GYNECOLOGY

Menstrual preconditioning for the prevention of major obstetrical syndromes in polycystic ovary syndrome

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The presence of multiple ovarian cysts, anovulation, and endometrial progesterone resistance in the neonate seems remarkably similar to ovarian and endometrial features of the polycystic ovary syndrome (PCOS) of adolescent and adult women. In fact, in the absence of cyclic menstruations after menarche, the neonatal progesterone resistance is likely to persist and adversely affect young women with PCOS at the time of pregnancy after induction of ovulation, because any persisting defect in progesterone response can interfere with the process of decidualization and trophoblast invasion. The primigravid woman with PCOS therefore is likely to be at risk of defective deep placentation as manifested by the increased risk of major obstetric syndromes. A recent, large epidemiologic study has demonstrated that the risk of preeclampsia and preterm delivery is elevated in the 13- to 15-year old group, although it does not persist in the 16- to 17-year old group. It is proposed therefore that induction of ovulation in the infertile nulligravid woman with PCOS should be preceded by a period of progesterone withdrawal bleedings to achieve full endometrial progesterone response by the time of pregnancy. The cyclic administration of clomiphene citrate for a period to be determined by vascular response may be an appropriate tool to reduce the risk of major obstetric syndromes by menstrual preconditioning.

Key words: clomiphene citrate, endometrium, metformin, polycystic ovary syndrome, preeclampsia, preterm birth, progesterone resistance

The polycystic ovary syndrome (PCOS) is among the most common female endocrine disorders, which occurs in 4-18% of reproductive-age women worldwide.[1] The syndrome is a complex metabolic and endocrine disorder that is associated with the presence of hyperandrogenemia, insulin resistance, obesity, infertility, and obstetric complications. As a consequence, it may have significant implications for the long-term physical and reproductive health of affected women. In view of the heterogeneity of the syndrome and the lack of understanding of its pathogenesis and mechanisms of action, it is not surprising that, even after 3 consensus meetings, the criteria to diagnose PCOS remain unsettled. Currently, PCOS is diagnosed by the presence of $\geq$2 of the following features: chronic oligo- or anovulation, clinical or biochemical evidence of androgen excess, and the presence of polycystic ovaries on sonographic examination.[2]

Generally, the belief is that, in most cases of PCOS, infertility results from the absence of ovulation; at the same time, it has also been recognized that anovulation may not be the only reason for the failure to conceive.[3] Indeed, there is evidence that infertility in women with PCOS cannot be attributed to anovulation only but also to endometrial dysfunction. A recent endometrial biopsy study by Lopes et al[4] showed that conventional doses of progesterone may not be enough to correct PCOS-associated changes in the endometrial histomorphologic condition and the receptivity markers. It is a fact that, despite the ability to correct ovulatory disorders in PCOS, pregnancy rates remain paradoxically low, and spontaneous pregnancy loss rates are high.[5] Once a woman with PCOS has conceived, her problems are not over because she will be at a higher risk of miscarriage, both after spontaneous or assisted conception (ART).[6] A recent Cochrane-based data review[7] on the use of metformin (an oral antidiabetic drug used to reduce insulin resistance) evidenced that (1) there is no conclusive evidence that metformin treatment before or during ART cycles improves the live birth rates in women with PCOS who undergo ovulation induction or in vitro fertilization and (2) its use increases clinical pregnancy rates and decreases the risk of ovarian hyperstimulation syndromes.

In this clinical opinion, we focus on a new theory of the pathogenesis of major obstetric complications that have been associated with PCOS to improve our understanding and potentially the management of these obstetric complications in women who are affected by PCOS.

Methods

The literature was searched via Scopus and PubMed for the following key words: *polycystic ovary syndrome*, *progesterone resistance*, and *metformin* in combination with *endometrium*, *menstrual preconditioning*, *pregnancy*, *trophoblast*,

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preeclampsia, or *preterm delivery*. In addition, the references were examined in published papers on related topics.

Pregnancy complications in PCOS

Several studies have documented an association between PCOS and major obstetric complications, particularly preeclampsia and preterm birth. A metaanalysis of pregnancy outcomes in women with PCOS demonstrated a significantly higher risk of the development of gestational diabetes mellitus, pregnancy-induced hypertension, preeclampsia, and preterm birth (Table 1).[8] An exhaustive review of the literature that assessed pregnancy outcomes and the effect of metformin treatment among women with PCOS by Ghazeeri et al[9] concluded that the weight of available evidence suggests that pregnant women with PCOS are at increased risk of the development of preterm birth and hypertensive disorders of pregnancy, with a prevalence of 6-15% for preterm birth, 10-30% for gestational hypertension, and 8-15% for preeclampsia. The authors concluded that metformin has proved to be effective in improving ovulation and pregnancy rates among patients who receive fertility-enhancing agents and supports its use among anovulatory women with PCOS. However, the continuation of metformin throughout pregnancy remains controversial.

