Human Reproduction Vol.20, No.2 pp. 390–396, 2005 doi:10.1093/humrep/deh625
Advance Access publication December 2, 2004

Recombinant human LH supplementation versus recombinant human FSH (rFSH) step-up protocol during controlled ovarian stimulation in normogonadotrophic women with initial inadequate ovarian response to rFSH. A multicentre, prospective, randomized controlled trial

G.De Placido[1], C.Alviggi[1,7], A.Perino[2], I.Strina[1], F.Lisi[3], A.Fasolino[4], R.De Palo[5], A.Ranieri[1], N.Colacurci[6] and A.Mollo[1] on behalf of the Italian Collaborative Group on Recombinant Human Luteinizing Hormone*

[1]Dipartimento Universitario di Scienze Ostetriche Ginecologiche e Medicina della Riproduzione, Area Funzionale di Medicina della Riproduzione ed Endoscopia Ginecologica, Università degli Studi di Napoli 'Federico II', Napoli, [2]Istituto Materno Infantile, Università di Palermo, Palermo, [3]Biogenesi, Casa di Cura Villa Europa all'EUR, Roma, [4]Unità Operativa Ginecologia e Ostetricia, A.O.San Giovanni di Dio e Ruggi D'Aragona, Salerno, [5]Dipartimento di Scienze Chirurgiche Generali e Specialistiche, Sezione Ginecologia e Ostetricia A, Università di Bari, Bari, [6]Dipartimento Universitario di Scienze Ginecologiche, Ostetriche e della Riproduzione, II Università di Napoli, Napoli

[7]To whom correspondence should be addressed at: via Luigi Caldieri 132, 80128, Napoli, Italy. E-mail: alviggi@unina.it

BACKGROUND: In ~12–14% of young normogonadotrophic women treated with a depot GnRH agonist long protocol, the initial ovarian response to recombinant human FSH (rFSH) can be suboptimal. We have tested the hypothesis that these women may benefit from recombinant human LH (rLH) supplementation in a multicentre, prospective, randomized trial compared with patients treated with an rFSH step-up protocol. METHODS: A total of 260 young normogonadotrophic women undergoing controlled ovarian stimulation with a GnRH agonist long protocol for IVF/ICSI were enrolled. The starting dose of rFSH was 225 IU. One hundred and thirty patients with serum estradiol levels <180 pg/ml and with at least six follicles with a mean diameter >5 mm but none >10 mm on both day 5 and day 8 of stimulation were randomly allocated to two groups. From the eighth day of stimulation, women in group A ($n = 65$) received 150 IU of rLH in addition to rFSH, while those in group B ($n = 65$) had an increase of 150 IU in the daily dose of rFSH (step-up protocol). One hundred and thirty normally responding women continued monotherapy with rFSH and served as a further control population (group C). RESULTS: The mean number of cumulus–oocyte complexes retrieved in group A (9.0 ± 4.3) was significantly higher ($P < 0.01$) compared with group B (rFSH 6.1 ± 2.6) but significantly lower compared with group C (10.49 ± 3.7, $P < 0.05$). Implantation and pregnancy rates were significantly lower ($P < 0.05$) in the rFSH step-up group (10.5 and 29.3% respectively) when compared with normal responders (18.1 and 47.3% respectively). CONCLUSIONS: rLH supplementation is more effective than increasing the dose of rFSH in terms of ovarian outcome in patients with an initial inadequate ovarian response to rFSH alone.

Key words: FSH/IVF/LH/recombinant human LH/poor responders

Introduction

The standard long protocol GnRH agonist regimen is a well-established strategy for controlled ovarian stimulation (COS) in young normogonadotrophic women undergoing IVF/ICSI (Hughes *et al.*, 1992; Tan *et al.*, 1992). In such a protocol, GnRH agonist administration is primarily followed by 'monotherapy' with highly purified urinary FSH (FSH-HP) or recombinant human FSH (rFSH). Thus, endogenous LH secretion is suppressed with no exogenous LH activity.

*Italian Collaborative Group on Recombinant Human Luteinizing Hormone: Antonino Abate, Francesco Abate, Maria Perino: Centro 'Riproduzione Umana', Messina; Erminia Alviggi, Francesco Bertocci, Salvatore Conforti, Roberto Clarizia, Maristella D'Uva, Emmanuel Fiore, Pasquale Granata, Tiziana Pagano, Maria Teresa Varricchio: Università degli Studi di Napoli 'Federico II', Napoli; Rosaria Schillaci: Istituto Materno Infantile, Università di Palermo, Palermo; Simon Fishel, Leonardo Rinaldi: Biogenesi, Roma; Mariateresa Capotorto, Luigi Selvaggi: Università di Bari, Bari; Daniela Mele, Argyro Tassou: II Università di Napoli, Napoli.

