Follicular Development and Hormonal Levels Following Highly Purified or Recombinant Follicle-Stimulating Hormone Administration in Ovulatory Women Undergoing Ovarian Stimulation After Pituitary Suppression for In Vitro Fertilization: Implications for Implantation Potential

JUAN BALASCH,^{1,2} FRANCISCO FÁBREGUES,¹ MONTSERRAT CREUS,¹ JOANA PEÑARRUBIA,¹ ESTHER VIDAL,¹ FRANCISCO CARMONA,¹ BIENVENIDO PUERTO,¹ and JUAN A. VANRELL¹

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Purpose: The main goal in the present study was to compare follicular development and estradiol levels after ovarian stimulation in pituitary suppressed normally ovulating women undergoing IVF, using highly purified urinary follicle stimulating hormone (FSH) (u-FSH-HP) and recombinant FSH (rec-FSH). A secondary variable in our study was embryo implantation potential, which is closely related to appropriate follicular development and oocyte competence.

Methods: For the main purpose of this study, 30 IVF patients (group 1) were treated during IVF consecutive cycles, using the same stimulation protocol, with u-FSH-HP in the first treatment study cycle and rec-FSH in the second one. As a control group (group 2) for implantation rates obtained in cycles treated with rec-FSH, 30 additional IVF patients were included who underwent a second IVF attempt again with u-FSH-HP.

Results: The total dose of FSH used and ovarian response obtained in terms of estradiol plasma levels and the total number of growing follicles on the day of human chorionic gonadotropin (HCG) injection were similar in both treatment cycles in group 1 but better follicular dynamics and oocyte maturity were obtained with rec-FSH. The implantation rate was significantly higher in rec-FSH treated cycles in patients in group 1 than in control women (group 2).

Conclusions: rec-FSH is more efficacious than u-FSH-HP when used in the same patient in inducing multiple follicular development in down-regulated cycles as indicated by ovarian performance and oocyte maturity. In addition, rec-FSH

yields significantly higher implantation rates than u-FSH-HP when used in patients undergoing their second IVF attempt.

KEY WORDS: IVF; induction of ovulation; oocyte maturity; recombinant FSH; urinary FSH.

INTRODUCTION

Ovulation inducing drugs, mainly gonadotropins, are being increasingly administered to normally ovulating women because of the high number of couples undergoing assisted reproduction technologies. Human menopausal gonadotropin (hMG) has been used effectively for ovulation induction in in vitro fertilization (IVF) patients, and for years this has been the only preparation available for clinical use. Because of advances in the purification techniques that have become available over the past recent years, hMG has been purified further. In the early 1980s, "pure" FSH (u-FSH) was made possible by the development of affinity purification processes using polivalent antibodies but the FSH hormonal extract was still contaminated with 95% urinary proteins (1,2). A further purification step using monoclonal antibodies and involving removal of urinary proteins led to the clinical availability of the third-generation gonadotropins known as highly purified FSH (u-FSH-HP), which is now the only marketed urinary FSH preparation (1,2). More recently, biotechnology has made available for the first time a recombinant human FSH (rec-FSH) preparation (3,4), which has been and is being marketed in a number of countries.

Since both hMG and FSH have been found to be effective in inducing follicular growth and maturation,

¹ Institute Clinic of Gynecology, Obstetrics and Neonatology, Faculty of Medicine, University of Barcelona, Hospital Clínic-Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

² To whom correspondence should be addressed at Institute Clinic of Gynecology, Obstetrics and Neonatology, Hospital Clínic, C/ Casanova 143, 08036 Barcelona, Spain.

the choice of gonadotropin for ovarian stimulation has been left to the physician. However, recent evidence comparing hMG and FSH and including the large database of IVF treatment cycles in France, a meta-analysis of randomized controlled trials comparing FSH with hMG in IVF, in vitro studies on luteinized granulosa cells and oocyte morphology studies, suggests that the wisdom of continuing to use hMG for ovarian stimulation in IVF treatment should be reevaluated (5). In addition, recent studies comparing u-FSH (6) or u-FSH-HP (7) with rec-FSH in IVF concluded that rec-FSH is more effective than urinary FSH in inducing multifollicular development.