A population-based cohort study of the risk of adverse pregnancy outcomes in women with PCOS found that, in singleton births, PCOS was associated strongly with preeclampsia (adjusted odds ratio, 1.45; 95% confidence interval [CI], 1.24−1.69) and very preterm birth (adjusted odds ratio, 2.21; 95% CI, 1.69−2.90).[10] A systematic review that involved 2544 patients with at least 2 features of the 2003 Rotterdam criteria for PCOS[11] and 89,848 patients without PCOS confirmed that women with the syndrome had significantly higher rates of gestational diabetes mellitus, pregnancy-induced hypertension, preeclampsia, preterm delivery, and small-for-gestational-age infants.[12] A 4-fold increase in the risk of pregnancy-induced hypertension linked to arterial wall stiffness has also been observed in these patients. The risk of preeclampsia, which is the most severe of all complications, is also 4 times higher in those who experience PCOS.[13]

TABLE 1
Major obstetric syndrome in women with polycystic ovary syndrome

Syndrome	Odds ratio	95% confidence interval
Pregnancy-induced hypertension	3.67	1.98−6.81
Preeclampsia	3.47	1.16−2.62
Preterm birth	1.75	1.16−2.62
Gestational diabetes mellitus	2.94	1.70−5.08
Perinatal death	3.07	1.03−9.21

Boomsma et al.[8]

Brosens. Menstrual preconditioning in polycystic ovary syndrome. Am J Obstet Gynecol 2015.

A MEDLINE search on relevant trials by Zheng et al[14] found that, in pregnant women with PCOS, the pooled odds ratio was 0.32 (95% CI, 0.19−0.55) for early pregnancy loss, 0.37 (95% CI, 0.25−0.56) for gestational diabetes mellitus, 0.53 (95% CI, 0.30−0.95) for preeclampsia, and 0.30 (95% CI, 0.13−0.68) for preterm delivery. The authors concluded that metformin therapy throughout pregnancy could decrease pregnancy-related complications in pregnant women with PCOS with no serious detrimental side-effects.

An epianalysis of 2 randomized, controlled trials that included 313 women with PCOS who were 18-42 years old and who had singleton pregnancies performed by Vanky et al[15] showed that the metformin-treated patients had fewer late miscarriages/preterm deliveries. At the same time, there was no difference in the prevalence of gestational diabetes mellitus and preeclampsia between the metformin and the placebo groups. The authors suggested that further randomized studies should be performed before firm conclusions can be drawn.

Endometrial progesterone resistance in PCOS

The concept of "progesterone resistance" implies that, in certain individuals, there is a decreased responsiveness of target tissues to bioavailable progesterone.[16] In recent years, the concept has been investigated particularly in women with endometriosis.[17] There is increasing evidence that an impaired progesterone response can be found in the endometrium of women with PCOS. Gregory et al[18] demonstrated that the expression of the p160 steroid receptor coactivators, which serve as transcriptional coactivators for a number of nuclear and nonnuclear receptors, is regulated in the endometrium during the menstrual cycle in normal fertile women but is over-expressed in the endometrium of women with PCOS. Cermik et al[19] investigated the up-regulation of the homeobox gene *HOXA10* that is necessary for the receptivity to embryo implantation. In vitro findings and endometrial biopsy specimens that were obtained from women with PCOS show that testosterone decreases HOXA10-messenger RNA, which leads to the conclusion that diminished uterine *HOXA10* expression may contribute to the diminished reproduction potential of women with PCOS. A review of endometrial aspects of the "window of implantation" in women with PCOS that focused mainly on adhesion molecules suggested that endometrial receptivity seems to be the major limiting factor for the establishment of pregnancy.[4]

