

The use of recombinant human LH (lutropin alfa) in the late stimulation phase of assisted reproduction cycles: a double-blind, randomized, prospective study

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BACKGROUND: The effect of recombinant human LH (r-hLH; lutropin alfa) in women undergoing controlled ovarian stimulation with recombinant human FSH (r-hFSH) prior to IVF was investigated. **METHODS:** After down-regulation with the GnRH agonist, buserelin, 114 normo-ovulatory women (aged 18–37 years) received r-hFSH alone until the lead follicle reached a diameter of 14 mm. Patients were then randomized in a double-blind fashion to receive r-hFSH in addition to r-hLH, 75 IU s.c., or placebo daily for a maximum of 10 days prior to oocyte retrieval and IVF. The primary end-point was the number of metaphase II oocytes. **RESULTS:** There were no significant differences between treatment groups for the primary end-point. Serum estradiol concentrations on the day of HCG administration were significantly higher in the group receiving r-hLH plus r-hFSH than in the group receiving r-hFSH alone ($P = 0.0001$), but there were no significant differences between the groups in dose and duration of r-hFSH treatment required, oocyte maturation, fertilization rate, pregnancy rate and live birth rate. **CONCLUSION:** In this patient population, the addition of r-hLH during the late follicular phase of a long GnRH agonist and r-hFSH stimulation cycle provides no further benefit in terms of oocyte maturation or other end-points.

Key words: FSH/IVF/LH/ovarian stimulation

Introduction

The validity of the two-cell, two-gonadotrophin hypothesis, which suggests that both LH and FSH are required for ovarian steroidogenesis (European Recombinant Human LH Study Group, 1998) in a gonadotrophin-deficient population [World Health Organization (WHO) I classification] is clear. However, there is still a considerable controversy on the need for additional LH supplementation in cycles of assisted reproductive techniques (ART) using a GnRH agonist. It has been proposed that ‘resting’ concentrations of LH are sufficient to maintain steroidogenesis and normal folliculogenesis (Chappel and Howles, 1991). Indeed, exposure of the developing follicle to inappropriately high LH concentrations may impair follicular and oocyte maturation (Hillier *et al.*, 1994; Shoham, 2002). This view is supported by a meta-analysis of eight, large, double-blind, randomized, controlled trials which demonstrated that, in IVF cycles, the use of FSH alone was associated with significantly higher rates of oocyte retrieval, embryo transfer and clinical pregnancies per cycle than HMG (Daya, 1995, 2000). However, a meta-analysis of six randomized controlled trials

reported a higher clinical pregnancy with HMG compared with recombinant human FSH (r-hFSH) following down-regulation in a long agonist protocol—though there were no differences in ongoing pregnancy or live birth rates (van Wely *et al.*, 2003).

In a small but controlled study in which LH activity was supplemented with a fixed daily dose (0 + 75 IU) of recombinant human LH (r-hLH) throughout (Sills *et al.*, 1999), pregnancy rates and stimulation characteristics did not improve. When considering the whole patient population, Marrs *et al.* (2004) also reported similar results following LH supplementation versus treatment with FSH alone. Only when stratification of the data by age was performed was there evidence for a benefit of LH supplementation, but only in those ≥ 35 years of age. In contrast, however, an open label, randomized, controlled study of 101 patients in each group found that the number of ampoules of FSH required for ovarian stimulation was higher in women receiving FSH alone than in those who also received HCG to supplement LH activity (Filicori *et al.*, 1999). These authors suggested that LH had a beneficial effect on follicular development during ovarian stimulation. In

another retrospective analysis, there were no differences [except in final estradiol (E_2) levels] in stimulation characteristics, but a five-fold increase in the risk of early pregnancy loss was noted in women with low plasma LH concentrations (<0.5 IU/l) after a standard stimulation protocol with GnRH agonist down-regulation and FSH treatment compared with patients with higher plasma LH concentrations (Westergaard *et al.*, 2000). These authors concluded that profound LH suppression can occur in a large number of patients during ovarian stimulation with FSH, resulting in impaired steroidogenesis and compromised treatment outcome.

LH suppression appears to be affected by the down-regulation regimen used. In another study by Westergaard *et al.* (2001), s.c. administration of buserelin produced significantly lower levels of serum LH on stimulation day 8 compared with intranasal administration.

The aim of the present study was to assess the need for additional LH by comparing the yield of metaphase II (MII) oocytes in infertile women undergoing assisted reproduction with and without treatment with supplementary r-hLH. To the best of our knowledge, this is the first double-blind study of its kind to be designed and performed to compare the efficacy in terms of stimulation outcome of r-hFSH alone or in addition to r-hLH in women undergoing ovarian stimulation with pituitary suppression utilizing a fixed dose of GnRH agonist prior to ART.

