Outlook

Gonadotrophin treatment in patients with polycystic ovary syndrome

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Abstract

Gonadotrophin treatment in clomiphene citrate resistant polycystic ovarian syndrome (PCOS) patients, using either low-dose step-up or low-dose step-down protocols, is highly effective to achieve singleton live births. Concomitant use of gonadotrophin releasing hormone analogues (GnRHa), which will block the endogenous feedback for monofollicular development during the low-dose step-up protocol, should not be employed. It is more difficult to induce ovulation in patients with more 'severe' PCOS, characterized by obesity and insulin resistance. There is need for optimization of starting doses for both the low-dose step-up and step-down protocols. Such optimization will prevent hyperstimulation due to a starting dose far above the FSH threshold, as well as minimize the time-consuming low-dose increments by starting with a higher dose in women with augmented FSH threshold. External validation of reported models for prediction of FSH response is warranted for tailoring and optimizing treatment for everyday clinical practice. Although preliminary, the partial cessation of follicular development, along with regression leading to atresia, lends support to the LH ceiling theory, emphasizing the delicate balance and need for both FSH and LH in normal follicular development. Future well-designed randomized controlled trials will reveal whether IVF with or without in-vitro maturation of the oocytes will improve safety and efficacy compared with classical ovulation induction strategies.

Keywords: *anovulation, gonadotrophins, infertility, ovulation induction, polycystic ovary syndrome*

Introduction

Polycystic ovary syndrome (PCOS) is the most common reproductive endocrine disorder. It is characterized by hyperandrogenism, chronic anovulation, and is related to infertility, hirsutism, increased risk of insulin resistance, type 2 diabetes, cardiovascular disease and endometrial cancer (Legro, 2002; Hardiman *et al*., 2003). Pathophysiology of the syndrome appears to be multifactorial and polygenic (Franks *et al*., 2001). Patients with PCOS display heterogeneity with respect to clinical presentation, biochemical features and metabolic abnormalities (Yildiz *et al*., 2003).

The diagnostic criteria of PCOS have been recently revised after a joint meeting of the European Society for Human Reproduction (ESHRE) and the American Society for Reproductive Medicine (ASRM) in Rotterdam in May 2003 (Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004). The new definition required the presence of at least two of the following three criteria: (i) oligo- and/or anovulation, (ii) hyperandrogenism (clinical and/or biochemical), and (iii) ultrasonographic appearance of polycystic ovaries (PCO) with the exclusion of other aetiologies. Furthermore, the diagnosis of PCO has been revised (Balen *et al*., 2003) to include at least one of the following: either 12 or more follicles measuring 2–9 mm in

diameter, or increased ovarian volume (>10 cm^3). If there is a follicle >10 mm in diameter, the scan should be repeated at a time of ovarian quiescence in order to calculate volume and area. The distribution of follicles and a description of the stroma are not required in the diagnosis. However, there is still room for improvement of the Rotterdam Criteria, which may carry the risk of misinterpretation and under- and overestimation of symptoms, as well as of overlooking other hyperandrogenic states (Geisthövel, 2003).

Ovulation induction

Since the main reason for infertility is anovulation in patients with PCOS, restoration of ovulation is undertaken when fertility is desired. The aim of ovulation induction in women with PCOS is the formation and ovulation of a single dominant follicle.

The following end-points should be considered when evaluating the safety and efficacy of ovulation induction in patients with PCOS: single follicle development rate, multiple follicle development rate, multiple pregnancy rate, ovarian hyperstimulation syndrome (OHSS) rate, ovulation rate per cycle, pregnancy rate per cycle, miscarriage rate, singleton live birth rate per cycle and cumulative singleton live birth rate. Singleton live birth rate per cycle and cumulative singleton live birth rate should be considered as the 'golden' outcome parameters for induction of ovulation.

There is currently no consensus on the best algorithm to induce ovulation in infertile patients with PCOS. Lifestyle modification may be a valuable option, not only to induce ovulation but also to prevent long-term metabolic risks (Norman *et al*., 2002). Obesity contributes to insulin resistance, and therefore anovulation. Although in practice not very successful, simple weight loss *per se* may restore ovulation in a subset of patients with PCOS (Crosignani *et al*., 2002).

Clomiphene citrate is commonly employed as the first-line treatment agent to induce ovulation in infertile patients with PCOS. Initial treatment with clomiphene citrate (highest daily dose of 150 mg for a maximum of six cycles) results in ovulation in 77% of patients (Eijkemans *et al*., 2003). Pregnancy is achieved in 47% of all patients and 61% of ovulatory patients, resulting in a cumulative singleton live birth rate of 41% and multiple live birth rate of 2%. Chances of ovulation (Imani *et al*., 1998, 2000) and pregnancy (Imani *et al*., 1999) may be predicted by age, body mass index (BMI), free androgen index [FAI = testosterone × 100/sex hormone binding globulin (SHBG)], cycle history and polycystic ovary appearance.

Although initial studies are promising for new alternative treatment options, including first-line treatment with insulin sensitizers (Lord *et al*., 2003) or aromatase inhibitors (Mitwally and Casper, 2003), their place in routine clinical practice remains uncertain until data from randomized controlled trials employing large series of well defined patient groups are available.

Resistance to clomiphene citrate covers a heterogenous group of patients including (i) patients who do not ovulate with clomiphene citrate and (ii) patients who ovulate but do not conceive with clomiphene citrate. Exogenous gonadotrophin treatment is usually undertaken as second-line treatment in patients with PCOS who are resistant to clomiphene citrate. Patients who fail to ovulate with clomiphene citrate may have a more 'serious' ovarian abnormality that is associated with a higher threshold dose of exogenous FSH (Imani *et al*., 2002). Patients who ovulate but do not conceive after clomiphene citrate may have a lower FSH threshold and may subsequently require a lower dose of exogenous FSH to reach sufficient ovarian stimulation (Imani *et al*., 2002).

Protocols for gonadotrophin treatment

While several approaches for ovulation induction with gonadotrophins have been described, the two most commonly used in clinical practice are the low-dose step-up and the low-dose step-down protocols. These chronic low-dose protocols should be employed for gonadotrophin treatment to minimize the risk of multiple follicular development. The two risks associated with multiple follicular development are multiple pregnancy and OHSS (Blankstein *et al*., 1987).

It is essential for the infertility practitioner to recognize the importance of the 'FSH threshold' concept as suggested by Brown (1978) for monofollicular development.

