The Effect of Exogenous Luteinizing Hormone (LH) on Oocyte Viability: Evidence from a Comparative Study Using Recombinant Human Follicle-Stimulating Hormone (FSH) Alone or in Combination with Recombinant LH for Ovarian Stimulation in Pituitary-Suppressed Women Undergoing Assisted Reproduction

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Purpose: *The purpose of this prospective, randomized study was to compare ovarian response and oocyte and embryo yields in women undergoing ovulation induction for IVF/ICSI using recombinant human FSH (rhFSH) alone or in combination with recombinant human LH (rhLH).*
Methods: *Patients were randomized to receive rhFSH alone (group F;* n = *13) or rhFSH + rhLH (group L;* n = *15). rhFSH was administered according to a step-down protocol; patients assigned to group L received rhLH at a fixed dose of 75 IU (1 ampoule) throughout the treatment period.*
Results: *The total dose of rhFSH, number of growing follicles, and serum concentrations of estradiol (E_2) on the day of hCG administration were similar in both treatment groups. However, the percentage of metaphase II oocytes and fertilization rate were significantly higher in group F than in group L. The lower fertilization rates associated with rhLH were also seen in a subgroup of patients from group L who had undergone a previous ART cycle stimulated with FSH only and thus acted as their own controls. However, when in vitro fertilization (IVF) and intracytoplasmic sperm injection cycles were considered separately, differences in fertilization rates were statistically significant only for oocytes treated by conventional IVF.*
Conclusions: *This study shows that the addition of recombinant LH to recombinant FSH in pituitary-suppressed women undergoing ART does not improve the ovarian response and even may have a negative impact on oocyte maturation and fertilization.*

KEY WORDS: IVF; LH; oocyte viability; recombinant LH.

INTRODUCTION

Follicular stimulation regimens in assisted reproductive technologies (ART) usually employ exogenous gonadotropins combined with gonadotropin releasing hormone (GnRH) agonists to prevent spontaneous LH surges and improve the follicular response (1). Human menopausal gonadotropin (hMG) was originally the only preparation available for clinical use. Advances in purification techniques, however, have led to the development of urinary FSH preparations (2). Both hMG and FSH have been found to be effective in inducing follicular growth and maturation. However, the relative importance of FSH and LH in this process is still being investigated, and considerable debate exists as to whether the LH activity

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contained in hMG preparations could affect the outcome of ART treatment in GnRH agonist down-regulated women (3–7).

Treatment with GnRH agonists does not usually result in total inhibition of LH secretion, and it is accepted that less than 1% of LH receptors need to be occupied to elicit a maximal steroidogenic response (8). However, LH concentrations in patients treated with GnRH agonists vary widely; it is possible that there may be a subgroup of patients with low LH concentrations in which ovarian responses and fertilization rates are influenced by these low concentrations during treatment with new urinary FSH preparations containing negligible LH activity (9). This is particularly important since such women cannot be identified in advance (4), the fertilization rate in ART is influenced by the hormonal milieu (10), and the recently available recombinant human FSH (rhFSH) preparations are totally devoid of LH activity (2).

The present pilot study was undertaken to compare the use of rhFSH alone or in combination with recombinant human LH (rhLH) for ovarian stimulation in down-regulated women undergoing ART. In addition to the prospective, randomized comparison, fertilization rates in patients receiving rhFSH plus rhLH were compared retrospectively with those occurring in the same patients in previous ART cycles during which patients were treated with highly purified urinary FSH (FSH-HP) alone.

MATERIALS AND METHODS

Patient Population

A total of 30 consecutive patients with primary infertility from our *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) programs was included in the present study, after approval by our Ethics Committee. All women were premenopausal (age 29–40 years) and were menstruating regularly; all had both ovaries and showed no evidence of occult ovarian failure as judged by a basal FSH concentration below 11 IU/L [Standard International Reference Preparation (IRP) 78/549]. No patient had polycystic ovarian disease. Each patient underwent a complete infertility evaluation, including laparoscopy when necessary and ultrasound examination of the ovaries. No woman had undergone more than two previous ART attempts. All patients provided informed consent to be included in the study.