However, any IVF general population is rather heterogeneous with respect to woman's age and ovarian reserve as well as indication for IVF, which in its turn may also affect the ovarian response to gonadotropin stimulation. Therefore, the use of the same treatment protocol applied to different gonadotropin drugs in the same patient as previously done by us (8,9) and others (10,11) seems the more appropriate study design when ovarian performance and hormonal levels are the objectives to be compared. This was done in the present study where the primary end point was to compare follicular development and estradiol levels after ovarian stimulation in pituitary suppressed normally ovulating women undergoing IVF, using u-FSH-HP and rec-FSH. To the best of our knowledge, such a comparative study has not been previously reported.

As a combined analysis of clinical trials comparing hMG and u-FSH with rec-FSH in IVF suggests that the latter yields significantly higher pregnancy rates than urinary gonadotropins (12), a secondary variable in our study was embryo implantation potential, which is closely related to appropriate follicular development and oocyte competence (13).

MATERIALS AND METHODS

Patients Studied and Design

In all, 60 primary infertility women from our IVF/intracytoplasmic sperm injection (ICSI) program were included in the present study. All of them were regularly menstruating premenopausal women aged 27–38 years. All of the women had both ovaries and no occult ovarian failure on the basis of their cycle days 3–4 FSH concentrations of <10 mIU/ml [standard International Reference Preparation (IRP) 78/549]. No woman had polycystic ovarian disease according to ultrasound examination of the ovaries or basal gonadotropin measurement. Patient indications for IVF/ICSI included

male factor, tubal infertility, and minimal or mild endometriosis. All patients had undergone their first IVF attempt at our institution, having three or four embryos replaced but no pregnancy following an appropriate ovarian response to gonadotropin stimulation using u-FSH-HP under pituitary suppression.

For the main purpose of this study, which was the comparison of ovarian performance and estradiol levels using u-FSH-HP and rec-FSH in IVF, 30 (group 1, or study group) consecutive patients fulfilling the above criteria and agreeing to be treated during their second IVF attempt with commercially available rec-FSH (Gonal-F; Serono S.A., Madrid, Spain) administered s.c., were treated using the same stimulation protocol. As a control group (group 2) for implantation rates obtained in cycles treated with rec-FSH, 30 additional IVF patients were included. The control women were selected as follows: a patient who fulfilled the above-mentioned inclusion requirements and underwent a second IVF attempt immediately after a group 1 patient but again receiving u-FSH-HP for ovarian stimulation (i.e., the closest consecutive cycle in temporal relationship to the index cycle). The time interval between treatment cycles in each woman was 6 to 13 months. Each patient underwent standard IVF or ICSI in both treatment cycles.

Stimulation Regimen

As reported previously (14), ovarian stimulation for IVF is routinely accomplished using gonadotropins under pituitary suppression with a GnRH agonist. Leuprolide acetate (Procrin; Abbott Laboratories S.A.,

Table I. Main Demographics and Baseline Characteristics of Patients in Groups 1 and 2^a

Variable	Group 1 (n = 30)	Group 2 (n = 30)	P
Age (y)	33.5 ± 0.52	33.6 ± 0.5	N.S. ^b
Body mass index (kg/m ²)	23.1 ± 0.3	23.8 ± 0.5	N.S.
No. with indicated cause of infertility			N.S.
Male factor	17 (56.1%)	15 (50%)	
Tubal factor	8 (27.1%)	9 (30%)	
Minimal/mild endometriosis	5 (17%)	6 (20%)	
Duration of infertility (yr)	4.9 ± 0.16	5.2 ± 0.21	N.S.
Interval between IVF cycles	9.2 ± 1.2	8.7 ± 1.0	N.S.
Day 2–4 HSH level (mIU/ml)			
1st IVF attempt	7.1 ± 0.3 ^c	6.9 ± 0.2 ^d	N.S.
2nd IVF attempt	7.0 ± 0.2 ^c	6.8 ± 0.2 ^d	N.S.
No. with ICSI	15 (50%)	14 (47%)	N.S.

