

## Review Article

# Effects of Metformin on Hyperinsulinemia, Hyperandrogenism and Reproduction in Women with Polycystic Ovarian Syndrome

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## Antidiabetic Drug-Metformin

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### Abstract

Polycystic Ovary Syndrome (PCOS) is characterized by ovulatory disturbances, hyperandrogenaemia and hyperinsulinemia secondary to increased insulin resistance. In PCOS, hyperinsulinemic insulin resistance is of interest because skeletal muscle may be resistant to insulin in terms of glucose metabolism, while the ovaries remain sensitive to insulin with regard to stimulation of testosterone biosynthesis. Insulin resistance and hyperinsulinemia are associated with reproductive failure such as early pregnancy loss, and cardiovascular risk and the development of diabetes mellitus later in life. Insulin-sensitizing agents such as metformin improve insulin sensitivity, thereby improving ovulatory cycles and fertility in women with PCOS. Metformin has also been shown to retard progression to type 2 diabetes in PCOS. This review addresses the effects of metformin on reproduction.

## INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder affecting at least 5% to 15% of women of reproductive age [1,2]. It is frequently associated with ovulatory disturbances and high estrogen, androgen and insulin levels in serum. Hyperinsulinemia is secondary to increased insulin resistance. Obesity often magnifies the clinical features of PCOS [1,2]. A major concern in women with PCOS is infertility, a consequence of chronic oligo- or anovulation [3]. However, even after ovulation is restored either pharmacologically [4-7]; or via lifestyle interventions [8], women with PCOS exhibit low reproductive potential with higher-than-expected rates of spontaneous miscarriage. During the first trimester, the rate of early pregnancy loss (EPL) is 30-50% in women with PCOS [9-11] compared to the 10-15% rate in overall pregnancies [11].

### Insulin resistance in PCOS

Increased insulin resistance and compensatory hyperinsulinemia play a critical role in the pathogenesis of hyperandrogenism and chronic anovulation [2,12,13]. Numerous studies have documented the presence of insulin resistance in both obese and lean women with PCOS [8,14-16]. Lean women with PCOS appear to have a form of insulin resistance that is

intrinsic to the syndrome [15,16]. Obese women with PCOS not only have this intrinsic form of insulin resistance, but they also have an added burden obesity-related insulin resistance [2,14,15].

### Effect of metformin Insulin resistance and hyperandrogenism.

Hyperinsulinemia stimulates testosterone biosynthesis in ovarian theca cells [17]. In PCOS, insulin appears to act via its own receptor in the theca cells, thereby increasing androgen production. This probably takes place by a signaling pathway different from that mediating the metabolic effects of insulin [17], i.e., by stimulation of ovarian cytochrome P450c17 $\alpha$  enzymes either directly and/or indirectly in response to increased LH release [3].

Hyperinsulinemia also increases free testosterone levels by decreasing hepatic synthesis of SHBG [18]. Insulin inhibits production of insulin-like growth factor binding protein-1 (IGFBP-1) in the liver, reducing the circulating levels and permitting greater local activity of IGF-I in the ovary. Increased free IGF-1 may stimulate ovarian androgen production [19,20].

Consistent with these findings improved insulin sensitivity

can be achieved by weight loss. This is followed by decreased free testosterone and increased SHBG and IGFBP-1 serum concentrations, and resumption of regular periods, ovulation, improvement of acne and decreased hirsutism [8,21,22]. Furthermore, after 4–8 weeks of metformin treatment, concurrently with the improvement of insulin sensitivity a significant decrease by 44% of serum-free testosterone levels has been observed in obese PCOS patients on metformin vs placebo. This suggests that reduction of serum insulin level by metformin can substantially decrease PCOS-related hyperandrogenism. The latter is also associated with a three-fold increase in SHBG [3,4].

In non-obese women with PCOS, metformin significantly reduces P450c17 $\alpha$  activity and hyperandrogenism [16]. In lean PCOS women, the administration of various insulin-sensitizing drugs, such as metformin and rosiglitazone, alone or in combination, has led to a significant decrease in serum androgens [6]. Overall, significant reduction of serum free testosterone has been found in 12 of the 18 studies that have evaluated this hormonal endpoint [2,12].