Protocol

As reported previously (11), in our IVF program ovarian stimulation is routinely accomplished by gonadotropin treatment after pituitary suppression with leuprolide acetate (Procrin; Abbott Laboratories S.A., 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 treatment is continued until the day of administration of human chorionic gonadotropin (hCG).

Gonadotropin stimulation of the ovaries was started when serum estradiol (E_2) concentrations declined to less than 30 pg/ml and a vaginal ultrasound scan showed an absence of follicles above 10 mm in diameter. Patients were allocated to a gonadotropin treatment group according to a computer-generated randomization table. Sealed envelopes for the randomization list were used. Patients in group F ($n = 14$) received s.c. rhFSH (Gonal F; Ares-Serono International S.A., Geneva, Switzerland) alone and patients in group L ($n = 16$) were treated with the combination of s.c. rhFSH and s.c. rhLH (Luveris; Ares-Serono International S.A). In both groups, rhFSH was administered according to a step-down regimen consisting of 450 IU (6 ampoules) on day 1, 300 IU (4 ampoules) on day 2, and 150 IU (2 ampoules) on days 3 to 5. From day 6 onward, rhFSH was administered in both treatment groups according to the ovarian response as objectively assessed by follicular development and E_2 levels. In no case did the ultrasonographer or the hormonal laboratory know the treatment groups in which the patients were included. Patients in group L received a fixed daily dose of 75 IU of rhLH throughout the treatment period. This dose of rhLH was selected because it has been shown to be effective in promoting follicular development in a recent dose-finding study (12).

Sequential transvaginal ultrasonography and serum E_2 measurements were performed from day 6 onward. hCG (5000 IU i.m.; Profasi; Serono S.A.) was administered when a consistent rise in serum E_2 concentration was observed in the presence of two or more follicles greater than 18 mm in diameter.

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 (13); embryos of Veeck grade 1 or 2 were considered high quality. Up to four embryos per patient were replaced and those remaining were

cryopreserved. Luteal-phase support was performed with hCG.

Hormone Analyses and Ultrasonography

Hormone concentrations were measured with commercially available kits. Serum concentrations of FSH were measured by an immunoenzymatic assay with two monoclonal antibodies (Immuno 1, Technicon, Bayer, Tarrytown, NY) and were expressed in terms of IRP 78/549. The sensitivity of the assay was 0.1 IU/L and the interassay coefficient of variation was 2.7%. E_2 concentrations were measured by a competitive immunoenzymatic assay (Immuno 1, Technicon, Bayer). The sensitivity of the assay was 10 pg/ml and the interassay coefficient of variation was 5%.

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. Statistical comparisons were performed by Student's *t* test, Mann–Whitney *U* test, and Fisher's exact test, as appropriate. Significance was assumed at $P < 0.05$.

RESULTS

Of the 30 patients initially included in the study, 1 woman in group F was withdrawn due to failure of leuprolide administration, and 1 woman in group L refused to undergo IVF after being randomized. Thus, a total of 28 patients was included in the analysis, 13 in the F group and 15 in the L group. The demographic and baseline characteristics of the patients were similar in both treatment groups (Table I).

Table I. Main Demographics and Baseline Characteristics of Patients in Groups F and L

Variable	Group F ($n = 13$)	Group L ($n = 15$)	*P*
Age (yr)	33.6 ± 0.8	34.8 ± 0.8	NS
Body mass index (kg/m^2)	22.4 ± 0.9	23.1 ± 0.7	NS
Duration of infertility (yr)	4.7 ± 0.5	5.7 ± 0.9	NS
No. with indicated cause of infertility			NS
Male factor	9 (69%)	8 (53%)	
Minimal/mild endometriosis	2 (15%)	3 (20%)	
Tubal factor	1 (8%)	3 (20%)	
Unexplained	1 (8%)	1 (7%)	
Day 3 FSH level (IU/L)	7.1 ± 0.4	6.9 ± 0.3	NS
No. with ICSI	10 (77%)	8 (53%)	NS

