International Journal of Medical and Health Research

International Journal of Medical and Health Research
ISSN: 2454-9142, Impact Factor: RJIF 5.54
www.medicalsciencejournal.com
Volume 2; Issue 10; October 2016; Page No. 26-31

Overcoming Infertility Caused By Anovulatory Polycystic Ovary Syndrome

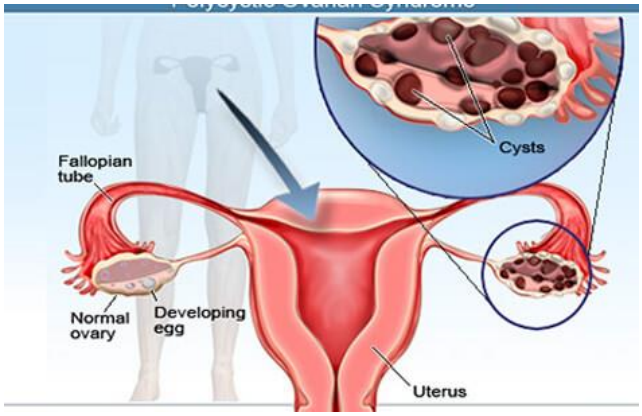
[1] Dr. Anil Batta, [2] Amandeep Kaur

[1] Prof & Head, Dept. of Medical Biochemistry GGS Medical College/Baba Farid University of Health Sciences, Faridkot, Punjab, India
[2] Senior Resident, Department of Anatomy, GGS Medical College, Faridkot, Punjab, India

Abstract
An ovarian cyst is a fluid-filled sac in the ovary and is common in women with regular periods. They are usually not cancerous however some are. Ovarian cyst is common in postmenopausal women. Most patients with ovarian cyst are asymptomatic with cysts being discovered incidentally or during USG or routine pelvic examination. Polycystic ovary syndrome represents 80% of anovulatory infertility cases. Treatment initially includes preconception guidelines, such as lifestyle changes (weight loss), folic acid therapy to prevent the risk of fetal neural tube defects and halting the consumption of tobacco and alcohol. The first-line pharmacological treatment for inducing ovulation consists of a clomiphene citrate treatment for timed intercourse. The second line Pharmacological treatment includes the administration of exogenous gonadotropins or laparoscopic ovarian surgery (ovarian drilling). Ovulation induction using clomiphene citrate or gonadotropins is effective with cumulative live birth rates of approximately 70%. Ovarian drilling should be performed when laparoscopy is indicated; this procedure is typically effective in approximately 50% of cases. Finally, a high-complexity reproduction treatment (*in vitro* fertilization or intracytoplasmic sperm injection) is the third-line treatment and is recommended when the previous interventions fail. This option is also the first choice in cases of bilateral tubal occlusion or semen alterations that impair the occurrence of natural pregnancy. Evidence for the routine use of metformin in infertility treatment of anovulatory women with polycystic ovary syndrome is not available. Aromatase inhibitors are promising and longer term studies are necessary to prove their safety.

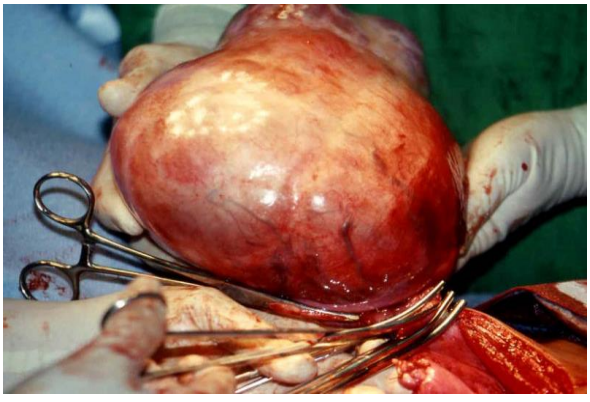
Keywords: polycystic ovary syndrome; infertility; clomiphene citrate; ovarian drilling; in vitro fertilization

