active component of Rhizoma coptidis, exists in a number of medicinal plants and displays a broad array of pharmacological effects [69]. In Chinese medicine, berberine has long been used for its antidiabetic effects. Recently, berberine has been shown to have positive effects on type 2 diabetes mellitus, IR, lipid metabolism, nitric oxide production and metabolic syndrome [70–73]. The mechanisms of berberine in treating PCOS are still unclear. The beneficial metabolic effects of berberine in diabetic animals and type 2 diabetes mellitus patients are through the activation of AMP-activated protein kinase (AMPK) [72, 74], which is similar to metformin.

14.3.4 Limitations of Studies on CHM

CHMs have been used as an alternative approach for subfertile women with PCOS [30]. However, efficacy and safety hamper the development of CHMs because there is minimal evidence that CHMs are safe and efficacious. Most of the trials have been small and thus inadequately powered to detect true differences. Most, not surprisingly, have been conducted mainly in Chinese populations and published in Chinese and thus are not easily accessible [34]. The studies have also tested a large number of varying preparations (most containing multiple components), and thus there has been little to no replication for individual preparations (Table 14.1).

The studies have been of poor methodological quality without adherence to existing Consolidated Standards of Reporting Trials (CONSORT) guidelines [34]. This is well illustrated by systematic reviews of CHM in subfertile women with PCOS [30] and patients with IGT [75] and type 2 diabetes mellitus [37], disorders

Table 14.1 Some of CHMs used to treat PCOS, their proposed mechanisms of action and their reported side effects [34]

Mechanism	Chinese name	Latin name	English name	Adverse effects
Improve insulin sensitivity	Baishao	*Radix paeoniae Alba*	White peony root	Uterine contractions, interfere with blood clotting
	Danggui	*Radix angelicae Sinensis*	Angelica	Uterine contractions
	Danshen	*Salvia miltiorrhiza Bunge*	Red sage	May interact and potentiate effects of warfarin
	Huang Lian	*Rhizoma coptidis*	Red sage	Hypertension, respiratory failure, paraesthesias
Induce ovulation (through oestrogenic effects)	Luole	*Ocimum basilicum*	Basil	Contains a chemical, estragole, which has caused liver cancer in mice
	Sanqi	*Radix no-toginseng*	Panax pseudo ginseng	Dry mouth, flushed skin, nervousness, sleep problems, nausea and vomiting
Inhibit androgen synthesis	Zelan	*Herba lycopi*	Bugleweed	Enlarged thyroid gland, hypoglycaemia
	Zexie	*Rhizoma alismatis*	Water plantain	Fresh rootstock may be poisonous
	Gancao	*Radix glycytthizae*	Licorice	Hypertension, fluid retention, hypokalaemia exacerbated kidney disease

related to PCOS because of the common underlying link of IR (Table 14.2). Although there is currently insufficient evidence about the safety and efficacy of CHM for the management of PCOS, a systematic review in this area was warranted. No systematic review on this topic has ever been done. What's more, there is limited evidence that the addition of CHM to CC have improved clinical pregnancy outcomes but no other evidence of an effect.

Besides, it is well known that not all herbs are risk free. There are concerns about adverse events, including allergic reactions and Chinese herbal nephropathy (CHN) [76–78]. For example, Gancao or licorice, given chronically or in excess, can cause an acquired form of apparent mineralocorticoid excess, as it is a potent inhibitor of 11β-hydroxysteroid dehydrogenase. This enzyme inactivates cortisol to cortisone, and decreased inactivation, especially in the kidney, can lead to excess cortisol cross-reacting with the mineralocorticoid receptor, which induces fluid retention, hypokalaemia and hypertension [79]. In addition, CHMs may interfere with the metabolism

Table 14.2 List of systematic reviews of CHM for treatment of PCOS and disorders of glucose metabolism including type 2 diabetes [34]

Topic	Total no. of studies retrieved	Total studies included in Cochrane review	Total no. of studies in Chinese	Total no. of subjects in included studies	Total no. of preparations tested in trials	Main conclusions
Subfertile PCOS [24]	267	4	4	334	6	Limited evidence that addition of CHM to clomiphene is associated with improved clinical pregnancy outcomes and no other evidence of any other effect. Methodology of RCTs was not adequately reported.
Impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) [61]	1926	16	15	1391	15	Some positive evidence favours CHM for treatment of IGT or IFG. Limited by the following factors: lack of trials that tested the same herbal medicine, lack of details on cointerventions, unclear methods of randomisation, poor reporting and other risks of bias.
Type 2 diabetes [31]	713	66	61	8302	69	Some herbal medicines show hypoglycaemic effect in type 2 diabetes. However, these findings are limited by low methodological quality, small sample size and limited number of trials.

of other drugs used to treat PCOS. For example, plantain has been proposed to interfere with many commonly prescribed medications such as digitoxin and tricyclic antidepressants, although at least one study shows no clinical interactions [80].

Conclusion

Some of the supplements may have positive effects on PCOS, but there was insufficient evidence to determine the efficacy of them. Furthermore, in daily life, women with PCOS can consume more vitamin D, vitamin B_{12} and EGCG or other components through dietetic invigoration, or they can visit a professional TCM doctor for treatment based on different syndrome. Last but not least, more well-designed larger trials should be carried out to ensure the efficacy and safety of these supplements. The scientists should also explore the mechanism of CHM.

References

1. Li R, Zhang Q, Yang D, et al. Prevalence of polycystic ovary syndrome in women in China: a large community-based study. Hum Reprod. 2013;28:2562–9.
2. Hahn S, Haselhorst U, Tan S, Quadbeck B, Schmidt M, Roesler S, Kimmig R, Mann K, Janssen OE. Low serum 25-hydroxyvitamin D concentrations are associated with insulin resistance and obesity in women with polycystic ovary syndrome. Exp Clin Endocrinol Diabetes. 2006;114:577–83.
3. Wehr E, Pilz S, Schweighofer N, Giuliani A, Kopera D, Pieber TR, Obermayer-Pietsch B. Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. Eur J Endocrinol. 2009;161:575–82.
4. Kotsa K, Yavropoulou MP, Anastasiou O, Yovos JG. Role of vitamin D treatment in glucose metabolism in polycystic ovary syndrome. Fertil Steril. 2009;92:1053–8.
5. Selimoglu H, Duran C, Kiyici S, Ersoy C, Guclu M, Ozkaya G, Tuncel E, Erturk E, Imamoglu S. The effect of vitamin D replacement therapy on insulin resistance and androgen levels in women with polycystic ovary syndrome. J Endocrinol Investig. 2010;33:234–8.
6. Rashidi B, Haghollahi F, Shariat M, Zayerii F. The effects of calcium-vitamin D and metformin on polycystic ovary syndrome: a pilot study. Taiwan J Obstet Gynecol. 2009;48:142–7.
7. Foroozanfard F, Jamilian M, Bahmani F, et al. Calcium plus vitamin D supplementation influences biomarkers of inflammation and oxidative stress in overweight and vitamin D-deficient women with polycystic ovary syndrome: a randomized double-blind placebo-controlled clinical trial. Clin Endocrinol. 2015;83:888–94.
8. Kaya C, Cengiz SD, Satiroglu H. Obesity and insulin resistance associated with lower plasma vitamin B12 in PCOS. Reprod Biomed Online. 2009;19:721–6.
9. Palomba S, Falbo A, Giallauria F, Russo T, Tolino A, Zullo F, Colao A, Orio F. Effects of metformin with or without supplementation with folate on homocysteine levels and vascular endothelium of women with polycystic ovary syndrome. Diabetes Care. 2010;33:246–51.
10. McKay DL, Blumberg JB. The role of tea in human health: an update. J Am Coll Nutr. 2002;21:1–13.
11. Chantre P, Lairon D. Recent findings of green tea extract AR25 (Exolise) and its activity for the treatment of obesity. Phytomedicine. 2002;9:3–8.
12. Dulloo AG, Duret C, Rohrer D, Girardier L, Mensi N, Fathi M, Chantre P, Chantre P. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. Am J Clin Nutr. 1999;70:1040–5.
13. Figueiroa MS, Cesar Vieira JS, Leite DS, Filho RC, Ferreira F, Gouveia PS, Udrisar DP, Wanderley MI. Green tea polyphenols inhibit testosterone production in rat Leydig cells. Asian J Androl. 2009;11:362–70.
14. Kao YH, Hiipakka RA, Liao S. Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. Endocrinology. 2000;141:980–7.
15. Sun F, Yu J. The effect of a special herbal tea on obesity and anovulation in androgen-sterilized rats. Proc Soc Exp Biol Med. 2000;223:295–301.

16. Chan CC, Koo MW, Ng EH, Tang OS, Yeung WS, Ho PC. Effects of Chinese green tea on weight, and hormonal and biochemical profiles in obese patients with polycystic ovary syndrome—a randomized placebo-controlled trial. J Soc Gynecol Investig. 2006;13:63–8.
17. Grant P. Spearmint herbal tea has significant anti-androgen effects in polycystic ovarian syndrome. A randomized controlled trial. Phytother Res. 2010;24:186–8.
18. Yu Ng EH, Ho PC. Polycystic ovary syndrome in asian women. Semin Reprod Med. 2008; 26:14–21.
19. Anderson RA, Broadhurst CL, Polansky MM, Schmidt WF, Khan A, Flanagan VP, Schoene NW, Graves DJ. Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. J Agric Food Chem. 2004;52:65–70.
20. Broadhurst CL, Polansky MM, Anderson RA. Insulin-like biological activity of culinary and medicinal plant aqueous extracts in vitro. J Agric Food Chem. 2000;48:849–52.
21. Jarvill-Taylor KJ, Anderson RA, Graves DJ. A hydroxychalcone derived from cinnamon functions as a mimetic for insulin in 3T3-L1 adipocytes. J Am Coll Nutr. 2001;20:327–36.
22. Qin B, Nagasaki M, Ren M, Bajotto G, Oshida Y, Sato Y. Cinnamon extract (traditional herb) potentiates in vivo insulin-regulated glucose utilization via enhancing insulin signaling in rats. Diabetes Res Clin Pract. 2003;62:139–48.
23. Wang JG, Anderson RA, Graham III GM, Chu MC, Sauer MV, Guarnaccia MM, Lobo RA. The effect of cinnamon extract on insulin resistance parameters in polycystic ovary syndrome: a pilot study. Fertil Steril. 2007;88:240–3.
24. Cussons AJ, Watts GF, Mori TA, Stuckey BG. Omega-3 fatty acid supplementation decreases liver fat content in polycystic ovary syndrome: a randomized controlled trial employing proton magnetic resonance spectroscopy. J Clin Endocrinol Metab. 2009;94:3842–8.
25. Kasim-Karakas SE, Almario RU, Gregory L, Wong R, Todd H, Lasley BL. Metabolic and endocrine effects of a polyunsaturated fatty acid-rich diet in polycystic ovary syndrome. J Clin Endocrinol Metab. 2004;89:615–20.
26. Mehri J, Maryamalsadat R, Zohreh FK, et al. Metabolic response to selenium supplementation in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Clin Endocrinol. 2015;82:885–91.
27. Foroozanfard F, Jamilian M, Jafari Z, et al. Effects of zinc supplementation on markers of insulin resistance and lipid profiles in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Exp Clin Endocrinol Diabetes. 2015;123:215–20.
28. Jamilian M, Asemi Z. Chromium Supplementation and the effects on metabolic status in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Ann Nutr Metab. 2015;67:42–8.
29. Zhang J, Zhou L, Tang L, et al. Chinese herbal medicine for subfertile women with polycystic ovarian syndrome. Cochrane Database Syst Rev. 2010;9:1399–400.
30. Sills ES, Perloe M, Tucker MJ, Kaplan CR, Genton MG, Schattman GL. Diagnostic and treatment characteristics of polycystic ovary syndrome: descriptive measurements of patient perception and awareness from 657 confidential self-reports. BMC Womens Health. 2001;1:3.
31. Lim CED, Liu J. Traditional chinese medicine for gynaecological diseases. J Aust Tradit Med Soc. 2011;17:17–20.
32. Zhang Y, Fu Y, Han F, et al. The effect of complementary and alternative medicine on subfertile women with in vitro fertilization. Evid Based Complement Alternat Med. 2014;68–78.
33. Raja-Khan N, Stener-Victorin E, Wu X, et al. The physiological basis of complementary and alternative medicines for polycystic ovary syndrome. Ajp Endocrinol Metabol. 2011;301:E1–E10.
34. Lim DC, Xue CC, Wong FW, et al. Acupuncture for polycystic ovarian syndrome. Cochrane Database Syst Rev. 2011;668:CD007689.
35. Bian ZX, Moher D, Dagenais S, Li YP, TX W, Liu L, Miao JX, Song L, Zhang HM. Improving the quality of randomized controlled trials in Chinese herbal medicine, part IV: applying a revised CONSORT checklist to measure reporting quality. Zhong Xi Yi Jie He Xue Bao. 2006;4:233–42.

36. Liu JP, Zhang M, Wang WY, Grimsgaard S. Chinese herbal medicines for type 2 diabetes mellitus. Cochrane Database Syst Rev. 2004;3:CD003642.
37. Ni HY, Gong J. Research progress on Chinese herbal medicine in treating PCOS. Liaoning J Trad Chinese Med. 2007;34:123–4.
38. Wang BQ, Ling M. Research development of Chinese herbal medicine for PCOS. Shandong J Trad Chinese Med. 2008;27:138–40.
39. Lau CH, Chan CM, Chan YW, Lau KM, Lau TW, Lam FC, Che CT, Leung PC, Fung KP, Ho YY, Lau CB. vitro antidiabetic activities of five medicinal herbs used in Chinese medicinal formulae. Phytother Res. 2008;22:1384–8.
40. Yang XB, Huang ZM, Cao WB, Chen HY, Wang JH, Xu L. Therapeutic and protective effects of water-ethanolic extract from Rhizoma alismatis on streptozotocin-induced diabetic mice. Xhongguo Shi Yian Fang Ji Xue Za Zhi. 2002;18:336–50.
41. Chen ZH, Li J, Liu J, Zhao Y, Zhang P, Zhang MX, Zhang L. Saponins isolated from the root of Panax notoginseng showed significant anti-diabetic effects in KK-Ay mice. Am J Chin Med. 2008;36:939–51.
42. Zhao L, Li W, Han F, Hou L, Baillargeon JP, Kuang H, Wang Y, Wu X. Berberine reduces insulin resistance induced by dexamethasone in theca cells in vitro. Fertil Steril. 2011;95:461–3.
43. Dvorak Z, Vrzal R. Berberine reduces insulin resistance: the roles for glucocorticoid receptor and aryl hydrocarbon receptor. Fertil Steril 95: e7.; author reply e8–e9, 2011.
44. Lee MJ, Lee HS, Park SD, Moon HI, Park WH. Protective effects of luteolin-7-O-beta-D-glucuronide methyl ester from the ethyl acetate fraction of Lycopi Herba against pro-oxidant reactive species and low-density lipoprotein peroxidation. J Enzyme Inhib Med Chem. 2010;25:702–7.
45. Xia W, Sun C, Zhao Y, Wu L. Hypolipidemic and antioxidant activities of Sanchi (Radix Notoginseng) in rats fed with a high fat diet. Phytomedicine. 2010;18:516–20.
46. Ning MH, Liu YJ, Ning XG. Clinical observation on yishenxiaozheng decoction for the treatment of 85 cases of polycystic ovarian disease. Hunan Guiding J TCM. 2004;10:27–8.
47. Yang ZW, You ZL, Zhang XH, Wang Y, Zeng M. Research on influence of Bushen Huoxue Method on menstrual cyclicity and reproductive hormone in PCOS. Chin J Tradit Med Sci Technol. 2006;13:5–6.
48. Zhang QP. Bushen Huoxue Method in treating PCOS. Chin J Inform TCM. 2004;11:1014–5.
49. Xia Y. Cangfu Daotan Soup in treating 30 obese cases with PCOS. Tianjin J Tradit Chin Med. 2004;21(2):169.
50. Cui FY, Liu XM. Gaoshao soup in treating 60 cases of PCOS. J Pract Tradit Chin Med. 2004;20:686–7.
51. Wang ZH, Yang YS, Zhang YL. Clinical study of Ganshao Capsule in treating clomiphene-resistant polycystic ovarian syndrome. Chin J Integr Tradit West Med. 2005;25:704–6.
52. Cong LX. Observation of Tiaojin Zhuyun Pellet combined with clomiphene in treating infertility caused by PCOS. J Pract Tradit Chin Med. 2006;22:290–1.
53. Liu Y, Lu XY. Traditional Chinese medicine in treating 12 PCOS. New J Tradit Chin Med. 2005;37:74–5.
54. Xue XW, Wang N. Effective observation on TCM combined with ultrasound in treating 56 PCOS. Chin Gen Pract. 2004;7:828.
55. Yuan XF. TCM periodical treatment on 38 PCOS. Fujian J Tradit Chin Med. 2003;34:22.
56. Lin Y. Combinative treatment of Chinese traditional and western medicine in 48 patients with sterility due to polycystic ovarian syndrome. Matern Child Health Care Chin. 2005;20:1642–3.
57. Ye LQ. TCM periodical therapy combined with metformin in treating 62 PCOS. Jiangxi J Tradit Chin Med. 2004;35:22–3.
58. Li CP. Effective observation on Bushen Tiaozhou Method in treating 30 infertility patients with PCOS. New J Tradit Chin Med. 2006;38:50–1.
59. Li XB, Li LY. Daotan Zhongzi Fang combined with clomiphene in treating PCOS. J Pract Med. 2000;16:330–1.
60. Li XB, Li LY, Huang JL, Liang XF. Effect of operation under celioscopy combined with kidney tonifying and phlegm removing herbal medicine for polycystic ovarian disease syndrome. Tradit Chin Drug Res Clin Pharmacol. 2002;13:75–6,131.

61. Liang RN, Liu J, Lu J, Zhang HF. Treatment of refractory polycystic ovary syndrome by Bushen Huoxue method combined with ultrasound guided follicle aspiration. Chin J Integr Tradit West Med. 2008;28:314–7.
62. Han J-Y, Fan J-Y, Horie Y, et al. Ameliorating effects of compounds derived from Salvia miltiorrhiza root extract on microcirculatory disturbance and target organ injury by ischemia and reperfusion. Pharmacol Ther. 2008;117:280–95.
63. Kang BY, Chung SW, Kim SH, et al. Inhibition of interleukin-12 and interferon-gamma production in immune cells by tanshinones from Salvia miltiorrhiza. Immunopharmacology. 2008;49:355–61.
64. Zhang Y, Jiang P, Ye M, et al. Tanshinones: sources, pharmacokinetics and anti-cancer activities. Int J Mol Sci. 2012;13:13621–66.
65. Yang X, Zhang Y, Wu X, et al. Cryptotanshinone reverses reproductive and metabolic disturbances in prenatally androgenized rats via regulation of ovarian signaling mechanisms and androgen synthesis. Am J Phys Regul Integr Comp Phys. 2011;300:R869–75.
66. Altschuler J, Casella S, MacKenzie T, Curtis K. The effect of cinnamon on A1C among adolescents with type 1 diabetes. Diabetes Care. 2007;30:813–6.
67. Khan A, Khattak KN, Safdar M, Anderson RA, Ali Khan MM. Cinnamon improves glucose and lipids of people with type 2 diabetes. Diabetes Care. 2003;26:3215–8.
68. Kort DH, Lobo RA. Preliminary evidence that cinnamon improves menstrual cyclicity in women with polycystic ovary syndrome: a randomized controlled trial. Am J Obstet Gynecol. 2014;211:487.e1–6.
69. Birdsall TC, Kelly GS. Berberine: therapeutic potential of an alkaloid found in several medicinal plants. Altern Med Rev. 1997;2:94–103.
70. Zhang Y, Li X, Zou D, Liu W, Yang J, Zhu N, Huo L, Wang M, Hong J, Wu P, Ren G, Ning G. Treatment of type 2 diabetes and dyslipidemia with the natural plant alkaloid berberine. J Clin Endocrinol Metab. 2008;93:2559–65.
71. Lee YS, Kim WS, Kim KH, Yoon MJ, Cho HJ, Shen Y, Ye JM, Lee CH, Oh WK, Kim CT, Hohnen-Behrens C, Gosby A, Kraegen EW, James DE, Kim JB. Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. Diabetes. 2006;55:2256–64.
72. Xu MG, Wang JM, Chen L, Wang Y, Yang Z, Tao J. Berberine-induced upregulation of circulating endothelial progenitor cells is related to nitric oxide production in healthy subjects. Cardiology. 2009;112:279–86.
73. Affuso F, Mercurio V, Ruvolo A, Pirozzi C, Micillo F, Carlomagno G, Grieco F, Fazio S. A nutraceutical combination improves insulin sensitivity in patients with metabolic syndrome. World J Cardiol. 2012;4:77–83.
74. Yin J, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. Metabolism. 2008;5:712–7.
75. Grant SJ, Bensoussan A, Chang D, Kiat H, Klupp NL, Liu JP, Li X. Chinese herbal medicines for people with impaired glucose tolerance or impaired fasting blood glucose. Cochrane Database Syst Rev. 2009;4:CD006690.
76. Lampert N, Xu Y. Chinese herbal nephropathy. Lancet. 2002;359:796–7.
77. Lord GM, Cook T, Arlt VM, Schmeiser HH, Williams G, Pusey CD. Urothelial malignant disease and Chinese herbal nephropathy. Lancet. 2001;358:1515–6.
78. Nortier JL, Martinez MC, Schmeiser HH, Arlt VM, Bieler CA, Petein M, et al. Urothelial carcinoma associated with the use of a Chinese herb (Aristolochia fangchi). N Engl J Med. 2000;342:1686–92.
79. Lin SH, Chau T. A puzzling cause of hypokalaemia. Lancet. 2002;360:224.
80. Dasgupta A, Davis B, Wells A. Effect of plantain on therapeutic drug monitoring of digoxin and thirteen other common drugs. Ann Clin Biochem. 2006;43:223–5.

Laparoscopic Ovarian Drilling

15

Hatem Abu Hashim

15.1 Introduction

Not surprisingly, polycystic ovary syndrome (PCOS) still poses a challenge not only for clinicians but also for researchers. Figure 15.1 represents an overview of the current magnitude of the problem of PCOS [1]. Historically speaking, surgical treatment of infertile women with PCOS by laparotomy and ovarian wedge resection was first reported by Stein and Leventhal in 1935 with promising results [2]. Three decades later, this procedure was abandoned because of the risk of postoperative pelvic adhesions and replaced by medical ovulation-inducing agents such as clomiphene citrate (CC) and gonadotrophins [3]. However, surgical treatment of infertile women with PCOS was successfully revived by Halvard Gjönnaess in 1984 in the form of laparoscopic ovarian drilling (LOD) with subsequent ovulation and pregnancy rates of 92% and to 80%, respectively [4]. Since that time, this minimally invasive and less traumatic modern version of ovarian wedge resection using either electrocautery (diathermy) or laser has continued to play an essential part in management of infertile women with PCOS [3].

Although LOD is recommended as a second-line treatment in infertile PCOS women who have CC resistance, LOD has been also proposed as first-line approach for PCOS-related anovulation or as an adjuvant therapy before in vitro fertilisation (IVF) cycles [3, 5, 6]. LOD seems to be as effective as gonadotrophin treatment without an increased risk of multiple pregnancy or ovarian hyperstimulation syndrome (OHSS) [3, 5, 6]. Owing to the remarkable advancement in information about the pathophysiology of PCOS in the last two decades, several oral ovulation induction drugs for CC-resistant PCOS were introduced such as metformin [7], CC plus metformin [8], CC plus tamoxifen [9], rosiglitazone plus CC [10] and aromatase

H. Abu Hashim
Department of Obstetrics and Gynaecology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
e-mail: hatem_ah@hotmail.com

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_15

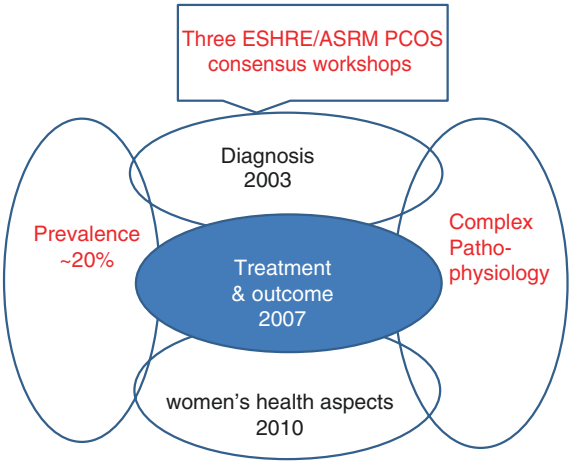


Fig. 15.1 An overview of the magnitude of the problem of PCOS. ESHRE, European Society of Human Reproduction and Embryology; ASRM, American Society of Reproductive Medicine. (Reprinted from Archives of Gynecology and Obstetrics, Volume 291, Issue 1, Hatem Abu Hashim, Predictors of success of laparoscopic ovarian drilling in women with polycystic ovary syndrome: an evidence-based approach, Page 12, ©Springer-Verlag Berlin Heidelberg 2014, with permission from Springer)

inhibitors [11, 12]. These agents vary in efficacy, treatment duration and patient compliance (see also Chaps. 9, 10 and 11). Moreover, with practicing LOD as a day-care procedure, we must ensure the primum non nocere principle, i.e. the intervention must be safer than potentially efficacy.

In this context, the focus of interest of this chapter is to evaluate the efficacy of LOD vs. oral ovulation induction treatments, assess predictors of LOD response, critically evaluate unilateral LOD, assess the efficacy of ovarian restimulation after LOD and finally address concerns with regard to possible risks of postoperative adhesions and damage on the ovarian reserve. Other aspects about potential mechanisms of action, surgical technique and dose response as well as other potential roles of LOD as a first-line or an adjuvant procedure before IVF will be also included.

15.2 Surgical Technique and Mechanism of Action

LOD is most commonly performed using a monopolar diathermy needle electrode [13]. The lowest effective number of ovarian drills sparked a heated discussion in the previous three decades [3]. In the original LOD technique, ovulation occurred more frequently if ten or more punctures were performed in the two ovaries together with each puncture having a diameter of ~3 mm and a depth of 2–4 mm using a power setting of 200–300 W for 2–4 s [4]. Subsequently, the occurrence of

postoperative pelvic adhesions and ovarian failure were reported as major shortcomings after increasing the number of drills [14, 15]. Armar et al. [16, 17] brought to light the widely adopted practice of delivering 640 Joules (J) per ovary (4 punctures × 4 s × 40 W) as the lowest effective dose with an ovulation and pregnancy rates of 86%. These findings were subsequently confirmed by Amer et al. [18]. An ovulation and pregnancy rates of 67% were reported using four punctures to deliver 600 J per ovary (4 punctures × 5 s × 30 W = 600 J) [18].

Various hypotheses have been proposed to explain the ovulation induction mechanism of LOD. It is strongly believed to be similar to ovarian wedge resection, i.e. the destruction of ovarian follicles and the ovarian stromal elements causes a fall in local and serum androgens as well as inhibin levels, leading to an increase in the follicle-stimulating hormone (FSH) secretion promoting follicular growth through negative feedback mechanisms [1, 3, 19]. A surgery-mediated increased ovarian blood flow releasing a cascade of local growth factors, such as insulin-like growth factor 1 (IGF1), interacting with FSH is thought to allow follicular growth, maturation and subsequent ovulation [1].

15.2.1 Unilateral vs. Bilateral LOD

Farquhar et al. [13] in a recent Cochrane review looked at five randomised controlled trials (RCTs) which compared unilateral LOD (ULOD) with bilateral LOD (BLOD) [20–24]. No significant differences were reported between unilateral and bilateral drilling with regard to ovulation rate [76% vs. 72%; odds ratio (OR) 1.20, 95% confidence interval (CI) 0.59–2.46], pregnancy rate (51.7% vs. 50.5%; OR 1.00, 95% CI 0.55–1.83), live birth rate (36.4% vs. 41%; OR 0.83, 95% CI 0.24–2.78) or miscarriage rate (9.2% vs. 9%; OR 1.02, 95% CI 0.31–3.33) [13]. Therefore, a suggested recommendation to apply a ULOD rather than a BLOD is generally concordant with these data.

15.2.2 Fixed vs. Dose-Adjusted Energy

The concept of using an adjusted thermal dose, i.e. to tailor the energy according to the preoperative ovarian volume, was recently tested in an RCT by Zakherah et al. [25] among 120 PCOS patients with CC resistance. The energy received by the adjusted thermal dose group was a mean dose calculated from four studies [16, 18, 26, 27], i.e. 625 J/10.8 cm^3 = 60 J/cm^3 of ovarian tissue. Consequently, the required number of ovarian drills was calculated by dividing the total individual ovarian dose (based on the measured preoperative ovarian volume with transvaginal sonography) to the dose delivered in each puncture point (1 puncture × 5 s × 30 W = 150 J). The authors reported a better reproductive outcome in the adjusted diathermy dose group compared with the fixed thermal dosage group who received 600 J per ovary through

four ovarian punctures regardless of ovarian volume. The ovulation and pregnancy rates were 81.8% vs. 62.2% and 51.7% vs. 36.8%, respectively. Also, more patients resumed regular cycles in the adjusted diathermy dose group (87.9% vs. 75.4%) [25].

More recently, the impact of a unilateral dose-adjusted LOD (using 60 J/cm^3 applied to the larger ovary) compared to BLOD (with fixed doses of 1200 J, i.e. 600 J per ovary) on reproductive outcome has been investigated [28, 29]. In a prospective longitudinal study, Sunj et al. [28] addressed this issue among 96 infertile PCOS women with CC resistance who were divided into a ULOD group (applied to right ovary, n. 49) and a BLOD group (n. 47). Patients in the ULOD group received various numbers of drills and varying thermal doses in the right ovary, while those in the BLOD group received the same number of drills (five punctures/ovary) and thermal doses in both ovaries (5 punctures × 4 s × 30 W = 600 J/ovary). The thermal dose received by the ULOD group was a mean dose calculated from three ULOD studies [21, 22, 24], i.e. 627 J/10 cm^3 or 60 J/cm^3. The number of punctures per ovary was calculated according to the following formula: 60 J/cm^3 divided by 30 W × 4 s. Both groups were followed up for 6 months to assess the ovulatory response [28].

A significantly higher ovulation rate during the first menstrual cycle after LOD was reported in the ULOD group than in the BLOD group [73% vs. 49%; absolute risk reduction (ARR) 20.25; 95% CI 20.44 to 20.03]. Meanwhile, the increase in the ovulation rate over the 6-month period after LOD in the ULOD group over that in the BLOD group was borderline (82% vs. 64%; ARR 20.18; 95% CI 20.35 to 0.02). In the ULOD group, a significantly increased ovulation rate was demonstrated in patients with a larger right ovary compared with those who had a smaller right ovary (100% vs. 36%; ARR 20.64; 95% CI 20.84 to 20.37). Noteworthy, the same observation was reported in the BLOD group (88% vs. 33%; ARR 20.55; 95% CI 20.73 to 20.28). The pregnancy rate was also significantly higher in patients with a larger right ovary in both treatment groups. The authors concluded that ULOD using adjusted thermal doses (60 J/cm^3) is more efficient in CC-resistant PCOS women than BLOD using fixed doses. They admitted the need for future research to examine the long-term differences in the ovulation beyond 6 months as well as to investigate whether ULOD treatment of the larger ovary, either left or right, would significantly increase the ovulation rate [28].

In a recent RCT, Rezk et al. [29] addressed the same question among 105 patients with CC-resistant PCOS. Dose-adjusted ULOD applied to the larger ovary following the formula described by Sunj et al. [28] had comparable ovulation and pregnancy rates to fixed-dose BLOD at 3-month follow-up period (65.4% vs. 77.3% and 15.4% vs. 26.4%, respectively) [29]. However, they were significantly higher in BLOD at 6-month follow-up period (58.5% vs. 32.7% and 49.1% vs. 11.5%, for ovulation and pregnancy rates, respectively), i.e. dose-adjusted ULOD applied to the larger ovary was associated with a reduction in its effectiveness after 6 months [29].

15.3 Indications and Efficacy Data

15.3.1 Efficacy Data in CC-Resistant PCOS

In a recent Cochrane review of subfertile PCOS women with CC resistance, Farquhar et al. [13] examined the efficacy of new treatment strategies, i.e. insulin-sensitising drugs and aromatase inhibitors, as compared to LOD. The authors looked at 25 RCTs. The primary outcomes were live birth and multiple pregnancy rates, while secondary outcomes were ovulation, pregnancy, miscarriage and OHSS rates. They reported no evidence of a significant difference in live births when LOD was compared with CC plus tamoxifen (OR 0.81, 95% CI 0.42–1.53) or compared with aromatase inhibitors (OR 0.84, 95% CI 0.54–1.31). Although there was evidence of significantly fewer live births following LOD compared with CC plus metformin (OR 0.44, 95% CI 0.24–0.82), there was no evidence of a significant difference in ovulation and pregnancy rates when LOD was compared to CC plus metformin (OR 0.89, 95% CI 0.27–2.93 and OR 0.79, 95% CI 0.53–1.18, respectively), CC plus tamoxifen (OR 1.34, 95% CI 0.68–2.63 and OR 0.97, 95% CI 0.59–1.59, respectively), aromatase inhibitors (OR for ovulation rate was not included; OR 0.89, 95% CI 0.58–1.37 for pregnancy rate) or rosiglitazone plus CC (OR 0.67, 95% CI 0.13–3.44 and OR 0.75, 95% CI 0.23–2.50, respectively) [13]. A significant benefit in favour of LOD in terms of pregnancy was only found when compared to metformin therapy alone (OR 2.47, 95% CI 1.05–5.81) [13, 30]. Notably, as regards the secondary outcomes, i.e. ovulation, pregnancy, miscarriage and OHSS rates, no difference was found when LOD was compared with any of these treatments [13]. Importantly, no significant difference in live birth, clinical pregnancy or spontaneous abortion rates was reported for women treated with LOD compared with gonadotrophins (OR 0.97, 95% CI 0.59–1.59; OR 1.01, 95% CI 0.72–1.32; and OR 0.73, 95% CI 0.40–1.33, respectively). However, the number of multiple pregnancies was lower after LOD compared with gonadotrophins (OR 0.13, 95% CI 0.03–0.52) [13].

In the light of the aforementioned evidence-based meta-analysis, these alternative medical choices should be tried first to induce ovulation in CC-resistant PCOS patients. Only when these regimens fail should a LOD be envisaged. Noteworthy, the final choice should be individualised considering what is available in a country or clinic as well as each woman's own perspective and circumstances, e.g. economics; side effects; the need to receive a laparoscopic approach for other reasons of infertility, e.g. tubal factor; endometriosis; etc. This is the essence for a successful evidence-based medicine practice being "the conscientious, explicit and judicious use of current best evidence in making decisions" [7, 31] (Fig. 15.2).

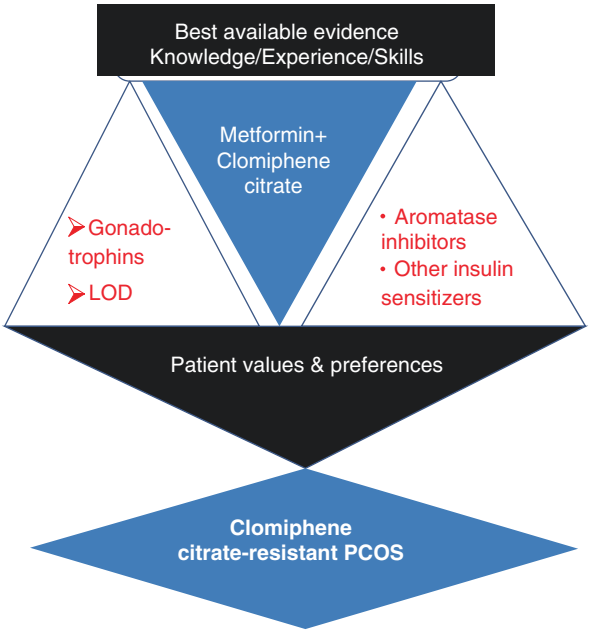


Fig. 15.2 Evidence-based decision making for clomiphene citrate-resistant infertile women with polycystic ovary syndrome. LOD, laparoscopic ovarian diathermy; PCOS, polycystic ovary syndrome. (Reprinted from Reproductive Biomedicine Online, Volume 32, Issue 1, Hatem Abu Hashim, Twenty years of ovulation induction with metformin for PCOS; what is the best available evidence?, Page 49, ©2015 Reproductive Healthcare Ltd. Published by Elsevier Ltd, with permission from Elsevier)

15.3.2 Other Potential Roles

LOD as a first-line therapy in PCOS may offer several theoretical advantages particularly resumption of mono-ovulatory cycles and avoiding of the anti-oestrogenic effects of CC. However, these suggested benefits were not clinically relevant when tested in comparison with six cycles of CC in an RCT [32]. In fact, no significant difference in the ovulation rate either per woman (64% vs. 76%) or per cycle (70% vs. 66%), pregnancy rate per woman (27% vs. 44%), cumulative pregnancy rate (52% vs. 63%) and live birth rate (46% vs. 56%) after 12-month follow-up.

Another merit suggested after LOD is increased responsiveness of the ovary to oral ovulation induction agents and gonadotrophins [33–35]. In a prospective study, we investigated the effect of ovarian restimulation by CC among 84 PCOS patients with CC resistance who remained anovulatory after LOD [36]. Ovulation and pregnancy were achieved in 35.7% and 15.5% of them, respectively. Noteworthy, hyperandrogenism and insulin resistance appeared to be negative predictors of ovulation [36]. Increased ovarian sensitivity to gonadotrophins after LOD was also reported in a retrospective study among 22 CC-resistant PCOS women who failed to achieve ovulation or conceive after LOD [37]. Ovulation and pregnancy rates increased significantly after LOD. In addition, a significant reduction of the number of ampules, daily effective dose and duration of the induction phase with gonadotrophins were reported [37].

Another argument is that LOD might work as an adjuvant procedure before IVF. In two recent retrospective studies, a lower incidence of OHSS was observed in PCOS patients undergoing IVF after LOD in comparison with untreated controls [38, 39]. Potential mechanisms proposed were reduction in ovarian blood flow velocity and

serum vascular endothelial growth factor (VEGF) concentrations following LOD [40–42]. This finding is in agreement with an RCT on 50 PCOS patients which showed that LOD can reduce the cancellation rate due to OHSS during controlled ovarian hyperstimulation (COH) for IVF treatment [43]. Nevertheless, the incidence of moderate or severe OHSS was not different between groups (4% vs. 16%; OR 0.22, 95% CI 0.02–2.11), as well as the pregnancy (36% vs. 32%; OR 1.20, 95% CI 0.37–3.86) and live birth (24% vs. 20%; OR 1.26, 95% CI 0.33–4.84) rates [13, 43].

15.4 Predictors for Poor Outcome

Lack of response to LOD has been reported in ~30% of anovulatory PCOS women. This is identified by lack of menstruation and persistent anovulation 8 weeks after the procedure as evidenced by the low mid-luteal serum progesterone levels and negative pregnancy test [33, 44]. Therefore, before embarking on LOD, it is of paramount importance to utilise the existing evidence-based data concerning its predictors of success to ensure better outcome as well as to avoid possible risk of impairment of ovarian reserve and other complications. In this context, it is worth remembering the quote of Sir Winston Churchill "failure to plan is planning to fail". In a recent publication, we evaluated different clinical, biochemical and ultrasonographic parameters that might help in predicting ovulation and/or pregnancy after LOD [45–55] (Table 15.1). We demonstrated that predictors of poor response to LOD in PCOS women with CC resistance and polycystic ovary morphology (PCOM) are a body mass index (BMI) higher than 25 kg/m^2, a duration of infertility

Table 15.1 Studies addressing predictors of ovulation and/or pregnancy after LOD in women with PCOS

Reference	Study design	Case	Control	Outcome
Baghdadi et al. (2012) [45]	Meta-analysis	879 PCOS (BMI < 25 kg/m^2)	905 PCOS (BMI > 25 kg/m^2)	Lean PCOS women respond better to LOD than obese ones (RR 1.43, 95% CI 1.22–1.66; RR 1.73, 95% CI 1.39–2.17 for ovulation and pregnancy rates, respectively)
Kirpalini et al. (2001) [46]	Prospective with multiple logistic regression analysis	70 (CC-resistant PCOS)	///////////////	Better pregnancy rates with: • Preoperative serum LH levels (>10 IU/L) • Short duration of infertility (<3 years) • Absence of pre-existing tubal disease

(continued)

Table 15.1 (continued)

Reference	Study design	Case	Control	Outcome
Ott et al. (2009) [47]	Retrospective with uni- and multivariate regression analysis	100 (CC-resistant PCOS, pretreated with metformin)	//////////////////	Preoperative serum high LH (≥12.1 IU/l) and androstenedione (≥3.26 ng/ml) levels are independent predictors of spontaneous ovulation within 3 months after LOD
Ott et al. (2014) [48]	Prospective with multivariate regression analysis	21 (CC-resistant PCOS)	8 (diagnostic laparoscopy for infertility)	• Significantly higher rates of postoperative spontaneous ovulation rates with higher preoperative androstenedione (OR 6.53), LH levels (OR 7.31) and secondary infertility (OR 5.40) • Intraoperative androstenedione kinetics are not useful predictors of postoperative ovulation
Van Willey et al. (2005) [49]	Prospective with multivariate logistic regression analysis	83 (CC-resistant PCOS)	///////////////	Poor ovulatory response to LOD if LH/FSH ratio < 2, menarche <13 years and glucose level <4.5 mmol/l
Amer et al. (2004) [50]	Retrospective with multiple logistic regression analysis	200 PCOS (161 CC resistant; 39 CC failure)	//////////////////	Marked obesity(BMI ≥35 kg/m^2), marked hyperandrogenism (serum T concentration ≥4.5 nmol/l, free androgen index ≥15) and/or long duration of infertility (>3 years) seems to predict poor response to LOD
Kato et al. (2007) [51]	Prospective study	19 CC-resistant PCOS women with high testosterone level, using a cut-off value of 50 ng/dl	13 CC resistant–PCOS women with normal testosterone level	Comparable rates of spontaneous ovulation (84.2% vs.69.2%) and pregnancy (42.1% vs. 76.9%) after LOD between both groups

Table 15.1 (continued)

Reference	Study design	Case	Control	Outcome
Alborzi et al. (2001) [52]	Comparative study	211 CC-resistant PCOS with ovarian volume >8 cm^3 or cross-sectional area >10 cm^2	160 CC-resistant PCOS with normal size ovary	Comparable rates of ovulation (90.99% vs. 88.75%) and pregnancy (73.45% vs.71.25%) in both groups, i.e. ovarian size is not a prognostic factor for LOD response in CC-resistant PCOS
Kong et al. (2011) [53]	Retrospective study	19 PCOS patients with metabolic syndrome	70 PCOS patients without metabolic syndrome	Comparable rates of ovulation (68% vs. 61%) and cumulative pregnancy (68% vs. 61%) in both groups, i.e. patients with metabolic syndrome should not be precluded from LOD
Amer et al. (2009) [54]	Prospective study with multivariate logistic regression analysis	29 anovulatory PCOS patients undergoing LOD	18 anovulatory PCOS patients had CC	Pretreatment circulating AMH level ≥7.7 ng/ml had a sensitivity of 78% and a specificity of 76% in the prediction of no ovulation after LOD
Elmashad (2011) [55]	Prospective study	23 CC-resistant PCOS patients undergoing LOD	20 healthy fertile women	Women who ovulated after LOD had a significantly lower preoperative AMH, compared with the nonresponders [median value and range; 6.3 (5.1–6.9) vs. 11.9 (11.1–13.6)]

AMH anti-Mullerian hormone, *BMI* body mass index, *CC* clomiphene citrate, *LH* luteinising hormone, *LOD* laparoscopic ovarian drilling, *PCOS* polycystic ovary syndrome, *RR* relative risk, *T* testosterone

longer than 3 years, basal luteinising hormone (LH) levels lower than 10 IU/L, marked biochemical hyperandrogenism (testosterone levels ≥4.5 nmol/L, free androgen index > 15) and high basal anti-Mullerian hormone (AMH) ≥7.7 ng/mL [1]. Other data show the best reproductive outcomes following LOD in normal-weight and young (≤30 years) patients with a short duration of infertility (≤3.5 years) [45].

15.5 Safety Concerns

The use of LOD did open an avenue for ovulation induction but with inherent risks of general anaesthesia and surgical risks of laparoscopy, e.g. visceral and vessel injuries, gas complications, etc. In addition, the main shortcomings of LOD are the risk of postoperative adhesions and the concern about a negative impact of the procedure on the ovarian reserve secondary to excessive ovarian damage [3, 27, 56]. Therefore, we must ensure the primum non nocere principle if LOD will be envisaged, i.e. it will not harm the patient by any iatrogenic complication.

15.5.1 Peritoneal Adhesions

The rate of periadnexal adhesions following LOD and the effect of these adhesions on pregnancy rates were evaluated in a recent review by Api [57]. The author looked at 16 articles in the period from 1984 to 2012. Postoperative adhesion rates were reported to be 0–100% (mean 35.5%, 95% CI 30.8 to 40.4), while pregnancy rates after the procedure in these articles were 35–87% (mean 64.3%, 95% CI 58.2 to 70.7) of the cases. Moreover, the rate of postoperative adhesions was not reduced by the different adhesion prevention measures utilised during or after the procedure. Therefore, it was concluded that the incidence of periadnexal adhesions after LOD does not represent a major constraint in its success story [57].

15.5.2 Ovarian Reserve

A possible risk of diminished ovarian reserve after LOD cannot be ignored. Flyckt and Goldberg [58] reported that these negative concerns have not been supported by the existing data. Other authors have pointed out that most of the changes in the ovarian reserve markers observed after LOD could be interpreted as normalisation of ovarian function rather than a reduction of ovarian reserve [59]. AMH is a glycoprotein related to the transforming growth factor-β (TGF-β) family. Being an exclusive product of granulosa cells of primary, pre-antral and small antral follicles (4–6 mm), serum AMH concentration has been considered as an important marker of ovarian reserve [60–63]. Serum AMH is two- to fourfold higher in women with PCOS than in healthy women [62–66]. This is because ovaries with PCOM exhibit an increased number of AMH-producing small antral follicles [60, 67] and increased production per granulose cells [68, 69]. Notably, AMH production from granulose cells was 75 times higher in anovulatory PCOS and only 4 times higher in ovulatory PCOS than normal ovaries [68]. The AMH overproduction of ovaries of PCO morphology (PCOM) has been inflicted for the anovulatory infertility in women with PCOS owing to its inhibitory effect on primordial follicle recruitment as well as lowering follicular sensitivity to the circulating FSH as shown in both mouse and human ovaries [70–72] (see also Chap. 8).

Noteworthy, the use of serum AMH as predictor of ovarian response to CC [73, 74], letrozole [75], gonadotrophins [76] and LOD [54, 55] in women with PCOS has recently garnered special interest of researchers. In an RCT, Amer et al. [54] evaluated this point among 29 anovulatory women with PCOS for whom LOD was performed as a first-line treatment compared with CC. The pretreatment median (range, ng/ml) plasma AMH concentrations in the LOD group were 6.1 (1.0–21.0). The authors found that women who ovulated after LOD had a significantly lower preoperative AMH [5.6 (1.0–21.0) ng/ml] compared with the nonresponders [9.0 (6.1–17.1) ng/ml]. In addition, they pointed out that plasma AMH ≥7.7 ng/mL was associated with a reduced chance of ovulation after treatment (60% vs. 95% in women with AMH <7.7 ng/ml, respectively; OR 0.08, 95% CI 0.01 to 0.89) [54]. Another prospective controlled study on little sample of PCOS women with CC resistance found also that those who ovulated after LOD had a significantly lower preoperative AMH, but not power Doppler indices compared with the nonresponders [55]. Moreover, that data were obtained in selected patients with PCOM and high AMH levels. Thus, LOD should be used with caution in case of PCOS women with AMH in normal (and low) ranges because the risk of ovarian damage can be high. Further large-scale well-designed studies are needed to find an absolute baseline serum level of AMH in CC-resistant PCOS above which women will not respond to LOD as well as the safest lower AMH levels to avoid ovarian damage if LOD is considered.

The impact of the dose-adjusted ULOD on the ovarian reserve compared with BLOD has been investigated [29, 77]. In a subsequent publication, Sunj et al. [77] addressed this issue in the same cohort of patients [28]. AMH, antral follicle count (AFC) and ovarian volume were measured before and after surgery (1 and 6 months). The authors reported that both groups experienced a decrease in AMH after LOD; however, it was significantly more vivid in the BLOD vs. ULOD group in the first follow-up month and remained as so at the 6-month follow-up period. As AMH is one of the most reliable markers of ovarian reserve, its significantly greater decrease in the BLOD than the ULOD group in this study could be explained by the greater ovarian tissue damage caused by BLOD owing to more punctures and greater total energy. On the other hand, the reduction in AMH can be considered as beneficial, i.e. regularisation of the AMH production from granulose cells of PCOM ovaries. Astoundingly, in the 6-month follow-up period, the ULOD showed a significantly greater increase in AFC and ovarian volume from baseline (preoperative value) compared with BLOD. To explain this finding, the authors hypothesised a possible subsequent compensatory reaction to ULOD which implies that ULOD normalising effects on ovarian function in PCOS patients may be short term [77]. In my opinion, these variables showed skewed distribution; therefore, its increase after surgery should be interpreted with caution as LOD is expected to reduce the PCOM, thus the AFC. Sunj et al. [77] concluded that the dose-adjusted ULOD (60 J/cm^3) does not have long-term effects on ovarian reserve and changes in ovarian reserve parameters can be regarded as normalisation, not as diminishing ovarian reserve.

In their recent RCT, Rezk et al. [29] reported a highly significant difference between ULOD and BLOD groups with regard to the AMH level at 3- and 6-month

follow-up periods with lower levels achieved in the BLOD group. AFC was comparable in the two groups after 3 months (15.2 ± 3.3 vs. 15.1 ± 3.2). However, it became significantly higher in the ULOD at 6-month follow-up period (18.6 ± 3.1 vs. 16.4 ± 3.2). Unlike the results of the aforementioned study [77], the reported postoperative values of AFC were still below its baseline (19.1 ± 5.4 and 18.9 ± 5.5 for ULOD and BLOD groups, respectively). This is in agreement with reduction in the PCOM and thus the AFC after LOD. These findings denote also that the dose-adjusted ULOD does not have long-term effects on ovarian reserve and postoperative changes can be regarded as normalisation rather than diminishing ovarian reserve [29].

Assessment of IVF outcomes in patients with prior LOD provides an opportunity to evaluate its risks on the ovarian reserve. Recently, in a retrospective study of 237 anovulatory infertile PCOS patients, a Chinese group investigated the effect of previous LOD on the cumulative ongoing pregnancy rates following IVF as compared to those without prior LOD treatment [38]. A lower number of retrieved oocytes, fewer available embryos and a lower number of cryopreserved embryos in the LOD group compared with the no-LOD groups were reported. The ongoing pregnancy rate per embryo transfer was found to be lower among patients in the LOD group in comparison with patients in the no-LOD; however, the live birth rate per fresh embryo transfer cycle did not differ between both groups. A lower ongoing cumulative pregnancy rate was observed among patients in the LOD group compared with patients in the no-LOD after including frozen embryo transfer cycles in the analyses of IVF outcomes. Logistic regression analysis demonstrated higher odds of cumulative pregnancy per initiated IVF cycle among PCOS patients without prior LOD in comparison with those with history of LOD (OR 1.98, 95% CI 1.10 to 3.58). The authors concluded that LOD could compromise cumulative ongoing pregnancy rates during subsequent IVF. However, they admitted the selection bias associated with the retrospective study design, the heterogeneous nature of included PCOS patients as well as the different surgical parameters under which LOD was carried out as major limitations for their study [38]. Another recent retrospective study reported the same findings in fresh embryo transfer cycles, i.e. significantly more obtained oocytes and embryos were demonstrated in CC-resistant PCOS women without LOD than women with prior LOD but with the same pregnancy rate in both groups [39].

Conclusion

Science, practice and evidence are dynamic processes. This is typically vivid in the surgical management of PCOS which has been successfully revived with the introduction of LOD by Gjönnaess in 1984. Subsequently, in view of the marvellous progress in the understanding of the pathophysiology and metabolic features of PCOS, different oral ovulation induction agents have also evolved over time competing with LOD for treatment of PCOS-related anovulation and, especially, for PCOS patients with CC resistance. It is of paramount importance that the final choice should be individualised considering what is available in a

country or clinic as well as each woman's own perspective and circumstances, e.g. economics; side effects; the need to receive a laparoscopic approach for other reasons of infertility, e.g. tubal factor; endometriosis; etc.

