Review

Role of androgens in the treatment of patients with low ovarian response

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Abstract

The objective of the current report was to provide a summary of knowledge concerning the treatment of women with poor ovarian response with androgens and androgen modulating agents. This involved a review of the literature. The literature search was performed using PubMed. Information concerning the role of androgens and androgen modulating agents in treating women with poor ovarian response is limited. The search of the literature yielded five studies and one case report concerning the treatment of poor responders with androgens. The variations in patient selection, type of androgens employed and the different duration of exposure preclude drawing any definite conclusions. Aromatase inhibitors block the conversion of androgens to oestrogens, thereby promoting an androgen-rich intrafollicular environment. The evidence presented in this review suggests a potential beneficial role for the use of aromatase inhibitors in treating women who have previously experienced failure of standard IVF protocols. The optimal dose and duration of this treatment is yet to be determined. Although the results of studies concerning LH supplementation in poor responders are conflicting, the latest Cochrane review on the use of recombinant LH for ovarian stimulation supports its use in poor responders, based on pooled pregnancy estimates.

Keywords: androgens, aromatase inhibitors, poor ovarian response

Introduction

The chance of achieving a successful ovarian stimulation cycle depends on the recruitment of multiple follicles. Failure to achieve this goal is evidence of poor ovarian response. Although no clear definition has been agreed, poor ovarian response in the context of IVF treatment is usually defined as failure to achieve at least three mature oocytes or a certain oestradiol concentration in response to ovarian stimulation. The incidence of low responders is estimated to be 10–25% of the stimulated population (Karande and Gleicher, 1999; Fasouliotis et al., 2000; Tarlatzis et al., 2003). These women comprise a heterogeneous group, in which routine markers, such as day 3 serum FSH, inhibin concentration, early follicular oestradiol and anti-Müllerian hormone, do not accurately and consistently predict poor ovarian response.

Treating poor responders is a difficult task. Studies focusing on this population have demonstrated that changing the concentration or type of gonadotrophins, employing different gonadotrophin-releasing hormone (GnRH) agonist flare-up protocols or GnRH antagonists (GnRHa), have yielded disappointing results (Tarlatzis et al., 2003).

Androgens and their ovarian receptors seem to play an important role in ovarian physiology and follicular growth. In a primate ovary model, androgen receptor gene expression correlates positively with follicular growth and granulosa cell proliferation (Weil et al., 1998). It has also been demonstrated that treating primates with androgens induces a significant increase in the number of preantral and antral follicles, independent of gonadotrophin effect (Vendola et al., 1998, 1999). These and other reports have
led to the assumption that poor responders may benefit from treatment with androgens or androgen-modulating drugs. The aim of this work was to review current studies on the use of androgens and androgen modulating agents in treating poor responders.

Literature search strategy

A systemic review spanning January 1980 to July 2009 was conducted, aimed at identifying all of the published studies dealing with the treatment of women with poor ovarian response with androgens, aromatase inhibitors or LH. The literature search was performed using PubMed. The basic keywords were ‘poor’ or ‘low’, or ‘bad’ with ‘responders’ or ‘ovarian response’ plus ‘dehydroepiandrosterone’ or ‘testosterone’ or ‘LH’ or ‘aromatase inhibitors’. The term ‘diminished ovarian function’ was also included.

Biosynthesis of androgens

The normal ovary secretes three major steroid androgens: androstenedione, testosterone and dehydroepiandrosterone. The latter is secreted mainly by the adrenal glands. The follicle is the basic functional unit of the ovary. A follicle contains a single oocyte, granulosa cells enclosed by a basal lamina and theca cells, which are located exterior to the basal lamina and are closely associated with the basal membrane. The principal function of the ovarian theca is steroid synthesis. Experiments with isolated theca cells by a cAMP-mediated mechanism that synthesizes androstenedione and testosterone from cholesterol. Androstenedione and testosterone diffuse across the basement membrane to the granulosa cells (Hillier et al., 1981, 1994). FSH stimulates granulosa cells by a cAMP-mediated mechanism that synthesizes androstenedione and testosterone from cholesterol. Androstenedione and testosterone diffuse across the basement membrane to the granulosa cells (Hillier et al., 1981, 1994). FSH stimulates granulosa cells by a cAMP-mediated mechanism. The granulosa cells can then convert the androstenedione and testosterone to oestrone and oestradiol respectively. LH is the key player in controlling androgen production and thus oestrogen production by the ovary. LH acts on theca cells to stimulate the enzymatic complex [cytochrome P450c17 including 17a-hydroxylase and C17–20-lyase activity], which converts pregnenolone and progesterone to androstenedione (Magoffin, 2005). Peak androgen production is achieved at moderate LH dosages, and higher doses induce no further rise (Gilling-Smith et al., 1994). Granulosa cells also participate in regulation of ovarian androgen production. Tanbo et al. demonstrated that in down-regulated women undergoing ovarian stimulation for IVF, production of androgens by theca cells increase significantly in response to FSH, implying that granulosa cell-derived paracrine factors participate in the control of androgen production (Tanbo et al., 2001). In addition, it has been shown that inhibin, produced by granulosa cells, acts synergistically with LH in a paracrine fashion to increase androgen production by the theca cells (Hillier et al., 1991; Nahuiii et al., 1995).

