A comparative randomized multicentric study comparing the step-up versus step-down protocol in polycystic ovary syndrome

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BACKGROUND: The aim of the study was to evaluate follicular development and ovulation comparing the low-dose step-up and the step-down protocols, in women with clomiphene citrate (CC)-resistant polycystic ovaries.

METHODS: Eighty-three women were randomized, and treated with recombinant (r) FSH (Puregon\textsuperscript{®}) using either the step-up (n = 44) or step-down (n = 39) protocol. They were followed up for three cycles unless pregnancy occurred. RESULTS: Monofollicular development occurred in 68.2\% of the 85 cycles in the step-up group, as compared with 32\% of the 72 cycles in the step-down group (P < 0.0001). Hyperstimulation was statistically less frequent using the step-up procedure (4.7 versus 36\%, P < 0.0001). Both protocols used the same number of FSH units per cycle (951\textpm 66 versus 967\textpm 66586 in step-up and step-down respectively, P = not significant). However, the duration of ovarian stimulation was statistically different (15.2 \textpm 7.0 days in step-up versus 9.7 \textpm 3.1 in step-down, P < 0.001). Ovulation was observed in 70.3\% of the cycles using the step-up procedure as compared with 51.3\% using the step-down procedure (P < 0.01). The cumulative rate of clinical gestations during the study did not differ between the two groups (38.6\% in the step-up versus 30.8\% in the step-down procedure). CONCLUSIONS: The step-up protocol using rFSH (Puregon\textsuperscript{®}), is more efficient in obtaining a monofollicular development and ovulation than the step-down protocol, in women with CC-resistant polycystic ovaries. Although the duration of stimulation is longer, the rate of ovarian hyperstimulation is much lower using the step-up protocol.

Key words: ovulation induction/polycystic ovary syndrome/recombinant FSH/step-down protocol/step-up protocol

Introduction

Polycystic ovary syndrome (PCOS) is a common cause of infertility in women (Franks, 1995). The first line therapy is usually clomiphene citrate (CC). However, \textapprox 20\% of PCOS women are CC-resistant and therefore need to benefit from gonadotrophins to achieve ovulation. During ovulation induction in these women, the two major risks are the occurrence of ovarian hyperstimulation syndrome (OHSS) and the development of multiple pregnancies. Therefore, in the past 10 years, two different protocols have been designed to reduce those risks, the so-called low-dose step-up protocol (Kamrava et al., 1982; Buvat et al., 1988; Sagle et al., 1991; Shoham et al., 1991; White et al., 1996) and the step-down protocol (Mizunuma et al., 1991; Fauser and Van Heusden, 1997).

Both protocols have been previously compared, giving conflicting results (Mizunuma et al., 1991; van Santbrink et al., 1997; Andoh et al., 1998; Balasch et al., 2001). However, these studies were performed in a small number of selected subjects which could limit the final conclusion.

We designed a randomized multicentric study in order to compare both efficacy and safety of the low-dose step-up and step-down protocols, using recombinant (r) FSH in clomiphene CC-women. The end points of this study were the ovulation rate, the pregnancy rate and the cycle outcome. In all cycles, women were treated with recombinant (r)FSH (Puregon\textsuperscript{®}, Organon, The Netherlands).

Patients and methods

Patients

Eighty-three patients with anovulatory infertility due to PCOS were included in the study. All women were seeking pregnancy. The mean (\textpm SD) age was 28.8 \textpm 3.2 years and the mean duration of infertility 2.9 \textpm 1.7 years. Patients had normal or slightly elevated body weight as the mean body mass...
index (BMI) was 23.5 ± 4.4 kg/m². Only 14.4 % had a BMI >30 kg/m². The study was approved by the various local ethics committees. Patients all signed an informed consent form.

The diagnosis of PCOS was made according to WHO type II criteria (Rowe, 1993). All patients presented with oligo/amenorrhoea or anovulatory cycles for at least 2 years. Vaginal ultrasound examination revealed at least eight follicles between 2–8 mm diameter with a stromal hypertrophy (Adams et al., 1985). All patients to be considered eligible had to have a normal concentration of serum prolactin (PRL) (<20 ng/ml). Serum FSH levels had to be <10 IU/l, with a testosterone levels <1ng/ml. Serum LH was not used as a diagnosis criteria. A normal hysterosalpingogram or laparoscopy had to have been recorded in the past 3 years prior to ovulation induction. Concerning the male factor, sperm count was considered abnormal if sperm density was <25 × 10⁶/ml, progressive motility measured at 1 h <40% and/or teratozoospermia >40%.