Table II. Ovarian Stimulation Characteristics in the Two Groups of Patients

Variable	Group F ($n = 13$)	Group L ($n = 15$)	*P*
Time for ovarian arrest	14.9 ± 0.6	14.3 ± 0.4	NS
Days of ovarian stimulation	11.5 ± 0.5	11.7 ± 0.3	NS
Total No. of ampoules of FSH	30.5 ± 1.6	32.4 ± 2.0	NS
Patients with hCG and ovum retrieval	13 (100%)	15 (100%)	NS
No. of >10-mm follicles on hCG day	15.9 ± 1.5	13.5 ± 1.3	NS
No. of ≥14-mm follicles on hCG day	13.0 ± 1.2	10.4 ± 0.9	0.09
E_2 (pg/ml) on hCG day	2503 ± 266	2247 ± 246	NS

The ovarian responses in the two groups are summarized in Table II. The time to ovarian arrest, duration of ovarian stimulation, total dose of rhFSH, number of patients receiving hCG and undergoing oocyte retrieval, total number of growing follicles, and serum concentrations of E_2 on the day of hCG administration were similar in both treatment groups. The number of follicles at least 14 mm in diameter on the day of hCG administration was higher in group F, but the difference did not reach statistical significance ($P = 0.09$).

As shown in Table III, a significantly higher percentage of metaphase II oocytes was found after treatment with rhFSH alone. The overall fertilization rate (defined by the presence of 2PN zygotes) was also significantly higher in group F than in group L. There were three patients with complete failure of fertilization and two additional patients with very poor fertilization rates (12.5 and 20%, respectively) in group L. However, when IVF and ICSI cycles were analyzed separately, the difference was statistically significant only for conventional IVF cycles. There were two

Table III. Ovum Retrieval and Outcome of IVF/ICSI in the Two Groups of Patients

Variable	Group F ($n = 13$)	Group L ($n = 15$)	*P*
No. of oocytes retrieved	10.1 ± 1.1	8.4 ± 0.9	NS
Oocytes at metaphase II (%)	84	74	<0.05
Fertilization rate			
Total	74.3%	50.4%	<0.001
IVF	75.0%	37.7%	<0.001
ICSI	74.1%	65.3%	NS
No. of embryos per replacement	3.3 ± 0.1	2.4 ± 0.4	NS
High-quality embryos replaced (%)	60.5	66.6	NS
No. of patients with 0 or 1 embryo replaced	0	5	<0.05

Table IV. Fertilization Rates in Seven Patients from Group L Having a Previous ART Cycle Stimulated with FSH-HP

ART cycles	No. of 2PN zygotes/No. of inseminated or injected oocytes		*P*
	FSH-HP cycle	FSH + LH cycle	
IVF ($n = 3$)	27/31 (87%)	13/23 (57.8%)	<0.01
ICSI ($n = 4$)	16/23 (69.5%)	17/31 (54.8%)	NS
All ($n = 7$)	43/54 (79.6%)	30/54 (55.5%)	<0.01

clinical pregnancies in group F (one of which ended in miscarriage during the first trimester), and no patients in group L became pregnant.

To investigate further the influence of the administered gonadotropin treatment on the fertilization rate, we compared fertilization rates during consecutive IVF/ICSI cycles in seven patients in group L who had undergone previous ART treatment in our program during which ovarian stimulation was performed with FSH-HP (Neo-Fertinorm, Serono S.A., Madrid) (Table IV). Thus, these patients served as their own controls in this analysis. Overall and IVF fertilization rates were significantly higher after treatment with FSH-HP cycles than after rhFSH plus rhLH; in ICSI cycles, the difference between the groups did not reach statistical significance. When data reported in Table IV were restricted to patients having had complete failure or a very poor rate of fertilization with the rhFSH + rhLH treatment, differences between treatment cycles became even more evident (Table V).

DISCUSSION

The optimal ratio of FSH-to-LH activity during ovarian stimulation has been a matter of debate since the early days of gonadotropin therapy (14,15), and recent years have seen renewed interest in this issue for several reasons. First, ovulation-inducing drugs are increasingly being administered to normally ovulating women. Second, purified urinary FSH (with less than 1% LH activity), highly purified FSH (less than 0.1% LH activity), and, more recently, rhFSH (completely devoid of LH activity) are now available. Third, GnRH agonists prevent the untimely LH surge but also suppress endogenous LH activity during the follicular phase. Thus, while the relative importance of LH in the follicular phase and its role in the stimulation of follicular growth and maturation have not been fully elucidated, the possible impact of LH on the outcome of ART has been widely discussed in the recent literature (3–7).