Savaris et al,[20] who compared gene expression between endometrial samples of normal fertile control subjects and women with PCOS, concluded that existing differences in gene expression provide evidence of progesterone resistance in midsecretory PCOS endometrium, independent of clomiphene citrate (CC). It can also explain differences that were observed in this group of

women in phenotypes of hyperplasia, cancer, and poor reproductive outcomes. In an in vitro experiment, Kajihara et al[21] investigated the effect of androgens on the expression of genes that are involved in oxidative stress resistance in decidualized human endometrial stromal cells. These cells that were isolated from hysterectomy specimens were decidualized with 8-bromo cyclic adenosine monophosphate and progesterone in the presence or absence of dihydrotestosterone at various concentrations. The authors 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. Recently, in the endometrium of patients with PCOS, Yan et al[22] showed differences in *FADD* (a gene that plays a role in cell proliferation, cycle regulation, and development) and *BCL-2* (a gene that encodes a protein that blocks the apoptotic death of some cells such as lymphocytes) expression during the window of implantation. They suggested that the decrease in cell apoptosis during the implantation window in patients with PCOS may be 1 of the causes of reduced endometrial receptivity.

Finally, a recent review of endometrial progesterone resistance in women with PCOS concluded that progesterone-mediated signaling pathways of expression, regulation, and signaling in the nucleus are involved.[23]

Neonatal progesterone response resistance

Throughout pregnancy, the fetus is exposed to high plasma concentrations of unbound estrogens and progesterone. Progesterone in the fetal circulation rises to reach much higher values than in the maternal circulation because of the dehydrogenase activity of the endothelial cells of the placental circulation.[24] Ober and Bernstein[25] carefully investigated neonatal ovaries and uteri in a series of 169 autopsies and observed that, in newborn infants, ovaries are frequently polycystic, but failed to show any sign of ovulation or corpus luteum formation. In the uteri, they described in detail the response of the fetal endometrium to the high circulating progesterone levels and classified this response as null (proliferative or inactive) in 68% of their cases, partial or early response (subnuclear vacuolization) in 27%, and full (decidualization or menstrual-like shedding) in only 5%. Thus, remarkably, at birth most neonates satisfy the current criteria for the diagnosis of PCOS by the presence of polycystic ovaries, anovulation, and progesterone-resistant endometrium.[26]

It can be speculated that the type of progesterone resistance that is present in the endometrium at birth is likely to persist until the onset of puberty when endogenous estrogens begin to stimulate endometrial cells.[26,27] Although full progesterone response with "neonatal menstruation" has been linked to pelvic endometriosis in premenarche and adolescence,[26,27] a persisting degree of progesterone resistance of the endometrium after menarche can be linked to defective deep placentation and major obstetric disorders, which include preeclampsia, fetal growth restriction, and preterm birth.[28,29]

Menstrual preconditioning reduces progesterone resistance

The concepts of "ontogenetic progesterone resistance" and of "menstrual preconditioning" infer that the human uterus may start out as a relatively immature organ that acquires the competence for deep placentation in response to dynamic remodeling events triggered by menstruations, miscarriage, or parturition.[30] Menstrual preconditioning implies that progesterone withdrawal bleedings or menstruations 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 that are associated with deep placentation.

It is conceivable that, in most young girls, ontogenetic progesterone resistance may persist until menarche and that full progesterone-responsiveness is achieved only gradually after the onset of cyclic menstruations. Al-Sabbagh et al[31] 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 such as endometriosis, is not only a consequence of perturbed progesterone signal transduction caused by chronic inflammation but also 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 physiologic preconditioning that prepares uterine tissue for the dramatic vascular remodeling that is associated with deep placentation. Indeed, deep placentation involves the remodeling of the spiral arteries in the placentation zone, which includes the endometrial and, most critically, the myometrial segments. It is well accepted that the pathogenesis of late onset preeclampsia in the primigravid woman is linked with defective deep placentation, which is defined by a restricted remodeling of the myometrial segments of the spiral arteries in the placental bed.[32]

Defective decidualization and trophoblast invasion in PCOS

Decidualization is described as the postovulatory process of endometrial remodeling in preparation for pregnancy, which includes secretory transformation of the uterine glands, influx of specialized uterine natural killer cells, and vascular remodeling. A more restricted definition of the decidual process denotes the morphologic and biochemical reprogramming of the endometrial stromal compartment. This differentiation process is dependent entirely on the convergence of cyclic adenosine monophosphate and progesterone signaling pathways that drives integrated changes at both the transcriptome and the proteome level.[33]

Decidualization of stromal cells precedes and regulates trophoblast invasion to resist inflammatory and oxidative insults and to dampen local maternal immune responses. Jindal et al[34] suggested that the spectrum of maternal and fetal complications that are associated with PCOS may be related to impaired trophoblast invasion in the placental bed.

