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Infertility in Polycystic Ovary Syndrome

Focus on Low-Dose Gonadotropin Treatment

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Polycystic ovary syndrome accounts for more than 75% of cases of anovulatory infertility. The mechanism of anovulation is uncertain but there is evidence that arrested antral follicle development is associated with the abnormal endocrine profile, in particular the interaction of insulin and LH on granulosa cell differentiation. In terms of management, induction of ovulation can be achieved in most cases by the use of antiestrogens. Treatment of clomiphene-resistant subjects is difficult; conventional doses of gonadotropins are associated with high rates of ovarian hyperstimulation syndrome and multiple pregnancy. On the other hand, low-dose gonadotropin therapy has proven effective in inducing unifollicular ovulation and, in this review, we present, in detail, a recent analysis the results from this center. The cumulative conception rate after six cycles was more than 50% and, importantly, the multiple pregnancy rate was only 3%. Weight reduction in obese subjects with PCOS not only increases the chance of fertility but may also improve the long-term prognosis with regard to development of diabetes. Insulin-sensitizing drugs such as metformin may also have a place in treatment of PCOS.

Key Words: Anovulation; luteinizing hormone; follicle-stimulating hormone; insulin; obesity

Introduction

Polycystic ovary syndrome (PCOS) is the commonest endocrine disorder in women and is the most prevalent cause of anovulatory infertility. Classically, PCOS *(1)* is characterized by the combination of menstrual disturbance and symptoms of excessive androgen secretion (hirsutism, acne, or androgenic alopecia) but it is typically heterogeneous in presentation *(2)*. The proceedings of the recent PCOS consensus conference held under the joint auspices of the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) have proposed revised diagnostic criteria for PCOS *(3)*. These recognize that polycystic ovaries may be present in women with regular, ovulatory, menses who have symptoms of hyperandrogenism alone (or indeed none of the classic endocrine symptoms of PCOS) *(2,3)*. Nevertheless, the primary cause of infertility in PCOS is anovulation. There is no clear evidence to suggest that women with polycystic ovaries and ovulatory cycles are less likely to be fertile but polycystic ovarian morphology is more common in women with ovulatory infertility than in the general population *(4)*, raising the possibility that there may be other factors (perhaps endometrial) that may contribute to reduced fertility in PCOS. Nevertheless, as discussed below, restoration of ovulatory cycles in anovulatory women with PCOS results in a normal (or near normal) pregnancy rate per cycle, emphasising that anovulation is the most important infertility factor. The major part of this article will therefore focus on anovulatory infertility in PCOS and its management.

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Diagnosis of PCOS

The diagnostic criteria suggested by the recent ESHRE/ASRM consensus conference recognize that the diagnosis of PCOS is largely a clinical one, supported by a small number relevant endocrine tests *(3)*. A patient presenting with irregular menses, oligomenorrhea, or amenorrhea (without estrogen deficiency) has a high chance of having PCOS, particularly if there are also signs of hyperandrogenism. No one test is diagnostic of the syndrome and choice of investigations is dictated by the clinical presentation. Serum LH levels are typically elevated in PCOS (FSH is normal) but up to 50% of women with all other clinical and biochemical features of the syndrome may have normal serum LH *(5,6)*. Measurement of LH is therefore of limited diagnostic value *(3)*. Pelvic ultrasonography will define the polycystic ovarian morphology but accurate assessment of the ovaries by ultrasound is a particular skill so that false negative results are not uncommon. Conversely, the presence of polycystic ovaries does not necessarily mean that the patient has polycystic ovary syndrome. Polycystic ovaries may be found coincidentally in women who have, for example, hypothalamic, estrogen-deficient amenorrhea.

Mechanism of Anovulation in PCOS

The mechanism of anovulation in PCOS remains unclear, but there is evidence that the arrested antral follicle growth that is typical of anovulatory women with PCOS reflects the abnormal endocrine environment *(7)*. There is evidence for premature advancement in the maturation of a subpopulation of follicles in the polycystic ovary *(8)*. The granulosa cells of these follicles appear to acquire responsiveness to LH at a much smaller size than in the normal cycle (typically 3–4 mm in diameter compared with 10 mm in the normal dominant follicle) *(8)*. They produce inappropriate levels of estradiol (and progesterone) for their size *(8)*, and we suggest that they suppress endogenous FSH levels, thereby preventing maturation of the small "healthy" follicles that are present within the same cohort *(9)*.