Based on the current evidence, oral ovulatory drugs should be tried first to induce ovulation in CC-resistant PCOS patients. Only when these regimens fail should an LOD be envisaged. In this context, it would be prudent to consider predictors of poor response before embarking to LOD in this subset of women. If LOD is elected for proper candidates, it is advisable to perform a dose-adjusted ULOD applied to the larger ovary (60 J/cm^3) rather than BLOD with fixed doses of 1200 J. Available evidence denotes that dose-adjusted ULOD achieves at least comparable ovulation and pregnancy rates to fixed-dose BLOD but without long-term effects on ovarian reserve. Interestingly, increased responsiveness of the ovary to oral ovulation induction agents and gonadotrophins has been reported after LOD. Noteworthy, the incidence of periadnexal adhesions after a careful technique of LOD does not represent a major constraint in its success story. Currently, there is no solid evidence of a diminished ovarian reserve associated with LOD in women with CC-resistant PCOS, and associated changes in ovarian reserve parameters with the use of an appropriate technique can be regarded as normalisation and not as diminishing ovarian reserve.

Acknowledgements The author would like to acknowledge the permissions granted to him free of charge both from Springer (for reuse of Fig. 15.1 and Table 15.1) and from Elsevier publishers (for reuse of Fig. 15.2).

References

1. Abu Hashim H. Predictors of success of laparoscopic ovarian drilling in women with polycystic ovary syndrome: an evidence-based approach. Arch Gynecol Obstet. 2015;291:11–8.
2. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol. 1935;29:181–91.
3. Abu Hashim H, Al-Inany H, De Vos M, Tournaye H. Three decades after Gjönnaess's laparoscopic ovarian drilling for treatment of PCOS; what do we know? An evidence-based approach. Arch Gynecol Obstet. 2013;288:409–22.
4. Gjönnaess H. Polycystic ovarian syndrome treated by ovarian electrocautery through the laparoscope. Fertil Steril. 1984;41:20–5.
5. Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Fertil Steril. 2008;89:505–22.
6. National collaborating centre for women's and children's health/national institute for clinical excellence. Fertility: assessment and treatment for people with fertility problems. Clinical guideline no. 156, RCOG Press, London. 2013. https://www.nice.org.uk/guidance/cg156
7. Abu Hashim H. Twenty years of ovulation induction with metformin for PCOS; what is the best available evidence? Reprod Biomed Online. 2016;32:44–53.
8. Abu Hashim H, Foda O, Ghayaty E. Combined metformin clomiphene in clomiphene-resistant polycystic ovary syndrome: a systematic review and meta-analysis of randomized controlled trials. Acta Obstet Gynecol Scand. 2015;9:921–30.

9. Zakherah MS, Nasr A, El Saman AM, Shaaban OM, Shahin AY. Clomiphene citrate plus tamoxifen versus laparoscopic ovarian drilling in women with clomiphene-resistant polycystic ovary syndrome. Int J Gynaecol Obstet. 2010;108:240–3.
10. Roy KK, Baruah J, Sharma A, Sharma JB, Kumar S, Kachava G, Karmakar D. A prospective randomized trial comparing the clinical and endocrinological outcome with rosiglitazone versus laparoscopic ovarian drilling in patients with polycystic ovarian disease resistant to ovulation induction with clomiphene citrate. Arch Gynecol Obstet. 2010;281:939–44.
11. Casper RF, Mitwally MF. Review: aromatase inhibitors for ovulation induction. J Clin Endocrinol Metab. 2006;91:760–71.
12. Franik S, Kremer JA, Nelen WL, Farquhar C. Aromatase inhibitors for subfertile women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2014;2:CD010287.
13. Farquhar C, Brown J, Marjoribanks J. Laparoscopic drilling by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. Cochrane Database Syst Rev. 2012;6:CD001122.
14. Dabirashrafi H, Mohamad K, Behjatnia Y, Moghadami-Tabrizi N. Adhesion formation after ovarian electrocauterization on patients with polycystic ovarian syndrome. Fertil Steril. 1991;55:1200–1.
15. Dabirashrafi H. Complications of laparoscopic ovarian cauterization. Fertil Steril. 1989;52:878–9.
16. Armar NA, McGarrigle HH, Honour J, Holownia P, Jacobs HS, Lachelin GC. Laparoscopic ovarian diathermy in the management of anovulatory infertility in women with polycystic ovaries: endocrine changes and clinical outcome. Fertil Steril. 1990;53:45–9.
17. Armar NA, Lachelin GC. Laparoscopic ovarian diathermy: an effective treatment for anti-oestrogen resistant anovulatory infertility in women with the polycystic ovary syndrome. Br J Obstet Gynaecol. 1993;100:161–4.
18. Amer SA, Li TC, Cooke ID. A prospective dose-finding study of the amount of thermal energy required for laparoscopic ovarian diathermy. Hum Reprod. 2003;18:1693–8.
19. Li RH, Ng EH. Management of anovulatory infertility. Best Pract Res Clin Obstet Gynaecol. 2012;26:757–68.
20. Sharma M, Kriplani A, Agarwal N. Laparoscopic bipolar versus unipolar ovarian drilling in infertile women with resistant polycystic ovarian syndrome: a pilot study. J Gynecol Surg. 2006;22:105–11.
21. Balen AH, Jacobs HS. A prospective study comparing unilateral and bilateral laparoscopic ovarian diathermy in women with the polycystic ovary syndrome. Fertil Steril. 1994;62:921–5.
22. Youssef H, Atallah MM. Unilateral ovarian drilling in polycystic ovarian syndrome: a prospective randomized study. Reprod Biomed Online. 2007;15:457–62.
23. Al-Mizyen E, Grudzinskas JG. Unilateral laparoscopic ovarian diathermy in infertile women with clomiphene citrate resistant polycystic ovary syndrome. Fertil Steril. 2007;88:1678–80.
24. Roy KK, Baruah J, Moda N, Kumar S. Evaluation of unilateral versus bilateral ovarian drilling in clomiphene citrate resistant cases of polycystic ovarian syndrome. Arch Gynecol Obstet. 2009;280:573–8.
25. Zakherah MS, Kamal MM, Hamed HO. Laparoscopic ovarian drilling in polycystic ovary syndrome: efficacy of adjusted thermal dose based on ovarian volume. Fertil Steril. 2011;95:1115–8.
26. Amer SA, Li TC, Cooke ID. Laparoscopic ovarian diathermy in women with polycystic ovarian syndrome: a retrospective study on the influence of the amount of energy used on the outcome. Hum Reprod. 2002;17:1046–51.
27. Felemban A, Tan SL, Tulandi T. Laparoscopic treatment of polycystic ovaries with insulated needle cautery: a reappraisal. Fertil Steril. 2000;73:266–9.
28. Sunj M, Canic T, Baldani DP, Tandara M, Jeroncic A, Palada I. Does unilateral laparoscopic diathermy adjusted to ovarian volume increase the chances of ovulation in women with polycystic ovary syndrome? Hum Reprod. 2013;28:2417–24.
29. Rezk M, Sayyed T, Saleh S. Impact of unilateral versus bilateral laparoscopic ovarian drilling on ovarian reserve and pregnancy rate: a randomized clinical trial. Gynecol Endocrinol. 2016;32:399–402.
30. Hamed HO, Hasan AF, Ahmed OG, Ahmed MA. Metformin versus laparoscopic ovarian drilling in clomiphene-and insulin-resistant women with polycystic ovary syndrome. Int J Gynaecol Obstet. 2010;108:143–7.

31. Sackett DL, Strauss SE, Richardson WS, Rosenberg W, Haynes RB. Evidence-based medicine: how to practice and teach EBM. 2nd ed. Edinburgh: Churchill Livingstone; 2000.
32. Amer SA, Li TC, Metwally M, Emarh M, Ledger WL. Randomized controlled trial comparing laparoscopic ovarian diathermy with clomiphene citrate as a first-line method of ovulation induction in women with polycystic ovary syndrome. Hum Reprod. 2009;24:219–25.
33. Bayram N, van Wely M, Kaaijk EM, Bossuyt PM, van der Veen F. Using an electrocautery strategy or recombinant follicle stimulating hormone to induce ovulation in polycystic ovary syndrome: randomised controlled trial. BMJ. 2004;328:192.
34. Kato M, Kikuchi I, Shimaniki H, Kobori H, Aida T, Kitade M, Kumakiri J, Takeuchi H. Efficacy of laparoscopic ovarian drilling for polycystic ovary syndrome resistant to clomiphene citrate. J Obstet Gynaecol Res. 2007;33:174–80.
35. Palomba S, Orio Jr F, Falbo A, Russo T, Caterina G, Manguso F, Tolino A, Colao A, Zullo F. Metformin administration and laparoscopic ovarian drilling improve ovarian response to clomiphene citrate (CC) in oligo-anovulatory CC-resistant women with polycystic ovary syndrome. Clin Endocrinol. 2005;63:631–5.
36. Abu Hashim H, El-Shafei M, Badawy A, Wafa A, Zaglol H. Does laparoscopic ovarian diathermy change clomiphene-resistant PCOS into clomiphene-sensitive? Arch Gynecol Obstet. 2011;284:503–7.
37. Farhi J, Soule S, Jacobs HS. Effect of laparoscopic ovarian electrocautery on ovarian response and outcome of treatment with gonadotropins in clomiphene citrate-resistant patients with polycystic ovary syndrome. Fertil Steril. 1995;64:930–5.
38. Cai J, Liu L, Sun L, Sha A, Jiang X, Ren J. Effects of previous ovarian drilling on cumulative ongoing pregnancy rates among patients with polycystic ovarian syndrome undergoing in vitro fertilization. Int J Gynaecol Obstet. 2016;134:272–7.
39. Eftekhar M, Deghani Firoozabadi R, Khani P, Ziaei Bideh E, Forghani H. Effect of laparoscopic ovarian drilling on outcomes of in vitro fertilization in clomiphene-resistant women with polycystic ovary syndrome. Int J Fertil Steril. 2016;10:42–7.
40. Amin AF, Abdel-Aal DE, Darwish AM, Mbeki AR. Evaluation of the impact of laparoscopic ovarian drilling on Doppler indices of ovarian stoma blood flow, serum vascular endothelial growth factor, and insulin-like growth factor-1 in women with polycystic ovary syndrome. Fertil Steril. 2003;79:938–42.
41. Parsanezhad ME, Bagheri MH, Alborzi S, Schmidt EH. Ovarian stromal blood flow changes after laparoscopic ovarian cauterization in women with polycystic ovary syndrome. Hum Reprod. 2003;18:1432–7.
42. El Behery MM, Diab AE, Mowafy H, Ebrahiem MA, Shehata AE. Effect of laparoscopic ovarian drilling on vascular endothelial growth factor and ovarian stromal blood flow using 3-dimensional power Doppler. Int J Gynaecol Obstet. 2011;112:119–21.
43. Rimington MR, Walker SM, Shaw RW. The use of laparoscopic ovarian electrocautery in preventing cancellation of in-vitro fertilization treatment cycles due to risk of ovarian hyperstimulation syndrome in women with polycystic ovaries. Hum Reprod. 1997;12:1443–7.
44. Seow KM, Juan CC, Hwang JL, Ho LT. Laparoscopic surgery in polycystic ovary syndrome: reproductive and metabolic effects. Semin Reprod Med. 2008;26:101–10.
45. Baghdadi LR, Abu Hashim H, Amer SA, Palomba S, Falbo A, Al-Ojaimi E, Ott J, Zhu W, Fernandez H, Nasr A, Ramzy AM, Clark J, Doi SA. Impact of obesity on reproductive outcomes after ovarian ablative therapy in PCOS: a collaborative meta-analysis. Reprod Biomed Online. 2012;25:227–41.
46. Kriplani A, Manchanda R, Agarwal N, Nayar B. Laparoscopic ovarian drilling in clomiphene citrate-resistant women with polycystic ovary syndrome. J Am Assoc Gynecol Laparosc. 2001;8:511–8.
47. Ott J, Wirth S, Nouri K, Kurz C, Mayerhofer K, Huber JC, Tempfer CB. Luteinizing hormone and androstenedione are independent predictors of ovulation after laparoscopic ovarian drilling: a retrospective cohort study. Reprod Biol Endocrinol. 2009;7:153.
48. Ott J, Mayerhofer K, Nouri K, Walch K, Seemann R, Kurz C. Perioperative androstenedione kinetics in women undergoing laparoscopic ovarian drilling: a prospective study. Endocrine. 2014;47:936–42.

49. van Wely M, Bayram N, van der Veen F, Bossuyt PM. Predictors for treatment failure after laparoscopic electrocautery of the ovaries in women with clomiphene citrate resistant polycystic ovary syndrome. Hum Reprod. 2005;20:900–5.
50. Amer SA, Li TC, Ledger WL. Ovulation induction using laparoscopic ovarian drilling in women with polycystic ovarian syndrome: predictors of success. Hum Reprod. 2004;19:1719–24.
51. Kato M, Kikuchi I, Shimaniki H, Kobori H, Aida T, Kitade M, Kumakiri J, Takeuchi H. Efficacy of laparoscopic ovarian drilling for polycystic ovary syndrome resistant to clomiphene citrate. J Obstet Gynaecol Res. 2007;33:174–80.
52. Alborzi S, Khodaee R, Parsanejad ME. Ovarian size and response to laparoscopic ovarian electro-cauterization in polycystic ovarian disease. Int J Gynaecol Obstet. 2001;74:269–74.
53. Kong GW, Cheung LP, Lok IH. Effects of laparoscopic ovarian drilling in treating infertile anovulatory polycystic ovarian syndrome patients with and without metabolic syndrome. Hong Kong Med J. 2011;17:5–10.
54. Amer SA, Li TC, Ledger WL. The value of measuring anti-Mullerian hormone in women with anovulatory polycystic ovary syndrome undergoing laparoscopic ovarian diathermy. Hum Reprod. 2009;24:2760–6.
55. Elmashad AI. Impact of laparoscopic ovarian drilling on anti-Müllerian hormone levels and ovarian stromal blood flow using three-dimensional power Doppler in women with anovulatory polycystic ovary syndrome. Fertil Steril. 2011;95:2342–6.
56. Mercorio F, Mercorio A, Di Spiezio Sardo A, Barba GV, Pellicano M, Nappi C. Evaluation of ovarian adhesion formation after laparoscopic ovarian drilling by second-look minilaparoscopy. Fertil Steril. 2008;89:1229–33.
57. Api M. Adhesions after laparoscopic ovarian drilling in the treatment of women with polycystic ovary syndrome: should it be a concern? J Minim Invasive Surg Sci. 2014;3:e10729.
58. Flyckt RL, Goldberg JM. Laparoscopic ovarian drilling for clomiphene-resistant polycystic ovary syndrome. Semin Reprod Med. 2011;29:138–46.
59. Api M. Is ovarian reserve diminished after laparoscopic ovarian drilling? Gynecol Endocrinol. 2009;25:159–65.
60. Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, Kramer P, Fauser BC, Themmen AP. Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod. 2004;10:77–83.
61. Visser JA, de Jong FH, Laven JSE, Themmen APN. Anti-Mullerian hormone: a new marker for ovarian function. Reproduction. 2006;131:1–9.
62. Laven JS, Mulders AG, Visser JA, Themmen APN, De Jong FH, Fauser BC. Anti-Müllerian hormone serum concentrations in normoovulatory and anovulatory women of reproductive age. J Clin Endocrinol Metab. 2004;89:318–23.
63. Pigny P, Merlen E, Robert Y, Cortet-Rudelli C, Decanter C, Jonard S, Dewailly D. Elevated serum level of anti-Mullerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest. J Clin Endocrinol Metab. 2003;88:5957–62.
64. Park AS, Lawson MA, Chuan SS, Oberfield SE, Hoeger KM, Witchel SF, Chang RJ. Serum anti-Mullerian hormone concentrations are elevated in oligomenorrheic girls without evidence of hyperandrogenism. J Clin Endocrinol Metab. 2010;95:1786–92.
65. Lie Fong S, Schipper I, de Jong FH, Themmen AP, Visser JA, Laven JS. Serum anti-Mullerian hormone and inhibin B concentrations are not useful predictors of ovarian response during ovulation induction treatment with recombinant follicle-stimulating hormone in women with polycystic ovary syndrome. Fertil Steril. 2011;96:459–63.
66. Bhide P, Dilgil M, Gudi A, Shah A, Akwaa C, Homburg R. Each small antral follicle in ovaries of women with polycystic ovary syndrome produces more antimüllerian hormone than its counterpart in a normal ovary: an observational cross-sectional study. Fertil Steril. 2015;103:537–41.
67. Jeppesen JV, Anderson RA, Kelsey TW, Christiansen SL, Kristensen SG, Jayaprakasan K, Raine-Fenning N, Campbell BK, Yding Andersen C. Which follicles make the most

anti-Mullerian hormone in humans? Evidence for an abrupt decline in AMH production at the time of follicle selection. Mol Hum Reprod. 2013;19:519–27.
68. Pellatt L, Hanna L, Brincat M, Galea R, Brain H, Whitehead S, Mason H. Granulosa cell production of anti-Müllerian hormone is increased in polycystic ovaries. J Clin Endocrinol Metab. 2007;92:240–5.
69. Pellatt L, Rice S, Mason HD. Anti-Mullerian hormone and polycystic ovary syndrome: a mountain too high? Reproduction. 2010;139:825–33.
70. Durlinger AL, Kramer P, Karels B, de Jong FH, Uilenbroek JT, Grootegoed JA, Themmen AP. Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary. Endocrinology. 1999;140:5789–96.
71. Durlinger AL, Gruijters MJ, Kramer P, Karels B, Kumar TR, Matzuk MM, Rose UM, de Jong FH, Uilenbroek JT, Grootegoed JA, Themmen AP. Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology. 2001;142:4891–9.
72. Pellatt L, Rice S, Dilaver N, Heshri A, Galea R, Brincat M, Brown K, Simpson ER, Mason HD. Anti-Mullerian hormone reduces follicle sensitivity to follicle stimulating hormone in human granulosa cells. Fertil Steril. 2011;96:1246–51.
73. Mahran A, Abdelmeged A, El-Adawy AR, Eissa MK, Shaw RW, Amer SA. The predictive value of circulating anti-Müllerian hormone in women with polycystic ovarian syndrome receiving clomiphene citrate: a prospective observational study. J Clin Endocrinol Metab. 2013;98:4170–5.
74. Xi W, Yang Y, Mao H, Zhao X, Liu M, Fu S. Circulating anti-mullerian hormone as predictor of ovarian response to clomiphene citrate in women with polycystic ovary syndrome. J Ovarian Res. 2016;9:3.
75. Mumford SL, Legro RS, Diamond MP, Coutifaris C, Steiner AZ, Schlaff WD, Alvero R, Christman GM, Casson PR, Huang H, Santoro N, Eisenberg E, Zhang H, Cedars MI. Baseline AMH level associated with ovulation following ovulation induction in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2016;101:3288–96.
76. Amer SA, Mahran A, Abdelmaged A, El-Adawy AR, Eissa MK, Shaw RW. The influence of circulating anti-Müllerian hormone on ovarian responsiveness to ovulation induction with gonadotrophins in women with polycystic ovarian syndrome: a pilot study. Reprod Biol Endocrinol. 2013;11:115.
77. Sunj M, Kasum M, Canic T, Karelovic D, Tandara M, Tandara L, Palada I. Assessment of ovarian reserve after unilateral diathermy with thermal doses adjusted to ovarian volume. Gynecol Endocrinol. 2014;30:785–8.

Inositols

16

John E. Nestler and Antonio Simone Laganà

16.1 Introduction

Evidence indicates that insulin resistance plays an important pathogenic role in the hyperandrogenism and anovulation of women affected by polycystic ovary syndrome (PCOS). Both lean and obese women with PCOS manifest insulin resistance that is intrinsic to the syndrome and associated with a compensatory hyperinsulinaemia (i.e. hyperinsulinaemic insulin resistance). Multiple in vivo [1–3] and in vitro [4, 5] studies demonstrate that hyperinsulinaemic insulin resistance increases ovarian androgen production and interferes with ovulation in women with PCOS. In addition, hyperinsulinaemia inhibits hepatic sex hormone-binding globulin (SHBG) production in women with PCOS [6], resulting in a marked increase in circulating free testosterone levels. Moreover, hyperinsulinaemia may alter physiologic gonadotrophin secretory dynamics, increasing luteinising hormone (LH) levels, which then act in concert with insulin to augment ovarian androgen production [4, 7]. The mechanism(s) for these effects remains unclear but may be related to one or more genetic defects that render PCOS women hypersensitive to the actions of insulin at these target sites. Insulin's actions in PCOS may include binding to the IGF-1 receptor on ovarian cells which may, in turn, adversely influence the ovulation process [5].

In addition, in PCOS there are profound metabolic consequences that affect fertility and pregnancy complications. In some women with PCOS, the compensatory hyperinsulinaemia is sufficient to maintain normal glucose tolerance, whereas in

J.E. Nestler
Department of Internal Medicine, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA, USA

A.S. Laganà (✉)
Unit of Gynecology and Obstetrics, Department of Human Pathology in Adulthood and Childhood "G. Barresi", University of Messina, Via C. Valeria 1, 98125 Messina, Italy
e-mail: antlagana@unime.it

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_16

other women the compensation is inadequate and glucose intolerance develops as a consequence. Insulin resistance is a major risk factor for the development of type 2 diabetes mellitus (DM), and 30–50% of obese PCOS women develop either impaired glucose tolerance or DM by the age of 30 years old [8, 9]. In addition, the prevalence of metabolic syndrome in women with PCOS is two to four times higher than in the general population, with the prevalence of metabolic syndrome in PCOS women between the ages of 30 and 40 years old is greater than 50% [10]. Women with PCOS develop a higher prevalence of cardiovascular risk factors at an earlier age, and studies suggest that women with PCOS may have a twofold higher risk for a cardiac event in the fifth and sixth decades of life [11–13].

Given the central pathogenic role of insulin resistance in the endocrine, reproductive and metabolic disturbances of PCOS, several pharmacological and non-pharmacological approaches have been proposed to counteract the hyperinsulinaemic insulin resistance typical of the syndrome. For example, an improvement in insulin sensitivity achieved through diet-induced weight loss has been shown to reduce circulating androgens and improve fertility. Similarly, insulin-sensitising drugs such as metformin, troglitazone and inositols have been studied and have proven beneficial for the treatment of infertility in women with PCOS.

Based on these considerations, also, the insulin-sensitising effects of inositol have been studied to assess their amelioration of the symptoms and signs of this syndrome, including the possibility of restoring the fertility of women with PCOS. One of the most important manifestations of PCOS is anovulation, which is the main contributor to infertility in affected patients. Considering the intimate connection among ovulatory dysfunction, hyperinsulinaemic insulin resistance and hyperandrogenism, clinical data on the efficacy of inositol alone or combined with other compounds have been assessed in humans with particular regard in women with PCOS. Indeed, the increasing interest for inositol action led to the International Consensus Conference on the Use of Inositol in Obstetrics and Gynaecology [40], which tried to shed light on the best practice about this topic. The aim of this chapter will be to summarise the available evidence regarding the role of inositols in ameliorating fertility in PCOS women.

16.2 Mechanisms of Action of Inositols

Inositol is synthesised by both prokaryotic and eukaryotic cells, but in mammals it is primarily obtained from dietary sources as inositol-6-phosphate. From a chemical point of view, inositol is a polyalcohol composed of six-carbon rings with a hydroxyl group attached to each carbon of the ring (Fig. 16.1). As summarised in a recent narrative review [14], there are nine possible stereoisomeric forms of inositol (myo-, scyllo-, muco-, epi-, neo-, allo-, D-chiro- and L-chiro-inositols and one that is not known to occur naturally, cis-inositol), related to the epimerisation of the six hydroxyl groups. Among these, the two isoforms myo-inositol (MI) and D-chiro-inositol (DCI) captured the attention of researchers for their insulin-sensitising actions, which configure them as proper candidates for the treatment of PCOS. We

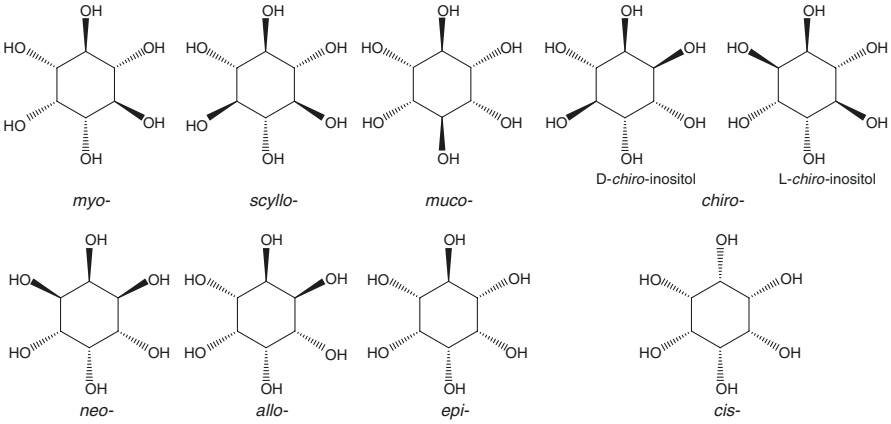


Fig. 16.1 The structures of the inositol isomers (Mills projection)

previously reported [15] that both MI and DCI function as insulin second messengers and mediate different actions of insulin in humans [16]. MI is converted to an inositolphosphoglycan (IPG) insulin second messenger (MI-IPG) involved primarily in cellular glucose uptake, whereas DCI is converted to an IPG insulin second messenger (DCI-IPG) involved primarily in glycogen synthesis (Fig. 16.1).

As will be discussed in detail, subsequently it has been demonstrated that the MI-IPG is involved in glucose uptake [15], whereas the DCI-IPG may mediate insulin's stimulation of ovarian androgen production [4] as well as improved target tissue sensitivity to insulin [17].

Inositol can be present within cells in a free form or as phosphatidylinositol (phosphoinositides, PtdIns), which can be further phosphorylated to form phosphatidylinositol phosphate (PIP) and biphosphate (PIP2). After stimulation by growth factors or other hormones, phospholipase-C (PLP-C) mediates the cleavage of PIP2 to form the precursor of inositol triphosphate (InsP3), which then acts as a second messenger to mediate different actions of insulin. As reviewed by Dinicola et al. [18], MI and PLP-C indirectly modulate LH/FSH activity and InsP3 release both in a mouse model [19] and humans [20]; in addition, InsP3 interacts with its respective receptors and controls intracellular Ca^{2+} release.

Within the ovary, the binding of InsP3 to its receptor 1 (IP3-R1) seems to be mandatory for oocyte maturation, especially in the final stages of development that are tightly calcium dependent [21]. Interestingly, accumulating evidence from animal models suggests that exogenous injection of InsP3 stimulates Ca^{2+} release from the ovary, thus allowing orderly oogenesis [22]. In addition, MI appears to promote meiotic progression of oocytes into fertilisation-competent eggs in the mouse model, whereas a depletion of MI intracellular stores within the ovary may alter the physiological processes previously described [23].

From a metabolic viewpoint, when insulin binds its own receptor, hydrolysis of glycosylphosphatidylinositol lipids located at the outer leaflet of the cell membrane occurs, and two IPGs, one incorporating DCI and the other MI, are released. These two IPGs play a pivotal role in glucose metabolism, activating key enzymes that

control the oxidative and non-oxidative metabolism of glucose. Specifically, studies in a mouse model suggested that the DCI-IPGs seem to be more effective in partially restoring insulin sensitivity and glycogen synthesis than the MI-IPGs [24]. Nevertheless, other studies conducted in obese rhesus monkeys [25] and postmenopausal women with metabolic syndrome [26] suggested a robust role as well for orally administered MI in improving insulin sensitivity, thereby reversing impaired glucose tolerance and improving serum lipid levels and blood pressure.

Within cells, MI can be converted to DCI through a process of epimerisation, and this intracellular epimerase enzyme is modulated by insulin [27]. Utilising this mechanism, every single organ can regulate the balance of inositol levels, and all organs have tissue-specific ratios of intracellular MI to DCI [28]. DCI levels are high in glycogen storage tissues, such as liver, muscle and fat, and low in tissues with high glucose utilisation, such as brain and heart. Insulin resistance has been associated with reduced availability of DCI, as demonstrated by Ortmeyer et al. [29], who reported low urinary excretion of IPGs incorporating DCI in diabetic rhesus monkeys, which was later confirmed in humans [30]. Congruent with these findings, diet supplementation of DCI reduced insulin resistance in diabetic rats or monkeys affected by hyperglycaemia [31].

In summary, accumulating evidence [32] suggests that metabolic derivatives of MI and DCI are mediators of insulin action that work in synergy with each other. MI-IPG induces glucose transporter type 4 (GLUT4) receptor translocation to the cell membrane [33, 34], thus enhancing cellular uptake of glucose, and MI derivatives inhibit adenylate cyclase enzyme, thus reducing release of free fatty acids from adipose tissues. Nevertheless, another study showed that lactate-induced translocation of GLUT1 is not mediated by PI3K in the mouse model, suggesting a different modulation with respect to the abovementioned GLUT4 [35].

DCI-IPG stimulates pyruvate dehydrogenase (PDH), thus supporting adenosine triphosphate (ATP) production through the Krebs cycle and stimulating glycogen synthase. Regarding this last point, DCI-IPG stimulates glycogen synthase, thus supporting glucose conversion to glycogen stored inside cells [24].

16.3 Role of Abnormal Epimerase Activity in PCOS

As discussed earlier, the circulating and/or intra-tissue balance between MI and DCI may play a pivotal role in modulating metabolic processes. Several studies have reported the lack of release of DCI-IPG (measured by a PDH phosphatase bioactivity assay) in the blood of DM subjects during a glucose tolerance test [36], lack of DCI-IPG release in women with PCOS during an insulin clamp [37] and lack of release of DCI-IPG from placental membranes obtained from women with preeclampsia in response to insulin administration in vitro [38].

Based on these data suggesting that an increased ratio of MI to DCI, due to a deficiency in DCI, is associated with insulin resistance, Larner's group proposed that this imbalance may be caused by a defective epimerisation of MI to DCI—i.e. an inversion of carbon 3 hydroxyl [39]. In order to investigate this possibility, they

demonstrated in the Goto-Kakizaki DM rat that in insulin-sensitive tissues (muscle, liver and fat) [^{3}H]MI conversion to [^{3}H]chiro-inositol was reduced from about 20–30% in control rats to under 5% in the DM rats [40]. Subsequently, they partially purified the MI to DCI epimerase from rat liver and demonstrated its absolute requirement for nucleotide, indicating that it acted via an oxido-reductive mechanism and suggesting reduced epimerase enzyme activity [41].

Reports suggest that as many as 50% of women with PCOS manifest hyperinsulinaemia and peripheral insulin resistance in adipose tissue and skeletal muscle. In distinct contrast, ovarian theca and granulosa cells in PCOS women do not develop insulin resistance and have been reported to be exquisitely sensitive to insulin [42]. Following up on this observation, Heimark et al. [27] recently studied well-characterised theca cells from normal cycling women with normal insulin sensitivity and theca cells from PCOS women with hyperinsulinaemic insulin resistance and examined the intracellular ratio of MI to DCI and the activity of the epimerase enzyme that converts MI to DCI. They reported that the ratio of MI to DCI in the theca cells from the PCOS women was lower in comparison with the high MI to DCI ratio in the theca cells from normal women. Similarly, thecal epimerase activity was increased in cells obtained from the PCOS women compared to that in theca cells of healthy women. The explanation for these observations is that the theca cells from the PCOS women manifested enhanced sensitivity to insulin, resulting in insulin-stimulated epimerase activity and increased conversion of MI to DCI [27].

In accordance with these data, it was suggested that epimerisation of MI to DCI is enhanced in patients with PCOS and hyperinsulinaemia, which in turn yields MI deficiency in the ovary that would impair FSH signalling, resulting in reduced oocyte quality and increased risk of ovarian hyperstimulation syndrome (OHSS) [43]. In order to explore this possibility, Unfer et al. [28] recently measured MI and DCI levels in the follicular fluid of a small sample of patients with PCOS, who manifested hyperinsulinaemic insulin resistance, and in a small sample of healthy women. They reported that the follicular ratio of MI to DCI was 100:1 in healthy women compared to only 0.2:1 in patients with PCOS, which was due to a dramatic reduction in follicular MI and increase in DCI in the PCOS women and consistent with insulin-stimulated epimerase activity.

The increased intra-follicular DCI resulting from insulin-stimulated epimerase activity would be incorporated into precursor glycophosphatidylinositol and/or precursor glycophosphatidylinositol-protein, which could then be cleaved into the DCI-IPG. As demonstrated with INS-2, a synthetic chiro-inositol-containing glycan, DCI-IPG could then act locally in the ovary to increase thecal androgen production [4].

16.4 Effects of Inositols in Women with PCOS

It is currently accepted that oral administration of MI alone, DCI alone or the combination of MI and DCI may alleviate many of the metabolic dysregulations typical of PCOS. Below we will report data on the effects of DCI and MI administration, alone and/or in combination, in women with PCOS.

16.4.1 DCI Administration

Since the initial report by Nestler et al. [17], several studies [44–46] have investigated the effects of oral DCI administration in women with PCOS.

Nestler and collaborators [17] performed a randomised, double-blind, placebo-controlled trial on 44 obese women with PCOS who received either 1200 mg DCI or placebo for 6–8 weeks. After treatment, there were significant decreases in the waist-to-hip ratio (WHR), systolic and diastolic blood pressure and plasma total cholesterol and triglyceride concentrations in women who received DCI compared with the placebo group. Regarding glucose metabolism, in the DCI group, the area under the plasma insulin curve after oral glucose administration decreased, although this decrease did not differ significantly from that in the placebo group [17]. In the same study [17], the leuprolide stimulation test [used to evaluate the changes from baseline of the serum LH and 17α-hydroxyprogesterone (17-OH-P) concentrations after leuprolide injection] demonstrated reduced early and late responses of serum LH and 17-OH-P only after DCI administration [17]. In keeping with these observations, administration of DCI was associated with a decrease in the serum free testosterone and dehydroepiandrosterone sulphate concentrations and an increase in the serum SHBG concentration. Finally, 86% of the women in the DCI group ovulated during treatment with DCI, as compared with only 27% of the women in the placebo group [17].

Subsequently, Iuorno et al. [44] performed a similar double-blind placebo-controlled randomised trial (RCT) using 600 mg of DCI once daily for 6–8 weeks to treat a small population of 20 lean women with PCOS. Both systolic and diastolic blood pressure, as well as serum total cholesterol and triglycerides, decreased significantly in women who received DCI, but not in the placebo group [44]. Neither the fasting plasma glucose nor fasting insulin concentration changed significantly in either group [44]. Conversely, both the area under the plasma glucose and insulin curves during the oral glucose tolerance test (OGTT) decreased significantly in the DCI group, and these decreases differed significantly compared with the lack of changes in the placebo group [44]. Furthermore, the composite whole-body insulin sensitivity index (ISIcomp) increased by 84% in the DCI group but did not change in the placebo group [44]. Regarding hormonal parameters, the administration of DCI was associated with significant declines in both serum total and free testosterone concentrations. Finally, 60% of women in the DCI group ovulated, compared with only 20% in the placebo group [44].

More recently, Laganà et al. [45] confirmed these data by performing a larger prospective cohort study. Forty-eight lean [body mass index (BMI) < 25 kg/m^2] women affected by oligo-anovulatory PCOS received 1 g of DCI plus 400 μg of folic acid orally once daily for 6 months. Significant decreases in systolic blood pressure, Ferriman-Gallwey score, plasma LH and the LH/FSH ratio, serum levels of total and free testosterone, Δ4-androstenedione and prolactin and the homeostatic model assessment (HOMA) index were observed after treatment. The DCI group also experienced significant increases in serum SHBG and the fasting glucose/insulin ratio. Finally, there was significant (62.5%) post-treatment menstrual cycle regularisation.

Similar results were reported by Genazzani et al. [46], who assessed the effects of DCI 500 mg orally once daily for 12 weeks on hormonal parameters and insulin sensitivity in a population of overweight/obese PCOS women (BMI > 26 kg/m^2). Plasma LH levels and the LH/FSH ratio decreased during the study, and consequently serum androstenedione, testosterone and 17-hydroxy-progesterone concentrations also declined significantly. Metabolically, at baseline, the obese PCOS women manifested a clear hyperinsulinaemic response to oral glucose administration that was essentially normalised by treatment. The area under the curve (AUC) of insulin and the maximal insulin response to a glucose load were both significantly reduced. Interestingly, these investigators performed low-dose gonadotrophin-releasing hormone (GnRH) stimulation tests in order to assess LH and FSH responses and reported that the LH response to GnRH bolus was significantly modified after the treatment and consistently reduced both in terms of AUC and maximal response to GnRH bolus.

Collectively, the data from these studies suggest that oral administration of DCI to women with PCOS can improve insulin sensitivity, reduce circulating insulin and androgens and improve ovulatory frequency reducing triglycerides and blood pressure—thus bringing ovarian function and metabolism toward normal homeostasis.

16.4.2 MI Administration

Papaleo et al. [47] explored whether oral administration of MI would improve insulin sensitivity in women with PCOS, thus restoring normal ovulatory function. The investigators enrolled 25 PCOS women of childbearing age whose infertility was solely due to oligo- or amenorrhoea, since no tubal defect or deficiency of male semen parameters was found. PCOS women were treated orally with MI 2 g plus folic acid 200 μg daily for 6 months or until a positive pregnancy test was obtained [47]. After the first month of treatment, 22 out of the 25 women (88%) had a menstrual cycle; 18 of these 22 patients menstruated monthly during the follow-up period [47]. All of these 22 women maintained monthly spontaneous ovulation activity, as documented by follicular growth and increased serum progesterone concentrations during the luteal phase [47]. Moreover, serum concentrations of total testosterone and free testosterone decreased significantly [47].

Concurrently, a double-blind, placebo-controlled RCT [48] assessed ovarian activity after 14 weeks of oral MI treatment in PCOS patients with oligo- or amenorrhoea. The daily dose of MI administered was 4 g MI plus 400 μg of folic acid, which was twice as large as that administered in the aforementioned study [47]. Ovulatory frequency and the number of ovulatory patients were significantly higher in the MI-treated group compared with the placebo, and the time to first ovulation was significantly briefer in the MI group [47]. Notably, the effect of MI administration on follicular maturation was rapid, since the oestradiol concentration increased over the first week of treatment [47]. In terms of metabolic outcomes, serum high-density lipoprotein (HDL) levels increased significantly in the women treated with MI, but there were no changes in concentrations of fasting glucose or fasting insulin

nor in the insulin responses to glucose challenge [47]. Women in the MI group experienced significant decreases in weight and leptin levels, whereas women in the placebo group gained weight [47].

Similar results were reported in another double-blind placebo-controlled RCT [49], which studied a smaller population (42 PCOS women) using a similar MI dosage of 4 g daily administered for 12–16 weeks. In this study, serum total testosterone, serum free testosterone, plasma triglycerides, systolic and diastolic blood pressure and the AUC for insulin after oral administration of glucose significantly decreased in the women treated with MI [49]; congruently with this last finding, insulin sensitivity (assessed by ISIcomp) increased significantly [49]. Moreover, the ovulation rate in the MI group was higher compared with the placebo group [49]. However, previous medical treatments (including oral contraceptives, insulin-sensitising agents and others) or a beta error effect due to the small population could have potentially influenced the results. Moreover, in agreement with these studies [48, 49], Pizzo et al. [50] also administered 4 g of MI plus folic acid to PCOS women and reported significant reductions in diastolic and systolic arterial pressure, LH, LH/FSH ratio, total testosterone, free testosterone, Δ4-androstenedione, prolactin and HOMA index. In these same patients, there were statistically significant increases in serum SHBG and fasting glucose/insulin ratio [50].

The ability of MI to improve ovulatory function has been tested against metformin, a drug that is commonly used to enhance ovulation in oligo-anovulatory women with PCOS. Raffone et al. [51] enrolled 120 patients and randomly treated them daily with metformin 1500 mg/day orally or 4 g MI plus 400 μg folic acid orally for 6 months or until pregnancy occurred. Fifty percent (50%) of the patients treated with metformin experienced restoration of spontaneous ovulation, and 18.3% of these women attained pregnancy. In contrast, spontaneous ovulation was restored in 65% of the patients treated with MI plus folic acid, and 30% of these women attained pregnancy [51]. The results between the metformin and MI groups were not statistically different [51]. Therefore, treatment with MI or metformin plus folic acid was equally effective. However, in this regard, it should be noted that the supplementation of folic acid to metformin may have played a contributory role, since folic acid can cause a subclinical alteration of homocysteine levels and may influence insulin sensitivity [52].

16.4.3 DCI plus MI Administration

Since both MI and DCI monotherapies had been shown to improve ovulatory function in PCOS, the efficacy of combined treatment with DCI plus MI in reducing the risk of metabolic syndrome and enhancing ovarian function, compared to treatment with MI monotherapy, has been studied. In an RCT [53], both plasma glucose and insulin concentrations decreased significantly in the combination DCI plus MI group, whereas no changes were observed in the group treated with MI alone. Furthermore, the decrement in total testosterone and the increment in serum SHBG were more robust in the combination DCI plus MI group compared with the MI

monotherapy group [53]. Arguably, overweight PCOS patients showed an inverse relationship between body mass and treatment efficacy [54], even if these data still need to be confirmed in lean patients.

In summary, the reviewed studies indicate that both isoforms of inositol (DCI and MI) are effective in improving ovarian function and metabolism in patients with PCOS, although MI appeared to have the most salutary effect on the metabolic profile, whereas DCI appeared superior in reducing circulating androgens [50].

16.5 Inositol as Fertility Drug

Women with PCOS have a high rate of infertility due in large part to anovulation and ovulatory dysfunction (see Chaps. 3 and 6). As detailed in previous chapters, hyperinsulinaemic insulin resistance and its consequent hyperandrogenaemia may play a pivotal role in causing this ovulatory dysfunction, and this serves as the rationale for the treatment of PCOS with insulin-sensitising drugs to restore metabolic and hormonal homeostasis and, consequently, physiologic ovulation and fertility. In this section, we will briefly review the available clinical data about fertility outcomes in PCOS women after inositol treatment, with or without the aim of assisted reproduction technologies (ART). In other specific chapters of the book, the controlled ovarian stimulation for IVF and non-IVF cycles has been detailed.

Spontaneous ovulation rate of women with PCOS treated with either oral DCI [17, 44, 45] or oral MI improved [47–51]. In addition, a 6-month prospective study on 50 PCOS patients with insulin resistance showed that the combined therapy with both DCI plus MI [18, 53, 54] and clomiphene citrate (CC) plus MI [55] achieve results, in terms of ovulation, superior to monotherapy. Similarly, a co-treatment consisting in oral 4 g MI plus 400 μg folic acid and recombinant FSH (37.5 IU/day) was explored in women with PCOS who failed to restore spontaneous ovulation under MI/folic acid therapy alone reporting a pregnancy rate of about 30% [51]. Despite the small population and the noncontrolled study designs, that data may open a new scenario wherein positive combination of MI and CC/FSH can induce mono-ovulation in anovulatory PCOS women.

It was recently reported [56] that MI administration improves oocyte quality and increases the number of oocytes collected after ovarian stimulation in PCOS patients undergoing IVF and, in euglycaemic PCOS patients undergoing ovulation induction for ICSI, the number of mature oocytes [57]. However, this result may have been influenced, at least in part, by the high dose of DCI administered on oocyte competence [58]. Similarly, a combination of inositol (1500 mg), lactoferrin (100 mg) and bromelain (20 mg) increased the pregnancy rate of about threefold in PCOS patients who received recombinant FSH for non-IVF cycles [59]. Nevertheless, there are several cautions regarding interpretation of this study: first, the report did not specify which isoform of inositol was administered; second, no information about sperm quality or other parameters that could influence conception during IVF were reported; and, finally, the duration of treatment was not standardised at study design.

Last but not least, it was recently reported that treatment with DCI 1 g daily decreases the production of reactive oxygen species (ROS) within the ovaries [60], which are known to play a detrimental role in PCOS [61], and that combined treatment with inositol plus metformin restores homeostasis at the level of thyroid-stimulating hormone in infertile PCOS patients affected by subclinical thyroid dysfunction [62]—a condition potentially associated with unfavourable reproductive outcomes [63].

Conclusion

Based on available evidence, both DCI and MI are able to improve the metabolic profile and fertility outcomes in PCOS patients. As showed in a comprehensive systematic review [64], data published so far allow us to attest that despite the relatively high number of clinical studies of inositol as a treatment in women with PCOS, only a few of them were designed as RCTs, and inositol mechanism of action appears to relate primarily to improving the insulin sensitivity of target tissues, resulting in a reduction in circulating insulinaemia, which in turn is responsible for positive effects on the reproductive axis (restoration of ovulation and improved oocyte quality) and on the hormonal milieu (reduction in serum androgens and improved lipid status).

Moreover, efficacy data are still preliminary and consist of small studies, frequently noncontrolled, including other active supplements. Also available experimental data are obtained in the presence of so many confounders that require the results to be confirmed in other settings and on well-selected samples. At the moment, there is no well-designed dose-finding study in order to define the best doses for DCI and MI.

In addition, doses used in the available studies are extremely variable. Similarly, there is no experimental or clinical study assessing the different doses for combining two drugs. Hence, there is a need for future properly controlled studies on larger cohorts of PCOS patients and with greater statistical power, which would more accurately clarify post-treatment fertility outcomes with the different inositol isoforms, establish optimal therapeutic strategies tailored to the pretreatment phenotype of the patient (i.e. “personalised dosage” based on patients’ clinical or biochemical features) and evaluate the variability of the long-term outcomes on the basis of these phenotypic parameters.

References

1. Nestler JE, Jakubowicz DJ. Decreases in ovarian cytochrome P450c17 alpha activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. N Engl J Med. 1996;335:617–23.
2. Nestler JE, Jakubowicz DJ. Lean women with polycystic ovary syndrome respond to insulin reduction with decreases in ovarian P450c17 alpha activity and serum androgens. J Clin Endocrinol Metab. 1997;82:4075–9.
3. Nestler JE, Barlascini CO, Matt DW, Steingold KA, Plymate SR, Clore JN, Blackard WG. Suppression of serum insulin by diazoxide reduces serum testosterone levels in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab. 1989;68:1027–32.

4. Nestler JE, Jakubowicz DJ, de Vargas AF, Brik C, Quintero N, Medina F. Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. J Clin Endocrinol Metab. 1998;83:2001–5.
5. Poretsky L, Kalin MF. The gonadotropic function of insulin. Endocr Rev. 1987;8:132–41.
6. Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Rittmaster RS, Clore JN, Blackard WG. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. J Clin Endocrinol Metab. 1991;72:83–9.
7. Buckler HM, McLachlan RI, MacLachlan VB, Healy DL, Burger HG. Serum inhibin levels in polycystic ovary syndrome: basal levels and response to luteinizing hormone-releasing hormone agonist and exogenous gonadotropin administration. J Clin Endocrinol Metab. 1988;66:798–803.
8. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev. 2012;33:981–1030.
9. Corbould A, Kim Y-B, Youngren JF, Pender C, Kahn BB, Lee A, Dunaif A. Insulin resistance in the skeletal muscle of women with PCOS involves intrinsic and acquired defects in insulin signaling. Am J Physiol Endocrinol Metab. 2005;288:E1047–54.
10. Apridonidze T, Essah PA, Iuorno MJ, Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2005;90:1929–35.
11. Tomlinson J, Millward A, Stenhouse E, Pinkney J. Type 2 diabetes and cardiovascular disease in polycystic ovary syndrome: what are the risks and can they be reduced? Diabet Med. 2010;27:498–515.
12. Wild RA, Carmina E, Diamanti-Kandarakis E, Dokras A, Escobar-Morreale HF, Futterweit W, Lobo R, Norman RJ, Talbott E, Dumesic DA. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the androgen excess and polycystic ovary syndrome (AE-PCOS) society. J Clin Endocrinol Metab. 2010;95:2038–49.
13. Shaw LJ, Bairey Merz CN, Azziz R, Stanczyk FZ, Sopko G, Braunstein GD, Kelsey SF, Kip KE, Cooper-Dehoff RM, Johnson BD, Vaccarino V, Reis SE, Bittner V, Hodgson TK, Rogers W, Pepine CJ. Postmenopausal women with a history of irregular menses and elevated androgen measurements at high risk for worsening cardiovascular event-free survival: results from the National Institutes of Health–National Heart, Lung, and Blood Institute sponsored Women's ischemia syndrome Evaluation. J Clin Endocrinol Metab. 2008;93:1276–84.
14. Thomas MP, Mills SJ, Potter BVL. The "other" inositols and their phosphates: synthesis, biology, and medicine (with recent advances in myo-inositol chemistry). Angew Chem Int Ed Eng. 2016;55:1614–50.
15. Nestler JE, Unfer V. Reflections on inositol(s) for PCOS therapy: steps toward success. Gynecol Endocrinol. 2015;31:501–5.
16. Larner J, Huang LC, Tang G, Suzuki S, Schwartz CF, Romero G, Roulidis Z, Zeller K, Shen TY, Oswald AS. Insulin mediators: structure and formation. Cold Spring Harb Symp Quant Biol. 1988;53(Pt 2):965–71.
17. Nestler JE, Jakubowicz DJ, Reamer P, Gunn RD, Allan G. Ovulatory and metabolic effects of D-chiro-inositol in the polycystic ovary syndrome. N Engl J Med. 1999;340:1314–20.
18. Dinicola S, Chiu TTY, Unfer V, Carlomagno G, Bizzarri M. The rationale of the myo-inositol and D-chiro-inositol combined treatment for polycystic ovary syndrome. J Clin Pharmacol. 2014;54:1079–92.
19. Matsuda M, Tsutsumi K, Kanematsu T, Fukami K, Terada Y, Takenawa T, Nakayama KI, Hirata M. Involvement of phospholipase C-related inactive protein in the mouse reproductive system through the regulation of gonadotropin levels. Biol Reprod. 2009;81:681–9.
20. Zacchè MM, Caputo L, Filippis S, Zacchè G, Dindelli M, Ferrari A. Efficacy of myo-inositol in the treatment of cutaneous disorders in young women with polycystic ovary syndrome. Gynecol Endocrinol. 2009;25:508–13.