^a Data are mean ± SE or n(%).

^b Not significant.

^c P = N.S.

^d P = N.S.

Table II. Ovarian Stimulation Characteristics in the Two Gonadotropin-Stimulated IVF Cycles in Group 1^a

Variable	FSH-HP cycles (n = 30)	rec-FSH cycles (n = 30)	P
Time for ovarian arrest	15.1 ± 0.4	15.2 ± 0.4	N.S. ^b
Days of ovarian stimulation	11.4 ± 0.4	11.6 ± 0.2	N.S.
Total No. of ampoules of FSH	31.5 ± 1.7	32.5 ± 2.0	N.S.
Patients with hCG and ovum retrieval	30 (100%)	30 (100%)	N.S.
No. of >10-mm follicles on hCG day	14.0 ± 1.1	14.6 ± 1.1	N.S.
No. of ≥14-mm follicles on hCG day	10.1 ± 0.4	11.4 ± 0.5	<0.05
Estradiol (pg/ml) on hCG day	2462 ± 211	2208 ± 101	N.S.

^a Values are mean ± SE or n(%).^b Not significant.

Madrid, Spain) suppression is started in the midluteal phase of the previous cycle at a daily dose of 1 mg s.c. This dose is reduced to 0.5 mg/day once ovarian arrest has been achieved and then is continued until the administration of hCG. Gonadotropin stimulation of the ovaries was started when serum estradiol levels declined to <50 pg/ml and a vaginal ultrasonographic scan showed an absence of follicles >10 mm in diameter. On days 1 and 2 of ovarian stimulation, 6 ampoules per day of u-FSH-HP (Neo-Fertinorm; Serono S.A.) or rec-FSH (Gonal-F; Serono S.A.) was administered subcutaneously. On days 3 to 5 of ovarian stimulation, 2 ampoules per day of u-FSH-HP or rec-FSH was

administered to each patient. Thus, for each patient in groups 1 and 2 the FSH dose during days 1 to 5 of gonadotropin stimulation was identical in both treatment cycles. From day 6 onward, u-FSH-HP or rec-FSH was administered on an individual basis according to the ovarian response. The criteria for human hCG administration (5000 IU i.m., Profasi; Serono S.A.) were the presence of two or more follicles >18 mm in diameter, with five or more follicles measuring ≥14 mm in association with a consistent rise in serum estradiol concentration.

Oocyte aspiration was performed by vaginal ultrasonography under local anesthesia 35–37 hr after hCG administration. The maturational status of the oocytes and embryo grading were recorded according to the criteria of Veeck (15). High-quality embryos were referred to having Veeck grade 1 or 2. Two to three days after oocyte recovery, three or four embryos per patient were replaced (according to patient age and embryo quality) and the remaining embryos were cryopreserved. Additional doses of 5000, 2500, and 2500 IU of hCG were given on the day of follicle aspiration and 2 and 5 days later, respectively, to supplement the luteal phase in all patients. Pregnancy was diagnosed by increasing serum concentrations of β-hCG 12 days after embryo transfer and the subsequent demonstration of an intrauterine gestational sac by ultrasonography.