Nevertheless, this approach is effective in almost all women, suggesting that residual circulating levels of endogenous LH are usually adequate to support multiple follicular growth and oocyte development (Loumaye *et al.*, 1997; Sills *et al.*, 1999). According to our experience (De Placido *et al.*, 2001, 2004), in ~12–14% of these patients treated with a depot GnRH agonist, an abnormal ovarian response profile to this protocol is observed. In particular, an initial follicular growth [at least six follicles ≥6 mm in diameter on the fifth day of stimulation in association with a ≥2-fold increase in estradiol (E_2) levels with respect to the value observed on the day of pituitary suppression assessment] is followed by a plateau phase, in which no significant change in follicular diameter and a suboptimal increase in E_2 levels are observed between days 5 and 8 of stimulation, despite the use of age and body mass index (BMI)-appropriate dosage increase of rFSH. This condition (steady response) usually leads to an increase in the rFSH daily dose and results in a suboptimal ovarian response with a large consumption of rFSH. It has been recently hypothesised that suboptimal ovarian response to rFSH may also be due to excessive pituitary LH suppression. In such cases, an improvement in the ovarian outcome from LH-containing gonadotrophin preparations would be expected (Laml *et al.*, 1999; Lèvy *et al.*, 2000; De Placido *et al.*, 2001, 2004). This hypothesis is consistent with our previous data demonstrating that in young normogonadotrophic patients undergoing a GnRH agonist long protocol, cycles displaying the above-mentioned pattern of initial steady response to rFSH are rescued by administering hMG, an extractive preparation of FSH and LH in a 1:1 ratio, from the eighth day of stimulation. The use of hMG as a source of LH supplementation results in an increase in the FSH dosage as well. Interestingly, women who benefited from exogenous LH supplementation showed endogenous hormonal levels during the early stimulation that were comparable with those observed in age- and BMI-matched subjects showing an adequate response to monotherapy with rFSH. Taken together, these data represented the first clinical evidence of the need for LH supplementation in specific patient groups, despite the lack of a priori indications. Furthermore, our findings were consistent with previous experimental and clinical lines of evidence indicating that immunoreactive LH is not necessarily representative of LH bioactivity (Mitchell *et al.*, 1995).

With the recent availability of recombinant human LH (rLH), clinicians have the opportunity of administering the two gonadotrophins independently. Thus, exogenous LH administration may be calibrated independently of rFSH. Our preliminary prospective randomized trial demonstrated that a daily rLH dose of 150 IU resulted in a significant increase in the mean number of oocytes retrieved when compared with 75 IU in women displaying steady response to rFSH (De Placido *et al.*, 2004). The present multicentre, prospective, randomized trial was designed in order to compare the efficacy of supplementation with 150 IU/die of rLH versus an rFSH step-up protocol in young normogonadotrophic women with an initial ovarian steady response to monotherapy with rFSH undergoing a GnRH agonist long protocol.

Materials and methods

Study design

A prospective, randomized controlled trial was conducted from February to December 2003 in a collaborative network of seven Italian IVF units. The study protocol was approved by the institutional review board of each centre and a consent form was signed by all patients before entering the study. On the basis of our previous studies (De Placido *et al.*, 2001, 2004) and having the mean number of cumulus–oocyte complexes (COC) retrieved as primary endpoint, it was calculated that a sample size of 55 patients in each treatment group would have 80% power to detect a mean difference of 2.0, using a one-way analysis of variance (ANOVA) with two-sided $P < 0.05$. To allow for drop-outs, a total of 130 patients with a 'steady response' to rFSH were randomized to two treatment groups (group A and group B). Randomization was done in blocks of four using computer-generated random number tables, and realized via a central telephone number. As our working hypothesis was that an ovarian steady response is due to inadequate LH activity and that it can be rescued to a 'normal response' by adding an adequate rLH dose, data from a further control population (group C) treated in the same centres and displaying a normal response to rFSH were collected. In particular, for each woman randomized in group A or B, one 'normal responder' (the first according to the chronological criterion) was enrolled (see Patients). Thus, in effect, two control populations were observed, one constituted by the randomization process (group B), the other selected on the basis of pre-constituted criteria (group C). The clinicians performing oocyte collection and the biologists did not know the stimulation protocol used for each patient.