21. Goud PT, Goud AP, Van Oostveldt P, Dhont M. Presence and dynamic redistribution of type I inositol 1,4,5-trisphosphate receptors in human oocytes and embryos during in-vitro maturation, fertilization and early cleavage divisions. Mol Hum Reprod. 1999;5:441–51.
22. Lowther KM, Weitzman VN, Maier D, Mehlmann LM. Maturation, fertilization, and the structure and function of the endoplasmic reticulum in cryopreserved mouse oocytes. Biol Reprod. 2009;81:147–54.
23. Chiu TTY, Rogers MS, Briton-Jones C, Haines C. Effects of myo-inositol on the in-vitro maturation and subsequent development of mouse oocytes. Hum Reprod. 2003;18:408–16.
24. Larner J. D-chiro-inositol--its functional role in insulin action and its deficit in insulin resistance. Int J Exp Diabetes Res. 2002;3:47–60.
25. Ortmeyer HK. Dietary myoinositol results in lower urine glucose and in lower postprandial plasma glucose in obese insulin resistant rhesus monkeys. Obes Res. 1996;4:569–75.
26. Giordano D, Corrado F, Santamaria A, Quattrone S, Pintaudi B, Di Benedetto A, D'Anna R. Effects of myo-inositol supplementation in postmenopausal women with metabolic syndrome: a perspective, randomized, placebo-controlled study. Menopause. 2011;18:102–4.
27. Heimark D, McAllister J, Larner J. Decreased myo-inositol to chiro-inositol (M/C) ratios and increased M/C epimerase activity in PCOS theca cells demonstrate increased insulin sensitivity compared to controls. Endocr J. 2014;61:111–7.
28. Unfer V, Carlomagno G, Papaleo E, Vailati S, Candiani M, Baillargeon J-P. Hyperinsulinemia alters myoinositol to D-chiroinositol ratio in the follicular fluid of patients with PCOS. Reprod Sci. 2014;21:854–8.
29. Ortmeyer HK, Bodkin NL, Lilley K, Larner J, Hansen BC. Chiroinositol deficiency and insulin resistance. I. Urinary excretion rate of chiroinositol is directly associated with insulin resistance in spontaneously diabetic rhesus monkeys. Endocrinology. 1993;132:640–5.
30. Asplin I, Galasko G, Larner J. Chiro-inositol deficiency and insulin resistance: a comparison of the chiro-inositol- and the myo-inositol-containing insulin mediators isolated from urine, hemodialysate, and muscle of control and type II diabetic subjects. Proc Natl Acad Sci U S A. 1993;90:5924–8.
31. Huang LC, Fonteles MC, Houston DB, Zhang C, Larner J. Chiroinositol deficiency and insulin resistance. III. Acute glycogenic and hypoglycemic effects of two inositol phosphoglycan insulin mediators in normal and streptozotocin-diabetic rats in vivo. Endocrinology. 1993;132:652–7.
32. Paul C, Laganà AS, Maniglio P, Triolo O, Brady DM. Inositol's and other nutraceuticals' synergistic actions counteract insulin resistance in polycystic ovarian syndrome and metabolic syndrome: state-of-the-art and future perspectives. Gynecol Endocrinol. 2016;32:431–8.
33. Kong AM, Horan KA, Sriratana A, Bailey CG, Collyer LJ, Nandurkar HH, Shisheva A, Layton MJ, Rasko JE, Rowe T, Mitchell CA. Phosphatidylinositol 3-phosphate [PtdIns3P] is generated at the plasma membrane by an inositol polyphosphate 5-phosphatase: endogenous PtdIns3P can promote GLUT4 translocation to the plasma membrane. Mol Cell Biol. 2006;26:6065–81.
34. Ijuin T, Takenawa T. Regulation of insulin signalling and glucose transporter 4 (GLUT4) exocytosis by the phosphatidylinositol 3,4,5-trisphosphate (PIP3) phosphatase, SKIP. J Biol Chem. 2012;287:6991–9.
35. Medina RA, Southworth R, Fuller W, Garlick PB. Lactate-induced translocation of GLUT1 and GLUT4 is not mediated by the phosphatidyl-inositol-3-kinase pathway in the rat heart. Basic Res Cardiol. 2002;97:168–76.
36. Shashkin PN, Shashkina EF, Fernqvist-Forbes E, Zhou YP, Grill V, Katz A. Insulin mediators in man: effects of glucose ingestion and insulin resistance. Diabetologia. 1997;40:557–63.
37. Baillargeon J-P, Iuorno MJ, Apridonidze T, Nestler JE. Uncoupling between insulin and release of a D-chiro-inositol-containing inositolphosphoglycan mediator of insulin action in obese women with polycystic ovary syndrome. Metab Syndr Relat Disord. 2010;8:127–36.
38. Scioscia M, Gumaa K, Kunjara S, Paine MA, Selvaggi LE, Rodeck CH, Rademacher TW. Insulin resistance in human preeclamptic placenta is mediated by serine phosphorylation of insulin receptor substrate-1 and -2. J Clin Endocrinol Metab. 2006;91:709–17.

39. Larner J, Craig JW. Urinary myo-inositol-to-chiro-inositol ratios and insulin resistance. Diabetes Care. 1996;19:76–8.
40. Pak Y, Hong Y, Kim S, Piccariello T, Farese RV, Larner J. In vivo chiro-inositol metabolism in the rat: a defect in chiro-inositol synthesis from myo-inositol and an increased incorporation of chiro-[3H]inositol into phospholipid in the Goto-Kakizaki (G.K) rat. Mol Cell. 1998;8:301–9.
41. Sun TH, Heimark DB, Nguygen T, Nadler JL, Larner J. Both myo-inositol to chiro-inositol epimerase activities and chiro-inositol to myo-inositol ratios are decreased in tissues of GK type 2 diabetic rats compared to Wistar controls. Biochem Biophys Res Commun. 2002;293:1092–8.
42. Dupont J, Scaramuzzi RJ. Insulin signalling and glucose transport in the ovary and ovarian function during the ovarian cycle. Biochem J. 2016;473:1483–501.
43. Carlomagno G, Unfer V, Roseff S. The D-chiro-inositol paradox in the ovary. Fertil Steril. 2011;95:2515–6.
44. Iuorno MJ, Jakubowicz DJ, Baillargeon J-P, Dillon P, Gunn RD, Allan G, Nestler JE. Effects of D-chiro-inositol in lean women with the polycystic ovary syndrome. Endocr Pract. 2002;8:417–23.
45. Laganà AS, Barbaro L, Pizzo A. Evaluation of ovarian function and metabolic factors in women affected by polycystic ovary syndrome after treatment with D-Chiro-inositol. Arch Gynecol Obstet. 2015;291:1181–6.
46. Genazzani AD, Santagni S, Rattighieri E, Chierchia E, Despini G, Marini G, Prati A, Simoncini T. Modulatory role of D-chiro-inositol (DCI) on LH and insulin secretion in obese PCOS patients. Gynecol Endocrinol. 2014;30:438–43.
47. Papaleo E, Unfer V, Baillargeon J-P, De Santis L, Fusi F, Brigante C, Marelli G, Cino I, Redaelli A, Ferrari A. Myo-inositol in patients with polycystic ovary syndrome: a novel method for ovulation induction. Gynecol Endocrinol. 2007;23:700–3.
48. Gerli S, Papaleo E, Ferrari A, Di Renzo G. Randomized, double blind placebo-controlled trial: effects of myo-inositol on ovarian function and metabolic factors in women with PCOS. Eur Rev Med Pharmacol Sci. 2007;11:347–54.
49. Costantino D, Minozzi G, Minozzi E, Guaraldi C. Metabolic and hormonal effects of myo-inositol in women with polycystic ovary syndrome: a double-blind trial. Eur Rev Med Pharmacol Sci. 2009;13:105–10.
50. Pizzo A, Laganà AS, Barbaro L. Comparison between effects of myo-inositol and D-chiro-inositol on ovarian function and metabolic factors in women with PCOS. Gynecol Endocrinol. 2014;30:205–8.
51. Raffone E, Rizzo P, Benedetto V. Insulin sensitiser agents alone and in co-treatment with r-FSH for ovulation induction in PCOS women. Gynecol Endocrinol. 2010;26:275–80.
52. Palomba S, Falbo A, Giallauria F, Russo T, Tolino A, Zullo F, Colao A, Orio F. Effects of metformin with or without supplementation with folate on homocysteine levels and vascular endothelium of women with polycystic ovary syndrome. Diabetes Care. 2010;33:246–51.
53. Nordio M, Proietti E. The combined therapy with myo-inositol and D-Chiro-inositol reduces the risk of metabolic disease in PCOS overweight patients compared to myo-inositol supplementation alone. Eur Rev Med Pharmacol Sci. 2012;16:575–81.
54. Unfer V, Porcaro G. Updates on the myo-inositol plus D-chiro-inositol combined therapy in polycystic ovary syndrome. Expert Rev Clin Pharmacol. 2014;7:623–31.
55. Kamenov Z, Kolarov G, Gateva A, Carlomagno G, Genazzani AD. Ovulation induction with myo-inositol alone and in combination with clomiphene citrate in polycystic ovarian syndrome patients with insulin resistance. Gynecol Endocrinol. 2015;31:131–5.
56. Ciotta L, Stracquadanio M, Pagano I, Carbonaro A, Palumbo M, Gulino F. Effects of myo-inositol supplementation on oocyte's quality in PCOS patients: a double blind trial. Eur Rev Med Pharmacol Sci. 2011;15:509–14.
57. Unfer V, Carlomagno G, Rizzo P, Raffone E, Roseff S. Myo-inositol rather than D-chiro-inositol is able to improve oocyte quality in intracytoplasmic sperm injection cycles. A prospective, controlled, randomized trial. Eur Rev Med Pharmacol Sci. 2011;15:452–7.

58. Isabella R, Raffone E. Does ovary need D-chiro-inositol? J Ovarian Res. 2012;5:14.
59. Morgante G, Orvieto R, Di Sabatino A, Musacchio MC, De Leo V. The role of inositol supplementation in patients with polycystic ovary syndrome, with insulin resistance, undergoing the low-dose gonadotropin ovulation induction regimen. Fertil Steril. 2011;95:2642–4.
60. De Leo V, La Marca A, Cappelli V, Stendardi A, Focarelli R, Musacchio MC, Piomboni P. Evaluation of the treatment with D-chiro-inositol on levels of oxidative stress in PCOS patients. Minerva Ginecol. 2012;64:531–8.
61. González F, Rote NS, Minium J, Kirwan JP. Reactive oxygen species-induced oxidative stress in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. J Clin Endocrinol Metab. 2006;91:336–40.
62. Morgante G, Musacchio MC, Orvieto R, Massaro MG, De Leo V. Alterations in thyroid function among the different polycystic ovary syndrome phenotypes. Gynecol Endocrinol. 2013;29:967–9.
63. Reid SM, Middleton P, Cossich MC, Crowther CA, Bain E. Interventions for clinical and subclinical hypothyroidism pre-pregnancy and during pregnancy. Cochrane Database Syst Rev. 2013;5:CD007752.
64. Unfer V, Carlomagno G, Dante G, Facchinetti F. Effects of myo-inositol in women with PCOS: a systematic review of randomized controlled trials. Gynecol Endocrinol. 2012;28:509–15.

Acupuncture

17

Elisabet Stener-Victorin, Anna Benrick, Romina Fornes, and Manuel Maliqueo

17.1 Introduction

Many women with polycystic ovary syndrome (PCOS) require prolonged treatment. Since the etiology of the syndrome is unclear, the treatment is most often symptom oriented and focused on reducing clinical and biochemical hyperandrogenism, restoring menstrual cycles, inducing ovulation, and improving reproductive outcome. Treatment should also address metabolic disturbances including hyperinsulinemia, insulin resistance, and obesity, which worsen many of the typical PCOS-related symptoms and affect long-term metabolic morbidity.

The use of acupuncture in gynecology and infertility is widespread although the evidence is not of highest level. In a recent Cochrane review, clinical data demonstrate that only a limited number of randomized controlled trials (RCTs) have been reported [1] and that, at present, there is insufficient evidence to support the use of acupuncture for treatment of ovulation disorders in women with PCOS. Given the fact that there is no inert acupuncture control situation, systematic reviews will

E. Stener-Victorin (✉) • R. Fornes
Department of Physiology and Pharmacology, Karolinska Institutet, 17177 Stockholm, Sweden
e-mail: Elisabet.stener-victorin@ki.se

A. Benrick
Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, 405 30 Gothenburg, Sweden

School of Health and Education, University of Skövde, 54128 Skövde, Sweden

M. Maliqueo
Department of Physiology and Pharmacology, Karolinska Institutet, 17177 Stockholm, Sweden

Endocrinology and Metabolism Laboratory, West Division, School of Medicine, University of Chile, 8320000 Santiago, Chile

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_17

always conclude low quality of acupuncture trials. This chapter will summarize current experimental and clinical acupuncture research within the field of PCOS, indicating that acupuncture can be a suitable alternative or complement to pharmacological treatment for different PCOS-related symptoms. Hypothetically, as acupuncture is associated with few side effects [1], the combination of acupuncture and pharmacological treatment decreases negative side effects of pharmacological treatment, although few severe are reported [2, 3].

17.2 Mechanisms of Acupuncture from a Western Medical Perspective

There is close interaction between the somato-autonomic reflexes and the endocrine system is of importance for homeostasis. As an example, the adrenal sympathetic efferent nerve activity controls catecholamine secretion from the adrenal medulla, and the pancreatic sympathetic and parasympathetic efferent nerve activity controls insulin secretion from pancreas [4]. Further, ovarian sympathetic nerves are involved in the regulation of ovulation and secretion of sex steroids [5].

A hypothesis of mechanism of action for acupuncture is showed in Fig. 17.1.

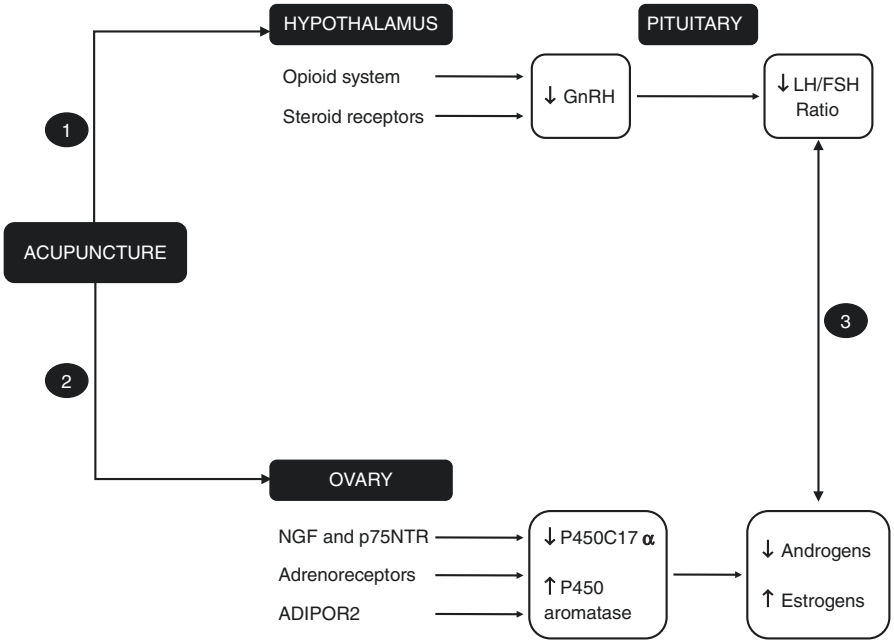


Fig. 17.1 Hypothetical mechanism of acupuncture. *1*. Acupuncture acts on the opioids and/or steroid signaling at hypothalamic levels reducing GnRH, modulating the pituitary secretion of gonadotrophins. *2*. Acupuncture reduces the ovarian sympathetic nerve activity, which decreases the P450C17α and increases P450 aromatase leading to normalization in the ovarian sex steroids synthesis. *3*. The hypothalamic improvements normalize the ovarian function or vice versa

17.2.1 Acupuncture Stimulation and Physiological Responses

Intramuscular needle insertion and stimulation cause a particular pattern of afferent activity in peripheral nerves [6]. Various fiber types, thick myelinated (Aβ), thin myelinated (Aδ), and thinner unmyelinated C fibers, have all been reported to be excited by acupuncture needle stimulation [7]. After insertion, acupuncture needles are stimulated by manual manipulation and/or by electrical stimulation, so-called electroacupuncture, for 20–40 min. During electroacupuncture, needles are attached to electrodes for passing an electric current. It has been suggested that low-frequency (1–15 Hz) electroacupuncture with repetitive muscle contraction activates physiological processes similar to those resulting from muscle contraction during physical exercise [8, 9].

Stimulation of acupuncture points in muscle tissue causes peripheral release of a number of neuropeptides, such as substance P (SP), calcitonin gene-related peptide (CGRP], vaso-intestinal peptide (VIP), and nerve growth factor (NGF), from peripheral nerve terminals into the surrounding area. The result is increased microcirculation in skeletal muscle [10] and glucose uptake, the latter most likely via a reflex response from muscle twitches during electrical stimulation [4].

Depending on the number and location of acupuncture needles and the intensity and type of stimulation (Table 17.1), activation of muscle afferents also modulates the transmission of signals in the spinal cord (segmental level) and in the central nervous system (CNS) [11]. Through sympathetic reflexes, acupuncture at the segmental (spinal) level may modulate the function of organs (e.g., ovaries, urinary bladder, heart) located in the same innervation area as the stimulated acupuncture points [12]. Simultaneously, the nervous system transfers signal to the brain, which generates a response that may further influence the organ. Both segmental (spinal)

Table 17.1 Acupuncture protocols in the treatment of PCOS for ovulation induction

Authors	Points
Jedel et al. (2011) [50]	Local points: CV3, 6—EA; ST29 bilateral—EA Distal points: SP6, 9 bilateral—EA; LI4 or PC6 bilateral—manual
Johansson et al. (2013) [54]	Alternated between two protocols every other treatment Protocol 1: Local points: CV3, 6—EA; ST 29 bilateral—EA Distal points: SP6, 9—EA; LI4 bilateral, GV20—manual
	Protocol 2: Local points: ST25, 29—EA; CV3, 6–manual Distal points: SP6, LR3 bilateral—EA, PC6 bilateral, GV20—manual

Classic acupuncture points are used and described as they are known to acupuncturists independent if they use the western medical approach or the classic Chinese medicine approach. The major difference is how acupuncture points are selected and in the two western medical trials described below acupuncture points in abdominal and leg points are selected in somatic segments innervating the ovaries

EA electroacupuncture, 2 Hz; *manual* de qi 3–4 times during 30 min

and central mechanisms of acupuncture most likely contribute to the total effect of acupuncture treatment. Since the CNS regulates the release of hormones from the pituitary, acupuncture may also modulate the endocrine system which in turn may affect the activity in the sympathetic nervous system.

Specifically, low-frequency electroacupuncture causes the release of a large number of neuropeptides, serotonin, endogenous opioids, and oxytocin in the CNS, which seem to be essential for inducing functional changes in different organ systems [9, 11, 13]. Of particular interest is β-endorphin, an endogenous opioid with high affinity for the μ-receptor [14]. The central hypothalamic β-endorphin system has a regulatory role in a variety of functions, including autonomic function [9, 15]. β-Endorphin is produced and released from the arcuate nucleus in the hypothalamus and the nucleus *tractus solitarius* in the brain stem, which projects to a number of sites within the brain, including all parts of the hypothalamus [16]. β-Endorphin is a key mediator of changes in autonomic functions, such as effects on the vasomotor center, which results in a general decrease of sympathetic tone, shown as regulation of blood pressure and as decreased muscle sympathetic nerve activity [9, 17]. β-Endorphin is also released into peripheral blood from the hypothalamus via the anterior pituitary [18], a process regulated by corticotropin-releasing factor (CRF), which is secreted from the paraventricular nucleus of the hypothalamus [19]. CRF promotes the release of β-endorphin, adrenocorticotropic hormone (ACTH), and melanocyte-stimulating hormone into the bloodstream in equimolar amounts by stimulating the synthesis of their precursor, pro-opiomelanocortin. β-Endorphin in plasma is thought to be related to the hyperinsulinemia response [20] and to stress [21]. It has been well documented that insulin can increase the sympathetic outflow, and it is suggested that hyperinsulinemia may contribute to sympathetic overdrive in obesity [22]. Stress increases the activity of the hypothalamic-pituitary-adrenal (HPA) axis and decreases reproductive functions among many others. Thus, hormones of the HPA axis are closely related to those of the hypothalamic-pituitary-gonadal (HPG) axis as well as sympathetic activity.

In healthy subjects, significant change in sympathetic and parasympathetic activity has been observed with different types of stimulation [23]. For example, stimulation of the first dorsal interosseous at the acupuncture point large intestine (LI9) decreased heart rate (mediated by sympathetic fibers) [23]. A recent study on acupuncture for relief of migraine showed reduction of migraine attacks of at least 50% and a reduction of the low-frequency component of heart rate variability (HRV), indicating decreased sympathetic activity [24]. Further, stimulation of acupuncture needles has been shown to correlate with increased parasympathetic levels during stimulation and post-stimulation and decrease in low-frequency/high-frequency luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio, indicating a normalizing effect of acupuncture [23].

Of note is that menstrual disturbances and high circulating androgens in women with PCOS are related to high activity in the sympathetic nervous system as further discussed below [25].

17.2.2 Autonomic Activity in PCOS and Modulation by Acupuncture

Many factors associated with PCOS—disturbed central and peripheral β-endorphin release, hyperandrogenemia, hyperinsulinemia, and insulin resistance, as well as abdominal obesity and cardiovascular disease—are also associated with increased activity in the sympathetic nervous system [26–30]. The involvement of the sympathetic nervous system in PCOS pathology is further supported by the greater density of catecholaminergic nerve fibers in ovaries with polycystic morphology (PCOM) [31, 32]. Increased ovarian sympathetic nerve activity might contribute to PCOS by stimulating androgen secretion [33]. Women with PCOS have enhanced ovarian production of NGF [34], a strong marker of sympathetic nerve activity. These results suggest that overproduction of ovarian NGF is a component of PCOS in humans. In a transgenic mouse model overexpressing NGF in the ovaries, a persistent elevation in plasma LH levels was required for morphological abnormalities to appear [34]. The strongest evidence for an augmented sympathetic nervous system has been demonstrated in a microneurography study. It showed that women with PCOS have high sympathetic nerve activity that may be relevant to the pathophysiology of the syndrome [25]. Interestingly, testosterone was the strongest independent factor explaining high sympathetic nerve activity in women with PCOS [25].

Recently it was demonstrated that repeated low-frequency electroacupuncture *and* physical exercise lower high sympathetic nerve activity in women with PCOS. Thus, treatment with low-frequency electroacupuncture or physical exercise to reduce sympathetic nervous activity may be of importance for women with PCOS [35].

Support for this observation can be found in experimental animal research. In an estradiol valerate (EV)-induced rat PCOM model, transection of the superior ovarian nerve reduces the steroid response, increases β_2-adrenoceptor expression to more normal levels, and restores estrus cyclicity and ovulation [5]. Also, blockade of endogenous NGF action restores the EV-induced changes in ovarian morphology and expression of the sympathetic markers α_1- and β_2-adrenoceptors, the p75 neurotrophin receptor, NGF-tyrosine kinase receptor A, and tyrosine hydroxylase. These data confirm the close interaction between NGF and the sympathetic nervous system in the pathogenesis of steroid-induced PCOM in rats [36]. In line with these observations, repeated low-frequency electroacupuncture reduces high ovarian concentrations of NGF [37, 38], CRF [39], and endothelin-1 [37] in EV-induced PCOM. It also modulates hypothalamic β-endorphin concentrations and immune function [40] in the same rat PCOM model.

To investigate the hypothesis that repeated low-frequency electroacupuncture treatments and physical exercise modulate sympathetic nerve activity in rats with EV-induced PCOM, we studied the expression of mRNA and protein of the α_{1a}-, α_{1b}-, α_{1d}-, and β_2-adrenoceptors, the p75 neurotrophin receptor, and tyrosine hydroxylase. Four weeks of physical exercise almost normalized ovarian morphology [41], and both electroacupuncture and exercise normalized the expression of NGF, NGF receptors, and α_1- and α_2-adrenoceptors [36, 41].

Further, in rats with dihydrotestosterone (DHT)-induced PCOS exhibiting reproductive and metabolic abnormalities, both low-frequency electroacupuncture and exercise improved ovarian morphology, as reflected by a higher proportion of healthy antral follicles and a thinner theca interna cell layer than in untreated PCOS [42]. This was accompanied by improved estrus cyclicity.

Low-frequency electroacupuncture also increased ovarian blood flow. The needles were placed in the abdominal and hind limb muscles, which have the same somatic innervation as the ovaries and uterus [43–45]. The response was mediated by ovarian sympathetic nerves as a reflex response controlled by supraspinal pathways (i.e., CNS) [43, 45]. Interestingly, electrical stimulation of the superior ovarian nerve affected the ovarian blood flow response and reduced ovarian estradiol secretion rate, an effect that was mediated via α_1-adrenoceptors in the regulation of ovarian function by electrical stimulation [46].

These findings support the theory that increased sympathetic activity contributes to the development and maintenance of PCOS and that the effects of electroacupuncture and exercise are mediated by modulation of sympathetic outflow to the adipose tissue and ovaries. Augmented sympathetic activity in PCOS may contribute to vascular risk factors associated with the condition. Thus, therapies aimed at reducing sympathetic activity in PCOS need to be studied.

17.3 Acupuncture in the Treatment of Ovarian Dysfunction

In uncontrolled trials, repeated acupuncture treatments decreased total testosterone and other sex steroid levels, reduced LH/FSH ratio, and improved menstrual frequency without negative side effects [47–49]. In a three-arm RCTs, 14 low-frequency electroacupuncture treatments (combination of electrical and manual stimulation) over 16 weeks, and 16 weeks of physical exercise, improved menstrual bleeding pattern and decreased high levels of circulating androgens compared with no intervention in women with PCOS [50]. Acupuncture was superior to physical exercise when compared directly after the treatment but did not differ from the exercise group at 4-month follow-up.

In a quasi-randomized study, daily abdominal acupuncture for 6 months improved menstrual frequency and decreased circulating testosterone more effectively than metformin over 6 months [51]. In another RCT, a course of 12 treatments of true acupuncture was compared with sham acupuncture for 8 weeks, and they found similar ovulation frequency and improvement in LH/FSH ratio in both groups of women with PCOS [52]. Thus, they were unable to demonstrate differences between true and sham acupuncture, and they did not include a nonintervention group. These results are in line with previous studies on different pain conditions and nausea caused by chemotherapy, demonstrating that true acupuncture is not more effective than sham acupuncture, although all these trials found a significant effect when compared with a nonintervention group [53]. These results indicate that sham acupuncture is not an inert method and highlight methodological difficulties in the design of acupuncture trials.

In a recent trial, the efficacy of low-frequency electroacupuncture in combination with manual stimulation, 30 min twice a week for 10–13 weeks (more intensive treatments than in the previous trials) in total of 20–26 treatments, was compared with equal time in meetings with a therapist (to control for attention and expectations) for ovulation induction in women with PCOS [54]. Women receiving acupuncture treatment displayed higher ovulation frequency compared with women meeting a therapist for an equal amount of time [54] (Fig. 17.2). Also the ovarian and adrenal sex steroid levels were reduced with no effect on LH secretion indicating that this regulation occurs at ovarian level. These results indicate that more frequent treatment results in more pronounced effects.

The molecular mechanism via which acupuncture could regulate the reproductive function has been studied in rat animal models induced by EV, DHT, and letrozole, an inhibitor of P450 aromatase [38, 42, 55]. Continuous exposure of these compounds results in disturbances in the estrous cycle (anovulation) and the presence of multiple ovarian cyst and elevated androgen levels in the DHT and letrozole models [38, 56, 57]. Acupuncture improves estrus cyclicity in these models [55, 58, 59]. In the DHT-induced PCOS model, electroacupuncture given three times per week for 4–5 weeks decreases the proportion of atretic antral follicles and results in

Control

- Meeting with a therapist: 2 times/week, 30 min per session
- Rest and listen to relaxing music

Acupuncture

- Acupuncture: 2 times/week, 30 min per session
- Rest and listen to relaxing music
- Alternating between two protocols with a combination of electrical (EA) and manual needle stimulation:
  - Protocol 1:
    - CV3 to CV6 – EA
    - ST29 bilateral – EA
    - SP6 to SP9 bilateral – EA
    - GV20 – manual
    - LI4 – manual
  - Protocol 2:
    - ST25 to ST29 bilateral – EA
    - CV3 and 6 – manual
    - SP6 to LR3 bilateral – EA
    - GV20 – manual
    - PC6 – manual

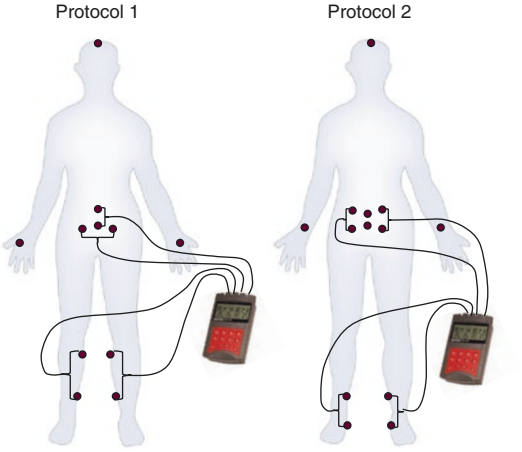


Fig. 17.2 Treatment was performed twice per week and all women rested and listened to relaxing music during treatment. The acupuncture group received acupuncture with a combination of both electrical with low-frequency [2 Hz] [EA] and manual needle stimulation. Placement of needles was chosen according to previous protocols and was in the muscle with the same innervation as the ovaries and uterus. Hypothetically this could directly influence sympathetic output to these organs. The attention control group did not receive acupuncture but was otherwise treated the same to control for the attention and time involved in the treatment. *CV* conception vessel, *GV* governor vessel, *LI* large intestinal, *LR* liver, *PC* pericardium, *SP* spleen, *ST* stomach

thinner theca interna cell layer compared to untreated DHT-exposed rats. Moreover, almost 50% of those rats treated with acupuncture showed fresh *corpora lutea* [42]. Similar to these observations, in rats exposed to letrozole, electroacupuncture in the acupuncture points CV-4 and CV-3 for 14 consecutive days demonstrated increased preantral and antral follicles, granular cell layers, and some *corpora lutea* [59].

Using the DHT-induced PCOS rat model, it was demonstrated that the recovery of estrous cyclicity induced by electroacupuncture treatment was associated with a decrease of gonadotrophin-releasing hormone (GnRH) and androgen receptor (AR) protein expression in the hypothalamus [58]. This suggests that electroacupuncture could regulate the release of GnRH and likely LH/FSH. Further, in the letrozole-induced PCOS models, low-frequency electroacupuncture reduced LH and LH/FSH ratio after 5–6 weeks of treatments supporting the concept that regulation of GnRH in turn regulates the pituitary gland function (Fig. 17.1) [55].

Acupuncture could regulate pathways associated with the steroid synthesis contributing to its positive effects on the estrous cyclicity and ovarian morphology. In this regard, rats exposed to DHT that respond to electroacupuncture showed a decrease in circulating androgen levels and increase in estrogen and progesterone [60]. Similar results were observed in letrozole-induced PCOS models, associated with an ovarian decrease of P450C17α and increase of P450 aromatase [59]. Interestingly, in the letrozole-induced PCOS model, electroacupuncture increased the expression of ovarian adiponectin receptor 2 protein and phosphorylation of ERK1/2, which has been associated with the synthesis of estrogen and progesterone in ovarian cells [55]. Interestingly, in an EV-induced PCOS model, low-frequency electroacupuncture decreases the NGF concentrations in the ovary and p75 neurotrophin receptors, and it normalizes the expression of ovarian adrenoreceptors [36, 38], suggesting that acupuncture reduces the ovarian responsiveness to sympathetic inputs, which potentially could improve the steroid synthesis (Fig. 17.1).

Taken together, from experimental and clinical data, there are strong indications that acupuncture treatment results in more regular cycles, acting on both ovarian and hypothalamic levels regulating sex steroid production and also the gonadotrophin secretion although those results are less clear. Whether acupuncture can be used for ovulation indication with the aim for pregnancy is currently under evaluation.

17.4 Acupuncture During Pregnancy

Pregnancy complications in women with PCOS are discussed in detail in Chap. 22. In brief, they are at an increased risk of adverse pregnancy outcomes such as gestational diabetes, preterm birth, and preeclampsia, and they are more likely to give birth to small for gestational age or large for gestational age infants. During pregnancy, women with PCOS are more likely to have elevated blood pressure independent of body mass index (BMI) [61], as well as impaired uterine artery blood flow, which is related to poor pregnancy outcomes [62, 63]. There is currently no treatment that prevents adverse obstetric outcomes in women with PCOS. Acupuncture with low-frequency electrical stimulation has been shown to increase ovarian and

uterine artery blood flow in rats and nonpregnant women undergoing in vitro fertilization, an effect that was mediated by the sympathetic nerve fibers innervating the ovary [37, 43, 45, 64]. In healthy pregnancies, acupuncture performed during third trimester decreases umbilical artery systolic/diastolic ratio, as measured using Doppler ultrasound, consistent with a decreased vascular resistance [65]. In the prenatal androgenized (PNA) rat PCOS model, the placenta and fetus are smaller and their offspring are usually born small for gestational age and develop a PCOS-like phenotype at prepubertal and adult age [66]. Therefore, the PNA model was used to elucidate if acupuncture has the potential to prevent the development of small placenta and promote fetal growth. Low-frequency electroacupuncture in control animals did not have any negative influence on any of the studied variables [67]. In contrast, electroacupuncture in pregnant dams exposed to testosterone increased blood pressure and impaired placental growth and function, leading to decreased fetal growth [67]. A previous study in normal Wistar rats, electroacupuncture given in the upper and hind limb and at sacral points during the whole pregnancy did not affect embryonic loss after implantation or resorption [68]. Nevertheless, the fetal weight was lower in the electroacupuncture groups compared with the no intervention and anesthetized group that served as control for electroacupuncture treatment [68]. Thus, acupuncture is not the method of choice to improve fetal growth.

During pregnancy, acupuncture is usually used with caution, because of concerns for miscarriages or preterm labor. However, there is no report indicating that acupuncture would cause miscarriage or preterm labor. The most common indication for acupuncture during pregnancy is nausea and vomiting, pelvic girdle pain, and pain relief during labor [69–71].

Although there are no reports indicating that the incidence or the extent of nausea or vomiting is more pronounced in women with PCOS, these problems are common in all women at early pregnancy, and there is a lack of strong evidence of any successful intervention for nausea or vomiting in early pregnancy [72]. An Australian clinical trial carried out in 593 pregnant women before 14 weeks of gestation demonstrated that acupuncture given at body points selected according to traditional Chinese medicine, or needling of pericardium 6 (PC6) in the forearm or sham acupuncture (needles inserted into an area close to, but not on, acupuncture points) during 4 weeks, reduces nausea and dry retching, but not vomiting compared with no acupuncture (control) [73]. There was no difference among the different acupuncture groups. A second report of that trial demonstrated that the obstetric outcomes did not differ among the groups, which suggests that acupuncture without electrical stimulation could be considered safe when it is performed during early pregnancy [74].

Acupuncture for pelvic girdle pain during pregnancy has been found to be superior to supervised exercise and a self-management group [70]. When acupuncture was compared with sham acupuncture, there was no significant difference between the groups [69], again indicating that the sham acupuncture procedures are not inert. Systematic reviews suggest that acupuncture improves pregnancy-related pelvic girdle pain, but not low back pain [75]; meanwhile others suggest that acupuncture and pelvic belts are the interventions that have the strongest evidence of their

positive effects in the relief of lumbo-pelvic pain compared with other physiotherapy interventions, such as exercise or osteopathic manual therapy [76].

Depression during pregnancy is relatively common, and acupuncture during 8 weeks has been shown to be more successful to reduce symptoms of depression during pregnancy than control acupuncture or massage [77]. Auricular acupuncture has been suggested as a safe and feasible treatment to assist mothers to reduce their methadone dose and length of treatment for neonatal abstinence syndrome [78].

Acupuncture has also been tested for induction of labor at pregnancy week 38 with no beneficial effects [79, 80]. During labor pain management, acupuncture with or without electrostimulation was less effective than sterile water injections [81]. Despite that, electroacupuncture decreased the need for epidural anesthesia [81] and the need for pharmacological and invasive methods during delivery [82]. Moreover, acupuncture has been demonstrated to decrease the length of the active phase of the labor [83]. Although limited evidence, acupuncture and acupressure may have a role for reducing pain and the use of pharmacological treatment during labor [84], but more research is needed.

17.5 Acupuncture for the Treatment of Infertility and Subfertility Cofactors

Infertility and subfertility cofactors in PCOS are discussed in detail in Chap. 6. Below the efficacy of the acupuncture for the treatment of these cofactors is described. Despite the high prevalence of insulin resistance, impaired glucose tolerance, and/or type 2 diabetes in women with PCOS, there is no consensus on the best long-term management of these conditions. First line of treatment is lifestyle changes including diet and exercise, while pharmacological treatments, including metformin, are symptom-oriented and usually effective but have unpleasant gastrointestinal side effects. Therefore, it is important to evaluate other non-pharmacological treatment strategies because most women with PCOS require long-term treatment. Acupuncture may improve symptoms by activating mechanisms relevant to weight loss and electroacupuncture can be used to improve insulin sensitivity.

17.5.1 Acupuncture and Obesity

A number of studies show that acupuncture activates mechanisms involved in body weight regulation and weight loss [85]. The hypothalamus regulates body weight via complex interactions between anorexigenic and orexigenic neuropeptides, and acupuncture can regulate these obesity-related neuropeptides. Acupuncture can also affect central β-endorphin and serotonin release, which in turn alters leptin and ghrelin signaling, which may have a favorable influence on food intake and obesity [86]. Acupuncture may also be involved in the regulation of the sympathetic-adrenal

cortex axis [35, 42]. Further, a number of studies have observed lipid-lowering effects i.e., decreased triglycerides, low-density lipoprotein [LDL], and total cholesterol [87, 88] in response to acupuncture.

Acupuncture mediates central mechanisms that could be relevant to short-term weight loss. One research team has shown effects in humans on body weight by manual, electrical stimulation and auricular acupuncture [88–90]. Although various meta-analyses suggest that acupuncture could have an effect on obesity, the evidences are inconclusive due to low numbers and quality [91, 92]. The majority of studies have been performed in China but studies performed outside China are of higher methodological quality [92]. One explanation for this may be that in China the traditional Chinese medicine focuses on personalized therapy, where symptoms and signs judged by the doctor form the basis of treatments, resulting in that patients with the same clinical diagnosis may be given different TCM treatments, often different modes of acupuncture in combination with Chinese herb therapy.

Whether acupuncture is a useful treatment for obesity in women with PCOS is unclear. In our experimental studies, acupuncture and electroacupuncture improved insulin sensitivity and adipose tissue function without influencing adipose tissue mass, BMI, or body weight [93–95]. Moreover, in our clinical studies, acupuncture did not result in any changes in body composition, BMI, or weight [50, 96]. However, more intense acupuncture treatment, 2–3 times a week for 3–6 months, decreased body weight and BMI [97, 98]. And, a randomized study with very intense treatment, once daily for 6 months, reduced BMI and waist-hip ratio as well as total cholesterol, triglyceride, and LDL [51].

In summary, multiple shortcomings have been identified in the systematic reviews on the effects of acupuncture on obesity. For example, several studies draw their conclusions from uncontrolled trials, and the design of the controlled trials suffers from methodological weaknesses and small sample size [99]. The same methodological limitations are present for studies including patients with PCOS. Further well-controlled, large studies are need if conclusive judgment regarding the effectiveness of acupuncture on obesity is to be drawn.

17.5.2 Acupuncture and Insulin Resistance

Reviews support the hypothesis that acupuncture has beneficial effects on insulin sensitivity in type 2 diabetes, with no negative side effects [91, 100]. For example, electroacupuncture 3 times per week for 3 months improved insulin sensitivity and lowered fasting insulin and glucose [97]. And, manual acupuncture, 2 times per week for 6 months, improved HOMA-IR [98]. However, the studies included in these reviews were underpowered and of poor methodological quality. Secondary analyses on metabolic variables in the RCT involving 74 women [50] found no effect of acupuncture on insulin sensitivity [101]. Though, in the experimental studies on DHT-induced PCOS rats that exhibit polycystic ovaries, irregular cycles, obesity, and insulin resistance [57, 58, 102], it was found that more frequent acupuncture treatments than we used in our RCT ameliorated insulin resistance [93]. When the

intensity was increased further to 5 times per week for 4–5 weeks, electroacupuncture completely restored insulin sensitivity and was equally effective as voluntary exercise [95].

This effect may involve regulation of adipose and skeletal muscle tissue signaling pathways because manual and electrical stimulation acupuncture each partly restore divergent gene and protein expression associated with insulin resistance, obesity, and inflammation in PCOS rats [93, 95]. Moreover, glucose transporter 4 increased in skeletal muscle after acupuncture, suggesting an increased glucose uptake capacity [94, 95]. Clinical studies support this as electroacupuncture increases serum adiponectin [97], an adipokine with insulin-sensitizing effects, plasminogen activator inhibitor 1 activity, and tissue plasminogen activator decrease after electroacupuncture [50], suggesting that is an effective strategy for improving a prothrombotic state in women with PCOS [50]. Furthermore, electroacupuncture has been shown to reduce plasma glucose levels by promoting insulin production and to improve insulin sensitivity by inducing secretion of endogenous β-endorphin in different rodent models of diabetes [103, 104]. Acute muscle contraction is a potent stimulator of glucose uptake even in insulin-resistant states, and acupuncture can reduce plasma glucose in obese and insulin-resistant women [105–107].

In experimental studies, muscle contractions elicited by electrical stimulation induce changes in skeletal muscle signaling pathways similar to changes induced by exercise [93–95]. The insulin-sensitizing effect seems to be mediated by activation of afferent nerves rather than muscle contraction per se [4, 108]. When afferent nerves in the electroacupuncture-treated hind limb were cut, the increased insulin responsiveness was lost [4], and, although electroacupuncture was superior to manual acupuncture in enhancing insulin sensitivity during stimulation, they were equally effective after stimulation [108]. Electroacupuncture, mimicking exercise by inducing muscle contractions, could therefore be used to improve insulin sensitivity in patients with a reduced ability to perform physical exercise.

In conclusion, acupuncture and particularly electroacupuncture of low-frequency (with most studies using 2 Hz) can improve insulin resistance by increasing insulin sensitivity. Even though data is based on a much smaller number of clinical studies compared to animal studies, it has the potential to be an effective treatment for many insulin-resistant conditions, not only PCOS. More studies are needed to evaluate the clinical relevance, and this is an important area to investigate because most women with PCOS require long-term treatment. There is one completed prospective pilot trial investigating the effect of 5 weeks of combined manual and electrical stimulation acupuncture (Clinicaltrials.gov: NCT01457209) and three ongoing trials including women with PCOS (ClinicalTrial.gov: NCT02026323, NCT02491333 and NCT02647827).

17.5.3 Acupuncture and Mental Health

In women with breast cancer, 12 weeks of acupuncture improved health-related quality of life (HRQoL) and sleep assessed with the Women's Health Questionnaire [109], general well-being assessed with the symptom checklist, and mood assessed

with the mood scale [110]. Similarly, acupuncture improved HRQoL assessed by the Short Form 36 (SF-36) in patients with chronic pain conditions, such as dysmenorrhea [111] and pain from osteoarthritis [112]. Acupuncture with manual stimulation [113, 114] and electroacupuncture have been shown to be effective in the treatment of major depression disorder in women [115–118] and in women with depression during pregnancy [77, 119] and postpartum [120]. Although there are a few studies indicating that acupuncture may improve HRQoL and symptoms of anxiety and depression in non-PCOS patients [121–123], only one study has investigated its effect as a secondary outcome measure in women with PCOS [124]. There was a modest improvement in HRQoL and depression and anxiety scores in women treated with acupuncture, with no significant difference compared with an untreated control group. Although a modest effect, these data suggest continued investigation of the effect of acupuncture on mental health in women with PCOS.

Conclusion

This chapter summarizes experimental and clinical data of acupuncture in the treatment of women with PCOS from a western medical perspective. Studies demonstrate that acupuncture regulates sex steroid production and also the gonadotrophin secretion resulting in more regular cycles. Whether acupuncture can be used for ovulation indication with the aim for pregnancy is currently under evaluation. In the recent Cochrane review, it was concluded that there is insufficient evidence for acupuncture in the treatment of ovulatory disorders [1]. The use of acupuncture for cofactors including metabolic dysfunction and mental health issues needs further investigation. Important to point out is that the control situation in acupuncture trials is under continuous debate, as the sham acupuncture procedures are not completely inert as they modulate CNS suggesting that control acupuncture evokes physiological responses as well [125].

References

1. Lim CE, Ng RW, Xu K, Cheng NC, Xue CC, Liu JP, et al. Acupuncture for polycystic ovarian syndrome. Cochrane Database Syst Rev. 2016;5:CD007689.
2. Domecq JP, Prutsky G, Mullan RJ, Sundaresh V, Wang AT, Erwin PJ, et al. Adverse effects of the common treatments for polycystic ovary syndrome: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2013;98:4646–54.
3. Al Khalifah RA, Florez ID, Dennis B, Neupane B, Thabane L, Bassilious E. The effectiveness and safety of treatments used for polycystic ovarian syndrome management in adolescents: a systematic review and network meta-analysis protocol. Syst Rev. 2015;4:125.
4. Higashimura Y, Shimoju R, Maruyama H, Kurosawa M. Electro-acupuncture improves responsiveness to insulin via excitation of somatic afferent fibers in diabetic rats. Auton Neurosci. 2009;150:100–3.
5. Barria A, Leyton V, Ojeda SR, Lara HE. Ovarian steroidal response to gonadotropins and beta-adrenergic stimulation is enhanced in polycystic ovary syndrome: role of sympathetic innervation. Endocrinology. 1993;133:2696–703.
6. Kagitani F, Uchida S, Hotta H, Aikawa Y. Manual acupuncture needle stimulation of the rat hindlimb activates groups I, II, III and IV single afferent nerve fibers in the dorsal spinal roots. Jpn J Physiol. 2005;55:149–55.

7. Sato A, Sato Y, Uchida S. Reflex modulation of visceral functions by acupuncture-like stimulation in anesthetized rats. Int Congr Ser. 2002;1238:111–23.
8. Kaufman MP, Waldrop TG, Rybycki KJ, Ordway GA, Mitchell JH. Effects of static and rythmic twitch contractions on the discharge of group III and IV muscle afferents. Cardiovasc Rec. 1984;18:663–8.
9. Andersson S, Lundeberg T. Acupuncture–from empiricism to science: functional background to acupuncture effects in pain and disease. Med Hypotheses. 1995;45:271–81.
10. Sato A, Sato Y, Shimura M, Uchida S. Calcitonin gene-related peptide produces skeletal muscle vasodilation following antidromic stimulation of unmyelinated afferents in the dorsal root in rats. Neurosci Lett. 2000;283:137–40.
11. Stener-Victorin E, Jedel E, Manneras L. Acupuncture in polycystic ovary syndrome: current experimental and clinical evidence. J Neuroendocrinol. 2008;20:290–8.
12. Sato A, Sato Y, Schmidt RF. The impact of somatosensory input on autonomic functions. Heidelberg: Springer-Verlag; 1997. p. 325.
13. Han J-S. Acupuncture and endorphins. Neurosci Lett. 2004;361:258–61.
14. Basbaum AI, Fields HL. Endogenous pain control systems: Brain-stem spinal pathways and endorphin circuitry. Annu Rev Neurosci. 1984;7:309–38.
15. Eyvazzadeh AD, Pennington KP, Pop-Busui R, Sowers M, Zubieta JK, Smith YR. The role of the endogenous opioid system in polycystic ovary syndrome. Fertil Steril. 2009;92:1–12.
16. Ferin M, Van Vugt D, Wardlaw S. The hypothalamic control of the menstrual cycle and the role of endogenous opioid peptides. Recent Prog Horm Res. 1984;40:441–85.
17. Yao T, Andersson S, Thoren P. Long-lasting cardiovascular depression induced by acupuncture-like stimulation of the sciatic nerve in unanaesthetized spontaneously hypertensive rats. Brain Res. 1982;240:77–85.
18. Crine P, Gianoulakis C, Seidah NG. Biosynthesis of beta-endorphin from beta-lipotropin and a larger molecular weight precursor in rat pars intermedia. Proc Natl Acad Sci U S A. 1978;75:4719–23.
19. Chan JS, Lu CL, Seidah NG, Chretien M. Corticotropin releasing factor [CRF]: effects on the release of pro-opiomelanocortin [POMC]-related peptides by human anterior pituitary cells in vitro. Endocrinology. 1982;111:1388–90.
20. Carmina E, Ditkoff EC, Malizia G, Vijod AG, Janni A, Lobo RA. Increased circulating levels of immunoreactive beta-endorphin in polycystic ovary syndrome is not caused by increased pituitary secretion. Am J Obstet Gynecol. 1992;167:1819–24.
21. Lobo RA, Granger LR, Paul WL, Goebelsmann U, Mishell Jr DR. Psychological stress and increases in urinary norepinephrine metabolites, platelet serotonin, and adrenal androgens in women with polycystic ovary syndrome. Am J Obstet Gynecol. 1983;145:496–503.
22. Gilchrist RB, Ritter LJ, Myllymaa S, Kaivo-Oja N, Dragovic RA, Hickey TE, et al. Molecular basis of oocyte-paracrine signalling that promotes granulosa cell proliferation. J Cell Sci. 2006;119:3811–21.
23. Haker E, Egekvist H, Bjerring P. Effect of sensory stimulation [acupuncture] on sympathetic and parasympathetic activities in healthy subjects. J Auton Nerv Syst. 2000;79:52–9.
24. Backer M, Grossman P, Schneider J, Michalsen A, Knoblauch N, Tan L, et al. Acupuncture in migraine: investigation of autonomic effects. Clin J Pain. 2008;24:106–15.
25. Sverrisdottir YB, Mogren T, Kataoka J, Janson PO, Stener-Victorin E. Is polycystic ovary syndrome associated with high sympathetic nerve activity and size at birth? Am J Physiol Endocrinol Metab. 2008;294:E576–81.
26. Fagius J. Sympathetic nerve activity in metabolic control--some basic concepts. Acta Physiol Scand. 2003;177:337–43.
27. Ojeda S, Lara H. In: Pirke KW, Schweiger U, editors. Role of the sympathetic nervous system in the regulation of ovarian function. Berlin: Springer-Verlag; 1989. p. 26–33.
28. Sir-Petermann T, Maliqueo M, Angel B, Lara HE, Perez-Bravo F, Recabarren SE. Maternal serum androgens in pregnant women with polycystic ovarian syndrome: possible implications in prenatal androgenization. Hum Reprod. 2002;17:2573–9.

29. Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities—the role of insulin resistance and the sympathoadrenal system. N Engl J Med. 1996;334:374–81.
30. Dissen GA, Garcia-Rudaz C, Ojeda SR. Role of neurotrophic factors in early ovarian development. Semin Reprod Med. 2009;27:24–31.
31. Semenova I. Adrenergic innervation of the ovaries in Stein-Leventhal syndrome. Vestn Akad Med Nauk SSSR. 1969;24:58–62.
32. Heider U, Pedal I, Spanel-Borowski K. Increase in nerve fibers and loss of mast cells in polycystic and postmenopausal ovaries. Fertil Steril. 2001;75:1141–7.
33. Greiner M, Paredes A, Araya V, Lara HE. Role of stress and sympathetic innervation in the development of polycystic ovary syndrome. Endocrine. 2005;28:319–24.
34. Dissen GA, Garcia-Rudaz C, Paredes A, Mayer C, Mayerhofer A, Ojeda SR. Excessive ovarian production of nerve growth factor facilitates development of cystic ovarian morphology in mice and is a feature of polycystic ovarian syndrome [PCOS] in humans. Endocrinology. 2009;150:2906–14.
35. Stener-Victorin E, Jedel E, Janson PO, Sverrisdottir YB. Low-frequency electroacupuncture and physical exercise decrease high muscle sympathetic nerve activity in polycystic ovary syndrome. Am J Phys Regul Integr Comp Phys. 2009;297:R387–95.
36. Manni L, Lundeberg T, Holmang A, Aloe L, Stener-Victorin E. Effect of electro-acupuncture on ovarian expression of alpha [1]- and beta [2]-adrenoceptors, and p75 neurotrophin receptors in rats with steroid-induced polycystic ovaries. Reprod Biol Endocrinol. 2005;3:21.
37. Stener-Victorin E, Lundeberg T, Cajander S, Aloe L, Manni L, Waldenstrom U, et al. Steroid-induced polycystic ovaries in rats: effect of electro-acupuncture on concentrations of endothelin-1 and nerve growth factor [NGF], and expression of NGF mRNA in the ovaries, the adrenal glands, and the central nervous system. Reprod Biol Endocrinol. 2003;1:33.
38. Stener-Victorin E, Lundeberg T, Waldenstrom U, Manni L, Aloe L, Gunnarsson S, et al. Effects of electro-acupuncture on nerve growth factor and ovarian morphology in rats with experimentally induced polycystic ovaries. Biol Reprod. 2000;63:1497–503.
39. Stener-Victorin E, Lundeberg T, Waldenstrom U, Bileviciute-Ljungar I, Janson PO. Effects of electro-acupuncture on corticotropin-releasing factor in rats with experimentally-induced polycystic ovaries. Neuropeptides. 2001;35:227–31.
40. Stener-Victorin E, Lindholm C. Immunity and beta-endorphin concentrations in hypothalamus and plasma in rats with steroid-induced polycystic ovaries: effect of low-frequency electroacupuncture. Biol Reprod. 2004;70:329–33.
41. Manni L, Cajander S, Lundeberg T, Naylor AS, Aloe L, Holmang A, et al. Effect of exercise on ovarian morphology and expression of nerve growth factor and alpha[1]- and beta[2]-adrenergic receptors in rats with steroid-induced polycystic ovaries. J Neuroendocrinol. 2005;17:846–58.
42. Manneras L, Cajander S, Lonn M, Stener-Victorin E. Acupuncture and exercise restore adipose tissue expression of sympathetic markers and improve ovarian morphology in rats with dihydrotestosterone-induced PCOS. Am J Phys Regul Integr Comp Phys. 2009;296: R1124–31.
43. Stener-Victorin E, Kobayashi R, Kurosawa M. Ovarian blood flow responses to electroacupuncture stimulation at different frequencies and intensities in anaesthetized rats. Auto Neurosci Basic Clin. 2003;108:50–6.
44. Stener-Victorin E, Kobayashi R, Watanabe O, Lundeberg T, Kurosawa M. Effect of electroacupuncture stimulation of different frequencies and intensities on ovarian blood flow in anaesthetised rats with steroid-induced polycystic ovaries. Reprod Biol Endocrinol. 2004;2:16.
45. Stener-Victorin E, Fujisawa S, Kurosawa M. Ovarian blood flow responses to electroacupuncture stimulation depend on estrous cycle and on site and frequency of stimulation in anesthetized rats. J Appl Physiol. 2006;101:84–91.
46. Kagitani F, Uchida S, Hotta H. The role of alpha adrenoceptors in the vascular and estradiol secretory responses to stimulation of the superior ovarian nerve. J Physiol Sci. 2011;61:247–51.

