A Randomized Prospective Cross-Over Study of Highly Purified Follicle-Stimulating Hormone and Human Menopausal Gonadotrophin for Ovarian Hyperstimulation in Women Aged 37–41 Years

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Purpose: In the present study, we investigated the benefits of highly purified FSH (H.P. FSH) in comparison with human menopausal gonadotrophin (hMG) in IVF patients aged 37–41 years.

Methods: Twenty patients experienced within a period of four months both preparations in subsequent cycles through a prospective randomized cross-over design. A standard hormonal treatment consisting of a flare-up protocol using gonadotrophin-releasing hormone agonist (GnRHa) in combination with the same starting dose of H.P. FSH or hMG was used in all cycles.

Results: Cycles stimulated with H.P. FSH resulted in a significantly higher mean number (10.3 ± 3) of oocytes retrieved with a significantly shorter (13 ± 2.3 days) duration of stimulation compared to cycles treated with hMG [mean number of oocytes 7.3 ± 5 ($P < 0.01$) and mean duration of stimulation 14.7 ± 3.9 days ($P < 0.02$)]. No detrimental effect of basal or exogenous LH on oocyte quality was observed in our study.

Conclusions: In our experience, H.P. FSH preparations seemed to be more effective than hMG preparations for ovarian stimulation in this group of women aged 37–41 years.

KEY WORDS: cross-over; FSH; hMG; IVF.

INTRODUCTION

Whatever types of gonadotrophins are used for ovarian stimulation, their mechanisms and sites of action are the same. All have proven effective in inducing multiple follicular development and growth before ovocyte collection for in vitro fertilization.

The hMG and FSH preparations contain similar amounts of FSH and LH only in the first preparation. Consequently, the value of exogenous LH administered in such preparations has been the subject of much debate. Too much circulating LH during the follicular phase has been linked to a detrimental effect on fertilization, cleavage, and embryo quality (1,2). Raised LH levels have also been associated with lower conception rates and a high rate of spontaneous abortion (3,4). To overcome the possible interference of unbalanced and/or untimely endogenous gonadotrophin secretion, combined therapy using agents suppressing hypothalamic-pituitary function together with gonadotrophins has been recommended (5,6,7). A higher incidence of poor ovarian response has been observed with advanced maternal age (6) and the flare-up short protocol has been advocated for such patients (8). As maternal gamete characteristics are clearly affected by advancing age (9,10), exogenous administration of LH during ovarian stimulation with a short protocol using hMG might be deleterious on such gametes in these patients. Highly purified follicle-stimulating hormone (H.P. FSH) derived from human urine is now available for ovarian hyperstimulation before IVF and has been used in randomized comparative studies (11). Its specific bioactivity is much higher compared to the classically available urinary gonadotrophins (12). The H.P. FSH preparation has no LH activity and its use during ovarian hyperstimulation avoids exogenous LH administration used with hMG preparations.

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The aim of this study was to compare the ovarian response using GnRHa/H.P. FSH and GnRHa/hMG stimulation regimens in the same women. Through a prospective, randomized, cross-over study, 20 patients aged between 37 and 41 years experienced, in subsequent cycles, the one and the other treatment for ovarian hyperstimulation before IVF. As the effect of aging on LH levels has been linked to poor gamete quality, we choose this group of patients in order to evaluate if they could benefit from the highly purified FSH preparation. From this point of view, the cross-over design was the most accurate way to determine how each gonadotrophin preparation would act on the same patient. Our main end-points were the comparison of the results of stimulation parameters (i.e., day of hCG administration, the total dose of gonadotrophin used, the E₂ levels on the day of hCG administration, the LH levels before and after gonadotrophin administration, the total number of oocytes retrieved) and embryo quality for each regimen of treatment.

MATERIALS AND METHODS

Twenty-seven patients undergoing their first IVF cycle were randomized for this trial. The Ethical Committee of St. Luc's University Hospital approved the study.

Inclusion criteria were patients aged between 37 and 41 years, normal cycles Day 3 FSH levels defined in our Laboratory as 8 mIU/ml, the presence of both ovaries, a body mass index between 18 and 28, and a cycle length between 26–32 days. The cut-off FSH value of 8 mIU/ml was established to have a homogeneous group in order to avoid poor responders in this age group.

