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REVIEW

Polycystic Ovary Syndrome: Implication for Drug Metabolism on Assisted Reproductive Techniques—A Literature Review

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ABSTRACT

Polycystic ovary syndrome (PCOS) affects 6–10% of women and could be considered one of the most common endocrine alterations in women of reproductive age. The syndrome is characterized by several hormonal and metabolic alterations, including insulin resistance and hyperandrogenism, which play a severe detrimental role in the patient's fertility. We aimed to offer an overview about drug metabolism in the PCOS population. Nevertheless, we did not find any study that directly compared drug metabolism between PCOS and healthy women. We therefore decided to summarize briefly how hormonal and insulin sensitizer drugs act differently in healthy and PCOS women, who show altered steroidogenesis by theca cells and metabolic imbalance, focusing especially on assisted reproductive techniques. To date, data about drug metabolism in the PCOS population appears to be extremely

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limited. This important gap could have significant implications for therapeutic approaches and future perspectives: the dosage of drugs commonly used for the treatment of PCOS women should be tailored according to each patient's characteristics; we should implement new clinical trials in order to identify the best pharmacologic strategy for PCOS patients undergoing in vitro fertilization (IVF); it would be advisable to create an international expert panel to investigate the drug metabolism in the PCOS population.

INTRODUCTION

Polycystic ovary syndrome (PCOS) affects 6–10% of women [1] and could be considered one of the most common endocrine alterations in women of reproductive age. This syndrome is characterized by clinical or biochemical hyperandrogenism, oligo-anovulatory cycles and polycystic ovaries. According to the Rotterdam criteria, it is necessary to have two of the three aforementioned criteria to diagnose PCOS [2]. On the other hand, 10 years ago the Androgen Excess-PCOS (AE-PCOS) Society Task Force stressed the importance of hyperandrogenism in PCOS presentation [3], and 3 years later confirmed the presence of hyperandrogenism together with ovarian dysfunction (oligo-anovulation and/or polycystic features) as diagnostic criteria, notwithstanding the necessity of accurate differential diagnosis for other disorders [1].

The exact pathogenetic mechanisms behind different PCOS features are still unclear; however, numerous studies have deepened our understanding, and the causes are linked to steroidogenesis enzyme function and metabolic disturbance related to insulin resistance. Considering these alterations in PCOS women, several drugs and other metabolically active compounds may have different effects in this population group compared with healthy women without PCOS as a result of hormonal and metabolic imbalance. In this literature review we aim to offer an overview about drug metabolism in women with PCOS.

After extensive literature review we did not find any study that evaluated drug metabolism in PCOS compared with healthy women. For this reason, we decided to summarize briefly different pathogenic mechanisms behind PCOS and how hormonal and insulin sensitizer medications act differently in healthy and PCOS women, especially with respect to assisted reproductive techniques.

METHODS

We searched the following electronic bibliographic databases: MEDLINE, EMBASE, Global Health, The Cochrane Library (Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, Cochrane Methodology Register), Health Technology Assessment Database, and Web of Science (science and social science citation index). We included only manuscripts published in the English language, without time restriction. Titles and/or abstracts of studies retrieved using the search strategy and those from additional sources were screened independently by two authors (V.L.L.R., S.G.V.) to identify studies that potentially meet the inclusion criteria outlined above and fall within the study aims. The full texts of these potentially eligible studies were retrieved and independently assessed for eligibility by other two authors (G.V., S.C.). Any disagreement between them over the eligibility of particular studies was resolved through discussion with a third author (M.B.). A standardised, pre-piloted form was used to extract data from the included studies for assessment of study quality and evidence synthesis. The searches were rerun just before the final analyses, in order to include further studies for inclusion.