Materials and methods

The trial was a double-blind, randomized, placebo-controlled, prospective study performed at six centres in four countries. It was conducted according to the principles of the Declaration of Helsinki, and was approved by local ethics committees at all centres.

Protocol

Women were eligible for inclusion in the study if they were aged between 18 and 37 years, had a normal uterus and two ovaries, and were scheduled to undergo controlled ovarian stimulation prior to IVF with ICSI. All women had normal ovulatory cycles of 24–35 days, with maximum FSH and prolactin concentrations of 12 IU/l and 1040 mIU/l, respectively, during the early follicular phase (days 2–6). No evidence of other gynaecological pathology (except tubal) was present in the women included in the study based on ultrasonography and laboratory investigations. Women in whom a previous IVF cycle had been unsuccessful due to a poor response (≤ 2 oocytes recovered) were not eligible for the study. Written informed consent was obtained from all patients prior to inclusion in the study.

Patients underwent pituitary down-regulation with buserelin (Suprefact®, Hoechst, Frankfurt, Germany), using a fixed daily dose of 200 mg s.c., according to the long agonist protocol, starting on day 2 of the normal menstrual cycle. Treatment with r-hFSH (Gonal-F®, Laboratoires Serono S.A., Aubonne, Switzerland) was then started in women with serum E_2 concentrations <200 pmol/l and no follicles >15 mm in diameter or ovarian cysts on ultrasonographic examination. The initial r-hFSH dose was 150 IU s.c. daily for 5 days, after which the dose was adjusted to a maximum of 450 IU per day according to the ovarian response. This starting dose of r-hFSH has been found to be effective in women of this age range (Bergh *et al.*, 1997; Frydman *et al.*, 2000; Schats *et al.*, 2000).

Once the leading follicle had reached a diameter of 14 mm, a stage of development associated with a heightened responsiveness to LH (Shaw *et al.*, 1989), patients were randomized to receive r-hLH (lutropin alfa; Luveris®, Laboratoires Serono S.A.), 75 IU s.c., or placebo

for a maximum of 10 days. A dose of 75 IU LH per day was chosen based on findings from a controlled, prospective, dose-finding study in gonadotrophin-deficient women (WHO I classification) (European Recombinant Human LH Study Group, 1998). Here, a dose of 75 IU LH per day was found to be sufficient to induce normal follicle development and E_2 secretion. Ovulation was induced by administration of HCG (Profasi®, Laboratoires Serono S.A.), 10 000 IU i.m. or s.c., when at least two follicles had reached a diameter of >17 mm.

Oocyte retrieval was performed by ultrasound-guided follicular aspiration techniques 34–38 h after administration of HCG. IVF and ICSI were performed according to standard practices at each centre. A maximum of four embryos were transferred 48 h after oocyte retrieval (ESHRE Committee on Good Clinical and Laboratory Practice, 1995). Patients received micronized progesterone, 600 mg/day, by vaginal administration for at least the first 3 weeks of pregnancy, beginning on the day of embryo transfer. Pregnancy was confirmed by the presence of a fetal sac and heart beat on ultrasonographic examination on day 35 after oocyte retrieval.

The primary efficacy end-point in this study was the mean number of MII oocytes retrieved in each group. In a previous study (Imthurn *et al.*, 1996), the mean number of MII oocytes retrieved was 13.3 ± 1.2 in the HMG group and 10.8 ± 0.9 in the group receiving high purity FSH (FSH-HP). Using these figures we calculated that, for this study, a sample size of 100 patients would provide 99% power to detect a difference of 2.5 in the mean number of MII oocytes between treatments, with a two-sided significance level of 0.05. Secondary efficacy end-points included the duration of ovarian stimulation and the dose of r-hFSH required for stimulation, the number of fertilized oocytes, the number of cleaved oocytes, the pregnancy rate and the live birth rate.

Statistical analysis was performed on a modified intent-to-treat population, considering all patients for whom the assessment on the number of MII oocytes was performed. Efficacy variables were analysed by analysis of variance, with factors for centre and treatment-centre interaction. Where the data were found to be asymmetrically distributed on graphical displays (normal plots or box plots), the analysis was repeated on square root-transformed data, under the assumption of a Poisson distribution.