Physiology of monofollicular development

Folliculogenesis is a sequence of complex and closely integrated events, which functionally link the hypothalamus, the pituitary gland and the ovary. Gonadotrophins, specifically FSH, are the essential drivers for folliculogenesis. The pre-antral stages of follicular development are not dependent on gonadotrophins; however, FSH can influence growth and survival of pre-antral follicles *in vitro* (Wright *et al*., 1999). Little is known about the local and/or endocrine factors that are responsible for initiation and progression of growth of these follicles (Franks *et al*., 2003). The later stages of follicle maturation, from antrum formation to ovulation, are gonadotrophin dependent and, in the final 2 weeks before ovulation, are highly regulated by the cyclical changes in LH and FSH (Franks *et al*., 2003). The great majority of oocytes are destined for atresia, whereas normally only one progresses to ovulation in each cycle in the human (Gougeon, 1996). The demise of the corpus luteum and a subsequent decrease in progesterone and oestradiol production lead to increase in FSH concentrations at the end of the luteal phase of the menstrual cycle (Roseff *et al*., 1989; le Nestour *et al*., 1993). Increased FSH concentration, which occurs during the luteofollicular transition, is a potent stimulus for follicular recruitment, and several (or cohort) early antral follicles start to enlarge beyond 2–5 mm (Hall *et al*., 1992). Subsequent development of this cohort during the follicular phase becomes dependent on continued stimulation by gonadotrophins (Macklon and Fauser, 2001). Oocytes in these recruited follicles have already completed their growth, acquired a zona pellucida, and are competent to resume meiosis (Trounson *et al*., 1998). Each

growing follicle has a 'threshold' level that should be passed to ensure ongoing preovulatory development (Brown, 1978; Van der Meer *et al*., 1994). This threshold level should be overcome for further preovulatory development. In the normo-ovulatory cycle, only one follicle will become responsive to FSH above this threshold and produce sufficient oestradiol by FSH induced aromatase from androgens produced in LH primed theca cells according to the 'two cell–two gonadotrophin model' (Dorrington and Armstrong, 1979). In response to negative feedback from rising oestradiol and inhibin concentrations, FSH concentrations fall in the late follicular phase. The dominant follicle remains 'sensitive and responsive' to the falling FSH concentrations through sufficiently developed FSH receptors, and continues growing (Bao *et al*., 1997; Fauser and Van Heusden, 1997). As FSH concentrations drop below the threshold for stimulation, less mature follicles from the cohort are driven to atresia. The duration of this 'FSH window' during which FSH concentrations are above the threshold required to stimulate ongoing development determines the number of follicles which can develop to the pre-ovulatory stage (Baird, 1987; Fauser and Van Heusden, 1997). These advanced stages of the follicular development are susceptible to therapeutic manipulation with exogenous FSH.

Conventional protocol

This protocol involves initial daily doses of 75–150 IU FSH treatment with increases of 75 IU FSH every 5–7 days, when needed. Supraphysiological doses of FSH in this protocol provoke initial development of a large cohort, stimulate additional follicles, and even rescue those follicles destined for atresia (Insler, 1988). While ovulation rates of 70% were achieved, multiple pregnancy rates were observed to occur in 36% of pregnancies, and potentially life-threatening OHSS in 14% (Dor *et al*., 1980). Reported cumulative pregnancy rates ranged from 21 to 75%; however, OHSS rates ranged between from 1.1 to 12% (Hamilton-Fairley and Franks, 1990; Farhi *et al*., 1993; Fauser and Van Heusden, 1997).

The conventional protocol, which is associated with unacceptably high multiple follicle development, multiple pregnancy and a significant incidence of OHSS, should not be employed to induce ovulation in patients with PCOS.

Chronic low-dose protocols

In order to minimize the rate of multifollicular development, chronic low-dose protocols should be employed. The chronic low-dose protocols include: low-dose step-up protocol, low-dose step-down protocol, sequential protocol and low-dose step up protocol.

Low-dose step-up protocol is the most commonly employed chronic low-dose regiment. This protocol is employed as follows (Yarali *et al*., 1999); after spontaneous or progesterone induced withdrawal bleeding, 75 IU of daily FSH treatment is commenced. The initial dose of 75 IU/day is maintained for up to 14 days unless follicle maturity is reached so that human chorionic gonadotrophin (HCG) could be administered. Ovarian response is initially monitored by serum oestradiol concentrations every 2 to 3 days. When serum oestradiol concentration exceeds 100 pg/ml (conversion factor = 3.671; 367 pmol/l), monitoring is continued by daily transvaginal ultrasonography and serum oestradiol concentrations. If no ovarian response is noted after 14 days of 75 IU/day therapy, the daily FSH dose is increased by 37.5 IU. Any further FSH increment thereafter is made by 37.5 IU at weekly intervals to a maximum of 225 IU/day. If a dominant follicle emerges, the dose of FSH (threshold dose) is maintained until the follicle reaches a mean diameter of 17 mm. At that point, HCG at a dose of 10,000 IU is administered. If there are more than three follicles of 15 mm or greater in diameter, the cycle is cancelled due to the risk of multiple pregnancy and/or OHSS. If there is no follicular response after 35 days of treatment, the cycle is cancelled. Blood is taken for measurement of progesterone 6–8 days after HCG administration and ovulation is confirmed when progesterone concentration exceeds 5 ng/ml (conversion factor = 3.18; 15.9 nmol/l). A serum pregnancy test is performed 14 days after administration of HCG. If, despite ovulation, pregnancy has not occurred during the first course of treatment, FSH is reintroduced at a subthreshold dose (37.5 IU less than the preceding threshold dose). Since the threshold dose within subjects varies from cycle to cycle, it is important to start with a subthreshold dose and adhere strictly to the stepwise increments. In women who develop multiple follicles using the above-mentioned protocol, the treatment is modified so that either a smaller starting dose (37.5 IU) is used and/or the increments are made less than usual (i.e. 18.7 IU).

The hypothalamo–pituitary–ovarian axis is intact during induction ovulation using the low-dose step-up protocol, which is essential to avoid multifollicular development (Van der Meer *et al*., 1996). Therefore, concomitant use of gonadotrophin releasing hormone analogues (GnRHa), which will block this endogenous feedback for monofollicular development (Van der Meer *et al*., 1996), is inadvisable during the low-dose step-up protocol. In accordance with this observation, a recent Cochrane Library meta-analysis reported a higher overstimulation rate when a GnRHa was added to gonadotrophin treatment [odds risk ratio: 3.15; 95% confidence intervals (95% CI) 1.48–6.70] (Nugent *et al*., 2003).

The multiple pregnancy rate may be minimized by strict adherence to the criteria for administering HCG. If HCG is withheld in the presence of more than three follicles >15 mm in diameter, the multiple pregnancy rate may be minimized (Ares-Serono, 1995). The pregnancy rates were 17, 26, 34 and 26% when 1, 2, 3 and >3 follicles exceeding 15 mm in diameter were present on HCG day. However, as might be expected multiple birth rate increased dramatically; the respective figures were 5, 12, 20 and 50%.