Patients

Among candidates for IVF–embryo transfer or ICSI cycles, only patients aged 18–37 years, with menstrual cycles ranging from 24 to 35 days (intra-individual variability ± 3 days), basal FSH (day 3 of a spontaneous menstrual cycle) concentrations ≤9 IU/l, and hysteroscopic evidence of a normal uterine cavity within the last 6 months were included. In addition, only women undergoing a GnRH agonist long protocol followed by rFSH administration were enrolled.

The following exclusion criteria were adopted: body mass index [BMI = weight (kg)/height (m^2)] <18.0 and >28.0, biochemical and/or ultrasonographic evidence of polycystic ovarian syndrome (PCOS), stage III–IV endometriosis according to the rAFS (1985), chromosomal abnormalities, endocrinological and/or autoimmune disorders, more than two previously unsuccessful IVF or ICSI cycles, and presence of only one ovary. On this basis, a total of 1389 patients were initially considered as potentially eligible. On the eighth day of stimulation, 130 patients showing a steady response (see Ovarian stimulation protocols) were randomized to two groups to receive rLH supplementation (group A) or an increase in the rFSH dose (group B). One hundred and thirty patients with an initial normal response to the ovarian stimulation protocol constituted group C.

Thus, a total of 260 women were enrolled. Six patients in group A and seven in group B had their cycle cancelled for an insufficient ovarian response. Eighteen women in group C dropped out from the study for cycle cancellation due to high risk of ovarian hyperstimulation syndrome ($n = 11$), and seven were protocol violators. Finally, 59 patients in group A, 58 in group B and 112 in group C were considered for efficacy analysis (Figure 1).

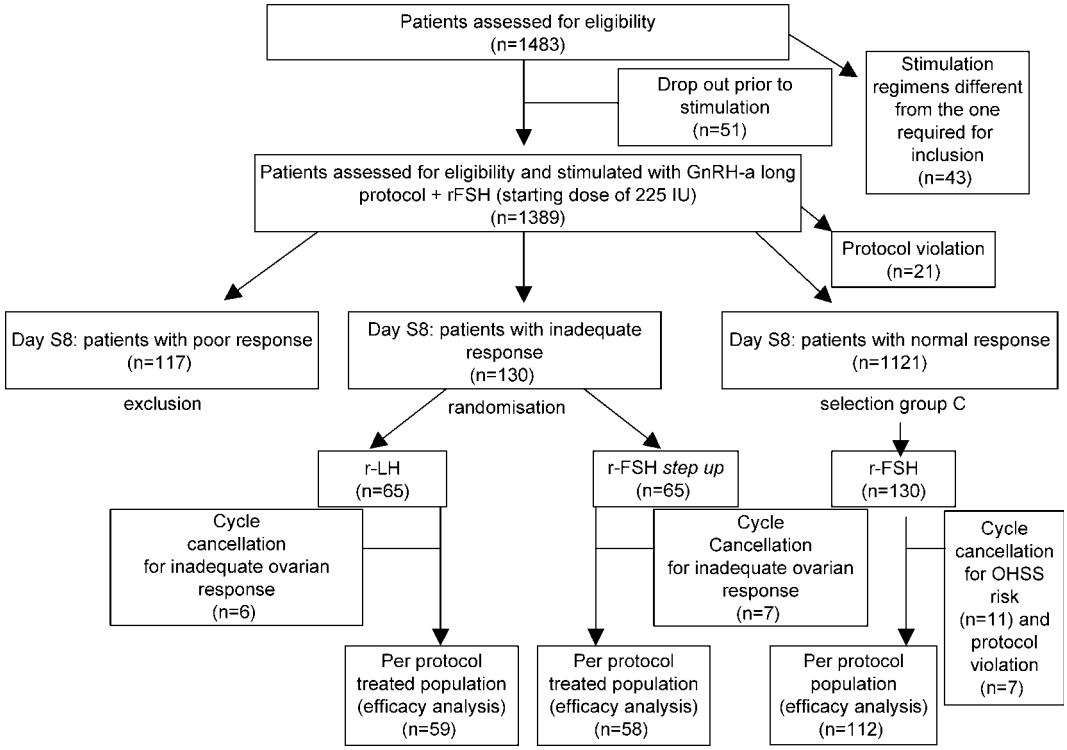

Figure 1. Flow diagram of the process through phases of the study.