There is evidence for an intrinsic abnormality in the early development of follicles in the polycystic ovary *(10,11)*—and that may contribute to abnormal follicle maturation—but we propose that the abnormal endocrine environment is the main reason for arrested antral follicle development. If serum concentrations of FSH are raised therapeutically, the small, healthy follicles within the cohort can be recruited and have the potential to reach full maturity. Indeed, it may take 10 d or more for a dominant follicle to appear during the first cycle of treatment with low-dose FSH (see below). What then is the reason for premature advancement of follicle maturation?

The cause of the inappropriate advancement of follicle maturation is not fully understood. Hypersecretion of LH may have a part to play but the hyperinsulinemia that is typical of anovulatory (but not ovulatory) women with PCOS *(5,12,13)* is likely to have important role. The steroidogenic response of granulosa cells to LH in vitro is greatly enhanced by the addition of insulin *(14)*, and this suggests that insulin is a key factor in producing prematurely advanced differentiation of granulosa cells. It may seem paradoxical to propose that hyperinsulinemia is producing an adverse effect on the follicle in a state of peripheral insulin resistance, but recent data indicate that insulin resistance in granulosa cells is signaling pathway specific. Insulin-mediated glucose uptake and lactate production is impaired in granulosa cells form anovulatory women with PCOS but steroidogenesis is not significantly affected *(15)*.

The effects of obesity and, conversely, calorie restriction on ovulatory function provide good clinical evidence of the effects of hyperinsulinemia on the ovary. Obese women with PCOS (who are invariably hyperinsulinemic) are more likely to have menstrual disturbances that lean PCO subjects *(16)*. Obese anovulatory women respond poorly to induction of ovulation compared with women with PCOS who are of normal weight *(17–19)*. Calorie restriction in obese women with PCOS results in a fall in circulating insulin levels and improvement of menstrual pattern and fertility *(20–23)*. Advice about diet and lifestyle is therefore an important facet of management of overweight PCOS patients with anovulatory infertility. Insulin-sensitizing drugs may also have a role to play. The role of diet, lifestyle changes, and insulin-sensitizing agents in management of PCOS is discussed in the next section and in detail elsewhere in this issue.

Recent studies have shown that anti-Mullerian hormone (AMH) is expressed in granulosa cells of follicles of all sizes in the human ovary and may have a role in abnormalities in early, preantral follicle development in PCOS *(46)*.

Management of Anovulatory Infertility in PCOS

Restoration of normal fertility is an achievable goal in most anovulatory women with PCOS. In overweight and obese subjects, the most important initial step in ensuring an optimal response to induction of ovulation is modification of diet and lifestyle. The recent medical literature (both scientific and popular publications) have made much of the potential use of insulin-modifying drugs in management of PCOS.

Insulin-Sensitizing Agents

The thiazolidinediones are a relatively new class of such drugs that has been introduced primarily for the control of type 2 diabetes. In a large randomized, multicenter study, troglitazone was shown to improve insulin sensitivity and menstrual cyclicity in obese women with polycystic ovary syndrome *(24)*. However, this drug has been withdrawn because of serious side-effects, and although there are newer, apparently safer, preparations available, there are concerns about the wisdom of administering thiazolidinediones in women of reproductive age.

Metformin is well established in the management of type 2 diabetes. Its mechanism of action is complex, and its effects include reduction of insulin resistance and insulin levels. Studies in the last 5 yr or so have suggested that metformin may be a safe and effective means of improving metabolic profile and reproductive function in both lean and obese women with polycystic ovary syndrome. Results have been encouraging but not conclusive; there are few randomized controlled trials, and these have produced conflicting results *(25)*. Intriguingly, a recently published controlled trial reports that metformin may be as effective and have fewer adverse effects that clomiphene when used as first-line treatment of lean, anovulatory women with PCOS *(26)*. Such results should, however, be interpreted with some caution because the dose of clomiphene used in this study was unusually high at 150 mg per day. A "standard" dose of 50 mg per day has been shown, in other studies, to be both effective and safe (see below) *(27)*.