Traditionally, the starting dose of FSH is 75 IU. Franks *et al*. published largest series of cases to date from a single centre (White *et al*., 1996), and concluded that it would be more appropriate when the starting dose was 52.5 IU for 14 days. They reported that in their initial series of patients, the routine starting dose was 75 IU, but this was subsequently modified because even at this initial dose, more than 20% of cycles had to be abandoned because of the subsequent development of multiple follicles. When they used a starting dose of 52.5 IU, they made the first incremental dose rise of 30% up to 75 IU for the third week and increments of 37.5 IU thereafter. A comparison of a starting dose of 75 IU with the starting dose of 52.5 IU had revealed an increase in the incidence of

monovulatory cycles with the latter (72 versus 84%). The rates of ovulation, pregnancy and miscarriage were similar.

When starting doses of 50 IU and 37.5 IU of rFSH, with increments of 50 IU and 37.5 IU respectively when necessary, were compared (Balasch *et al*., 2000), similar monofollicular development rates were achieved. The authors concluded that even a starting dose of 37.5 IU of rFSH may be adequate to induce follicular growth.

The prediction of the threshold FSH dose is essential for optimization of ovulation induction using the low-dose step-up protocol. Body mass index (BMI) is an important prognostic factor that not only influences cycle cancellation due to no ovarian response (White *et al*., 1996), but also threshold FSH dose (Imani *et al*., 2002). White *et al*. reported that in women with a BMI greater than 25 kg/m^2, the number of abandoned cycles was higher than that in women of normal weight (31 versus 15%) (White *et al*., 1996). Imani *et al*. established a model for prediction of FSH threshold using multivariate analysis, and significant predictors for monofollicular development were BMI, presence of clomiphene citrate resistance, initial free insulin-like growth factor-1 and initial serum FSH concentrations (Imani *et al*., 2002).

It is believed that the strict adherence to a 14-day starting period using a persistent dose is essential to minimize the risk of multifollicular development. Homburg *et al*. has compared a starting dose of 75 IU of FSH for 14 days with the same dose for 7 days before employing an incremental dose rise of 37.5 IU if necessary (Homburg and Howles, 1999). The 7-day starting regimen significantly decreased the amount of FSH required (mean 17.1 versus 22.1 ampoules) and the mean duration of treatment (13.1 versus 17.4 days) compared with the 14-day starters. There was no difference in the daily effective dose, number of cancelled cycles, oestradiol concentrations and number of large and intermediate follicles. Pregnancy rates for the 7-day and 14-day protocols were similar (56 and 40% per patient and 33 and 19% per completed cycle respectively). There were no multiple pregnancies in the group of 14-day starters but the 7-day starter group had a multiple pregnancy rate of 24%.

Chances for multiple follicular development during FSH induction of ovulation may be predicted by a model in which initial serum androstenedione, ovarian response during preceding clomiphene citrate treatment and number of antral follicles on initial screening are represented (Van Der Meer *et al*., 1998; Van Santbrink *et al*., 2002; Jonard *et al*., 2003; Mulders *et al*., 2003b). As multiple follicular growth is associated with increased risk of both multiple pregnancy and OHSS (Blankstein *et al*., 1987), patients at risk may be identified using this prediction model.

The results of experience on low-dose step-up protocol using recombinant FSH (rFSH) in 122 patients (252 cycles) are given in **Tables 1** and **2**.

Conventional and low-dose step-up protocols yield comparable pregnancy rates (Homburg *et al*., 1995; Hedon *et al*., 1998). However, the major advantage of the low-dose step-up protocol is the achievement of high rate of monofollicular development which is ~69% (54–88%) (Homburg and Insler, 2002). The reported fecundity per cycle is around 20% (12–45%); the multiple pregnancy rate is ~6% and OHSS is 0.14% (0–2.4%) (Homburg and Howles, 1999).

White *et al*. (1996) reported the cumulative conception rate as 57% after six cycles; 75% of pregnancies occurred in the first three cycles of treatment and only one pregnancy occurred after the sixth cycle. IVF may be offered to those who fail to conceive after a maximum of six cycles of gonadotrophin treatment.

Table 1. Clinical and endocrine features of PCOS patients (ongoing study). Values are means ± SD. Range given in parentheses.

No. of patients	122
No. of cycles	252
Duration of infertility (years)	6.7 ± 4.1 (1–17)
Mean female age (years)	28.4 ± 4.7 (19–40)
Body-mass index (kg/m^2)	27.4 ± 4.9 (18–43)
LH (IU/l)	11.7 ± 6.6 (5–29)
Testosterone (ng/dl)	1.03 ± 0.69 (0.27–4)
Anovulatory with clomiphene citrate (%)	51

Table 2. Results of the low-dose step-up protocol (ongoing study). Values are means ± SD. Range given in parentheses unless otherwise stated.

Cycle cancellation (%)	23 (9)
Hyper-response (%)	7 (3)
No response (%)	16 (6)
Threshold FSH dose (IU/day)	112.8 ± 45.4 (38–300)
Duration of stimulation (day)	14.1 ± 6.1 (5–36)
Oestradiol on day of HCG (pg/ml)	510 ± 447 (101–1500)
No. of ovulatory cycles (%)	214 (85)
No. of monofollicular cycles[a] (%)	128 (56)
No. of pregnancies	53
Per cycle attempted (%)	21
Per ovulatory cycle (%)	25
Cumulative pregnancy (%)	
1st cycle	17.1
2nd cycle	36.3
3rd cycle	46.0
No. of multiple pregnancies (%)	4 (8)
No. of miscarriages (%)	14 (26)

[a]Only single follicle ≥14 mm in diameter on the day of HCG.

Low-dose step-down protocol

In an attempt to mimic physiology more closely in women with PCOS, a stimulation regimen has been described by Fauser's group, which involves reducing (instead of increasing) the dose of gonadotrophins administered during the period of follicular development (Van Santbrink and Fauser, 1997). Monitoring of a step-down cycle may need more experience compared with a low-dose step-up regimen.

In the step-down protocol, 150 IU FSH daily is started shortly after a spontaneous or progesterone-induced bleed and continued until a dominant follicle (≥10 mm) is seen on transvaginal ultrasonography. The dose is then decreased to 112.5 IU per day followed by a further decrease to 75 IU per day 3 days later, which is continued until HCG is administered to induce ovulation (Van Santbrink *et al*., 1995; Van Santbrink and Fauser, 1997).