Role of androgens in the different stages of follicular maturation and atresia

The primordial follicle, a single oocyte surrounded by a single layer of pre-granulosa cells, represents the earliest stage of follicular maturation in the primate ovary. The factors that initiate the maturation of primordial follicles are poorly understood. Treating Rhesus monkeys with androgens resulted in a highly significant increase in insulin-like growth factor-1 (IGF1) and IGF1 receptor mRNAs in oocytes of primordial follicles (Vendola et al., 1999). IGF1 receptors are abundant in oocytes of all species; they stimulate maturation in vitro and may trigger primordial follicle growth (Yoshimura et al., 1996). Androgen treatment, mediated by IGF1, also results in a significant increase in the number of primary follicles, as well as preantral and antral follicles, and increases granulosa and theca cell proliferation in the primate ovary (Vendola et al., 1998, 1999). Despite the suppression of gonadotrophins induced by testosterone treatment, testosterone-treated women have increased numbers of developing follicles, implying that androgens induce maturation of early follicles independent of gonadotrophin stimulation (Futterweit and Deligdisch, 1986; Spinder et al., 1989).

Any factor that alters intracellular cAMP concentrations is a potential modulator of granulosa cell differentiation, and hence follicular development. Androgen appears to modulate gonadotrophin action on granulosa cells through amplification of cAMP-mediated post-receptor signalling. In a normal cycle, during early and intermediate stages of follicular development, locally produced androgens act via granulosa cell androgen receptors (AR) to promote FSH-induced granulosa cell differentiation. However, during late pre-ovulatory follicular development, higher concentrations of cAMP due to LH stimulation suppress granulosa cell proliferation and down-regulate some of the genes induced by FSH at earlier stages of pre-ovulatory development, including aromatase activity (Hillier and Tetsuka, 1997), contributing to the mechanism that determines which follicle will become dominant and which will undergo atresia. In ovarian stimulation cycles, the addition of androgens might be synergistic to FSH in follicular recruitment and granulosa cell proliferation, while continuous exogenous exposure to FSH protects such recruited follicles from atresia. In addition, there are some reports demonstrating that androgens are likely to act on folliculogenesis by increasing the number of FSH receptors expressed in granulosa cells (Weil et al., 1999). The mechanism whereby androgen administration increases granulosa FSH receptor gene expression is as yet unclear.

Androgen concentrations and ovarian stimulation

The increase in androgen concentrations during gonadotrophin treatment and until the day of human chorionic gonadotrophin (HCG) administration is well established. Cedars et al. (1990) reported that human menopausal
gonadotrophin (HMG) treatment induced a two-fold increase in plasma androgen concentrations. Fanchin and collaborators confirmed that the elevation in androgen concentrations associated with ovarian stimulation peaked 12–15 h after injection of HMG (Fanchin et al., 1997). Other studies in which the effect of FSH administration was examined demonstrated a significant increase in testosterone, androstenedione and DHEAS concentrations during administration of FSH in ovarian stimulation, which was significantly related to oestradiol concentrations. It was concluded that FSH, through granulosa-derived paracrine factors, initiates thecal androgen synthesis and secretion independent of LH stimulation (Tanbo et al., 2001). Based on these observations, it is reasonable to assume that the administration of HCG to induce ovulation would elevate androgen concentrations even further. However, a small sample study conducted on six normally ovulating but infertile women failed to demonstrate a further increase in androgen concentrations after administration of HCG during ovarian stimulation (Fanchin et al., 2000).

There is an age-dependent decrease in the follicular pool, such that the relative proportion of ovarian follicular cells decreases with age, while that of stroma cells increases. As a result, changes may occur in the contributions of the two cell compartments with regard to oestrogen biosynthesis. Traditionally, evaluation of ovarian reserve, as well as predictors of IVF cycle success, focused on hormonal parameters that reflect granulosa cell function. The majority of these studies focused on parameters such as baseline serum FSH concentrations, FSH concentrations after a challenge test, or oestrogen concentrations at different stages of an IVF cycle. Such tests have been shown to have high specificity, but lack the desired sensitivity, thus they fail to predict low response to gonadotrophin stimulation in a substantial percentage of low responders (Scott and Hofmann, 1995). Recently, some attention had been paid to the assessment of the theca cell component as a predictor of IVF success. A study conducted on 43 normal ovulatory women enrolled in an IVF programme examined the predictive value of serum androgen concentrations on IVF outcome (Frattarelli and Peterson, 2004). It was shown that basal testosterone concentrations were negatively related to some stimulation parameters, such as the number of ampoules used and number of stimulation days, and were closely related to day 3 oestradiol concentrations. This study demonstrated that a low baseline testosterone value was predictive of poor cycle outcome, with a threshold estimated at <20 ng/dl. Women with baseline testosterone values <20 ng/dl were five times less likely to achieve pregnancy. A larger prospective study was performed by Frattarelli and Gerber to confirm these results (Frattarelli and Gerber, 2006). Data were collected on 117 infertile women between the ages of 19 and 42, independent of their diagnosis, who were enrolled in an IVF programme. In this study, ovarian day 3 androgen concentrations were significantly related to several IVF stimulation parameters, most significantly with peak oestradiol concentrations, follicle number and oocyte number. In contrast to the smaller retrospective study by Frattarelli and Peterson (2004), ovarian androgen concentrations were not predictive of pregnancy outcome.