Clomiphene

Despite the increasing popularity of metformin (and other insulin-sensitizing drugs) as first-line treatment, the weight of evidence supports the use of antiestrogens as first choice

for induction of ovulation; the most commonly used is clomiphene citrate. Clomiphene is given orally, usually at a starting dose of 50 mg/d for 5 d, usually starting on d 2 or 3 after the onset of spontaneous or progestin-induced menses. It stimulates endogenous FSH secretion, leading to development of a dominant follicle and ovulation in about 75% of cases. The fecundity rate after clomiphene-induced ovulation (around 70% of women conceive within six cycles) is close to normal *(27)*. Although clomiphene treatment is relatively straightforward, it is important that ultrasound and endocrine monitoring is performed—at least in the first cycle of treatment—so that the dose can be adjusted, if necessary, in subsequent cycles. Possible problems range from failure to develop a preovulatory follicle to hyper-stimulation syndrome—a potentially dangerous complication which, although more commonly associated with gonadotropin therapy, can certainly occur following antiestrogen treatment. Predictors of ovulation in response to clomiphene include body weight and free androgen index *(17,27–29)*.

Gonadotropin Treatment

In patients who fail to ovulate in response to clomiphene, the main options for treatment are exogenous FSH or laparoscopic ovarian diathermy. There are pros and cons of each of these modes of therapy (see below), but, in this section, we focus on gonadotropin therapy and present the results of our experience with low-dose FSH for induction of ovulation at a single center.

Induction of ovulation by conventional doses of exogenous gonadotropins is associated with lower rates of ovulation and pregnancy than in hypogonadotropic women but with an increased chance of multiple follicle development (and an associated increase in the risk of hyperstimulation syndrome and of multiple pregnancy) and a higher rate of miscarriage compared with gonadotropin-deficient patients *(30–32)*. There is an increasing awareness of the adverse medical and sociological implications of multiple gestation, and it should be the aim of an induction of the ovulation program to achieve unifollicular ovulation.

Low-Dose Gonadotropin Therapy for PCOS

A low-dose regimen for induction of ovulation with gonadotropins in women with PCOS has been adopted in a number of centers. This approach was based on the work of Brown and colleagues *(33,34)* in women who were given human pituitary gonadotropin (HPG). He observed that by making small, stepwise increments in the dose of FSH of about 30% at 5 d intervals, it was possible to define a "threshold" dose at which a significant rise in estrogen excretion was detectable and beyond which multiple folliculogenesis was more likely. Subsequently, Kamrava et al. *(35)* reported successful induction of ovulation in two patients with PCOS following the administration of urinary-derived FSH at a fixed daily dose of only one ampoule (75 IU). In our center, we have adapted this low-dose regimen not only to include a low starting dose but also to allow for small stepwise increments with the aid of regular ultrasound monitoring to determine the threshold dose for a single dominant follicle to develop and ovulate.

A variation of this "step-up" low-dose gonadotropin regimen is the so-called "step-down" protocol *(36)*. Both produce similar results but the latter is generally considered more difficult to institute because very careful monitoring is required in the first few days of treatment and there is an increased risk of multiple follicle development *(37)*.

In our unit, before commencing treatment, all women have a baseline scan and, if the endometrium is thicker than 8 mm, a short course of a progestin (medroxyprogesterone acetate, 5 mg/d) is given to induce a withdrawal bleed. Gonadotropin treatment [human menopausal gonadotropin (hMG) or FSH] is started at a dose of 75 IU (or more recently) 50 IU by daily intramuscular injection following the onset of spontaneous or progestin-induced menses *(19)*. Monitoring of response is primarily by ultrasound scanning, which is performed initially at 3–4 d intervals and, in the preovulatory phase, on alternate days (or daily, if indicated). Decisions about changes in treatment are usually made on the basis of ultrasound scanning with retrospective analysis of LH and estradiol measurements when necessary. The initial dose of hMG or FSH is maintained for up to 14 d in the first cycle and the dose is increased to 75 IU only if no follicle >10 mm is observed by that stage. Further increments of 25 or 37.5 IU (half ampoule) are made, if necessary, at weekly intervals, to a maximum of 225 IU/d. If a dominant follicle emerges, that dose of hMG (the "threshold" dose) is continued until the follicle has reached a diameter of at least 18 mm. More recently, the availability of ampoules of recombinant human FSH containing 50 IU FSH has made administration of the chosen doses somewhat easier.