Van Santbrink and Fauser (1997), in their small prospective randomized controlled trial, reported comparable clinical outcomes with stepdown and low-dose step-up regimens in 37 clomiphene citrate-resistant women. However, in the step-down group, a substantially reduced stimulation period (9 days versus 18 days) was required with a more physiological late-follicular serum FSH profile. This resulted in more monofollicular cycles (88 versus 56%), coinciding with more cycles in which serum oestradiol concentrations were within the physiological range. However, no multiple pregnancies were noted in either group.

Balasch *et al*. (2001), using a crossover study design, compared the low-dose step-up protocol with a modified step-down protocol in which patients received 300 IU of rFSH on cycle day 3 and no treatment was given on the next 3 days (cycle days 4–6). rFSH was reinitiated on cycle day 7 (treatment day 5) by administering 75 IU daily. This dose was maintained until cycle day 9 and then the protocol was exactly the same as that in the low-dose step-up protocol. The total number of follicles that were larger than 10, 14 and 17 mm in diameter on the day of HCG, and thus cycles cancelled were significantly increased with the step-up protocol. The authors concluded that a 'physiological' step-down approach might be more appropriate than the step-up protocol to avoid multifollicular development.

However, in a recent multicentric study enrolling 83 patients in 11 centres, the step-up protocol using rFSH was reported to be significantly more efficient in obtaining monofollicular development (68 versus 32%) and ovulation (70 versus 62%) than the step-down protocol (Christin-Maitre and Hugues, 2003). Treatment results showed a relatively high cancellation rate (38 versus 15%) in the step-down compared with the step-up group. Pregnancy rates were comparable, but there was a clear tendency for hyperstimulation in the step-down group. Although the duration of stimulation was longer (15 days versus ~10 days), the rate of ovarian hyperstimulation was much lower (2 versus 11%) using the step-up protocol. Some differences in patient population between this study and that of Van Santbrink and Fauser's study (Van Santbrink and Fauser, 1997) may account for the conflicting results. PCOS is a heterogeneous disorder and patients included in the study by Christin-Maitre and Hugues (2003) presented with 'milder' form of PCOS; they had normal mean BMI (23.5 ± 4.4 kg/m^2), did not have hyperandrogenism (testosterone <1 ng/ml) and had sonographic 'mild' PCO appearance, and hence can be expected to have low FSH threshold doses. Therefore, employment of a step-down protocol with a starting dose of 100 IU to such a 'milder' subset of patients with PCOS may account for the observed high overstimulation rate in the step-down group. The finding that 55–73% of the patients randomized to the step-up protocol did not need a dose increment during their entire cycle further confirms this contention.

These findings underline the need for optimization of the starting doses for both the step-up and step-down protocols. Such optimization will prevent hyperstimulation due to a starting dose far above the FSH threshold as well as minimize the time-consuming low-dose increments by starting with a higher dose in women with augmented FSH threshold. Imani *et al*. (2002) developed a model using the initial patient and treatment characteristics (BMI, ovarian response during preceding clomiphene citrate cycle, insulin-like growth factor-1 (IGF-1) and basal FSH concentrations) to predict the FSH response. External validation of such models is obviously warranted for widespread everyday clinical use.

Van Santbrink and Fauser (2003) reported that 31% of their patients undergoing ovulation induction using the low-dose step-up protocol developed a preovulatory follicle without a need for dose increment. It may be speculated that these women would hyper-respond if a step-down protocol had been offered with a relatively 'high' starting dose. Hence, in order to avoid this hyper-response, Fauser's group advocate offering first a 'dose-finding' low-dose step-up induction cycle during which the FSH threshold dose is determined (Van Santbrink and Fauser, 2003). After the first cycle, step-down cycles are undertaken with a starting dose 37.5 IU above the threshold dose in the initial low-dose step-up cycle. A fixed dose is applied in the following cycle if the patient did not require any dose increase in the initial low-dose step-up cycle.

Sequential step-up and step-down protocol

The FSH dependence of the leading follicle decreases as the follicle grows. This decrease in FSH threshold contributes to the escape of the leading follicle from atresia when FSH concentrations start to decrease due to negative feedback of rising oestrogen concentrations during the follicular phase. Hugues *et al*. (1996), using this physiological principle, developed the so-called 'sequential step-up and step-down protocol'. In this protocol, a step-up approach is utilized, and thereafter the FSH dose is reduced by 37.5 IU FSH when the leading follicle reaches a diameter of 14 mm. In a prospective randomized controlled trial, they compared the low-dose step-up protocol (38 cycles) with the sequential protocol (35 cycles). At the time of HCG administration, cycles treated with sequential protocol exhibited significantly lower oestradiol concentrations and the number of medium-sized (14–15 mm) follicles was significantly reduced (0.3 ± 0.1 versus 0.8 ± 0.2) compared with cycles treated with the low-dose step-up protocol. The authors concluded that decreasing the FSH dose following step-up follicular selection might be an alternative method to avoid multifollicular development.

Type of gonadotrophins

Although induction of ovulation with gonadotrophins proved efficacious, there is still an ongoing controversy as to which gonadotrophin preparations are more convenient. Gonadotrophin preparations in the form of urinary derived human menopausal gonadotrophins (HMG), which contain FSH, LH, HCG and large quantities of urinary proteins, have been used for decades. Improvements in purification techniques led to increasing relative amounts of the active ingredients, and the first urine derived preparation containing only urinary FSH (uFSH) became available in 1983. The development and application of the production techniques based on immuno-affinity chromatography with monoclonal antibodies enabled the production of highly purified uFSH. In the 1990s, recombinant DNA technology led to the development and clinical introduction of human recombinant FSH (rFSH) by stable transfection of the common α- and β-FSH subunit into a Chinese hamster ovary cell line. This development promised not only unlimited availability, but also improved purity and batch-to-batch consistency than urinary derived products. Highly purified (HP) HMG is the latest addition to this family of infertility drugs.

A small comparative randomized study of low dose HMG versus 'purified' uFSH was carried out in 30 women with PCOS over 75 cycles (Sagle *et al*., 1991). In this small study with no power analysis, ovulation and pregnancy rates and the number of monovulatory cycles induced by the two preparations were comparable. The authors suggested that the success of treatment depended on the low dose of gonadotrophin used, rather than the presence or absence of LH in the preparation.

A trial comparing HMG (eight patients, 26 cycles) with rFSH (14 patients, 40 cycles) using the low-dose step-up protocol in women who had suffered severe OHSS with previous conventional treatment did not demonstrate any difference in efficacy. No further cases of OHSS were noted with either preparation (Aboulghar *et al*., 1996).