1. Introduction
Infertility due to ovulation disorders is the most common reason for women to seek counseling or treatment. These women are treated by stimulating the ovulation with medication, so-called 'ovulation induction'. This is usually done with hormonal tablets; 'clomiphene citrate', as the first line of treatment [13]. Women who do not ovulate on clomiphene citrate require second-line ovulation induction strategies. The most common second-line treatment in these women is ovulation induction with gonadotropins, which are Injectable drugs. Various types of gonadotropin have been developed: urinary-derived products such as urofollitropin (FSH) and human menopausal gonadotrophin (HMG), available in purified (FSH-P) and highly purified (FSH-HP and HP-HMG) forms. Finally, recombinant FSH (rFSH) was developed to obtain even higher purity. Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies, affecting 5–10% of women of reproductive age [12]. The syndrome is surrounded by controversies regarding both its diagnosis and treatment. The need to establish universally accepted diagnostic criteria led to the Rotterdam meeting in 2003, during which experts in PCOS from all over the world, arrived at a consensus regarding the diagnosis of the syndrome. Criteria proposed for the diagnosis of PCOS were set in order to allow the performance of properly designed trials with good external validity in PCOS patients [11]. These trials would assist in defining the various phenotypes of the syndrome, in discovering its genetic origins, in evaluating its long-term consequences and in describing its optimal treatment. Obesity is common in women with PCOS and is linked to failure or delayed response to the various treatments proposed, such as administration of CC (Imani *et al*., 1998, 1999), gonadotrophins (Mulder*et al*., 2003; Balen *et al*., 2006) and laparoscopic ovarian diathermy (Gjonnaess, 1994) [16]. Weight loss is recommended as first-line therapy in obese women with PCOS seeking pregnancy [10]. This recommendation is based on extrapolation from the benefits of weight loss seen in multiple other conditions, such as diabetes and cardiovascular disease, as well as recognition of obesity's association with poor reproductive outcome. However, it should be noted that there is a paucity of studies suggesting that weight loss prior to conception improves live birth rate in obese women with or without PCOS (Moran *et al*.2006). On the other hand, multiple observational studies have noted that weight loss is associated with improved spontaneous ovulation rates in women with PCOS (Pasquali *et al*., 2003; Moran *et al*., 2006), while pregnancies have been reported after losing as little as 5% of initial body weight (Kiddy *et al*., 1992). The treatment of obesity is multifaceted and involves behavioral counseling, lifestyle therapy (diet and exercise), pharmacological treatment and bariatric surgery (Yanovski and Yanovski, 2002) [14]. The effects of calorie restriction, increased physical activity and pharmacological and weight loss agents in the periconceptional period are unknown and potentially harmful on the goal of live birth (Morris *et al*., 2006; Tsagareli *et al*., 2006) [15]. These interventions should be conducted prior to pregnancy, not concurrently with infertility treatment, until the risk benefit ratio of these therapies on pregnancy are better understood.