In a case-control study, Rabaglino et al[35] used a bioinformatics approach and found evidence for impaired endometrial maturation in early pregnancy in women who subsequently experienced preeclampsia. Palomba et al[36] in an experimental case-control study collected trophoblastic and decidual tissue after pregnancy termination during the week 12 of gestation in women with and without PCOS. The rate of implantation site vessels with endovascular trophoblast invasion and the extent of endovascular trophoblast invasion were significantly lower in patients with PCOS, compared with healthy non-PCOS subjects.

In a macroscopic and microscopic study, Palomba et al[37] investigated the placenta from women with PCOS, excluding obese patients who achieved a pregnancy after the use of ovulation induction or ART. They showed that placental weight, thickness, density, and volume were significantly inferior in women with PCOS, compared with those without PCOS. Also, the percentage of patients with placental lesions and the mean number of these lesions were higher in the PCOS group than in the control group.

A third study by the same group attempted a matched-control evaluation of the type of phenotype of PCOS that is associated with placentation disorder, again excluding obese patients who achieved a pregnancy after the use of ovulation induction or ART.[38] They found that placental weight, thickness, density, and fetoplacental weight ratio were significantly different in the full-blown PCOS and nonpolycystic ovary phenotypes vs the ovulatory and nonhyperandrogenic phenotypes. The incidence of macroscopic placental lesions was only significantly different between control subjects and the full-blown and nonpolycystic phenotypes. The overall incidence of microscopic placental lesions was significantly different among PCOS phenotypes and was significantly higher in the full-blown and nonpolycystic phenotypes than in the ovulatory and nonhyperandrogenic phenotypes.

A major limitation of these placental studies is that (1) they are based on the basal plate of the placenta, which represents the battlefield between decidua and trophoblast, and, as such, is rather a poor area for assessing deep invasion, and (2) biopsy specimens from the center of the placenta may not be representative for deep invasion because decreased invasion is not observed in the central but in the paracentral region.[39]

TABLE 2
Comparison of preeclampsia and preterm delivery in polycystic ovary syndrome and teenager groups

Variable	Polycystic ovary syndrome[a]	13-15 years old[b]	16-17 years old[b]
Preeclampsia	3.5 (1.9–6.2)	2.5 (1.1–5.8)	0.7 (0.5–1.0)
Preterm delivery	1.8 (1.2–2.7)	3.0 (1.6–5.7)	1.1 (0.9–1.5)

Data presented as odds ratio (95% confidence interval).

[a] Boomsma et al[8]; [b] Leppälathi et al.[40]

Brosens. Menstrual preconditioning in polycystic ovary syndrome. Am J Obstet Gynecol 2015.

Menstrual preconditioning to improve pregnancy outcome

As stated, preeclampsia and preterm birth are major obstetric risks in women with PCOS and are characterized by defective deep placentation.[39] It has been shown that insufficient or defective maturation of endometrium and decidual natural killer cells during the secretory phase and early pregnancy precede the development of preeclampsia[35]; in addition, the defective or restrictive trophoblast invasion of the spiral arteries can be explained by the progesterone resistance in women with anovulatory PCOS.[36,37] Therefore, it seems plausible that, in young women with PCOS, the presence of ontogenetic progesterone resistance, combined with the absence of menstrual preconditioning constitutes a risk factor for preeclampsia and preterm delivery. A recent large epidemiologic study demonstrated that the risk of preeclampsia and preterm delivery is high in 13- to 15-year-old pregnant teenagers and is normalized in the 16- to 17-year-old pregnant teenager[40] (Table 2). This is in agreement with the gradual increase of ovulatory cycles from 49% at 1 year to 86% at 5 years after the menarche.[41] Therefore, the high risk of preeclampsia and preterm birth in PCOS after induction of ovulation in young subjects can be explained by the absence of menstrual preconditioning and the persistence of ontogenetic progesterone resistance at the time of ovulation induction.

TABLE 3
Prospective trials of clomiphene citrate vs metformin

Authors	Trial	Medication	Ovulation, %	Recommendation[a]
Palomba et al[43]	PnRT	MF + CC vs CC	62.9 vs 67.0	Both first-line options
Moll et al[44]	RT	MF + CC vs CC	64 vs 72	Both effective; higher incidence of side-effects with MF
Legro et al[3]	RT	CC vs MF	49 vs 29	CC superior to MF
		MF + CC	60.4	
Zain et al[45]	RT	CC vs MF	59 vs 23.7	CC the first-line treatment for induction of ovulation
		MF + CC	68.1	

CC, clomiphene citrate; *MF*, metformin; *PnRT*, prospective non-randomized trial; *RT*, randomized trial.