In view of the conflicting data regarding the value of androgen concentrations in predicting the response to gonadotrophin stimulation, a stimulation test to examine ovarian theca reserve could be important. The only thecal stimulation test reported in the literature was conducted on 44 female volunteers (aged 20–44 years). All subjects underwent HCG stimulation 2–5 days after spontaneous menstrual bleeding. The ovarian capacity to secrete oestrogen precursors in response to HCG was clearly decreased in women older than 30 years. Similarly, the negative correlations between age and area under the curve found in that study, implies a decreasing androgen secretion capacity with age (Piltonen et al., 2003). Despite the decreases in serum androgen concentrations, oestradiol concentrations remained unchanged in women aged 20–44 years, implying that the first sign for ovarian ageing may be the decreased capacity to produce androgens in response to a stimulation test.

**Observations favouring androgen supplementation in poor responders**

Women with hyper-androgenic states such as polycystic ovarian disease (PCOD) have an increased number of follicles with an ability to respond to FSH (Buyalos and Lee, 1996). In fact, it is not uncommon for women with PCOD to hyper-respond to stimulation with gonadotrophins due to the large cohort of early follicles existing in their ovaries. In addition, accumulating evidence that androgen concentrations may play a role in predicting response to ovarian stimulation, supported by the fact that plasma androgen concentrations decrease in elderly women (Piltonen et al., 2003), has led to the assumption that treating low responders with androgens might improve the response to ovarian stimulation.

The first study to address the issue of the potential benefit of androgen supplementation on ovarian sensitivity to FSH was conducted by Casson et al., 2000. In this study five women, aged 35–40, with a basal FSH concentration lower than 20 mIU/ml and a diagnosis of unexplained infertility, and who had poorly responded to previous gonadotrophin stimulation, were given 80 mg/day DHEA for 2 months. The subjects’ baseline mean FSH concentration was 10.7 IU/ml, DHEA was 122 μg/dl and testosterone was 34.2 ng/dl. After 2 months of treatment, stimulation with recombinant FSH and intrauterine insemination (IUI) were performed after induction of ovulation with HCG. Each woman served as her own control. After DHEA supplementation all women had increased responsiveness to FSH, peak oestradiol concentrations increased 3.1-fold, and the mean number of mature follicles also increased from a mean of 12.2. One woman conceived and delivered twins. The authors concluded that DHEA supplementation appeared to augment ovulation induction in poor responders who have normal FSH concentrations and are 35–40 years old (Casson et al., 2000).

A recently published, prospective, therapeutic, self-controlled clinical trial examined the effect of administering transdermal testosterone on ovarian response (Balasch...
et al., 2006). The subjects were 25 regularly menstruating, premenopausal women, aged 31–39 years, with a normal basal FSH concentration of <10 IU/l. In this trial, transdermal testosterone was administered using a patch with a delivery rate of 2.5 mg per day. Each patient received 20 µg/kg of testosterone, thus increasing peak testosterone serum concentration to about four-fold higher than the upper limit for normal reproductive-age women. All participants had a background of cancelled first and second IVF treatment cycles due to poor follicular response. In their third IVF attempt, all patients received transdermal application of testosterone preceding gonadotrophin ovarian stimulation. In the first IVF treatment cycle, ovarian stimulation was carried out with recombinant human FSH under pituitary suppression with GnRH agonist, according to a routinely used protocol. In the second IVF treatment cycle, high-dose gonadotrophin in association with a reduced dose of GnRH agonist was used. In the third IVF attempt, the patients received exactly the same standard, long-down-regulation protocol used in their first IVF treatment cycle, with the addition of testosterone treatment 5 days preceding gonadotrophin administration. Basal hormone measurements were similar in all three cycles assessed. In this study, peak serum oestradiol concentrations and the number of follicles recruited were significantly higher in the third treatment cycle compared with the two preceding IVF treatments. Antral follicle count increased during testosterone treatment, and the number of follicles available for recruitment and development at the time of FSH initiation was higher in non-c_cancelled versus cancelled cycles in the third IVF treatment cycle. In the third cycle, there were five early cancelled cases (20%), and 20 patients (80%) who had received HCG underwent oocyte retrieval. There was an average of 8.5 follicles aspirated, with six clinical pregnancies, including three pairs of twins for a pregnancy rate of 24% per started cycle, a 30% pregnancy rate per oocyte retrieval and a 16.6% implantation rate. As many as 80% of patients having their first two IVF cycles cancelled because of poor follicular response, and in whom testosterone treatment was used in a third attempt, produced a fair number of oocytes, generated two or three embryos for transfer, and achieved an acceptable clinical pregnancy rate of 30% per oocyte retrieval.