2. Observation

It is generally agreed that energy restriction is required for weight loss [7]. In fact, early improvements in reproductive function, in the absence of apparent weight loss, were probably due to energy restriction per se. However, there is little agreement on what constitutes the optimal diet for women with PCOS (Marsh and Brand-Miller, 2005). The resurgence of the Atkins' diet has generated considerable interest in very low calorie diets in recent years, and these can lead to significantly decreased body weight in PCOS (12% in 24 weeks) and improve reproductive outcome (Moran *et al*., 2004) [9]. A range of dietary approaches has been shown to be effective in weight loss and in improving reproductive function, but only two randomized controlled trials (RCTs) have compared the effect of different diets in women with PCOS (Moran *et al*., 2003; Stamets *et al*., 2004). However, these studies did not show that dietary patterns differentially affect weight loss and reproductive outcomes. Increasing evidence in women without PCOS suggest that diets with reduced glycemic load may be beneficial in alleviating hyperinsulinaemia and its metabolic consequences (Reaven, 2005). This is of particular relevance to women with PCOS, due to the close association between insulin resistance and reproductive health[8]. In the absence of level I evidence, the recommended diet for obese women with PCOS is any hypocaloric diet (with a 500 Kcal/day deficit) with reduced glycemic load and, failing that, any calorie restricted diet with which patients can comply and achieve a 5% weight loss. Insufficient physical activity might explain why women with PCOS have a tendency towards overweight/obesity. Baseline activity levels by self-report were less in women with PCOS compared with control women (Wright *et al*., 2004). In the Nurses' Health Study, vigorous activity was associated with a reduced relative risk of anovulatory infertility (Rich-Edwards *et al*., 2002). Few studies have examined the role of exercise alone in improving reproductive function in PCOS. In a pilot trial examining exercise and nutritional counseling in PCOS, women were assigned to nutritional counseling alone or in combination with exercise. No differences were seen between groups with respect to weight loss or restoration of menstruation (Bruner *et al*., 2006) [7]. The available literature supports the adjuvant use of bariatric surgery and pharmacological weight loss for the treatment of obesity in PCOS, although large clinical trials are needed. In morbidly obese women, the PCOS phenotype appears to be very frequent (Alvarez-Blasco *et al*., 2006). Most importantly, this disorder has been found to improve markedly after sustained weight loss following bariatric surgery (Escobar-Morreale *et al*., 2005). Anti-obesity pharmacological agents have been used in obese women with PCOS, although few quality studies have been published (Sabuncu *et al*., 2003; Jayagopal *et al*., 2005). Both orlistat, which blocks intestinal absorption of fat (Jayagopal *et al*., 2005), and sibutramine, an appetite suppressant (Sabuncu *et al*., 2003), have displayed a weight loss-independent effect on androgens and insulin resistance. Gonadotrophins and GnRH analogues. The aim of ovulation induction for women with anovulatory PCOS is to restore fertility and achieve a singleton live birth. The method of ovulation induction using gonadotrophin therapy is based on the physiological concept that initiation and maintenance of follicle growth may be achieved by a transient increase in FSH above a threshold dose for sufficient duration to generate a limited number of developing follicles. Application of this concept is essential when ovulation induction is conducted in women with PCOS, because they are specifically prone to excessive multiple follicle development (Brown, 1978; Baird, 1987). It has been suggested that increased luteinizing hormone (LH) secretion in PCOS may interfere with fertility. The mechanisms include premature oocyte maturation, through inhibition of oocyte maturation inhibitor (Jacobs and Homburg, 1990) and deleterious LH effect on granulosa cell steroidogenesis (Willis *et al.*, 1996, 1998). In addition, elevated LH levels may be associated with an increased pregnancy loss (Homburg *et al*., 1988; Regan *et al*., 1990; Balen *et al.*, 1993; Tarlatzis *et al.*, 1995), although more recent data are not consistent with this assumption (Rai *et al*., 2000; Mulders *et al*., 2003; Oliveira *et al.*, 2007). The concomitant use of a GnRH agonist with gonadotrophin administration to improve pregnancy rates in patients undergoing ovulation induction has not been firmly established (Fleming *et al*., 1985, 1988; Dodson *et al*., 1987). Moreover, combined therapy was associated with an increased risk of OHSS (Charbonnel *et al.*, 1987; Homburg *et al.*, 1990; Scheele *et al*., 1993; Buckler *et al*., 1993; van der Meer *et al.*, 1996), while there are insufficient data to draw solid conclusions on miscarriage and multiple pregnancy rates (*Bachus et al.*, 1990; Homburg *et al*., 1993; Clifford *et al.*, 1996). Therefore, the significantly higher hyperstimulation rate, the associated risk of multiple pregnancies and the additional inconvenience and cost of concomitant GnRH agonist administration, in the absence of documented increases in pregnancy success, do not justify the routine use of GnRH agonists during ovulation induction with gonadotrophins in PCOS patients. The question of whether LH suppression by a GnRH antagonist during gonadotrophin-based ovulation induction is of benefit to women with PCOS has not yet been addressed by RCTs.