Table V. Fertilization Rates in Four Patients Having Fertilization Failure or a Very Poor Rate of Fertilization in the rhFSH + rhLH-Treated Cycle and Having a Previous ART Cycle Stimulated with FSH-HP

Patient No.	ART	No. of 2PN zygote/No. of inseminated or injected oocytes		*P*
		FSH-HP cycle	FSH + LH cycle	
1	IVF	6/7[a]	0/3	
2	IVF	8/10	1/8	
3	ICSI	3/3	1/5	
4	ICSI	4/6	0/4	
All		21/26 (80.7%)	2/20 (10%)	<0.0001
1 and 2	IVF	14/17 (82.3%)	1/11 (9%)	<0.0001
3 and 4	ICSI	7/9 (78%)	1/9 (11%)	<0.0001

[a] Four embryos replaced and a single term pregnancy.

Experimental and clinical experience indicates that LH is not required for follicular growth, but exogenously administered LH plays a primary role in complete maturation of the follicle and oocyte competence in patients with long-standing hypogonadotropic hypogonadism (16–18). It is not clear, however, whether the resting levels of LH after pituitary suppression with GnRH agonists are sufficient to fulfill these requirements in patients displaying different levels of LH activity and whether these low endogenous LH levels may in some cases amplify any possible differences in outcome during treatment with hMG and FSH preparations. Nevertheless, the idea has persisted that elevated concentrations of LH (whether endogenous or resulting from the use of hMG) during follicular development and in the periovulatory phase may have detrimental effects on oocyte health and subsequent fertilization and implantation rates (3,8). Hence, a number of studies have compared FSH and hMG for ovulation induction in patients undergoing ART (see Refs. 5–7 for review). Several studies did not identify any substantial differences in outcome between different gonadotropin preparations; conversely, other reports suggested that the use of FSH-only preparations may be clinically advantageous, whereas some have suggested that hMG preparations may be superior to those that contain only FSH.

A recent review (5), however, identified four lines of evidence to suggest that treatment with FSH alone is clinically advantageous. First, data from the large database of IVF treatment cycles in France (FIVNAT) showed that FSH use was associated with higher pregnancy rates. Second, the largest randomized trial so far published comparing the two gonadotropins demonstrated higher clinical pregnancy rates with FSH administration, an effect that was confirmed in a meta-analysis of 10 randomized trials.

Interestingly, higher pregnancy rates were observed with FSH irrespective of whether GnRH agonists were used and regardless of the GnRH agonist protocol used. Third, a cumulative meta-analysis indicated that, for IVF treatment, no further comparative study of the two gonadotropins was necessary. Finally, although no major differences in oocyte quality were observed, complete failure of fertilization was more likely with hMG.

The present report represents the first study to investigate the influence of rhLH, given during the whole preovulatory period, on follicular and oocyte maturation and subsequent fertilization in down-regulated women treated with rhFSH. The finding that there tended to be fewer mature follicles on the day of hCG injection in patients receiving rhLH could be related to the intriguing hypothesis recently postulated in hypogonadotropic hypogonadal women treated with rhFSH and rhLH (12) and suggest that a LH ceiling effect may exist, i.e., some secondary follicles undergo atresia due to their high sensitivity to LH (19).

The results of this study also indicate that the use of rhLH may have detrimental effects on oocyte maturation and fertilization. In macaques treated with rhFSH with and without rhLH following 90 days of GnRH antagonist (Antide) treatment, the percentage of metaphase II oocytes and fertilization rate were higher with rhFSH alone (20), which suggests that the addition of rhLH during the preovulatory interval impairs gametogenic events in the periovulatory period. In a subsequent study, however, the same authors suggested that exposure to rhLH may improve embryo viability and implantation rate (21).