### **Effects of metformin Insulin resistance and ovulation.**

In women with PCOS, insulin resistance-related anovulation, oligomenorrhea and their related hormonal abnormalities can be reversed by weight loss or drugs enhancing insulin sensitivity, [4,8,20-23]. Indeed, improvement of ovulatory cycles in PCOS women has been achieved with metformin treatment (4) and with other insulin-sensitizing drugs, such as rosiglitazone, metformin and d-chiroinositol [3-6,8]. Moreover, metformin treatment has been found to decrease the incidence of miscarriage in women with PCOS [23]. Collectively, these findings suggest that, in women with PCOS, insulin resistance with compensatory hyperinsulinemia and hyperandrogenemia appear to lie behind both anovulation and EPL [2].

### **Insulin resistance and oocyte maturation**

Appropriate maturation of the oocyte is the key factor in fertilization, embryonic development, and maintenance of pregnancy. Poor oocyte quality may be associated with failure of fertilization, delayed embryonic development, abnormal blastocyst formation, fetal growth retardation, and increased fetal loss [24]. Insulin is an important hormone influencing oocyte maturation. Insulin exerts its effects on the oocyte via receptors in the granulosa cells. In insulin resistance, defects in glucose metabolism in the granulosa cells may adversely affect oocyte competence [25].

Insulin, acting in concert with insulin-like growth factors -1 (IGF-1) and -2 (IGF-2), LH, follicle stimulating hormone (FSH), and other intra-ovarian growth factors, has an effect on steroidogenesis, mitogenic activity, and glucose metabolism in the granulosa cells leading to follicular development and maturation [26]. Phosphorylation of Insulin Receptor Substrate-1 (IRS-1) by insulin is the key step in insulin mediated metabolic effects in the granulosa cells. These include glucose uptake, glycogen synthesis, synthesis of pyruvate and lactate and de novo purine synthesis via stimulation of the pentose phosphate pathway [27-29]. The mitogenic effects of insulin include activation of the Meiosis Promoting Factor (MPF) in the cumulus oophorus cells,

activation of cell differentiation of the granulosa cells, and oocyte maturation. These are mediated via stimulation of IRS-2, another insulin post-receptor substrate [27].

Many studies have reported selective impairment of insulin-stimulated glucose uptake in ovarian granulosa cells of PCOS women [30,31]. Compared with ovulatory women with follicles at a similar stage of development, the follicles from PCOS women have a decreased concentration of IRS-1 (metabolic) in granulosa cells but an increased level of IRS-2 (mitogenic) in theca interna cells [30]. A significant decrease of insulin-stimulated glucose incorporation into glycogen has been reported in ovarian cells from PCOS women. By contrast, stimulation of thymidine incorporation by IGF-1 is greater in PCOS cells compared to normal ovarian cells, indicating greater responsiveness to mitogenic stimuli in PCOS. Moreover, troglitazone, an insulin sensitizing drug, reverses the expression imbalance between IRS-1 and IRS-2 in PCOS cells, and treatment with troglitazone increases the IGF-1 and insulin-induced glycogen synthesis in granulosa cells but decreases the mitogenic over-responsiveness to IGF-1 in the PCOS ovary [31].

Due to selective insulin resistance at the ovarian level the defects in glucose metabolism could adversely affect the flow of glucose, lactate, pyruvate, purines, and cAMP in the oocyte, affecting meiosis [32] and oocyte maturation. These mechanisms may be involved in anovulatory disturbances and of miscarriage in women with PCOS. Nevertheless, the effects of metformin have not been explored in these *in vitro* experiments.

### **Insulin resistance and its role in blastocyst apoptosis**

Insulin and IGF-1 are important for the maintenance of pregnancy as they stimulate glucose uptake in the pre-implantation blastocyst. These effects are mediated via the IGF-1 receptor, which mediates translocation towards the cell membrane of GLUT 8, an insulin-regulated glucose transporter in the blastocyst [33]. High insulin and/or IGF-1 levels surrounding the pre-implantation blastocyst may down-regulate the IGF-1 receptor, leading to decreased glucose uptake and attenuated cell growth. Impaired glucose uptake may then result in increased apoptosis [34,35]. It has been suggested that hyperinsulinemia and hyperglycemia may induce expression of apoptosis-related caspases, enzymes that attack the blastocyst and trigger the cascade of programmed cell death [35].