Women were defined as CC-resistant if they had failed to ovulate after three cycles with CC (100 mg/day for 5 days) or had failed to conceive after six cycles with this treatment.

**Methods**

**Study design**

This was a prospective, randomized, multicentric study covering a total of 11 centres. Each principal investigator in each centre received numbered sealed envelopes that had to be used in numerical order. Randomization occurred after patients agreed to inclusion in the study (Figure 1). Women after randomization were treated with the same protocol for three consecutive cycles unless pregnancy was achieved.

**Step-up protocol**

Puregon® 50 IU (1 vial) was administered s.c. starting on day 3 to 5 of a spontaneous cycle or after a withdrawal bleeding induced by a short course of progestin. Serial vaginal

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**Figure 1.** Participant flow through trial.
ultrasound scanning and serum estradiol measurements were routinely performed. The daily starting dose (50 IU) was maintained for up to 14 days of the first cycle. If no dominant follicle (>9 mm maximum diameter) was present, the dose was increased to 75 IU. Any further increments were by 25 IU (1/2 ampoule) doses, at weekly intervals up to 100 IU during the first cycle of treatment. The starting dose of the second and third cycles could be increased to 75 IU if no follicular development occurred before the dose of 100 IU, in the first cycle. In the second and third cycles of treatment, the stepwise increase could reach a maximum of 125 IU of rFSH daily.

**Step-down protocol**

Puregon® 100 IU (two vials) was administered s.c. starting on day 3 to 5 of a spontaneous cycle or after a withdrawal bleed induced by a short course of progestin. As soon as a follicular development (>9 mm maximum diameter) could be detected, the dose was firstly decreased to 75 IU for 3 days and secondly to 50 IU until the day prior to hCG administration. In the absence of follicular development after 5 days of treatment, the initial dose of rFSH was increased to 150 IU. When follicular development occurred (>9 mm maximum diameter), the FSH dose was decreased to 125 IU for 3 days, 100 IU for 3 days and 75 IU until the day prior to hCG administration.

**hCG administration**

In both protocols, a single dose of hCG 5000 IU was administered i.m. or s.c. when the leading follicle reached >18 mm. The hCG administration was withheld if four or more follicles >16 mm in diameter were present and/or if the serum estradiol level was ≥1000 pg/ml. In this study, the luteal phase was not supported. Treatment with Puregon® was withheld in the absence of follicular development after 21 days of stimulation or in case of cyst development. Ovulation was assessed by a progesterone plasma level >8 ng/ml (30 nmol/l) 7–10 days after hCG administration and/or pregnancy at the end of the treatment cycle.

**Statistical analysis**

Data were analysed by software using the software SAS. Results are expressed as means ± SD. χ² or Fisher’s tests were used, as appropriate.

**Results**

A total of 83 women was randomized between the step-up (n = 44) and the step-down (n = 39) protocols. They were followed up for a total of 85 and 72 cycles in the step-up and step-down protocols respectively. Patients were comparable among the two groups (Table I). No statistical difference could be detected for age, BMI, duration of infertility, number of cycles per year nor the LH, estradiol and testosterone plasma levels, among the two groups of patients. The number of previous pregnancies were similar (11 in the step-up versus nine in the step-down protocol). Transvaginal sonographies performed in the initial screening were not different between the two groups,
Regarding the ovarian volume and the number of follicles <8 mm diameter. Ultrasound data were missing on the day of hCG administration for three treatment cycles in the step-up protocol and two cycles in the step-down protocol. In the step-up protocol, one patient dropped out on treatment cycle 3 whereas in the step-down one patient dropped out on the second cycle of treatment.

As shown in Table II, the mean duration of treatment was significantly longer in the step-up than in the step-down protocol (P < 0.001). This difference was constant in consecutive treatment cycles. However, the total amount of rFSH used in the treatment cycles was similar in the two protocols. For patients treated with the step-up protocol, the rFSH daily dose of 50 IU was maintained in 55 and 73% of patients during the first and the second cycle of treatment respectively.