Ovarian stimulation protocols

All patients underwent a GnRH agonist long protocol, as previously described (De Placido *et al.*, 2001). Pituitary desensitization was induced with the administration of the GnRH agonist triptorelin (Decapeptyl 3.75 mg depot; Ipsen S.P.A, Italy) on the first day of the menstrual cycle. After 15 days, patients underwent transvaginal ultrasound evaluation and biochemical evaluations. Subjects showing serum E_2 level ≤ 40 pg/ml, endometrial thickness ≤ 5 mm and arrested follicular development started gonadotrophin administration. Women with delayed suppression (including subjects who developed ovarian cysts after the GnRH agonist administration) were excluded from the study. A fixed daily dose of 225 IU of rFSH (Gonal-F®; Serono Pharma, Italy) was administered s.c. On the fifth day of stimulation, serum E_2 levels were measured and the daily rFSH dose was reduced by 75 IU in patients showing concentrations > 180 pg/ml. Serum E_2 concentrations were measured and follicular growth was monitored with a transvaginal scan on the eighth day of stimulation, and thereafter on alternate days until hCG was administered. On the eighth day of stimulation, patients with serum E_2 levels < 180 pg/ml and ultrasound evidence of at least six follicles ranging between 6 and 10 mm, but with no follicle with a mean diameter > 10 mm ('steady response') from day 5 to day 8, were randomized into two groups. The choice of this E_2 threshold value was based on receiver-operating characteristic analysis performed during the course of our preliminary experience (data not published). This value gave the most accurate discrimination between normal and abnormal (no follicle with a mean diameter > 10 mm) ovarian response to rFSH on the eighth day of stimulation. In group A, a fixed daily dose of 150 IU of rLH (Luveris®; Serono Pharma) was added. Patients in group B received an increase in the rFSH daily dose of 150 IU. Patients satisfying inclusion and exclusion criteria and characterized by a tripling of serum E_2 levels between days 5 and 8, and with more than four follicles > 10 mm in diameter on day 8, constituted group C. These patients continued their monotherapy with the current rFSH dose.

Patients who displayed one to four follicles of diameter > 10 mm or no follicle > 10 mm but serum E_2 levels ≥ 180 pg/ml on day 8 of stimulation were outside the criteria for inclusion in either groups A, B or C and were therefore excluded from the present study. The ovarian response was defined 'inadequate' when less than four follicles of 12 mm in diameter were observed on day 12 of stimulation. On the basis of this criterion, six and seven stimulation cycles were cancelled in groups A and B respectively. In group C, seven women were not considered for the efficacy analysis because of protocol violation and 11 cycles were cancelled because of an increased risk for ovarian hyperstimulation syndrome. The ovulatory dose (10 000 IU i.m.) of hCG (Gonasi®; AMSA SRL, Italy) was administered when three follicles showed a mean diameter of ≥ 17 mm. Oocytes were retrieved by transvaginal ultrasound-guided aspiration 35 h after the hCG injection. Serum concentrations of LH were measured on the day of pituitary suppression assessment and on the eighth day of stimulation (just before randomization into groups A and B).

Luteal phase supplementation

Patients began progesterone, 50 mg in oil (Prontogest®; AMSA SRL), luteal phase supplementation with daily i.m. administration (50 mg/day) on the day of oocyte retrieval.

Hormone measurement

Serum concentrations of E_2 and LH were measured using an enzyme-linked fluorescent assay technique (Vidas oestradiol II and Vidas LH respectively; BioMérieux SA, France). The detection limits, defined as the lowest concentration that is significantly different from zero with a probability of 95%, were 9.0 pg/ml for E_2 and 0.1 IU/l for LH. The intra- and inter-assay coefficients of variation (CV) were $< 8\%$ for both E_2 and LH assays. Serum FSH was

determined by an immunometric assay based on enhanced luminescence (Amerlite FSH assay; Amersham International PLC, Amersham Pharmacia Biotech, UK). The detection limit was 0.5 IU/l. The intra- and inter-assay CV were <7%.

Statistical analysis

The results are reported as the mean ± SD. Data were analysed with the SPSS version 12.0 (SPSS Inc., USA). Normal distribution has been inferred to continuous variables whose skewness ranged between +1.0 and −1.0 in each single group. In such a case, one-way ANOVA was used to determine the effect of the stimulation protocol. The *post hoc* Fisher least-significant-difference method was used to assess differences between groups. The Mann–Whitney *U*-test was applied to test differences between groups for continuous variables with non-parametric distributions. χ^2-Statistics were used to compare discontinuous data. $P < 0.05$ was considered statistically significant.

Results

In the present study, 229 IVF cycles were analysed. The two study groups and the control population were comparable for age, BMI, basal serum FSH and LH concentration, and serum LH levels after pituitary suppression. Mean duration of infertility and indication for IVF were also similar in the three groups (Table I). In group A, 14 women underwent IVF and 45 ICSI; in group B, 16 women underwent IVF and 42 ICSI; in group C, 33 women underwent IVF and 79 ICSI.