However, the non-transformed analysis was also performed since the study protocol did not foresee the use of transformations. In these cases, the results of both (transformed and untransformed analyses) are provided. Except for the variables time of r-hLH/placebo to urinary-HCG (u-HCG) administration and number of follicles ≥ 14 mm at HCG day, all variables showed consistency of the results obtained with the raw data or the square root transformation. Dichotomous variables were analysed by Mantel-Haenszel tests, with adjustment for centre. All statistical tests were performed using SAS (version 6.12) software and P values below 0.05 were considered significant.

Assignment

Each centre was allocated a block of 22 consecutive patient numbers and patients were randomized according to their treatment number by means of a computer program generated by Serono International S.A., Corporate Biometrics Department, Geneva, Switzerland. Twenty-two patients were allocated to each centre to allow for drop-outs and respect the randomization procedure in each centre.

Blinding

Each patient's medication was provided in a treatment box containing ampoules of diluent and study medication. r-hLH and placebo ampoules were identical in appearance. The investigator was provided with sealed envelopes containing details of the randomized treatment. These were to be opened only in case of emergency.

Results

Participant flow and follow-up

A total of 123 patients were enrolled in the study, 114 of whom were randomized to treatment. Of these, 112 received HCG. The reasons for withdrawal prior to randomization were: failure of GnRH treatment ($n = 5$); patient request ($n = 2$); ovarian cysts ($n=1$); and an allergic reaction to treatment ($n = 1$). The reason for the two withdrawals after randomization was poor response to stimulation (resulting in no oocytes being retrieved).

The progress of patients through the study programme is shown in Fig. 1. The demographic characteristics of the patients in the two groups were similar (Table I). Although patients undergoing both IVF and ICSI were recruited, only those patients undergoing ICSI were eligible for primary efficacy end-point analysis (15 patients were switched from IVF to ICSI because of problems on the day of oocyte retrieval; seven patients and eight patients, respectively for r-hFSH alone and r-hFSH + r-hLH).

The minimum time interval required for pituitary down-regulation was 12 days for the r-hFSH group and 11 days for the r-hFSH + r-hLH group, and the corresponding mean durations were 18.75 and 19.04 days, respectively for the r-hFSH and the r-hFSH + r-hLH groups. The distributions of the elapsed time from the first day of r-hFSH to the point when the leading follicle reached >14 mm ranged from 3 to 14 days, and were similar in both groups, with median 7 days.

Table I. Demographic characteristics of patients randomized to treatment

	r-hFSH	r-hFSH plus r-hLH
Number of patients	59	55
Age (years)	30.3 ± 3.6	30.5 ± 3.5
Body mass index (kg/m ²)	22.9 ± 3.1	23.0 ± 3.0
Duration of infertility (years)	5.0 ± 3.7	5.6 ± 2.9
Primary/secondary infertility (%)	64.3/37.7	54.5/45.5
Cause of infertility (%):		
tubal factor	33.9	38.2
male factor	61.0	50.9
Semen characteristics (%)		
normal	66.1	76.4
abnormal	32.2	21.8

Data are presented as mean ± SD. r-hFSH = recombinant human FSH; r-hLH = recombinant human LH.

Analysis

The adjusted difference of 0.59 (95% confidence interval: -1.28; 2.45) in the mean number of MII oocytes retrieved was not statistically significant. Details of ovarian stimulation and outcome in the two groups are summarized in Tables II and III. Serum E₂ concentrations on the day of HCG administration were significantly higher in the group receiving r-hLH in addition to r-hFSH than in the group receiving r-hFSH alone ($P < 0.0001$).

Table II. Ovarian stimulation characteristics of the two treatment groups

	r-hFSH plus placebo	r-hFSH plus r-hLH
Number of patients receiving HCG	57	55
Cancellations due to poor response rate	2 (3.4%)*	0 (0%)
Stimulation duration (days)	9.9 ± 3.2	9.7 ± 2.3
Days until lead follicle >14 mm	7.5 ± 2.1	7.4 ± 2.3
r-hFSH ampoules (75 IU) required	23.4 ± 9.1	24.5 ± 10.7
Number of follicles >14 mm on day of HCG administration	8.4 ± 5.1	9.1 ± 3.8
Serum estradiol on HCG day (pmol/l)	1539 ± 723	1901 ± 1073 ^a

Results are presented as mean ± SD, or as number of patients and percentages. *Based on 59 patients randomized. ^a $P = 0.0001$. r-hFSH = recombinant human FSH; r-hLH = recombinant human LH.