Ovulation is triggered by a single, intramuscular dose of hCG, 5000 IU, and hMG is stopped. hCG is given routinely because, although some patients will have a spontaneous LH surge, it is not possible to predict this with any reliability. Treatment is discontinued if there are more than three follicles of 15 mm (or larger) in diameter, to minimize the risk of hyperstimulation and/or multiple pregnancy. Serum progesterone is measured 5–8 d after hCG.

If further cycles of treatment are necessary, hMG is reintroduced at a dose below the previous threshold (and no more than 75 IU/d). In the second and subsequent cycles, the first increment in dose is usually made after 7 d, rather than 14 d. In women who develop multiple follicles, either a smaller starting dose (i.e., 25 or 37.5 IU) is given or the dose is increased by only 25 IU.

Low-Dose Gonadotropin Therapy—The St. Mary's Experience

Patients

In this review, new data from 199 patients, treated between 5th January 1990 and 28th February 2002 have been

Table 1
Overall Results for 199 Women with PCOS Treated with Low-Dose Gonadotropin

Number of patients	199
Total number of cycles	916
Number of ovulatory cycles	657 (72)[a]
Number of anovulatory cycles	128 (14)
Number of completed cycles	785 (86)
Number of unifollicular cycles	667 (73)
Number of unifollicular ovulatory cycles	562 (61)
Number of abandoned cycles (multiple follicles)	131 (14)

[a]Figures in parentheses are percentages.

Table 2
Outcome of Induction of Ovulation in 199 Women with PCOS Treated with Low-Dose Gonadotrophin

Number of patients	199
Number of pregnancies	91 (46)[a]
Number of multiple pregnancies*	3 (3)
Number of miscarriages	21 (23)

[a]Figures in parentheses are percentages.
*All multiple pregnancies were twins.

analyzed (Table 1). The patients all presented at infertility clinics at St Mary's hospital, London and all had a diagnosis of anovulatory PCOS as the primary cause of their infertility. The criteria for diagnosing PCOS were in accord with the ESHRE/ASRM consensus crtiteria. All had polycystic ovaries on ultrasound scan and, by definition, had anovulatory cycles or amenorrhea.

The mean age of the women treated was 30.34 yr (range: 20–42) yr and their average BMI was 24.21 (range: 17.38–45.86). The average pretreatment basal LH was 11.63 IU/L (range: 1.9–37.10) and 43.34% of women were found to have a basal LH greater than normal (where a normal LH is considered to be less than or equal to 11 IU/L). The mean FSH was 6.02 IU/L (range: 1.7–16.0) and all but one woman (FSH elevated on one occasion at 16 IU/L) had a normal FSH (that is, less than or equal to 11 IU/L). Of the 193 patients who had pretreatment T levels measured, the mean value was 2.8 nmol/L (range: 0.9–7.0 nmol/L); 41% of women had a T greater than 2.7 nmol/L (the upper limit of normal in our laboratory). Of the 199 couples treated, 66 (33.17%) had additional factors that may have had a bearing on their fertility. In 51 patients (25.63%) the male partner had a sperm density <20 million/mL, although the majority of these had a normal sperm separation ("swim-up"), i.e., ≥3 million sperm/mL.

Treatment Protocol

All women who entered the low-dose gonadotropin treatment program had been previously treated either at St. Mary's hospital or elsewhere with clomiphene citrate and had failed to become pregnant. Once this was performed, the women were ready to begin treatment. In this series FSH was administered in the form of human menopausal gonadotropin (hMG), but we have previously shown that there are no significant differences between the effects of hMG and purified FSH on outcome of treatment *(38)*. All doses of hMG were given as daily intramuscular (im) injections according to the protocol outlined above.