Hormone Analyses and Ultrasonography

Hormone measurements and ultrasonographic scans were carried out as reported previously (14). Hormones

Table III. Follicular Dynamics During Gonadotropin Ovarian Stimulation in the Two IVF Cycles in Group 1

Day of ovarian stimulation	Follicular size (mm)	No. of follicles ^a		P
		FSH-HP cycles (n = 30)	rec-FSH cycles (n = 30)	
6	>10	3.0 ± 0.4	2.6 ± 0.4	N.S. ^b
8	>10	8.4 ± 1.2	8.0 ± 0.8	N.S.
	>10 to <14	6.7 ± 0.9	6.3 ± 0.6	N.S.
	≥14 to <18	1.7 ± 0.5	1.5 ± 0.3	N.S.
10	>10	13.4 ± 1.2	14.7 ± 0.9	N.S.
	>10–14	8.7 ± 1.0	8.4 ± 0.7	N.S.
	≥14 to <18	4.6 ± 0.6	6.3 ± 0.5	<0.05
hCG day ^c	>10	14.0 ± 1.1	14.6 ± 1.1	N.S.
	>10 to <14	3.8 ± 0.5	3.3 ± 0.2	N.S.
	≥14 to <18	5.8 ± 0.5	5.4 ± 0.4	N.S.
	≥18	4.4 ± 0.5	5.9 ± 0.3	<0.01

^a Values are mean ± SE.^b Not significant.^c Mean (±SE) diameters of the two leading follicles in FSH-HP (19.4 ± 0.2 and 18.8 ± 0.2 mm) and rec-FSH (20.9 ± 0.2 and 19.9 ± 0.2 mm) treatment cycles were significantly different (*P* < 0.005) on this day.

Table IV. Assessment of Oocyte and Embryological Characteristics in the Two Gonadotropin-Stimulated IVF Cycles in Group 1^a

Parameter	FSH-HP cycles (<i>n</i> = 30)	rec-FSH cycles (<i>n</i> = 30)	<i>P</i>
No. of oocytes retrieved	10.3 ± 1.0	9.9 ± 0.6	N.S. ^b
Oocyte maturity (%)			
Metaphase II	4.4 ± 0.8	7.9 ± 0.6	<0.005
Metaphase I	5.3 ± 0.5	1.8 ± 0.1	<0.05
Prophase	0.13 ± 0.0	0	N.S.
Degenerative	0.1 ± 0.0	0	N.S.
No. of 2PN oocytes on day 1	6.8 ± 0.6	6.9 ± 0.5	N.S.
Fertilization rate on day 1 (%)	69.6 ± 2.8	71.1 ± 3.5	N.S.
No. of cleaved embryos on day 2	4.8 ± 0.4	5.1 ± 0.4	N.S.
No. of embryos cryopreserved	1.2 ± 0.4	1.5 ± 0.4	N.S.
No. of embryos per replacement	3.3 ± 0.09	3.4 ± 0.09	N.S.
High quality embryos replaced (%)	76.8 ± 6.1	76.6 ± 6.0	N.S.
Day of embryo transfer	2.5 ± 0.09	2.8 ± 0.06	<0.05

^a Values are mean ± SE.^b Not significant.

were measured with the use of commercially available kits. Serum levels of FSH were measured by an immunoradiometric assay (Immunotech International, Marseilles, France) and data are expressed in terms of IRP 78/549. The sensitivity of the assay was 0.5 IU/L and the intraassay and interassay coefficients of variation were <3.5 and 5.2%, respectively. Estradiol levels in serum were estimated by direct RIA (BioMérieux, Marcy l'Etoile, France). Intraassay and interassay coefficients of variation were <4.5 and 5.5%, respectively. Ultrasonographic scans were performed with a 5-MHz vaginal transducer attached to an Aloka sector scanner (Model SSD-620; Aloka, Tokyo).

Statistics

Data were analyzed by SPSS statistical software using the chi-square test with Yates' correction when necessary, the Wilcoxon matched-pairs signed-rank test, the McNemar test, and the unpaired Student *t*-test, as appropriate. Data are presented as mean ± SE.