### **Insulin resistance and endometrial glucose uptake**

Insulin induces translocation of GLUT 4 to the surface of endometrial cells, facilitating glucose uptake in the cells and improving endometrial receptivity [36]. Studies have shown that the GLUT 4 content is significantly lower in endometrial cells of hyperinsulinemic and obese PCOS women compared with that of normo-insulinemic controls [36]. These results indicate that hyperinsulinemia in PCOS women may have a harmful effect on endometrial receptivity [36].

### **Insulin resistance and implantation: Roles of glycolipin and IGFBP-1 and effects of metformin**

Compelling evidence suggests a role of insulin resistance in PCOS-related miscarriage. This is based on the observations that, in women with hyperinsulinemic PCOS, the circulating levels

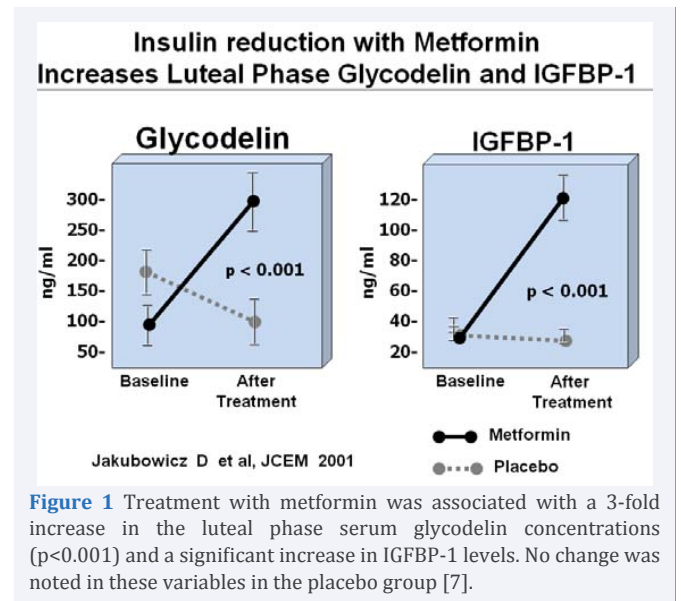
of endometrial stromal IGFBP-1 and epithelial glycodelin are subnormal [7] and the levels are increased by treatment with metformin. These two endometrial secretory proteins are likely to play a role in implantation and maintenance of pregnancy.

Glycodelin is produced in secretory/decidualized endometrial glands during the luteal phase of the cycle and early pregnancy. Its concentration is increased during implantation, and its immunosuppressive properties are believed to play a part in fetomaternal defense [37-39]. The maximum increase in the level of glycodelin takes place at 10-12 weeks' gestation [37,40]. Women with unexplained infertility, recurrent EPL, and retarded endometrial maturation have significantly lower levels of glycodelin in uterine flushings and serum compared with normal fertile women. [40,41].

IGFBP-1, is produced in the liver and endometrial stromal cells. It facilitates the adhesion process at the fetomaternal interface and maintains adequate uteroplacental blood flow, and thus plays a central role in the peri-implantation period [42-44]. Secretion of IGFBP-1 is down-regulated by insulin [45]. During pregnancy, IGFBP-1 is a major secretory product of the decidual stroma [46]. It is thought to act locally by signaling through  $\alpha_5\beta_1$  integrin, influencing endovascular trophoblastic invasion of the spiral arteries, primarily during the first trimester of pregnancy. This leads to remodeling of uteroplacental arteries into dilated, low resistance, non-elastic tubes, with loss of maternal vasomotor control. Maternal blood flow and uteroplacental perfusion increase to meet the requirements of the fetus [43,44].

The mechanisms by which hyperinsulinemic insulin resistance contributes to miscarriage in PCOS are not known. In PCOS, the levels of endometrial glycodelin and IGFBP-1 production are low [7,47]. In support of this, treatment with metformin 1500 mg daily during 4 weeks in PCOS women, was associated with a 20-fold increase in serum glycodelin concentration in the follicular phase and a 3-fold increase in the luteal phase ( $p < 0.001$ ). There also was a significant increase in the IGFBP-1 levels in the metformin group. These changes in the metformin group were accompanied by a substantial decrease in serum insulin and glucose concentrations, a 37% decrease in serum free testosterone levels, and a significant increase in SHBG. Along with these changes penetration of uterine vasculature increased (a 20% decrease in the resistance index). No similar changes were noted in the placebo group (see Figure 1) [7].