Statistical Analysis of Data

Data were stored using the Macintosh program "Filemaker pro 5.5." Statistical analysis was performed using "SPSS 11 for Windows."

Results

In 73% of treatment cycles, the threshold dose of gonadotropin required to produce a dominant follicle was between 37.5 and 75 IU (37.5 IU: 5%; 50 IU: 45%; 75 IU: 23%). A dose of hMG in excess of 150 IU was required in only 4% of cycles. Patients were treated with hMG for on average 16 d per cycle, and they visited the clinic for assessment an average of six times per completed cycle.

Overall data from all the treatment cycles has been summarized in Table 2. The table shows that of 916 cycles, 657 (72%) were ovulatory and 562 (61%) were unifollicular ovulatory cycles. This means that 86% of ovulatory cycles were unifollicular and only 14% were multifollicular; 128 cycles (14%) were anovulatory, and 131 cycles (14%) had to be abandoned because of multiple follicle development before completion.

Cumulative conception (CCR) rates were calculated for the results of all patients and also separately for the patients who were clomiphene citrate resistant and for patients who were not resistant to clomiphene (i.e., had ovulated on clomiphene but had not conceived). The CCR was calculated for either the first six cycles (whether or not ovulation occurred), or the first six ovulatory cycles (Fig. 1). The CCR for all the patients after six cycles was 55%. If only the first six ovulatory cycles were considered, the CCR was 59%. The CCR rate appears higher in the clomiphene-resistant patients than those patients who had previously ovulated (but not conceived) after clomiphene treatment. There was an obvious trend toward a higher CCR in the clomiphene-resistant group, although life table analysis and Kaplan–Meier survival analysis (performed on the data from the ovulatory cycles) showed no statistically significant differences in CCR between the two groups.

Factors Affecting Outcome of Treatment

The influence of various clinical and endocrine features on outcome of treatment was considered. Only nine patients in this series (two of whom conceived) were 38 yr or older and, not surprisingly, logistic regression analysis showed no significant effect of age on outcome in this cohort. Body mass index (BMI), however, did have a significant effect on the threshold dose of FSH, ovulation, and conception

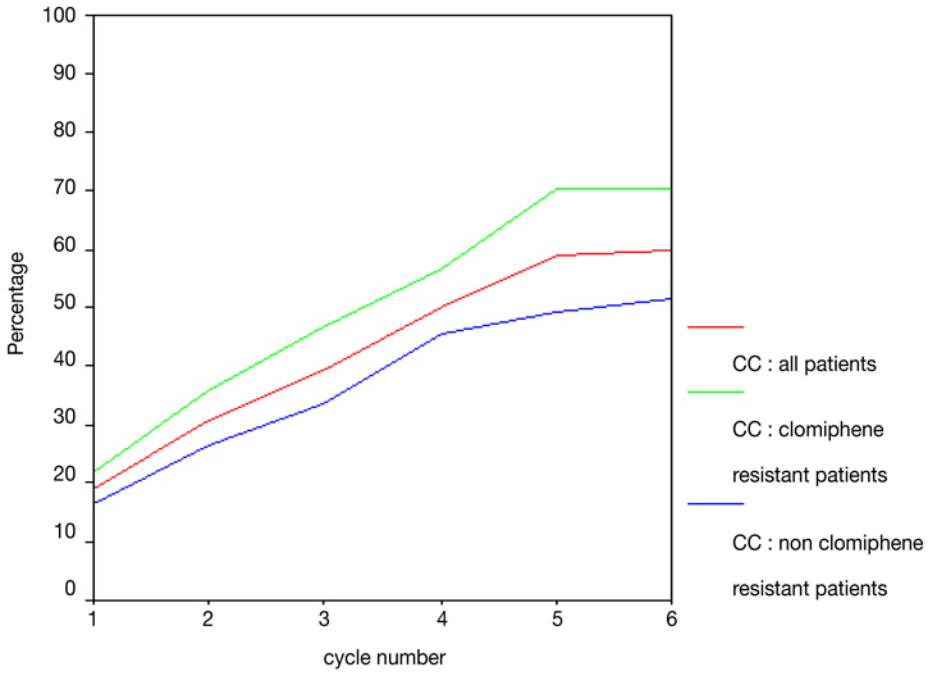

Fig. 1. Cumulative conception data (calculated by life table analysis) in women ovulating in response to low-dose gonadotropin therapy.