RESULTS

The results are summarized in Tables I to V and Fig. 1. As shown in Table I, the main demographic and baseline characteristics of the patients in groups 1 and 2 were similar, including age, body mass index, cause and duration of infertility, interval between IVF treatment cycles, and FSH level in the early follicular phase. It is noteworthy that basal FSH concentrations preceding the first and second IVF attempts were almost-identical within groups.

Table II shows the data regarding ovarian response in the two gonadotropin stimulated IVF cycles in group 1. Time for ovarian arrest, days of ovarian stimulation, total dose of FSH administered, total number of growing follicles, and serum concentration of estradiol on the day of hCG injection were similar in both treatment cycles. However, the number of follicles measuring ≥14 mm in diameter on the hCG day was significantly higher in the rec-FSH-treated cycles.

Table V. Embryo Transfer and Pregnancy in the Two IVF Cycles in Groups 1 and 2^a

Parameter	Group 1 (<i>n</i> = 30)		Group 2 (<i>n</i> = 30) ^b		<i>P</i>
	FSH-HP cycle	rec-FSH cycle	1st cycle	2nd cycle	
No. of embryos per replacement	3.3 ± 0.09	3.4 ± 0.09	3.4 ± 0.1	3.3 ± 0.1	N.S.
Day of embryo transfer	2.5 ± 0.09	2.8 ± 0.06	2.4 ± 0.09	2.7 ± 0.08	<0.05
Clinical pregnancies					
Number (%)	—	12 (40%)	—	7 (23%)	0.1
Implantation rate (%)	—	17.7 ± 4.0	—	8.0 ± 2.7	<0.05

^a Values are mean ± SE or *n* (%).^b Both IVF cycles treated with FSH-HP.

Fig. 1. Mean estradiol serum levels and mean number of growing follicles related to the FSH treatment days in the two gonadotropin-treated cycles in patients in group 1. rec-FSH, recombinant FSH; FSH-HP, highly purified urinary FSH. (—○—) FSH-HP; (—■—) rec-FSH.

Table III and Fig. 1 are presented to analyze further follicular dynamics and estradiol levels during gonadotropin ovarian stimulation with u-FSH-HP and rec-FSH in patients in group 1. The serum estradiol concentration and the mean number of total growing follicles (>10 mm) related to the FSH treatment day are depicted in Fig. 1; no significant differences were observed between gonadotropin treatment cycles with respect to any of both parameters. However, the number of follicles measuring ≥ 14 and ≥ 18 mm on FSH treatment day 10 and on the day of hCG injection, respectively, were significantly higher in the rec-FSH

cycles (Table III). Accordingly with final follicular development, a significantly higher number of metaphase II oocytes was found with rec-FSH treatment (Table IV). No other significant differences were found between u-FSH-HP and rec-FSH treatment cycles regarding other oocyte and embryological characteristics summarized in Table IV. However, the interval between IVF and embryo transfer was longer in the rec-FSH treatment cycles (Table IV).

An analysis of the secondary end points (pregnancy and implantation rates) is presented in Table V, which compares embryo transfer and pregnancy in the two IVF treatment cycles in groups 1 and 2. The number of embryos per replacement was almost-identical in the four IVF cycles analyzed. There were 12 (40%) clinical pregnancies including one triplet and two pairs of twins with rec-FSH therapy. There was a first-trimester miscarriage in a patient having a single gestational sac in this study group.

There were no significant differences between the two IVF cycles in patients in group 2 with regard to ovarian response, ovum retrieval, and IVF outcome (data not shown). However, as with group 1, the interval between insemination of the oocytes and transfer of the embryos was significantly longer in the second IVF attempt in the control group of patients (group 2). There were seven (23%) clinical pregnancies in the latter group of women including a pair of twins. One of the six singleton pregnancies ended in spontaneous early abortion.

The comparison of pregnancy rates between the second IVF treatment cycles in groups 1 and 2 suggested a trend in favor of rec-FSH. The difference was statistically significant ($P < 0.05$) when implantation rates were compared (Table V). No patient developed ovarian hyperstimulation syndrome throughout the study.