[a] As first choice for the induction of ovulation in women with anovulatory polycystic ovary syndrome.

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Several randomized studies have demonstrated the efficiency of CC in comparison with metformin for the induction of ovulation in oligo- or anovulatory women (Table 3). Based on the results of a randomized, double-blind clinical trial, Moll et al[44] proposed to use CC as a primary method for the induction of ovulation rather than metformin or to add metformin to CC. Zain et al[45] confirmed in an Asian randomized-controlled study that CC is superior to metformin in inducing ovulation in anovulatory women with PCOS.

When deciding on the best method to induce ovulatory cycles in young patients with PCOS, several considerations are in order. First, treatment with CC is relatively safe, although it has been questioned whether its long-term use may alter the risk of ovarian cancer. Some 20 years ago, Rossing et al,[46] who evaluated a cohort of 3837 women who had been treated for infertility over an 11-year period, found a relative risk of invasive or borderline malignant ovarian tumors of 2.3 (95% CI, 0.5−11.4). In a further analysis, they found that the use of the drug during ≥12 cycles was associated with an increased risk of ovarian tumors among both women with ovarian abnormalities and those without apparent abnormalities (relative risk, 11.1; 95% CI, 1.5−82.3). In contrast, taking CC for <1 year did not lead to an increased risk. Some 10 years later, another large retrospective cohort study by Brinton et al[47] observed a rate ratio of 0.82 (95% CI, 0.4−1.5) in ever users of CC. This rate increased, although in a nonsignificant way, with long follow-up; after ≥15 years, it became 1.48 (95% CI, 0.7−3.2). Finally, a very recent study by Bjørnholt et al[48] analyzed data from a cohort of 96,545 women with fertility problems from all Danish fertility clinics for the years 1963-2006. They found that the overall risk for borderline ovarian tumors was not associated with the use of CC (relative risk, 0.96; 95% CI, 0.64−1.44).

Second, the aforementioned study by Moll et al[44] concluded that metformin may be a relatively safe medication but that it is associated with a high incidence of side-effects. At the same time, recent preliminary studies have demonstrated that metformin has the potential to reduce the risk of adverse pregnancy outcomes in women with PCOS.[15,49]

Third, the question arises how to best monitor the use of CC or metformin to induce ovulatory cycles in achieving full maturation of progesterone response in the spiral arteries before attempting pregnancy. The most direct method at present is the estimation of blood flow in the spiral arteries at their origin in the myometrial junctional zone and in the endometrium. Yang et al[42] were the first group to use a modified color Doppler technique to determine the outcome of in vitro fertilization by measuring endometrial blood flow. Women with adequate endometrial thickness, but a small intraendometrial power Doppler area, tended to have an unfavorable reproductive outcome. For clinical applications, Malhotra et al[50] recommended to estimate by color Doppler sonography the junctional zone vascular response during the mid-luteal phase of the induced ovulatory menstrual cycle. In a prospective clinical study, Kim et al[51] demonstrated that 3-dimensional−power Doppler ultrasound scanning was useful for the evaluation of endometrial and subendometrial neovascularization in intrauterine insemination cycle. A recent prospective study confirmed that the presence of subendometrial-endometrial blood flow improved cycle outcome in frozen-thawed embryo transfer cycles.[52] The clinical studies suggest that 3-dimensional−power Doppler ultrasound scanning can be used during induced ovulatory cycles to monitor the stage of progesterone response by the presence and extent of subendometrial-endometrial blood flow.

Conclusion

In the human, menstruations or cyclic progesterone withdrawal bleedings may play a role in the preconditioning or maturation of the endometrial progesterone response. Therefore, the hypothesis has been formulated that the woman with full blown anovulatory PCOS who attempts a first pregnancy in the absence of preceding cyclic menstruations is likely to be exposed to ontogenetic endometrial progesterone resistance with increased risk of miscarriage, preeclampsia, and preterm delivery.

It is suggested that a period of induced cyclic progesterone withdrawal bleedings by CC, rather than metformin, may mature the endometrial progesterone response.

Therefore, it should be investigated by prospective studies whether uterine progesterone response can be matured by a period of cyclic menstruations before an attempt of the induction of ovulation for the treatment of infertility. ■

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