47. Chen BY, Yu J. Relationship between blood radioimmunoreactive beta-endorphin and hand skin temperature during the electro-acupuncture induction of ovulation. Acupunct Electrother Res. 1991;16:1–5.
48. Gerhard I, Postneek F. Auricular acupuncture in the treatment of female infertility. Gynecol Endocrinol. 1992;6:171–81.
49. Stener-Victorin E, Waldenstrom U, Tagnfors U, Lundeberg T, Lindstedt G, Janson PO. Effects of electro-acupuncture on anovulation in women with polycystic ovary syndrome. Acta Obstet Gynecol Scand. 2000;79:180–8.
50. Jedel E, Labrie F, Oden A, Holm G, Nilsson L, Janson PO, et al. Impact of electro-acupuncture and physical exercise on hyperandrogenism and oligo/amenorrhea in women with polycystic ovary syndrome: a randomized controlled trial. Am J Phys. 2011;300:E37–45.
51. Lai MH, Ma HX, Yao H, Liu H, Song XH, Huang WY, et al. Effect of abdominal acupuncture therapy on the endocrine and metabolism in obesity-type polycystic ovarian syndrome patients. Zhen Ci Yan Jiu. 2010;35:298–302.
52. Pastore LM, Williams CD, Jenkins J, Patrie JT. True and sham acupuncture produced similar frequency of ovulation and improved LH to FSH ratios in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2011;96:3143–50.
53. Enblom A, Lekander M, Hammar M, Johnsson A, Onelov E, Ingvar M, et al. Getting the grip on nonspecific treatment effects: emesis in patients randomized to acupuncture or sham compared to patients receiving standard care. PLoS One. 2011;6:e14766.
54. Johansson J, Redman L, Veldhuis PP, Sazonova A, Labrie F, Holm G, et al. Acupuncture for ovulation induction in polycystic ovary syndrome: a randomized controlled trial. Am J Phys. 2013;304:E934–43.
55. Maliqueo M, Benrick A, Alvi A, Johansson J, Sun M, Labrie F, et al. Circulating gonadotropins and ovarian adiponectin system are modulated by acupuncture independently of sex steroid or beta-adrenergic action in a female hyperandrogenic rat model of polycystic ovary syndrome. Mol Cell Endocrinol. 2015;412:159–69.
56. Maliqueo M, Sun M, Johansson J, Benrick A, Labrie F, Svensson H, et al. Continuous administration of a P450 aromatase inhibitor induces polycystic ovary syndrome with a metabolic and endocrine phenotype in female rats at adult age. Endocrinology. 2013;154:434–45.
57. Manneråås L, Cajander S, Holmäng A, Seleskovic Z, Lystig T, Lönn M, et al. A new rat model exhibiting both ovarian and metabolic characteristics of polycystic ovary syndrome. Endocrinology. 2007;148:3781–91.
58. Feng Y, Johansson J, Shao R, Manneras L, Fernandez-Rodriguez J, Billig H, et al. Hypothalamic neuroendocrine functions in rats with dihydrotestosterone-induced polycystic ovary syndrome: effects of low-frequency electro-acupuncture. PLoS One. 2009;4:e6638.
59. Sun J, Jin C, Wu H, Zhao J, Cui Y, Liu H, et al. Effects of electro-acupuncture on ovarian P450arom, P450c17alpha and mRNA expression induced by letrozole in PCOS rats. PLoS One. 2013;8:e79382.
60. Feng Y, Johansson J, Shao R, Manneras-Holm L, Billig H, Stener-Victorin E. Electrical and manual acupuncture stimulation affect oestrous cyclicity and neuroendocrine function in an 5alpha-dihydrotestosterone-induced rat polycystic ovary syndrome model. Exp Physiol. 2012;97:651–62.
61. Hu S, Leonard A, Seifalian A, Hardiman P. Vascular dysfunction during pregnancy in women with polycystic ovary syndrome. Hum Reprod. 2007;22:1532–9.
62. Palomba S, Falbo A, Russo T, Battista L, Tolino A, Orio F, et al. Uterine blood flow in pregnant patients with polycystic ovary syndrome: relationships with clinical outcomes. BJOG. 2010;117:711–21.
63. Falbo A, Rocca M, Russo T, D'Ettore A, Tolino A, Zullo F, et al. Changes in androgens and insulin sensitivity indexes throughout pregnancy in women with polycystic ovary syndrome [PCOS]: relationships with adverse outcomes. J Ovarian Res. 2010;3:23.
64. Stener-Victorin E, Waldenstrom U, Andersson SA, Wikland M. Reduction of blood flow impedance in the uterine arteries of infertile women with electro-acupuncture. Hum Reprod. 1996;11:1314–7.

65. Zeisler H, Eppel W, Husslein P, Bernaschek G, Deutinger J. Influence of acupuncture on Doppler ultrasound in pregnant women. Ultrasound Obstet Gynecol. 2001;17:229–32.
66. Sir-Petermann T, Codner E, Perez V, Echiburu B, Maliqueo M, Ladron de Guevara A, et al. Metabolic and reproductive features before and during puberty in daughters of women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2009;94:1923–30.
67. Fornes R, Hu M, Maliqueo M, Kokosar M, Benrick A, Carr D, et al. Maternal testosterone and placental function: effect of electroacupuncture on placental expression of angiogenic markers and fetal growth. Mol Cell Endocrinol. 2016;433:1–11.
68. Guerreiro da Silva AV, Nakamura MU, Cordeiro JA, Guerreiro da Silva JB, Mendes GEF, Burdmann EA. The effects of so-called 'forbidden acupuncture points' on pregnancy outcome in wistar rats. Forsch Komplementmed. 2011;18:10–4.
69. Elden H, Fagevik-Olsen M, Ostgaard HC, Stener-Victorin E, Hagberg H. Acupuncture as an adjunct to standard treatment for pelvic girdle pain in pregnant women: randomised double-blinded controlled trial comparing acupuncture with non-penetrating sham acupuncture. BJOG. 2008;115:1655–68.
70. Elden H, Ladfors L, Olsen MF, Ostgaard HC, Hagberg H. Effects of acupuncture and stabilising exercises as adjunct to standard treatment in pregnant women with pelvic girdle pain: randomised single blind controlled trial. BMJ. 2005;330:761.
71. Smith CA, Cochrane S. Does acupuncture have a place as an adjunct treatment during pregnancy? A review of randomized controlled trials and systematic reviews. Birth. 2009;36:246–53.
72. Matthews A, Haas DM, O'Mathuna DP, Dowswell T. Interventions for nausea and vomiting in early pregnancy. Cochrane Database Syst Rev. 2015;9:CD007575.
73. Smith C, Crowther C, Beilby J. Acupuncture to treat nausea and vomiting in early pregnancy: a randomized controlled trial. Birth. 2002;29:1–9.
74. Smith C, Crowther C, Beilby J. Pregnancy outcome following women's participation in a randomised controlled trial of acupuncture to treat nausea and vomiting in early pregnancy. Compl Therap Med. 2002;10:78–83.
75. Liddle SD, Pennick V. Interventions for preventing and treating low-back and pelvic pain during pregnancy. Cochrane Database Syst Rev. 2015;9:CD001139.
76. Gutke A, Betten C, Degerskar K, Pousette S, Olsen MF. Treatments for pregnancy-related lumbopelvic pain: a systematic review of physiotherapy modalities. Acta Obstet Gynecol Scand. 2015;94:1156–67.
77. Manber R, Schnyer RN, Lyell D, Chambers AS, Caughey AB, Druzin M, et al. Acupuncture for depression during pregnancy: a randomized controlled trial. Obstet Gynecol. 2010;115:511–20.
78. Janssen PA, Demorest LC, Kelly A, Thiessen P, Abrahams R. Auricular acupuncture for chemically dependent pregnant women: a randomized controlled trial of the NADA protocol. Subst Abuse Treat Prev Policy. 2012;7:48.
79. Ajori L, Nazari L, Eliaspour D. Effects of acupuncture for initiation of labor: a double-blind randomized sham-controlled trial. Arch Gynecol Obstet. 2013;287:887–91.
80. Andersen Bodil B, Knudsen B, Lyndrup J, Fælling Anni E, Illum D, Johansen M, et al. Acupuncture and/or sweeping of the fetal membranes before induction of labor: a prospective, randomized, controlled trial. J Perinat Med. 2013;41:555.
81. Vixner L, Schytt E, Stener-Victorin E, Waldenstrom U, Pettersson H, Martensson LB. Acupuncture with manual and electrical stimulation for labour pain: a longitudinal randomised controlled trial. BMC Complement Altern Med. 2014;14:187.
82. Borup L, Wurlitzer W, Hedegaard M, Kesmodel US, Hvidman L. Acupuncture as pain relief during delivery: a randomized controlled trial. Birth. 2009;36:5–12.
83. Allameh Z, Tehrani HG, Ghasemi M. Comparing the impact of acupuncture and pethidine on reducing labor pain. Adv Biomed Res. 2015;4:46.
84. Smith CA, Collins CT, Crowther CA, Levett KM. Acupuncture or acupressure for pain management in labour. Cochrane Database Syst Rev. 2011;7:CD009232.
85. Belivani M, Dimitroula C, Katsiki N, Apostolopoulou M, Cummings M, Hatzitolios AI. Acupuncture in the treatment of obesity: a narrative review of the literature. Acupunct Med. 2013;31:88–97.

86. Cabioglu MT, Ergene N. Changes in serum leptin and beta endorphin levels with weight loss by electroacupuncture and diet restriction in obesity treatment. Am J Chin Med. 2006;34:1–11.
87. Cabioglu MT, Ergene N. Electroacupuncture therapy for weight loss reduces serum total cholesterol, triglycerides, and LDL cholesterol levels in obese women. Am J Chin Med. 2005;33:525–33.
88. Abdi H, Abbasi-Parizad P, Zhao B, Ghayour-Mobarhan M, Tavallaie S, Rahsepar AA, et al. Effects of auricular acupuncture on anthropometric, lipid profile, inflammatory, and immunologic markers: a randomized controlled trial study. J Altern Complement Med. 2012;18:668–77.
89. Darbandi S, Darbandi M, Mokarram P, Owji AA, Zhao B, Ghayor-Mobarhan M, et al. Effects of body electroacupuncture on plasma leptin concentrations in obese and overweight people in Iran: a randomized controlled trial. Altern Ther Health Med. 2013;19:24–31.
90. Gucel F, Bahar B, Demirtas C, Mit S, Cevik C. Influence of acupuncture on leptin, ghrelin, insulin and cholecystokinin in obese women: a randomised, sham-controlled preliminary trial. Acupunct Med. 2012;30:203–7.
91. Cho SH, Lee JS, Thabane L, Lee J. Acupuncture for obesity: a systematic review and meta-analysis. Int J Obes. 2009;33:183–96.
92. Sui Y, Zhao HL, Wong VC, Brown N, Li XL, Kwan AK, et al. A systematic review on use of Chinese medicine and acupuncture for treatment of obesity. Obes Rev. 2012;13:409–30.
93. Manneras L, Jonsdottir IH, Holmang A, Lonn M, Stener-Victorin E. Low-frequency electroacupuncture and physical exercise improve metabolic disturbances and modulate gene expression in adipose tissue in rats with dihydrotestosterone-induced polycystic ovary syndrome. Endocrinology. 2008;149:3559–68.
94. Johansson J, Manneras-Holm L, Shao R, Olsson A, Lonn M, Billig H, et al. Electrical vs manual acupuncture stimulation in a rat model of polycystic ovary syndrome: different effects on muscle and fat tissue insulin signaling. PLoS One. 2013;8:e54357.
95. Johansson J, Yi F, Shao R, Lonn M, Billig H, Stener-Victorin E. Intense acupuncture normalizes insulin sensitivity, increases muscle GLUT4 content, and improves lipid profile in a rat model of polycystic ovary syndrome. Am J Physiol Endocrinol Metab. 2010;299:E551–E9.
96. Johansson J, Redman L, Veldhuis PP, Sazonova A, Labrie F, Holm G, et al. Acupuncture for ovulation induction in polycystic ovary syndrome: a randomized controlled trial. Am J Physiol Endocrinol Metab. 2013;304:E934–43.
97. Yu L, Liao Y, Wu H, Zhao J, Wu L, Shi Y, et al. Effects of electroacupuncture and Chinese kidney-nourishing medicine on polycystic ovary syndrome in obese patients. J Tradit Chin Med. 2013;33:287–93.
98. Zheng YH, Wang XH, Lai MH, Yao H, Liu H, Ma HX. Effectiveness of abdominal acupuncture for patients with obesity-type polycystic ovary syndrome: a randomized controlled trial. J Altern Complement Med. 2013;19:740–5.
99. Esteghamati A, Mazaheri T, Vahidi Rad M, Noshad S. Complementary and alternative medicine for the treatment of obesity: a critical review. Int J Endocrinol Metab. 2015;13:e19678.
100. Liang F, Koya D. Acupuncture: is it effective for treatment of insulin resistance? Diabetes Obes Metab. 2010;12:555–69.
101. Stener-Victorin E, Baghaei F, Holm G, Janson PO, Olivecrona G, Lonn M, et al. Effects of acupuncture and exercise on insulin sensitivity, adipose tissue characteristics, and markers of coagulation and fibrinolysis in women with polycystic ovary syndrome: secondary analyses of a randomized controlled trial. Fertil Steril. 2012;97:501–8.
102. van Houten EL, Kramer P, McLuskey A, Karels B, Themmen AP, Visser JA. Reproductive and metabolic phenotype of a mouse model of PCOS. Endocrinology. 2012;153(6):2861–9.
103. Chang SL, Lin JG, Chi TC, Liu IM, Cheng JT. An insulin-dependent hypoglycaemia induced by electroacupuncture at the Zhongwan [CV12] acupoint in diabetic rats. Diabetologia. 1999;42:250–5.
104. Chang S-L, Lin K-J, Lin R-T, Hung P-H, Lin J-G, Cheng J-T. Enhanced insulin sensitivity using electroacupuncture on bilateral Zusanli acupoints [ST 36] in rats. Life Sci. 2006;79:967–71.
105. Wang Y, Liu ZC, Xu B. Efficacy analysis on type 2 diabetes mellitus treated with acupuncture in females. Zhongguo Zhen Jiu. 2014;34:21–4.

106. Belivani M, Lundeberg T, Cummings M, Dimitroula C, Belivani N, Vasilakos D, et al. Immediate effect of three different electroacupuncture protocols on fasting blood glucose in obese patients: a pilot study. Acupunct Med. 2015;33:110–4.
107. Garcia-Vivas JM, Galaviz-Hernandez C, Becerril-Chavez F, Lozano-Rodriguez F, Zamorano-Carrillo A, Lopez-Camarillo C, et al. Acupoint catgut embedding therapy with moxibustion reduces the risk of diabetes in obese women. J Res Med Sci. 2014;19:610–6.
108. Benrick A, Maliqueo M, Johansson J, Sun M, Wu X, Manneras-Holm L, et al. Enhanced insulin sensitivity and acute regulation of metabolic genes and signaling pathways after a single electrical or manual acupuncture session in female insulin-resistant rats. Acta Diabetol. 2014;51:963–72.
109. Frisk J, Kallstrom AC, Wall N, Fredrikson M, Hammar M. Acupuncture improves health-related quality-of-life [HRQoL] and sleep in women with breast cancer and hot flushes. Support Care Cancer. 2011;20:715–24.
110. Nedstrand E, Wyon Y, Hammar M, Wijma K. Psychological well-being improves in women with breast cancer after treatment with applied relaxation or electro-acupuncture for vasomotor symptom. J Psychosom Obstet Gynaecol. 2006;27:193–9.
111. Witt CM, Reinhold T, Brinkhaus B, Roll S, Jena S, Willich SN. Acupuncture in patients with dysmenorrhea: a randomized study on clinical effectiveness and cost-effectiveness in usual care. Am J Obstet Gynecol. 2008;198:166.e1–8.
112. Witt CM, Jena S, Brinkhaus B, Liecker B, Wegscheider K, Willich SN. Acupuncture in patients with osteoarthritis of the knee or hip: a randomized, controlled trial with an additional nonrandomized arm. Arthritis Rheum. 2006;54:3485–93.
113. Allen JJ, Schnyer RN, Chambers AS, Hitt SK, Moreno FA, Manber R. Acupuncture for depression: a randomized controlled trial. J Clin Psychiatry. 2006;67:1665–73.
114. Roschke J, Wolf C, Muller MJ, Wagner P, Mann K, Grozinger M, et al. The benefit from whole body acupuncture in major depression. J Affect Disord. 2000;57:73–81.
115. Luo H, Meng F, Jia Y, Zhao X. Clinical research on the therapeutic effect of the electro-acupuncture treatment in patients with depression. Psychiatry Clin Neurosci. 1998;52(Suppl):S338–40.
116. Luo HC, Jia YK, Li Z. Electro-acupuncture vs. amitriptyline in the treatment of depressive states. J Tradit Chin Med. 1985;5:3–8.
117. Yeung WF, Chung KF, Tso KC, Zhang SP, Zhang ZJ, Ho LM. Electroacupuncture for residual insomnia associated with major depressive disorder: a randomized controlled trial. Sleep. 2011;34:807–15.
118. Gronier H, Letombe B, Collier F, Dewailly D, Robin G. Focus on intrauterine contraception in 15 questions and answers. Gynecol Obst Fertil. 2012;40:37–42.
119. Manber R, Schnyer RN, Allen JJ, Rush AJ, Blasey CM. Acupuncture: a promising treatment for depression during pregnancy. J Affect Disord. 2004;83:89–95.
120. Hardy OT, Wiecha J, Kim A, Salas C, Briceno R, Moody K, et al. Effects of a multicomponent wellness intervention on dyslipidemia among overweight adolescents. J Pediat Endocrinol Metabol. 2012;25:79–82.
121. Pilkington K. Anxiety, depression and acupuncture: A review of the clinical research. Auton Neurosci. 2010;157:91–5.
122. Pilkington K, Kirkwood G, Rampes H, Cummings M, Richardson J. Acupuncture for anxiety and anxiety disorders--a systematic literature review. Acupunct Med. 2007;25:1–10.
123. Sniezek DP, Siddiqui IJ. Acupuncture for treating anxiety and depression in women: a clinical systematic review. Med Acupunct. 2013;25:164–72.
124. Stener-Victorin E, Holm G, Janson PO, Gustafson D, Waern M. Acupuncture and physical exercise for affective symptoms and health-related quality of life in polycystic ovary syndrome: Secondary analysis from a randomized controlled trial. BMC Complement Altern Med. 2013;13:131.
125. MacPherson H, Hammerschlag R, Coeytaux RR, Davis RT, Harris RE, Kong JT, et al. Unanticipated insights into biomedicine from the study of acupuncture. J Altern Complement Med. 2016;22:101.

Part IV

Controlled Ovarian Stimulation and In Vitro Oocyte Maturation

Intrauterine Insemination

18

Madelon van Wely

18.1 Introduction

Treatment of women with polycystic ovary syndrome (PCOS) is symptom oriented. Infertility due to anovulation is the most common reason for women with PCOS to seek treatment. In the previous chapters, many treatment options have been described, like ovulation induction with metformin, clomiphene citrate (CC), aromatase inhibitors and gonadotrophins. All these treatments can be combined with intrauterine insemination (IUI). Controlled ovarian (hyper)stimulation (COH) refers to the use of gonadotrophins to induce maturation of multiple ovarian follicles. These multiple follicles can be retrieved for use in in vitro fertilisation (IVF) or be given time to ovulate. Sometimes IVF was planned but the ovarian hyperstimulation resulted in only one or two dominant follicles. In such a case, a conversion to IUI is an option. Poor ovarian response is however an unexpected finding in women with PCOS. There is no specific data on the effectiveness of conversion to IUI after COH in women with PCOS. In an ovulatory population of women with poor ovarian response and less than four dominant follicles, IVF was found to be more effective and also more cost-effective than IUI [1, 2]. Conversion to IUI bears the risk on multiple pregnancies; thus, conversion to IUI should only be done in case of one or two dominant follicles [3].

COH in women with PCOS planned to undergo IUI assumes that we aim at the ovulation of a multiple number of oocytes. In the past, this was indeed a standard practice that resulted in many cancellations of cycles, a high multiple pregnancy rate and unacceptable ovarian hyperstimulation syndrome (OHSS) risks. A recent

M. van Wely
Center for Reproductive Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
e-mail: m.vanwely@amc.uva.nl

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_18

case-control study found that compared to natural conception, the use of fertility drug was strongly associated with multiple births, and this association was even stronger when IUI was used [4].

Nowadays we aim at the prevention of multiple pregnancies as well as the prevention of OHSS. This has resulted in low-dose stimulation regimens and close monitoring. The goal is to not exceed the gonadotrophin concentration above which more than one follicle will respond. When ovulatory cycles do not result in conception, slightly looser criteria might be considered. In ovulatory women that fail to conceive without any other reason for subfertility, two follicles were more effective than one follicle [3]. As this approach is focussed on mono- or maximally bifollicular growth, ovulation induction is the better term here. Ovulation induction has been extensively described in the previous chapters.

The more general question in this chapter is therefore whether we should use IUI or not in women that undergo ovulation induction.

18.2 IUI in Women with PCOS

IUI is a relatively simple technique that was developed long ago for male factor infertility [5]. The first publication on IUI in humans was in 1799 by Everard Home. His brother-in-law inseminated a woman whose husband had severe hypospadias. The rationale behind IUI was to increase sperm density at the site of fertilisation by depositing motile sperm in close proximity of the oocyte [6]. Over the decades, IUI was increasingly applied in couples with unexplained subfertility, human immunodeficiency virus (HIV)-discordant couples, sperm donation treatment and finally PCOS. Potential indications and contraindications for IUI in PCOS are summarised in Table 18.1.

In IUI, a small volume of processed semen is injected trans-cervically into the uterine cavity around the expected time of ovulation. Processing involves a washing procedure to improve chances of fertilisation by purifying the progressively motile and morphologically normal spermatozoa and removing the seminal fluid. The oldest method is the washing and centrifuging of spermatozoa through culture medium. The *swim-up* is a commonly used technique when the semen sample has a normal

Table 18.1 Indication and contraindications for IUI in women with PCOS

Indications
Sexual dysfunction
Requiring donor sperm
Failure to conceive following six ovulatory cycles
Male subfertility (pre-wash total motile sperm count between 5 and 12 million per ml)
Mild endometriosis
In case of HIV infection
Contraindications
Both tubes blocked
Male infertility
Genital tract infection

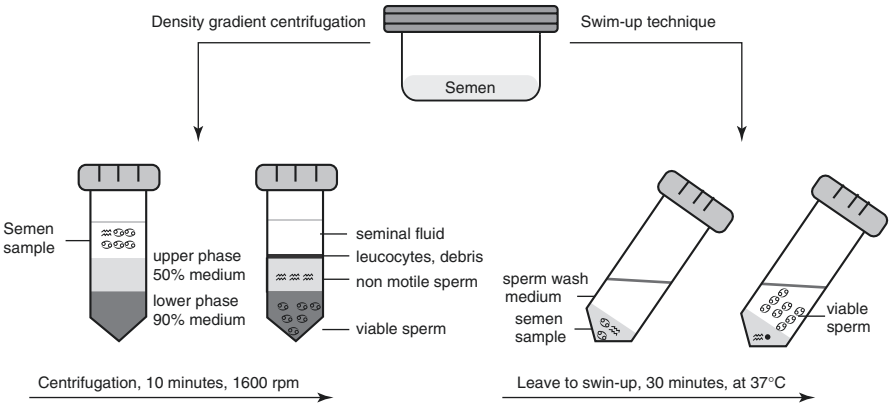


Fig. 18.1 Graphical presentation of density gradient centrifugation and the swim-up technique

number of good sperms (normozoospermia). By this technique, the sperms are selected on their motility and the capability to swim out of the seminal plasma (Fig. 18.1). Alternatively, a density gradient centrifugation can be used (Fig. 18.1). This is probably the preferred technique to select the greater number of motile spermatozoa when there is no normozoospermia and theoretically the safest technique in case of viral infections. By this method, good-quality sperms can be separated from dead sperms, leukocytes and the other components of the seminal plasma using a density discontinuous gradient. These semen preparation techniques have been compared in a review, but studies were too small and limited in number to detect a difference in pregnancy chance following IUI [7]. Semen washing has been shown to prevent virus transmission in HIV-discordant couples desiring children, regardless of viral suppression in the male partner [8].

Before the insemination, women with PCOS will undergo ovulation induction with either CC, CC plus metformin, letrozole or gonadotrophins (see Chaps. 9, 10, 11, and 12). By ultrasound evaluation of follicular growth and endometrial thickness on days 11–14 of the cycle, nonresponse can be identified as well as multiple follicle development. Monitoring may help in timing natural intercourse or IUI, but solid evidences are lacking (especially for oral ovulation inductors). An ovulation-triggering dose of human chorionic gonadotrophin (hCG) at mid-cycle is often done in IUI cycles though there is no evidence that it improved pregnancy chance. National Institute for Health and Clinical Excellence (NICE) guidelines suggest that no more than six cycles with CC should be done. This guideline is not based on solid evidence. A cohort study suggests that half of the women ovulating on CC without a conception after 6 cycles can reach an ongoing pregnancy when continuing ovulation induction with CC up to 12 cycles [9].

In case of gonadotrophins, daily low-dose injections of gonadotrophins are combined with concurrent blood and ultrasound monitoring after 4 days of medication. Subsequent monitoring visits will depend on the actual follicle development. At the moment, however, there is no data suggesting that an intensive (and combined)

monitoring is more effective and safer versus non-intensive (and ultrasonographic alone) monitoring. When required, adjustments in the dose can be made. It can be difficult to stimulate the development of a single dominant follicle. Because of the inherent nature of exogenous gonadotrophin treatment, multifollicular development is not uncommon, despite careful dose adjustment and monitoring, and it may be necessary to cancel the cycle to prevent multiple pregnancies and overstimulation. Ovulation is usually triggered with a single injection of hCG 5000 units when at least one follicle of at least 17 mm in its largest diameter has developed. To reduce the risks of multiple pregnancy and OHSS, hCG should not be administered if a total of three or more follicles larger than 14 mm in diameter have developed. In overstimulated cycles, hCG is withheld and the patient is counselled about the risks and advised to refrain from sexual intercourse [10].

Once the dominant follicle has reached the appropriate size, hCG is administered to trigger ovulation. This is usually done when the leading follicle has reached a size of at least 18 mm. There is low-grade evidence suggesting that the diameter of the leading follicle should be larger in CC cycles than in gonadotrophin cycles, i.e. at least 20 mm in CC cycles and at least 18 mm in gonadotrophin cycles [11].

The IUI procedure will be performed around the time of ovulation, typically about 32–36 h following hCG gift [12, 13]. The actual insemination procedure is done with a catheter through which the post-washed spermatozoa are directly placed into the uterus.

In IUI a single insemination is standard. There is no evidence that multiple inseminations result in more pregnancies though such has not been evaluated in a randomised setting. Several randomised trials have evaluated the effectiveness of single versus double insemination in women with unexplained subfertility. Pooling this evidence in a meta-analysis suggested that single insemination resulted in the same pregnancy rate as double insemination [14].

18.3 Efficacy Data in PCOS

IUI is theoretically expected to result in higher pregnancy rates than intercourse, although this has never been compared head to head. IUI requires extra laboratory work and more hospital visits and will therefore be more expensive. The rationale behind IUI is bypassing the cervical mucus barrier and bringing the semen closer to the released oocyte. In addition, washing and preparation of semen increases the density of motile, morphologically normal spermatozoa at the site of conception.

IUI is often applied in women with PCOS undergoing ovulation induction though its effectiveness compared to timed/regular intercourse has not been settled. Pregnancy rates after IUI as well as after timed intercourse (TI) have been registered to range between 6% and 15% per cycle.

Aiming to find all available, preferably randomised, studies on the effectiveness of IUI, a literature search was done up to the 15th of June 2016. We searched Medline, Embase and the trial registries. MeSH terms used were intrauterine

insemination, ovulation induction and pregnancy. The search was directed at women with World Health Organization (WHO) II anovulation and/or women with PCOS. In woman with PCOS undergoing ovulation induction, IUI was compared to natural conception or TI in only two studies.

The first one was a randomised trial [15]. In this trial, 188 women had been allocated to three consecutive cycles of ovulation induction with CC and IUI (93 women, 259 cycles) or three consecutive cycles of CC with TI (95 women, 266 cycles). There were 18 live births (19%) following CC + IUI and 18 live births (18%) following CC alone. The clinical pregnancy rates were 23% versus 22%. Two twin pregnancies occurred in each group. This small study suggested IUI did not improve pregnancy outcomes in women with PCOS undergoing ovulation induction with CC.

The second study was a retrospective cohort study [16]. Couples were women with PCOS and men with normal semen analysis. Ovulation induction was done with CC, letrozole or gonadotrophins with or without IUI. Of a total 265 cycles, 151 cycles were with IUI and 114 others with TI. Clinical pregnancy rates were 17% in the IUI group and 18% in the TI group. This study did not adjust for confounders like type of ovulation induction and female age. The quality of the evidence was very low but did not suggest differences in pregnancy outcomes in ovulation induction cycles with or without IUI.

On the basis of the available studies, it seems there is no direct evidence in favour of IUI in a general population of couples with PCOS undergoing ovulation induction, whether with CC, aromatase inhibitors or gonadotrophins. We are presently awaiting the results of the Movin trial. In this randomised controlled trial (RCT), 660 women were randomised to receive ovulation induction with CC or gonadotrophins with or without IUI [17].

18.4 Efficacy Data in Couples with Unexplained Subfertility or Male Subfertility

In view of the limited evidence for IUI in women with PCOS, it is interesting to evaluate the evidence of IUI in other populations. Most evidence on IUI is available for couples with unexplained subfertility and couples with mild male subfertility. Though these populations are different from couples with PCOS, the results may provide insight into the potential effectiveness of IUI in women with PCOS.

18.4.1 Unexplained Subfertility

Couples with unexplained subfertility do not have identifiable problems to explain their subfertility. Unexplained infertility is usually defined as a lack of conception after 1 to 2 years of regular sexual intercourse in couples for whom the results of a standard infertility evaluation are normal. This population presents a proxy for the population of women with PCOS that did not conceive the following six ovulatory cycles.

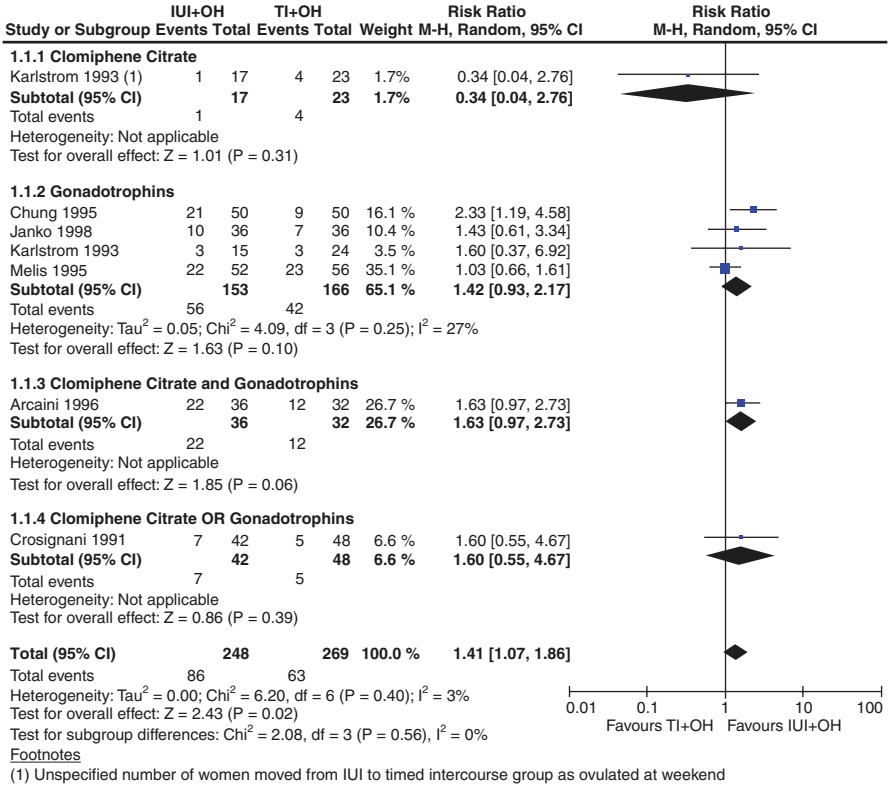


Fig. 18.2 IUI versus TI or expectant policy in couples with unexplained subfertility that underwent the same ovarian stimulation in both arms. Study-specific and pooled relative risks with corresponding 95% confidence interval are presented. Results were summarised using a Mantel-Haenszel random effect model. Review Manager 5.3 software was used to calculate the results and create the forest plots

The effectiveness of IUI in couples with unexplained subfertility undergoing ovarian stimulation has recently been evaluated in a Cochrane review [18]. The authors found 14 RCTs that compared IUI and expectant management or TI in couples with or without ovarian stimulation with CC, letrozole or gonadotrophins. We were only interested in the seven trials that compared IUI and TI or expectant policy in couples that underwent the same ovarian stimulation in both arms as these trials provide the best evidence to evaluate the effectiveness of IUI. All these trials provided data on clinical pregnancy but most did not have data on live birth rate. Only the data on clinical pregnancy rate are shown here as an indication for the potential effectiveness of IUI.

The clinical pregnancies per woman for the women undergoing IUI versus TI or expectant policy in stimulated cycles are depicted in Fig. 18.2 for each individual study. On the right side of this figure, the relative risks are given per study. The results were summarised using meta-analysis, both per type of stimulation and as a total. The individual studies were of small and of low quality. The 95% boundaries largely overlap indicating low heterogeneity in results across studies as is reflected by the inconsistency measure I^2. Overall IUI resulted in more clinical pregnancies

than either TI or expectant management [relative risk (RR) 1.4, 95% confidence interval (CI) 1.07–1.86]. In view of the limited available data on live birth rate and the limited quality of the available studies, no solid conclusions can yet be drawn on the effectiveness of IUI. However, the available data do suggest IUI improves pregnancy chances in women with unexplained subfertility.

18.4.2 Mild Male Subfertility

When the male partner of a woman with PCOS has mild male subfertility, IUI is indicated according to the NICE guidelines. Couples with mild male subfertility are diagnosed following abnormal semen parameters, usually according to WHO guidelines [19]. IUI has not been evaluated in a clinical study in couples with both PCOS and mild male subfertility. However, there have been several randomised studies that evaluated IUI in couples with mild male subfertility without PCOS.

The effectiveness of IUI in couples with mild male subfertility has also recently been summarised in a Cochrane review [20]. The authors found ten RCTs that compared IUI and expectant management or TI in couples with or without ovarian stimulation with CC, letrozole or FSH. We were only interested in the trials that compared IUI and TI or expectant policy in couples that underwent the same or no ovarian stimulation in both arms.

Four RCTs comparing IUI with TI had pregnancy data such that a RR could be calculated (Fig. 18.3). One trial compared IUI and TI in natural cycles and found no evidence of a difference in pregnancy rates between IUI versus TI (RR 3.73, 95% CI 0.22–64). Three trials compared IUI and TI in stimulated cycles and found no evidence of a difference in pregnancy rates between IUI versus TI (RR 1.37, 95%

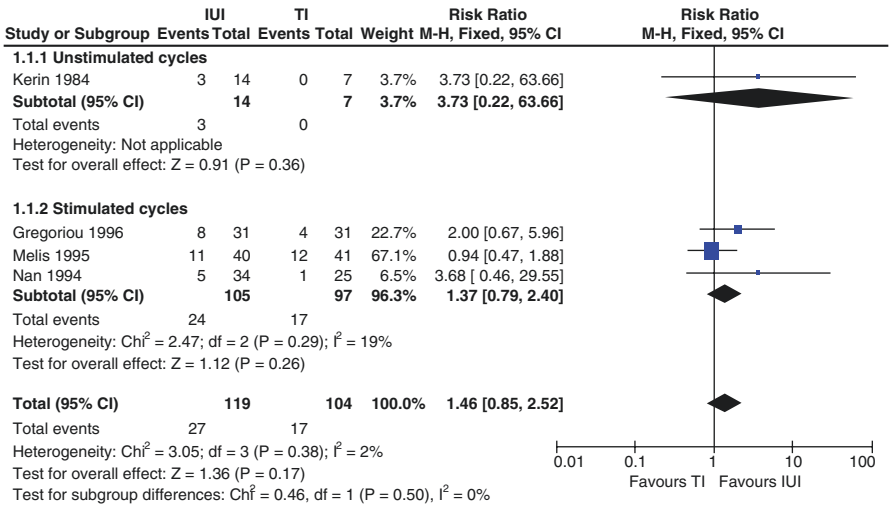


Fig. 18.3 IUI versus TI or expectant management in couples with mild male subfertility in unstimulated and in stimulated cycles. Study-specific and pooled relative risks with corresponding 95% confidence interval are presented. Results were summarised using a Mantel-Haenszel random effect model. Review Manager 5.3 software was used to calculate the results and create the forest plots

CI 0.79–2.40). The direction of the effects seems comparable to that observed in the trials done in couples with unexplained subfertility. However, the available studies were too small to draw conclusions on.

Conclusion

Although IUI is widely used in women with PCOS undergoing ovulation induction, the evidence underpinning the efficacy is scarce.

When IUI is not required, there is no reason to use it, for instance, when the ovulation disorder is the only obstacle for conception. NICE guidelines recommend ovulation induction with IUI in women with PCOS and associated male factor infertility and in women who ovulate on ovulation induction treatment but do not conceive following six ovulatory cycles (National Institute for Health and Clinical Excellence Fertility Guidelines [21]). This recommendation seems to be consistent with the suggestion that IUI appears to be beneficial in unexplained subfertility and in mild male subfertility. On the other hand, this advice is of low-grade evidence, while good-quality evidence is lacking. At this moment, we do not really know whether women with PCOS who ovulate upon ovulation induction treatment benefit from IUI.

IUI has often been compared to TI. TI interferes with natural intercourse. Some authors have argued that TI may therefore be less effective than expectant management [22, 23]. There are no studies that directly compared TI with expectant management. One review summarised the trials of IUI of male partner's prepared semen among subfertile couples according to whether the control group had TI or expectant management [24]. The authors found no evidence of a difference but did suggest that TI resulted in slightly lower pregnancy rates than both IUI and expectant management.

One RCT in the Netherlands evaluated IUI in this population and has just finished enrolment of women into the trial. This trial allocated women with PCOS that ovulate on CC but did not conceive after six ovulatory cycles to one of four study arms: CC or gonadotrophins followed by IUI or an expectant policy, i.e. advising the couple to have regular intercourse. Women were treated for six cycles or until an ongoing pregnancy [17]. The results of this trial are expected to become available in 2017 and will hopefully provide more solid evidence on the effectiveness of IUI.

References

1. Quinquin M, Mialon O, Isnard V, Massin N, Parinaud J, Delotte J, Bongain A. In vitro fertilization versus conversion to intrauterine insemination in bologna-criteria poor responders: how to decide which option? Fertil Steril. 2014;102:1596–601.
2. Yu B, Mumford S, Royster IV GD, Segars J, Armstrong AY. Cost-effectiveness analysis comparing continuation of assisted reproductive technology with conversion to intrauterine insemination in patients with low follicle numbers. Fertil Steril. 2014;102:435–9.
3. van Rumste MM, Custers IM, van der Veen F, van Wely M, Evers JL, Mol BW. The influence of the number of follicles on pregnancy rates in intrauterine insemination with ovarian stimulation: a meta-analysis. Hum Reprod Update. 2008;14:563–70.

4. Chaabane S, Sheehy O, Monnier P, et al. Association between ovarian stimulators with or without intrauterine insemination, and assisted reproductive technologies on multiple births. Am J Obstet Gynecol. 2015;213:511.
5. Barwin BN. Intrauterine insemination of husband's semen. J Reprod Fertil. 1974;36:101–6.
6. Ombelet W, Dhont N, Thijssen A, Bosmans E, Kruger T. Semen quality and prediction of IUI success in male subfertility: a systematic review. Reprod Biomed Online. 2014;28:300–9.
7. Boomsma CM, Heineman MJ, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination. Cochrane Database Syst Rev. 2007;4:CD004507.
8. Zafer M, Horvath H, Mmeje O, van der Poel S, Semprini AE, Rutherford G, Brown J. Effectiveness of semen washing to prevent human immunodeficiency virus (HIV) transmission and assist pregnancy in HIV-discordant couples: a systematic review and meta-analysis. Fertil Steril. 2016;105:645–55.
9. Weiss NS, Braam S, König TE, Hendriks ML, Hamilton CJ, Smeenk JM, Koks CA, Kaaijk EM, Hompes PG, Lambalk CB, van der Veen F, Mol BW, van Wely M. How long should we continue clomiphene citrate in anovulatory women? Hum Reprod. 2014;29:2482–6.
10. Balen AH, Morley LC, Misso M, Franks S, Legro RS, Wijeyaratne CN, Stener-Victorin E, Fauser BC, Norman RJ, Teede H. The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. Hum Reprod Update. 2016;22:687–708.
11. Shalom-Paz E, Marzal A, Wiser A, Hyman J, Tulandi T. Does optimal follicular size in IUI cycles vary between clomiphene citrate and gonadotrophins treatments? Gynecol Endocrinol. 2014;30:107–10.
12. ESHRE Capri Workshop Group. Intrauterine insemination. Hum Reprod Update. 2009;15(3):265–77.
13. Ragni G, Somigliana E, Vegetti W. Timing of intrauterine insemination: where are we? Fertil Steril. 2004;82:25–6.
14. Polyzos NP, Tzioras S, Mauri D, Tatsioni A. Double versus single intrauterine insemination for unexplained infertility: a meta-analysis of randomized trials. Fertil Steril. 2010;94:1261–6.
15. Abu Hashim H, Ombar O, Abd EI. Intrauterine insemination versus timed intercourse with clomiphene citrate in polycystic ovary syndrome: a randomized controlled trial. Acta Obstet Gynecol Scand. 2011;90:344–50.
16. Wiser A, Shalom-Paz E, Reinblatt SL, Holzer H, Tulandi T. Controlled ovarian hyperstimulation in women with polycystic ovarian syndrome with or without intrauterine insemination. Gynecol Endocrinol. 2012;28:502–4.
17. Nahuis MJ, Weiss NS, van der Veen F, Mol BW, Hompes PG, Oosterhuis J, Lambalk NB, Smeenk JM, Koks CA, van Golde RJ, Laven JS, Cohlen BJ, Fleischer K, Goverde AJ, Gerards MH, Klijn NF, Nekrui LC, van Rooij IA, Hoozemans DA, van Wely M. The M-OVIN study: does switching treatment to FSH and/or IUI lead to higher pregnancy rates in a subset of women with world health organization type II anovulation not conceiving after six ovulatory cycles with clomiphene citrate – a randomised controlled trial. BMC Womens Health. 2013;13:42.
18. Veltman-Verhulst SM, Hughes E, Ayeleke RO, Cohlen BJ. Intra-uterine insemination for unexplained subfertility. Cochrane Database Syst Rev. 2016;2:CD001838.
19. Jungwirth A, Diemer T, Dohle GR, Giwercman A, Kopa Z, Krausz C, Tournaye H. Guideline on male infertility. Euro Assoc Urol. 2015. http://uroweb.org/wp-content/uploads/17-Male-Infertility_LR1.pdf.
20. Cissen M, Bensdorp A, Cohlen BJ, Repping S, de Bruin JP, van Wely M. Assisted reproductive technologies for male subfertility. Cochrane Database Syst Rev. 2016;2:CD000360.
21. National Institute for Clinical Excellence Fertility Guidelines. https://www.nice.org.uk/guidance/cg156.
22. Nulsen J, Wheeler C, Ausmanas M, Blasco L. Cervical mucus changes in relationship to urinary luteinizing hormone. Fertil Steril. 1987;48:783–6.
23. Wilcox AJ, Weinberg CR, Baird DB. Timing of intercourse in relation to ovulation: effects on the probability of conception, survival of the pregnancy and sex of the baby. N Engl J Med. 1995;333:1517–21.
24. Snick HK, Collins JA, Evers JLH. What is the most valid comparison treatment in trials of intrauterine insemination, timed or uninfluenced intercourse? A systematic review and meta-analysis of indirect evidence. Hum Reprod. 2008;23:2239–45.

Controlled Ovarian Stimulation for In Vitro Fertilisation Cycles

19

Raoul Orvieto

19.1 Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy among women of reproductive age [1, 2]. Women with PCOS are at increased risk for the metabolic syndrome but also of infertility [3]. The optimal treatment for infertile women with PCOS has not yet been defined. The recognition of the controversies surrounding the treatment has led to the European Society of Human Reproduction and Embryology (ESHRE)/American Society of Reproductive Medicine (ASRM) consensus that addressed the therapeutic challenges raised in women with infertility and PCOS, the various treatments available and their efficacy as well as their safety [4].

In vitro fertilisation (IVF) and embryo transfer (ET) are an effective therapy for PCOS patients and result in pregnancy rates that are comparable with those for women with tubal factor infertility [5, 6]. Moreover, because the number of multiple pregnancies can be kept to a minimum by transferring small numbers of embryos, IVF-ET became a reasonable option to PCOS patients who are refractory to conventional infertility modalities or who have coexisting infertility factors [4, 5]. Eijkemans et al. [7] demonstrated a 72% cumulative singleton live birth rate following ovulation induction using clomiphene (CC) as first-line treatment and gonadotrophins as second-line treatment, meaning that 28% of PCOS patients should be practically referred to IVF.

R. Orvieto
Department of Obstetrics and Gynecology, Chaim Sheba Medical Center (Tel Hashomer), Ramat Gan, and Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Infertility and IVF Unit, Department of Obstetrics and Gynecology, Chaim Sheba Medical Center (Tel Hashomer), Ramat Gan 52621, Israel
e-mail: raoul.orvieto@sheba.health.gov.il

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_19

PCOS patients are known to demonstrate numerous clinical presentations with clinical heterogeneity as a rule. Part of this heterogeneity results from the PCOS definition established at the Rotterdam conference, using 12 follicles of 2–9 mm diameter per ovary for the polycystic ovarian morphology (PCOM) [1] (see Chaps. 2 and 7). Accordingly, the ovarian response of PCOS patients to controlled ovarian hyperstimulation (COH) correlates with ovarian morphology, which varies between hyperresponse, to patients who are collectively referred to as "poor responders" or "low responders". Moreover, since the ovarian overresponse is the result of follicular excess, which correlates with serum anti-Müllerian hormone (AMH) levels [8], AMH level might be a useful tool in categorising PCOS patients according to their expected ovarian response to COH.

Many COH strategies have been offered for the treatment of patients with PCOS undergoing IVF [5, 9–12]. Nevertheless, no compelling advantage for one stimulation protocol over another has been hitherto established, and the optimal stimulation protocol is still under debate [4].

The ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group has argued for the need to perform further randomised controlled trials (RCTs) comparing follicle-stimulating hormone (FSH) stimulation protocols with the use of GnRH agonist versus GnRH antagonist. However, in an era where IVF success, individualisation and careful tailoring of the COH protocol and patient's safety are interrelated and mandatory, such RCT would raise several ethical and legal issues.

Severe ovarian hyperstimulation syndrome (OHSS) is a serious life-threatening complication of ovulation induction and should be of an important consideration in PCOS patients. Moreover, while meta-analyses have yielded conflicting results for pregnancy rate, with a tendency towards a better outcome utilising the long GnRH-agonist suppressive protocol than the GnRH-antagonist protocol [13], the GnRH-agonist COH protocol resulted in an increase incidence of severe OHSS [14, 15]. It was therefore suggested that in patients at high risk for severe OHSS (such as the PCOS patients), the use of the GnRH antagonist should be the preferred COH protocol during their first IVF attempt, since it enables the use of GnRH agonist, instead of human chorionic gonadotrophin (hCG), to trigger ovulation, with the consequent elimination of severe OHSS [16].

19.2 Metformin in COH Cycles

Metformin, an orally active biguanide, enhances insulin sensitivity by the inhibition of hepatic glucose production and by increasing glucose uptake and utilisation into muscle tissue (see Chap. 11). For approximately two decades, metformin has been also used in PCOS patients to improve insulin resistance and reduce hyperinsulinemia with the subsequent improvement in PCOS metabolic and hyperandrogenic disturbances [17].

A systematic review by Costello et al. [18] demonstrated that while the co-administration of metformin to gonadotrophin ovulation induction and IVF does not improve ovulation, pregnancy or live birth rates, it does consistently affect ovarian

response during ovulation induction with variable effects on the length of ovarian stimulation, total dose of FSH used, peak serum oestradiol (E_2) level and the number of oocytes collected and significantly reduces the risk of OHSS. Moreover, while addressing the therapeutic challenges raised in women with infertility and PCOS, the ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group [3] has therefore concluded that there is no evidence for improved live birth rates with the use of metformin, and metformin should be, therefore, restricted only to those patients with glucose intolerance.

We therefore recommend that every PCOS with impaired glucose tolerance, or insulin resistance, should be treated with metformin, starting 6–8 weeks prior to COH, in an attempt to improve COH variable and to reduce the risk of developing severe OHSS.

19.3 Long GnRH Agonist Protocol Versus Multiple-Dose GnRH Antagonist Protocol in COH Cycles

When the ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group has argued for the need to perform further RCTs comparing COH protocols with the use of GnRH agonist versus GnRH antagonist, it relies on Griesinger et al. [11] meta-analysis. Four RCTs were eligible for analysis in PCOS patients. No differences in outcomes were found, except a significantly shorter duration of stimulation, when GnRH-antagonist multiple-dose protocol and GnRH-agonist long protocol were compared [11]. However, due to the sample sizes (118 and 107 patients in the GnRH-antagonist and agonist COH protocols, respectively) and the inadequate power to detect subtle differences, they emphasised that their observation was limited [11].

The addition of the hitherto published RCTs to Griesinger et al. [11] meta-analysis revealed a tendency towards a better outcome using the GnRH-agonist protocol (Table 19.1) [19–26]. This observation was consistently supported by our previous retrospective studies, demonstrating that PCOS patients undergoing COH utilising the long GnRH-agonist suppressive protocol showed a significantly higher

Table 19.1 Clinical pregnancy rate from studies comparing the GnRH antagonist to the GnRH-agonist protocols in PCOS patients

Source	Year	Antagonist	Agonist
Kim	2004	7/21	7/20
Hwang	2004	10/27	10/29
Bahceci	2005	34/73	41/75
Ashrefi	2005	5/30	6/30
Lainas	2007	15/26	32/52
Kurzawa	2008	20/37	21/37
Vrtacnik-Bokal	2009	3/10	3/10
Lainas	2010	58/110	68/110
Combined		152/334 (45.5%)	188/363 (51.8%)

clinical pregnancy rate, as compared to the GnRH-antagonist protocol [27]. Moreover, COH with the use of the long GnRH-agonist suppressive protocol yielded the higher pregnancy rate in lean PCOS patients [28], as well as in those with high basal LH/FSH ratio [29].

A recently published meta-analysis by Lin et al. [30] included nine RCTs that examined PCOS patients undergoing IVF/ICSI. The meta-analysis consisted of 588 women who underwent the long agonist protocols and 554 women who underwent the GnRH-antagonist protocols. While the clinical pregnancy rate, number of days of stimulation and number of oocytes retrieved were similar in the two groups, those undergoing the GnRH-agonist COH protocol required a higher dose of gonadotrophin. However, since the occurrence of OHSS was found to be unexpectedly comparable in Lin et al. meta-analysis [30], it might be speculated that it reflects the use of step-down/coasting or other used measures to prevent OHSS using the GnRH-agonist COH protocol, with the consequent observed decrease, and therefore comparable, in IVF outcome.