Another interesting case report demonstrated the possible benefit of long DHEA supplementation. In this report, a 43-year-old woman with normal baseline FSH was seeking embryo cryobanking for post-thaw aneuploidy screen to preserve an option for future pregnancy (Barad and Gleicher, 2005). After her first cycle, she began self-administration of 75 mg/day DHEA, as well as weekly sessions of acupuncture. In her first cycle peak oestradiol was 1211 pmol/ml. After 7 months of DHEA supplementation her peak oestradiol was 18,000 pmol/ml. The number of oocytes retrieved, as well as the number of embryos available for cryopreservation, increased linearly following initiation of DHEA. Her oocyte yield increased from one before initiation of DHEA to a peak of 18 oocytes after 9 months of treatment. This case report may be an example of a possible advantage of prolonged DHEA supplementation, keeping in mind the time required for follicular recruitment and growth. In view of the extraordinary response to DHEA seen in this patient, the same group of researchers recently published their study on prolonged DHEA supplementation in 25 women with significantly diminished ovarian reserve, based on baseline oestradiol or FSH concentrations (Barad and Gleicher, 2006). They had all experienced a prior IVF cycle, and had produced fewer than four oocytes, with uniformly poor embryo quality. All patients underwent both their pre- and post-DHEA treatment IVF cycles at the same centre. If clinically possible, patients received approximately 16 weeks of DHEA treatment before any post-treatment IVF cycle. Ovulation induction was accomplished using norethindrone acetate, 10 mg for 10 days, starting on day 2 of menses, followed 3 days later by 50 µg of leuprolide acetate, twice daily, and, after another 3 days, by 450 IU of recombinant FSH and 150 IU of HMG. Analysis involved the paired evaluation of 25 study subjects who underwent both pre- and post-DHEA IVF cycles. Outcomes of cycles were compared between pre- and post-DHEA IVF cycles. After treatment with DHEA, patients produced an average of 1.04 ± 0.46 more oocytes (P < 0.05), had higher fertilization rates (P < 0.001), had higher average day 3 blastomere counts (P = 0.01) and had higher grade embryos on day 3 (P = 0.02). The cycle cancellation rate was 8/25 cycles (32%) in pretreatment and 1/25 (4%) in post-DHEA treatment (P = 0.02). The medication was well tolerated by all patients. In 2007, The same group published a case control study of 190 women with diminished ovarian function, defined as FSH 12 mIU/ml, or a baseline oestradiol concentration 75 pg/ml, or FSH concentration >12 mIU/ml but greater than the 95% of the mean value for the patient’s age group (Barad et al., 2007). A group of 101 patients were treated with IVF as soon as possible. The other 89 patients were placed on 75 mg per day DHEA supplementation, and represented the study group. The study group was treated for 4 months, unless they became pregnant earlier. At the end of 4 months these patients were treated with IVF. The study patients were slightly older, and had higher baseline oestradiol concentrations. The study group, representing a more severe degree of diminished ovarian function, produced fewer oocytes, normal day 3 embryos and transferred embryos. Overall clinical pregnancy rates were significantly higher in the study group. Implantation rates showed a similar trend, but failed to reach statistical significance.

Observations demonstrating failure of androgen treatment

In order to assess the effects of testosterone application on the ovarian response to FSH in a subgroup of patients who had previously experienced poor ovarian response to ovarian stimulation, a double-blind, prospective, randomized study was set up (Massin et al., 2006). The design included paired comparison of the ovarian parameters recorded in two consecutive cycles, each woman serving as her own control. The first cycle enabled the selection of patients according to the inclusion criteria. The second cycle was performed following application of a gel containing testosterone or its identical placebo, according to a randomization list. Women applied 1 g of gel (10 mg of testosterone) once daily on the external side of the thigh. Both testosterone and placebo gels were applied for 15–20 days in the period preceding the second
stimulation for IVF or intracytoplasmic sperm injection (ICSI). The number of patients to be included was estimated at 28 in each group. Women were enrolled if two of the following criteria were met: a poor response to ovarian stimulation in a previous IVF or ICSI attempt, defined as plasma oestradiol concentration <1200 pg/ml at HCG day, total number of retrieved oocytes less than or equal to five, and evidence of a decreased ovarian reserve. The latter defined as plasma hormonal values (FSH, oestradiol or inhibin B) outside the normal range of the local standard on day 3 of a spontaneous cycle. Only 53 women met the inclusion criteria and were enrolled in the study, due to difficulties in recruiting women who met the strict inclusion criteria. A total of 26 patients were allocated to placebo and 27 to testosterone gel application. In each group, one patient stopped treatment for personal reasons. In the testosterone-treated group, two patients were discontinued by investigator decision, and they were excluded from analysis. The number of small antral follicles (3–9 mm in diameter) was not significantly modified by gel application in both testosterone- and placebo-treated patients. Comparative analysis of the number of follicles according to their size did not show any significant difference between the two treatment groups. Criteria for HCG administration were met, and oocyte retrievals were performed in 16 women treated with testosterone (67%) and 20 women treated with placebo (80%). A paired comparison was performed between the ovarian parameters in the first and second cycles of the control and testosterone groups. There was a trend towards an increase in the number of follicles, number of retrieved oocytes and number of embryos generated in both groups. Comparison between the number of follicles, oocytes and embryos, in placebo- and testosterone-treated patients did not show any significant difference, although one and four clinical pregnancies were observed in placebo and testosterone-treated patients respectively. However, the low number of patients enrolled in each group precluded any statistical comparison.

Aromatase inhibitors

Aromatase is an enzyme member of the cytochrome p450 family. It catalyses the rate-limiting step in the production of oestrogens: conversion of androstenedione and testosterone to oestrone and oestradiol respectively (Akhtar et al., 1993). This enzyme is found in many tissues, including the ovaries. According to their site of action and biochemical configuration, aromatase inhibitors can be classified as inhibitors with steroid configuration and inhibitors with non-steroidal configuration. The steroidal configured inhibitors, like exemestane, are analogues of androstendione that bind irreversibly to the androgen-binding site of the aromatase enzyme to block its action. The non-steroidal configured inhibitors, namely anastazol and letrozole, bind to the haem moiety of the enzyme to inhibit its function. Anastrazole and letrozole are non-competitive aromatase inhibitors that suppress oestrogen production by 97–99%. These drugs have excellent oral bioavailability, with a half-life of approximately 45 h. Similar to clomiphene citrate, administration of aromatase inhibitors in the early phase of the menstrual cycle increases gonadotrophin secretion (due to reduced oestrogen negative feedback) and enhances follicular development (Casper and Mitwally, 2006).