rates. Patients with a BMI > 25 were statistically more likely ($p < 0.05$) to require a greater threshold dose of hMG than patients with a lower BMI. Logistic regression was performed to examine the effects of BMI on the likelihood of conception. The logistic regression analysis (a form of binary multiple regression analysis) was performed using the pregnancy outcome as the dependent variable and the values for age or BMI (or, as below for testosterone and LH) as covariates. Patients with a BMI > 25 were ($p < 0.05$) less likely to conceive than those with a BMI ≤ 25.

The effects of baseline (pretreatment) serum concentrations of testosterone (T) and LH on threshold dose of hMG required to produce a dominant follicle and on pregnancy rate were examined. The mean threshold dose of hMG was significantly greater ($p < 0.05$) in patients with baseline T > 3 nmol/L than in those with a serum T ≤ 3 nmol/L (89 vs 78 IU/d), and in those with a baseline LH > 11 IU/L compared with patients with LH ≤11 IU/L (92 vs 73 IU/d).

Comment

The key findings from this review of our experience with low-dose FSH are the high rate of unifollicular ovulation and the very low rates of multiple pregnancy and hyperstimulation syndrome. The overall pregnancy rate is comparable with the results of treatment with conventional doses of gonadotropin and the miscarriage rate is little greater than that observed in spontaneous conception cycles. The pregnancy rate per cycle may be somewhat lower than that resulting from conventional gonadotropin therapy but the much lower frequency of serious complications is a strong argument for the use of the low-dose regimen.

There was a trend toward a higher cumulative conception rate for patients who have previously been found to be resistant to clomiphene citrate compared with that in women who had ovulated but not conceived. The obvious explanation for such a discrepancy is that clomiphene-resistant subjects ovulate anew in response to FSH, whereas those who had previously ovulated but did not conceive may well have other (occult) factors contributing to their subfertility.

In terms of prediction of outcome of treatment, BMI was the major clinical factor affecting the dose of FSH and pregnancy rate. Neither basal LH nor basal T affected the pregnancy rate, but those with elevated levels of either hormone required higher doses of gonadotropin. These findings are consistent with those of a previous report from this center *(19)*. This supports data from several previous studies, which have suggested that obesity reduces fertility in patients with PCOS *(20–23)*. It is important when managing obese patients with PCOS to encourage weight loss before embarking upon fertility treatment. Metformin may well have a place in management of clomiphene-resistant women with PCOS *(25)*, but, at the time of writing, there are too few double-blind, randomized controlled trials to draw upon to be able to reach definitive conclusion about the routine use in induction of ovulation.

In this series, we have reported the results of treatment with FSH given in the form of urinary-derived hMG. It has been claimed that FSH is more effective than hMG in the management of patients with PCOS, but careful examination of the literature does not support this contention. In the few, prospective, randomized, controlled trials that have

been conducted, there have been no significant differences between FSH and hMG treatment with regard to rates of ovulation and pregnancy *(38,39)*. The results of a recent Cochrane Database review support this conclusion *(40)*. It has also been suggested that human recombinant FSH is safer and more effective than urinary FSH for induction of ovulation in PCOS *(41)*; a recent Cochrane review concluded that there were insufficient data to demonstrate that recombinant FSH has a significant advantage in efficacy or safety over urinary-derived gonadotropins in management of PCOS *(42)*. Nevertheless, it makes sense, in the long term, to replace urinary gonadotrophins with pure recombinant preparations.