Based on the above findings and to further confirm the hypothesis of adverse effects of insulin resistance in PCOS [47], we conducted a study in 134 pregnant women - 72 with PCOS and 62 pregnant controls - during the first trimester, assessing serum glycodelin and IGFBP-1 levels. The serum concentrations of glycodelin and IGFBP-1 were both markedly lower in women with PCOS as compared with the controls. Specifically in women with PCOS, serum glycodelin was 56% lower during weeks 3-5 of pregnancy, 23% lower during weeks 6-8, but during weeks 9-11 the levels were similar between the two groups. Likewise, the IGFBP-1 levels in women with PCOS were 60-70% lower during weeks 3-5 and 6-8 of pregnancy, and 39% lower during weeks 9-11. Insulin sensitivity was markedly lower and serum total testosterone was significantly higher in women with PCOS



**Figure 1** Treatment with metformin was associated with a 3-fold increase in the luteal phase serum glycodelin concentrations ( $p < 0.001$ ) and a significant increase in IGFBP-1 levels. No change was noted in these variables in the placebo group [7].

throughout the first trimester. Moreover, women with PCOS had significantly more miscarriages compared to women without PCOS (14% vs. 3%, respectively). In the PCOS group, serum glycodelin and IGFBP-1 levels were significantly lower in those women who miscarried [47].

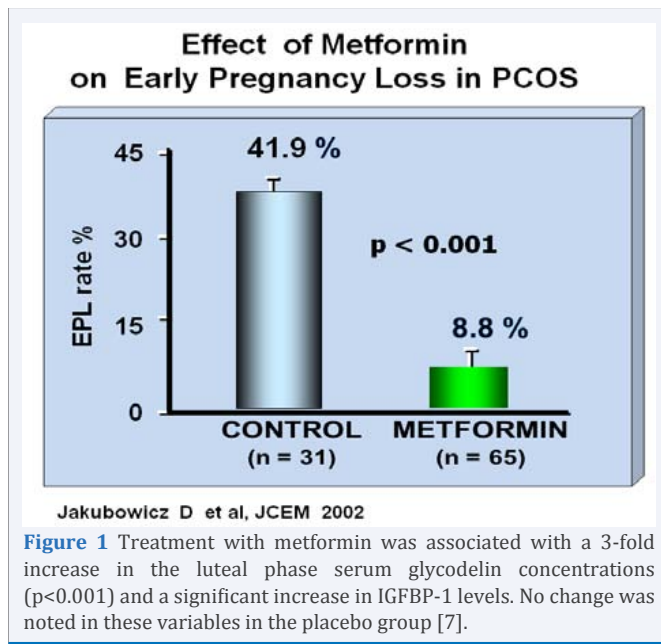
Several other studies have documented decreased endometrial levels of IGFBP-1 and glycodelin during the first trimester of pregnancy in various insulin resistant and hyperinsulinemic states including PCOS [23,48,49]. Decreased secretion of IGFBP-1 from the secretory endometrium has been associated with retarded endometrial development, abnormal trophoblastic invasion, disturbed remodeling of spiral arteries, and recurrent miscarriage. Lower circulating concentrations of IGFBP-1 in the first half of pregnancy have also been associated with intrauterine growth restriction (IUGR) and pre-eclampsia [48,50,51].

Given the consistency among these studies, it is assumed that insulin resistance is associated with reduced levels of these endometrial proteins, and this may contribute to a more hostile environment for implantation and fetal growth, leading to EPL in extreme cases.

### Insulin sensitizing drugs prevent miscarriage in PCOS pregnancy

Numerous studies suggest that insulin resistance contributes to endometrial dysfunction, infertility, and to miscarriage in PCOS. Insulin sensitizing drugs may thus offer a therapeutic option for these women. However, the safety of such treatments in early pregnancy remains an issue (see below).

A prospective cohort pilot study compared a group of women with PCOS treated with metformin throughout pregnancy to historical controls consisting of pregnant women who did not take metformin. The rate of early pregnancy loss was 39% in the historical controls, whereas in the metformin group it was only 11%. In the subcohort with metformin, the miscarriage rate decreased from 73% to 10% ( $p < 0.002$ ) [52]. Subsequently, a larger uncontrolled study confirmed that EPL in PCOS women



decreased from 62% to 26% ( $p < 0.0001$ ) in the women who took metformin was throughout pregnancy. No teratogenic events were found in their children [53].