Over recent years, there have been accumulating reports demonstrating the successful use of aromatase inhibitors for ovulation induction in anovulatory women, after clomiphene citrate (CC) failure and for induction of ovulation (Mitwally and Casper, 2000a,b; Sammour et al., 2001; Al-Fozan et al., 2004). In their review on the role of aromatase inhibitors for ovulation induction, Casper and Mitwally (2006) concluded that these medications are effective for ovulation induction in infertile women, particularly in cases with recurrent CC failure. In addition to the central effect of aromatase inhibitors, a few peripheral effects may contribute to its potential use in poor responders. By blocking the conversion of androgen substrate to oestrogen, accumulation of intra-ovarian androgens occurs. Elevated intra-ovarian androgen concentrations may in turn, as previously discussed, improve follicular recruitment and development.

During the course of an ovarian stimulation cycle, supraphysiological concentrations of oestradiol are reached. Oestradiol concentrations >9000 pmol/l on the day of HCG injection were related to reduced endometrial receptivity and implantation rates (Simon et al., 1995). The fact that high-dose exogenous oestrogen could be used successfully as a post-coital contraceptive supports the theory that supraphysiological concentrations of oestrogen have a detrimental effect on fertilization and implantation. Many mechanisms have been suggested, including a direct deleterious effect on endometrial development, changes in timing of pinopode formation, impaired endometrial blood flow and reduced integrin expression (Mitwally et al., 2005). In view of the accumulating data that aromatase inhibitors can successfully induce ovulation in anovulatory women, it was only logical to examine its effect on ovarian stimulation cycles that used gonadotrophins. A few studies addressing the issue have demonstrated that the addition of aromatase inhibitors to gonadotrophins reduced the FSH dose required for optimum ovarian stimulation, without concomitant adverse anti-oestrogenic effects or change in outcome, despite lower oestradiol concentrations (Biljan et al., 2002; Healey et al., 2003; Mitwally and Casper, 2004). The use of an aromatase inhibitor (letrozole) in conjunction with FSH was compared with the use of CC in conjunction with FSH or FSH alone. Co-treatment with an aromatase inhibitor significantly reduced the FSH dose required during COH to the same extent as CC. Letrozole was not associated with anti-oestrogenic effects, as demonstrated by a significantly lower endometrial thickness noted with CC treatment despite a significantly higher oestradiol concentration. In addition, pregnancy rate with an aromatase inhibitor and FSH was equivalent to FSH alone, and almost twice the rate achieved with CC and FSH treatment (Mitwally and Casper, 2003). The co-administration of aromatase inhibitors (letrozole), during a GnRHa IVF/ICSI protocol was examined in a prospective randomized trial (Verpoest et al., 2006). The results of that study demonstrated a significant increase in LH concentrations, and reduced serum oestradiol concentrations in the group receiving aromatase inhibitor, resulting in an increase in oocytes retrieved and endometrial thickness.
Aromatase inhibitors and low responders

The first observational cohort study addressing the issue of aromatase inhibitors for low responders was published by Mitwally and Casper, in 2002. In this study, 12 patients with unexplained infertility and a poor response to ovarian stimulation, defined as fewer than three follicles >18 mm in diameter on the day of the LH surge or HCG administration, in at least two cycles, were studied. In the experimental cycles, letrozole was given at a dose of 2.5 mg from days 3 to 7 and FSH injections were started on day 7 of the menstrual cycle at a dose of 50–225 IU/day. During cycles applying FSH plus letrozole stimulation, the mean number of mature follicles was 3.3, which was significantly higher than in FSH-only cycles (3.3 versus 1.9 follicles). The amount of FSH required was significantly lower in the letrozole plus FSH cycles and three women conceived a clinical pregnancy with the combined letrozole and FSH treatment. Another randomized, controlled, single-blind trial was conducted to assess the use of letrozole as part of a low-cost IVF protocol for poor responders (Goswami et al., 2004). This study included 38 women over 35 years of age, who had failed one to three IVF attempts due to poor ovarian response, defined as the recruitment of fewer than two dominant follicles in response to a conventional stimulation protocol. All women had normal basal FSH concentrations. Thirteen women were randomly selected and treated with the proposed low-cost IVF protocol consisting of letrozole and recombinant FSH (rFSH), while the remaining 25 subjects were treated with the conventional long down-regulated protocol with incremental doses of rFSH (300–450 IU).

The letrozole group received letrozole at a dose of 2.5 mg daily orally from days 3 to 7 of the menstrual cycle, and rFSH was administered at a dose of 75 IU/day on days 3 and 8 of the menstrual cycle. The letrozole group received a 20-fold lower total dose of FSH and had significantly decreased peak oestradiol concentrations. The two groups did not differ significantly with respect to the number of matured follicles, the number of retrieved oocytes, the number of transferable embryos and endometrial thickness.