In a randomized controlled trial, the efficiency of uFSH (35 patients, 64 cycles) and rFSH (16 patients, 32 cycles) was compared using the low-dose step-up protocol (Yarali *et al*., 1999). Cumulative ovulation rates in three cycles were 89.3 and 93.1% for the uFSH and rFSH groups respectively. The threshold and total doses of FSH and duration of stimulation were similar between the two groups. Significantly more monofollicular development was noted in the rFSH group (48 versus 76%, $P < 0.05$). The clinical pregnancy rates for uFSH and rFSH groups were comparable (23 and 28%). Hugues *et al*. (2001), in a retrospective controlled study, reported that while the number of selected follicles was similar, the mean daily FSH dose required to achieve the threshold of follicular selection was significantly lower with rFSH than uFSH. The total FSH dose was also significantly less in the rFSH group.

In a recent Cochrane Library meta-analysis, the effectiveness of HMG and uFSH was compared with induced ovulation in infertile patients with PCOS (Nugent *et al*., 2003). Twenty-three randomized controlled trials were identified; only 14 were eligible for meta-analysis. Gonadotrophin protocols in the included studies were not uniform; apart from low-dose step-up protocol, conventional protocol was also utilized. Concomitant GnRHa was administered in four trials. No significant differences were demonstrated in any of the studied outcomes, except for a reduction in the risk of OHSS [odds ratio (OR) 0.33; 95% CI 0.16–0.65] with uFSH in the seven trials reporting this outcome. Separating the studies into those with (two trials) and without (five trials) concomitant GnRHa administration, the beneficial effect of FSH versus HMG for OHSS was only present where no analogue was used (OR 0.20; 95% CI: 0.08–0.46), halving the incidence from 12 to 6%.

Bayram *et al*. (2003), in a recent Cochrane Library meta-analysis, compared the safety and effectiveness of uFSH and rFSH in terms of ovulation, pregnancy, miscarriage, multiple pregnancy and OHSS rates. Only four randomized controlled trials were included in the meta-analysis. No significant differences were demonstrated for the relevant outcomes. The odds ratio for ovulation rate was 1.19 (95% CI: 0.78–1.80), for pregnancy rate 0.95 (95% CI: 0.64–1.41), for miscarriage rate 1.26 (95% CI: 0.59–2.70), for multiple pregnancy rate 0.44 (95% CI: 0.16–1.21) and for OHSS 1.55 (95% CI: 0.50–4.84). The authors concluded that, although there were not sufficient data, both rFSH and uFSH appeared to be equally applicable for ovulation induction in women with PCOS.

Good prognostic parameters for ovulation and pregnancy after treatment with gonadotrophins

Prognostic factors which may affect outcome for gonadotrophin treatment are female age (McClure *et al*., 1993; Mulders *et al*., 2003a), obesity (Hamilton-Fairley *et al*., 1992; White *et al*., 1996; Vicino *et al*., 2000; Mulders *et al*., 2003b), insulin resistance (Dale *et al*., 1998; Mulders *et al*., 2003b), hyperandrogenaemia (Mulders *et al*., 2003b), polycystic ovaries (Jonard *et al*., 2003) and preceding response to clomiphene citrate (Imani *et al*., 2002).

One of the major problems after ovulation induction in patients with PCOS is the high rate of early pregnancy loss, particularly in overweight women with the syndrome. The contributing factors for this increased early pregnancy loss may include errors in folliculogenesis, meiotic competence and oocyte maturation due to an abnormal endocrine environment and/or intrinsic abnormalities of the oocyte (Franks *et al*., 2003).

Mulders *et al*. (2003a) recently reported the prognostic factors for success or complications for gonadotrophin ovulation induction in women with normogonadotrophic anovulatory infertility. One hundred and fifty-four women with normogonadotrophic anovulatory infertility for whom clomiphene citrate induction of ovulation had been unsuccessful were included. In most patients, a dose finding low-dose step-up regimen was applied during the first treatment cycle in order to identify the individual FSH response dose. In all subsequent cycles, a step-down protocol was employed. A mean of 3.5 stimulation cycles resulted in an ovulation rate of 82%, a cumulative pregnancy rate of 56%, a singleton live birth rate of 43% and a multiple live birth rate of 5%. Initial serum concentrations of LH, testosterone and

androstenedione were significant predictors for the probability of multifollicular development. Comparing those women who did, versus those who did not, achieve an ongoing pregnancy in a multivariate Cox regression analysis, initial serum IGF-I, testosterone and women's age entered into the final model (AUC = 0.67). The authors concluded that individual treatment outcome following gonadotrophin induction of ovulation might be predicted by initial screening characteristics.

The same group, through a meta-analysis, reported patient predictor factors for outcome of gonadotrophin ovulation induction in women with normogonadotrophic anovulatory infertility (Mulders *et al*., 2003b). Thirteen studies in which pretreatment screening characteristics (BMI, serum LH and androgen concentrations, insulin sensitivity and ultrasound appearance of the ovaries) were related to outcome parameters (such as total amount of FSH administered, cancellation, ovulation, pregnancy and miscarriage) were included. A positive association was found in all studies between obesity and total amount of FSH administered. Pooled OR between obesity, cancellation and ovulation were 1.86 (95% CI: 1.13–3.06) and 0.44 (95% CI: 0.31–0.61) respectively. However, no significant association was noted between obesity and pregnancy rate. The pooled OR for obese versus non-obese women and miscarriage rate was significant [3.05 (95% CI: 1.45–6.44)]. Association measures between insulin resistance (definition applied as assessed by individual studies) and total amount of FSH administered produced a weighted mean difference of 351 (95% CI: 73–630) IU. A significant association was found between insulin resistance with pregnancy rate [OR = 0.29 (95% CI: 0.10–0.80)]. However, no significant association was noted between insulin resistance (hyperinsulinaemia versus normoinsulinaemia) and miscarriage. A pooled OR of 1.04 (95% CI: 1.01–1.07) was found for LH with pregnancy rate. The pooled OR for LH and miscarriage rate was not significant. The authors concluded that the most clinically useful predictors of gonadotrophin ovulation induction outcome in normogonadotrophic women were obesity and insulin resistance.

In a small randomized controlled trial, no difference in any outcome was observed when metformin was co-administered during low-dose step-up gonadotrophin treatment of anovulatory PCOS patients (Yarali *et al*., 2002).

LH ceiling theory

Recent evidence points to a central role for LH in monofollicular selection and dominance in the normal ovulatory cycle (Shoham, 2002; Loumaye *et al*., 2003).

Preclinical evidence suggests that developing follicles have finite requirements for exposure to LH, beyond which normal maturation ceases (Hillier, 1994). This has given rise to the concept of an 'LH ceiling', which defines an upper limit of stimulation.