19.4 PCOS Patients at Their First IVF Attempt

PCOS patients undergoing their first IVF cycle attempt should be offered the GnRH-antagonist COH protocol (Fig. 19.1).

19.4.1 GnRH Antagonist Co-Treatment and GnRH Agonist Trigger

In patients who vigorously respond to COH and are at risk to develop severe OHSS [31], COH which combines GnRH-antagonist co-treatment and GnRH agonist trigger has already become a common tool aiming to eliminate severe early OHSS and to support the concept of an OHSS-free clinic [32, 33]. However, due to the reported significantly reduced clinical pregnancy and increased first trimester pregnancy loss [34, 35], three different optional strategies were suggested aiming to improve outcome: freeze-all policy, fresh transfer and intensive luteal support and fresh transfer with low-dose hCG supplementation. While the first two options eliminate OHSS, the ability of the addition of low-dose (1500 IU) hCG bolus to eliminate OHSS is still debatable as below discussed.

19.4.1.1 One Bolus of 1500 IU hCG 35 h After the Triggering Bolus of GnRH Agonist

The administration of 1500 IU hCG 1 h after oocyte retrieval [36, 37] was demonstrated to rescue the luteal phase, resulting in a reproductive outcome comparable with that of hCG triggering and with no increased risk of OHSS [38]. However, when applied to patients at high risk to develop severe OHSS, 26% developed severe early OHSS requiring ascites drainage and hospitalisation [39], a figure that is comparable to the acceptable 20% prevalence of severe OHSS in ostensibly high-risk patients [40].

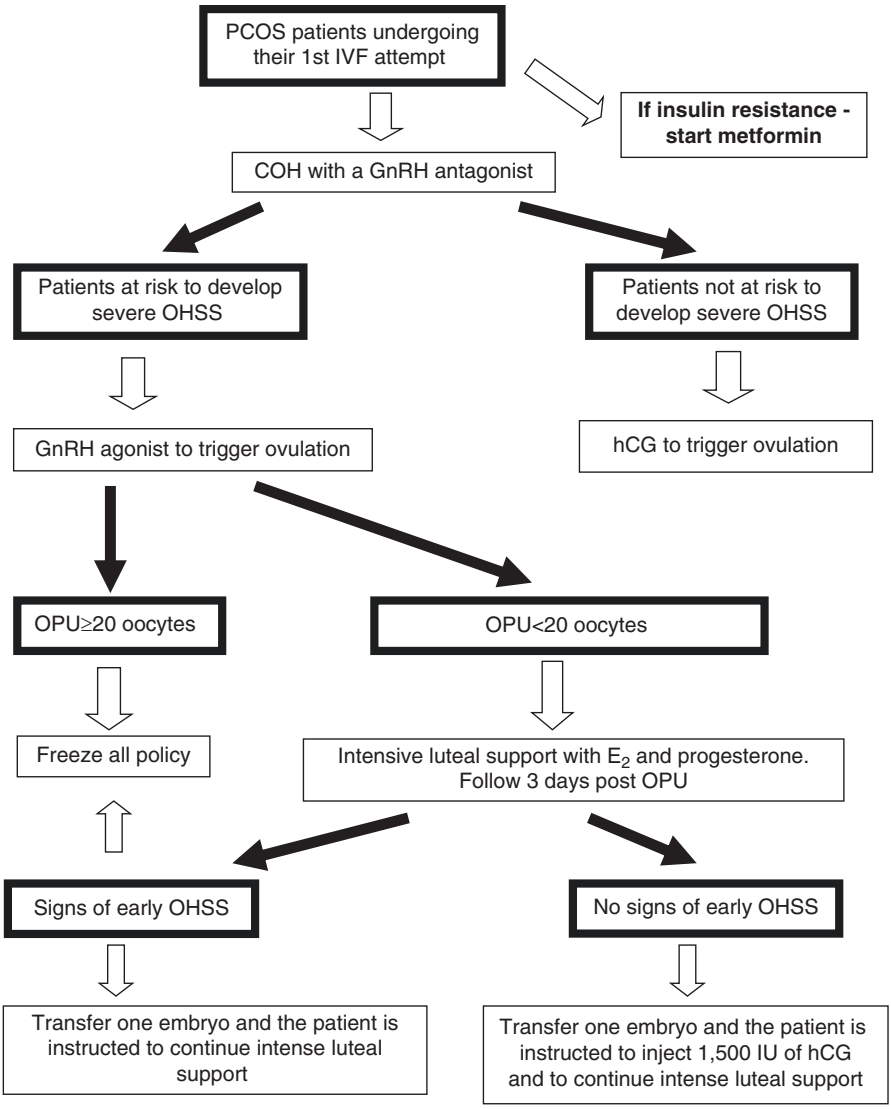


Fig. 19.1 IVF treatment in PCOS patients undergoing their first IVF attempt

19.4.1.2 One Bolus of 1500 IU hCG Concomitant with GnRH Agonist (Dual Trigger)

The administration of 1500 IU hCG 34–36 h before oocyte retrieval was suggested as a method which improves oocyte maturation, while providing more sustained support for the corpus luteum than can be realised by the GnRH-agonist-induced LH surge alone [41, 42]. While acceptable rates of fertilisation, implantation, clinical pregnancy, ongoing pregnancy rates and early pregnancy loss were achieved in

high responders after dual trigger [41, 42], the incidence of clinically significant OHSS was not eliminated but rather reduced to 0.5% [42].

19.4.1.3 One Bolus of 1500 IU hCG 5 days After the Triggering Bolus of GnRH Agonist

While the freeze-all policy was applied to all patients yielding more than 20 oocytes, those triggered with GnRH agonist, who achieved less than 20 oocytes, were instructed to start an intensive luteal support with E_2 and progesterone, the day following OPU, and were re-evaluated 3 days after oocyte retrieval (on the day of embryo transfer) for signs of early or moderate OHSS (ultrasonographic signs of ascites as reflected by the appearance of fluid surrounding the uterus/ovaries and/or haematocrit levels >40% for the degree of haemoconcentration) [43, 44]. If no early signs of OHSS developed, one embryo was transferred, and the patients were instructed to inject 1500 IU of hCG [43, 44]. By deferring the hCG bolus by 3 days (5 days following GnRH agonist trigger), the corpus luteum was rescued, with an observed extremely high mid-luteal progesterone levels [44] and reasonable pregnancy rate, with no patient developing severe OHSS. However, while these preliminary results are promising, the small sample size mandates further large RCTs [44].

19.4.2 Combined Oral Contraceptive (COC) Pretreatment

COCs were shown to induce significant reduction of hormonal and clinical parameters in PCOS patients, e.g., a significant inhibition of gonadotrophin release, reducing androgenic milieu, Ferriman-Gallwey score and ovarian volume [45]. However, while COC pretreatment in patients undergoing the GnRH-antagonist COH protocol resulted in better synchronised response and a scheduled cycle, it also caused significantly longer duration of the stimulation, higher gonadotrophin consumption and, possibly, a lower ongoing pregnancy rate. The detrimental effect of pretreatment COCs was related to the potential negative effect of its gestagen component on the endometrium, or the low endogenous LH levels induced by COCs, with their deleterious impact on oocyte competence or endometrial receptivity.

Recently, anti-androgenic COC pretreatment was shown to result in a significantly higher oocyte yield in oocyte donors, as compared to donors receiving androgenic gestagens, and a comparable yield to donors without COC pretreatment. These differences were maintained after adjustments for donor age and total FSH dose used in ovulation induction [46].

19.5 PCOS Patients After First IVF Attempt

Following the first failed IVF attempt, and in cases where an additional IVF cycle attempt is required, patients may be offered either the GnRH-antagonist or agonist COH protocols. In cases demonstrating a previous vigorous response to daily

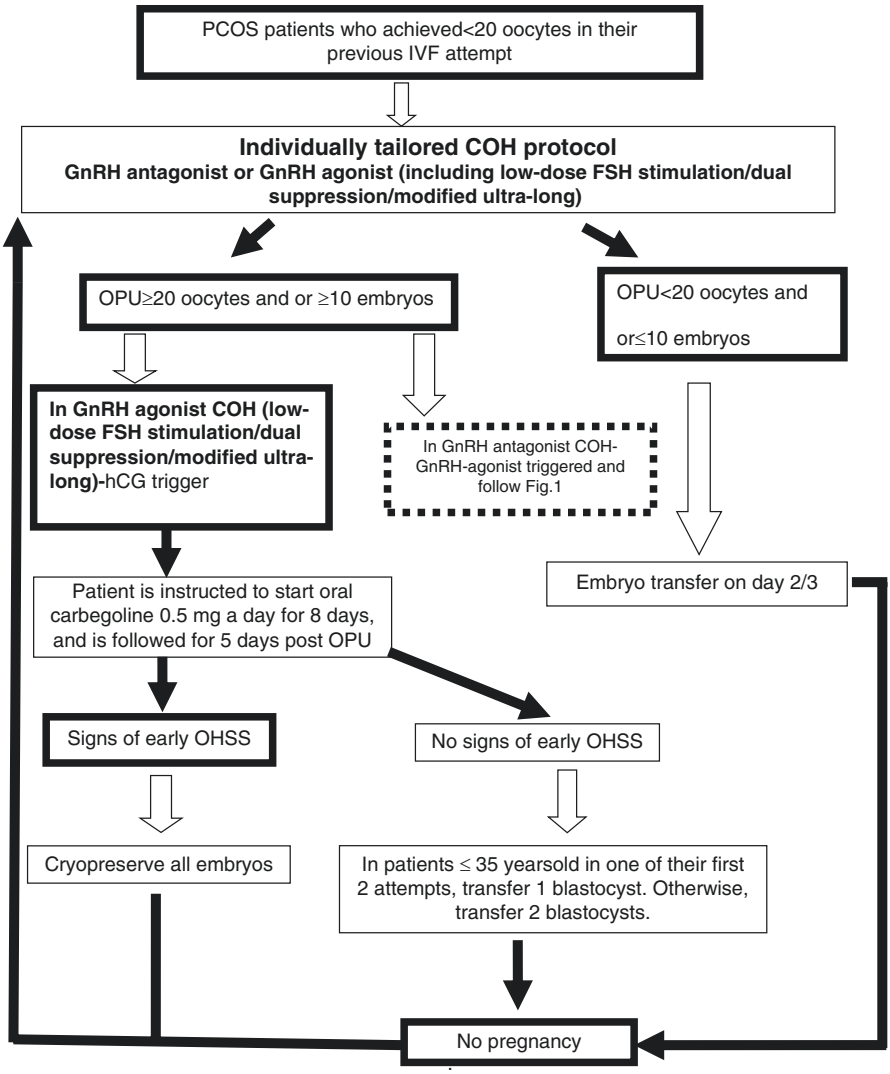


Fig. 19.2 IVF treatment in PCOS patients undergoing their second and more IVF attempt

low-dose gonadotrophin stimulation, the GnRH-antagonist protocol would be probably the protocol of choice, enabling the substitution of hCG with GnRH agonist for final follicular maturation (Fig. 19.2).

Otherwise, and specifically in those in whom <20 oocytes were retrieved in the first IVF cycle attempt, the long GnRH-agonist suppressive protocol might be offered in one of the regimens below detailed.

19.5.1 Low-Dose FSH Stimulation Protocol

Marci et al. [47] have assessed the benefits of a low-dose FSH stimulation protocol in 61 patients with PCOM, who have presented previously, with a very high ovarian response to a standard long GnRH-agonist COH protocol with human menopausal gonadotrophin (hMG). A subsequent long GnRH-agonist COH protocol using a daily low-dose FSH protocol was offered with an initial dose of 75 IU FSH a day, which was increased by steps of 37.5 IU every 4 days in case of no response. The low-dose protocol revealed significantly lower number of ampules used, peak E_2 and the number of oocytes retrieved with high implantation (21.8%) and clinical pregnancy (38.4%) rates and cumulative deliveries per cycle started and per patient of 41.6% and 52.5%, respectively. Moreover, while none of the patients using the low-dose protocol suffered from severe OHSS, five patients using the standard protocol did.

19.5.2 Dual Suppression with COCs and GnRH Agonists

Damario et al. [12] offered the dual-suppression protocol to patients who had previously exhibited clinical features suggestive of a heightened sensitivity to exogenous gonadotrophin therapy, which included previous cycles of ovulation induction or IVF with peak E_2 concentrations exceeding 2500 pg/mL or evidence of a previous high response to gonadotrophin (i.e., excessive number of oocytes retrieved and/or OHSS).

The dual-suppression protocol consists of COC pretreatment following the onset of a spontaneous menstrual period or progestin-induced withdrawal bleeding. COCs were taken daily for 25 days. Daily GnRH agonist was started on day 21, overlapping the COCs for 5 days. Thereafter, low-dose gonadotrophin stimulation was initiated on the third day of subsequent withdrawal bleeding, at which time the daily dose of the GnRH agonist was halved. The daily gonadotrophin dosage was decreased in an incremental step-down fashion once initial follicular recruitment was established and further adjusted individually according to each patient's follicular response [12].

A review of 99 cycles obtained in 73 high-responder patients using the dual-suppression protocol revealed only 13 cancellations prior to embryo transfer (13.1%), with clinical and ongoing pregnancy rates per initiated cycle of 46.5 and 40.4%, respectively. Only eight patients experienced mild-moderate OHSS following treatment [12].

When compared to patients who had undergone previous IVF-ET cycles at their centre, significant improvements were noted in oocyte fertilisation rates, embryo implantation rates and clinical/ongoing pregnancy rates with the dual-suppression protocol.

19.5.3 Modified Ultra-Long Downregulation Protocol

Gong et al. [48] offered the modified ultra-long GnRH-agonist protocol to PCOS patients. The protocol consists of two 1.5–1.875 mg depot GnRH-agonist intramuscular injection, applied on day 20 of the patient's first menstrual cycle and again on

day 21 of the following menstrual cycle. Two to three weeks following the last injection and after confirmation of pituitary-ovarian suppression, human menopausal gonadotrophin was started. When the ultra-long was retrospectively compared to the conventional long GnRH-agonist protocols, PCOS patients yielded thicker endometrium and lower peak progesterone levels and achieved significantly higher implantation and pregnancy rates, with comparable gonadotrophin dosages required, number of oocytes retrieved, good quality embryo, cancellation, OHSS and fertilisation rates.

The prolonged suppression probably contributes to the optimisation of the clinical and hormonal profiles at the start of ovarian stimulation. As was already emphasised [45], prolonged GnRH-agonist suppression in PCOS patients was shown to be effective in reducing androgenic milieu, Ferriman-Gallwey score, ovarian volume and antral follicle count.

These might be further improved when GnRH agonist was combined regimen with COCs [45], suggesting that the modified ultra-long GnRH-agonist protocol is one of the COH protocol that should be offered to PCPS patients aiming to improve IVF outcome.

If the aforementioned long GnRH-agonist COH protocols (triggered with hCG) yield >20 oocytes, or >10 embryos develop, the patient should be instructed to start oral cabergoline 0.5 mg a day for 8 days [49] and is followed for 5 days after oocyte retrieval for signs of early OHSS (see above). If early signs develop, embryo transfer is withheld and all resulting embryos cryopreserved. This approach limits early OHSS, if it appears, to a milder and shorter form. If it does not appear, we transfer one blastocyst, with the consequent decrease in the risk of multiple pregnancy to almost zero, thereby eliminating the risk of late OHSS.

19.6 Luteal Support Following IVF

Luteal phase defect is always present following COH for IVF, either due to the luteolytic effect of the GnRH analogues used during COH or as a consequent of the supra-physiologic levels of E_2 that may both alter endometrial responsiveness to progesterone and inhibit LH release by the negative feedback mechanism [50]. A recent Cochrane meta-analysis regarding luteal phase support for ART cycles confirmed that progesterone exerts a significant positive effect on clinical, ongoing pregnancy rates and pooled live birth [51].

Conclusion

PCOS patients undergoing IVF-ET cycles and COH utilising the mid-luteal long GnRH-agonist suppressive protocol are probably associated with a higher clinical pregnancy rate than the multidose GnRH-antagonist protocol, specifically in lean patients or those high basal LH/FSH ratio. However, since PCOS patients are at high risk to develop severe OHSS, it would be prudent, in the first IVF cycle attempt, to offer these patients the GnRH-antagonist COH protocol, with its inherent lower risk of OHSS. Moreover, it enables the substitution of hCG

with GnRH agonist to trigger ovulation, with the consequent elimination of severe OHSS. Further large studies are needed to clarify the role of these two GnRH analogues in the different PCOS phenotypes. These studies may help fertility specialists in tailoring of the COH protocol, for optimising IVF success and without endangering the patients with severe OHSS.

References

1. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81:19–25.
2. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol. 1935;29:181–91.
3. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes. 1989;38:1165–74.
4. Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Fertil Steril. 2008;89:505–22.
5. Buyalos RP, Lee CT. Polycystic ovary syndrome: pathophysiology and outcome with in vitro fertilization. Fertil Steril. 1996;65:1–10.
6. Ashkenazi J, Farhi J, Orvieto R, Homburg R, Dekel A, Feldberg D. Polycystic ovary syndrome patients as oocyte donors: the effect of ovarian stimulation protocol on the implantation rate of the recipient. Fertil Steril. 1995;64:564–7.
7. Eijkemans MJ, Imani B, Mulders AG, Habbema JD, Fauser BC. High singleton live birth rate following classical ovulation induction in normogonadotrophic anovulatory infertility (WHO 2). Hum Reprod. 2003;18:2357–62.
8. Dumont A, Robin G, Catteau-Jonard S, Dewailly D. Role of Anti-Müllerian Hormone in pathophysiology, diagnosis and treatment of polycystic ovary syndrome: a review. Reprod Biol Endocrinol. 2015;13:137.
9. Dor J, Shulman A, Levran D, Ben-Rafael Z, Rudak E, Mashiach S. The treatment of patients with polycystic ovarian syndrome by in-vitro fertilization and embryo transfer: a comparison of results with those of patients with tubal infertility. Hum Reprod. 1990;5:816–8.
10. Urman B, Fluker MR, Yuen BH, Fleige-Zahradka BG, Zouves CG, Moon YS. The outcome of in vitro fertilization and embryo transfer in women with polycystic ovary syndrome failing to conceive after ovulation induction with exogenous gonadotropins. Fertil Steril. 1992;57:1269–73.
11. Griesinger G, Diedrich K, Tarlatzis BC, Kolibianakis EM. GnRH-antagonists in ovarian stimulation for IVF in patients with poor response to gonadotrophins, polycystic ovary syndrome, and risk of ovarian hyperstimulation: a meta-analysis. Reprod Biomed Online. 2006;13:628–38.
12. Damario MA, Barmat L, Liu HC, Davis OK, Rosenwaks Z. Dual suppression with oral contraceptives and gonadotrophin releasing-hormone agonists improves in-vitro fertilization outcome in high responder patients. Hum Reprod. 1997;12:2359–65.
13. Orvieto R, Patrizio P. GnRH agonist versus GnRH antagonist in ovarian stimulation: an ongoing debate. Reprod Biomed Online. 2013;26:4–8.
14. Al-Inany H, Abou-Setta AM, Aboulghar M. Gonadotrophin releasing hormone antagonists for assisted conception: a Cochrane review. Reprod Biomed Online. 2007;14:640–9.
15. Al-Inany HG, Youssef MA, Aboulghar M, Broekmans F, Sterrenburg M, Smit J, Abou-Setta AM. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. Cochrane Database Syst Rev. 2011;5:CD001750.

16. Orvieto R. Can we eliminate severe ovarian hyperstimulation syndrome? Hum Reprod. 2005;20:320–2.
17. Velazquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ. Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism. 1994;43:647–54.
18. Costello MF, Chapman M, Conway U. A systematic review and meta-analysis of randomized controlled trials on metformin co-administration during gonadotrophin ovulation induction or IVF in women with polycystic ovary syndrome. Hum Reprod. 2006;21:1387–99.
19. Kim CH, Lee YJ, Hong SH. Efficacy of a GnRH antagonist during early and late controlled ovarian hyperstimulation period in women with polycystic ovary syndrome undergoing IVF-ET. Hum Reprod. 2004;19:105–9.
20. Hwang JL, Seow KM, Lin YH, Huang LW, Hsieh BC. Ovarian stimulation by concomitant administration of cetrorelix acetate and HMG following Diane-35 pre-treatment for patients with polycystic ovary syndrome: a prospective randomized study. Hum Reprod. 2004;19:1993–2000.
21. Bahceci M, Ulug U, Ben-Shlomo I, Erden HF, Akman MA. Use of a GnRH antagonist in controlled ovarian hyperstimulation for assisted conception in women with polycystic ovary disease: a randomized, prospective, pilot study. J Reprod Med. 2005;50:84–90.
22. Ashrafi M, Moini A, Mohammadzadeh A, Ezabadi Z, Zafarani F. A comparative study of GnRH antagonist and GnRH agonist in PCO patients undergoing IVF/ICSI cycles. Iran J Reprod Med. 2005;3:14–8.
23. Lainas TG, Petsas GK, Zorzovilis IZ, Iliadis GS, Lainas GT. Initiation of GnRH antagonist on day 1 of stimulation as compared to the long agonist protocol in PCOS patients. A randomized controlled trial: effect on hormonal levels and follicular development. Hum Reprod. 2007;22:1540–6.
24. Kurzawa R, Ciepiela P, Baczkowski T, Safranow K, Brelik P. Comparison of embryological and clinical outcome in GnRH antagonist vs. GnRH agonist protocols for in vitro fertilization in PCOS non-obese patients. A prospective randomized study. J Assist Reprod Genet. 2008;25:365–74.
25. Vrtacnik-Bokal E, Virant Klun I, Verdenik I. Follicular oestradiol and VEGF after GnRH antagonists or GnRH agonists in women with PCOS. Reprod Biomed Online. 2009;18:21–8.
26. Lainas TG, Sfontouris IA, Zorzovilis IZ, Petsas GK, Lainas GT. Flexible GnRH antagonist protocol versus GnRH agonist long protocol in patients with polycystic ovary syndrome treated for IVF: a prospective randomized controlled trial (RCT). Hum Reprod. 2010;25:683–9.
27. Orvieto R, Meltcer S, Homburg R, Nahum R, Rabinson J, Ashkenazi J. What is the preferred GnRH-analogue for polycystic ovary syndrome patients undergoing controlled ovarian hyperstimulation for in-vitro fertilization? Fertil Steril. 2009;91:1466–8.
28. Orvieto R, Nahum R, Meltcer S, Homburg R, Rabinson J, Anteby EY, Ashkenazi J. Controlled ovarian hyperstimulation in polycystic ovary syndrome patients: the role of body mass index. Reprod Biomed Online. 2009;18:333–6.
29. Orvieto R, Meltcer S, Liberty G, Rabinson J, Anteby EY, Nahum R. Does day-3 LH/FSH ratio influence in vitro fertilization outcome in PCOS patients undergoing controlled ovarian hyperstimulation with different GnRH-analogue? Gynecol Endocrinol. 2012;28:422–4.
30. Lin H, Li Y, Li L, Wang W, Yang D, Zhanget Q. Is a GnRH antagonist protocol better in PCOS patients? A meta-analysis of RCTs. PLoS One. 2014;9:e91796.
31. The Practice Committee of the American Society for Reproductive Medicine (ASRM). Ovarian hyperstimulation syndrome. Fertil Steril. 2008;90:188–93.
32. Orvieto R. Can we eliminate severe ovarian hyperstimulation syndrome? Hum Reprod. 2005;20:320–2.
33. Devroey P, Polyzos NP, Blockeel C. An OHSS-free clinic by segmentation of IVF treatment. Hum Reprod. 2011;6:2593–7.

34. Griesinger G, Diedrich K, Devroey P, Kolibianakis EM. GnRH agonist for triggering final oocyte maturation in the GnRH antagonist ovarian hyperstimulation protocol: a systematic review and meta-analysis. Hum Reprod Update. 2006;12:159–68.
35. Orvieto R, Rabinson J, Meltzer S, Zohav E, Anteby E, Homburg R. Substituting HCG with GnRH agonist to trigger final follicular maturation–a retrospective comparison of three different ovarian stimulation protocols. Reprod Biomed Online. 2006;13:198–201.
36. Humaidan P, Bungum L, Bungum M, Yding AC. Rescue of corpus luteum function with periovulatory HCG supplementation in IVF/ICSI GnRH antagonist cycles in which ovulation was triggered with a GnRH agonist: a pilot study. Reprod Biomed Online. 2006;13:173–8.
37. Humaidan P, Bredkjaer HE, Westergaard LG, Andersen CY. 1,500 IU human chorionic gonadotropin administered at oocyte retrieval rescues the luteal phase when gonadotropin-releasing hormone agonist is used for ovulation induction: a prospective, randomized, controlled study. Fertil Steril. 2010;93:847–54.
38. Humaidan P, Papanikolaou EG, Kyrou D, Alsbjerg B, Polyzos NP, Devroey P, Fatemi HM. The luteal phase after GnRH-agonist triggering of ovulation: present and future perspectives. Reprod Biomed Online. 2012;24:134–41.
39. Seyhan A, Ata B, Polat M, Son WY, Yarali H, Dahan MH. Severe early ovarian hyperstimulation syndrome following GnRH agonist trigger with the addition of 1500 IU hCG. Hum Reprod. 2013;28:2522–8.
40. Orvieto R, Ben-Rafael Z. Role of intravenous albumin in the prevention of severe ovarian hyperstimulation syndrome. Hum Reprod. 1998;13:3306–9.
41. Shapiro BS, Daneshmand ST, Garner FC, Aguirr M, Thomas S. Gonadotropin-releasing hormone agonist combined with a reduced dose of human chorionic gonadotropin for final oocyte maturation in fresh autologous cycles of in vitro fertilization. Fertil Steril. 2008;90:231–3.
42. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C. Comparison of “triggers” using leuprolide acetate alone or in combination with low-dose human chorionic gonadotropin. Fertil Steril. 2011;95:2715–7.
43. Orvieto R. Ovarian hyperstimulation syndrome – an optimal solution for an unresolved enigma. J Ovarian Res. 2013;6:77.
44. Haas J, Kedem A, Machtinger R, Dar S, Hourovitz A, Yerushalmi G, Orvieto R. HCG (1500IU) administration on day 3 after oocytes retrieval, following GnRH-agonist trigger for final follicular maturation, results in high sufficient mid luteal progesterone levels – a proof of concept. J Ovarian Res. 2014;7:35.
45. Genazzani AD, Petraglia F, Battaglia C, Gamba O, Volpe A, Genazzani AR. A long-term treatment with gonadotropin-releasing hormone agonist plus a low-dose oral contraceptive improves the recovery of the ovulatory function in patients with polycystic ovary syndrome. Fertil Steril. 1997;67:463–8.
46. Barad DH, Kim A, Kubba H, Weghofer A, Gleicher N. Does hormonal contraception prior to in vitro fertilization (IVF) negatively affect oocyte yields? A pilot study. Reprod Biol Endocrinol. 2013;11:28.
47. Marci R, Senn A, Dessole S, Chanson A, Loumaye E, De Grandi P, Germond M. low-dose stimulation protocol using highly purified follicle-stimulating hormone can lead to high pregnancy rates in in vitro fertilization patients with polycystic ovaries who are at risk of a high ovarian response to gonadotropins. Fertil Steril. 2001;75:1131–5.
48. Gong F, Li X, Zhang S, Ma H, Cai S, Li J, Lin GE, Lu G. A modified ultra-long pituitary downregulation protocol improved endometrial receptivity and clinical outcome for infertile patients with polycystic ovarian syndrome. Exp Ther Med. 2015;10:1865–70.
49. Soares SR. Etiology of OHSS and use of dopamine agonists. Fertil Steril. 2012;97:517–22.
50. Palomba S, Santagni S, La Sala GB. Progesterone administration for luteal phase deficiency in human reproduction: an old or new issue? J Ovarian Res. 2015;19:77.
51. Van der Linden M, Buckingham K, Farquhar C, Kremer JAM, Metwally M. Luteal phase support for assisted reproduction cycles. Cochrane Database Syst Rev. 2015;7:CD009154.

In Vitro Oocyte Maturation

20

Melanie L. Walls

20.1 Introduction

Human IVM was first reported more than 50 years ago [1]; however, with the introduction of gonadotrophins to stimulate multi-follicular growth [2], research into IVM treatment became less popular. This introduction of ovarian hyperstimulation also introduced the side effect of OHSS [3], which is a significant clinical consequence of gonadotrophin stimulation, resulting in patient discomfort in the mild stages and significant morbidity or mortality in the major forms of the condition [4]. Even though IVM provided a viable alternative to avoid ovarian hyperstimulation syndrome (OHSS), stimulated IVF treatment expanded worldwide, and it wasn't until 1991 when the first live birth was recorded after IVM following collection by ovary biopsy [5]. Following this, in 1994 IVM success was achieved following a transvaginal oocyte aspiration (TVOA) [6], and its reported use in the literature has continued at a steady pace ever since. IVM treatment has now expanded to treat a range of conditions including gamete donation and follicle-stimulating hormone (FSH)-resistant ovaries and for fertility preservation. However, the predominant patient cohort who are suitable for IVM treatment are those diagnosed with polycystic ovarian morphology (PCOM) and/or polycystic ovary syndrome (PCOS). Additionally and most importantly, IVM is currently the only treatment option which completely eliminates the risk of OHSS [7].

This chapter will outline the use of IVM as an effective treatment method for patients with PCOS and PCOM, as well as summarise the different protocols for treatment regimes, hormonal priming and culture conditions with resulting reproductive

M.L. Walls
School of Women's and Infant's Health, The University of Western Australia, Perth, WA 6009, Australia

The Fertility Specialists of Western Australia, 25 Queenslea Drive, Claremont, Perth, WA 6010, Australia
e-mail: melanie@fertilitywa.com.au; melanie.walls@uwa.edu.au

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_20

outcomes. It will also address the limited current evidence available for the outcomes of children born following IVM and where IVM research is heading in the future.

20.2 IVM in the Treatment of Women with PCOM/PCOS

The defining feature for increased IVM success rates is an increased antral follicle count (AFC) [8]. Only PCOS patients with PCOM (more than 12 antral follicles) and PCOS patients with a high antral follicle (although not so high to be classified as PCOM) count could have best benefit from IVM. As the AFC is a determining factor for IVM treatment, patients with fewer than five antral follicles should not be considered for this treatment [9]. Considering women with PCOM/PCOS who typically have a very high AFC, they therefore respond better to IVM treatment and have more oocytes collected from IVM cycles than patients without the condition. While those patients with PCOM may still benefit from IVM treatment, a recent meta-analysis showed that implantation and clinical pregnancy rates are highest in patients with PCOS [10].

20.3 Treatment Regimes and Hormonal Priming

In theory, IVM requires no exogenous gonadotrophin administration as the immature oocytes complete their final stages of maturation under the influence of suitable culture conditions, tailored to mimic the intra-follicular environment. However, hormonal priming using follicle-stimulating hormone (FSH) or human menopausal gonadotrophin (hMG) and/or human chorionic gonadotrophin (hCG) is often used to 'prime' the follicles prior to oocyte aspiration. The results of these protocols are contradictory and difficult to evaluate, due to differences in priming and culture conditions. FSH priming plays an important role in increasing follicular growth and contributes to higher rates of oocytes collected, increased maturation [9], fertilisation, embryo development and implantation [9], and the highest rates of IVM success have been achieved with 3–5 days of FSH priming without hCG triggering when the dominant follicle is less than 12 mm [11]. Protocols for hCG priming achieve the best result with a 38 h interval from 10,000 IU hCG triggering to oocyte collection [12] when the dominant follicle is less than 14 mm [13].

The predominant controversial issue is the use of hCG or gonadotrophin-releasing hormone (GnRH) agonist triggers in IVM protocols, as they induce nuclear maturation in vivo [14] and, hence, are at odds with the core concept of IVM, where maturation by definition takes place in vitro. Additionally, as hCG can induce oocyte maturation in vivo in follicles greater than 9 mm [15], this methodology is logistically problematic, as oocytes are at varying stages of development when they are collected. This in turn leads to multiple insemination times for the same patient and subsequent variations in embryo culture stages. Recently, an effort to change the clinical definition of IVM has been suggested to categorise the different protocols currently employed around the world [16]. The authors suggested four definitions of IVM treatment protocols: (1) IVM without triggering, (2) natural cycle IVF with early triggering combined with IVM, (3) IVM with short gonadotrophin stimulation and (4) modified natural cycle IVF with early triggering combined with IVM. However, these definitions are confusing and still allow for a category of

patients receiving both FSH priming and hCG triggering which others have suggested be termed 'truncated IVF' [17, 18].

More widely accepted definitions have been recommended to include three treatment groups, 'truncated IVF'(where priming includes both FSH and hCG) and 'hCG-primed IVM' (where there is no FSH administered and patients receive an hCG or agonist trigger), and the definition of 'IVM' is suggested to be reserved for those cycles with or without FSH/FSH-analogue priming, without the use of 'gonadotrophins that are intended to trigger oocyte maturation *in vivo*, such as hCG or GnRH agonists' [18]. This is a necessary development for IVM both clinically and for research purposes, to avoid comparisons between outcomes of what some consider to be true IVM vs an abridged version of standard, stimulated IVF treatment. This will aid to avoid confusion for clinicians, patients and health professionals when IVM is discussed.

20.4 Immature Oocyte Collection

Similarly to priming protocols, procedures for immature oocyte collection in IVM cycles show considerable variation. They are mostly centred around a standard TVOA procedure with modifications that enable the collection of oocytes from small follicles. Clinicians may use a double or single lumen needle, depending on whether the protocol employs follicular flushing. Some clinics have reported flushing each follicle up to three times [19], and others do not employ follicular flushing [20]. Follicular flushing solutions include HEPES [21] or Hartmann's supplemented with heparin [19]. Additionally, the aspiration pressure used in TVOA for IVM has been reported in varying ranges, from 7 kpa (52.5 mmHg) [22] to 200 mmHg [21].

Such as in standard IVF, germinal vesicle (GV) stage oocytes can be identified by sight [11, 19], which involves additional training of the embryologist, or by filtering follicular aspirates through a mesh cell strainer [20, 23]. Figure 20.1 represents a GV stage oocyte located during an IVM oocyte collection. Once the cumulus-oocyte complex is removed from the follicle, there is a significant drop in the amount of gap junction communication between the cumulus cells and the oocyte and a dramatic decrease in cyclic adenosine monophosphate (cAMP)

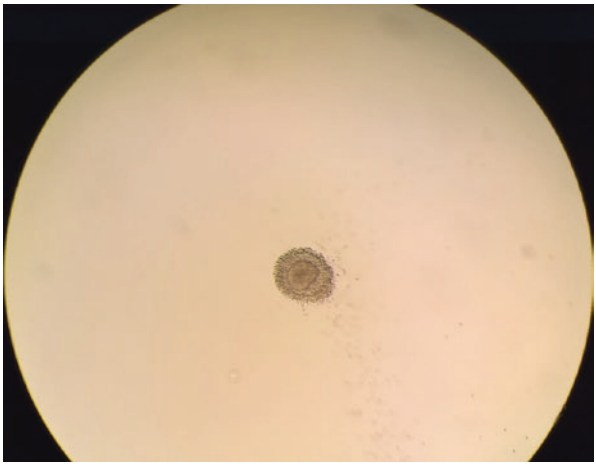


Fig. 20.1 A GV stage oocyte surrounded by compact cumulus oophorus, following follicular aspiration (800× magnification, SXZ12 stereo microscope, Olympus)

activity [24]. Following removal from the follicle, some oocytes may undergo spontaneous nuclear maturation, evidence by the progression to metaphase II (MII) status [25]. However, this does not necessarily correlate to cytoplasmic maturity, whereby the oocyte gains the capacity for activation and the resumption of meiosis, leading to successful fertilisation and ongoing embryo development [26]. This needs to be managed effectively in order to prevent spontaneous maturation and asynchrony between nuclear and cytoplasmic maturation, which can be detrimental to embryo growth, and this is predominantly managed through the development of specialised IVM culture systems.

20.5 Oocyte Maturation and Culture

IVM culture differs depending on the base media, culture timing and hormone and serum concentrations. A range of culture media have been formulated for use in IVM. The two mostly widely used commercially available IVM base media are Sage (CooperSurgical, USA) and MediCult (Origio, Denmark), which have shown similar success rates [27]. Media specifically formulated for blastocyst culture have been used successfully [11, 28] and have comparable success rates compared to Sage IVM media [29].

Unlike priming protocols, hormonal additives are common components for culture media in human IVM. Luteinising hormone (LH) and hCG are important mediators of oocyte maturation as they act on a common granulosa cell receptor to induce the intracellular rise in cAMP activity within the oocyte [30]. The cAMP cascade then promotes the resumption of meiosis and germinal vesicle breakdown [26]. Recombinant LH or hCG are added in concentrations from 0.1 IU/mL [23] to 0.75 IU/mL [31]. FSH is used to promote cumulus-oocyte complex (COC) expansion and a subsequent rise in cAMP activity leading to increased oocyte maturation [32, 33]. The concentration of FSH added is relatively consistent across IVM protocols at 0.075 IU/mL [23, 34] or 0.1 IU/mL [11, 19].

20.5.1 Other Culture Media Additives

Other additives have been suggested to be beneficial to IVM culture media by aiding in either maturation, fertilisation or embryo development. These include insulin-like growth factor (IGF-I) [35, 36], epidermal growth factor (EGF) [35, 37, 38], meiosis-activating sterols (MAS) [39, 40] and activin [41]; however, these are rarely used in routine culture.

20.5.2 Serum or Follicular Fluid in Culture

Human follicular fluid (HFF), inactivated autologous patient serum (maternal serum) or foetal bovine serum (FBS) are the three protein additives used most commonly in human IVM. HFF supplementation has been used in concentrations

ranging from 30% [42] up to 70% [21]. Heat-inactivated FBS has also been used at either 10% [43] or 20% [14, 44]. Maternal serum is the most common protein supplement, used in concentrations of 10% [23, 45] or 20% [22, 46]. The use of human serum in IVM culture media may provide a number of nutrients and growth factors that are involved in the maturation process. There are, however, a number of negative aspects associated with using serum in embryo culture including contributing to ammonia formation, which can be damaging to embryo development through mitochondrial disruption [47], and the interruption of the aromatisation process involved in the conversion of androgens to oestrogens in granulosa cells [48].

Additionally, concentrations of amino acids, lipids, hormones, antibodies and other immunological mediators vary between women according to diet, genetics and infection status, such that serum can be highly variable in its potency and toxicity. Additionally, there is the risk of contributing unknown contaminants (microbial and otherwise) to the cultured embryos when using serum or HFF preparations. However, it is not clear whether the negative effects of serum relevant to embryo culture also relate to in oocyte maturation culture. In most circumstances, oocytes are only exposed to maternal serum for the first 24–48 h of in vitro culture, depending on the protocol employed, and are then moved into commercial embryo culture media prior to insemination. Regardless, more research is needed into the effects of protein additives to IVM culture.

Recent research has investigated the benefits of supplementing IVM culture media with cAMP modulators. These include cilostamide and forskolin, which are designed to prevent the loss of gap cell junctions following removal of the oocyte from the follicle and the subsequent decrease in cAMP activity [49]. cAMP modulators may also prove beneficial during a short pre-IVM period, immediately after removal from the follicle, known as simulated physiological oocyte maturation (SPOM) [50], although in humans, the benefits of this pretreatment are inhibited by heparin, which is most often used during the collection procedure [51]. Initial testing of another cAMP modulator 3-isobutyl-1-methylxanthine (IBMX) in human participants was shown to be a safe additive, in terms of the incidence of embryo chromosomal aneuploidy rates [52]. Further large-scale trials into its effect on human IVM success rates are required.

20.5.3 Culture Timing

Similarly to priming protocols and culture media contents, there are considerable variations in oocyte maturation culture time reported in the literature. Following the oocyte collection procedure and transport to the embryology laboratory, oocytes are placed into maturation culture for at least 24 h [19]; however, culture periods of up to 40 h have also been reported [53]. Additionally, if a hCG trigger is used, there will be variations in culture timings such as at 24 and 30 h [54] for the same oocyte cohort. At the initiation of maturation culture in non-hCG-primed cycles, the GV stage oocytes will have a tightly compacted cumulus oophorus (Figs. 20.2, 20.3 and 20.4), and after maturation culture, expansion of the coronal cells should be visible (Figs. 20.5, 20.6 and 20.7).

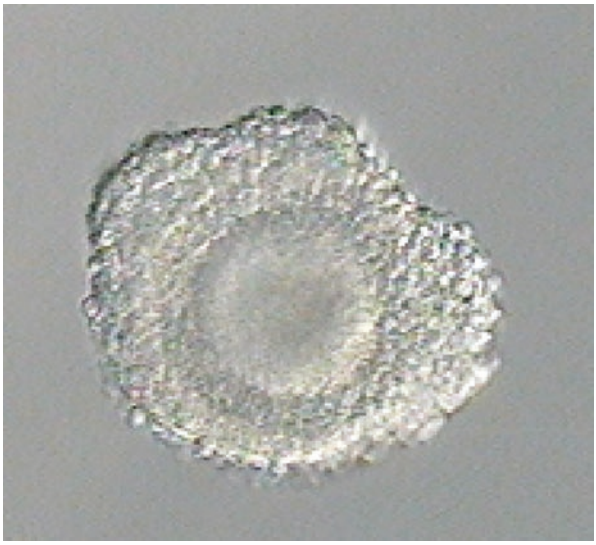


Fig. 20.2 Compact coronal cells surrounding GV stage oocyte following oocyte collection

Fig. 20.3 Compact coronal cells surrounding GV stage oocyte following oocyte collection

Fig. 20.4 Compact coronal cells surrounding GV stage oocyte following oocyte collection

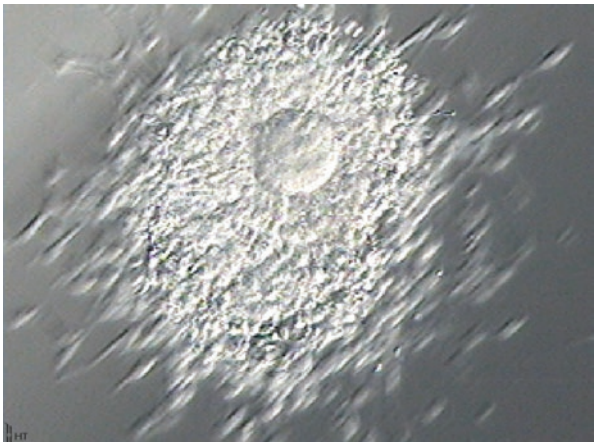


Fig. 20.5 Expanded coronal cells following 24 h in maturation culture

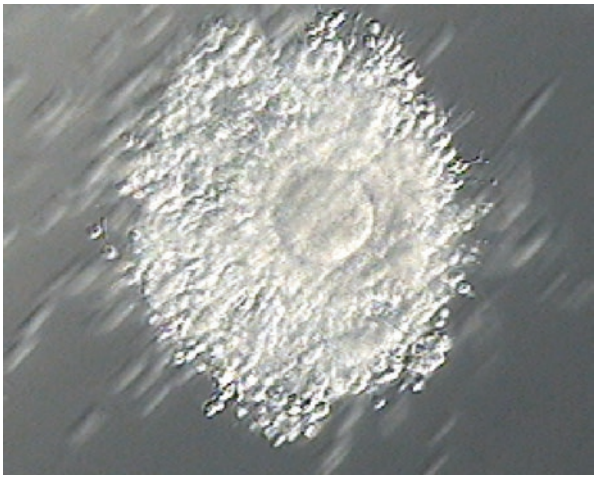


Fig. 20.6 Expanded coronal cells following 24 h in maturation culture

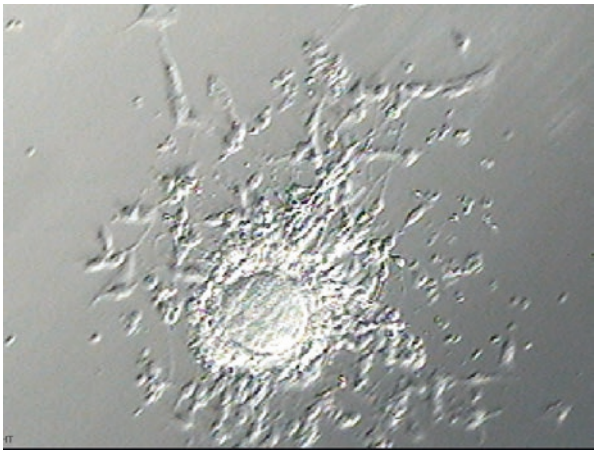


Fig. 20.7 Expanded coronal cells following 24 h in maturation culture

20.6 Fertilisation, Embryo Culture

IVM fertilisation is predominantly performed using intracytoplasmic sperm injection (ICSI) as fertilisation and implantation rates were originally reported as significantly lower than IVF-inseminated oocytes following IVM [23]. However, following a small, sibling oocyte study, no significant differences were seen between those fertilised by ICSI and IVF in outcomes of fertilisation, blastocyst development or implantation rates [28]. When using IVF as a fertilisation technique, it is important to consider that there is a potential conflict in the timing at which maturation/fertilisation check is performed between ICSI and IVF. IVF oocytes are allowed an additional 16–20 h prior to denudation and, therefore, additional time to undergo spontaneous and late maturation which may skew fertilisation results if they are calculated per oocyte matured.

Following fertilisation, embryo culture practices generally do not differ from standard IVF, and culture timing should reflect what is normally performed in any treating clinic. The majority of reports of clinical IVM culture perform 3 days of culture and transfer or freezing of cleavage stage embryos [22, 23, 44]; however, as with standard IVF treatment, blastocyst culture in IVM has become more prevalent [55]. Blastocyst culture may also be preferable in IVM as embryo development may be impaired during the early cleavage stages, with higher rates embryo arrest reported during the second and third cell cycles; however, embryo arrest during compaction and blastulation as well as kinetic time points were no different from standard ICSI treatment [56]. Blastocyst culture would therefore enable the deselection of embryos which may appear to be of suitable quality at the cleavage stage but fail to progress further.

20.7 Transfer/Cryopreservation

Endometrial priming is required if IVM-derived embryos are intended to be transferred in fresh cycles, and this is achieved using oestrogen and progesterone supplementation. This methodology shown to be beneficial when administration begins at the mid-follicular timing of the cycle [57] with at least 6 days of oestrogen necessary for endometrial receptivity [58]. High rates of implantation and live birth can be achieved by oestrogen supplementation 2 days prior and progesterone supplementation commencing on the day of oocyte collection, extending to the day of pregnancy test [11]. However, significantly higher rates of miscarriage and early pregnancy loss were seen following transfers in fresh IVM cycles compared to fresh IVF cycles, and this is not evident following frozen transfers [19]. Therefore, a freeze-all approach may provide the best outcomes for patients following IVM treatment.

20.8 Reproductive Outcomes

A recent systematic review and meta-analysis of treatment strategies for PCOS was inconclusive in regard to IVM as no RCTs were identified [59]. Success rates from non-RCT publications vary considerably in reported outcomes, and the results are difficult to compare due to the differences in treatment protocols. Reports of implantation rates for IVM range from 0% [9] to 34.5% [23] for cycles with no hormonal priming, 21.6% [9] to 47.7% [11] for cycles with recombinant FSH priming only, 8.9% [22] to 26.8% [60] for cycles with hCG priming only and 9.7% [46] for cycles with both FSH and hCG administered.

One of the primary reasons for the limited use of IVM around the world is that traditionally it is significantly less successful than standard IVF. There are only three reports of clinical IVM outcomes which include an IVF control group, all of which report lower live birth rates in PCOM/PCOS patients from fresh cycles [28, 34, 61]. In addition, miscarriage rates following fresh IVM cycles are significantly higher than in IVF and ICSI cycles [62], although this may be influenced by PCOS status and not the IVM procedure itself and/or insufficient endometrial conditions following fresh cycles, as this is not seen following frozen embryo transfer cycles [28]. The small size of these studies as well as the large variation in results further highlights the need for a large-scale, randomised clinical trial for IVM treatment.

20.9 Birth Outcomes

It is estimated that more than 3000 births have been achieved worldwide following IVM treatment, and although there is very limited research on the outcomes of these children, that which is available demonstrates a very positive outlook. Only six

Table 20.1 Neonatal outcomes of live births following IVM treatment

Reference	Singleton	Twins	Congenital birth defects (%)	Mean birthweight grammes (twins)	Preterm birth <37 weeks (twins)	IVF control group
[63]	24	4	2 (7.1)	3252 ± 516 (2361 ± 304)	1 2 (one set)	No
[64]	46	2	1 (2.1)	3720	1 2 (one set)	No
[65]	40	6	0 (0)	3550 ± 441 (2622 ± 194)	2 (4)	No
[66]	153	43	10, 2 from twin births (5.1)	3269 ± 616 (2311 ± 577)	26 (15)	Yes
[67]	34	4	Not reported	3119.5 ± 871	Not reported	No
[19]	33	0	1 (3.0)	3364 ± 590 (N/A)	2 (N/A)	Yes

publications to date have reported on neonatal outcomes from IVM births. The reported incidence rates of congenital birth defects include 0% [11, 65], 2.1% [64], 7.1% [63], 5.1% [66] and 3% [19] (Table 20.1). Additionally, Walls et al. and Fadini et al. included IVF controls and found no difference in congenital birth defects between the two treatments. However, the number of live births included within these data sets was small, with some including multiple births, and therefore, further large-scale studies are needed to determine the true impact of IVM treatment on congenital malformation.

Other measurements of neonatal health including Apgar scores have been reported in multiple studies and are within normal ranges [65] or show no significant difference to controls for singleton live births [66–68]. The incidence of adverse outcomes is often confounded by multiple births as many IVF centres worldwide still routinely transfer multiple embryos, though evidence suggests this is not best practice [69]. Regardless of multiple birth outcomes, the incidence of congenital birth defects, preterm birth and low birth weight, which are often associated with ART treatments (especially in PCOS patients), is low following IVM treatment.

Such as for birth outcomes, long-term outcomes from children born following IVM are unknown, and there are currently no long-term data on children born from IVM. However, the limited reports of the follow-up to children born from this technique do not demonstrate any adverse outcomes. The first study published on the development of children following IVM reported follow-up at 6, 12 and 24 months after birth and found that physical growth at all stages as well as neurological and neuropsychological outcomes were normal [65]. There were minor developmental delays in 8/43 children at 12 months (19%), but this decreased to 3% at 24 months. Their findings did not include IVF controls; however, they were within normal ranges for the general population.

Very few reports of early childhood outcomes are available employing standard IVF controls for IVM treatment. However, in one such study, it was reported that no differences in height or weight were found in 6 and 24 months of age in IVM infants

compared to standard IVF controls [68]. Additionally, there was no difference in mental developmental index and psychomotor scores between the two groups according to the Bayley Scales of Infant Development. Following these reports, in a cohort of French children, female infants born from IVM treatment displayed increased mean weight, height, body mass index (BMI) and head circumference at birth compared to those born following standard IVF treatment with ICSI. These outcomes remained significantly higher than the control group after 2 years of follow-up [67]. It is yet to be determined whether these findings are related to underlying infertility and PCOS rather than the IVM procedure itself. It appears that growth and development of IVM children falls within normal limits; however, further research is crucial to determine outcomes into early childhood, adolescence and adulthood.

20.10 The Risks of Aneuploidy and Epigenetic Variation

There is very little evidence of the effects of IVM on the risk of embryo chromosomal aneuploidy. Two case-control studies utilising fluorescence in situ hybridisation (FISH) reported no difference in the incidence of chromosomal abnormality between IVF- and IVM-derived embryos [70, 71]. Only one study has reported on the use of array comparative genomic hybridisation (aCGH) with IVM-derived embryos. This study involved the addition of the phosphodiesterase inhibitor IBMX into the culture media, and rates of aneuploidy were found to be similar to the researchers' previously published data from standard IVF treatments [72]. The limited data available in regard to aneuploidy and IVM highlights the need for more research in this area.