The rate of monofollicular development (i.e., only one follicle >16 mm diameter at the time of hCG administration) was significantly higher in patients treated with the step-up protocol than in those treated with the step-down protocol (68.2% and 32% of treatment cycles respectively, P < 0.0001). Regarding the percentage of cycles with one and two follicles that reached >16 mm diameter, the difference remained statistically significant between the step-up and the step-down protocols (83.5% versus 55.6% respectively, P < 0.0001). Moreover, the rate of multifollicular development (at least three follicles >16 mm diameter) was significantly higher in the step-down protocol cycles than in the step-up cycles (36% and 4.7% respectively, P < 0.0001). Finally, the estradiol plasma levels on the day of hCG administration were significantly higher (P < 0.05) in the step-down than in the step-up protocol, mainly in the first two cycles of treatment (Table II).

hCG was administered in 84.6% of the step-up versus 61.8% in the step-down cycles (P = 0.001). The rate of ovarian hyperstimulation was significantly higher in patients treated with the step-up protocol than in step-up cycles (P < 0.001). Only three cases of OHSS, staged as moderate hyperstimulation by the investigators, occurred during the study. The absence of ovarian response was not statistically different between the step-up and the step-down protocols.

The ovulation rate was significantly higher in the step-up group than in the step-down protocol (P = 0.02). During the study, 17 and 12 gestations occurred in the step-up and in the step-down treatment groups respectively, resulting in a similar fecundity rate per cycle for both regimens. At the end of the first treatment cycle, the number of clinical pregnancies was 10 (22.7%) and 7 (15.4%), in the step-up and step-down protocols respectively. The cumulative rate of gestations during the 3 months study was not significantly different (38.6 and 30.8%) as well as the miscarriage rate (12.5 and 16.7%), in the step-up and step-down regimens respectively. No ectopic pregnancy was observed during the study. Two twin pregnancies occurred in each group of treatment and a triple pregnancy occurred in the step-down protocol. Each twin pregnancy resulted in the birth of two children. However, the triple pregnancy ended at 26 weeks gestation after premature delivery.

Discussion

Induction of ovulation in women with polycystic ovaries is currently associated with a high incidence of OHSS and multiple pregnancies (Sagle et al., 1991; Homburg et al 1995). Therefore, administration of low doses of FSH in a stepwise fashion (step-up, step-down protocol or a combination of step-up and step-down) has been suggested and proved to be effective to significantly reduce those risks. Therefore, the step protocols are currently used to induce ovarian stimulation in CC-resistant women with PCOS. However, in the literature to date, only few studies have compared the efficacy and safety of the step-up and step-down protocols within the same study (Mizunuma et al., 1991; van Santbrink and Fauser, 1997; Andoh et al., 1998; Balasch et al., 2001). Furthermore, those studies included a small number of subjects and gave conflicting results. Thus, we designed a large multicentric randomized study in order to compare both efficacy and safety of a low-dose step-up protocol versus a step-down protocol in women treated with recombinant human FSH.

Our study shows that the chronic low-dose regimen of rFSH administration is as effective but safer than the step-down regimen. Indeed, the rate of monofollicular development is significantly higher and the rate of multifollicular development is significantly lower with the step-up protocol as compared with the step-down regimen. Those results are in agreement with data from Andoh’s study (Andoh et al., 1998) but differ from other reports (van Santbrink and Fauser, 1997; Balasch et al., 2001). However, many discrepancies exist in the design of those studies and could explain the differences observed. Several factors are known to be critical in order to get a limited number of growing follicles in stimulation protocols, such as the daily FSH starting dose, the regimen of FSH dose adjustment and the type of gonadotrophins used.