Later, we confirmed these findings in a larger, controlled, retrospective study of 96 pregnant PCOS women [23]. Among the 65 women who became pregnant while taking metformin and who continued taking metformin throughout pregnancy, there were a total of 68 pregnancies, of which 6 (8.8%) ended in EPL. In contrast, among the 31 control PCOS women who did not take metformin, 13, or 41.9% ended in EPL. The 41.9% rate of EPL in the control group is compatible with the 30–50% rate reported in the literature for women with PCOS (see Figure 2) [9-11].

In contrast, the 8.8% rate of EPL in the women treated with metformin is similar to the 10–15% rate reported for clinically recognized normal pregnancies [11]. These results suggest that metformin treatment decreases an independent risk for miscarriage conferred by the disorder itself. Moreover, metformin treatment resulted in a 57% decrease in free testosterone levels and it improved insulin sensitivity. These findings suggest that metformin treatment prior to conception and during pregnancy can decrease the rate of miscarriage in women with PCOS [23].

Among the insulin-sensitizing drugs, metformin is designated as a Class B drug for use during pregnancy, which means that there are no evidences of animal or fetal toxicity or teratogenicity. Metformin is probably the safest therapeutic option in its class given that thiazolidinediones (TZDs) belong to pregnancy class C drugs due to the potential risk of causing fetal growth restriction in animal experiments.

Insulin sensitizing drugs, especially metformin, may prove to be unique among therapeutic options to decrease the rate of miscarriage in women with PCOS. However, the duration of metformin treatment during pregnancy in women with PCOS is still controversial, and more long-term prospective studies are required in the future.

The incidence of complications in the second and the third trimester of pregnancy among PCOS patients need more studies. PCOS has been associated with gestational diabetes mellitus, hypertensive disorders of pregnancy, pre-eclampsia, intrauterine growth restriction (IUGR), and premature delivery. Hyperinsulinemic insulin resistance may contribute to most of these complications [54-56].

Metformin treatment during pregnancy has been shown to reduce severe pregnancy and post-partum complications [56]. It also has become increasingly evident that foundations of good health are built in utero. For instance, infants surviving IUGR are at an increased risk for health problems such as hypertension, dyslipidemia, obesity, diabetes, precocious adrenarche, and infertility [57-60].

These observations suggest that interventions to reduce insulin resistance during pregnancy will not only reduce the risk for spontaneous miscarriage, but they may also exert beneficial health effects on the offspring later in life [60, 61].

### Effects of metformin in patients with PCOS Systematic reviews of the reproductive system.

Systematic reviews on the therapeutic effect of metformin in PCOS have shown that metformin in obese and lean women with PCOS women is beneficial in improving menstrual cyclicity, the ovulatory frequency when is taken in combination with clomiphene and improved also the pregnancy rates along with reduction of testosterone levels [62-65]. However when obese and lean women were analyzed separately metformin appears significantly more beneficial in lean vs obese women with PCOS. High insulin levels stimulating ovarian androgen production and inadequate reduction by metformin of the insulin concentration in obese women with PCOS were confirmed in these reports. However, these reviews did not demonstrate that metformin reduces the miscarriage rate.

A recent study reported increased ovulation rates in PCOS women who took metformin with clomiphene treatment, but the live birth rates were not significantly different between the groups with combined therapy and clomiphene alone [66]. Again, the study showed that obesity poses a significant negative impact on the cumulative live birth rate, and that adverse pregnancy complications such as pre-eclampsia and gestational diabetes were high among the obese women with PCOS [61,66].

### FINAL COMMENTS AND CAVEATS

Current evidence emphasizes the beneficial effects of weight loss on reproductive, metabolic and pregnancy outcomes in obesity-related PCOS. Because metformin is less effective in these women, the lifestyle modification remains the first line management of their infertility [67]. Recent reports have uncovered diabetogenic/insulin resistance-enhancing effects among diuretics, some oral contraceptives and statins [12,68-70]. Therefore, concomitant use of such drugs may decrease the beneficial effects of metformin. Finally, given the metabolic derangements in PCOS [71], any treatment that reduces insulin resistance may also reduce its long term consequences.

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