Similar to the risks of aneuploidy, concerns have been raised about the possible interference of IVM with epigenetic mechanisms and in particular with genomic imprinting. A systemic review of the risks of imprinting defects following oocyte culture in animal studies shows reassuring evidence of correct imprinted DNA methylation while highlighting the need for further research [73]. Additionally, research into the impacts of IVM on epigenetic variation in human oocytes is limited, and there is currently no information available on a genome-wide scale. Instead, researchers have focused on the analysis of selected imprinting genes and their error rates following IVM treatment. In one study of IVM, it was found that for the selected genes LIT1, SNRPN, PEG3 and GTL2, there were no significant increases in imprinting mutations [74]. In a more recent study, researchers compared six imprinting, five tumour suppressors, two pluripotency and two metabolic genes from cord blood and chorionic villus samples. Two repetitive elements were included to detect genome-wide DNA methylation changes in both, to detect allele methylation errors and found no difference in epigenetic changes between samples from 11 IVM and 19 IVF control neonates [75]. Therefore, while there is a clear need for further research, the limited data available so far is reassuring with respect to the continued use of IVM as a treatment for infertility.

Conclusions

With significant improvements to success rates in recent years, IVM may be considered a valuable treatment option for ART clinics. This is especially important for patients with PCOS who are at a significantly higher risk of developing OHSS. There have been significant milestones made in animal models from the investigation of oocyte-secreted factors growth-differentiation factor nine (GDF9) and bone morphogenic protein 15 (BMP15). These form part of the transforming growth factor β (TGFβ) superfamily and are necessary components for functional fertility [76, 77]. These factors produced by the oocyte act through paracrine signalling as regulators of granulosa/cumulus cell expansion and differentiation [78, 79]. The addition of recombinant forms of these factors to IVM culture media and/or other additives such as cAMP modulators may help improve success rates even further.

However, there still remains a clear need for large-scale randomised controlled studies to validate IVM success compared to standard IVF. Additionally, further research into the long-term outcomes of children born following IVM is necessary, even though initial assessments of children born from the technique show promising results. Finally, in order for IVM to become a more widely accepted treatment method worldwide, there needs to be a more standardised approach to protocols which will enable clinics to more easily implement this important treatment option.

References

1. Edwards RG. Maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. Nature. 1965;208:349–51.
2. Porter RN, Smith W, Craft IL, Abdulwahid NA, Jacobs HS. Induction of ovulation for in-vitro fertilisation using buserelin and gonadotropins. Lancet (London). 1984;2:1284–5.
3. Rizk B, Smitz J. Ovarian hyperstimulation syndrome after superovulation using GnRH agonists for IVF and related procedures. Hum Reprod. 1992;7:320–7.
4. Saul T, Sonson JM. Ovarian hyperstimulation syndrome. Am J Emerg Med. 2009;27:250.e3–4.
5. Cha KY, Koo JJ, Ko JJ, Choi DH, Han SY, Yoon TK. Pregnancy after in vitro fertilization of human follicular oocytes collected from nonstimulated cycles, their culture in vitro and their transfer in a donor oocyte program. Fertil Steril. 1991;55:109.
6. Trounson A, Wood C, Kausche A. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertil Steril. 1994;62:353.
7. Lindenberg S. New approach in patients with polycystic ovaries, lessons for everyone. Fertil Steril. 2013;99:1170–2.
8. Tan SL, Child TJ, Gulekli B. In vitro maturation and fertilization of oocytes from unstimulated ovaries: predicting the number of immature oocytes retrieved by early follicular phase ultrasonography. Am J Obstet Gynecol. 2002;186:684–9.
9. Mikkelsen A, Lindenberg S. Benefit of FSH priming of women with PCOS to the in vitro maturation procedure and the outcome: a randomized prospective study. Reproduction. 2001;122:587–92.
10. Siristatidis C, Sergentanis TN, Vogiatzi P, Kanavidis P, Chrelias C, Papantoniou N, et al. In vitro maturation in women with vs. without polycystic ovarian syndrome: a systematic review and meta-analysis. PLoS One. 2015;10:e0134696.

11. Junk SM, Yeap D. Improved implantation and ongoing pregnancy rates after single-embryo transfer with an optimized protocol for in vitro oocyte maturation in women with polycystic ovaries and polycystic ovary syndrome. Fertil Steril. 2012;98:888–92.
12. Son W-Y, Chung J-T, Chian R-C, Herrero B, Demirtas E, Elizur S, et al. A 38 h interval between hCG priming and oocyte retrieval increases in vivo and in vitro oocyte maturation rate in programmed IVM cycles. Hum Reprod. 2008;23:2010–6.
13. Son W-Y, Chung J-T, Herrero B, Dean N, Demirtas E, Holzer H, et al. Selection of the optimal day for oocyte retrieval based on the diameter of the dominant follicle in hCG-primed in vitro maturation cycles. Hum Reprod. 2008;23:2680–5.
14. Chian RC, Buckett WM, Tulandi T, Tan SL. Prospective randomized study of human chorionic gonadotrophin priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. Hum Reprod. 2000;15:165–70.
15. Gougeon A. Human ovarian follicular development: from activation of resting follicles to preovulatory maturation. Ann Endocrinol. 2010;71:132–43.
16. Dahan MH, Tan SL, Chung J, Son W-Y. Clinical definition paper on in vitro maturation of human oocytes. Hum Reprod. 2016;31:1383–6.
17. Coticchio G. IVM in need of clear definitions. Hum Reprod. 2016;31:1387–9.
18. De Vos M, Smitz J, Thompson JG, Gilchrist RB. The definition of IVM is clear – variations need defining. Hum Reprod. 2016;31:2411–5. Invited Commentary
19. Walls ML, Hunter T, Ryan JP, Keelan JA, Nathan E, Hart RJ. In vitro maturation as an alternative to standard in vitro fertilization for patients diagnosed with polycystic ovaries: a comparative analysis of fresh, frozen and cumulative cycle outcomes. Hum Reprod. 2015;30:s88–96.
20. Hreinsson J, Rosenlund B, Fridén B, Levkov L, Ek I, Suikkari AM, et al. Recombinant LH is equally effective as recombinant hCG in promoting oocyte maturation in a clinical in-vitro maturation programme: a randomized study. Hum Reprod. 2003;18:2131–6.
21. Yoon H-G, Yoon S-H, Son W-Y, Lee S-W, Park S-P, Im K-S, et al. Clinical assisted reproduction: pregnancies resulting from in vitro matured oocytes collected from women with regular menstrual cycle. J Assist Reprod Genet. 2001;18:325–9.
22. Child TJ, Abdul-Jalil AK, Gulekli B, Tan SL. In vitro maturation and fertilization of oocytes from unstimulated normal ovaries, polycystic ovaries, and women with polycystic ovary syndrome. Fertil Steril. 2001;76:936–42.
23. Söderström-Anttila V, Mäkinen S, Tuuri T, Suikkari A-M. Favourable pregnancy results with insemination of in vitro matured oocytes from unstimulated patients. Hum Reprod. 2005;20:1534–40.
24. Sasseville M, Gagnon MC, Guillemette C, Sullivan R, Gilchrist RB, Richard FJ. Regulation of gap junctions in porcine cumulus-oocyte complexes: contributions of granulosa cell contact, gonadotropins, and lipid rafts. Mol Endocrinol. 2009;23:700–10.
25. Escrich L, Grau N, Mercader A, Rubio C, Pellicer A, Escribá M-J. Spontaneous in vitro maturation and artificial activation of human germinal vesicle oocytes recovered from stimulated cycles. J Assist Reprod Genet. 2011;28:111–7.
26. Eppig JJ. Coordination of nuclear and cytoplasmic oocyte maturation in eutherian mammals. Reprod Fertil Dev. 1996;8:485–9.
27. Pongsuthirak P, Vutyavanich T. Comparison of medicult and sage media for in vitro maturation of immature oocytes obtained during cesarean deliveries. J Fertil In Vitro IVF-Worldw Reprod Med Genet Stem Cell Biol. 2015;3:136. doi:10.4172/2375-4508.1000136.
28. Walls M, Junk S, Ryan J, Hart R. IVF versus ICSI for the fertilization of in-vitro matured human oocytes. Reprod Biomed Online. 2012;25:603–7.
29. Pongsuthirak P, Songveeratham S, Vutyavanich T. Comparison of blastocyst and Sage media for in vitro maturation of human immature oocytes. Reprod Sci. 2015;22:343–6.
30. Conti M. Specificity of the cyclic adenosine 3′,5′-monophosphate signal in granulosa cell function. Biol Reprod. 2002;67:1653–61.
31. Le Du A, Kadoch IJ, Bourcigaux N, Doumerc S, Bourrier M-C, Chevalier N, et al. In vitro oocyte maturation for the treatment of infertility associated with polycystic ovarian syndrome: the French experience. Hum Reprod. 2005;20:420–4.

32. Downs SM, Daniel SAJ, Eppig JJ. Induction of maturation in cumulus cell enclosed mouse oocytes by follicle stimulating hormone and epidermal growth factor: evidence for a positive stimulus of somatic cell origin. J Exp Zool. 1988;245:86–96.
33. Guoliang X, Byskov AG, Andersen CY. Cumulus cells secrete a meiosi inducing substance by stimulation with forskolin and dibutyric cyclic adenosine monophosphate. Mol Reprod Dev. 1994;39:17–24.
34. Gremeau A-S, Andreadis N, Fatum M, Craig J, Turner K, McVeigh E, et al. In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case–control study of 194 treatment cycles. Fertil Steril. 2012;98:355–60.
35. Gomez E, Tarin J, Pellicer A. Oocyte maturation in humans: the role of gonadotropins and growth factors. Fertil Steril. 1993;60:40–6.
36. Pawshe C, Appa Rao K, Totey S. Effect of insulin like growth factor I and its interaction with gonadotropins on in vitro maturation and embryonic development, cell proliferation, and biosynthetic activity of cumulus oocyte complexes and granulosa cells in buffalo. Mol Reprod Dev. 1998;49:277–85.
37. Das K, Stout L, Hensleigh H, Tagatz G, Phipps W, Leung B. Direct positive effect of epidermal growth factor on the cytoplasmic maturation of mouse and human oocytes. Fertil Steril. 1991;55:1000.
38. Goud PT, Goud AP, Qian C, Laverge H, Van der Elst J, De Sutter P, et al. In-vitro maturation of human germinal vesicle stage oocytes: role of cumulus cells and epidermal growth factor in the culture medium. Hum Reprod. 1998;13:1638–44.
39. Smitz J, Picton HM, Platteau P, Rutherford A, Cortvrindt R, Clyde J, et al. Principal findings from a multicenter trial investigating the safety of follicular-fluid meiosis-activating sterol for in vitro maturation of human cumulus-enclosed oocytes. Fertil Steril. 2007;87:949–64.
40. Grøndahl C, Hansen TH, Marky-Nielsen K, Ottesen JL, Hyttel P. Human oocyte maturation in vitro is stimulated by meiosis-activating sterol. Hum Reprod. 2000;15(Suppl 5):3–10.
41. Alak BM, Coskun S, Friedman CI, Kennard EA, Kim MH, Seifer DB. Activin A stimulates meiotic maturation of human oocytes and modulates granulosa cell steroidogenesis in vitro. Fertil Steril. 1998;70:1126–30.
42. Son W-Y, Lee S-Y, Lim J-H. Fertilization, cleavage and blastocyst development according to the maturation timing of oocytes in in vitro maturation cycles. Hum Reprod. 2005;20:3204–7.
43. Suikkari A-M, Tulppala M, Tuuri T, Hovatta O, Barnes F. Luteal phase start of low-dose FSH priming of follicles results in an efficient recovery, maturation and fertilization of immature human oocytes. Hum Reprod. 2000;15:747–51.
44. Cha KY, Han SY, Chung HM, Choi DH, Lim JM, Lee WS, et al. Pregnancies and deliveries after in vitro maturation culture followed by in vitro fertilization and embryo transfer without stimulation in women with polycystic ovary syndrome. Fertil Steril. 2000;73:978–83.
45. Mikkelsen AL, Smith SD, Lindenberg S. In-vitro maturation of human oocytes from regularly menstruating women may be successful without follicle stimulating hormone priming. Hum Reprod. 1999;14:1847–51.
46. Lin YH, Hwang JL, Huang LW, Mu SC, Seow KM, Chung J, et al. Combination of FSH priming and hCG priming for in-vitro maturation of human oocytes. Hum Reprod. 2003;18:1632–6.
47. Gardner DK, Lane M. Amino acids and ammonium regulate mouse embryo development in culture. Biol Reprod. 1993;48:377–85.
48. Salha O, Nugent D, Dada T, Kaufmann S, Levett S, Jenner L, et al. The relationship between follicular fluid aspirate volume and oocyte maturity in in-vitro fertilization cycles. Hum Reprod. 1998;13:1901–6.
49. Y-m S, H-t Z, Ren Z, G-l Z, Liang X-y, Shen H-w, et al. Effects of cilostamide and forskolin on the meiotic resumption and embryonic development of immature human oocytes. Hum Reprod. 2008;23:504–13.
50. Albuz F, Sasseville M, Lane M, Armstrong D, Thompson J, Gilchrist R. Simulated physiological oocyte maturation (SPOM): a novel *in vitro* maturation system that substantially improves embryo yield and pregnancy outcomes. Hum Reprod. 2010;25:2999–3011.

51. Zeng H-T, Ren Z, Guzman L, Wang X, Sutton-McDowall ML, Ritter LJ, et al. Heparin and cAMP modulators interact during pre-in vitro maturation to affect mouse and human oocyte meiosis and developmental competence. Hum Reprod. 2013;28:1536–45.
52. Spits C, Guzman L, Mertzanidou A, Jacobs K, Ortega-Hrepich C, Gilchrist RB, et al. Chromosome constitution of human embryos generated after in vitro maturation including 3-isobutyl-1-methylxanthine in the oocyte collection medium. Hum Reprod. 2015;30:653–63.
53. Son W-Y, Chung J-T, Dahan M, Reinblatt S, Tan SL, Holzer H. Comparison of fertilization and embryonic development in sibling in vivo matured oocytes retrieved from different sizes follicles from in vitro maturation cycles. J Assist Reprod Genet. 2011;28:539–44.
54. Farsi MM, Kamali N, Pourghasem M. Embryological aspects of oocyte in vitro maturation. Int J Mol Cell Med. 2013;2:99–109.
55. Barnes FL. Blastocyst development and birth after in-vitro maturation of human primary oocytes, intracytoplasmic sperm injection and assisted hatching. Hum Reprod. 1995;10:3243–7.
56. Walls ML, Ryan JP, Keelan JA, Hart R. In vitro maturation is associated with increased early embryo arrest without impairing morphokinetic development of useable embryos progressing to blastocysts. Hum Reprod. 2015;30:1842–9.
57. Russell JB, Knezevich KM, Fabian KF, Dickson JA. Unstimulated immature oocyte retrieval: early versus midfollicular endometrial priming. Fertil Steril. 1997;67:616–20.
58. Navot D, Anderson TL, Droesch K, Scott RT, Kreiner D, Rosenwaks Z. Hormonal manipulation of endometrial maturation. J Clin Endocrinol Metab. 1989;68:801–7.
59. Kollmann M, Martins WP, Lima ML, Craciunas L, Nastri CO, Richardson A, et al. Strategies to improve the outcomes of assisted reproduction in women with polycystic ovarian syndrome: a systematic review and meta-analysis. Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol. 2016;48:709–18.
60. Son WY, Lee SY, Yoon SH, Lim JH. Pregnancies and deliveries after transfer of human blastocysts derived from in vitro matured oocytes in in vitro maturation cycles. Fertil Steril. 2007;87:1491–3.
61. Child TJ, Phillips SJ, Abdul-Jalil AK, Gulekli B, Tan SL. A comparison of in vitro maturation and in vitro fertilization for women with polycystic ovaries. Obstet Gynecol. 2002;100:665–70.
62. Buckett WM, Chian R-C, Dean NL, Sylvestre C, Holzer HEG, Tan SL. Pregnancy loss in pregnancies conceived after in vitro oocyte maturation, conventional in vitro fertilization, and intracytoplasmic sperm injection. Fertil Steril. 2008;90:546–50.
63. Cha KY, Chung HM, Lee DR, Kwon H, Chung MK, Park LS, et al. Obstetric outcome of patients with polycystic ovary syndrome treated by in vitro maturation and in vitro fertilization–embryo transfer. Fertil Steril. 2005;83:1461–5.
64. Mikkelsen AL. Strategies in human in-vitro maturation and their clinical outcome. Reprod Biomed Online. 2005;10:593–9.
65. Söderström-Anttila V, Salokorpi T, Pihlaja M, Serenius-Sirve S, Suikkari A-M. Obstetric and perinatal outcome and preliminary results of development of children born after in vitro maturation of oocytes. Hum Reprod. 2006;21:1508–13.
66. Fadini R, Mignini Renzini M, Guarnieri T, Dal Canto M, De Ponti E, Sutcliffe A, et al. Comparison of the obstetric and perinatal outcomes of children conceived from in vitro or in vivo matured oocytes in in vitro maturation treatments with births from conventional ICSI cycles. Hum Reprod. 2012;27:3601–8.
67. Foix-L'Helias L, Grynberg M, Ducot B, Frydman N, Kerbrat V, Bouyer J, et al. Growth development of French children born after in vitro maturation. PLoS One. 2014;9:e89713.
68. Shu-Chi M, Jiann-Loung H, Yu-Hung L, Tseng-Chen S, Ming-I L, Tsu-Fuh Y. Growth and development of children conceived by in-vitro maturation of human oocytes. Early Hum Dev. 2006;82:677–82.
69. Pandian Z, Marjoribanks J, Ozturk O, Serour G, Bhattacharya S. Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection. Cochrane Database Syst Rev. 2013;7:CD003416.

70. Zhang XY, Ata B, Son W-Y, Buckett WM, Tan S-L, Ao A. Chromosome abnormality rates in human embryos obtained from in-vitro maturation and IVF treatment cycles. Reprod Biomed Online. 2010;21:552–9.
71. Requena A, Bronet F, Guillén A, Agudo D, Bou C, García-Velasco JA. The impact of in-vitro maturation of oocytes on aneuploidy rate. Reprod Biomed Online. 2009;18:777–83.
72. Mertzanidou A, Wilton L, Cheng J, Spits C, Vanneste E, Moreau Y, et al. Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos. Hum Reprod. 2013;28:256–64.
73. Anckaert E, De Rycke M, Smitz J. Culture of oocytes and risk of imprinting defects. Hum Reprod Update. 2013;19:52–66.
74. Kuhtz J, Romero S, De Vos M, Smitz J, Haaf T, Anckaert E. Human in vitro oocyte maturation is not associated with increased imprinting error rates at LIT1, SNRPN, PEG3 and GTL2. Hum Reprod. 2014;29:1995–2005.
75. Pliushch G, Schneider E, Schneider T, El Hajj N, Rösner S, Strowitzki T, et al. In vitro maturation of oocytes is not associated with altered deoxyribonucleic acid methylation patterns in children from in vitro fertilization or intracytoplasmic sperm injection. Fertil Steril. 2015;103:720–7.e1.
76. Galloway SM, McNatty KP, Cambridge LM, Laitinen MP, Juengel JL, Jokiranta TS, et al. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nat Genet. 2000;25:279–83.
77. Dong J, Albertini DF, Nishimori K, Kumar TR, Lu N, Matzuk MM. Growth differentiation factor-9 is required during early ovarian folliculogenesis. Nature. 1996;383:531–5.
78. Gilchrist RB, Lane M, Thompson JG. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Hum Reprod Update. 2008;14:159–77.
79. Otsuka F, McTavish KJ, Shimasaki S. Integral role of GDF-9 and BMP-15 in ovarian function. Mol Reprod Dev. 2011;78:9–21.

Part V

Integrated Strategies, Complications of Pregnancy, and Outlook

Integrated Strategies for Enhancement of Fertility in PCOS

21

Edwina Coghlan and Roger J. Hart

21.1 Introduction

Polycystic ovary syndrome (PCOS) is a medical condition characterised by metabolic disturbance with reproductive consequences. The aetiology of PCOS is underpinned by insulin resistance (IR) which is with compensatory hyperinsulinaemia being a prominent feature of PCOS affecting approximately 65–80% of women with PCOS. It is well established that there are a number of intra- and extra-ovarian factors which negatively affect the reproductive performance of women with PCOS, which interfere with oocyte maturation and ovulation [1–4]. Hyperinsulinaemia leads to an increased androgen microenvironment within the ovary, directly related to both ovarian androgen biosynthesis and a decrease in hepatic sex hormone-binding globulin (SHBG) synthesis. The excess in local ovarian androgen production augmented by hyperinsulinaemia causes premature follicular atresia and anovulation [5]. Systemic hyperinsulinaemia is further exacerbated by the central obesity that affects 50% of women with PCOS, which further exacerbates the situation [6], as obesity leads to a further reduction in serum levels of SHBG, increased levels of total testosterone, free androgen index, fasting glucose, fasting insulin and a more adverse lipid profile when compared to normal weight women with PCOS [6].

E. Coghlan
King Edward Memorial Hospital, 374 Bagot Road, Subiaco, Perth, WA 6008, Australia

R.J. Hart (✉)
School of Women's and Infants' Health, University of Western Australia,
Perth, WA 6008, Australia

Fertility Specialists of Wetern Australia, Bethesda Hospital, 25 Queenslea Drive, Claremont,
Perth, WA 6010, Australia

School of Women's and Infants' Health, King Edward Memorial Hospital,
374 Bagot Road, Subiaco, Perth, WA 6008, Australia
e-mail: roger.hart@uwa.edu.au

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_21

Further to the reduction in ovulatory frequency, multiple serum, follicular factors and cytokines in patients with PCOS are believed to adversely affect oocyte quality [3] (see Fig. 21.1), and adverse changes within the endometrium in association with IR lead to a reduction in embryo implantation [7] and an increase in miscarriage rate [8] (see Chap. 5). Again all these effects are exacerbated by coexisting obesity [9].

Women who are overweight are less likely to ovulate or spontaneously conceive and once pregnant are more likely to miscarry [10]. Furthermore, maternal obesity has a number of adverse effects on both pregnancy and delivery including pre-eclampsia, gestational diabetes, premature delivery, stillbirth [11] and congenital malformations in the offspring [12]. Consequently, therapies that are directed towards weight loss are of particular importance for women with PCOS.

This chapter discusses the interventions that are recommended for the induction of ovulation in women with PCOS, and a proposed systematic approach to the management although very limited data exists to justify the order of drug therapies, as there exists some difficulty in accessing clomiphene citrate in some jurisdictions, letrozole is 'off label' in some countries and gonadotrophin medication may be prohibitively expensive in some countries but cost-free to the patient in other jurisdictions. Hence, treatment should be individualised and may differ from one country to the next, and when further studies are performed to develop a systematic

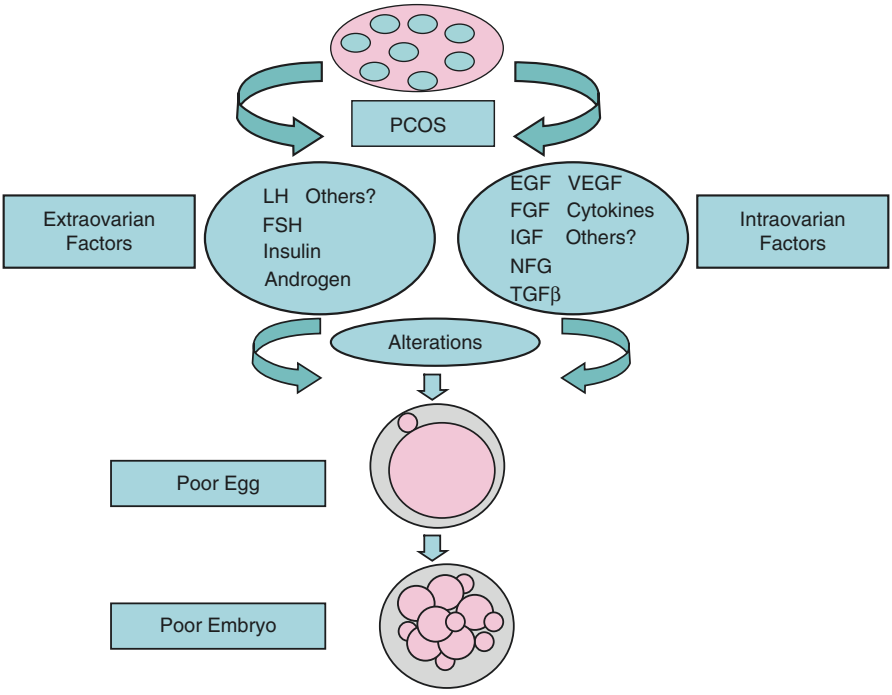


Fig. 21.1 Some of the intra- and extra-ovarian factors that are associated with the pathology of PCOS that may negatively influence oocyte and subsequent embryo quality with permission from Qiao and Feng [3]

approach to treatment, it is essential that the end point studied is a live birth, and ideally a singleton pregnancy [13]. However, it is essential whenever ovarian stimulation is performed that close monitoring of the response is implemented with the aim of inducing mono-follicular ovulation, with the ideal monitoring performed by a combination of serum monitoring and ultrasound examination to limit the number of multiple pregnancies (see Fig. 21.1).

21.2 Effective Lifestyle Interventions

Lifestyle therapy as a first-line treatment aims to promote weight loss and prevent weight gain where required [14] (see Chap. 13). Excess weight adversely affects *all* features of PCOS including reproductive [15, 16], psychological and metabolic features of the disease [17]. Women affected by PCOS are known to have a higher level of weight gain than non-affected women [18].

Effective lifestyle interventions to optimise body mass index (BMI) and improve overall insulin resistance remain the recommended first-line strategy to improve fertility and reproductive outcomes [9, 18]. A particular challenging situation often arises for the overweight woman with PCOS, where there is the further negative influence of the patient's age on the chances of conceiving. In this situation an individualised approach to her management is often required. Hence, a woman over 35 years of age may be encouraged to address lifestyle changes for 3 months, whereas a younger woman may be encouraged to try a more protracted period of lifestyle intervention, before resorting to ovulation induction therapy.

21.2.1 Weight Loss

Overall weight loss of as little as 5–15% can lead to improvements in biochemical hyperandrogenism, menstrual cyclicity, ovulation and fasting insulin and glucose levels [19]. The exact mechanism by which weight loss restores reproductive function is not fully understood; however, it is thought that improvements are a result of increased insulin sensitivity from weight loss [20].

21.2.2 Dietary Interventions

There has been increasing focus on the macronutrient composition of the dietary interventions in lifestyle modification for women with PCOS to aid with weight loss. Current evidence suggests that it is the overall caloric restriction rather than the macronutrient composition, carbohydrates, protein and the fat content, which is effective for weight loss leading to the subsequent clinical benefits [17, 18, 21]. More evidence is needed to ascertain if there is an overall diet composition that is more beneficial to patients with PCOS [18].

21.2.3 Exercise

Exercise has significant benefits to both the metabolic and reproductive outcomes of PCOS independent of diet-related weight loss [18]. The long-term metabolic benefits of exercise suggest that all women with PCOS should incorporate exercise into their regular lifestyle. The mechanism by which exercise improves reproductive outcomes is not fully understood, although it is thought to be related to an improvement in IR [14]. There is no current recommendation on the best exercise type, duration or regime for women with PCOS and subfertility to suggest that one approach is better than another [14, 22]. Women who are overweight with BMI in excess of 25 kg/m^2 should undertake at least 150 min of exercise per week, with 90 min of this at moderate to high intensity level to improve fertility outcomes [18].

In a small randomised controlled trial (RCT) that compared exercise directly with a low caloric diet [23], exercise was shown to be more effective than diet, with respect to the regulation of menstrual cycles and ovulation when compared to diet alone. The study demonstrated no improvements in anthropometry, metabolic health, fertility or overall quality of life, although the intervention of exercise led to significant improvement in IR for ovulatory women [23]. A recent observational study agreed with these finding that as metabolic disorder is often present in lean women with PCOS lifestyle, intervention with exercise is still beneficial at improving these adverse metabolic and reproductive features [24]. However, a recent multicentre randomised controlled trial of lifestyle intervention for obese infertile women (not with PCOS per se) failed to demonstrate a benefit of the lifestyle intervention, which consisted of calorie restriction and exercise, on the chance of a live birth after 6 months of lifestyle intervention followed by 18 months of fertility treatment [25].

21.2.4 Behaviour Modification

It is a challenge for all individuals attempting to embrace a lifestyle change. For women with PCOS, it has been suggested that optimising psychological factors, including health coaching principles with appropriate education addressing achievable goals, risk perception and patient-driven goal setting, may assist with motivation and engagement in the implementation of successful lifestyle changes [18].

21.2.5 Bariatric Surgery

The use of bariatric surgery can be considered for obesity treatment if lifestyle interventions thus far have been unsuccessful. Bariatric surgery is associated with a reduced rate of gestational diabetes and large-for-gestational-age infants. However, it is also associated with intrauterine growth restriction, preterm birth and an increased risk of stillbirth or neonatal death [26]. A significant concern for women

who undergo bariatric surgery is the potential for subsequent nutritional deficiencies; a recent study reported no significant effect of bariatric surgery on the overall risk of congenital malformations [27]. Current recommendation for women who undergo bariatric surgery is that they should delay attempts to conceive for at least 1 year after surgery [26], as it is only then that their reproductive chances have substantially increased, the obstetric risk is reduced and the long-term cardiometabolic consequences for the child have improved. The benefit of bariatric surgery for weight loss for subfertile women is demonstrated by a meta-analysis of almost 600 women as over two-thirds of infertile women spontaneously conceived after surgery [28]. Based on current evidence, bariatric surgery should be used with caution following appropriate patient selection and counselling, after intensive lifestyle interventions have been exhausted.

In summary, lifestyle modification (dietary intervention and increased physical activity) (Figs. 21.2 and 21.3) remains the optimal treatment strategy for overweight/obese women with PCOS [22]. Weight loss of 5–10% achieved with long-term caloric restrictions (5–6 months) improves IR, hyperandrogenism, menstrual function and fertility [22] and will result in significant benefits for long-term metabolic health. There are no large RCTs of lifestyle intervention studies with the end point of live birth rate [22, 29], although lifestyle interventions will lead to an increase in ovulation rates. Lifestyle management (including single or combined weight loss), exercise and behavioural techniques should be first-line techniques for women to prevent weight gain and promote weight loss where required. These interventions are cost-effective and can be commenced at the primary care level [17, 18] (see Figs. 21.2 and 21.3).

Weight loss	As little as 5-10% weight loss may help with spontaneous ovulation
Diet composition	Hypo-caloric diet is advised to achieve weight loss. More research is needed on specific diet composition for women with PCOS
Exercise	Exercise independent of weight loss has overall benefits to metabolic component of POCS specifically IR. The duration and best type of exercise to undertake is unknown
Bariatric surgery	Consider if lifestyle treatment has failed. Patients should avoid pregnancy for 12 months
Behaviour modification	Improves engagement and success in all lifestyle interventions

Fig. 21.2 Lifestyle modification summary

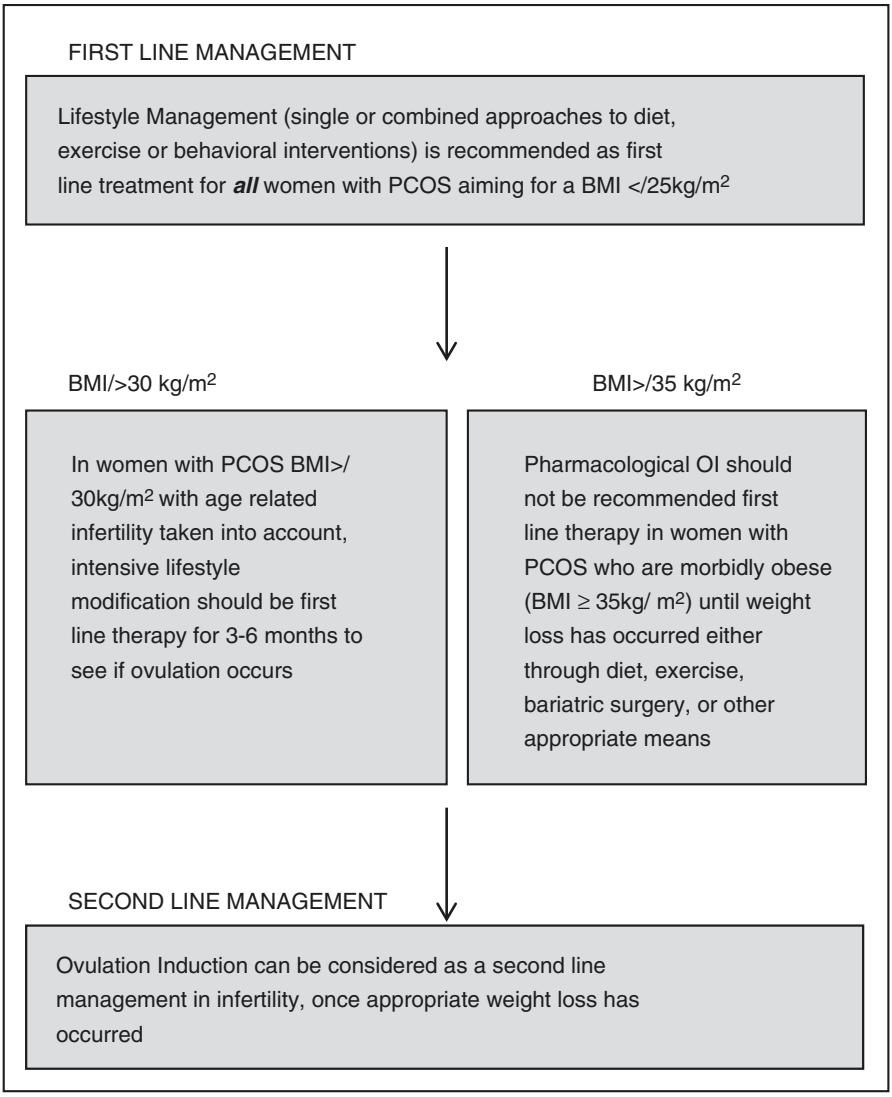


Fig. 21.3 Lifestyle algorithm management of BMI. Adapted from PCOS Australian Alliance and Jean Hailes Foundation for Women's Health [18]

21.3 Ovulation Induction Methods

21.3.1 Clomiphene Citrate (CC)

CC is considered the first line of pharmacological management to induce ovulation in women with PCOS [30, 31] (see Fig. 21.4). Its use since the 1960s has reassured clinicians on the reliable safety and efficacy profile of this medication [32]. CC has

an ovulation rate of 60–85% and a pregnancy rate of 30–50% after 6 ovulatory cycles [18, 32]. The discrepancy between these two rates has been attributed to the antioestrogenic effects of CC on the endometrium and cervical mucus [18, 32]. Rates of twin pregnancy and triplets with clomiphene citrate are 5–7% and 0.3%, respectively, but can be reduced with very close cycle monitoring with ultrasound examination. Importantly the incidence of ovarian hyperstimulation syndrome is less than 1% [33].

CC is commenced on day 2–5 of the menstrual cycle for 5 days starting with 50 mg/day and increasing to maximum of 150 mg/day [18]. CC resistance is reached if ovulation is not achieved on the maximum dose, and therapy should then be reviewed. If pregnancy cannot be achieved after six ovulatory cycles with clomiphene citrate, then the patient is described as having CC failure [34]. It is best practice to limit a patient's lifetime exposure to 12 treatment cycles, as there is conferred risk of borderline ovarian tumours with additional cycles [35]. Furthermore, as this chapter goes to press, there is a concern regarding the ongoing supply of the drug clomiphene worldwide.

21.3.2 Metformin

The association of insulin resistance contributing to anovulation in PCOS has led to the introduction of insulin-sensitising drugs in an attempt to restore ovulation and enhance pregnancy rates. Metformin can be introduced as either first-line monotherapy or to compliment other therapies to induce ovulation [18, 30] (see Chap. 11). Metformin has been the most widely studied hypoglycaemic drug in women with PCOS and has the most reassuring safety profile [30, 36]. Metformin can have some mild gastrointestinal-related side effects; thus, patients need to be counselled on these prior to commencing treatment [18].

There is no difference between the effectiveness of metformin and CC as first-line monotherapy for ovulation induction in nonobese women's BMI $\leq$30 kg/m^2 with PCOS [18, 37, 38]. However, if a patient is using first-line CC therapy and is deemed CC resistant, metformin should be added to improve fertility outcomes rather than persisting with further treatment with CC alone [18, 30]. However, for obese women (BMI $\geq$30 kg/m^2), the pregnancy and live birth rate appears to be overwhelmingly higher for CC vs. metformin [30, 38, 39] and should be considered first-line treatment in this instance.

21.3.3 Letrozole

Letrozole is the most commonly aromatase inhibitor used as an oral ovulation-inducing drug. This class of drugs were first proposed as new ovulation-inducing agents in 2001 to avoid some of the undesired antioestrogenic side effects of CC therapy [32] (see Chap. 10). The most commonly used aromatase inhibitor is letrozole [40]. The primary mode of action is to inhibit oestrogen production by

inhibiting the enzyme aromatase and stopping the conversion of androgens to oestrogens leading to a substantial reduction in oestrogen levels [41]. This method of action avoids oestrogenic-based negative feedback in the hypothalamus and increases follicle-stimulating hormone (FSH) secretion by the pituitary [32]. The accumulation of androgens in the ovary allows an increase in follicular sensitivity to FSH [42]. It is currently an off-label drug for ovulation induction and is generally prescribed for 5 days at the beginning of the follicular phase at doses of 2.5–7.5 mg/day [43].

Current meta-analysis from letrozole use shows no difference in ovulation rates of letrozole vs. clomiphene. However, live birth rate and pregnancy rate are thought to be significantly greater in those patients using letrozole compared with CC [32]. There are no differences in the multiple pregnancy and miscarriage rates among different therapy groups. It is postulated that the improved live birth and pregnancy rates observed among the patients that used letrozole are explained by differences in the pharmacodynamics between the two drugs [32].

The evidence about the risk of congenital abnormalities with the use of aromatase inhibitors remains unclear [18, 32], and whilst there is increasing evidence about the effectiveness of aromatase inhibitors, current recommendation is that it may be considered as a first-line therapy for ovulation induction. However, there are detractors who view the data on the efficacy of letrozole in a circumspect manner [44]. As letrozole appears to be more effective in obese women than CC, North American studies will have a favourable bias towards letrozole in terms of efficacy, and potential genetic differences across populations require the convincing North American [45] to be replicated in other populations, and furthermore it is not clear from the literature what 'letrozole resistance or failure' is; hence, more work is required [44].

21.3.4 Gonadotrophin Therapy

Gonadotrophin therapy can also be implemented as second-line pharmacological therapy to induce ovulation (see Chap. 12). Evidence would suggest that induction of ovulation with gonadotrophin administration is highly effective and leads to more rapid conception than using clomiphene, the drugs are more expensive, and the intervention requires more cycle monitoring. Evidence would suggest that using gonadotrophins [46] may lead to a higher rate of multiple pregnancies, cycle cancellation and ovarian hyperstimulation syndrome compared with clomiphene citrate as first-line treatment, and this can be minimised by expert cycle monitoring [18]. It is generally considered appropriate to proceed to the second-line approach of gonadotrophin therapy after 3–6 months of documented ovulation, without conception, or in a woman who has documented CC resistance despite increasing the starting dose and the addition of metformin. It is recognised that this process can be very frustrating for patients as the treatment regime often appears protracted. However, if it is explained to patients that a woman with a normal menstrual cycle would generally

expect to try for several months prior to seeking fertility assistance, it will assist her to rationalise the protracted regime that she has embarked upon.

As detailed in Chaps. 12 and 18, to minimise the risk of multiple follicle development, a 'low-dose step-up' protocol is well established in fertility practice [47, 48]. Gonadotrophin administration is commenced at 25, 37.5, 50 or occasionally 75 IU per day of FSH for 7–10 days, and then the dose is increased incrementally by 25–37.5 IU every week if there is no development of a follicle ≥12 mm in size. Ovulation is triggered when there is development of a solitary follicle ≥18 mm in size in the absence of any other follicles in excess of 14 mm in size. Alternatively, a 'step-down' protocol can be used with a starting dose of 150 IU of FSH until a dominant follicle develops, although this is less favoured. The dose of gonadotrophins is then decreased until the triggering of ovulation with human chorionic gonadotrophin (hCG) [49].

The addition of intrauterine insemination (IUI) is not appropriate for women who purely require ovulation induction therapy unless the partner is absent, hence requiring frozen sperm, or for a single woman, or if she were in a same-sex relationship, where donor sperm would be required. The use of IUI may also be required if there exists a minor impairment of the semen parameters or intercourse is not feasible, either due to difficulties with maintenance of an erection or with vaginismus. It may also be appropriate to consider IUI treatment in a young woman who has not conceived after six cycles of documented ovulation, when it could be considered that a degree of unexplained infertility is now evident. In this situation it would be appropriate to consider ovarian hyperstimulation to develop two dominant follicles as there must now be a further impediment to conception in addition to a lack of ovulation. For more details about the use of controlled ovarian stimulation for IUI, see also Chap. 18.

21.3.5 Ovarian Drilling

Ovarian drilling should be considered a second-line treatment indicated for infertile, anovulatory women with CC-resistant PCOS [50]; it may have a role as first-line treatment if laparoscopy is indicated in the patient for another reason [18] (see Chap. 15).

The mechanism by which ovarian drilling works is not clearly understood; however, it is thought that it may involve destruction of the ovarian stroma that produces the androgens [50]. The following results always occur: a decline in plasma luteinising hormone (LH) and in pulsations, a temporary fall in inhibin B, a (moderate) rise in gonadotrophins and sex hormone-binding globulin and a constant fall in androgens (especially testosterone) [50].

When ovarian drilling is compared to gonadotrophin use, there is no difference in live birth rate, pregnancy rate and ovulation rate per patient or miscarriage rate per pregnancy, with a reduced multiple pregnancy rate. Further benefits of ovarian drilling include a significantly less financial burden and lack of requirement for

cycle monitoring for the patients compared with gonadotrophin use [51]. The evidence comparing ovarian drilling to metformin for live birth rate per patient, ovulation rate per cycle, pregnancy rate per cycle, pregnancy rate per patient and miscarriage rate per pregnancy is conflicting [18]. It is important to consider that ovarian surgery, although performed using a laparoscopic approach, is associated with both increased intraoperative and postoperative risks, especially in women who are overweight or obese [18]. It may also be appropriate if a laparoscopy is being performed for another indication such as mild endometriosis for a woman with ovulatory disorder, with patent fallopian tubes who have a partner with normal semen parameters. However, unless the patient had religious, ethical or financial reasons to prevent her proceeding with IVF treatment, a woman over 35 years with ovulatory disorder in the presence of endometriosis would be best advised to proceed to IVF treatment (see Fig. 21.4).

21.4 IVM and Its Role in PCOS

A proportion of women who do not respond to first- and second-line therapies will ultimately proceed on to IVF, or as they may have a primary indication for IVF, such as fallopian tube compromise or due to male-related factors [52] (see Chap. 20). It is generally considered appropriate to consider proceeding to either IVM or IVF treatment after 6–12 cycles of ovulation induction therapy that were unsuccessful. In women with PCOS, the supraphysiological doses of gonadotrophins used for controlled ovarian hyperstimulation can provoke the development of a large cohort of follicles of uneven quality [52, 53]. This may result in the retrieval of immature oocytes leading to poor fertilisation and lower cleavage, pregnancy and live birth rates. Furthermore, such women also face significant risk of and the potentially life-threatening complication of ovarian hyperstimulation syndrome (OHSS) [52, 53].

IVM was first reported as an alternative treatment to overcome these challenges in 1991 and involves the retrieval of immature oocytes (at the germinal vesicle stage) followed by growth in culture up to the metaphase II stage, replacing the maturation process that would normally occur in the ovary [54, 55]. The number of oocytes retrieved in IVM is higher in women with PCOS than women without PCOS women due to the higher antral follicle count. This newer method of assisted reproductive technology (ART) is useful intervention for women with PCOS-related subfertility allowing oocytes to retain their maturational and developmental competence [55] and avoiding the complication of OHSS [56].

There are no RCTs to guide clinicians on whether it is more beneficial to perform IVM than IVF in women with PCOS [53, 55]. However, with exceptionally high pregnancy rates in experienced hands, the process of IVM offers an opportunity for young women with PCOS who are at a significant risk of OHSS the ability to have a successful oocyte retrieval avoiding the discomfort associated with IVF [57]. Furthermore, it enables them to return home without the need for close observation, which has particular benefits for patients in a remote environment, such as Western Australia [56]. Recent observational data using both fresh and frozen transfer cycles

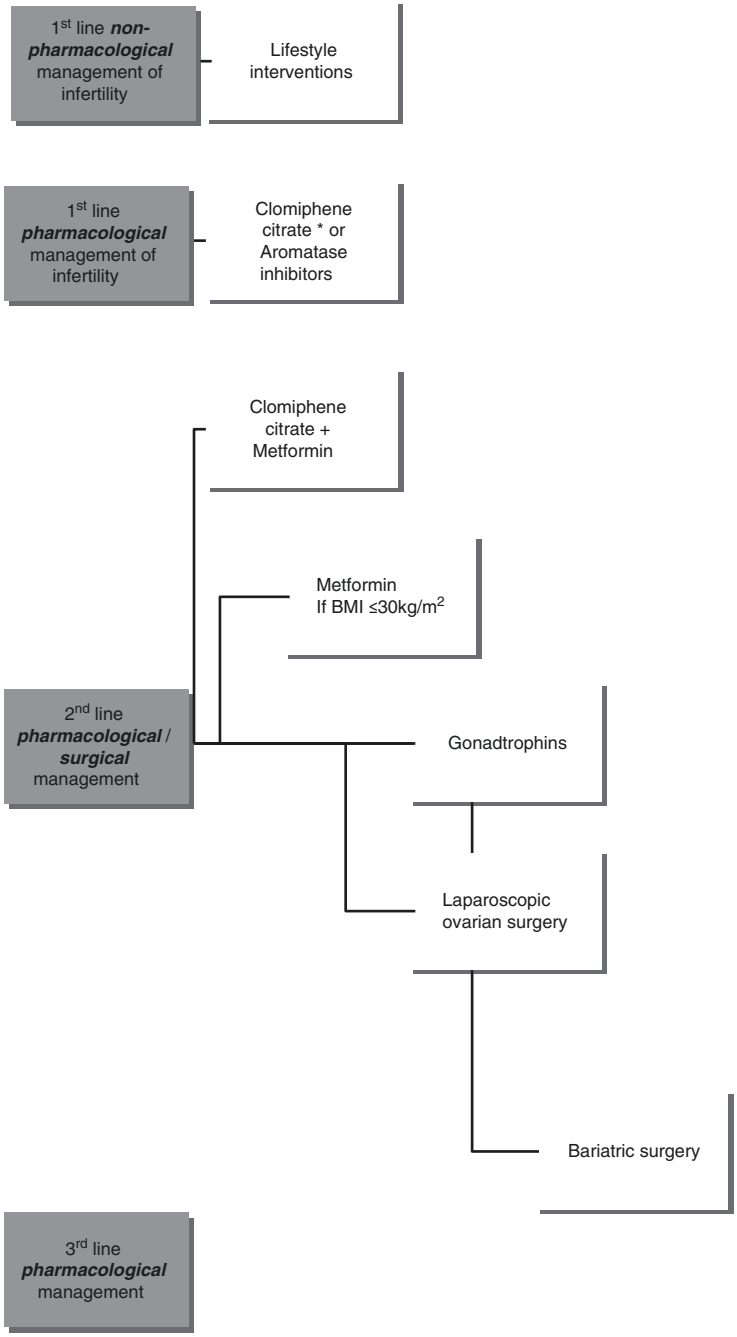


Fig. 21.4 Management of ovulation induction [18, 44]

demonstrates that the cumulative biochemical pregnancy, clinical pregnancy and live birth rates are significantly lower in IVM compared with conventional IVF [53, 55, 58]; however, for frozen embryo transfer cycles, there were no differences in the biochemical pregnancy, clinical pregnancy, live birth or miscarriage rates between the two treatment groups [56]. The rate of OHSS was 0% in the IVM group compared with 7–11% in the IVF group [56, 59]. IVM also has a significantly lower treatment burden to the patient with less consumption of gonadotrophin medication, decreased need for cycle monitoring and acceptable rates of blastocyst development [58] and is ideally suited to the young woman with PCOS who is at particular risk of OHSS.

21.5 Managing IVF Cycles in Patients with PCOS

If a woman with PCOS undertakes IVF, there are a number of strategies that can be incorporated into her treatment to minimise her risk of developing OHSS (see Chap. 19). The use of a GnRH antagonist protocols with the use of a GnRH agonist trigger has emerged as an alternative to the traditional GnRH agonist protocol and triggering of oocyte maturation with hCG [60] and leads to a significantly reduced risk of OHSS [61], without negatively affecting clinical pregnancy rate or miscarriage rates, when an embryo is replaced in a subsequent cycle [62]. Additionally, the concurrent use of metformin for women with PCOS during IVF cycles reduces their risk of OHSS by fourfold [63] (see Chaps. 11 and 19). Cabergoline is a dopamine receptor agonist that is now also increasingly used during IVF cycles in women with PCOS. It reduces the risk of OHSS by disrupting the follicular fluid hormone microenvironment, as it acts on the VEGF receptor within the vascular system, and should be prescribed at a dose of 0.5 mg per day for 5 days from the trigger injection prior to oocyte retrieval [64, 65]. Furthermore, the adoption of a ‘freeze-all’ approach for women with PCOS can be implemented which further decreases the overall risk of OHSS without negative impacts on miscarriage or clinical pregnancy rates as this enables the ready use of a GnRH trigger in an antagonist cycle and avoids the late-onset OHSS established by the release of hCG from the implanted embryo [66] (see Chap. 19).

Conclusion

PCOS is a common medical condition and, consequently, is often encountered in any fertility practice. Women with PCOS have an excellent prognosis for conception; however, the treating clinician is encouraged to adopt a systematic approach to their treatment. This often involves addressing lifestyle issues and treating concurrent comorbidities prior to embarking on ovulation induction. Care should be individualised and often will involve addressing weight loss; however, in the older patient delay in commencing treatment may not ultimately be in the patient’s best interest, and if the couple have a further factor limiting conception, pharmacological induction of ovulation may not be the best approach and either IUI, IVM or IVF/ICSI may well be more appropriate. Adopting a

careful approach to ovulation induction to minimise multiple pregnancies with ovulation induction is imperative, and for women who require IVF treatment ensuring that strategies to minimise OHSS are adopted is essential.