The hypothesis that over-dosing with recombinant human LH (rLH) during the late follicular phase would suppress the development of follicles was studied in hyper-responsive WHO II group anovulatory patients (Loumaye *et al*., 2003). The eligibility criteria were a hyper-response to FSH treatment defined as the presence of >4 follicles that were >8 mm and <13 mm in diameter, no larger follicles and an endometrial thickness of >8 mm. The FSH treatment was then stopped and the patients were randomized into one of three blinded arms: 1) rLH at a dose of 225 IU/day, 2) rLH at a dose of 450 IU/day, or 3) placebo. Five out of 12 patients presented follicular growth arrest in the rLH groups, but none in the placebo group. The mean number of follicles >10 mm was 4.6 ± 1.8 for the placebo group, 2.5 ± 1.9 for the rLH 225 IU group and 4.2 ± 1.4 in the rLH 450 IU group (not significant). Although preliminary, the partial cessation of follicular development along with regression leading to atresia lend support to the LH ceiling theory, emphasizing the delicate balance and need for both FSH and LH in normal follicular development. HMG preparations contain HCG, which contributes to circulating bioactive LH concentrations (Rodgers *et al*., 1994). Due to pharmacokinetics (Balasch *et al*., 2003) and contribution of both LH and HCG, LH exposure is high with HMG; this may have implications for the LH ceiling when HMG is used.

Strategies to convert inadvertent multiple ovulation to IVF: results

Despite development of low-dose protocols to induce ovulation induction in patients with PCOS, multifollicular development could not be totally eliminated mainly due to violation of rules for low-dose step up protocol as a result of impatience of both patient and physician. Cancellation of the treatment cycle by withholding HCG usually reduces the risk of, but does not entirely prevent, the development of OHSS. This whole process involves considerable frustration and disappointment as well as a financial burden, in addition to loss of patient trust in the physician.

Conversion of the gonadotrophin cycle to IVF with fresh embryo transfer or IVF with cryopreservation of all of the embryos with transfer in a subsequent thaw cycle (Wada *et al*., 1992; Chen *et al*., 2003) are the alternatives to withholding HCG administration in hyper-responders including but not limited to patients with PCOS. Lessing *et al*. (1991) reported conversion of 12 high responsive ovulation induction cycles to IVF fresh embryo transfer; they reported a pregnancy rate of 42% and three cases of OHSS (two mild and one moderate OHSS). Bergh *et al*. (1998) reported conversion of 25 cycles to IVF out of 154 WHO group II gonadotrophin stimulation cycles intended for ovulation induction. The pregnancy and delivery rates in the IVF-converted cycles were 50 and 41%, respectively, and 31 and 22% when gonadotrophin stimulation was followed by intercourse. The cancellation rate, including both ovulation induction and IVF cycles, was 15% and the multiple pregnancy rate was 30%, mainly twins.

Wada *et al*. (1992) reported cryopreservation of all embryos at the pronucleate stage to minimize the risk of developing OHSS in 78 infertile women with a serum oestradiol concentration >3500 pg/ml on the day of the ovulatory trigger. A median of 19 oocytes (range 7–43) was obtained and 12 embryos (range 1–37) frozen per cycle. No differences were found for any of the following criteria: aetiology of infertility, age, total dose of HMG, number of oocytes, fertilization rate or freeze–thaw survival of embryos. The overall freeze–thaw survival and implantation rates per embryo were 71.8 and 11.7% respectively. These findings underline that the risk of OHSS is not totally eliminated even following conversion to IVF with cryopreservation of all the available embryos.

Conclusion

It may be concluded that gonadotrophin treatment is highly effective to achieve singleton live birth in infertile patients with PCOS, even in the current era of assisted reproduction (Van Santbrink and Fauser, 2003). It is more difficult to induce ovulation in patients with more 'severe' PCOS, characterized by obesity, insulin resistance and PCO appearance. However, there is still room for improvement for prediction models to tailor and optimize the treatment outcomes. Future well designed randomized controlled trials will reveal whether IVF with or without in-vitro maturation of the oocytes will improve safety and efficacy compared with classical ovulation induction strategies.

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References

Aboulghar MA, Mansour RT, Serour GI *et al*. 1996 Recombinant follicle-stimulating hormone in the treatment of patients with history of severe ovarian hyperstimulation syndrome. *Fertility and Sterility* **66**, 757–760.

Ares-Serono 1995 A phase III, open, randomized, multicentre study to compare the safety and efficacy of recombinant human follicle stimulating hormone (Gonal-F) administered subcutaneously with that urinary human follicle stimulating hormone (Metrodin) given intramuscularly, to induce ovulation in WHO Group II anovulatory infertile women. Ares-Serono, Internal Report.

Baird DT 1987 A model for follicular selection and ovulation: lessons from superovulation. *Journal of Steroid Biochemistry* **27**, 15–23.

Balasch J, Fabregues F, Creus M *et al*. 2000 Recombinant human follicle-stimulating hormone for ovulation induction in polycystic ovary syndrome: a prospective, randomized trial of two starting doses in a chronic low-dose step-up protocol. *Journal of Assisted Reproduction and Genetics* **17**, 561–565.

Balasch J, Fabregues F, Creus M *et al*. 2001 Follicular development and hormone concentrations following recombinant FSH administration for anovulation associated with polycystic ovarian syndrome: prospective, randomized comparison between low-dose step-up and modified step-down regimens. *Human Reproduction* **16**, 652–656.

Balasch J, Fabregues F, Casamitjana R *et al*. 2003 A pharmacokinetic and endocrine comparison of recombinant follicle-stimulating hormone and human menopausal gonadotrophin in polycystic ovary syndrome. *Reproductive BioMedicine Online* **6**, 296–301.

Balen A, Laven J, Tan SL, Dewailly D 2003 Ultrasound assessment of the polycystic ovary: international consensus definitions. *Human Reproduction Update* **9**, 505–514.

Bao B, Garverick HA, Smith GW *et al*. 1997 Changes in messenger ribonucleic acid encoding luteinizing hormone receptor, cytochrome P450-side chain cleavage, and aromatase are associated with recruitment and selection of bovine ovarian follicles. *Biology of Reproduction* **56**, 1158–1168.

Bayram N, van Wely M, van Der Veen F 2003 Recombinant FSH versus urinary gonadotrophins or recombinant FSH for ovulation induction in subfertility associated with polycystic ovary syndrome. *Cochrane Database Systematic Review*, CD002121.

Bergh C, Bryman I, Nilsson L, Janson PO 1998 Results of gonadotrophin stimulation with the option to convert cycles to in vitro fertilization in cases of multifollicular development. *Acta Obstetrica et Gynecologica Scandinavica* **77**, 68–73.

Blankstein J, Shalev J, Saadon T *et al*. 1987 Ovarian hyperstimulation syndrome: prediction by number and size of preovulatory ovarian follicles. *Fertility and Sterility* **47**, 597–602.