On the basis of the aim of the current paper, we first searched "polycystic ovary syndrome AND drug metabolism", "polycystic ovary syndrome AND pharmacokinetics", "polycystic ovary syndrome AND pharmacodynamics":

from this first search, we did not identify any relevant study comparing directly drug metabolism between PCOS and healthy women. Subsequently, we searched "polycystic ovary syndrome AND substrate" and we found 135 items that were not pertinent to our topic; in addition, searching "pharmacokinetics OR pharmacokinetics AND polycystic ovary syndrome" afforded 173 items, but only one study pertinent to our topic, a study of pregnant women affected by PCOS [4]. Surprisingly, we did not find any study that directly compared drug metabolism between PCOS and healthy women. We therefore decided to summarize briefly how hormonal and insulin sensitizer drugs act differently in healthy and PCOS women, who show intrinsic altered steroidogenesis by theca cells and metabolic imbalance, focusing especially on assisted reproductive techniques.

This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

Steroidogenesis Alteration in PCOS

Usually androgens have only been considered as detrimental hormones in oocyte maturation; however, recently their in follicle maturation has been re-evaluated. New evidence showed that androgen, via genomic and non-genomic signal transduction, induced oocyte maturation, especially in the early stage of follicle maturation [5, 6]. Androgen acts synergistically with follicle-stimulating hormone (FSH), modifying steroidogenic enzymes and metabolism in granulosa cells [6]. To confirm these results, treatment of follicles with anti-androgenic drugs was shown to alter and arrest oocyte meiotic maturation. Nevertheless, excess androgen has detrimental effects on oocyte maturation.

Hyperandrogenism occurs in up to 70–80% women affected by PCOS and it is extremely frequent during adolescence. The aetiology of hyperandrogenism during PCOS is not completely understood. In normal women, androgens are synthesized by ovarian theca cells and adrenal glands, contributing half and half to circulating testosterone during reproductive age

[7]. Interestingly, hyperandrogenism in PCOS appears to be related to dysregulation of steroidogenesis in both ovary and adrenal glands. Luteinising hormone (LH) also plays a pivotal role in the regulation of ovarian androgen production, whereas adrenocorticotropic hormone (ACTH) regulates androgen production by adrenal glands. In addition, several enzymes of the cytochrome P450 family are involved in hormonal balance [8] including cholesterol side-chain cleavage enzyme (P450 scc), aromatase (P450 arom), 3β-hydroxysteroid dehydrogenase type II (3βHSD-II) and steroid acute regulatory protein.

Several studies confirmed the reduction of aromatase activity in ovaries from hyperandrogenic PCOS women with respect to normoandrogenic ones [9, 10], leading to a significant reduction of oestradiol/testosterone ratio. Interestingly, aromatase activity is enhanced by oestrogens and inhibited by androgens [10]. One study found a reduction of 3βHSD-II activity in hyperandrogenic PCOS women compared to normoandrogenic PCOS ones [11]. Other data showed increased 11β-hydroxysteroid dehydrogenase II (11β-HSDII) activity to convert dehydroepiandrosterone (DHEA) into androstenedione in hyperandrogenic PCOS women compared to normoandrogenic ones and controls [9].

Steroidogenesis is overactive at multiple steps in PCOS, especially concerning 17,20-lyase activity [12]: indeed, accumulating evidence suggests that androstenedione/17-OH progesterone ratio in response to gonadotropin-releasing hormone (GnRH) stimulation is lower in PCOS women compared with healthy controls, probably as a result of the lower activity of 17α-hydroxylase [13].