The average number of COC retrieved in group A was significantly higher when compared with that observed in group B (9.0 ± 4.3 and 6.1 ± 2.6, $P < 0.01$). The difference between groups A and C (10.5 ± 3.7) was also statistically significant ($P < 0.05$; Table II). The mean number of mature oocytes (data evaluated only in ICSI cycles) was also significantly higher in group A when compared with group B (7.8 ± 4.3 versus 4.7 ± 1.6 respectively, $P < 0.01$). For this parameter, results of groups A and C (9.0 ± 3.1) were comparable ($P = 0.052$).

The average number of rFSH ampoules was significantly lower in group C (34.4 ± 7.50) when compared with groups A (41.1 ± 7.1, $P < 0.01$) and B (56.7 ± 8.0, $P < 0.01$). The difference between the two study groups, A and B, was also statistically significant ($P < 0.01$; Table II).

Mean serum E_2 levels on the day of hCG administration were significantly higher in the group of patients receiving rLH (1778.9 ± 778.6 pg/ml) when compared with group B (1248.0 ± 472.4 pg/ml, $P < 0.001$). Mean levels observed in the control group, group C (2376.5 ± 722.8 pg/ml), remained significantly higher ($P < 0.001$) than those measured in both groups A and B (Table II, Figures 2 and 3).

Fertilization rates (number of two-pronuclei cells/number of mature oocytes) were comparable in the three groups (71.8, 69.2 and 73.2% respectively).

Implantation rates (number of gestational sacs/number of transferred embryos) were 14.2, 10.5 and 18.1% in groups A, B and C respectively; the only statistically significant difference was observed between groups B and C ($P < 0.05$). The cumulative pregnancy rate (positive serum βhCG per started cycle) was also significantly lower in group B (29.3%) with respect to group C (47.3%, $P < 0.05$), whereas in patients treated with rLH, the pregnancy rate was 37.2%. Abortion rates were 17.0, 22.0 and 13.2% in groups A, B and C respectively (not significant). The ongoing pregnancy rates (number of pregnancies reaching week 12/cycle) were 32.5, 22.0 and 40.2% in the three groups respectively. Also for this parameter the only statistically significant difference was revealed between groups B and C ($P < 0.05$). Serum LH levels on the day of assessment of pituitary suppression and on day 8 of stimulation were comparable in the three groups (Table II).

When the IVF outcome was evaluated according to the actual method of fertilization, standard IVF or ICSI, women who underwent standard IVF in group C showed a significantly higher ($P < 0.05$) implantation rate (21.2%) when compared with those of group B (7.7%).

Furthermore, the IVF patients' pregnancy rate in group C (58.1%) was significantly higher than that observed in both group A (28.6%) and group B (23.1%).

Table I. Patients' characteristics and indications for IVF

Characteristics	Group A ($n = 57$)	Group B ($n = 58$)	Group C ($n = 112$)
Age (years)	31.5 ± 3.9	30.4 ± 4.1	30.4 ± 3.5
Body mass index (kg/m^2)	23.0 ± 2.6	23.1 ± 2.2	23.7 ± 3.0
Duration of infertility (years)	3.1 ± 0.9	3.0 ± 1.0	2.9 ± 1.1
Basal FSH (IU/l)	6.9 ± 2.1	6.2 ± 1.5	5.9 ± 1.8
Basal LH (IU/l)	4.5 ± 2.1	4.5 ± 1.7	4.6 ± 2.1
Basal estradiol (pg/ml)	51.2 ± 20.1	49.8 ± 14.6	46.4 ± 18.3
Indication for IVF (%)			
Tubal factor	21.7	25.6	27.5
Male factor	51.5	48.4	52.5
Combined	20.1	21.8	15.7
Other[a]	6.7	4.2	4.3

[a]Other factors refer to endometriosis and idiopathic infertility.
Values are mean ± SD. One-way ANOVA was adopted for continuous variables. The *post hoc* Fisher least-significant-difference test assessed differences between groups. No statistically significant difference was revealed between groups for each of the variables analysed.