3. Discussion

Ultrasound assessment of the ovary can be performed at baseline prior to the initiation of each cycle. Serial ovarian ultrasound is an excellent method of determining follicle growth and development in response to gonadotrophin stimulation. In particular, documentation of all follicles >10 mm may be helpful to predict the risk of multiple pregnancies. Adherence to the chronic low-dose regimen of FSH administration in women with PCOS should markedly reduce the likelihood of excessive ovarian stimulation and OHSS. However, before ovulation induction with gonadotrophins, it is mandatory to counsel the patient about the risks associated with higher-order multiple pregnancies following polyovulation. In most previous studies, cycle cancellation has been advised when more than three follicles of 16 mm or larger were observed (White *et al*., 1996; Homburg and Howles, 1999; Calaf *et al*., 2003a) in order to prevent OHSS and multiple pregnancies. In some studies, the limit was four or more follicles >14 mm (Kamrava *et al.*, 1982; Hugues *et al*., 2006) [4]. Recently, more stringent criteria have been recommended for ovarian stimulation in unexplained infertility: no more than two follicles >14 mm (Farhi *et al.*, 1996) or no more than three or four follicles >10 mm (Tur *et al.*, 2001; Dickey *et al*., 2005). In addition, recent data stress the need for taking into account the overall number of follicles and cycle cancellation may be considered in the presence of more than three follicles >14 mm. It should be noted that the definition of a monofollicular cycle has usually been a single follicle of 16 mm or higher without any information on the number of smaller follicles, except in the study by Leader (2006), which defined a cycle as monoovulatory when a single follicle of 16 mm or higher was present with no other follicle 12 mm or higher. Measurements of circulating E_2 levels have been used to cancel ovulation induction cycles using gonadotrophins (due to over- or under-response) or to adjust the dose of gonadotrophins used either upwards or, more frequently, downwards, in order to minimize the risk of multiple pregnancies or OHSS. While specific normative thresholds vary, in 2006 the Practice Committee of the ASRM suggested that caution was indicated when a rapidly rising serum E_2 level or an E_2 concentration in excess of 2500 pg/ml was present during gonadotrophin ovulation induction (Practice Committee of the American Society for Reproductive Medicine, 2006). However, in other studies (Tur *et al*., 2001; Dickey *et al*., 2005) the threshold E_2concentration was much lower, <1000 pg/ml, which seems to be more realistic according to the number of growing follicles. It would seem prudent to withhold hCG administration in the presence of more than two follicles ≥16 mm or more than one follicle ≥16 mm and two additional follicles ≥14 mm, in order to minimize the risk of multiple pregnancies in women with PCOS under the age of 38 without any other infertility factors [17].

4. Results

The recognition of an association between PCOS and hyperinsulinaemia has led to the use of insulin-sensitizing agents in ovulation induction. Metformin, the most widely studied agent used in PCOS, is a biguanide insulin-sensitizing agent that acts by inhibiting hepatic glucose production and increasing peripheral glucose uptake [15]. It does not stimulate secretion of insulin or cause hypoglycemia. Many earlier studies examining the use of metformin alone or in conjunction with CC in ovulation induction showed promising results [12, 16] but most of these studies had relatively small sample sizes. Pregnancy rates were not significantly better with metformin than with placebo (OR 2.76; 95% CI 0.85 to 8.98), but an improvement was seen with metformin plus CC over CC alone (OR 4.4; CI 1.96 to 9.85).43 A more recent meta-analysis published in April 200844 comparing CC and metformin, both alone and in combination, found that metformin alone increased the odds of ovulation compared with placebo (OR 2.94; 95% CI 1.43 to 6.02) but did not result in a statistically significant difference in pregnancy rates (OR 1.56; 95% CI 0.74 to 3.33). When CC and metformin were compared with CC alone, both ovulation and pregnancy rates were statistically increased to 4.39 (95% CI 1.94 to 9.96) and 2.67 (95% CI 1.45 to 4.94), respectively. This meta-analysis also included studies that reported live birth rates. Ng *et al*. 45 in 2001 compared metformin and placebo in 20 women and found that women who received metformin were less likely to achieve a live birth, although this difference did not reach statistical significance (OR 0.44; 95% CI 0.03 to 5.88). Four of the included trials examined live birth rates with CC and metformin versus CC alone.41,46–48 Collectively, the combination of CC and metformin suggested an increase in live birth rate over CC alone, but this increase was not statistically significant (OR 1.74; 95% CI 0.79 to 3.86). The only trial adequately powered to assess live birth rates was the large randomized controlled trial published by Legro *et al*. in 2007.46This trial included 626 patients and demonstrated that although the live birth rate following up to 6 months of treatment with metformin and CC was increased (26.8%), it was not significantly different from that with CC alone (22.5%). Live birth rates using CC alone or with metformin were significantly higher than rates with metformin alone (7.2%). The evidence supports the use of clomiphene citrate over metformin as first-line pharmacologic therapy following lifestyle modification in women with PCOS. However, there may be a role for the addition of metformin to CC in women who are clomiphene-resistant. Siebert *et al* [9]. Examined 6 trials in which metformin was randomized with either placebo or CC in clomiphene-resistant patients and found an overall statistically significant improvement in ovulation with combination therapy (OR 6.82; 95% CI 3.59 to 12.96). Further, a recent study also suggested that women with PCOS who are older and have increased visceral obesity may benefit from the additional use of metformin.50 Patients on metformin often experience unpleasant side effects of nausea, bloating, cramps, and diarrhea, and they should be counseled to start with 250 mg to 500 mg PO daily and increase as tolerated to the optimal daily dose of 500 mg to 750 mg 3 times daily with food. Metformin can also be dosed 850 mg PO twice daily or a long-acting formulation (Glumetza) can be used to improve compliance. Although some studies have shown that continuing metformin in pregnancy may decrease the spontaneous abortion rate [2, 5, 3] none of these are prospective, randomized trials. Randomized controlled trials are needed in this area before sustained metformin treatment throughout pregnancy can be recommended [3]. Metformin combined with clomiphene citrate may increase ovulation rates and pregnancy rates but does not significantly improve the live birth rate over that of clomiphene citrate alone.(I-A) Metformin may be added to clomiphene citrate in women with clomiphene resistance who are older and who have visceral obesity. (I-A) GONADOTROPINS Use of intramuscular gonadotropins