The number of pregnancies in the present study was too small to investigate implantation rates. In fact, the study was discontinued because of poor results obtained in group L after the first 30 patients were included in our protocol. In a previous study, rhFSH was administered alone and in combination with rhLH from day 6 onward in patients undergoing ICSI (22). The implantation rate was reduced in a subgroup of patients with serum LH levels above 1.5 IU/L on the day down-regulation was achieved. In that study, as in the present report, the ovarian response was similar in both treatment groups; furthermore, the total dose of rhFSH, number of follicles, oocytes retrieved, and E_2 levels on the day of hCG administration were similar. In that study (22), however, there were no significant differences in oocyte maturation and fertilization rates. A recent study (23) compared the use of FSH-HP alone (17 patients) and in combination with rhLH (14 patients), given under down-regulation conditions similar to ours. As in the present study, rhLH was given at a dose of 75 IU throughout the gonadotropin treatment period and FSH was given according to a step-down protocol. A trend toward lower implantation and clinical pregnancy rates was seen in women receiving rhLH, but no differences in metaphase II oocytes and fertilization rates were observed between the groups. Results with respect to fertilization rates in those previous reports (22,23), where as many as 64% (23) to 100% (22) of patients underwent ICSI, are in agreement with findings in the present study indicating statistically significant differences in fertilization rates only for oocytes treated by conventional IVF. Thus, it is tempting to speculate that ICSI can overcome, at least in part, an apparent defect which may be present in oocytes derived from cycles treated with rhLH.

LH receptors have not been identified in oocytes to date. However, excessive LH may disrupt granulosa cell communication in the cumulus oophorus, which is critical to maintain the oocyte in the late diplotene stage of meiosis until ovulation (7). In addition, recent immunohistochemical studies have demonstrated that the LH receptor is also initially expressed in cumulus cells during follicular development, suggesting that LH might exert an effect throughout the oocyte's growth phase (24). Furthermore, LH plays a fundamental role in androgen production by theca cells from the earliest stages of follicle growth and elaboration of excess androgen secondary to LH has been associated with cell death of both oocytes and granulosa cells (25,26). These findings are consistent with those of a recent study (18) analyzing the *in vitro* maturation of a well-defined class of mouse preantral follicles, in which metaphase II oocytes were obtained only when rhLH, rhFSH, or a combination of both hormones was added to a standard rich culture medium. Theca cells played a fundamental role in follicle survival when rhLH was added as the only supplement, and the addition of this hormone to rhFSH significantly improved the completion of the first meiotic division up the metaphase II stage. Thus, LH apparently creates conditions favoring the oocyte's meiotic maturation, while theca cells play an important role in providing modulators of *in vitro* gonadotropin action. It remains open to speculation, however, whether the improved conditions for completion of meiosis were due to a more appropriate steroid environment enhancing meiosis-activating substances or to other effects concurrent with the effects of LH on follicle differentiation (18).

Interestingly, the highest dose of LH resulted in more frequent gamete degeneration and nonprogression of meiosis than lower LH doses, which suggests that the optimal LH dose might have been exceeded (18). Similarly, it has been stressed that a threshold concentration of rhLH would exist for optimal oocyte fertilization and subsequent embryonic development when rhLH is administered in combination with rhFSH in primates (20).

The above evidence suggests that rhLH might have a potentially deleterious effect on the oocyte when administered from the beginning of ovarian stimulation, as in the present study. However, potential detrimental effects on oocyte maturation, fertilization, and embryo quality with the use of high doses of hMG for multiple follicular recruitment in ART are a matter of controversy (3–7). In this regard, it should be noted that the LH activity of hMG is due mainly to the presence of hCG (27). As a product derived from urine, hCG is associated with problems of variability of source material, quality control, and possible batch-to-batch variation (28). In contrast, like rhFSH (29), rhLH is produced under the most stringent manufacturing conditions. Because the source (Chinese hamster ovary cells) is constant and the manufacturing process is quality assured, rhLH is highly consistent from batch to batch and its bioavailability is markedly increased in comparison with the LH activity of hMG.

In conclusion, the present study suggests that the addition of rhLH to rhFSH from the first day of controlled ovarian hyperstimulation in down-regulated women may result in potentially deleterious effects on follicle and oocyte maturation and fertilization rates. Further comparative studies, including more patients and different rhLH doses, are necessary to confirm these results and to analyze the impact of such effects in terms of pregnancy rates after ART.

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