Brown JB 1978 Pituitary control of ovarian function – concepts derived from gonadotrophin therapy. *Australian and New Zealand Journal of Obstetrics and Gynaecology* **18**, 46–54.

Chen D, Burmeister L, Goldschlag D, Rosenwaks Z 2003 Ovarian hyperstimulation syndrome: strategies for prevention. *Reproductive BioMedicine Online* **7**, 43–49.

Christin-Maitre S, Hugues JN 2003 A comparative randomized multicentric study comparing the step-up versus step-down protocol in polycystic ovary syndrome. *Human Reproduction* **18**, 1626–1631.

Crosignani PG, Vegetti W, Colombo M, Ragni G 2002 Resumption of fertility with diet in overweight women. *Reproductive BioMedicine Online* **5**, 60–64.

Dale PO, Tanbo T, Haug E, Abyholm T 1998 The impact of insulin resistance on the outcome of ovulation induction with low-dose follicle stimulating hormone in women with polycystic ovary syndrome. *Human Reproduction* **13**, 567–570.

Dor J, Itzkowic DJ, Mashiach S *et al*. 1980 Cumulative conception rates following gonadotropin therapy. *American Journal of Obstetrics and Gynecology* **136**, 102–105.

Dorrington JH, Armstrong DT 1979 Effects of FSH on gonadal functions. *Recent Progress in Hormone Research* **35**, 301–342.

Eijkemans MJ, Imani B, Mulders AG *et al*. 2003 High singleton live birth rate following classical ovulation induction in normogonadotrophic anovulatory infertility (WHO 2). *Human Reproduction* **18**, 2357–2362.

Farhi J, Homburg R, Lerner A, Ben-Rafael Z 1993 The choice of treatment for anovulation associated with polycystic ovary syndrome following failure to conceive with clomiphene. *Human Reproduction* **8**, 1367–1371.

Fauser BC, Van Heusden AM 1997 Manipulation of human ovarian function: physiological concepts and clinical consequences. *Endocrine Reviews* **18**, 71–106.

Franks S, Gharani N, McCarthy M 2001 Candidate genes in polycystic ovary syndrome. *Human Reproduction Update* **7**, 405–410.

Franks S, Roberts R, Hardy K 2003 Gonadotrophin regimens and oocyte quality in women with polycystic ovaries. *Reproductive BioMedicine Online* **6**, 181–184.

Geisthövel F 2003 A comment of the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine consensus of the polycystic ovarian syndrome. *Reproductive BioMedicine Online* **7**, 602–605.

Gougeon A 1996 Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocrine Reviews* **17**, 121–155.

Hall JE, Schoenfeld DA, Martin KA, Crowley WF Jr 1992 Hypothalamic gonadotropin-releasing hormone secretion and follicle-stimulating hormone dynamics during the luteal–follicular transition. *Journal of Clinical Endocrinology and Metabolism* **74**, 600–607.

Hamilton-Fairley D, Franks S 1990 Common problems in induction of ovulation. *Baillière's Clinical Obstetrics and Gynaecology* **4**, 609–625.

Hamilton-Fairley D, Kiddy D, Watson H *et al*. 1992 Association of moderate obesity with a poor pregnancy outcome in women with polycystic ovary syndrome treated with low dose gonadotrophin. *British Journal of Obstetrics and Gynaecology* **99**, 128–131.

Hardiman P, Pillay OC, Atiomo W 2003 Polycystic ovary syndrome and endometrial carcinoma. *Lancet* **361**, 1810–1812.

Hedon B, Hugues JN, Emperaire JC *et al*. 1998 A comparative prospective study of a chronic low dose versus a conventional ovulation stimulation regimen using recombinant human follicle stimulating hormone in anovulatory infertile women. *Human Reproduction* **13**, 2688–2692.

Hillier SG 1994 Current concepts of the roles of follicle stimulating hormone and luteinizing hormone in folliculogenesis. *Human Reproduction* **9**, 188–191.

Homburg R, Howles CM 1999 Low-dose FSH therapy for anovulatory infertility associated with polycystic ovary syndrome: rationale, results, reflections and refinements. *Human*