began in the 1960s. These preparations, from the purified urine of postmenopausal women, contained both FSH and LH. SOGC Clinical Practice Guideline 498 MAY JOGC MAI 2010 over the last decade, recombinant human FSH has been the main preparation, and it can be self-administered subcutaneously.[5] Gonadotropins are used when PCOS patients fail either to ovulate or to conceive with oral ovulation inducing medications. Daily injections of gonadotropins are combined with concurrent blood and ultrasound monitoring with the aim of monofollicular growth and development. However, because of the inherent nature of exogenous gonadotropin treatment, multifollicular development is not uncommon, despite careful dose adjustment and monitoring. Once the dominant follicle has reached the appropriate size, HCG is administered to trigger ovulation. Injectable gonadotropins are very expensive and require frequent monitoring, with serum estradiol and ultrasound assessments to minimize the risks from excessive follicular growth and development.[3] Because of the high number of antral follicles in women with PCOS, it is not uncommon that treatment is cancelled to minimize the occurrence of multiple pregnancy and also of ovarian hyperstimulation syndrome [15]. Pregnancy rates with gonadotropins are 20% to 25% per cycle.54 Drawbacks to gonadotropin treatment, as mentioned earlier, are requirements for intensive monitoring, cost, multiple pregnancy, and ovarian hyperstimulation. Gonadotropins should be administered by physicians with specific training in reproductive medicine and with ready access to ultrasound monitoring and rapid hormone testing.