Laparoscopic Ovarian Diathermy

Laparoscopic ovarian diathermy (LOD) is now a well-established alternative method of treating infertility in clomiphene-resistant women with PCOS. Although it is an "invasive" procedure and carries the risk of pelvic adhesion formation, it is usually both effective and well tolerated. In a prospective, controlled study comparing LOD with low-dose gonadotropin therapy, the rates of ovulation, pregnancy, and miscarriage were similar in the two groups. Subsequent questionnaires revealed that most women preferred a single surgical procedure to repeated gonadotropin injections and monitoring *(43)*. A recent study also compared low-dose FSH with LOD in a randomized controlled trial *(44)*. The cumulative conception rate was similar in the two groups, but the rate of multiple pregnancy was much higher in the gonadotropin-treated group. On the face of it, the results of this study favor the use of LOD over FSH treatment, but it should be noted that the rate of multiple pregnancy in that series (16%) is far higher than in most centers using the low-dose FSH regimen and that 54% of the patients who underwent LOD required adjuvant therapy with clomiphene or FSH following surgery in order to ovulate. Nevertheless, it is the policy in our center to offer clomiphene "resistant" patients the choice of LOD and low-dose FSH.

In Vitro Fertilization

The outcome of treatment following superovulation and in vitro fertilization (IVF) in women with PCOS is similar to those with normal ovaries with respect to successful pregnancy rates *(45)*. However, women with PCOS are at increased risk of OHSS after superovulation, and IVF is not advocated as first-line therapy in those who present with anovulation but no other infertility factors. In vitro fertilization should usually be reserved for management of couples with additional causes of infertility.

References

1. Zawadzki, J. K. and Dunaif, A. (1992). In: *Polycystic ovary syndrome*. Dunaif, A., Givens, J. R., Haseltine, F. P., and Merriam, G. R. (eds.), Blackwell Scientific Publications, Oxford, pp. 59–69.
2. Franks, S. (1995). *N. Engl. J. Med.* **333,** 853–861.
3. The Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. (2004). *Hum. Reprod.* **19,** 41–47.
4. Kousta, E., White, D. M., Cela, E., McCarthy, M. I., and Franks, S. (1999). *Hum. Reprod.* **14,** 2720–2723.
5. Conway, G. S., Honour, J. W., and Jacobs, H. S. (1989). *Clin. Endocrinol. (Oxf.)* **30,** 459–470.
6. Franks, S. (1989). *Clin. Endocrinol. (Oxf.)* **31,** 87–120.
7. Franks, S., Mason, H., and Willis, D. (2000). *Mol. Cell. Endocrinol.* **163,** 49–52.
8. Willis, D. S., Watson, H., Mason, H. D., Galea, R., Brincat, M., and Franks, S. (1998). *J. Clin. Endocrinol. Metab.* **83,** 3984–3991.
9. Chavez-Ross, A., Franks, S., Mason, H. D., Hardy, K., and Stark, J. (1997). *J. Math. Biol.* **36,** 95–118.
10. Webber, L. J., Stubbs, S., Stark, J., et al. (2003). *Lancet* **362,** 1017–1021.
11. Maciel, G. A., Baracat, E. C., Benda, J. A., et al. (2004). *J. Clin. Endocrinol. Metab.* **89,** 5321–5327.
12. Dunaif, A., Graf, M., Mandeli, J., Laumas, V., and Dobrjansky, A. (1987). *J. Clin. Endocrinol. Metab.* **65,** 499–507.
13. Robinson, S., Kiddy, D., Gelding, S. V., et al. (1993). *Clin. Endocrinol. (Oxf.)* **39,** 351–355.
14. Willis, D., Mason, H., Gilling-Smith, C., and Franks, S. (1996). *J. Clin. Endocrinol. Metab.* **81,** 302–309.
15. Rice, S., Christoforidis, N., Gadd, C., et al. (2005). *Hum. Reprod.* **20,** 373–381.
16. Kiddy, D. S., Sharp, P. S., White, D. M., et al. (1990). *Clin. Endocrinol. (Oxf.)* **32,** 213–220.
17. Polson, D. W., Kiddy, D. S., Mason, H. D., and Franks, S. (1989). *Fertil. Steril.* **51,** 30–34.
18. Hamilton-Fairley, D., Kiddy, D., Watson, H., Paterson, C., and Franks, S. (1992). *Br. J. Obstet. Gynaecol.* **99,** 128–131.
19. White, D. M., Polson, D. W., Kiddy, D., et al. (1996). *J. Clin. Endocrinol. Metab.* **81,** 3821–3824.
20. Pasquali, R., Antenucci, D., Casimirri, F., et al. (1989). *J. Clin. Endocrinol. Metab.* **68,** 173–179.
21. Kiddy, D. S., Hamilton-Fairley, D., Bush, A., et al. (1992). *Clin. Endocrinol. (Oxf.)* **36,** 105–111.
22. Clark, A. M., Ledger, W., Galletly, C., et al. (1995). *Hum. Reprod.* **10,** 2705–2712.
23. Hickey, T., Chandy, A., and Norman, R. J. (2002). *J. Clin. Endocrinol. Metab.* **87,** 161–165.
24. Azziz, R., Ehrmann, D., Legro, R. S., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 1626–1632.
25. Lord, J. M., Flight, I. H., and Norman, R. J. (2003). *BMJ* **327,** 951–953.
26. Palomba, S., Orio, F. Jr., Falbo, A., et al. (2005). *J. Clin. Endocrinol. Metab.* **90,** 4068–4074.
27. Kousta, E., White, D. M., and Franks, S. (1997). *Hum. Reprod. Update* **3,** 359–365.
28. Imani, B., Eijkemans, M. J., te Velde, E. R., Habbema, J. D., and Fauser, B. C. (1998). *J. Clin. Endocrinol. Metab.* **83,** 2361–2365.
29. Imani, B., Eijkemans, M. J., Faessen, G. H., Bouchard, P., Giudice, L. C., and Fauser, B. C. (2002). *Fertil. Steril.* **77,** 83–90.
30. Hamilton-Fairley, D. and Franks, S. (1990). *Baillieres Clin. Obstet. Gynaecol.* **4,** 609–625.
31. Balen, A. H., Braat, D. D., West, C., Patel, A., and Jacobs, H. S. (1994). *Hum. Reprod.* **9,** 1563–1570.
32. Tadokoro, N., Vollenhoven, B., Clark, S., et al. (1997). *Hum. Reprod.* **12,** 1939–1944.
33. Brown, J. B., Evans, J. H., Adey, F. D., Taft, H. P., and Townsend, L. (1969). *J. Obstet. Gynaecol. Br. Commonw.* **76,** 289–307.
34. Brown, J. B. (1978). *Aust. N Z J Obstet. Gynaecol.* 18, 46–54.
35. Kamrava, M. M., Seibel, M. M., Berger, M. J., Thompson, I., and Taymor, M. L. (1982). *Fertil. Steril.* **37,** 520–523.
36. van Santbrink, E. J., Donderwinkel, P. F., van Dessel, T. J., and Fauser, B. C. (1995). *Hum. Reprod.* **10,** 1048–1053.