Determining the most appropriate starting dose has been shown to be critical to reduce the rate of hyperstimulation. Therefore, in those patients with PCOS and at high risk of multifollicular development, we decided to use a low starting dose in both protocols. Our study shows that a starting dose of 50 IU is adequate, in step-up protocol. These results are in agreement with previous reports (White et al., 1996; Hayden et al., 1999) but in those studies the risk of hyperstimulation was not entirely suppressed. The 50 IU dose may explain the high rate of monofollicular development observed in this study (68.2%) compared with those reported in studies using a 75 IU starting dose (56 and 46% respectively in van Santbrink and Fauser, 1997; Balasch et al., 2001). As the ability of clinicians to choose an appropriate starting FSH dose for a given patient could considerably improve treatment outcome, prediction of the individual FSH threshold is clearly needed. For that purpose, Imani et al. studied 90 women treated with a chronic low-dose protocol and showed that BMI, cycle history, ovarian response to CC as well as basal serum FSH values are significantly correlated with the individual FSH response (Imani et al., 2002). Therefore, a strict adjustment of the FSH starting dose on the basis of initial screening characteristics is required to improve the safety and convenience of low-dose regimens.
In patients treated with a step-down protocol, we observed that, even using a 100 IU starting dose, the risk of multifollicular development still exists with some patients overresponding after only 3 days of treatment. These results differ from two previous reports (van Santbrink and Fauser, 1997; Balasch et al., 2001) stating that high FSH starting doses do not expose to the risk of hyperstimulation. However, many discrepancies exist among those studies: some anovulatory patients without PCOS features were included (van Santbrink and Fauser, 1997) or a 3 day coasting period of FSH administration was used (Balasch et al., 2001). From these reports, a key issue of any step-down regimen is to estimate the FSH threshold of follicular development in order to determine the FSH starting dose.

Another major issue of the step protocols is the duration of the initial dose and the FSH dose adjustment. Regarding the step-up protocols, many studies have clearly shown that a chronic administration of low FSH doses for 14 days according to the so called ‘Chronic Low Dose’ is safer than a regimen with FSH dose adjustment after 7 days (Hedon et al., 1998; Homburg and Howles, 1999). Therefore, a strict adherence to a 14-day starting period using a persistent dose seems to be critical to prevent the risk of hyperstimulation. Concerning the step-down protocols, the timing of the FSH dose reduction is also a major determinant of the number of developing follicles. Indeed, by decreasing the circulating FSH level, the number of medium size follicles is significantly reduced (Schoot et al., 1992). The beneficial effect of closing the FSH window was also demonstrated in a sequential step-up–step-down protocol (Hugues et al., 1996). It may account for the results observed in the study of Balasch et al. where a coasting period was performed from day 3 to 5 of the stimulation (Balasch et al., 2001). However, the long half-life of FSH preparations precludes an adequate control of follicular development in PCOS patients who are highly sensitive to FSH. Therefore, in those patients, a careful assessment of early follicular development is helpful to timely adjust the FSH dose.

Another difference between studies is related to the type of gonadotrophin used during the ovulation induction protocol. Many previous studies (Mizunuma et al., 1991; White et al., 1996; van Santbrink and Fauser, 1997; Andoh et al., 1998) have been performed using urinary-derived preparations, hMG or purified FSH. A meta-analysis based on several studies showed that administration of FSH is safer than hMG by reducing the risk of OHSS in those patients with high endogenous LH secretion (Hughes et al., 2000). By contrast, comparative studies between urinary and recombinant FSH preparations could not show any difference in terms of safety. A higher efficiency of recombinant preparations previously reported (Coelingh Bennink et al., 1998) could explain the high rate of multifollicular development observed in our study as well as in other studies using recombinant preparations (Hayden et al., 1999; Balasch et al., 2001). Therefore, whatever the protocol used, clinicians must take into account the choice of gonadotrophin to determine the starting FSH dose and to adjust the dose adequately.

In conclusion, this study represents the largest randomized study so far, comparing the step-up versus the step-down protocol, using recombinant human FSH. In the population of CC-resistant women studied, the step-up protocol is safer than the step-down protocol. Indeed, the rate of ovarian hyperstimulation is lower using the step-up protocol. The major inconvenience of step-up protocols is a longer duration of FSH administration. Therefore, in PCOS patients, highly sensitive to FSH, a chronic step-up low-dose protocol could be the first line therapy in order to determine the individual FSH threshold. The starting FSH dose should be chosen according to predictive factors. For the next cycle, a step down protocol may be used with a starting dose defined just below the individual FSH threshold.

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