Only 20 patients subsequently completed the two regimens of treatment within a period of four months. Three patients were excluded because of the occurrence of pregnancy during the first cycle, and they were unable to enter the cross-over arm. Two patients (one in each treatment group) ended without ovocyte retrieval because of the absence of ovarian response to gonadotrophin therapy. Two patients were excluded because they did not wish to participate in the study in their subsequent cycles.

Study Design

This was a prospective, randomized, cross-over study. Two protocols of treatment using GnRHa with either highly purified FSH (Metrodin HP®, Serono,

Geneva, Switzerland) or hMG (Pergonal®, Serono, Geneva, Switzerland) were compared during ovarian stimulation for IVF in women aged 37–41 years. After they had given their informed consent, patients starting their first IVF attempt were allocated randomly to either GnRHa/hMG or GnRHa/FSH treatment. In the absence of pregnancy, the patients were allocated to the other arm of treatment for a subsequent cycle.

Hormonal Treatment

A short version of combined GnRHa therapy was used. Women began GnRHa on the first day of the cycle in the form of Buserelin nasal spray, 900 µg/24 h (Suprefact®, Hoechst, Brussels, Belgium) and continued this treatment up to the day of hCG administration. On the third day of the cycle, administration of 300 IU of gonadotrophins (hMG or FSH according to randomization) was commenced for 3 days. The hMG preparation was administered intramuscularly and the H.P. FSH preparation subcutaneously as recommended by the manufacturer. Vaginal ultrasound with serum estradiol level measurement was initiated from the sixth day, and gonadotrophin doses were adapted according to the ovarian response. Human chorionic gonadotrophin was administered (10,000 IU)(Profasi®, Serono, Geneva, Switzerland) in the presence of more than 2 follicles over 18 mm in diameter with an estradiol level of 150 pg/ml per follicle over 15 mm in diameter. The criteria for hCG administration were the same in both treatment groups.

Ovocyte Retrieval, In Vitro Culture, and Embryo Evaluation

Thirty-five hr after hCG injection, oocytes were retrieved by transvaginal ultrasound guidance. Aspirated oocytes were cultured in IVF medium (Medicult, Copenhagen, Denmark) in air containing 5% CO₂ and 100% humidity. Oocytes were inseminated 3 hr after retrieval either by adding 100,000 spermatozoa to the culture medium or by ICSI if indicated. Each oocyte was checked for fertilization 18 hr after insemination and then transferred to fresh IVF medium. Forty-eight hr after retrieval, the developmental stage of the embryo was checked under an inverted microscope and scored according to the classification previously reported (13).

Embryo Transfer and Luteal Phase Support

After embryo evaluation, patients were given the choice between 2 or 3 embryos for transfer. The trans-

fer was performed in the gynecological position using a Frydman catheter (CCD, Paris, France). Each patient was given luteal support in the form of 30 mg of dydrogesterone (Duphaston®, Duphar, Brussels, Belgium) daily.

Statistical Analysis

Statistical analysis was carried out using the paired student *t* test to calculate the mean and the χ^2 Fisher exact test (small number of patients) for percentages. A *P* value < 0.05 was considered significant. The end-points of the study were the comparison of stimulation parameters, the total dose of gonadotrophins administered, the number of oocytes recovered, the fertilization rate observed, and the quality of embryos obtained.

RESULTS

Twenty-seven patients were randomized for this trial. The analysis represents data from 20 patients who completed either the first or the second regimen of treatment. Indeed, seven patients were excluded either because of the occurrence of pregnancy during the first cycle (*N* = 3), or the absence of ovarian response (*N* = 2) or drop-out from the study between the first and second cycles (*N* = 2). The first group was allocated to purified urinary FSH treatment (group 1: GnRHa/Metrodin HP treatment) and the second group to hMG treatment (group 2: GnRHa/hMG treatment). Forty cycles were included in the statistical analysis. The overall clinical pregnancy rate was 20%/cycle (8 pregnancies/40 cycles) and 40%/patient (8/20 patients, respectively).

The mean age of the patients was 37.6 ± 2.5 years (range: 37–41). The stimulation parameters and IVF outcome results are listed in Table I.