Recent data confirmed increased activities of steroidogenic enzymes in hyperandrogenic PCOS women, although the regulation of delta (Δ)-4 and Δ-5 enzymes seems different. On the one hand, the 17α-hydroxylase [14] and 17,20-lyase [8, 14] activities in the Δ-4 pathway of hyperandrogenic PCOS women lead to higher production rates of 17α-hydroxyprogesterone from progesterone, and androstenedione from 17α-hydroxyprogesterone, with respect to normoandrogenic PCOS women; on the other hand,

both hyperandrogenic and normoandrogenic PCOS women have similar activity of the Δ-5 pathway [9, 14]. In partial agreement with these results, others showed that oestrogens may activate 17,20-lyase, increasing only the production rate of androstenedione (Δ-4 pathway) [15], or both androstenedione and DHEA (Δ-4 and Δ-5 pathways) [10], even though not all studies confirmed this hypothesis [14]. Nevertheless, after diagnostic criteria standardization, robust data proved that the phosphorylation of serine/threonine residues, as well as cytochrome b_5 and cytochrome c allosteric actions were all involved in 17,20-lyase regulation [16, 17]. Not surprisingly, the dysregulation of the cytochrome P450 17α-hydroxylase (P450c17α) and consequent increase in 17,20-lyase activities were observed only in PCOS.

Hyperinsulinemia in PCOS

Hyperinsulinemia plays key role in the pathogenesis of PCOS and related hyperandrogenism. In particular, some studies showed a decrease of insulin sensitivity from 35% to 40% [18, 19]. Despite the typical insulin resistance of PCOS, previous studies found that ovarian theca cells had hypersensitivity to insulin stimulation and increase androgen production in response to it [20–22]. This point is particularly debated: some studies showed that insulin alone is able to stimulate testosterone production by cultured theca cells from normal and PCOS patients [22]; conversely, others found that insulin alone is not able to stimulate testosterone production [20]. Similarly, porcine theca cell culture showed increased expression of cytochrome P450 17alpha-hydroxylase/17,20-lyase (CYP17) after insulin and LH stimulation [23], whereas others failed to confirm these results [24].

Interestingly, many studies showed that prolonged suppression of insulin in PCOS women by weight loss [25], diazoxide [26], statins or metformin [27–29] results in significant reduction of androgen production. Diazoxide-mediated reduction of insulin level was also found even in lean normoinsulinemic PCOS women [30], but the same did not occur for testosterone levels [31].

The elevated LH in PCOS women is able to stimulate steroidogenesis within theca cells. In addition, theca cells were shown to be abnormally sensitive to LH stimulation, although downregulation of LH receptors may occur [32].

Therapeutic Approaches

Assisted Reproductive Techniques

Female infertility has increased in the last decades: recent data from the Centers for Disease Control (CDC) National Survey of family growth show that 6% of women aged between 15 and 44 years of childbearing age are infertile and 12% have impaired fertility [33]. Aging is one of the principal factors that play a detrimental role in female fertility as a result of reduced quality of oocytes, ovulatory dysfunction and other gynaecological disorders [34, 35]. Several cohort studies on large populations found that oocyte quality and embryo development may be severely affected by PCOS [36]. In particular, accumulating evidence suggests that the number of small preantral follicles is increased in anovulatory PCOS women: the excess of these types of follicles may be due to accelerated follicle growth and/or prolonged survival (reduced atresia) of small follicles [37]. Interestingly, others found that granulosa cells from ovulatory PCOS women have similar response to FSH with respect to healthy women; conversely, granulosa cells isolated from some small to medium-sized antral follicles obtained from anovulatory PCOS women showed increased oestradiol production in response to FSH and premature responsiveness to LH [38, 39]. This process seems to allow overexpression of LH receptors and premature luteinisation of granulosa cells [40], which could be considering a leading cause of the arrest of follicular development in anovulatory PCOS women. Nevertheless, some authors found that follicles from PCOS women undergoing GnRH analogue/FSH stimulation for in vitro fertilization (IVF) show normal luteinisation [40].

Recently, PCOS phenotypes were further investigated and this approach has improved our knowledge about hormonal/metabolic dysregulation, especially in ovulatory/

hyperandrogenic PCOS women and ovulatory/normoandrogenic ones [38]. According to recent evidence, hyperandrogenic women with PCO on ultrasound are at high risk of developing hyperstimulation syndrome during ovulation induction [41]. In addition, ovulatory women with PCO did not show worse IVF/intracytoplasmic sperm injection (ICSI) outcomes (including live birth rate) compared to healthy controls [42].