References

1. DeUgarte CM, Bartolucci AA, Azziz R. Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. Fertil Steril. 2005;83:1454–60.
2. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes. 1989;38:1165–74.
3. Qiao J, Feng HL. Extra- and intra-ovarian factors in polycystic ovary syndrome: impact on oocyte maturation and embryo developmental competence. Hum Reprod Update. 2011;17:17–33.
4. Stepto NK, Cassar S, Joham AE, Hutchison SK, Harrison CL, Goldstein RF, Teede HJ. Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic-hyperinsulaemic clamp. Hum Reprod. 2013;28:777–84.
5. Costello MF, Eden JA. A systematic review of the reproductive system effects of metformin in patients with polycystic ovary syndrome. Fertil Steril. 2003;79:1–13.
6. Lim SS, Norman RJ, Davies MJ, Moran LJ. The effect of obesity on polycystic ovary syndrome: a systematic review and meta-analysis. Obes Rev. 2013;14:95–109.
7. Dumesic DA, Abbott DH. Implications of polycystic ovary syndrome on oocyte development. Semin Reprod Med. 2008;26:53–61.
8. Setji TL, Brown AJ. Polycystic ovary syndrome: update on diagnosis and treatment. Am J Med. 2014;127:912–9.
9. Domecq JP, Prutsky G, Mullan RJ, Hazem A, Sundaresh V, Elamin MB, Phung OJ, Wang A, Hoeger K, Pasquali R, Erwin P, Bodde A, Montori VM, Murad MH. Lifestyle modification programs in polycystic ovary syndrome: systematic review and meta-analysis. J Clin Endocrinol Metab. 2013;98:4655–63.
10. Norman RJ, Clark AM. Obesity and reproductive disorders: a review. Reprod Fertil Dev. 1998;10:55–63.
11. Schummers L, Hutcheon JA, Bodnar LM, Lieberman E, Himes KP. Risk of adverse pregnancy outcomes by prepregnancy body mass index: a population-based study to inform prepregnancy weight loss counseling. Obstet Gynecol. 2015;125:133–43.
12. Stothard KJ, Tennant PW, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. JAMA. 2009;301:636–50.
13. Barnhart KT. Live birth is the correct outcome for clinical trials evaluating therapy for the infertile couple. Fertil Steril. 2014;101:1205–8.
14. Moran LJ, Pasquali R, Teede HJ, Hoeger KM, Norman RJ. Treatment of obesity in polycystic ovary syndrome: a position statement of the Androgen Excess and Polycystic Ovary Syndrome Society. Fertil Steril. 2009;92:1966–82.
15. Balen AH, Conway GS, Kaltsas G, Techatrasak K, Manning PJ, West C, Jacobs HS. Polycystic ovary syndrome: the spectrum of the disorder in 1741 patients. Hum Reprod. 1995;10:2107–11.
16. Kiddy DS, Sharp PS, White DM, Scanlon MF, Mason HD, Bray CS, Polson DW, Reed MJ, Franks S. Differences in clinical and endocrine features between obese and non-obese subjects with polycystic ovary syndrome: an analysis of 263 consecutive cases. Clin Endocrinol. 1990;32:213–20.
17. Moran L, Teede H. Metabolic features of the reproductive phenotypes of polycystic ovary syndrome. Hum Reprod Update. 2009;15:477–88.
18. PCOS Australian Alliance and Jean Hailes Foundation for Women's Health. Evidence-based guideline for the assessment and management of polycystic ovary syndrome. National Health and Medical Research Council. 2015. https://jeanhailes.org.au/contents/documents/Resources/

Tools/PCOS_evidence-based_guideline_for_assessment_and_management_pcos.pdf. Accessed 14 June 2016.
19. Norman RJ, Davies MJ, Lord J, Moran LJ. The role of lifestyle modification in polycystic ovary syndrome. Trends Endocrinol Metab. 2002;13:251–7.
20. Lim SS, Clifton PM, Noakes M, Norman RJ. Obesity management in women with polycystic ovary syndrome. Womens Health. 2007;3:73–86.
21. Moran LJ, Ko H, Misso M, Marsh K, Noakes M, Talbot M, Frearson M, Thondan M, Stepto N, Teede HJ. Dietary composition in the treatment of polycystic ovary syndrome: a systematic review to inform evidence-based guidelines. J Acad Nutr Diet. 2013;113:520–45.
22. Moran LJ, Hutchison SK, Norman RJ, Teede HJ. Lifestyle changes in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2011;16:CD007506.
23. Palomba S, Giallauria F, Falbo A, Russo T, Oppedisano R, Tolino A, Colao A, Vigorito C, Zullo F, Orio F. Structured exercise training programme versus hypocaloric hyperproteic diet in obese polycystic ovary syndrome patients with anovulatory infertility: a 24-week pilot study. Hum Reprod. 2008;23:642–50.
24. Kogure GS, Miranda-Furtado CL, Silva RC, Melo AS, Ferriani RA, DES MF, Reis RM. Resistance exercise impacts lean muscle mass in women with polycystic ovary syndrome. Med Sci Sports Exerc. 2016;48:589–98.
25. Mutsaerts MA, van Oers AM, Groen H, Burggraaff JM, Kuchenbecker WK, Perquin DA, Koks CA, van Golde R, Kaaijk EM, Schierbeek JM, Oosterhuis GJ, Broekmans FJ, Bemelmans WJ, Lambalk CB, Verberg MF, van der Veen F, Klijn NF, Mercelina PE, van Kasteren YM, Nap AW, Brinkhuis EA, Vogel NE, Mulder RJ, Gondrie ET, de Bruin JP, Sikkema JM, de Greef MH, ter Bogt NC, Land JA, Mol BW, Hoek A. Randomized trial of a lifestyle program in obese infertile women. NEJM. 2016;374:1942–53.
26. Guelinckx I, Devlieger R, Vansant G. Reproductive outcome after bariatric surgery: a critical review. Hum Reprod Update. 2009;15:189–201.
27. Johansson K, Cnattingius S, Naslund I, Roos N, Trolle Lagerros Y, Granath F, Stephansson O, Neovius M. Outcomes of pregnancy after bariatric surgery. NEJM. 2015;372:814–24.
28. Milone M, De Placido G, Musella M, Sosa Fernandez LM, Sosa Fernandez LV, Campana G, Di Minno MN, Milone F. Incidence of successful pregnancy after weight loss interventions in infertile women: a systematic review and meta-analysis of the literature. Obes Surg. 2016;26:443–51.
29. Legro RS, Dodson WC, Kris-Etherton PM, Kunselman AR, Stetter CM, Williams NI, Gnatuk CL, Estes SJ, Fleming J, Allison KC, Sarwer DB, Coutifaris C, Dokras A. Randomized controlled trial of preconception interventions in infertile women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2015;100:4048–58.
30. Johnson N. Metformin use in women with polycystic ovary syndrome. Ann Transl Med. 2014;2:23–32.
31. The Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Hum Reprod. 2008;23:462–77.
32. Roque M, Tostes AC, Valle M, Sampaio M, Geber S. Letrozole versus clomiphene citrate in polycystic ovary syndrome: systematic review and meta-analysis. Gynecol Endocrinol. 2015;31:917–21.
33. Kafy S, Tulandi T. New advances in ovulation induction. Curr Opin Obstet Gynecol. 2007;19:248–52.
34. Palomba S, Falbo A, Zullo F. Management strategies for ovulation induction in women with polycystic ovary syndrome and known clomifene citrate resistance. Curr Opin Obstet Gynecol. 2009;21:465–73.
35. Rossing MA, Daling JR, Weiss NS, Moore DE, Self SG. Ovarian tumors in a cohort of infertile women. NEJM. 1994;331:771–6.
36. Palomba S, Falbo A, Zullo F, Orio Jr F. Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. Endocr Rev. 2009;30:1–50.

37. Johnson N. Metformin is a reasonable first-line treatment option for non-obese women with infertility related to anovulatory polycystic ovary syndrome – a meta-analysis of randomised trials. Aust N Z J Obstets Gynaecol. 2011;51:125–9.
38. Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. Cochrane Database Syst Rev. 2012;5:5.
39. Zain MM, Jamaluddin R, Ibrahim A, Norman RJ. Comparison of clomiphene citrate, metformin, or the combination of both for first-line ovulation induction, achievement of pregnancy, and live birth in Asian women with polycystic ovary syndrome: a randomized controlled trial. Fertil Steril. 2009;91:514–21.
40. Elizur SE, Tulandi T. Drugs in infertility and fetal safety. Fertil Steril. 2008;89:1595–602.
41. Pavone ME, Bulun SE. Clinical review: the use of aromatase inhibitors for ovulation induction and superovulation. J Clin Endocrinol Metab. 2013;98:1838–44.
42. Requena A, Herrero J, Landeras J, Navarro E, Neyro JL, Salvador C, Tur R, Callejo J, Checa MA, Farre M, Espinos JJ, Fabregues F, Grana-Barcia M. Use of letrozole in assisted reproduction: a systematic review and meta-analysis. Hum Reprod Update. 2008;14:571–82.
43. Pritts EA. Letrozole for ovulation induction and controlled ovarian hyperstimulation. Curr Opin Obstet Gynecol. 2010;22:289–94.
44. Palomba S. Aromatase inhibitors for ovulation induction. J Clin Endocrinol Metab. 2015;100:1742–7.
45. Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P, Christman GM, Huang H, Yan Q, Alvero R, Haisenleder DJ, Barnhart KT, Bates GW, Usadi R, Lucidi S, Baker V, Trussell JC, Krawetz SA, Snyder P, Ohl D, Santoro N, Eisenberg E, Zhang H. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. NEJM. 2014;371:119–29.
46. Homburg R, Hendriks ML, Konig TE, Anderson RA, Balen AH, Brincat M, Child T, Davies M, D'Hooghe T, Martinez A, Rajkhowa M, Rueda-Saenz R, Hompes P, Lambalk CB. Clomifene citrate or low-dose FSH for the first-line treatment of infertile women with anovulation associated with polycystic ovary syndrome: a prospective randomized multinational study. Hum Reprod. 2012;27:468–73.
47. Birch Petersen K, Pedersen NG, Pedersen AT, Lauritsen MP, la Cour Freiesleben N. Monoovulation in women with polycystic ovary syndrome: a clinical review on ovulation induction. Reprod Biomed Online. 2016;32:563–83. doi:10.1016/j.rbmo.2016.03.006.
48. Messinis IE. Ovulation induction: a mini review. Hum Reprod. 2005;20:2688–97.
49. Macklon NS, Fauser BC. Gonadotrophins in ovulation induction. Reprod Biomed Online. 2005;10:25–31.
50. Fernandez H, Morin-Surruca M, Torre A, Faivre E, Deffieux X, Gervaise A. Ovarian drilling for surgical treatment of polycystic ovarian syndrome: a comprehensive review. Reprod Biomed Online. 2011;22:556–68.
51. Farquhar C, Brown J, Marjoribanks J. Laparoscopic drilling by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. Cochrane Database Syst Rev. 2012;6:4.
52. Siristatidis C, Sergentanis TN, Vogiatzi P, Kanavidis P, Chrelias C, Papantoniou N, Psaltopoulou T. In vitro maturation in women with vs. without polycystic ovarian syndrome: a systematic review and meta-analysis. PLoS One. 2015;10:0134696.
53. Siristatidis C, Vrachnis N, Creatsa M, Maheshwari A, Bhattacharya S. In vitro maturation in subfertile women with polycystic ovarian syndrome undergoing assisted reproduction. Cochrane Database Syst Rev. 2013;10:3.
54. Cha KY, Koo JJ, Ko JJ, Choi DH, Han SY, Yoon TK. Pregnancy after in vitro fertilization of human follicular oocytes collected from nonstimulated cycles, their culture in vitro and their transfer in a donor oocyte program. Fertil Steril. 1991;55:109–13.
55. Sauerbrun-Cutler M, Vega M, Keltz M, McGovern PG. In vitro maturation and its role in clinical assisted reproductive technology. Obstet Gynecol Survey. 2015;70:45–57.

56. Walls ML, Hunter T, Ryan JP, Keelan JA, Nathan E, Hart RJ. In vitro maturation as an alternative to standard in vitro fertilization for patients diagnosed with polycystic ovaries: a comparative analysis of fresh, frozen and cumulative cycle outcomes. Hum Reprod. 2015;30:88–96.
57. Junk SM, Yeap D. Improved implantation and ongoing pregnancy rates after single-embryo transfer with an optimized protocol for in vitro oocyte maturation in women with polycystic ovaries and polycystic ovary syndrome. Fertil Steril. 2012;98:888–92.
58. Walls ML, Ryan JP, Keelan JA, Hart R. In vitro maturation is associated with increased early embryo arrest without impairing morphokinetic development of useable embryos progressing to blastocysts. Hum Reprod. 2015;30:1842–9.
59. Child TJ, Phillips SJ, Abdul-Jalil AK, Gulekli B, Tan SL. A comparison of in vitro maturation and in vitro fertilization for women with polycystic ovaries. Obstet Gynecol. 2002;100: 665–70.
60. O'Neill KE, Senapati S, Dokras A. Use of gonadotropin-releasing hormone agonist trigger during in vitro fertilization is associated with similar endocrine profiles and oocyte measures in women with and without polycystic ovary syndrome. Fertil Steril. 2015;103:264–9.
61. Lin H, Li Y, Li L, Wang W, Yang D, Zhang Q. Is a GnRH antagonist protocol better in PCOS patients? A meta-analysis of RCTs. PLoS One. 2014;9:0091796.
62. Mancini F, Tur R, Martinez F, Coroleu B, Rodriguez I, Barri PN. Gonadotrophin-releasing hormone-antagonists vs long agonist in in-vitro fertilization patients with polycystic ovary syndrome: a meta-analysis. Gynecol Endocrinol. 2011;27:150–5.
63. Tso LO, Costello MF, Albuquerque LE, Andriolo RB, Macedo CR. Metformin treatment before and during IVF or ICSI in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2014;11:3.
64. Guvendag Guven ES, Dilbaz S, Duraker R, Mentese A, Cinar O, Ozdegirmenci O. The effect of cabergoline on folicular microenviroment profile in patients with high risk of OHSS. Gynecol Endocrinol. 2013;29:749–53.
65. Tang H, Hunter T, Hu Y, Zhai SD, Sheng X, Hart RJ. Cabergoline for preventing ovarian hyperstimulation syndrome. Cochrane Database Syst Rev. 2012;2:2.
66. Boothroyd C, Karia S, Andreadis N, Rombauts L, Johnson N, Chapman M. Consensus statement on prevention and detection of ovarian hyperstimulation syndrome. Aust N Z J Obstet Gynaecol. 2015;55:12406.

Complications of Pregnancy

22

Stefano Palomba and Bart C.J.M. Fauser

22.1 Introduction

The primary endpoint in reproductive medicine should be the healthy mother and offspring, and all other (clinical and/or biological) endpoints should be considered as surrogates [1, 2]. Nonetheless, most publications of infertility clinical trials do not show clear data about the harms of medical, surgical and biological procedures for enhancing fertility [3]. In fact, only 4.8% and 5.7% of randomised controlled trials (RCTs) on infertility treatments are reported on neonatal and maternal outcomes, respectively [4]. In part, this is due to difficulty in obtaining data since obstetric and neonatal care are delivered by other providers, and patients are lost to follow-up.

In recent literature a shift in attention can be observed towards the causes of this increased obstetric risk, in particular it seems to be influenced by three main determinants: multiple gestation [5, 6], patients and/or couples' characteristics and comorbidities [7–9] and infertility treatments and biological manipulations [6, 10]. However, it is very difficult to precisely estimate the amount of the risk of specific reproductive disorders on individual pregnancy outcomes due to a lack of high-quality data and to heterogeneity of the studied populations, often mixing assisted reproduction technologies (ART) and spontaneous conceptions. Finally, infertility itself is considered a risk factor for obstetric complications, creating an inherent bias in studies of fertility treatment [3].

S. Palomba (✉)
Unit of Gynecology and Obstetrics, IRCCS–Arcispedale Santa Maria Nuova of Reggio Emilia, Via Risorgimento 80, 42123 Reggio Emilia, Italy
e-mail: stefanopalomba@tin.it

B.C.J.M. Fauser
Department of Reproductive Medicine and Gynecology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_22

The polycystic ovary syndrome (PCOS), a heterogeneous condition characterised by metabolic alterations and infertility [11], has been also closely linked to maternal, neonatal and obstetric complications. At the moment, many systematic reviews with [12–14] and without data synthesis [7] demonstrated an increased risk of pregnancy complication in women with PCOS. The current chapter will summarise the current knowledge regarding pregnancy complications in women with PCOS and its potential pathophysiology.

22.2 Clinical Data

Three main systematic reviews with meta-analyses [12–14] are available in literature and comparing pregnancy outcomes in women with PCOS versus controls (Table 22.1). Moreover, the available meta-analytic results were not adjusted for body mass index (BMI) or other confounders. In addition, they were the results of the inclusion of retrospective studies and the included longitudinal prospective data regarded relatively small populations.

Available data on the risk of miscarriage in women with PCOS are conflicting [15]. The increased miscarriage rate observed in women with PCOS seems to be closely dependant on BMI [16]. The data synthesis of nine randomised controlled trials (RCTs) comparing women with and without PCOS undergoing IVF demonstrated, without statistical heterogeneity, no difference in miscarriage rates (odds ratio (OR) 0.9, 95% coefficient interval (CI) 0.5–1.8) [17]. Moreover, recent observational clinical data [18], in which patients with PCOS were matched to controls by age and BMI, demonstrated an increased risk of miscarriage of about 70% (adjusted OR (aOR) 1.70, 95%CI 1.56–1.84).

Gestational diabetes mellitus (GDM) is the most common pregnancy complication observed in women with PCOS. Recent prospective studies [19–22] showed an incidence of GDM in women with PCOS from 14.7% to 22%. Aggregated data

Table 22.1 Main pregnancy complications in women with polycystic ovary syndrome (PCOS)

Outcome	Boomsma et al. [12]	Kjerulff et al. [13]	Qin et al. [14]
Maternal			
PIH	3.67 (1.98–6.81)	4.07 (2.75–6.02)	3.07 (1.82–5.18)
PE	3.47 (1.95–6.17)	4.23 (2.77–6.46)	3.28 (2.06–5.22)
GDM	2.94 (1.70–5.08)	2.82 (1.94–4.11)	2.81 (1.99–3.98)
Preterm delivery	1.75 (1.16–2.62)	2.20 (1.59–3.04)	1.34 (0.56–3.23)
Neonatal			
SGA	1.16 (0.31–5.12)	2.62 (1.35–5.10)	–
LGA	–	1.56 (0.92–2.64)	–
Macrosomia	1.13 (0.73–1.75)	–	–

Data are odds ratios (95% confidence intervals)
GMD gestational diabetes mellitus, *LGA* large for gestational age, *PE* pre-eclampsia, *PIH* pregnancy-induced hypertension, *SGA* small for gestational age
From Palomba et al. [7]

demonstrated that the risk of GDM is about three times higher in women with PCOS [12–14]. After adjusting data for confounders (including age, BMI, etc.), the incidence of GDM resulted more than twofold higher in pregnant women with PCOS demonstrating that PCOS is an independent risk factor for GDM [16, 23]. In a recent nationwide population-based study [24], the risk to develop GDM (aOR 2.15, 95%CI 1.96–2.37) was confirmed more than twofold higher in women with PCOS when compared to women without a PCOS diagnosis after adjusting for economic status and comorbidities. The increased risk of GDM in PCOS population has been also more recently confirmed [18].

Meta-analysis data [12–14] reported in women with PCOS an overall increased risk of three to four times of pregnancy-induced hypertension (PIH) and of developing preeclampsia (PE) during pregnancy. A large cohort study on 3787 women with PCOS and 1,191,336 controls confirmed an increased incidence of PE (aOR 1.45, 95%CI 1.24–1.69) in PCOS also after adjusting data for BMI and the use of ARTs [23]. That increased risk, irrespectively from BMI, has also been confirmed in prospective case-control studies [20, 21] and large observational trials [18]. This seems true especially for women with severe PCOS phenotypes. In fact, the PE/PIH risk is reduced but did not disappear in hyperandrogenic women with PCOS (OR 2.41, 95%CI 1.26–4.58) [25].

Data on the risk of delivery by caesarean section in women with PCOS are controversial. A significantly higher caesarean delivery risk (OR 1.56, 95%CI 1.20–2.02) was observed only in one meta-analysis [12] but not on the others [13, 14]. Recent evidences demonstrate a higher incidence of caesarean sections (aOR 1.13, 95%CI 1.05–1.21) in mothers with PCOS [18], whereas no significant influence of PCOS on the risk of assisted vaginal delivery was observed [12, 13].

Data on foetal and perinatal outcomes in women with PCOS are also inconclusive. The risk of preterm delivery (PTD) resulted twofold increased in two meta-analyses [12, 13], whereas in the third and more recent meta-analysis no effect was demonstrated [14]. In largest available cohort study [23], infants born to mothers with PCOS were more frequently delivered prematurely (aOR 2.21, 95%CI 1.69–2.90) and had an increased risk of meconium aspiration (aOR 2.02, 95%CI 1.13–3.61). A very interesting retrospective study [26], in which data were controlled for maternal (including maternal diabetes and obesity) and perinatal characteristics, confirmed that women with PCOS are at higher risk of PTD (aOR 1.74, 95%CI 1.53–1.98), perinatal mortality (aOR 1.49, 95%CI 1.02–2.18) and postnatal hospital admissions (aOR 1.21, 95%CI 1.05–1.40). Of particular interest are the data regarding the offspring that resulted at increased risk not only of hospitalisation for various diseases (including metabolic, nervous system, asthma) but also for congenital anomalies [26]. The risk of overall congenital anomalies (aOR 1.20, 95%CI 1.03–1.40) was significantly increased with particular regard for cardiovascular (aOR 1.37, 95%CI 1.01–1.87) and urogenital (aOR 1.36, 95%CI 1.03–1.81) defects [26]. Also recent well-conducted study seems to confirm that women of PCOS have a higher risk of PTD (aOR 1.25, 95%CI 1.1–1.43), neonatal jaundice (aOR 1.20, 95%CI 1.03–1.39) and respiratory complications (aOR 1.20, 95%CI 1.06–1.37), although the incidence of adverse outcomes resulted significantly attenuated [18].

It is unclear whether the presumed increased risk in PTD was related to induced or spontaneous PTD. A retrospective cohort study of 11,726 women demonstrated that the clinical presentation of PTD changes according to BMI subgroup [27]. Spontaneous PTD resulted less frequently in class I obese (aOR 0.7, 95%CI 0.5–1.0), and the risk of PTD due to preterm premature rupture of the membranes was increased in class II women (aOR 1.7, 95%CI 1.1–2.7), while medically indicated PTD were increased both in class III obese (aOR 2.2, 95%CI 1.4–3.4) and moderately underweight (aOR 2.9, 95%CI 1.3–6.3) patients [27].

Available findings on the neonatal risk of being small for gestational age (SGA) are conflicting. The risk was twofold increased in one meta-analysis [13] but not statistically different in another [12]. Also more recent studies seem to generate different results [25, 28]. The incidence of large for gestational age (LGA) neonates and/or macrosomia is rarely reported in literature. In consideration of the high incidence of GDM, no effect of PCOS was unexpectedly observed on the risks of LGA [13] and macrosomia [12]. The moderate risk for LGA neonates observed after adjusting data for confounders [23] is probably influenced by BMI because its incidence in PCOS increase enormously in presence of obesity [28]. However, longitudinal data on BMI-matched populations of women with and without PCOS demonstrated differences in the foetal growth with higher incidences of SGA and LGA [20]. On the other hand, the risk of admission to the neonatal intensive care units (NICU) for neonates of mother with PCOS is twofold increased [12–14], a low Apgar score is more frequently observed (OR 1.41, 95%CI 1.09–1.83) [23], and the perinatal mortality is three times higher (OR 3.07, 95%CI 1.03–9.21) [12].

22.3 Pathophysiological Mechanisms and Hypotheses

The increased incidence of pregnancy complications in women with PCOS can be the result of several coinciding factors that independently or in concert can play a role in its pathophysiology (Fig. 22.1).

Iatrogenic multiple pregnancies are the most important cause of the increased obstetric and perinatal morbidity [6]. In comparison with singletons, twin pregnancies have a tenfold increased risk of SGA newborns, a sixfold increased risk of PTD, threefold increased risk of admission to a NICU and an incidence of perinatal mortality sixfold higher [29, 30]. In consideration of the ovarian response difficult to control in case of PCOS and of the good prognosis, infertile patients with PCOS could be considered a subgroup at high risk for multiple pregnancies [31]. Twin pregnancies in women with PCOS have a higher risk of PTD (risk ratio (RR) 1.96, 95%CI 1.05–1.36), very PTD (RR 1.82, 95%CI 1.30–2.53) and delivery with low birth weight (RR 1.39, 95%CI 1.10–1.76) [32]. That increased risk lost the statistical and clinical significance after adjusting for BMI and gestational age, suggesting again a crucial role of body weight in the determinism of pregnancy complications in patients with PCOS [32].

The three main criteria for the diagnosis of PCOS [33, 34], i.e. clinical and/or biochemical hyperandrogenism, oligo-amenorrhoea and polycystic ovary

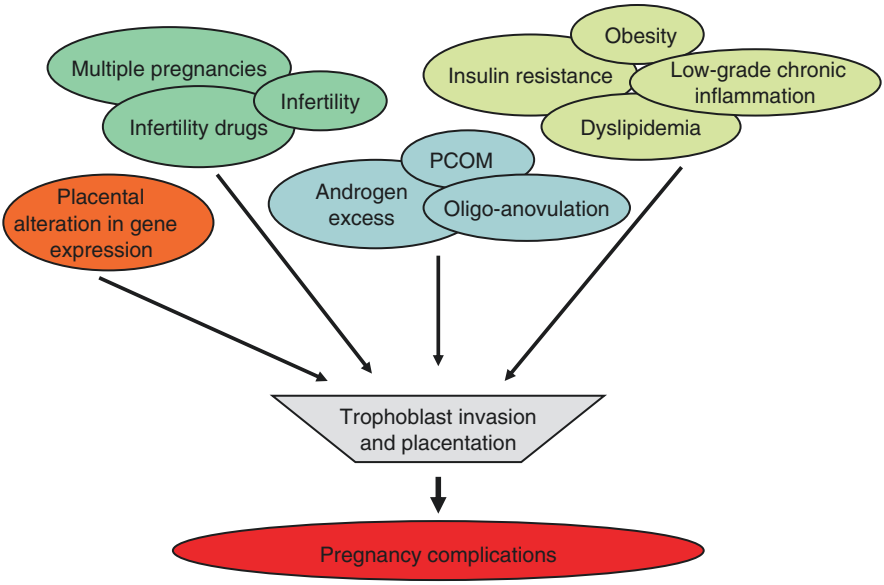


Fig. 22.1 Potential causes of the increased risk in pregnancy complications in women with PCOS. All factors shown in the figure can increase the risk of obstetric/neonatal complications directly and/or through an altered trophoblast invasion and placentation. *PCOM* polycystic ovarian morphology. From Palomba et al. [7]

morphology (PCOM), have been related, singularly or in concert as PCOS phenotypes, to pregnancy and neonatal complications. Notwithstanding the evidence that more severe PCOS phenotypes seem to present a higher incidence of pregnancy complications when compared with mild phenotypes due to their hormonal and metabolic correlates [35], a recent retrospective study [36] reported that the prevalence of adverse maternal and neonatal outcomes in women with PCOS does not vary according to phenotypes and definitions. Moreover, that data [36] were biased by retrospective nature and regarded matched cohorts (selection bias) that make the studied populations very homogeneous and not representative [37].

Hyperandrogenic states during pregnancy (i.e. pregnancy luteoma and hyperreactio luteinalis) have been frequently associated with adverse pregnancy outcomes [38, 39], and pregnant women with PCOS exert androgen levels higher compared to controls [40, 41]; they increase significantly throughout pregnancy probably due to abnormal steroidogenic function of the placenta [41–43]. In pregnant women with PCOS, the incidence and extension of microscopic alterations in early trophoblast invasion and placentation are strongly influenced by hyperandrogenism [44–46], and the hyperandrogenism is the PCOS feature related with the highest risk of adverse pregnancy outcomes [35]. The alterations in endovascular trophoblast invasion and placentation may be the result of a suboptimal implantation process due to the direct effect of androgens on the endometrium and/or to a specific tissue susceptibility [47, 48]. Recent data suggest that androgens can increase the incidence of

adverse pregnancy outcomes by acting on cervical remodelling and myometrial function [39].

In women with PCOS, the oligo-amenorrhoea is related with a risk of adverse pregnancy outcomes fivefold higher, whereas no effect on the PCOM has been observed [35]. Although a proportion of women with PCOS may achieve pregnancy without intervention [49], the great majority shows anovulation that is a main cause of infertility, i.e. a time-to-pregnancy (TTP) longer than 12 months. The effect of TTP longer than 2 months on pregnancy and neonatal complications has been assessed in two systematic reviews with meta-analyses [10, 50]. According to the included studies, natural singleton conceptions had an odd of PTD ranging from 39% to 31% higher after a TTP >12 months than after a TTP less than 12 months [10]. Women conceiving after long TTP also had increased odds of giving birth to children with low birth weight (OR 1.34, 95%CI 1.21–1.48), whereas no effect was detected on the risk of SGA foetus [51]. A large retrospective trial [52] of 40,773 pregnancies demonstrated also an increased incidence of developing GDM ranging from 50% to 39% according to the adjusting (age, pre-pregnancy BMI or lifestyle characteristics) [52]. Of note, ovulations disorders were associated with a 50% increased incidence of GDM (RRa 1.52, 95%CI 1.23–1.87) [52]. An infertility diagnosis, defined as a TTP higher than 12 months despite regular unprotected sexual intercourse with or without amenorrhoea, PCOM and ovaries or tubal damage, was related with increased risk of adverse outcomes in singleton pregnancies, irrespectively from the infertility treatment, with particular regard for PE (adjusted relative risk (aRR) 1.18, 95%CI 1.02–1.37), antepartum haemorrhage (aRR 1.32, 95%CI 1.18–1.47) and very early PTD (aRR 1.96, 95%CI 1.53–2.49) [53].

In infertile women with PCOS, an effect of the treatments for enhancing fertility on the risk of pregnancy complications cannot be excluded, although specific data for infertile patients with PCOS are limited. It is well known that a non-pharmacological intervention consisting of a combination of hypocaloric diet, increased physical activity and individualised behavioural modification plan can be effective in obese/overweight women with PCOS to lose weight and to improve natural and/or artificial reproductive outcomes [54]. A recent large RCT [55] assessing the effect of 6-month lifestyle intervention programme before infertility treatment on 574 infertile obese patients demonstrated more an odd of natural conception higher in the lifestyle intervention programme arm (RR 1.61, 95%CI 1.16–2.24) [55]. Moreover, as below detailed, data are still confusing and contradictory. Ovarian stimulation, with or without intrauterine insemination (IUI), represents an attractive therapeutic option for couples with anovulatory infertility or unexplained infertility, and several medications are used to treat anovulation or to enhance natural conceptions in subfertile women with PCOS. Unfortunately, the use of fertility drugs for inducing ovulation is associated with adverse neonatal outcome [10]. In fact, considering women who delivered singletons after TTP less than 12 months, the risk of PTD was significantly higher (OR 1.45, 95%CI 1.21–1.74) after the use of ovulation induction when compared to women who conceived without ovulation induction [10]. PRAMS survey's data confirmed that ovarian stimulation is associated

with a twofold increased odd of PTD at <34 weeks (OR 2.05, 95%CI 1.28–3.27) in singleton pregnancies compared with women who never used fertility treatments [56]. These data have been confirmed also for PTD at <37, <35 and <32 weeks of gestation [57]. The drug more frequently and significantly related with an increased risk of adverse pregnancy outcome is the clomiphene citrate (CC), that is, the traditional first choice ovulation induction treatment in women with PCOS. A 60% increased risk (OR 1.6, 95%CI 1.2–2.4) for SGA has been detected in women who conceived after CC administration followed by IUI compared with women who conceived after IUI in natural cycles [58]. However, recent RCTs [59, 60] have demonstrated reassuring data on its administration on the pregnancy and neonatal risk. At the moment, longitudinal data from three large RCTs showed a very low rate of maternal and neonatal complications not only in patients treated with CC but also in those who received metformin [59], metformin plus CC [59], letrozole [60, 61] or gonadotrophins [61]. At the moment, only one study extension of a RCT [62] demonstrated no difference in pregnancy complications after 9–12 years of follow-up in CC-resistant PCOS patients treated with laparoscopic ovarian drilling (LOD) or gonadotrophins. Finally, the use of ART treatments, including medical treatments and biological manipulations, can increase significantly the risk of pregnancy complications with particular regard for PTD risk [10, 63]. Recent data [64] on singleton in vitro fertilisation (IVF)/intracytoplasmic sperm injection (ICSI) pregnancies of women with PCOS have demonstrated a higher risk of developing GDM (aOR 3.15, 95%CI 1.35–7.33), PIH/PE (aOR 4.25, 95%CI 1.94–9.32), PTD (aOR 2.30, 95%CI 1.07–4.97) and LGA newborns (aOR 2.77, 95%CI 1.21–6.35) after adjusting data for age, parity, BMI and TTP. Very interesting data suggest that in infertile patients with PCOS the increased risk of PTD is closely dependent to the development of PIH/PE, whereas those of LGA are independent from GDM [64].

An elegant cohort study [65] on a total of 1,953,932 term singleton pregnancies recently demonstrated that the effect of the infertility treatments on the pregnancy and neonatal risks disappeared after propensity score matching analysis, which included multiple maternal baseline covariates, such as maternal age, ethnicity, socioeconomic status, parity, year of birth and pre-existent diseases, demonstrating that the risk could be mainly due to maternal characteristics. To this regard, it is well known that women with PCOS have specific anthropometric and metabolic characteristics that can influence enormously the obstetric and perinatal risk.

Firstly, the comorbidity most closely related to PCOS is the obesity, which is more prevalent in the more severe PCOS phenotypes and aggravates the reproductive phenotype [66]. The effect of obesity on human reproduction is well known [67]. Increased BMI is strongly correlated with pregnancy complications [68–70] including a higher risk of miscarriage (OR 1.31, 95%CI 1.18–1.46) [71], foetal death, stillbirth and infant death [72]. Also recently, a very large cohort study [73] confirmed a risk of neonatal and postnatal mortality higher in obese patients from 30% to 70% according to pre-pregnancy BMI. An increased risk of malformation in the offspring such as neural tube defects [74], congenital heart defects [75] and omphalocele [76] has been also detected. The high increased incidence of symptoms of sleep-disordered breathing [34, 77] observed in obese patients with PCOS

is another independent risk factors for pregnancy complications [78]. Obesity is an independent risk factor for the development of type 2 diabetes or GDM [79], and this risk is positively associated with BMI before conception [80, 81]. The risk of pregnancy and neonatal complications is higher in women with PCOS and GDM than in patients with GDM alone [82, 83]. The risk of PIH (aOR 4.43, 95%CI 1.17–16.72), PTD (aOR 1.92, 95%CI 1.12–3.42) and neonatal hyperbilirubinemia (aOR 3.18, 95%CI 1.14–8.82) is significantly higher in patients with both PCOS and GDM compared to women who only developed GDM [82]. Pregnant women with PCOS gain more weight than in BMI-matched controls [20, 21], and the risk of weight gain in pregnancy is another risk factor, independently from obesity [84].

Secondly, the insulin resistance with compensatory hyperinsulinaemia, one of the cornerstones in the pathogenesis of PCOS [85], can play a crucial role for the increased risk for adverse pregnancy outcomes in PCOS. A state of hyperinsulinaemic insulin resistance is crucial in order to ensure constant metabolic supplies to the growing foetus in physiologic pregnancies [86]. However, insulin resistance is related with a risk of spontaneous abortion more than eightfold higher [87], and higher serum insulin levels are observed in women with PIH/PE than in those with an uncomplicated pregnancy [88–91]. Insulin resistance with compensatory hyperinsulinaemia can influence the risk of pregnancy complication in pregnant women with PCOS through several direct and indirect mechanisms of action, although it is plausible an action on the extent of endovascular trophoblast invasion [44].

Although visceral fat accumulation and hyperlipidaemia are considered metabolic adaptations to support foetal growth [92], more and more data suggest that lipid abnormalities are associated with increased risk of adverse obstetric or neonatal outcomes, especially with PIH and PE [93]. Women with PCOS have higher serum low-density lipoprotein (LDL) and triglyceride (TG) concentrations before and during pregnancy compared to healthy controls, and serum LDL and TG levels are directly and independently related to pregnancy complications [21] probably inducing an endothelial dysfunction due to oxidative stress from free radicals, lipid peroxides and vascular damage [94–96].

In pregnancy, many inflammatory changes, including along with activation of peripheral blood leucocytes, increased white blood cells (WBC), ferritin and C-reactive protein (CRP) levels, are observed probably to modulate the maternal immunocompetence [97, 98]. Moreover, an abnormal low-grade inflammatory state during pregnancy has been associated with vascular damage and development of adverse pregnancy and neonatal outcomes with particular regard for PIH/PE and GDM [99–101]. To this regard, recent clinical data demonstrated that, during pregnancy, several markers of low-grade chronic inflammation are higher in women with PCOS than in healthy controls, suggesting that PCOS can enhance the inflammatory changes typical of the pregnancy, and closely related with adverse obstetric and neonatal outcomes [20].

The placenta may be the final and common target of all aberrations observed during pregnancy in women with PCOS (Fig. 22.1). Hormone-independent alterations, crucial for the regulation of placenta nutrient transport for foetal growth, have been detected in women with PCOS [102]. However, trophoblast and placental

tissue of women with PCOS are hyperandrogenic and/or insulin resistant microenvironment targets of epigenetic factors, including infertility treatments [46]. The macroscopic and microscopic analysis of the placenta of women with PCOS, also in uncomplicated pregnancies, shows clear alterations [45, 46, 103]. Those histological changes, including chronic villitis/intervillositis and increased thickness of stem villi arterial walls, seem compatible with local microvascular and inflammatory damage, and their severity vary according to the PCOS phenotype [45, 46]. In women with PCOS with an uncomplicated pregnancy, the potential compensatory morphometric adaptations of the placenta seem already maximised to improve the maternofoetal oxygen and nutrient transfer [45]. Moreover, it is possible to hypothesise that in pregnant women with PCOS further compensatory adaptations of the placental tissue to external noxae cannot be act with the subsequent development of pregnancy complications [44, 45].

From a pathogenetic point of view, an abnormal inflammatory and metabolic pattern can induce an abnormal endovascular trophoblast invasion with altered vascular structure and a subsequent hypoxic state with abnormalities of physiological changes and remodelling of spiral vessels. These abnormalities of the uteroplacental circulation have been confirmed by Doppler velocimetry in pregnant women with PCOS [44, 104]. The crucial role of the endometrium has been also recently highlighted in a retrospective study demonstrating that the incidence of aneuploidy in miscarriage in women with PCOS is more than twofold lower than in non-PCOS controls suggesting that as maternal factors are pivotal in the influence of the endometrial receptivity and competence [105] (see Chap. 5). A further hypothesis to explain the increased risk of pregnancy complications in women with PCOS regards the concepts of "ontogenetic progesterone resistance" and of "menstrual preconditioning" (see Chap. 5). According to this hypothesis [106], the uterus and the endometrium can become competent for deep trophoblast invasion and placentation only after menstruations. This hypothesis opens new preventive strategies for reducing pregnancy complications consisting of the induction of regular menstrual cycles before pregnancy. On the other hand, few and controversial clinical data are at the moment available at regard [107].

22.4 Prevention and Management of Pregnancy Complications in Women with PCOS

Although the risks of obesity in pregnancy are widely known, there is no evidence-based strategy to guide preconception weight loss. The efficacy of preconception weight loss as intervention for preventing pregnancy complications is limited, although a lower risk of obstetric and neonatal adverse outcomes have been observed in normal weight women compared with overweight/obese subjects [108]. General recommendations include dietary modification and exercise, without specification of the exact timing of the intervention or rate of weight loss [109]. Nevertheless, losing weight before conception can be effective in reducing maternal and foetal/perinatal complications, as well as the risk of congenital anomalies [110]. In

addition, diet and/or physical activity during pregnancy can reduce the gestational weight gain and the associated risks [108, 111]. In particular, a significant effect on the risks of PE (RR 0.74, 95%CI 0.60–0.92) and shoulder dystocia (RR 0.39, 95%CI 0.22–0.70) has been detected [111]. Diet alone significantly reduced the risk of PE (relative risk (RR) 0.67, 95%CI 0.53–0.85), PIH (RR 0.30, 0.10–0.88), GDM (RR 0.39, 95%CI 0.23–0.69) and PTD (RR 0.68, 0.48–0.96) compared with any other intervention [111]. On the other hand, a recent large RCT [55] demonstrated worse results (rate ratio (RaRa) 0.77, 95%CI 0.60–0.99) in obese patients who received 6-month lifestyle intervention programme before infertility treatment in terms of the vaginal birth of a healthy singleton at 37 weeks or more [55]. No effect of the lifestyle intervention programme before infertility treatment was detected in any maternal and neonatal outcome [55]. According to international guidelines [112], gestational weight gain in obese women from 5 to 9 kg is recommended. Two meta-analyses indicate a small increased risk of PTB when weight gain is above or below the recommended range [113, 114], although gestational weight gain below the recommended range reduces lightly the risk of PIH/PE [113].

Recent guidelines [115] suggest to pay great attention in women with PCOS with multiple risk factors for adverse pregnancy outcome, including impaired glucose tolerance (IGT) and metabolic syndrome. Patients should be screened and treated for hypertension and diabetes mellitus and counselled about weight loss prior to attempting conception [115]. However, at the moment, no clear and evidence-based recommendation can be given. The screening for type 2 diabetes in all women who are planning a pregnancy, with special regard to women with PCOS [15, 116], could reduce the cost and health burden associated with GDM especially in women with other additive risk factors, such as obesity, advanced age and a particular ethnicity [81, 116]. Similarly, a screening is recommended at the first prenatal visit [117, 118].

In infertile women with PCOS, the TTP should be reduced, even if the use of high technologies for enhancing fertility increases similarly to the risk of pregnancy and neonatal complications [50]. Multiple pregnancies [32] should be also avoided in infertile patients with PCOS using strategies and/or drugs that induce monoovulation and always using the elective single embryo transfer in ART cycles.

At present, no indication for a specific management of pregnant women with PCOS women is available, and very few data on instrumental pregnancy monitoring have been published. Throughout gestation, special attention should be paid to early changes in acute-phase proteins, to dyslipidaemia and to abnormally low haemoglobin and haematocrit levels because these biochemical markers are all related to a higher risk for pregnancy complications in women with PCOS [20, 21]. A recent systematic review [119] demonstrated that five proteomic biomarkers (including transferrin, fibrinogen α, β and γ chain variants, kininogen-1, annexin 2 and peroxiredoxin 2) are expressed both in women with PE and PCOS differentially from controls suggesting that in the future these biomarkers could be useful from an academic and clinical point of view. Routine assessment of uterine artery Doppler indices during the early phases of pregnancy could be useful to select PCOS patients at high risk of adverse pregnancy and perinatal outcomes [104].

Most of the data available on pharmacological measures to propose in women with PCOS in order to reduce the obstetric and neonatal risks regard metformin (see Chap. 11). Certainly, metformin is effective and safe for the treatment of GDM, although subjects with multiple risk factors for insulin resistance may require supplementary insulin [120, 121]. Metformin use (when compared to insulin) in GDM is associated with a reduced weight gain, better neonatal outcomes (including less visceral fat) and patient compliance [121, 122]. However, the beneficial effects of metformin on GMD are more significant in non-RCTs [123].

In women with PCOS, metformin leads to a hormonal pattern and ovarian dynamic similar to spontaneous cycles in normo-ovulating controls and has no abnormal effects on follicular growth and vascularisation or endometrial competence markers [124]. Metformin could enhance trophoblast invasion and/or placentation exerting favourably effects on the endometrial receptivity through enhanced endometrial thickness and volume, endometrial and subendometrial vascularity, improved markers of endothelial activation and coagulation and immunomodulation [28, 124]. Notwithstanding that data, metformin administration does not reduce the risk for spontaneous miscarriage when used alone or combined with other fertility drugs for treating anovulation [20, 124, 125]. On the other hand, metformin reduces moderately the risk of miscarriage in hyperstimulated IVF/ICSI cycles [126]. A meta-analysis [127] found that metformin administration in pregnant women with PCOS is associated with reduced incidence of miscarriage (ORs 0.32 (95%CI, 0.19–0.55), GDM (0.37 95%CI, 0.25–0.56), PE (0.53 95%CI, 0.30–0.95) and PTD (0.30 95%CI, 0.13–0.68). Also more recently [128] aggregate data from five studies demonstrated a significantly lower risk of miscarriage (RR 0.32, 95%CI 0.19–0.56) and PTD (RR 0.40, 95%CI 0.18–0.91), whereas no effect was detected on the incidence of GDM and PE. At the moment, also high-quality studies seem to demonstrate no or little effect of metformin on pregnancy complications in PCOS and non-PCOS patients. Vanky and colleagues performed two RCTs on metformin use in pregnant women with PCOS, showing contrasting results [22], although a per-protocol reanalysis [129] of data from two previous RCTs demonstrated a reduction of the PTD of about threefold. Moreover, the potential mechanism of metformin is unclear. Metformin exerts no effect on the cervical length in women with PCOS but could act minimising the effects of androgens on uterine contractility [130]. A recent large retrospective study [24] showed that the use of oral hypoglycaemic agent, such as metformin, before pregnancy does not reduce the risk of GDM. Conversely, a recent double-blind placebo-controlled RCT in obese pregnant women, at high risk but unselected for PCOS, showed lower maternal gestational weight gain and a risk of PE lower of about 80% in women treated with metformin from 12 to 16 weeks' gestation until delivery [131]. Data regarding the potential effect of metformin on the prevention of PIH/PE are also scarce. Initial data demonstrated a reduction of the uterine artery impedance between 12 and 19 weeks of gestation [132], but that data have been refuted in another sub-analysis of RCTs [133].

The potential effect of other drugs has been studied for preventing and treating pregnancy complications in women with PCOS. Low molecular weight heparin

(LMWH) and acetylsalicylic acid (ASA), as monotherapy or a combined scheme, prevented miscarriage and recurrent pregnancy loss in patients with PCOS and hyperhomocysteinaemia [134]. LMWH, alone or combined with metformin, reduced the incidence of miscarriage in a little sample of women with PCOS and coagulation disorders [135].

Data on acupuncture are in progress (see Chap. 17). Unfortunately, initial animal data [136] seem to demonstrate that low-frequency electroacupuncture in hyperandrogenic animal model increases blood pressure and impairs placental growth and function during pregnancy.

Conclusion

Available clinical data demonstrate that women with PCOS are at increased risk of pregnancy and neonatal complications, and that risk increases significantly in the presence of comorbidities such as obesity, insulin resistance and dyslipidaemia. The specific mechanisms involved in the pathogenesis of the increased incidence of adverse maternal and perinatal outcomes remain unclear. PCOS-related features, such as hyperandrogenism, oligo-amenorrhoea, insulin resistance, obesity, dyslipidaemia and chronic low-grade inflammation, may play a crucial role in the first phases of pregnancy, i.e. during trophoblast invasion and placentation. Pregnancy in PCOS patients can worsen that risk, increasing abnormally the metabolic and inflammatory changes observed during pregnancy. The increased incidence of pregnancy complications can again increase in the presence of infertility (TTP higher than 12 months), the use of fertility drugs and/or procedures for enhancing fertility (IUI, ART, etc.), including biological manipulation of gametes and/or embryos.

The prevention of pregnancy complications in women with PCOS remains uncertain. All comorbidities present before pregnancy in women with PCOS should be identified and, whenever possible, treated before conception. In fact, pharmacological and non-pharmacological intervention strategies during pregnancy for reducing the incidence of complications are still experimental, and results reported in the literature vary. The pregnancy in patients with severe PCOS phenotype and many other risk cofactors should be always considered at high risk for obstetric and/or neonatal complications because potential diagnostic tools to identify high-risk patients in pregnant populations with PCOS are still lacking.

Complications developed during pregnancy in women with PCOS should be clearly reported since the obstetric history could act as a sensitive screening tool to identify subgroups of young women with PCOS at risk for subsequent cardiovascular and metabolic diseases. Long-term follow-up by specialist referral should be suggested [137, 138].

References

1. Silver R. Infertility trial outcomes: healthy moms and babies. Fertil Steril. 2014;101:1209–16.
2. Barnhart KT. Live birth is the correct outcome for clinical trials evaluating therapy for the infertile couple. Fertil Steril. 2014;101:1205–8.

3. Barnhart KT. Assisted reproductive technologies and perinatal morbidity: interrogating the association. Fertil Steril. 2013;99:299–302.
4. Braakhekke M, Kamphuis EI, van Rumste MM, Mol F, van der Veen F, Mol BW. How are neonatal and maternal outcomes reported in randomised controlled trials (RCTs) in reproductive medicine? Hum Reprod. 2014;29:1211–7.
5. Fauser BC, Devroey P, Macklon NS. Multiple birth resulting from ovarian stimulation for subfertility treatment. Lancet. 2005;365:1807–16.
6. Sunderam S, Kissin DM, Crawford SB, Folger SG, Jamieson DJ, Warner L, et al. Assisted reproductive technology surveillance – United States, 2013. MMWR Surveill Summ. 2015;64:1–25.
7. Palomba S, de Wilde MA, Falbo A, Koster MP, La Sala GB, Fauser BC. Pregnancy complications in women with polycystic ovary syndrome. Hum Reprod Update. 2015;21:575–92.
8. Palomba S, Santagni S, Gibbins K, La Sala GB, Silver RM. Pregnancy complications in spontaneous and assisted conceptions of women with infertility and factors of subfertility. A comprehensive review. Reprod Biomed Online. 2016;33:612–28.
9. Joham AE, Palomba S, Hart R. Polycystic ovary syndrome, obesity, and pregnancy. Semin Reprod Med. 2016;34:93–101.
10. Pinborg A, Wennerholm UB, Romundstad LB, Loft A, Aittomaki K, Söderström-Anttila V, et al. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. Hum Reprod Update. 2013;19:87–104.
11. National Institute of Health. Evidence-based methodology workshop on polycystic ovary syndrome. December 3–5, 2012. https://prevention.nih.gov/docs/programs/pcos/FinalReport.pdf.
12. Boomsma CM, Eijkemans MJ, Hughes EG, Visser GH, Fauser BC, Macklon NS. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. Hum Reprod Update. 2006;12:673–83.
13. Kjerulff LE, Sanchez-Ramos L, Duffy D. Pregnancy outcomes in women with polycystic ovary syndrome: a metaanalysis. Am J Obstet Gynecol. 2011;204:558.e1–6.
14. Qin JZ, Pang LH, Li MJ, Fan XJ, Huang RD, Chen HY. Obstetric complications in women with polycystic ovary syndrome: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2013;11:56.
15. Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Consensus on women's health aspects of polycystic ovary syndrome (PCOS). Hum Reprod. 2012;27:14–24.
16. Joham AE, Ranasinha S, Zoungas S, Moran L, Teede HJ. Gestational diabetes and type 2 diabetes in reproductive-aged women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2014;99:447–52.
17. Heijnen EM, Eijkemans MJ, Hughes EG, Laven JS, Macklon NS, Fauser BC. A meta-analysis of outcomes of conventional IVF in women with polycystic ovary syndrome. Hum Reprod Update. 2006;12:13–21.
18. Rees DA, Jenkins-Jones S, Morgan CL. Contemporary reproductive outcomes for patients with polycystic ovary syndrome: a retrospective observational study. J Clin Endocrinol Metab. 2016;101:1664–72.
19. de Wilde MA, Veltman-Verhulst SM, Goverde AJ, Lambalk CB, Laven JS, Franx A, Koster MP, Eijkemans MJ, Fauser BC. Preconception predictors of gestational diabetes: a multicentre prospective cohort study on the predominant complication of pregnancy in polycystic ovary syndrome. Hum Reprod. 2014;29:1327–36.
20. Palomba S, Chiossi G, Falbo A, Orio F, Tolino A, Colao A, La Sala GB, Zullo F. Low-grade chronic inflammation in pregnant women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2014;99:2942–51.
21. Palomba S, Falbo A, Chiossi G, Muscogiuri G, Orio F, Tolino A, Colao A, La Sala GB, Zullo F. Lipid profile in pregnant women with polycystic ovary syndrome. Steroids. 2014;88C:36–43.

22. Vanky E, Stridsklev S, Heimstad R, Romundstad P, Skogøy K, Kleggetveit O. Metformin versus placebo from first trimester to delivery in polycystic ovary syndrome: a randomized, controlled multicenter study. J Clin Endocrinol Metab. 2010;95:448–55.
23. Roos N, Kieler H, Sahlin L, Ekman-Ordeberg G, Falconer H, Stephansson O. Risk of adverse pregnancy outcomes in women with polycystic ovary syndrome: population based cohort study. BMJ. 2011;343:d6309.
24. Pan ML, Chen LR, Tsao HM, Chen KH. Relationship between polycystic ovarian syndrome and subsequent gestational diabetes mellitus: a nationwide population-based study. PLoS One. 2015;10:e0140544.
25. Naver KV, Grinsted J, Larsen SO, Hedley PL, Jørgensen FS, Christiansen M, Nilas L. Increased risk of preterm delivery and pre-eclampsia in women with polycystic ovary syndrome and hyperandrogenaemia. BJOG. 2014;121:575–81.
26. Doherty DA, Newnham JP, Bower C, Hart R. Implications of polycystic ovary syndrome for pregnancy and for the health of offspring. Obstet Gynecol. 2015;125:1397–406.
27. Lynch AM, Hart JE, Agwu OC, Fisher BM, West NA, Gibbs RS. Association of extremes of prepregnancy BMI with the clinical presentations of preterm birth. Am J Obstet Gynecol. 2014;210:428–32.
28. Han AR, Kim HO, Cha SW, Park CW, Kim JY, Yang KM, Song IO, Koong MK, Kang IS. Adverse pregnancy outcomes with assisted reproductive technology in non-obese women with polycystic ovary syndrome: a case-control study. Clin Exp Reprod Med. 2011;38:103–8.
29. Rao A, Sairam S, Shehata H. Obstetric complications of twin pregnancies. Best Pract Res Clin Obstet Gynaecol. 2004;18:557–76.
30. Society of Obstetricians and Gynaecologists of Canada. Pregnancy outcomes after assisted human reproduction. J Obstet Gynaecol Can. 2014;36:64–83.
31. Johnston J, Gusmano MK, Patrizio P. Preterm births, multiples, and fertility treatment: recommendations for changes to policy and clinical practices. Fertil Steril. 2014;102:36–9.
32. Løvvik TS, Wikström AK, Neovius M, Stephansson O, Roos N, Vanky E, Magnussen EB, Vatten LJ. Pregnancy and perinatal outcomes in women with polycystic ovary syndrome and twin births: a population-based cohort study. BJOG. 2016;122:1295–302.
33. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19:41–7.
34. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, Welt CK. Diagnosis and treatment of polycystic ovary syndrome: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2013;98:4565–92.
35. Palomba S, Falbo A, Russo T, Tolino A, Orio F, Zullo F. Pregnancy in women with polycystic ovary syndrome: the effect of different phenotypes and features on obstetric and neonatal outcomes. Fertil Steril. 2010;94:1805–11.
36. Kollmann M, Klaritsch P, Martins WP, Guenther F, Schneider V, Herzog SA, Craciunas L, Lang U, Obermayer-Pietsch B, Lerchbaum E, Raine-Fenning N. Maternal and neonatal outcomes in pregnant women with PCOS: comparison of different diagnostic definitions. Hum Reprod. 2015;30:2396–403.
37. Palomba S, La Sala GB. Pregnancy complications in women with polycystic ovary syndrome: importance of diagnostic criteria or of phenotypic features? Hum Reprod. 2016;31:223–4.
38. Kaňová N, Bičíková M. Hyperandrogenic states in pregnancy. Physiol Res. 2011;60:243–52.
39. Makieva S, Saunders PTK, Norman JE. Androgens in pregnancy: roles in parturition. Hum Reprod Update. 2014;20:542–59.
40. Sir-Petermann T, Maliqueo M, Angel B, Lara HE, Pérez-Bravo F, Recabarren SE. Maternal serum androgens in pregnant women with polycystic ovarian syndrome: possible implications in prenatal androgenization. Hum Reprod. 2002;17:2573–9.