5. Recommendation

Gonadotropin should be considered second-line therapy for fertility in anovulatory women with PCOS. The treatment requires ultrasound and laboratory monitoring. High costs and the risk of multiple pregnancy and ovarian hyperstimulation syndrome are drawbacks of the treatment. (II-2A) OVARIAN DRILLING Surgical ovarian wedge resection by open laparotomy was one of the first treatments for anovulation due to PCOS [16]. It was thought to induce ovulation by decreasing the ovarian theca and thus reducing androgen production. Because of the operative morbidity of the procedure and the risk of postoperative adhesions [17]. Ovarian wedge resection by laparotomy has largely been abandoned as more effective medical therapies for ovulation induction have become available. With the popularity of minimally invasive surgery, laparoscopic ovarian drilling is thought to be less destructive to the ovary and has a lower risk of adhesion formation. Laparoscopic ovarian drilling uses either cautery or laser to create approximately 10 superficial perforations per ovary [8]. A Cochrane review published in 2007 examined 16 randomized controlled trials evaluating ovulation induction in clomiphene-resistant PCOS with LOD. The dose at which clomiphene resistance was defined ranged from 100 mg to 200 mg in the various studies. Approximately 80% of PCOS patients will become ovulatory after LOD. There was no difference found in the rates of miscarriage, ongoing pregnancy, or live birth between patients who underwent LOD and patients treated with gonadotropins for ovulation induction. There were significantly fewer multiple pregnancies in the LOD than in the gonadotropin treatment groups (1% vs. 16%; OR 0.13; 95% CI 0.03 to 0.59).58 In one of the included trials, adjuvant therapy with CC or gonadotropins was required to achieve equivalent pregnancy and live birth rates in patients remaining anovulatory 8 weeks after LOD or those who subsequently became anovulatory.59 Despite this evidence that LOD may be equivalent to gonadotropins in achieving ovulation, the effects of LOD on postoperative adhesion formation remain a concern,60 although it has been shown that in women who respond to this treatment, the rate of cessation of ovulation is low [16] Recommendation [5]. Laparoscopic ovarian drilling may be considered in women with clomiphene-resistant PCOS, particularly when there are other indications for laparoscopy. (I-A) Surgical risks need to be considered in these patients. (III-A). Aromatase inhibitors have been used for the last decade as adjunctive treatments in breast cancer [6]. They block the conversion of testosterone and androstenedione to estradiol and estrone, respectively, and hence inhibit the estrogen-negative feedback on the hypothalamic–pituitary axis. This leads to increased gonadotropin secretion, which in turn leads to ovarian follicular growth and development [12]. The use of aromatase inhibitors in ovulation induction was first introduced in 2001 [13]. Ovulation and pregnancy rates with aromatase inhibitors such as letrozole and anastrozole appear to be promising, and these agents appear to have less anti-estrogen effect on the endometrium, but the evidence on endometrial effects is conflicting, and most studies show equivalence with clomiphene citrate [12–15]. In 2005, however, Health Canada and the manufacturing company of letrozole issued a "Physician Warning Letter" on the off-label use of letrozole for fertility and the possibility of embryotoxicity, fetotoxicity, and teratogenicity found in rats [16]. This followed preliminary research findings by Biljan *et al* [6]. comparing congenital malformations in babies conceived with letrozole with or without gonadotropins with those in babies born to a low-risk population of women Ovulation Induction in Polycystic Ovary Syndrome MAY JOGC MAI 2010 499 without known fertility treatments. These findings reported a higher incidence of both cardiac and bone abnormalities in the letrozole group [12]. More recently, however, Tulandi *et al* [18]. retrospectively evaluated [11] newborns from letrozole and CC pregnancies. They found a 2.4% incidence of congenital malformations and chromosomal abnormalities in the letrozole group versus 4.8% in the CC group.68 However, until aromatase inhibitors have been approved for ovulation induction by Health Canada, they should be used with caution, and patients should be carefully counseled, given potential medico–legal implications. *In Vitro* Fertilization, Ivf, with or without intracytoplasmic sperm injection, is the next treatment

option for women with PCOS who fail to conceive with gonadotropin treatment or in the presence of other indications for advanced reproductive technologies? In IVF, gonadotropins are administered to achieve multifollicular development for oocyte retrieval and generation of embryos for transfer into the uterus. Pregnancy rates can approach 40% to 50% per cycle with IVF, but, as with fertility in general, success is significantly influenced by the women's age[15] PCOS patients achieve pregnancy and live birth rates similar to those of non-PCOS patients during conventional IVF cycles. Side effects include multiple pregnancies when multiple embryos are transferred, and a higher risk of ovarian hyperstimulation; however, the risk of multiple pregnancies is more easily controlled with IVF than with ovulation induction with gonadotropins, because the number of embryos transferred into the patient's uterus can be limited and surplus good quality embryos cryopreserved for future transfer. *In vitro* fertilization should be reserved for women with PCOS who fail gonadotropin therapy or who have other indications for IVF treatment.

6. Summary

Patients with polycystic ovary syndrome commonly present with a history of infertility due to oligo-ovulation or anovulation. First-line management of infertility should always include weight loss and exercise and lifestyle modifications in the overweight patient. This is beneficial in the patient's overall health, it may lead to spontaneous ovulation, and it will improve response to ovulation-induction medications. Clomiphene citrate has been used for many years and remains the first-line medication despite potential anti-estrogenic effects on the endometrium and cervical mucus. Recent evidence indicates that insulin-sensitizing agents should not be used as a first-line therapy, although they may be beneficial in PCOS patients who are older and who have increased visceral obesity as assessed by increased waist-to-hip ratios, and in those who have failed to ovulate on clomiphene citrate alone.