Discussion

The results of this prospective randomized trial support the hypothesis that an exogenous LH supplementation in the form of rLH is effective in improving the ovarian response in a specific subset of young normogonadotrophic patients, who show an initially inadequate ovarian response to rFSH during concomitant treatment with depot GnRH agonist. These women have to be distinguished from the classical poor responders, in whom the detection of a few antral follicles during the early stages of stimulation is followed by development of one to four follicles with a mean diameter >10 mm. In contrast, women investigated in the present study displayed an apparently normal follicular reserve (as expected in young normogonadotrophic women) and at least six follicles ≥6 mm in diameter were evident from day 5 of stimulation: nevertheless, the employment of age- and BMI-appropriate rFSH dosages did not result in a further clinically significant follicular growth, whereas on day 8 of stimulation,

Table II. Cycle outcomes

	Group A ($n = 59$)	*P* value (A versus B)	Group B ($n = 58$)	*P* value (B versus C)	Group C ($n = 112$)	*P* value (A versus C)
Suppressed LH[a]	1.2 (0.2–4.1)		1.2 (0.5–4.6)		1.5 (0.4–7.3)	
Duration of stimulation (days)[b]	14.5 ± 1.3		15.0 ± 1.1	< 0.01	11.1 ± 1.4	< 0.01
No. of rFSH ampoules[b]	41.9 ± 7.1	< 0.001	56.7 ± 8.0	< 0.001	34.4 ± 7.5	< 0.001
No. of rLH ampoules	12.9 ± 2.9					
Total no. of ampoules of rLH + rFSH[b]	54.2 ± 7.4	0.077	56.7 ± 8.0	< 0.001	34.4 ± 7.5	< 0.001
Day 5 E_2 concentration (pg/ml)[b]	48.3 ± 16.5		52.2 ± 17.7	< 0.001	227.7 ± 166.8	< 0.001
Day 8 LH concentration (IU/l)[a]	0.7 (0.1–3.6)		0.7 (0.1–4.0)		4.3 (0.1–4.4)	
Day 8 E_2 concentration (pg/ml)[b]	116.8 ± 43.6		122.0 ± 42.4	< 0.001	887.7 ± 544.8	< 0.001
E_2 at hCG day (pg/ml)[b]	1778.9 ± 778.6	< 0.001	1248.0 ± 472.4	< 0.001	2376.5 ± 722.8	< 0.001
No. of COC retrieved[b]	9.0 ± 4.3	< 0.01	6.1 ± 2.6	< 0.01	10.5 ± 3.7	< 0.05
No. of mature oocytes (MII)[b,c]	7.8 ± 4.3	< 0.01	4.7 ± 1.6	< 0.01	9.0 ± 3.1	0.052
Fertilization rate (%)[d]	71.8		69.2		73.2	
No. of transferred embryos[a]	3.5 (0–4)		3 (0–4)		4 (0–4)	
Cumulative implantation rate (%)[d]	14.2		10.5	< 0.05	18.1	
Cumulative pregnancy rate (%)[d]	37.2		29.3	< 0.05	47.3	
Cumulative abortion rate (%)[d]	17.0		22.0		13.2	
Cumulative ongoing pregnancy rate (%)[d]	32.5		22.0	< 0.05	40.2	

[a]Values are median ± ranges; non-parametric statistics (Mann–Whitney *U*-test).
[b]Values are mean ± SD; one-way ANOVA. The *post hoc* Fisher least-significant-difference method tested for differences between groups.
[c]Data concerning only ICSI cycles (group A: $n = 45$; group B: $n = 42$; group C: $n = 79$).
[d]χ^2-Statistics.
Blank fields: no statistically significant difference.
E_2 = estradiol; COC = cumulus–oocyte complexes; MII = metaphase II.

none of those follicles overcame a mean diameter of 10 mm and E_2 levels were ≤180 pg/ml. We arbitrarily defined this particular profile of ovarian response as 'steady response'.

The hypothesis of the present study was that this particular profile of initial ovarian response is due to insufficient LH activity following desensitization with a depot GnRH agonist. If this hypothesis is correct, the addition of rLH would reverse this condition and consequently result in a similar ovarian response to that in 'normally responding patients'. This is the reason why a further control population, represented by women treated in the same centres during the same period and showing a good profile of initial response to rFSH, was included.

A significant increase in the number of COC (primary endpoint) was observed in patients with ovarian steady response who received rLH in the course of COS, when compared with those undergoing an increase in rFSH dose. Not surprisingly, this parameter was significantly higher in patients with a good initial ovarian response (group C). Nevertheless, it is interesting to observe that the mean number of mature oocytes, implantation and pregnancy rates of these normal responders were similar to those observed in women treated with rLH. Furthermore, in both groups these parameters were significantly better than those observed in group B. This evidence supports the hypothesis that women with a 'steady' response are potential normal responders, whose ovarian and IVF outcome is significantly improved by LH supplementation.