Cycles stimulated with H.P. FSH showed a significantly (*P* = 0.01) shorter length of stimulation (13 days) than cycles treated with hMG. The mean number of oocytes retrieved per cycle was 10.3 ± 3 and 7.3 ± 5 for the first and second groups respectively. This difference was statistically significant (*P* = 0.02). In group 1, the mean LH level on Day 3 before gonadotrophin administration was higher than in the second group. This level remained higher on the sixth day in the same group and it reached a significant level (*P* < 0.03). The mean total number of embryos replaced was similar in the two groups.

Patients treated with H.P. FSH presented with a higher total number of embryos and a higher number of good quality embryos available for transfer. The results are detailed in Table II. In the first group, 73.6% of embryos available were of excellent or good quality, compared to 58% in the second group.

Out of 34 embryos replaced in group 1, 8 implanted compared to 3 out of the 34 placed in group 2. This represents an implantation rate of 23.5% for patients treated with H.P. FSH compared to 9% for those treated with hMG. However, this difference was not statistically significant (*P* = 0.09).

In the first and second groups, 13 cycles ended in embryo transfer. The clinical pregnancy rates according to each group are listed in Table 3. In 65% of cycles, an embryo transfer was possible in each group; however, in the group treated with H.P. FSH, 57 embryos were available for transfer compared to only 43 in the group treated with hMG. The pregnancy rate/ET was respectively 46% and 15% in the first and the second groups and the pregnancy rate per cycle was respectively 30% and 10%. This difference, though statistically significant, could not be taken into consideration because of the cross-over study design. Pregnancies obtained during first cycles need to be added. This gives a total number of 21 cycles in the H.P. FSH group with 14

Table I. Results of the Stimulation Parameters in Each Group

	Group 1 (Met.HP) nb = 20	Group 2 (hMG) nb = 20	Paired <i>t</i> test <i>P</i>
Day of hCG administration	13 ± 2.7^a	14.7 ± 3.9	0.01
Total dose of gonadotrophins (nb amp.)	40.9 ± 4.2	50 ± 6.2	0.08
E ₂ on the day of hCG administration (pg/ml)	2299 ± 1.586	1860 ± 1.127	0.2
E ₂ per oocyte retrieved (pg/ml)	226.6 ± 89	332 ± 213	0.055
LH Day 3 (before gonadotrophin administration) (mIU/ml)	4.3 ± 0.7	3.3 ± 0.6	0.1
LH Day 6 (mIU/ml)	3.1 ± 0.4	2.1 ± 0.35	0.03
Total number of oocytes	10.3 ± 5	7.3 ± 5.6	0.02
Total number of embryos	3.3 ± 3	2.4 ± 2	ns

^a Results are expressed as mean $\pm$ SD.

Table II. Embryo Quality and Implantation Rates in Each Group

	Group 1 (Met. HP) N = 20	Group 2 (hMG) N = 20	X ² test (Fisher exact test) P
Total nb of embryos obtained (nb) ^a	57	43	ns
Grade I, II embryos replaced (nb) ^a (%)	42 (73.6%)	25 (58%)	ns
Grade III embryos replaced (nb) ^a (%)	15 (26.3%)	18 (42%)	ns
Gestational sacs (nb) ^a	8	3	ns
Total nb of embryos transferred ^a	34	34	ns
Implantation rates (nb) ^a (%)	8/34 (23.5%)	3/34 (9%)	0.09

^a nb = number.

transfers and 22 cycles in the hMG group with 15 transfers. Thus, the clinical pregnancy rates were 7 out of 14 transfers (50%) and 7 out of 21 cycles (33.3%) in the first group, compared to 4 out of 15 transfers (26%) and 4 out of 22 cycles (18%) in the second group. These differences were not statistically significant.

DISCUSSION

Many investigators have compared FSH and hMG and concluded that no differences exist (14,15,16). Recently, Daya *et al.* (17) reported that previous conclusions may have been premature and suggested, through their meta-analysis of randomized studies, that FSH is associated with a higher clinical pregnancy rate. The present study is different from previous ones because each patient experienced both gonadotrophin treatments at the same dose and within an interval of 4 months. Also, in order to determine whether there is any benefit from the use of purified FSH, we selected patients aged 37–41 years with normal FSH levels on cycle Day 3.