Table III. Pregnancy outcome characteristics of the two treatment groups

	r-hFSH plus placebo	r-hFSH plus r-hLH
Number of patients with retrieved oocytes	57	55
Number of oocytes retrieved	9.8 ± 7.0	10.1 ± 5.4
Number of patients undergoing ICSI	52	47
Number of MII oocytes retrieved a	6.2 ± 4.8	6.9 ± 4.9
Number of two pronuclei oocytes b	5.0 ± 3.8	5.6 ± 3.5
Number of embryos transferred b	2.9 ± 1.5	3.2 ± 1.5
Number of viable pregnancies (%/cycle)	14 (23.7%) ^c	9 (16.4%)
Number of miscarriages (%/pregnancy)	4 (28.6%)	3 (33.3%)
Number of patients giving birth (% live birth rate)	10 (16.9%)*	6 (10.9%)
Number of live births	16	8

Results are presented as mean ± SD, or as number of patients and percentages. ^aFor patients who underwent ICSI; ^bFor patients with retrieved oocytes; ^cBased on 59 patients randomized. r-hFSH = recombinant human FSH; r-hLH = recombinant human LH.

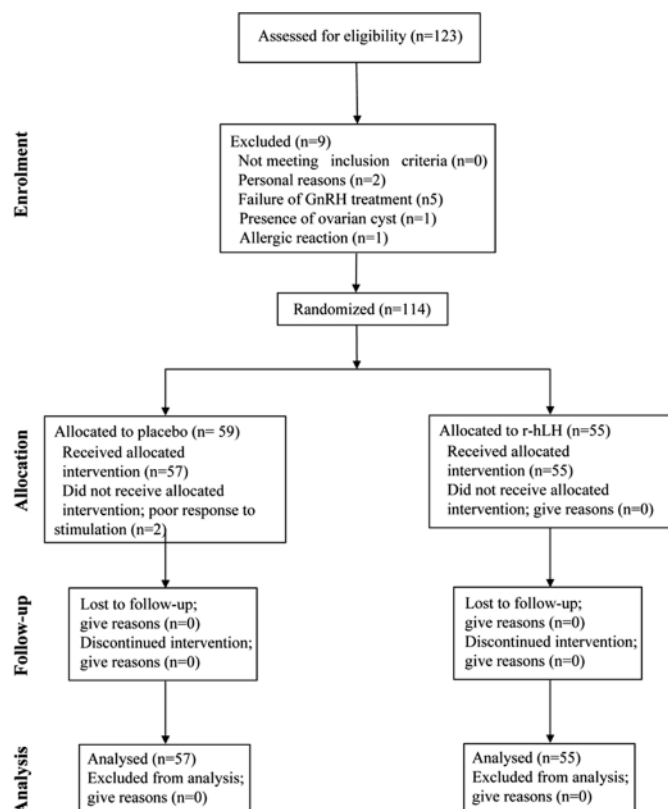


Figure 1. Progress of patients through the study programme.

In the two groups, 52 and 47 patients, respectively, underwent ICSI. There were no significant differences between the groups in the dose and duration of r-hFSH treatment, oocyte maturation, fertilization rate, pregnancy rate and live birth rate. In 46.6% of the patients in the FSH alone group, the dose of FSH remained unchanged, while the dose was increased or decreased in 43.1% and 10.3%, respectively. The corresponding proportions in the r-hFSH + 75 IU r-hLH group were 37.7%, 58.8% and 9.4%. No statistical difference was observed between the two groups ($P = 0.3$, Permutation Exact Test).

The number of days from leading follicles attaining a diameter of 14 mm until the day of HCG administration in both groups ranged from 0 to 5. In the r-hFSH plus r-hLH group, 89.1% of patients received r-hLH for between 1 and 3 days. In the control group 83.1% of patients received placebo for between 1 and 3 days. The mean time patients received randomized treatment prior to ovulation induction was 2.1 ± 1.1 days in the placebo-treated group and 2.4 ± 1.1 days in the group given r-hLH. These differences were not statistically significant ($P = 0.0824$).

Ovarian cysts were the most common adverse events during this study, occurring in 18 patients receiving r-hFSH alone and in 22 patients receiving both r-hFSH and r-hLH. Mild and transient injection-site reactions occurred in three and six patients, respectively. Ovarian hyper stimulation syndrome (OHSS) occurred in three patients receiving r-hFSH alone; these patients required hospitalization. No patient in the r-hLH group developed OSS. Gynaecological bleeding occurred in two patients in each group.

Discussion

This randomized, double-blind trial compared the efficacy of r-hFSH alone and with the addition of r-hLH in the late follicular phase of ovarian stimulation in patients undergoing ART. The results showed that the addition of r-hLH at a critical time of follicular development and in the face of low endogenous levels of LH due to the long agonist regimen produces no further benefit in a relatively young (mean age 30.5 years) patient population in terms of the primary end-point—the number of MII oocytes retrieved.