Results concerning the effects of this treatment on ovarian outcome are consistent with our previous data (De Placido *et al.*, 2001): in that study, the administration of exogenous LH in the form of hMG rescued the ongoing COS cycle in

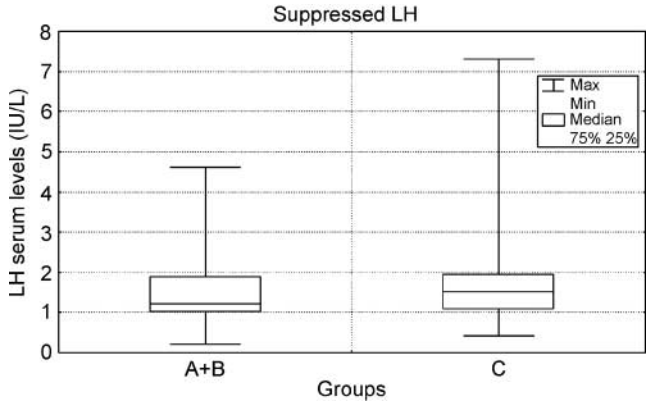

Figure 2. LH serum concentrations in the two groups of women with steady response (A + B) and in normal responders (C) on the day of pituitary suppression assessment. Mann–Whitney *U*-test: differences were not statistically significant.

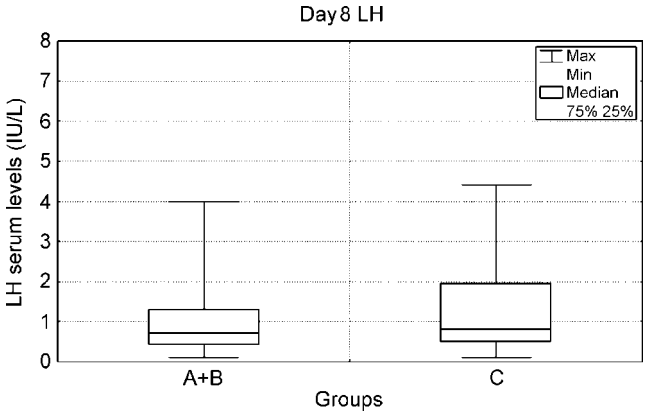

Figure 3. LH serum concentrations in the two groups of women with steady response (A + B) and in normal responders (C) on the eighth day of stimulation. Mann–Whitney *U*-test: differences were not statistically significant.

the same subset of patients, resulting in a significant increase in the number of oocytes retrieved when compared with the classical rFSH step-up protocol. The relevance of confirming this observation by using rLH is related to the advantage that in this case no change in the rFSH regimen is required. Firstly, it is to be considered that according to our previous data (De Placido *et al.*, 2004), an rLH daily dose of 150 IU is more effective than 75 IU in improving the ovarian outcome in this subgroup of patients. Notwithstanding, in our present and previous studies, the administration of such a daily rLH dose resulted in a significant decrease in the cumulative rFSH amount and in a trend toward a reduction of the stimulation length. The hypothesis that a higher rLH daily dose (i.e. 225 IU) would have improved the ovarian outcome cannot be excluded. Nevertheless, considering our previous study (De Placido *et al.*, 2004), the daily dose of 150 IU seemed to be the most suitable in order to improve the ovarian response to that commonly reported in normal responders. Recent studies have underlined the relevance of LH activity during follicular growth (Filicori and Cognini, 2001; Filicori, 2003). Nevertheless, clinical trials have shown that rLH supplementation in unselected young normogonadotrophic patients undergoing COS with rFSH does not improve either the ovarian or the IVF outcome when compared with rFSH monotherapy (Sills *et al.*, 1999; Ben-Amor *et al.*, 2000; Marrs *et al.*, 2004). These data indicate that residual LH levels after GnRH agonist-mediated pituitary desensitization are usually adequate to support multiple follicular development when rFSH is administered, and prompted investigators to identify specific subgroups of women who may benefit from exogenous LH administration (Humaidan *et al.*, 2004). Lisi *et al.* (2001) have recently reported that women who had required a high rFSH dosage in a previous cycle showed a significant increase in implantation and pregnancy rates in a subsequent stimulation performed with rFSH and rLH. In this context, it could be considered that in the present study patients with initial steady response who underwent treatment with increased doses of rFSH required a mean cumulative dose >4000 IU. Taken together, our results and data from Lisi *et al.* (2001) seem to identify a specific subset of women who cannot be classified either as 'poor responders', due to the achievement of a normal ovarian response (i.e. more than five oocytes retrieved), or as 'normal responders', due to the high cumulative rFSH requirement. This subgroup shows suboptimal ovarian and IVF outcomes and seems to benefit from rLH administration. These considerations lead to the hypothesis that an early identification of women at risk for requiring a high rFSH dose may allow an adequately timed integration with rLH, which, in turn, may rescue the ovarian response and improve the IVF outcome. Thus, a certain number of re-stimulations may be potentially avoided. The question is which is the most reliable criterion for identifying subjects who require exogenous LH. The evidence that these patients benefit from an rLH supplementation leads to the hypothesis that endogenous levels of the hormone may fall below a hypothetical threshold in the course of COS due to the combined action of the GnRH agonist and rising levels of E_2 (and/or other factors secreted from developing follicles). Interestingly, our studies failed to demonstrate any significant correlation between circulating levels of endogenous LH and initial response to rFSH: patients with initial steady response (groups A and B) and patients in group C had comparable serum LH levels on the day of the assessment of suppression after GnRH agonist administration and on the eighth day of stimulation (before the first rLH dose in group A). This finding is consistent with previous studies demonstrating that levels of LH circulating during COS do not correlate with any ovarian outcome parameter (Westergaard *et al.*, 2000; Balasch *et al.*, 2001). In addition, available data from the literature are controversial and insufficient to recommend LH supplementation in women undergoing IVF on the basis of the analysis of LH values during COS (Fleming *et al.*, 1998; Westergaard *et al.*, 2000; Balasch *et al.*, 2001; Humaidan *et al.*, 2002). The contradictory results reported by different investigators may be related to differences in the assays used to measure LH. A two-site immunoradiometric assay (IRMA) may be most accurate, but whatever assay is used there is no guarantee that the LH measured is actually bioactive. Additionally, the type of GnRH agonist and the mode of administration seem to play a role in ovarian response and each may affect the endogenous LH activity (Westergaard *et al.*, 2001).