7. Treatment of Infertility

If tests determine that lack of ovulation is the cause of infertility, several treatment options are available. These treatments work best in women who are not obese. The primary treatment for women who are unable to become pregnant and who have PCOS is weight loss. Even a modest amount of weight loss may allow the woman to begin ovulating normally. In addition, weight loss can improve the effectiveness of other infertility treatments. Clomiphene is an oral medication that stimulates the ovaries to release one or more eggs. It triggers ovulation in about 80 percent of women with PCOS, and about 50 percent of these women will become pregnant. Some studies have shown that live birth rates are higher in obese women with PCOS when they are treated with letrozole rather than clomiphene. A few studies have shown that taking metformin in addition to clomiphene increases the rate of ovulation; other studies have shown no additional benefit of adding metformin to clomiphene treatment [1]. In addition, it is not clear if metformin is safe during pregnancy (but metformin is FDA category B in pregnancy, which is generally interpreted as reasonably safe); women who take metformin before pregnancy are usually advised to stop it once they become pregnant. If a woman does not ovulate or is unable to conceive with clomiphene, gonadotropin therapy (follicle-stimulating hormone [FSH] injections) may be recommended. Ovulation occurs in almost all women with PCOS who use gonadotropin therapy; approximately 60 percent of these women become pregnant.

8. References

1. Palomba S, Falbo A, Zullo F, Orio F Jr. Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. Endocr Rev. 2009; 30:1-50. doi: 10.1210/er.2008-0030.
2. Palomba S, Falbo A, Russo T, Orio F, Tolino A, Zullo F. Systemic and local effects of metformin administration in patients with polycystic ovary syndrome (PCOS): relationship to the ovulatory response. Hum Reprod. 2010; 25:1005-1013. doi: 10.1093/humrep/dep466.
3. Falbo A, Orio F, Venturella R, Rania E, Materazzo C, Tolino A *et al*. Does metformin affect ovarian morphology in patients with polycystic ovary syndrome? A retrospective cross-sectional preliminary analysis. J Ovarian Res. 2009; 31:2-5.
4. 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; 335:617-623. doi: 10.1056/NEJM199608293350902.
5. Sonnatag B, Götte M, Wülfing P, Schüring AN, Kiesel L, Greb RR. Metformin alters insulin signaling and viability of human granulosa cells. Fertil Steril. 2003; 84:1173-1179.
6. Tosca L, Chabrolle C, Uzbekova S, Dupont J. Effects of metformin on bovine granulosa cells steroidogenesis: possible involvement of adenosine 5′ monophosphate-activated protein kinase (AMPK) Biol Reprod. 2007; 76:368-378. doi: 10.1095/biolreprod.106.055749
7. Palomba S, Russo T, Orio F Jr, Falbo A, Manguso F, Sammartino A, *et al*. Uterine effects of metformin administration in anovulatory women with polycystic ovary syndrome. Hum Reprod. 2006; 21:457-465.
8. Palomba S, Falbo A, La Sala GB. Effects of metformin in women with polycystic ovary syndrome treated with gonadotropins for *in vitro* fertilization and intracytoplasmatic sperm injection cycles: a systematic review and meta-analysis of randomized controlled trials. BJOG. 2013; 120:267-276. doi: 10.1111/1471-0528.12070.
9. Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Hum Reprod. 2008; 23:462-477.
10. Beall SA, DeCherney A. History and challenges surrounding ovarian stimulation in the treatment of infertility. Fertil Steril. 2012; 97:795-801. doi: 10.1016/j.fertnstert.2012.02.030.
11. 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; 21:1387-1399. doi: 10.1093/humrep/dei501.
12. Moher D, Liberati A, Tetzlaff J, Altman DG, Altman D, Antes G. PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA

statement. BMJ. 2009; 339:b2535. doi: 10.1136/bmj.b2535.
13. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K *et al*. International Committee for Monitoring Assisted Reproductive Technology; World Health Organization; International Committee for Monitoring Assisted Reproductive Technology; World Health Organization. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology. Fertil Steril. 2009; 92:1520-1524. doi: 10.1016/j.fertnstert.2009.09.009
14. Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011] The Cochrane Collaboration, 2011. (The Cochrane Collaboration).
15. Review Manager Version, 5.
16. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst. 1959; 4:719-748.
17. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003; 327:557-560. doi: 10.1136/bmj.327.7414.557.