Another study addressing the issue of letrozole treatment for poor responders was conducted on 147 women (Garcia-Velasco et al., 2005). This study included a total of 147 IVF cycles performed in 147 low responders, defined as those patients who had at least one previous cancelled IVF attempt in which four or fewer follicles, 16 mm in diameter, were obtained and/or serum oestradiol concentrations were below 500 pg/ml. Basal FSH concentrations were 12 IU/ml or lower. The cancelled cycle was stimulated with a long protocol combined with high doses of FSH/HMG. The study group included 71 patients treated with a high-dose regimen and letrozole. The control group comprised of 76 patients who were given a high-dose regimen alone. The protocol for ovarian stimulation was initiated with the administration of an oral contraceptive pill followed by recombinant FSH and highly purified HMG. GnRH antagonist was added when the leading follicles reached 14 mm in mean diameter. No significant differences among groups were found in terms of days of stimulation, mean FSH/HMG dose administered, mean serum oestradiol concentrations on the day of HCG, endometrial thickness and cancellation rate due to low response. On the other hand, the number of oocytes retrieved was significantly higher in the study group, with a similar fertilization rate between the groups. Implantation rate was significantly higher in the letrozole group. Pregnancy rate per transfer was higher in the letrozole group when compared with the control group, but this finding was not significant.

The largest study evaluating the use of letrozole for the treatment of poor responders was published by Schoolcraft et al., 2008. This prospective study was conducted on 534 infertile women. The major flow of the article was that most patients were recruited based on clinical trials and not on past IVF cycle results. Inclusion criteria included at least one of the following: day 3 serum FSH concentration >10 mIU/ml, fewer than six total antral follicles, prior cycle cancellation, prior poor response to ovarian stimulation (peak oestradiol <500 pg/ml and/or fewer than six oocytes retrieved), and age >41. Patients were assigned in a 2:1 ratio to either a GnRH agonist flare protocol or GnRH antagonist/letrozole protocol. A total of 355 patients were assigned to the flare protocol. All of these patients received 14–21 days of an oral contraceptive. A total of 179 patients were assigned to the GnRH antagonist/letrozole protocol. Oral contraceptives were not used in this regimen. There were no significant differences in age, number of prior failed IVF cycles, ovarian reserve testing, or the indication for IVF between the two groups. There were no differences in duration or doses of gonadotrophins required, numbers of oocytes, or percentage of mature oocytes obtained. A trend towards higher cycle cancellation rates that did not reach statistical significance was experienced among patients assigned to letrozole. Fertilization rates and degree of day 3 embryo quality were similar between the two groups. The mean number of available embryos was also similar. Ongoing pregnancy rates in the letrozole group were significantly lower. A trend towards lower implantation rates, which did not achieve statistical significance, was also noted in this group.

Recently, a large retrospective, case-control study was electronically published by Yarali et al. (2009). In this study, the effect of GnRH antagonist/letrozole protocol was compared with the microdose GnRH agonist flare-up protocol in 885 patients, suspected of having, or with a history of poor ovarian response. The microdose GnRH agonist flare-up protocol was used in 673 patients (1026 cycles), and the GnRH antagonist/letrozole protocol was employed in the remaining 212 patients (357 cycles). The two groups were comparable regarding the mean female age and body mass index. The basal antral follicle count and the rate of first ICSI attempt were significantly less with the letrozole protocol. The total gonadotrophin consumption, duration of stimulation and oestradiol concentration on the day of HCG administration, and number of oocytes retrieved were significantly lower with the letrozole protocol. On the other hand, the fertilization rate and the rate of at least one top-quality embryo transferred were higher in the GnRH antagonist/letrozole protocol. Although it seems that the more severe poor ovarian responders were allocated to the letrozole group (as reflected by the significantly lower basal antral
The use of aromatase inhibitors for ovulation induction has raised some safety issues regarding teratogenicity. Recently, an abstract was presented during a meeting of the American Society for Reproductive Medicine (ASRM) (Biljan et al., 2005), raising the concern that the use of letrozole for infertility treatment might be associated with a higher risk of congenital cardiac and bone malformations in newborns. The abstract was based on examination of 130 babies from 130 pregnancies achieved after letrozole treatment, compared with a large control group of spontaneous conceptions. As a result of this study, Novartis Pharmaceutical issued a statement advising that letrozole is contraindicated for ovulation induction. In 2006, the results on 931 babies born from women who conceived following CC or letrozole treatment at five fertility centres in Canada were published (Tulandi et al., 2006). In this study, data were obtained from 514 babies of mothers who conceived after letrozole treatment and 397 babies of women who conceived after CC. There were no significant differences in the ages of the two groups. The birth weights of the newborns and the incidence of multiple births among the groups were also similar. Overall, congenital malformations or chromosomal abnormalities were found in 14 of 514 newborns in the letrozole group (2.4%) and in 19 of 397 newborns in CC group (4.8%). The rates of minor congenital malformations were not different. The authors of this study in a cohort of infertile women concluded that letrozole treatment for ovulation induction does not increase the risk of congenital malformations, as compared with ovulation induction with CC. In view of the fact that the half-life of letrozole is only 45 h, the drug is already completely eliminated by the time of conception, and therefore teratogenic potential seems unlikely.

**LH supplementation during ovarian stimulation**

GnRHa, long protocol, has become the mainstay for ovarian stimulation in young normoresponders in most IVF units. The widespread use of monotherapy with recombinant FSH causes a decline in LH concentrations. In the late stages of the stimulation protocol, LH concentrations may decline dramatically below 0.5 IU/l. Despite the fact that LH activity seems to be crucial for follicular development, utilizing such an ovarian stimulation protocol is well established in the literature, with excellent results. A meta-analysis comparing r-FSH with HMG in normal responders was conducted in 2003 (Van Wely et al., 2003). The authors failed to demonstrate any advantage for the use of HMG. Most studies comparing the use of rFSH versus rFSh plus rLH for the general IVF population have come up with the same results. The use of GnRH antagonists to block the premature surge of LH is well established in the literature. Although its use causes a profound block in LH, to undetectable concentrations, a recent randomized trial failed to demonstrate any advantage for LH supplementation during GnRHa cycles in an unselected IVF population (Humaidan et al., 2004). Thus, for patients who respond well to ovarian stimulation, the addition of LH during ovarian stimulation in cycles applying either GnRH agonist or antagonist is not mandatory for an adequate outcome.