41. Falbo A, Rocca M, Russo T, D'Ettore A, Tolino A, Zullo F, Orio F, Palomba S. Changes in androgens and insulin sensitivity indexes throughout pregnancy in women with polycystic ovary syndrome (PCOS): relationships with adverse outcomes. J Ovarian Res. 2010;3:23.
42. Escobar JC, Patel SS, Beshay VE, Suzuki T, Carr BR. The human placenta expresses CYP17 and generates androgens de novo. J Clin Endocrinol Metab. 2011;96:1385–92.
43. Maliqueo M, Lara HE, Sánchez F, Echiburú B, Crisosto N, Sir-Petermann T. Placental steroidogenesis in pregnant women with plycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol. 2013;166:151–5.
44. Palomba S, Russo T, Falbo A, Di Cello A, Amendola G, Mazza R, Tolino A, Zullo F, Tucci L, La Sala GB. Decidual endovascular trophoblast invasion in women with polycystic ovary syndrome: an experimental case-control study. J Clin Endocrinol Metab. 2012;97:2441–9.
45. Palomba S, Russo T, Falbo A, Di Cello A, Tolino A, Tucci L, La Sala GB, Zullo F. Macroscopic and microscopic findings of the placenta in women with polycystic ovary syndrome. Hum Reprod. 2013;28:2838–47.
46. Palomba S, Falbo A, Chiossi G, Tolino A, Tucci L, La Sala GB, Zullo F. Early trophoblast invasion and placentation in women with different polycystic ovary syndrome phenotypes. Reprod Biomed Online. 2014;29:370–81.
47. Cakmak H, Taylor HS. Implantation failure: molecular mechanisms and clinical treatment. Hum Reprod Update. 2011;17:242–53.
48. Kajihara T, Tanaka K, Oguro T, Tochigi H, Prechapanich J, Uchino S, Itakura A, Sućurović S, Murakami K, Brosens JJ. Androgens modulate the morphological characteristics of human endometrial stromal cells decidualized in vitro. Reprod Sci. 2013;21:372–80.
49. Hudecova M, Holte J, Olovsson M, Sundström PI. Long-term follow-up of patients with polycystic ovary syndrome: reproductive outcome and ovarian reserve. Hum Reprod. 2009;24:1176–83.
50. Messerlian C, Maclagan L, Basso O. Infertility and the risk of adverse pregnancy outcomes: a systematic review and meta-analysis. Hum Reprod. 2013;28:125–37.
51. Jaques AM, Amor DJ, Baker HWG, Healy DL, Ukoumunne OC, Breheny S, Garrett C, Halliday JL. Adverse obstetric and perinatal outcomes in subfertile women conceiving without assisted reproductive technologies. Fertil Steril. 2010;7:2674–9.
52. Tobias DK, Chavarro JE, Williams MA, Buck Louis GM, Hu FB, Rich-Edwards J, Missmer SA, Zhang C. History of infertility and risk of gestational diabetes mellitus: a prospective analysis of 40,773 pregnancies. Am J Epidemiol. 2013;178:1219–25.
53. DoPierala AL, Bhatta S, Raja EA, Bhattacharya S, Bhattacharya S. Obstetric consequences of subfertility: a retrospective cohort study. BJOG. 2015;3:1–9.
54. Norman RJ, Noakes M, Wu R, Davies MJ, Moran L, Wang JX. Improving reproductive performance in overweight/obese women with effective weight management. Hum Reprod Update. 2004;10:267–80.
55. Mutsaerts MA, van Oers AM, Groen H, Burggraaff JM, Kuchenbecker WK, Perquin DA, et al. Randomized trial of a lifestyle program in obese infertile women. N Engl J Med. 2016;374:1942–53.
56. Stanford JB, Simonsen SE, Baksh L. Fertility treatments and adverse perinatal outcomes in a population-based sampling of births in Florida, Maryland, and Utah: a cross-sectional study. BJOG. 2016;123:718–29.
57. Messerlian C, Platt RW, Tan S-L, Gagnon R, Basso O. Low-technology assisted reproduction and the risk of preterm birth in a hospital-based cohort. Fertil Steril. 2015;103:81–8.
58. Malchau SS, Loft A, Henningsen AK, Nyboe Andersen A, Pinborg A. Perinatal outcomes in 6,338 singletons born after intrauterine insemination in Denmark, 2007 to 2012: the influence of ovarian stimulation. Fertil Steril. 2014;102:1110–6.
59. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, Steinkampf MP, Coutifaris C, McGovern PG, Cataldo NA, Cooperative Multicenter Reproductive Medicine Network, et al. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. N Engl J Med. 2007;356:551–6.

60. Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P, Christman GM, Huang H, Yan Q, Alvero R, NICHD Reproductive Medicine Network, et al. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. N Engl J Med. 2014; 371:119–29.
61. Diamond MP, Legro RS, Coutifaris C, Alvero R, Robinson RD, Casson P, NICHD Reproductive Medicine Network, et al. Letrozole, gonadotropin, or clomiphene for unexplained infertility. N Engl J Med. 2015;373:1230–40.
62. Nahuis MJ, Oude Lohuis EJ, Bayram N, Hompes PG, Oosterhuis GJ, van der Veen F, et al. Pregnancy complications and metabolic disease in women with clomiphene citrate-resistant anovulation randomized to receive laparoscopic electrocautery of the ovaries or ovulation induction with gonadotropins: a 10-year follow-up. Fertil Steril. 2014;101:270–4.
63. Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. Hum Reprod Update. 2012;18:485–503.
64. Sterling L, Liu J, Okun N, Sakhuja A, Sierra S, Greenblatt E. Pregnancy outcomes in women with polycystic ovary syndrome undergoing in vitro fertilization. Fertil Steril. 2016;105: 791–7.
65. Ensing S, Abu-Hanna A, Roseboom TJ, Repping S, van der Veen F, Mol BW, Ravelli AC. Risk of poor neonatal outcome at term after medically assisted reproduction: a propensity score-matched study. Fertil Steril. 2015;104:384–90.
66. Moran LJ, Norman RJ, Teede HJ. Metabolic risk in PCOS: phenotype and adiposity impact. Trends Endocrinol Metab. 2015;26:136–43.
67. Michalakis K, Mintziori G, Kaprara A, Tarlatzis BC, Goulis DG. The complex interaction between obesity, metabolic syndrome and reproductive axis: a narrative review. Metabolism. 2013;62:457–78.
68. Cedergren MI. Maternal morbid obesity and the risk of adverse pregnancy outcome. Obstet Gynecol. 2004;103:219–24.
69. Lawlor DA, Relton C, Sattar N, Nelson SM. Maternal adiposity: a determinant of perinatal and offspring outcomes? Nat Rev Endocrinol. 2012;8:679–88.
70. Marchi J, Berg M, Dencker A, Olander EK, Begley C. Risks associated with obesity in pregnancy, for the mother and baby: a systematic review of reviews. Obes Rev. 2015;16:621–38.
71. Boots C, Stephenson MD. Does obesity increase the risk of miscarriage in spontaneous conception: a systematic review. Semin Reprod Med. 2011;29:507–13.
72. Aune D, Saugstad OD, Henriksen T, Tonstad S. Maternal body mass index and the risk of fetal death, stillbirth, and infant death: a systematic review and meta-analysis. JAMA. 2014;311:1536–46.
73. Declercq E, MacDorman M, Cabral H, Stotland N. Prepregnancy body mass index and infant mortality in 38 U.S. States, 2012–2013. Obstet Gynecol. 2016;127:279–87.
74. Rasmussen SA, Chu SY, Kim SY, Schmid CH, Lau J. Maternal obesity and risk of neural tube defects: a metaanalysis. Am J Obstet Gynecol. 2008;198:611–9.
75. Cai GJ, Sun XX, Zhang L, Hong Q. Association between maternal body mass index and congenital heart defects in offspring: a systematic review. Am J Obstet Gynecol. 2014;211:91–117.
76. Sirimi N, Dimistrios GC. Obesity in pregnancy. Hormones (Athens). 2010;9:299–306.
77. Shreeve N, Cagampang F, Sadek K, Tolhurst M, Houldey A, Hill CM, Brook N, Macklon N, Cheong Y. Poor sleep in PCOS; is melatonin the culprit? Hum Reprod. 2013;28:1348–53.
78. Bisson M, Sériès F, Giguère Y, Pamidi S, Kimoff J, Weisnagel SJ, Marc I. Gestational diabetes mellitus and sleep-disordered breathing. Obstet Gynecol. 2014;123:634–41.
79. Yogev Y, Catalano PM. Pregnancy and obesity. Obstet Gynecol Clin N Am. 2009;36:285–300.
80. Horvath K, Koch K, Jeitler K, Matyas E, Bender R, Bastian H, Lange S, Siebenhofer A. Effects of treatment in women with gestational diabetes mellitus: systematic review and meta-analysis. BMJ. 2010;340:c1395.

81. Torloni MR, Betrán AP, Horta BL, Nakamura MU, Atallah AN, Moron AF, Valente O. Prepregnancy BMI and the risk of gestational diabetes: a systematic review and meta-analysis. Obes Rev. 2009;10:194–203.
82. Alshammari A, Hanley A, Ni A, Tomlinson G, Feig DS. Does the presence of polycystic ovary syndrome increase the risk of obstetrical complications in women with gestational diabetes? J Matern Fetal Neonatal Med. 2010;23:545–9.
83. Palomba S, Falbo A, Russo T, Rivoli L, Orio M, Cosco AG, Vero R, Capula C, Tolino A, Zullo F, et al. The risk of a persistent glucose metabolism impairment after gestational diabetes mellitus is increased in patients with polycystic ovary syndrome. Diabetes Care. 2012;35:861–7.
84. Sentilhes L, Sénat MV, Boulogne AI, Deneux-Tharaux C, Fuchs F, Legendre G, et al. Shoulder dystocia: guidelines for clinical practice from the French College of Gynecologists and Obstetricians (CNGOF). Eur J Obstet Gynecol Reprod Biol. 2016;203:156–61.
85. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev. 2012;33:981–1030.
86. Hodson K, Man CD, Smith FE, Thelwall PE, Cobelli C, Robson SC, Taylor R. Mechanism of insulin resistance in normal pregnancy. Horm Metab Res. 2013;45:567–71.
87. Tian L, Shen H, Lu Q, Norman RJ, Wang J. Insulin resistance increases the risk of spontaneous abortion after assisted reproduction technology treatment. J Clin Endocrinol Metab. 2007;92:1430–3.
88. Mikola M, Hiilesmaa V, Halttunen M, Suhonen L, Tiitinen A. Obstetric outcome in women with polycystic ovarian syndrome. Hum Reprod. 2001;16:226–9.
89. Haakova L, Cibula D, Rezabek K, Hill M, Fanta M, Zivny J. Pregnancy outcome in women with PCOS and in controls matched by age and weight. Hum Reprod. 2003;18:1438–41.
90. Seely EW, Solomon CG. Insulin resistance and its potential role in pregnancy-induced hypertension. J Clin Endocrinol Metab. 2003;88:2393–8.
91. Lorentzen B, Henriksen T. Plasma lipids and vascular dysfunction in preeclampsia. Semin Reprod Endocrinol. 1998;16:33–9.
92. Herrera E, Ortega-Senovilla H. Maternal lipid metabolism during normal pregnancy and its implications to fetal development. Clin Lipidol. 2010;5:899–911.
93. Vrijkotte TG, Krukziener N, Hutten BA, Vollebregt KC, van Eijsden M, Twickler MB. Maternal lipid profile during early pregnancy and pregnancy complications and outcomes: the ABCD study. J Clin Endocrinol Metab. 2012;97:3917–25.
94. Kaaja R, Tikkanen MJ, Viinikka L, Ylikorkala O. Serum lipoproteins, insulin, and urinary prostanoid metabolites in normal and hypertensive pregnant women. Obstet Gynecol. 1995;85:353–6.
95. Hubel CA. Dyslipidemia, iron, and oxidative stress in preeclampsia: assessment of maternal and feto-placental interactions. Semin Reprod Endocrinol. 1998;16:75–92.
96. Sattar N, Berry C, Greer IA. Essential fatty acids in relation to pregnancy complications and fetal development. BJOG. 1998;105:1248–55.
97. Valsamakis G, Kumar S, Creatsas G, Mastorakos G. The effects of adipose tissue and adipocytokines in human pregnancy. Ann N Y Acad Sci. 2010;1205:76–81.
98. Cao C, O'Brien KO. Pregnancy and iron homeostasis: an update. Nutr Rev. 2013;71:35–51.
99. Wolf M, Sandler L, Hsu K, Vossen-Smirnakis K, Ecker JL, Thadhani R. First-trimester C-reactive protein and subsequent gestational diabetes. Diabetes Care. 2003;26:819–24.
100. Sacks GP, Seyani L, Lavery S, Trew G. Maternal C-reactive protein levels are raised at 4 weeks gestation. Hum Reprod. 2004;19:1025–30.
101. Parchim NF, Wang W, Iriyama T, Ashimi OA, Siddiqui AH, Blackwell S, Sibai B, Kellems RE, Xia Y. Neurokinin 3 receptor and phosphocholine transferase: missing factors for pathogenesis of C-reactive protein in preeclampsia. Hypertension. 2015;65:430–9.
102. Maliqueo M, Sundstrom-Poromaa I, Vanky E, Fornes R, Benrick A, Akerud H, Stridsklev S, Labrie F, Jansson T, Stener-Victorin E. Placental STAT3 signaling is activated in women with polycystic ovary syndrome. Hum Reprod. 2015;30:692–700.

103. Koster MP, de Wilde MA, Veltman-Verhulst SM, Houben ML, Nikkels PG, van Rijn BB, Fauser BC. Placental characteristics in women with polycystic ovary syndrome. Hum Reprod. 2015;30:2829–37.
104. Palomba S, Falbo A, Russo T, Battista L, Tolino A, Orio F, Zullo F. Uterine blood flow in pregnant women with polycystic ovary syndrome: relationships with clinical outcomes. BJOG. 2010;117:711–21.
105. Wang Q, Luo L, Lei Q, Lin MM, Huang X, Chen MH, Zeng YH, Zhou CQ. Low aneuploidy rate in early pregnancy loss abortuses from patients with polycystic ovary syndrome. Reprod Biomed Online. 2016;33:85–92. pii: S1472-6483(16)30069-4
106. Brosens I, Benagiano G. Menstrual preconditioning for the prevention of major obstetrical syndromes in polycystic ovary syndrome. Am J Obstet Gynecol. 2015;213:488–93.
107. Palomba S, La Sala GB. Menstrual preconditioning for the prevention of pregnancy complications in women with polycystic ovary syndrome (PCOS): clinical opinion or viewpoint-this is the question. Am J Obstet Gynecol. 2016;214:417–8.
108. Agha M, Agha RA, Sandell J. Interventions to reduce and prevent obesity in pre-conceptual and pregnant women: a systematic review and meta-analysis. PLoS One. 2014;9:e95132.
109. Matusiak K, Barrett HL, Callaway LK, Nitert MD. Periconception weight loss: common sense for mothers, but what about for babies? J Obes. 2014;2014:204295.
110. American College of Obstetricians and Gynecologists. Committee opinion no. 549: obesity in pregnancy. Obstet Gynecol. 2013;121:213–7.
111. Thangaratinam S, Rogozinska E, Jolly K, Glinkowski S, Roseboom T, Tomlinson JW, Kunz R, Mol BW, Coomarasamy A, Khan KS. Effects of interventions in pregnancy on maternal weight and obstetric outcomes: meta-analysis of randomised evidence. BMJ. 2012;344:e2088.
112. Institute of Medicine (IOM) and National Research Council (NRC). Weight gain during pregnancy: reexamining the guidelines. Washington, DC: The National Academies Press; 2009. p. 1–854.
113. Kapadia MZ, Park CK, Beyene J, Giglia L, Maxwell C, McDonald SD. Can we safely recommend gestational weight gain below the 2009 guidelines in obese women? A systematic review and meta-analysis. Obes Rev. 2015;16:189–206.
114. Faucher MA, Hastings-Tolsma M, Song JJ, Willoughby DS, Bader SG. Gestational weight gain and preterm birth in obese women: a systematic review and meta-analysis. BJOG. 2016;123:199–206.
115. Goodman NF, Cobin RH, Futterweit W, Glueck JS, Legro RS, Carmina E. American Association of Clinical Endocrinologists, American College of Endocrinology, and Androgen Excess and PCOS Society Disease state clinical review: guide to the best practices in the evaluation and treatment of polycystic ovary syndrome – part 2. Endocr Pract. 2015;21:1415–26.
116. Peterson C, Grosse SD, Li R, Sharma AJ, Razzaghi H, Herman WH, Gilboa SM. Preventable health and cost burden of adverse birth outcomes associated with pregestational diabetes in the United States. Am J Obstet Gynecol. 2015;212:74.e1–9.
117. American Diabetes Association. Standards of medical care in diabetes - 2011. Diabetes Care. 2011;34:11–61.
118. International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, Dyer AR, Leiva AD, Hod M, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010;33:676–82.
119. Khan GH, Galazis N, Docheva N, Layfield R, Atiomo W. Overlap of proteomic biomarkers between women with pre-eclampsia and PCOS: a systematic review and biomarker database integration. Hum Reprod. 2015;30:133–48.
120. Rowan JA, Hague WM, Gao W, Battin MR, Moore MP, Investigators MGT. Metformin versus insulin for the treatment of gestational diabetes. N Engl J Med. 2008;358:2003–15.

121. Lautatzis ME, Goulis DG, Vrontakis M. Efficacy and safety of metformin during pregnancy in women with gestational diabetes mellitus or polycystic ovary syndrome: a systematic review. Metabolism. 2013;62:1522–34.
122. Sivalingam VN, Myers J, Nicholas S, Balen AH, Crosbie EJ. Metformin in reproductive health, pregnancy and gynaecological cancer: established and emerging indications. Hum Reprod Update. 2014;20:853–68.
123. Zhuo Z, Wang A, Yu H. Effect of metformin intervention during pregnancy on the gestational diabetes mellitus in women with polycystic ovary syndrome: a systematic review and meta-analysis. J Diabetes Res. 2014;2014:381231.
124. Palomba S, Falbo A, Zullo F, Orio Jr F. Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. Endocr Rev. 2009;30:1–50.
125. Palomba S, Falbo A, Orio F, Zullo F. Effect of preconceptional metformin on abortion risk in polycystic ovary syndrome: a systematic review and meta-analysis of randomized controlled trials. Fertil Steril. 2009;92:1646–58.
126. Palomba S, Falbo A, La Sala GB. Metformin and gonadotropins for ovulation induction in patients with polycystic ovary syndrome: a systematic review with meta-analysis of randomized controlled trials. Reprod Biol Endocrinol. 2014;12:3.
127. Zheng J, Shan PF, Gu W. The efficacy of metformin in pregnant women with polycystic ovary syndrome: a meta-analysis of clinical trials. J Endocrinol Investig. 2013;36:797–802.
128. Feng L, Lin XF, Wan ZH, Hu D, Du YK. Efficacy of metformin on pregnancy complications in women with polycystic ovary syndrome: a meta-analysis. Gynecol Endocrinol. 2015;31:833–9.
129. Vanky E, Isaksen H, Moen MH, Carlsen SM. Breastfeeding in polycystic ovary syndrome. Acta Obstet Gynecol Scand. 2008;87:531–5.
130. Shetelig Løvvik T, Stridsklev S, Carlsen SM, Salvesen Ø, Vanky E. Cervical length and androgens in pregnant women with polycystic ovary syndrome: has metformin any effect? J Clin Endocrinol Metab. 2016;101:2325–31.
131. Syngelaki A, Nicolaides KH, Balani J, Hyer S, Akolekar R, Kotecha R, Pastides A, Shehata H. Metformin versus placebo in obese pregnant women without diabetes mellitus. N Engl J Med. 2016;374:434–43.
132. Salvesen KA, Vanky E, Carlsen SM. Metformin treatment in pregnant women with polycystic ovary syndrome–is reduced complication rate mediated by changes in the uteroplacental circulation? Ultrasound Obstet Gynecol. 2007;29:433–7.
133. Stridsklev S, Carlsen SM, Salvesen Ø, Clemens I, Vanky E. Midpregnancy Doppler ultrasound of the uterine artery in metformin-versus placebo-treated PCOS women: a randomized trial. J Clin Endocrinol Metab. 2014;99:972–7.
134. Chakraborty P, Goswami SK, Rajani S, Sharma S, Kabir SN, Chakravarty B, Jana K. Recurrent pregnancy loss in polycystic ovary syndrome: role of hyperhomocysteinemia and insulin resistance. PLoS One. 2013;8:e64446.
135. Ramidi G, Khan N, Glueck CJ, Wang P, Goldenberg N. Enoxaparin-metformin and enoxaparin alone may safely reduce pregnancy loss. Transl Res. 2009;153:33–43.
136. Fornes R, Hu M, Maliqueo M, Kokosar M, Benrick A, Carr D, Billig H, Jansson T, Manni L, Stener-Victorin E. Maternal testosterone and placental function: effect of electroacupuncture on placental expression of angiogenic markers and fetal growth. Mol Cell Endocrinol. 2016;433:1–11.
137. Spaan J, Peeters L, Spaanderman M, Brown M. Cardiovascular risk management after a hypertensive disorder of pregnancy. Hypertension. 2012;60:1368–73.
138. Cusimano MC, Pudwell J, Roddy M, Cho CK, Smith GN. The maternal health clinic: an initiative for cardiovascular risk identification in women with pregnancy-related complications. Am J Obstet Gynecol. 2014;210:438.e1–9.

Conclusive Remarks and Future Perspectives

23

Stefano Palomba

23.1 Introduction

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous syndrome related in different ways with the infertility. Previous chapters have focused the attention on several aspects regarding the relationship between PCOS and infertility. Data reported and discussed have underlined that a great effort has been performed to optimise the diagnostic and therapeutic work-up for infertile women with PCOS during the last years. Unfortunately, many are the questions still unanswered about the relationship between the PCOS and the infertility. In the present and conclusive chapter, the available clinical evidences about the management of infertility in patients with PCOS will be summarised, and the main future perspectives will be discussed.

23.2 Infertility and PCOS

The presence of infertility in women with PCOS is an evident feature related to the syndrome (Chap. 1). International consensus suggests that oligo-anovulation is certainly associated with infertility in PCOS. Moreover, the exact aetiology of anovulation in PCOS has not yet been defined, and various factors (including alterations in anti-Mullerian hormone (AMH) and, recently, kisspeptin levels) may help us to understand the mechanisms involved in the PCOS-related anovulation and suggest that the ovulation in PCOS, also when present, can be probably perturbated (Chaps. 3, 4, 6, and 8).

S. Palomba
Department of Gynecology and Obstetrics, IRCCS–Arcispedale Santa Maria Nuova of Reggio Emilia, Via Risorgimento 80, 42123 Reggio Emilia, Italy
e-mail: stefanopalomba@tin.it

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1_23

At the present, there are not data about the stratification of this risk according to PCOS phenotypes (Chaps. 7 and 21). This could be due to the still recent discussion about the diagnostic criteria and the need to assess completely all diagnostic PCOS characteristics (e.g. oligo-anovulation, hyperandrogenemia, clinical hyperandrogenism, polycystic ovarian morphology (PCOM)) in order to obtain the diagnosis of a specific phenotype for each patient also in the clinical practice (Chap. 2).

In the next future, the availability of robust assays for the AMH and of new very accurate and automatised three-dimensional ultrasonographic machines will make necessary a profound change of the diagnostic criteria specially for the PCOM (Chap. 8).

The need or not of complete assessment of all diagnostic criteria as well as of all PCOS-related characteristics (including obesity, glucose metabolism, lipid pattern impairment, etc.) that influence the severity of the syndrome also in terms of reproductive outcomes notwithstanding they are not diagnostic of the syndrome is a crucial topic. Recent evidences suggest that the contributor of these characteristics, as well as of the diagnostic PCOS features, is very relevant in terms of reproductive potential (Chaps. 4 and 5). To this regard, endometrial and oocyte competence is obviously altered in obese patients with PCOS and with metabolic/hormonal dysfunction (Chap. 4), while the true effect of the PCOS and its phenotypes is only partially known and seems clinically little. However, data about the abnormalities of the endometrium in women with PCOS and their contribution of the infertility in PCOS are stronger than those regarding the oocyte, but surprisingly many data are available about potential interventions for improving oocyte quality but not for optimising the endometrial competence (Chap. 5). Finally, the knowledge of the specific PCOS phenotype would be important also in view of a correct counselling about the future risks for the mother and the baby when the pregnancy is achieved (Chap. 22).

23.3 Diagnostic Work-Up in Infertile Patients with PCOS

Another point of discussion, partially related to the previous, is whether patients with PCOS need a specific diagnostic work-up for infertility. In infertile patients, a careful diagnosis of PCOS can be certainly useful for optimising the treatment and for a correct counselling, and other causes of anovulation should be always excluded (Chap. 6), but there are not sufficient data to suggest at the moment a diagnostic wok-up specific for infertile women with PCOS.

Basal characteristics of women with PCOS enrolled in large clinical trials have demonstrated that women with PCOS have several additive “characteristics” with potential and negative effect on the reproductive (Chap. 6). Infertile patients with PCOS seem to have alterations of the libido and a reduction of the sexual intercourses, unsafe lifestyle attitudes, obese partners, and so on. Thus, a very carefully and detailed history could provide crucial suggestions to drive to an effective management strategy.

New data seem to suggest a role of diet (micro- and macronutrients) factors in the PCOS pathogenesis (Chaps. 14 and 16). Alterations of the vitamin D and

inositol concentrations have been observed in women with PCOS, and in the next future, specific assays aimed to detect specific diet insufficiency could select subjects sensible to appropriate dietary supplement intake.

23.4 Management of Infertility in Women with PCOS

There is a consensus about the crucial role of lifestyle modification programmes, including not only weight loss and physical activity but also correct and safe diet and stop of smoking, as initial management of infertile women with PCOS to enhance the natural fertility (Chap. 13). Moreover, strong evidence-based data are still lacking specially for patients with PCOS, commonly considered patients having a low compliance to interventions.

In all oligo-amenorrhoeic patients with PCOS, the restoration of regular ovarian function and endometrial shedding would be a primary aim of the physicians in consideration of the relationship between the endometrial function and the increased risk of pregnancy complications (Chaps. 5 and 22). That risk is particularly high also in patients with a long time to pregnancy (TTP); thus, a short diagnostic phase is needed to start quickly the further treatment(s). Lifestyle modification programmes; insulin-sensitising drugs, such as metformin or inositol; or specific diet supplements should be quickly started as initial attempt to normalise the ovarian function (Chaps. 11, 13, and 16) before the reproductive desire and to avoid the maternal-foetal risk related to the infertility treatments (Chap. 22).

Many data seem to suggest a central role of letrozole as first-line pharmacological treatment of anovulation in women with PCOS (Chap. 10). However, the areas of uncertain are many and include its off-label use, the lack of appropriate studies addressing the selection of patients with PCOS, the optimal dosages of administration, how many cycles would be repeated before to change treatment, and long-term follow-up on the offspring. The use of clomiphene citrate (CC) is still largely considered since the clinical experience about its efficacy and safety is well known (Chap. 9). However, notwithstanding its use for more than 60 years in gynaecology, several unexplored areas are also present specially about the clinical and biochemical predictors to select the patients. A high proportion of infertile women with PCOS who receive CC administration remains anovulatory or did not achieve a pregnancy. In these patients, the use of letrozole or the combination of CC plus metformin has been demonstrated effective in a good percentage of cases (Chaps. 10 and 11). In women with PCOS under metformin administration, the insulin-sensitising drug should be not suspended in consideration of the high efficacy of the metformin plus CC or plus letrozole (Chap. 11), of the reduced risk of multiple follicular development (and of cancelled cycles), and of ovarian hyperstimulation syndrome (OHSS) in case of further gonadotrophin treatment for mono- or multiple-ovulatory cycles for free or timed intercourses or for in vitro fertilisation (IVF) procedures, respectively (Chaps. 11, 12, and 19).

A proportion of anovulatory patients with PCOS unresponsive and/or intolerant to oral ovulation inductors will benefit of gonadotrophin administration or of the

laparoscopic ovarian drilling (LOD). In fact, both step-up protocols using low or very low starting dose of gonadotrophins and personalised LOD are two efficacy interventions for treating patients with PCOS-related anovulatory infertility (Chaps. 12 and 15). Moreover, both treatments require a higher expertise to sustain an acceptable safety for the patient. In addition, their direct and indirect costs limit their use as initial managing steps. In the clinical practice, these concerns can be respectively overtaken in case of mild male factor of infertility requiring an intrauterine insemination (IUI) and a close ovarian monitoring (Chap. 18) or in case of pelvic factors of subfertility (such as adhesions, endometriosis, fibroids, etc.) that require a laparoscopic surgery (Chaps. 15 and 21). However, further data are needed to confirm or to rebut the hypothesis that women with PCOS ovulating under ovulation inductors for six or more cycles would be considered as patients with unexplained infertility and that can benefit from IUI (Chap. 18).

The failure of the previous interventions in anovulatory women with PCOS and the presence of male and/or tubal cofactors of infertility are indications for assisted reproductive technologies (ART), including IVF cycles with or without in vitro maturation (IVM) of oocytes (Chaps. 19 and 20). New controlled ovarian hyperstimulation (COH) protocols including multiple dose gonadotrophin-releasing hormone (GnRH) antagonist and multiple ovulation triggering with GnRH agonists have demonstrated a high safety that can be implemented by single-embryo transfer in subsequent frozen-thawed cycles (Chap. 19). Further comparative data are waited about the efficacy-safety ratio of that new protocols with traditional GnRH-agonist COH protocols supplemented with metformin. However, the biological manipulation of oocytes aimed to complete their final stages of maturation in the laboratory will be certainly in the next future a totally safe option of treatment for infertile patients with PCOS (Chap. 20). Furthermore, the optimisation and the standardisation of the clinical and biological protocols are needed before this procedure could be considered a real and routine treatment option.

Finally, inositols, dietary supplements, phytotherapy, Chinese herbal medicine, and acupuncture certainly influence the ovarian function, although their role as specific interventions in the management of the infertility in women with PCOS should be still explored with well-designed large clinical trials (Chaps. 14 and 17).

Conclusion

The management of women with PCOS is still a challenging issue that requires a deep knowledge of the pathogenesis of the PCOS, of its diagnostic criteria, and of available interventions. An integrated strategy (Chap. 21), including also aggressive interventions, such as the bariatric surgery for severely obese patients, is crucial for the main goal in reproductive medicine, i.e. to achieve a healthy baby in arm preserving the health of the mother (Chap. 22). A proposal of integrated strategy of treatment of infertility in PCOS is shown in Fig. 23.1.

Future pharmacogenetic studies are needed to identify patients with good or poor response to each specific and/or combined treatment and open new opportunities for individualised and minimally invasive treatments.

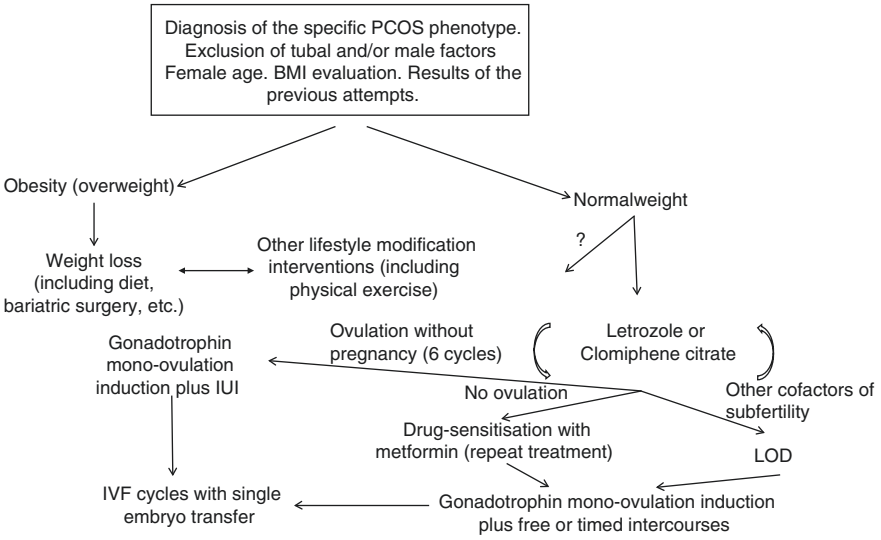


Fig. 23.1 Integrated strategy of treatment of infertility in PCOS. A conclusive and summarising proposal. *BMI* body mass index, *IUI* intrauterine insemination, *IVF* in-vitro fertilization, *LOD* laparoscopic ovarian drilling

Index

A
Abdominal acupuncture, 232
Acupuncture
  autonomic activity, 231–232
  daily abdominal acupuncture, 232
  DHT-induced PCOS rat model, 234
  hypothetical mechanism, 228
  in infertility and subfertility cofactors, 236–239
  and insulin resistance, 237–238
  intramuscular needle insertion and stimulation, 229
  low-frequency electroacupuncture, 230, 231, 233
  and mental health, 238–239
  modulation, 231–232
  molecular mechanism, 233
  and obesity, 236–237
  in ovarian dysfunction, 232–234
  during pregnancy, 234–236
  protocols, 229
  randomized controlled trials, 227
  stimulation and physiological responses, 229–230
  Western medical perspective, 228–232
Androgen excess (AE)-PCOS Society criteria, 13, 16
Aneuploidy, 33, 34, 37, 281, 313
Anovulation, 8, 24–25
  AMH, 26–28
  gonadotropin release abnormalities, 25
  hyperandrogenism, 25–26
  infertility, metformin
    clinical pregnancy rates, 142
    pretreatment, 141, 143
    randomised controlled studies, 141, 143
    therapy-naïve infertile patients, 142
  placenta, aromatase activity, 25
  sex hormone-binding hormone activity, 25
Antiestrogens. *See* Selective estrogen receptor modulators (SERMs)
Anti-Müllerian hormone (AMH)
  assay, 6
  foetal life, 26
  follicle excess
    clinical application, 95
    immuno-analysers, 96
    inter-laboratory variability, 96
    molecular heterogeneity, 95–96
    serum concentration, 94–95
  and FSH concentrations, 27
  functions, 26, 27
  levels, 15
  and LH concentrations, 28
  male sexual differentiation, 94
  mechanisms, 27
  ovarian reserve, indirect reflection, 94
  in ovary, 94, 95
  role, PCOS anovulation, 26, 27
  transforming growth factor-beta family, 26
Antral follicular count (AFC), 5
Aromatase inhibitors (AI)
  aminoglutethimide, 120
  anastrozole, 120
  breast cancer and fertility preservation, 128
  CC resistance, 124–125
  clinical studies, 123–125
  exemstane, 120
  foetal safety and teratogenic effects, 126–127
  formestane, 120
  half-life, 121
  history, 120
  hormonal balance and interactions, 123
  letrozole, 119
  LOD, 125
  mechanism of action, 122–123
  oestrogen biosynthesis, 119
  OHSS prevention, 129

S. Palomba (ed.), *Infertility in Women with Polycystic Ovary Syndrome*,
https://doi.org/10.1007/978-3-319-45534-1

Aromatase inhibitors (AI) (*cont.*)
pharmacodynamics, 121–122
pharmacokinetics, 121–122
protocols and doses, 125
side effects, 126
unexplained infertility, 127
Assisted reproductive technologies (ARTs), 3, 7–8, 43, 122, 126, 221, 267
biological manipulations, 311
elective single embryo transfer, 314
and lifestyle interventions, 173, 174, 176–178
luteal phase support, 267
male/tubal cofactors, infertility, 328
maturational and developmental competence, 298
medical treatments, 311
vitamin D supplementation, 173

C
Chinese herbal medicine (CHM)
Berberine, 187
Cinnamon, 187
formula, 186–187
glucose metabolism, 188, 189
limitations, 187–189
mechanisms of action, 187, 188
physiological mechanisms, 185–186
plantain, 189
side effects, 187, 188
Tanshinones, 187
TCM, 184–185
therapeutic strategies, 186
Chronic stress exposure adaptation, 171
Classic PCOS (phenotypes A and B), 83–84
Clomiphene citrate (CC)
adjuvant infertility therapies, 113–114
adverse events, 111–112
anovulatory infertility treatment, 110
CC-resistant patients, metformin
co-treatment, 144–145
gonadotrophins, 145–147
pretreatment, 145
treatment, 143–144
chemical structure, 110
congenital birth defects, 112
efficacy, 110–111
estrogenic effect, 110
hormone-dependent breast cancer, 109
induced withdrawal bleed, 113
mechanism of action, 110, 115
predictive factors, 110, 111
protocols, 112–113
uncertainty, 114
Combined oral contraceptive (COC) pretreatment, 35, 264, 266, 267, 274
Controlled ovarian hyperstimulation (COH), 259
after first IVF
dual-suppression protocol, 266
GnRH-agonist suppressive protocol, 265
low-dose FSH stimulation protocol, 266
low-dose gonadotrophin stimulation, 264–265
modified ultra-long GnRH-agonist protocol, 266–267
first IVF cycle
COC pretreatment, 264
GnRH antagonist co-treatment, 262–264
GnRH *vs.* multiple-dose GnRH antagonist protocol, 261–262
luteal phase defect, 267
metformin, 260–261
ovarian morphology, 260
strategies, 260
Cord blood androgen studies, 25
Couples with mild male subfertility, 255
Couples with unexplained subfertility, 255–256

D
Daily abdominal acupuncture, 232
Defective deep placentation
classification, 42
vs. normal placental bed, 43
Diagnostic criteria
AE-PCOS Society, 16
AMH assay, 19
ESHRE/ASRM, 14–16
follicle distribution, 18, 19
limitations, 16, 17
NIH, 12–14
patient characterisation, 17
Dietary supplements, 171–173
beneficial effects, 181
cinnamon extract, 183
green and spearmint tea, 182–183
micronutrients, 184
polyunsaturated fatty acids, 183–184
vitamin B_{12} and folate, 182
vitamin D deficiency, 182
ω-3 fatty acids, 183–184

E
Embryo culture, 272, 275, 278
Embryo transfer (ET), 57, 128, 206, 259, 264, 266, 279

Endometrial abnormalities, 4
Endometrial receptivity
abnormal endometrial competence, 51–53
cell adhesion molecules, 48
clinical protocol, 57
decidualization and placentation, 53–55
differential gene regulation, 47
embryo implantation, 56
endometrial apolipoprotein A1 expression, 47
ER expression, 48
folliculogenesis, 46
and functions, 44–48
and glucose metabolism, 55
glucose transporter 4, 48
HOXA10 expression, 46
impairment, subfertile women, 44
implantation, 44
micronized progesterone, 47
molecular analysis, 56
ontogenetic progesterone resistance, 44
progesterone, role, 46
sequential gene expression, 44
subfertility pathogenesis, 45
treatment, 55
Endometrial scratching, 56
Endometrium competence, 8
Epidemiological findings, fertility, 6
Epigenetics, 49, 281, 313
ESHRE/ASRM criteria, 13–16

F
Ferriman-Gallwey scoring system, 12, 13
Fertility
enhancement, PCOS
bariatric surgery, 292–293
behaviour modification, 292
dietary interventions, 291
exercise, 292
insulin resistance, 289
intra-and extra-ovarian factors, 290, 291
IVF cycles management, 300
IVM, 298–300
lifestyle algorithm management, 293, 294
lifestyle therapy, 291
mono-follicular ovulation, 291
ovulation induction methods, 294–298
weight loss, 291
evaluation
BBT measurements, 71
laboratory test, 71–72
medical and reproductive history, 70–71
physical examination, 71
semen analysis, 72–73
transvaginal ultrasound, 72
tubal patency, 72
Follicle excess
AMH regulation, 90, 91, 94–96
clinical role
diagnostic performance, 96, 97
infertility treatment, 98–99
PCOS phenotypes, 97–98
elevated serum anti-Müllerian hormone level, 90
intraovarian hyperandrogenism, 91
ovarian reserve, 89
pathophysiology, 89–91
Rotterdam classification, 91, 93, 99
ultrasound
B-mode ultrasonography, 92–93
magnetic resonance imaging, 94
ovarian polycystic aspect, 91, 92
three-dimensional ultrasonography, 93
Follicular apoptosis defect, 90
Folliculogenesis, 35

G
Gene expression, 41
Genetic approach, oocyte quality, 33–34
Genome-wide association studies (GWAS), 85
Gonadotrophins
administration, 3–4, 124
CC-resistant patients, 145–147
COH after first IVF, 264–265
in human ovarian physiology, 154–156
infertility treatment, 153
metformin, 145–146
mono-ovulation induction, 153
in mono-ovulatory induction, 146–147, 160–161
in multiple ovulatory cycles, IVF procedures, 147
oocyte retrieval, 128
ovarian response, predictive markers, 161–162
release abnormalities, 25
single ovulation, 153
step-down protocol, 159, 160
step-up protocol, 158–160
treatment, women, 156–157
types, 157–158
in unexplained infertility, 127

H
Health-related quality of life (HRQoL), 170–171
Healthy diet, 171–173
Hirsutism, 24, 171, 183, 186
  AE-PCOS, 16
  chronic treatments, 109
  Ferriman-Gallwey score, 12, 13, 71
  NIH/NICHD criteria, 14
  2003 Rotterdam criteria, 15
  signs, 11
Homeostatic model assessment (HOMA) index, 218
Hormonal priming, 271–273
Human ovarian physiology, 154–156
Hyperandrogenemia, 3
Hyperandrogenism, 3, 12, 14, 17, 25–26, 44, 47, 66, 81–85, 94, 97, 98
  anovulation and infertility, 65
  biochemical, 7, 83, 203, 227, 291, 308–309
  central obesity, 66
  definition, 12
  evaluation, 65
  and insulin resistance, 67–68, 200, 213
  metformin, 138
  occurrence, 65
  ovarian environment, 65
  PCOS-related factors, 65
  reproductive abnormalities, 111
  tanshinone, 187
Hyperinsulinaemic insulin resistance, 43, 213, 214, 217, 221, 312

I
Immature oocyte collection, 273–274
Infertility
  AMH assay, 326
  definition, 63
  diagnostic criteria, 326–327
  endometrial receptivity, 68–69
  endometriosis, 64
  evaluation, 63, 64
  fertility evaluation, 70–73
  hyperandrogenism, 64
  integrated strategy of treatment, 328, 329
  irregular menses, 64
  management, 327–328
  oligo-anovulation, 64, 325
  oocytes and embryo quality, 69
  ovulatory disorder, 64
  PCOS-related factors
    anovulation, 325
    characteristics, 64
    hyperandrogenism, 65
    insulin resistance, 67–68
    obesity, 66–67
    phenotypes, 85
  pelvic inflammatory disease, 64
  treatment, follicle excess
    clomiphene citrate, 98
    LOD, 99
    in vitro stimulation, 98–99
  vitamin D/calcitriol, 69–70
Inositols
  abnormal epimerase activity, 216–217
  data efficacy, 222
  effects, women with PCOS
    D-chiro-inositol administration, 218–219
    myo-inositol administration, 219–220
    myo-inositol and D-chiro-inositol monotherapies, 220–221
  as fertility drug, 221–222
  insulin-sensitising effects, 214
  mechanisms of action, 214–216
  post-treatment fertility, 222
  structure, 214, 215
Insulin-sensitising drugs. *See* Metformin
Intrauterine insemination (IUI), 8
  contraindications, 250
  data efficacy, 252–253
  density gradient centrifugation, 251
  indications, 250
  mild male subfertility couples, 255
  swim-up technique, 251
  vs. timed intercourse, 254, 255
  unexplained subfertility couples, 255–256
  in women, PCOS, 250–252
In vitro fertilisation (IVF) cycles, 8, 259
  after first IVF
    dual-suppression protocol, 266
    GnRH-agonist suppressive protocol, 265
    low-dose FSH stimulation protocol, 266
    low-dose gonadotrophin stimulation, 264–265
    modified ultra-long GnRH-agonist protocol, 266–267
  fertility enhancement, 300
  first IVF cycle
    COC pretreatment, 264
    GnRH antagonist co-treatment, 262–264
  luteal phase defect, 267
In vitro oocyte maturation (IVM)
  aneuploidy, 281

birth outcomes, 279–281
embryo culture, 278
epigenetic variation, 281
fertilisation, 278
fertility enhancement, 298, 300
hormonal priming, 272–273
immature oocyte collection, 273–274
multi-follicular growth, 271
oocyte maturation and culture, 274–278
reproductive outcomes, 279
stimulated IVF treatment, 271
transfer/cryopreservation, 279
treatment method, PCOM/PCOS, 272

L
Laparoscopic ovarian drilling (LOD)
AMH assay, 99
in clomiphene citrate resistant patients, 125, 145, 311
efficacy, 196
evidence-based data, 201
fixed *vs.* dose-adjusted energy, 197–198
indications and data efficacy
in CC-resistant PCOS, 199, 200
OHSS, 200, 201
theoretical advantages, 200
mechanism of action, 196–198
metformin, 144, 145
vs. oral ovulation induction treatments, 196
oral ovulatory drugs, 207
ovarian adhesions, 125
ovulation induction, 99, 125
ovulation/pregnancy predictors, 201–203
PCOS-related anovulation, 195
reproductive outcomes, 203
safety concerns
IVF outcomes, 206
ovarian reserve, 204–206
periadnexal adhesions, 204
surgical technique, 196–198
surgical treatment, infertility, 195
unilateral *vs.* bilateral, 197
Lifestyle interventions
bariatric surgery, 176
clinical effectiveness, 174–175
definition, 174
methodological aspects, 177
and natural and assisted reproduction (*see* Assisted reproductive technologies (ARTs))
obesity and infertility, 176–177
structured lifestyle program, 176
therapeutic intervention, 177–178
therapeutic procedure, 176
weight loss, 174
Luteal phase defect, 50, 51, 84, 113, 219, 262, 267
Luteinising hormone/follicle-stimulating hormone (LH/FSH) ratio, 12

M
Metformin
co-treatment, 144–145
data efficacy
in anovulatory infertility, 141–143
in CC-resistant patients, 143–147
infertility diagnosis prevention, 141
drug safety profile, 139–140
gonadotrophins
for mono-ovulatory cycles, 146–147
in multiple ovulatory cycles, IVF procedures, 147
ovarian gluconeogenesis, 138
patients selection, 140
pharmacology, 136–138
pretreatment, 145
therapeutic regimens, 138–139
treatment, 143–144
Morphological approach, oocyte quality, 32–33

N
National Institutes of Health (NIH) criteria, 12–14
Neonatal morbidity, 7
Normoandrogenic PCOS (phenotype D), 84

O
Obesity, 3–4
adiponectin, 67
characteristics, 66
leptin, 66–67
SHBG, 66
Oligo-anovulation, 3
OMICS technologies, oocyte quality, 34–35
Ontogenetic progesterone resistance, 52
Oocyte/embryo
cryopreservation, 128, 206, 267, 279
maturation and culture
coronal cells, 275–278
culture media additives, 274
culture timing, 275–278
hormonal additives, 274
serum/follicular fluid, 274–275

Oocyte/embryo (*cont.*)
quality, 8
body mass index, 36
embryonic development, 32
fecundation, 32
fertilization, 32
genetic approach, 33–34
implantation, 32
meiotic maturation, 32
meiotic/mitotic cell cycle pathway, 37
molecular specificities, 36
morphological approach, 32–33
nuclear maturation status, 32
OMICS technologies, 34–35
pregnancy, 32
pregnancy rates, 32
Ovarian hyperstimulation syndrome (OHSS), 264, 266, 267, 327
complications rates, 114
drainage and hospitalisation, 262
follicle excess, 98
gonadotrophin protocols, 146, 158
IVM, 298, 300
LOD, 195, 200
occurrence, 262
ovarian hyperstimulation, 271
ovulation induction, 260
predictive markers, 99
prevention, 129, 250, 262
Ovulation induction methods
with antiestrogens (*see* Selective estrogen receptor modulators (SERMs))
clomiphene citrate, 294–295
gonadotrophin therapy, 296–297
letrozole, 295–296
management, 298, 299
metformin, 295
ovarian drilling, 297–298
Ovulatory PCOS (phenotype C), 84

P
PCOS phenotypes
classic (A and B), 83–84
and infertility, 85
normoandrogenic (D), 84
ovulatory (C), 84
pathogenesis, 84–85
prevalence, 82–83
Rotterdam criteria, 82
PCOS-related ovulatory dysfunction, 8
Pharmacology, metformin
pharmacodynamics, 136–138
pharmacokinetics pathway, 136
Phytotherapy, 181, 185, 328
Pipelle for Pregnancy (PIP), 56
Placenta function, 7
Polycystic ovarian morphology (PCOM), 3
Pregnancy
acupuncture
depression, 236
labor induction, 236
low-frequency electrical stimulation, 234–235
for pelvic girdle pain, 235
prenatal androgenized rat PCOS model, 235
complications
abnormal inflammatory and metabolic pattern, 313
acetylsalicylic acid, 315–316
causes, 308, 309
clinical data, 306–308
foetal and perinatal outcomes, 307
gestational diabetes mellitus, 306
hormone-independent alterations, 312
hyperandrogenic states, 309
iatrogenic multiple pregnancies, 308
incidence, 308
large for gestational age, 308
lipid abnormalities, 312
low-frequency electroacupuncture, 316
low molecular weight heparin, 315–316
management, 313–316
metformin administration, 315, 316
miscarriage, 306
non-pharmacological intervention, 310
oligo-amenorrhoea, 310
pathophysiology, 308–313
pharmacological measures, 315
pregnancy-induced hypertension, 307
prevention, 313–316
sleep-disordered breathing, 311–312
small for gestational age, 308
Pregnancy in Polycystic Ovary Syndrome I and II (PPCOS-I and PPCOS-II) trials, 5
Preterm delivery (PTD), 7, 52, 307, 308, 310–312, 314, 315
Progesterone resistance, 48–50
abnormal endometrial competence, 51–53
decidualization and placentation, 53–54
embryonic implantation, 51
late-onset preeclampsia, 52
luteal dysfunction, 50
ovulation/corpus luteum formation, 52
ovulatory cycles, 53
subnuclear vacuolization, 52
Psychological disorders, 170–171

R
Raloxifene, 109, 114
Reproductive medicine, 6, 305, 328
Rotterdam or Androgen Excess-PCOS Society criteria, 3, 15

S
Selective estrogen receptor modulators (SERMs)
  clinical role, 109
  clomiphene citrate, 109–114
  raloxifene, 109, 114, 115
  tamoxifen, 109, 110, 114, 115
Semen analysis, 72–73
Signal transduction pathways, 41
Steroidogenesis, 41
Subfertility. *See* Infertility

T
Tamoxifen, 109, 110, 114, 115, 120, 124, 128
Traditional Chinese Medicine (TCM), 184–185
Transvaginal ultrasound (TVS), 12, 72
Tubal disease, 72
Two cell-two gonadotrophin theory, 155