Some studies found that PCOS women undergoing IVF had lower FSH, the oocyte retrieval was higher and the fertilization rate slightly lower compared with controls, without any significant difference regarding embryo arrest (EA) rate [43], regardless of stimulation protocol used and testosterone level. Although others confirmed that there is no difference in EA, clinical abortion rate in PCOS women seems to be higher than in healthy controls [44].

To date, it is widely accepted that hyperandrogenism, high LH level and insulin resistance may all play a detrimental role in oocyte quality [45–47]: these alterations seem to be particularly pronounced in the zona pellucida of oocytes from PCOS women and this may account, at least in part, for the lower fertilization rate observed during ICSI [48].

However, robust data about this topic are still lacking and the evidence published so far does not allow one to draw a firm conclusion about the best management of hormonal/metabolic dysregulation in PCOS patients undergoing IVF. Although a recent meta-analysis [49] demonstrates similar pregnancy and live birth rates per cycle (despite an increased cancellation rate, but more oocytes retrieved per retrieval and a lower fertilization rate) in PCOS women undergoing IVF, we truly think that future pharmacologic strategies for assisted reproductive technologies should be tailored according to the patient's characteristics and, most importantly, PCOS phenotype.

Insulin Sensitizer Drugs

Recent data suggested that the reduction of insulin level with diazoxide or insulin sensitizer drugs such as metformin, thiazolidinediones (PPAR-γ agonist) and inositols also reduces hyperandrogenism [50–52]. Because insulin resistance and secondary hyperinsulinemia play an important role in hyperandrogenism, anovulation or irregular cycles, and metabolic alterations in both lean and obese PCOS patients, insulin sensitizers are currently receiving increasing attention for pharmacologic management of this syndrome [53]. Metformin's effect appears to be mediated by reduction of insulin level, both in normal weight and obese women, improving also hyperandrogenism [54–57]. Some authors showed that metformin acts directly on ovarian steroidogenesis in a dose-dependent manner: gonadotropin and insulin stimulate progesterone and oestradiol production by theca cells, whereas metformin inhibits androgen production with no effect on progesterone [57].

Beside metformin, to date inositols have also received increasing attention owing to their safety profile and key role in modulating secondary messenger activity in insulin signal transduction pathways [58]. Inositol is a polyalcohol, it is classified as an insulin sensitizer and it is a secondary messenger in insulin signalling [58, 59]. We know that two different stereoisomers are currently used in the treatment of PCOS: myo-inositol (MI) and D-chiro-inositol (DCI). Both stereoisomers have an insulin-like action in vivo. In humans, accumulating evidence suggests that MI and DCI have clear and beneficial effects on oocyte maturation and metabolic pathways [59, 60]. Confirming the latter role, others found that DCI levels were about 50% lower in tissue and urinary samples from patients with type 2 diabetes compared to those in healthy controls [61]. Both MI and DCI were therefore evaluated in PCOS women and shown to ameliorate hormonal and metabolic parameters [62, 63]. Although the last Cochrane systematic review about the topic does not offer a robust recommendation about the best treatment for PCOS among insulin sensitizer medications [64], a recent study did not find any significant difference between metformin and MI in lowering body mass index, ameliorating insulin sensitivity and improving the menstrual cycle [65].

Robust data underline the importance of MI in oocyte differentiation and the possibility to improve IVF outcomes in PCOS women [66–68]:

in particular, the direct correlation between oocyte quality and follicular fluid concentration of MI was demonstrated both in animal models and humans [69, 70]. Unfer et al. compared MI with DCI in patients with PCOS, finding that MI had the capacity to ameliorate the quality of oocytes, to decrease the number of immature oocytes and to improve the number of good quality embryos compared to DCI treatment during an ovarian stimulation protocol [67]; others found that in patients under 35 years old, MI plus DCI improved the fertilization rate [68].