DISCUSSION

To obtain multiple follicular development is a basic goal in IVF. This is accomplished with the combination of GnRH agonists and gonadotropins in most IVF programs around the world. To stimulate follicular growth most IVF centers have used a supposedly equal mixture of FSH and LH in the form of hMG which also contains urinary proteins. Apart from problems with urinary protein contamination, hMG may contain hCG to achieve an LH/FSH ratio of roughly 1:1. This hCG may accumulate and would explain some cases of premature luteinization and progesterone rise with

potential detrimental effects on the ova or the endometrium (16).

The use of hMG in IVF was based on traditional ovulation induction in cases of amenorrhea due to hypothalamic–pituitary failure and gonadotropin deficiency and on the belief that stimulation of the ovaries under pituitary suppression would require supplementation with LH (17). The availability of u-FSH, u-FSH-HP, and, more recently, rec-FSH made it possible to discriminate better the relative roles of FSH and LH in the ovulation process, underlining the key role of FSH in folliculogenesis. It is now well established that in women with normal pituitary function, treatment with FSH alone after pituitary suppression of endogenous gonadotropin with a GnRH agonist results in normal increases in estradiol, ovulation, and fertilization, and thus supplementation with LH is not needed (17). Accordingly, in previous randomized clinical trials we showed that urinary FSH (either purified or highly purified) alone was as effective as hMG for ovarian stimulation under pituitary desensitization (18).

rec-FSH versus u-FSH with a GnRH agonist in a long protocol has been analyzed in previous studies (6,19). In a multicenter study including a total of 981 patients, Out *et al.* (6) found that a significantly higher number of oocytes was retrieved with a lower total dose of FSH over a shorter treatment period among patients receiving rec-FSH, but there were no differences between the two groups in implantation rates or clinical pregnancy rates. In the study by Hedon *et al.* (19) including 99 patients, results were in the same direction but the higher efficacy of rec-FSH compared with u-FSH was apparently not significant, probably because of the small sample size. This is further supported by a meta-analysis including three comparative studies on rec-FSH and urinary gonadotropins which indicated that the ongoing pregnancy rate per started cycle was significantly higher for rec-FSH (22.9 versus 17.9%) (12). However, in one of the trials the active agent was hMG and no pituitary desensitization was used (12).

At present, the point is whether rec-FSH is more effective and may yield higher pregnancy rates in down-regulated IVF patients than u-FSH-HP, which is now the only marketed urinary FSH preparation and a product with a specific activity that approaches that of rec-FSH and that can also be injected subcutaneously. Two recent studies deal with this topic (7,20) and both concluded that the mean number of oocytes recovered was significantly higher in the rec-FSH group, despite that the treatment duration and number

of 75 IU ampoules were both significantly lower in this treatment group. However, in both studies a 11% treatment difference with respect to the number of patients receiving hCG was observed in favor of rec-FSH (7,20). This was due to a high incidence of canceled cycles because of low ovarian response in the u-FSH-HP group, where also the collection of only one or two oocytes at retrieval was higher than expected (7). Accordingly, a higher proportion of patients had only one embryo replaced in the u-FSH-HP group. Despite this fact, however, there were no significant differences between the rec-FSH and the u-FSH-HP groups in the pregnancy rate per started cycle in the study by either Bergh *et al.* (7) or Frydman *et al.* (20).

In these previously reported studies (7,20) the starting dose for both gonadotropin preparations was 150 IU (2 ampoules) daily during the first 6 days. This dosage may be too low in some patients, mainly those receiving u-FSH-HP, if it definitely proves to be less effective than rec-FSH in inducing multiple follicular development. During the stimulation process, both the timing and the dose of FSH administered determine the number of follicles recruited and selected (21). Follicular recruitment takes place in the late luteal and early follicular phase of the menstrual cycle and each follicle in the cohort has a “threshold” beyond which it is stimulated by FSH to start preovulatory development, otherwise it becomes atretic (22). The so-called intercycle increase in bioactive FSH secretion plays an essential role in this respect in the spontaneous menstrual cycle.