Reproduction Update **5**, 493–499.
Homburg R, Insler V 2002 Ovulation induction in perspective. *Human Reproduction Update* **8**, 449–462.
Homburg R, Levy T, Ben-Rafael Z 1995 A comparative prospective study of conventional regimen with chronic low-dose administration of follicle-stimulating hormone for anovulation associated with polycystic ovary syndrome. *Fertility and Sterility* **63**, 729–733.
Hugues JN, Cedrin-Durnerin I, Avril C *et al*. 1996 Sequential step-up and step-down dose regimen: an alternative method for ovulation induction with follicle-stimulating hormone in polycystic ovarian syndrome. *Human Reproduction* **11**, 2581–2584.
Hugues JN, Bstandig B, Bry-Gaillard H *et al*. 2001 Comparison of the effectiveness of recombinant and urinary FSH preparations in the achievement of follicular selection in chronic anovulation. *Reproductive BioMedicine Online* **3**, 195–198.
Imani B, Eijkemans MJ, te Velde ER *et al*. 1998 Predictors of patients remaining anovulatory during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *Journal of Clinical Endocrinology and Metabolism* **83**, 2361–2365.
Imani B, Eijkemans MJ, te Velde ER *et al*. 1999 Predictors of chances to conceive in ovulatory patients during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *Journal of Clinical Endocrinology and Metabolism* **84**, 1617–1622.
Imani B, Eijkemans MJ, de Jong FH *et al*. 2000 Free androgen index and leptin are the most prominent endocrine predictors of ovarian response during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *Journal of Clinical Endocrinology and Metabolism* **85**, 676–682.
Imani B, Eijkemans MJ, Faessen GH *et al*. 2002 Prediction of the individual follicle-stimulating hormone threshold for gonadotropin induction of ovulation in normogonadotropic anovulatory infertility: an approach to increase safety and efficiency. *Fertility and Sterility* **77**, 83–90.
Insler V 1988 Gonadotropin therapy: new trends and insights. *International Journal of Fertility* **33**, 85–86, 89–97.
Jonard S, Robert Y, Cortet-Rudelli C *et al*. 2003 Ultrasound examination of polycystic ovaries: is it worth counting the follicles? *Human Reproduction* **18**, 598–603.
le Nestour E, Marraoui J, Lahlou N *et al*. 1993 Role of estradiol in the rise in follicle-stimulating hormone levels during the luteal–follicular transition. *Journal of Clinical Endocrinology and Metabolism* **77**, 439–442.
Legro RS 2002 Polycystic ovary syndrome. Long term sequelae and management. *Minerva Ginecologica* **54**, 97–114.
Lessing JB, Amit A, Libal Y *et al*. 1991 Avoidance of cancellation of potential hyperstimulation cycles by conversion to in vitro fertilization-embryo transfer. *Fertility and Sterility* **56**, 75–78.
Lord JM, Flight IH, Norman RJ 2003 Insulin-sensitising drugs (metformin, troglitazone, rosiglitazone, pioglitazone, D-chiro-inositol) for polycystic ovary syndrome. *Cochrane Database Systematic Review*, CD003053.
Loumaye E, Engrand P, Shoham Z *et al*. 2003 Clinical evidence for an LH 'ceiling' effect induced by administration of recombinant human LH during the late follicular phase of stimulated cycles in World Health Organization type I and type II anovulation. *Human Reproduction* **18**, 314–322.
Macklon NS, Fauser BC 2001 Follicle-stimulating hormone and advanced follicle development in the human. *Archives of Medical Research* **32**, 595–600.
McClure N, McDonald J, Kovacs GT *et al*. 1993 Age and follicular phase estradiol are better predictors of pregnancy outcome than luteinizing hormone in menotropin ovulation induction for anovulatory polycystic ovarian syndrome. *Fertility and Sterility* **59**, 729–733.
Mitwally MF, Casper RF 2003 Aromatase inhibitors for the treatment of infertility. *Expert Opinion in Investigative Drugs* **12**, 353–371.
Mulders AG, Eijkemans MJ, Imani B, Fauser BC 2003a Prediction of chances for success or complications in gonadotrophin ovulation induction in normogonadotrophic anovulatory infertility. *Reproductive BioMedicine Online* **7**, 170–178.
Mulders AG, Laven JS, Eijkemans MJ *et al*. 2003b Patient predictors for outcome of gonadotrophin ovulation induction in women with normogonadotrophic anovulatory infertility: a meta-analysis. *Human Reproduction Update* **9**, 429–449.
Norman RJ, Davies MJ, Lord J, Moran LJ 2002 The role of lifestyle modification in polycystic ovary syndrome. *Trends in Endocrinology and Metabolism* **13**, 251–257.
Nugent D, Vandekerckhove P, Hughes E *et al*. 2003 Gonadotrophin therapy for ovulation induction in subfertility associated with polycystic ovary syndrome. *Cochrane Database Systematic Review*, CD000410.
Rodgers M, McLoughlin J, Peers N *et al*. 1994 Accumulation of human chorionic gonadotrophin in the serum of patients during in-vitro fertilization treatment cycles with Pergonal. *Human Reproduction* **9**, 638–642.
Roseff SJ, Bangah ML, Kettel LM *et al*. 1989 Dynamic changes in circulating inhibin levels during the luteal–follicular transition of the human menstrual cycle. *Journal of Clinical Endocrinology and Metabolism* **69**, 1033–1039.
Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Human Reproduction* **19**, 41–47.
Sagle MA, Hamilton-Fairley D, Kiddy DS, Franks S 1991 A comparative, randomized study of low-dose human menopausal gonadotropin and follicle-stimulating hormone in women with polycystic ovarian syndrome. *Fertility and Sterility* **55**, 56–60.
Shoham Z 2002 The clinical therapeutic window for luteinizing hormone in controlled ovarian stimulation. *Fertility and Sterility* **77**, 1170–1177.
Van der Meer M, Hompes PG, Scheele F *et al*. 1994 Follicle stimulating hormone (FSH) dynamics of low dose step-up ovulation induction with FSH in patients with polycystic ovary syndrome. *Human Reproduction* **9**, 1612–1617.
Van der Meer M, Hompes PG, Scheele F *et al*. 1996 The importance of endogenous feedback for monofollicular growth in low-dose step-up ovulation induction with follicle-stimulating hormone in polycystic ovary syndrome: a randomized study. *Fertility and Sterility* **66**, 571–576.
Van der Meer M, Hompes PG, De Boer JA *et al*. 1998 Cohort size rather than follicle-stimulating hormone threshold level determines ovarian sensitivity in polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* **83**, 423–426.
Van Santbrink EJ, Fauser BC 1997 Urinary follicle-stimulating hormone for normogonadotropic clomiphene-resistant anovulatory infertility: prospective, randomized comparison between low dose step-up and step-down dose regimens. *Journal of Clinical Endocrinology and Metabolism* **82**, 3597–3602.
Van Santbrink EJ, Fauser BC 2003 Is there a future for ovulation induction in the current era of assisted reproduction? *Human Reproduction* **18**, 2499–2502.
Van Santbrink EJ, Donderwinkel PF, van Dessel TJ, Fauser BC 1995 Gonadotrophin induction of ovulation using a step-down dose regimen: single-centre clinical experience in 82 patients. *Human Reproduction* **10**, 1048–1053.
Van Santbrink EJ, Eijkemans MJ, Macklon NS, Fauser BC 2002 FSH response-dose can be predicted in ovulation induction for normogonadotropic anovulatory infertility. *European Journal of Endocrinology/European Federation of Endocrine Societies* **147**, 223–226.
Vicino M, Loverro G, Bettocchi S *et al*. 2000 Predictive value of serum androstenedione basal levels on the choice of gonadotropin or laparoscopic ovarian electrocautery as ovulation induction in clomiphene citrate-resistant patients with polycystic ovary syndrome. *Gynecological Endocrinology* **14**, 42–49.
Wada I, Matson PL, Troup SA *et al*. 1992 Outcome of treatment subsequent to the elective cryopreservation of all embryos from women at risk of the ovarian hyperstimulation syndrome. *Human Reproduction* **7**, 962–966.

White DM, Polson DW, Kiddy D *et al*. 1996 Induction of ovulation with low-dose gonadotropins in polycystic ovary syndrome: an analysis of 109 pregnancies in 225 women. *Journal of Clinical Endocrinology and Metabolism* **81**, 3821–3824.

Wright CS, Hovatta O, Margara R *et al*. 1999 Effects of follicle-stimulating hormone and serum substitution on the in-vitro growth of human ovarian follicles. *Human Reproduction* **14**, 1555–1562.

Yarali H, Bukulmez O, Gurgan T 1999 Urinary follicle-stimulating hormone (FSH) versus recombinant FSH in clomiphene citrate-resistant, normogonadotropic, chronic anovulation: a prospective randomized study. *Fertility and Sterility* **72**, 276–281.

Yarali H, Yildiz BO, Demirol A *et al*. 2002 Co-administration of metformin during rFSH treatment in patients with clomiphene citrate-resistant polycystic ovarian syndrome: a prospective randomized trial. *Human Reproduction* **17**, 289–294.

Yildiz BO, Chang W, Azziz R 2003 Polycystic ovary syndrome and ovulation induction. *Minerva Ginecologica* **55**, 425–439.

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