Information concerning LH supplementation of the GnRHa flare-up protocol in poor responders is conflicting. The biggest randomized trial addressing the issue was performed on 145 women going through a flare-up protocol (Berkkanoglu et al., 2007). They were divided into three groups: group A received rFSH, group B rFSH plus rLH and group C rFSH plus a microdose of HCG. The outcome of all three groups was the same. The authors concluded that exogenous LH is not beneficial in either form.

Nevertheless, there are a few small studies supporting the use of LH during a GnRHa long protocol for poor responders. Ferrari and colleagues (Ferrari et al., 2002) examined the effect of HCG supplementation in 25 poor responders based on their previous long protocol cycle. He found that HCG supplementation resulted in better pregnancy rates and higher oestradiol concentrations. Another small study supporting the use of LH during a long GnRHa analogue protocol was published in 1999 by Laml et al. (1999). In a retrospective cohort study on 141 GnRHa antagonist cycles initiated in poor responders, Chung et al. (2005) demonstrated that the group treated with exogenous LH had fewer oocytes retrieved and fewer fertilized embryos. Despite the retrospective nature of this study, it casts doubt on the common practice of LH supplementation during GnRHa antagonist treatment. In the latest Cochrane review on rLH for ovarian stimulation in assisted reproductive cycles (Mochtart et al., 2007) the authors concluded that although there was no evidence of a statistical difference in pregnancy outcomes when rLH was used, all pooled pregnancy estimates point towards a beneficial effect of co-treatment with rLH, in particular with respect to poor responders.

**Conclusions**

Modulating intra-follicular androgen concentrations may have a stimulating effect on the process of follicular development. However, relatively little attention has been paid so far to theca cell assessment and stimulation in women who have a poor response to ovarian stimulation. Only recently has it been demonstrated that androgen concentrations may have a predictive value in assessing the success of ovarian stimulation. The information concerning the role of androgens and androgen modulating agents, as well as LH, for treating women with poor ovarian response is limited. The search of the literature on this subject yielded four studies and one case report concerning the treatment of poor responders with androgens. The only prospective randomized trial, demonstrated that testosterone supplementation had no beneficial effect. The other three publications were case-control studies, with patients serving as their own controls (Table 1). The variations in patient selection, type of androgens employed and the different duration of exposure precludes from drawing any definite conclusions.

The use of aromatase inhibitors blocks the conversion of androgens to oestrogens, thereby promoting an androgen-rich intrafollicular environment. Recent data supports the...
Table 1. Comparison of the studies on the impact of androgen supplementation in low responders.

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of study</th>
<th>No. of participants</th>
<th>Age (years)</th>
<th>Evidence of low ovarian reserve</th>
<th>Type of androgen used</th>
<th>Period of treatment</th>
<th>Peak androgen concentration</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casson et al., 2000</td>
<td>Prospective self-controlled</td>
<td>5</td>
<td>35–40</td>
<td>FSH &lt; 20 IU/l</td>
<td>DHEA 80 mg/day</td>
<td>8 weeks</td>
<td>Mean testosterone 67.7 ng/dl</td>
<td>Increased E2 concentrations, increased no. of follicles recruited</td>
</tr>
<tr>
<td>Massin et al., 2006</td>
<td>Prospective randomized</td>
<td>Placebo 25, testosterone 24</td>
<td>Not over 42, mean 37.1</td>
<td>FSH &gt; 12 IU/l, E2 &gt; 70 pg/ml, Inhibin B &lt; 45 pg/ml</td>
<td>Testosterone gel 10 mg/day</td>
<td>15–20 days before stimulation</td>
<td>Testosterone &gt; 155 ng/dl</td>
<td>No difference in any parameter</td>
</tr>
<tr>
<td>Balasch et al., 2006</td>
<td>Prospective self-controlled</td>
<td>25</td>
<td>31–39, mean 35.6</td>
<td>FSH &lt; 10 IU/l, mean E2 41.5 pg/ml</td>
<td>Testosterone patch 20 μg/kg</td>
<td>5 days</td>
<td>Testosterone &lt; 300 and &gt; 250 ng/ml</td>
<td>Increase in no. of antral follicles higher peak E2 increased no. of oocytes retrieved by 30%, higher pregnancy rate</td>
</tr>
<tr>
<td>Barad and Gleicher, 2006</td>
<td>Prospective self-controlled</td>
<td>25</td>
<td>Mean 39.9</td>
<td>FSH &gt; 10 IU/l, E2 &gt; 75 pg/ml</td>
<td>DHEA 75 mg/day</td>
<td>&gt;16 weeks</td>
<td>Unknown</td>
<td>Increased in no. of oocytes retrieved higher fertilization rate, higher grade of embryos on day 3</td>
</tr>
<tr>
<td>Brad et al., 2007</td>
<td>Case control</td>
<td>IVF 101, DHEA 89</td>
<td>Mean; study 41.6, control 40</td>
<td>FSH ≥ 12, E2 ≥ 75, FSH ≥ 95% for patient’s age</td>
<td>DHEA 75 mg/day</td>
<td>Up to 4 months</td>
<td>Unknown</td>
<td>Clinical pregnancy rates increased</td>
</tr>
</tbody>
</table>
Table 2. Comparison of the studies on the impact of aromatase inhibitors in low responders.