It has been shown that for successful induction of multiple folliculogenesis in normally ovulating women there is a critical period during the early follicular phase of the cycle when FSH values should remain above the physiological level to stimulate follicle recruitment maximally in the primary cohort, and follicles recruited by exogenous FSH require an FSH threshold level that is higher than that in the natural cycle (23). Interestingly, such a threshold level was reached in that study with a single injection of 6 ampoules of u-FSH on cycle day 2 and further growth of the follicles was obtained with extra FSH from cycle day 4 onward at a daily dose of 2 ampoules (23), an approach which mimics that one used in the present study. Therefore, our gonadotropin treatment regimen seems more appropriate than those used in previous IVF studies comparing rec-FSH and u-FSH-HP in order to exceed individual follicular threshold requirements for FSH and thereby stimulate multiple follicular development in most IVF patients.

On the other hand, it is important to emphasize that marked interindividual variation exists in FSH thresholds as well as in FSH metabolic clearance and ovarian sensitivity to FSH (24–26). Thus, from the beginning of IVF it has been known that healthy women with comparable weights and a normal menstrual cycle may react differently to the same dose of gonadotropins. Therefore, as discussed above, the use of the same treatment protocol applied to different drugs in the same patient as done in the present study seems more appropriate to compare ovarian performance and hormonal levels. Thus, a feature of our study is that each woman was her own control for ovarian activity and hormonal changes.

Our results indicate that both gonadotropin preparations are clinically useful but rec-FSH may have a higher biopotency compared with u-FSH-HP. The following facts support this contention. First, the fact that the treatment cycles with u-FSH-HP and rec-FSH in patients in group 1 were almost-identical with respect to days of ovarian stimulation, total dose of FSH used, total number of growing follicles and estradiol levels throughout the stimulation period, number of patients with hCG and ovum retrieval, and number of oocytes retrieved indicates that the stimulation procedures were carried out in a similar way. However, a significantly higher number of potentially mature follicles (≥ 14 mm) was obtained with the use of rec-FSH. Second, the number of follicles ≥ 18 mm in diameter on the hCG day was significantly higher in the rec-FSH cycles. Third, the mean diameter of the two leading follicles on the day of hCG injection was significantly larger with the use of the recombinant preparation. Fourth, more metaphase II oocytes were obtained with rec-FSH treatment.

Finally, the implantation rate was significantly higher in rec-FSH treated cycles in patients in group 1 than in a control group of women undergoing their second IVF and embryo transfer attempt following ovarian stimulation with u-FSH-HP as done in their first IVF treatment cycle. This could be associated with better follicular dynamics and oocyte competence obtained with rec-FSH. The significantly longer interval between IVF and embryo transfer observed in the second IVF treatment cycle cannot explain the results obtained given that this was true for both groups of patients. Furthermore, previous studies showed that pregnancy rates are similar when transferring embryos on either day 2 or day 3 (27,28), a fact which is in agreement with data from our center (unpublished observation).

In summary, this study has demonstrated that rec-FSH is more efficacious than u-FSH-HP when used in the same patient in inducing multiple follicular development in down-regulated cycles as indicated by ovarian performance and oocyte maturity obtained using the same amount of FSH. In addition rec-FSH yields significantly higher implantation rates than u-FSH-HP when used in patients undergoing their second IVF attempt. Further studies appropriately designed comparing both gonadotropin preparations in the same patient are warranted to show the superiority of rec-FSH over u-FSH-HP in terms of pregnancy rates after assisted reproductive techniques. Considering the difference in pregnancy rates between the second treatment cycles in groups 1 and 2 obtained in our study, a sample size of 256 patients would be necessary in order to provide an 80% statistical power of avoiding a type II error and a 5% chance of making a type I error.

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