37. Balasch, J., Fabregues, F., Creus, M., Puerto, B., Penarrubia, J., and Vanrell, J. A. (2001). *Hum. Reprod.* **16,** 652–656.
38. Sagle, M. A., Hamilton-Fairley, D., Kiddy, D. S., and Franks, S. (1991). *Fertil. Steril.* **55,** 56–60.
39. Tanbo, T., Dale, P. O., Kjekshus, E., Haug, E., and Abyholm, T. (1990). *Fertil. Steril.* **53,** 798–803.
40. Hughes, E., Collins, J., and Vandekerckhove, P. (2000). *Cochrane Database Syst. Rev.* CD000087.
41. Fulghesu, A. M., Apa, R., Belosi, C., et al. (2001). *Horm. Res.* **55,** 224–228.
42. Bayram, N., van Wely, M., and van Der Veen, F. (2001). *Cochrane Database Syst. Rev.* CD002121.
43. Farquhar, C. M., Williamson, K., Gudex, G., Johnson, N. P., Garland, J., and Sadler, L. (2002). *Fertil. Steril.* **78,** 404–411.
44. Bayram, N., van Wely, M., Kaaijk, E. M., Bossuyt, P. M., and van der Veen, F. (2004). *BMJ* **328,** 192.
45. Franks, S., Roberts, R., and Hardy, K. (2003). *Reprod. Biomed. Online* **6,** 181–184.
46. Stubbs, S. A., Hardy, K., DaSilva-Buttkus, P., et al. (2005). *J. Clin. Endocrinol. Metab.* **90,** 5536–5543.