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of study</th>
<th>No. of patients</th>
<th>Age (years)</th>
<th>Evidence of ovarian failure</th>
<th>Type of aromatase inhibitor</th>
<th>Cycle day of treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitwally and Casper, 2002</td>
<td>Prospective self-controlled</td>
<td>12</td>
<td>24–41, mean 35.5</td>
<td>FSH 5–10.65 IU/l, prior poor response to FSH</td>
<td>Letrozole 2.5 mg</td>
<td>Days 3–7</td>
<td>Reduced total FSH used. Increased no. of mature follicles, no change in endometrium</td>
</tr>
<tr>
<td>Goswami et al., 2004</td>
<td>Randomized controlled trial</td>
<td>38, 13 in letrozole arm</td>
<td>Over 35</td>
<td>Normal basal FSH concentrations, previous cancelled cycle</td>
<td>Letrozole 2.5 mg</td>
<td>Days 3–7</td>
<td>20-fold lower total dose of FSH. No change in other parameters</td>
</tr>
<tr>
<td>Garcia-Velasco et al., 2005</td>
<td>Randomized controlled trial</td>
<td>147, 71 in letrozole arm</td>
<td>Mean 37.1 years</td>
<td>Basal FSH 12 IU/ml or lower, previous cancelled cycle</td>
<td>Letrozole 2.5 mg</td>
<td>Days 3–7</td>
<td>Increased no. of oocytes retrieved, similar fertilization rate with higher implantation rate</td>
</tr>
<tr>
<td>Schoolcraft et al., 2008</td>
<td>Prospective controlled trial</td>
<td>534, 179 in letrozole arm</td>
<td>Unknown 38% &gt;41 years</td>
<td>FSH &gt;10 IU/l, total antral follicles &lt;6, age &gt;41</td>
<td>Letrozole 2.5 mg</td>
<td>Days 3–7</td>
<td>Pregnancy rates were lower in the letrozole group. Trend towards increased implantation and lower cancellation rates in the control group</td>
</tr>
<tr>
<td>Yarali et al., 2009</td>
<td>Retrospective, case-control flare-up protocol, 212 in letrozole arm</td>
<td>Mean 36</td>
<td>FSH &gt;10, total antral follicles &lt;6, E2 &gt;60 or history of poor response</td>
<td>Letrozole 2.5 mg</td>
<td>Days 2–6</td>
<td>Gonadotrophin consumption, duration of stimulation, E2 concentration and no. of oocytes retrieved were lower Higher fertilization rate and the rate of at least one top-quality embryo transferred in aromatase inhibitor protocol</td>
<td></td>
</tr>
</tbody>
</table>
effectiveness of letrozole as a safe option for ovulation induction (Table 2). The evidence presented in this review suggests a potential beneficial role for the use of aromatase inhibitors in treating women who have previously failed standard IVF protocols. The optimal dose and duration of this treatment is yet to be determined. It might be possible that prolonged use of letrozole, as done with androgen supplementation, may increase the cohort of early follicles, leading to better follicular yield.

In a natural cycle, LH is essential in maintaining adequate steroidogenesis and follicular development. Since the development and wide use of recombinant FSH as a single gonadotrophin for ovarian stimulation, the question of LH supplementation has been debated. Despite the fact that the common practice of LH supplementation for poor responders has no statistically proven benefit, the latest Cochrane review on rLH for controlled ovarian hyperstimulation support its use in poor responders, based on pooled pregnancy estimates.

It is suggested that a subgroup of poor responders have theca cell failure but retain a relatively preserved granulosa cell function. Identifying this particular population and modulating their intra-follicular androgen environment may improve their ovarian response. It seems that in spite of the dearth of information in this area, patients for whom other modalities of treatment have failed may consider this option.

References


Barad DH, Gleicher N 2005 Increased oocyte production after treatment with dehydroepiandrosterone. Fertility and Sterility 84, 756, e1-e3.

Barad DH, Gleicher N 2006 Effect of dehydroepiandrosterone on oocyte and embryo yields, embryo grade and cell number in IVF. Human Reproduction 21, 2845–2849.


Biljan MM, Hemmings R, Brassard N 2005 The outcome of 150 babies following the treatment with letrozole or letrozole and gonadotropins. Fertility and Sterility 84, O-231, Abstract 1033.

Biljan MM, Tan SL, Tulandi T 2002 A prospective randomized trial comparing the effects of 2.5 and 5.0 mg of letrozole (LE) on follicular development, endometrial thickness and pregnancy rate in patients undergoing super-ovulation. Fertility and Sterility 78, S55.


Mitwally MF, Casper RF 2002 Aromatase inhibition improves ovarian response to follicle-stimulating hormone in poor responders. Fertility and Sterility 77, 776–780.


Mitwally MF, Casper RF, Diamond MP 2005 The role of aromatase inhibitors in ameliorate deleterious effects of ovarian stimulation on outcome of infertility treatment. Reproductive Biology and Endocrinology 3, 54.


Sammour A, Biljun MM, Tan SL et al. 2001 Prospective randomized trial comparing the effects of letrozole (LE) and clomiphene citrate (CC) on follicular development, endometrial thickness and pregnancy rate in patients undergoing super-ovulation prior to intrauterine insemination (IUI). Fertility and Sterility 76, S110.


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