A clinical history of requirement of high rFSH doses in a previous cycle may prompt the addition of rLH in a subsequent one. Analogously, our data also suggest that an ongoing stimulation cycle with specific characteristics of initially inappropriate ovarian response to rFSH may be rescued by rLH supplementation. Thus, following confirmation of these data, the ovarian response to rFSH (in ongoing or previous cycles) may reveal a practical and reliable criterion for identifying women to undergo rLH treatment.

The observation that subsets of women showing an improvement in their ovarian response by rLH administration display apparently normal circulating levels of the endogenous hormone remains to be explained. It could be hypothesized that in these subjects, a less biologically active LH is present. In other words, serum concentrations of the 'immunoreactive' molecule may be not representative of the effective bioactivity of the hormone (Huhtaniemi *et al.*, 1999; Jiang *et al.*, 1999; Ropelato *et al.*, 1999). Similar clinical findings have been previously reported in the literature. In particular, discrepancies between immunoreactive and bioactive LH are manifest as an age-related phenomenon in men (Mitchell *et al.*, 1995). The presence of a less bioactive LH may also explain the observation of the statistically significant increase ($\sim$50%) in the number of oocytes retrieved in women with a steady response treated with rLH when compared with the rFSH group. Another possible explanation may involve a change at the level of the receptor (Huhtaniemi *et al.*, 1999). Finally, it is to be underlined that the use of different GnRH agonist preparations and/or administration modalities may result in different degrees of endogenous LH suppression (Westergaard *et al.*, 2001). In particular, the use of depot preparation may lead to a higher degree of suppression, which in turn may favour

the manifestation of a steady response. Conversely, it could be hypothesized that the use of daily s.c. or intranasal preparation could reduce the probability of such a phenomenon.

In conclusion, our results suggest that: (i) specific subgroups of young normogonadotrophic women showing an initial abnormal ovarian response to monotherapy with rFSH require an LH supplementation during COS; (ii) in this subset of women, the initial clinical response to rFSH—more so than to LH serum levels—during COS seemed to be most predictive of the exogenous LH requirement; (iii) in agreement with previous findings, the eighth day of stimulation seemed to be appropriate for starting rLH supplementation. Finally, larger prospective randomized trials are required to evaluate the effects of rLH supplementation in these subsets of patients on IVF outcome, according to the method of fertilization (IVF or ICSI).

Acknowledgements

We would like to thank Gianluca Ficca for advice concerning statistical analysis and Colin Howles for his helpful suggestions and comments. This study was realised with grants from the *Ministero dell'Istruzione, dell'Università e della Ricerca* (*PRIN annualità 2003 prot. 2003065093_002*).

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Submitted on August 6, 2004; accepted on October 29, 2004