Administration of either MI (4 g/day) or DCI (1 g/day) together with folic acid reduced androgens, LH level, LH/FSH ratio and insulin sensitivity in PCOS women [71–73]. MI seems to have the most marked effect on metabolic parameters, whereas DCI seems to have the most marked effect on hyperandrogenism [74–79]. According to several studies, MI is able to increase oocyte quality, reducing the rate of immature oocytes, during IVF [62, 63, 74]. Last but not least, MI seems able to reduce the days of FSH stimulation, oestradiol level before human chorionic gonadotropin administration and risk of hyperstimulation syndrome [63]. Once again, we take the opportunity to underline that future pharmacologic strategies should target insulin resistance selectively according to each patient's characteristics. To date we are able to measure insulin resistance, for example using the homeostasis model assessment, so that we would solicit future tailored treatment of PCOS women.

To date we have a number of studies on insulin sensitizer therapies to assess fertility outcomes in women with PCOS; there were no significant differences in live birth or fetal outcomes to recommend their use in routine clinical practice.

New Possible Targets

A recent mathematical review of microarray data in patients with PCOS showed that 504 genes are differently expressed, accounting for the altered hormonal/metabolic pathways in this syndrome [80]. Some authors found that the level of serological thymidine kinase 1 (STK1), a protein kinase involved in liver metabolism of metformin, was strictly related to the ovulation response after treatment with metformin and, additionally, the response differs according to PCOS phenotypes [81]. Similarly, other gene expressions may play pivotal roles in addressing altered drug action(s) during PCOS [82]. For example, the adipokine chemerin physiologically binds it receptor, chemochine receptor like-1, and inhibits FSH-induced steroidogenesis; according to recent evidence from a mouse model, chemerin is able to suppress FSH-induced progesterone and oestrogen secretion in preantral follicles and granulosa cells, also inhibiting aromatase expression [83]. Another possible strategy could be to target phosphatidylinositide 3-kinase (PI3K): indeed, recent data showed that the flavonoid quercetin may have a beneficial effect in PCOS by virtue of inhibition of PI3K, which was attributed to a decrease in the expression of CYP17A1 gene, and thereby has a key role in steroidogenesis [84].

CONCLUSIONS

To date, PCOS could be considered as a multifactorial disease due to concurrent hormonal and metabolic alterations that influence each other. Insulin resistance represents one of the key elements of the syndrome and causes multiple effects: on the one hand it increases androgen production by theca cells, on the other hand it alters the follicle maturation and reduces oocyte quality. On the basis of this scenario, insulin resistance rapidly became an important target and several trials found beneficial effects of insulin sensitizer drugs on hormonal, metabolic and even reproductive outcomes in the PCOS population. Despite these important therapeutic improvements, we should consider that the PCOS population may have significantly different responses to several classes of drugs as a result of a unique hormonal/metabolic environment. The situation could be even more complicated if we consider that PCOS could not be identified as a single syndrome, but as different phenotypes

according to clinical and biochemical characteristics. To date, data about drug metabolism in the PCOS population appears to be almost non-existent or at least extremely limited. This important gap in the literature could have significant implications for therapeutic approaches and future perspectives: first of all, the dosage of drugs commonly used for the treatment of PCOS women should be tailored according to each patient's characteristics; second, we should implement new clinical trials in order to identify the best pharmacologic strategy for PCOS patients undergoing IVF; finally, it would be advisable to create an international expert panel to investigate the drug metabolism in the PCOS population.

In addition, we take the opportunity to solicit new research aimed to also explore how different classes of drugs influence the epigenetic up- and downregulation of genes related to steroidogenesis and insulin transduction signalling, especially through genome-wide association studies (GWAS).

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Compliance with Ethics Guidelines. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

Data Availability. Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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