Similar results have been reported recently from other randomized trials comparing r-hFSH alone or in combination with r-hLH (Lisi *et al.*, 2002; Humaidan *et al.*, 2004; Marrs *et al.*, 2004). In the study by Marrs *et al.* (2004), the addition of r-hLH had no significant effect in the overall study population on the number of MII oocytes retrieved or on implantation. Among women over 35 years of age, however, the implantation rate was markedly higher in women who received r-hLH compared with those patients receiving r-hFSH alone (Marrs *et al.*, 2004; Humaidan *et al.*, 2004). This might suggest that there is a subgroup of older women who may benefit from treatment with r-hLH. It is noteworthy that the present study involved principally younger women (mean age 30.5 years).

In WHO Type I anovulatory patients under circumstances of profound gonadotrophin deficiency, the synergistic effect of

LH on FSH-driven folliculogenesis is clearly demonstrated (Couzinet *et al.*, 1988; Shoham *et al.*, 1991; European Recombinant Human LH Study Group, 1998). Exogenous LH also appears to have a beneficial effect on endometrial thickness in patients with WHO type I anovulation (European Recombinant Human LH Study Group, 1998).

It has been widely demonstrated that, during ovarian stimulation with FSH and concomitant administration of a GnRH agonist, endogenous levels of LH decrease—reaching lowest values during the late stimulation phase (Howles *et al.*, 1994; Loumaye *et al.*, 1997; Westergaard *et al.*, 2000). Thus, it would seem logical that if LH supplementation is to have any benefit, then the late follicular phase would be the appropriate time for its administration especially if, as has been reported, ~50% of agonist/FSH-treated women are LH deficient (plasma LH concentration <0.5 IU/l) (Westergaard *et al.*, 2000).

This is consistent with current concepts of the relative roles of FSH and LH in folliculogenesis, according to which LH plays an essential role in the final stages of maturation (Hillier, 2001; Zeleznik, 2001). Sills *et al.* (1999) in a small prospective study found that implantation and pregnancy rates actually tended to be higher in patients who received r-hFSH alone compared with those who received supplementary r-hLH, although the differences did not reach statistical significance.

Other recent studies have reported a significant reduction in the efficacy of r-hFSH when LH (r-hLH or LH activity derived from HMG) is co-administered, reflected in an increase in the number of vials of FSH needed to achieve ovarian stimulation (Balasch *et al.*, 2001, 2003; Westergaard *et al.*, 2001). It has been reported that the addition of LH can have either beneficial or detrimental effects on oocyte yield and quality in egg donors, depending on the level of endogenous LH (Tesarik and Mendoza, 2002). Beneficial effects were seen in donors with plasma LH concentrations below 1 IU/l prior to ovarian stimulation, whereas detrimental effects were seen in patients with higher concentrations.

The dose of r-hLH given in this study (75 IU/day) was lower than that used in the study of Marrs *et al.* (150 IU/day) (Marrs *et al.*, 2004); we also started treatment with r-hLH slightly later in the cycle. The dose used by Marrs *et al.* (2004) was calculated to be sufficient to achieve a maximum concentration of 1.2 IU/l, which in turn was reported to be the minimum required for achievement of pregnancy in hypogonadotrophic women (Marrs *et al.*, 2004). Thus, it is possible that a more pronounced effect of r-hLH on follicle development would have been observed with a higher dose and/or earlier administration of r-hLH.

The assumption defined for calculating the sample size needed for this study was under-estimated because the SDs of the measure 'number of MII oocytes retrieved' obtained for the two groups were higher than expected (4.8 and 4.9, respectively for r-hFSH alone and r-hFSH + r-hLH). This compares with values of 1.2 and 0.9, respectively, for the HMG and FSH-HP groups used to calculate the sample size for this study (Imthurn *et al.*, 1996). Using these figures, ~100 patients per group (200 in total) would have been required to demonstrate a statistically significant difference of 2.5 MII oocytes between the two groups using 95% power.

The number of embryos transferred in the current study is relatively high considering the trend towards single embryo transfer and the increasing awareness of the problem of multiple pregnancies resulting from IVF. This trend, however, was not so prominent when the study was designed.

In summary, this was the first double-blind, prospective, randomized study examining the impact of LH supplementation in the late FSH stimulation phase of long agonist ART cycles. LH supplementation resulted in higher E_2 levels on the day of HCG administration. However, this study was unable to detect any benefit on stimulation outcome of additional LH in this relatively young patient population. Consistent with these findings, recent data (Humaidan *et al.*, 2004; Marrs *et al.*, 2004) suggest that LH supplementation might be beneficial in a subgroup of older patients.

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