The use of highly purified urinary FSH was associated with a significantly shorter stimulation length (13

days) and a higher mean number of oocytes recovered (10.3 ± 5) after puncture compared to cycles treated with hMG. The mean total dose of FSH administered per cycle was lower in the first group (3.067 IU) than the total dose of hMG used in the second group (3.750 IU). These results are in accordance with the results of parallel randomized studies on patients treated with H.P. FSH/hMG after down regulation (2,17,18,19). However, in our study, the difference did not reach significant levels. This could be a result of the small number of cycles. The fertilization rates per oocyte retrieved were similar in the two groups and nontransferred cycles with failures of fertilization were mainly associated with male factors and were not associated with high LH levels. Several studies have demonstrated that high LH concentrations predispose patients to lower fertilization rates (1,7,20,21). It has been suggested that higher LH concentrations may prematurely allow resumption of meiotic maturation (22) inducing a recovery of postmature oocytes that would probably fail to fertilize. This effect was not observed in our study. The LH serum levels on Day 3 and Day 6 of the cycle were determined during each treatment in the same woman. As shown in Table I, LH concentrations were similar on Day 3 but, surprisingly, they

Table III. Results of IVF Outcome According to Each Regimen of Treatment

	Group 1 (Met.HP) N = 20	Group 2(hMG) N = 20	χ ² test P
Nb of cycles with transfer (%)	13 (65%)	13 (65%)	ns
Total number of embryos	57	43	ns
Clinical pregnancies (cross-over study)	6	2	
	46%/transfer 30%/cycle	15.4%/transfer 10%/cycle	
Clinical pregnancies (first cycle)	1	2	
Total of clinical pregnancies ^a	7/14	4/15	
	50%/transfer 33.3%/cycle	26.6%/transfer 18%/cycle	

^a This takes into consideration the results of first cycles, the number thus rising to 21 cycles and 14 transfers for Group 1 and 22 cycles and 15 transfers for Group 2.

were higher on Day 6 in cycles treated with purified urinary FSH. This difference, though statistically significant, should be interpreted with caution. Patients in group 1 had a higher mean concentration of LH before gonadotrophin administration. Also, the difference observed between the two groups in mean LH concentrations on Day 6 ($\Delta\text{LH Day 6} = 1 \pm 0.05$) was similar to the difference observed on Day 3 ($\Delta\text{LH Day 3} = 1 \pm 0.01$). In addition, the significantly higher mean LH concentrations found in H.P. FSH-treated patients may suggest that exogenous LH in hMG preparations is not detected in blood circulation with our dosage technique or that these LH amounts were not sufficient to influence endogenous secretion and so were not detected in blood circulation. Also, the endogenous pulses of LH vary considerably and contribute to the total amount of LH present during follicular development. This endogenous effect may explain why the same patients had a different LH level in each cycle and reflect an intercycle variation (23). In a recent report, Westergaard *et al.* (16) did not find any detrimental effect of the LH activity of hMG on the clinical outcome of IVF in GnRHa down-regulated women. Even if an LH effect could not be ruled out in our study because patients were not down-regulated, the similar number of embryos obtained per ovocyte inseminated strongly suggests the absence of such a detrimental effect. This was also demonstrated in the study by Westergaard *et al.* (16) and Fleming *et al.* (21). On the other hand, in a randomized, prospective study, Daya *et al.* (2,17) compared the effects of FSH (Metrodin) and hMG in IVF and found, in contrast to the present results, a statistically higher fertilization rate in patients treated with FSH compared to hMG. They concluded that the LH activity of hMG accounted for the proper results in the hMG group compared to the FSH group. This is at odds with our results, and we believe that the small amount of LH present in hMG preparations may not influence the fertilization process.

The beneficial effect of FSH over hMG was only visible on the duration of stimulation and on follicular recruitment. The explanation of such an effect, in theory at least, may be related to the injection site and biological properties of the H.P. FSH preparation. The FSH isoforms in Metrodin HP are more acidic than other urinary gonadotrophin preparations (20). Acidic isoforms have a longer half-life than basic FSH isoforms. It might be expected that such acidic forms stay longer and accumulate to higher concentrations in serum than do other urinary gonadotrophins. Such a biological profile may sustain the growth of a larger

number of developing follicles. The higher number of oocytes obtained in cycles treated with H.P. FSH compared to cycles treated with hMG may explain the higher number of embryos of good quality obtained in the H.P. FSH-treated group, giving the possibility of transferring better embryos which may lead to a higher pregnancy rate. Staessen *et al.* (24) and, later, our group (13) demonstrated that the number of embryos transferred correlated better with the pregnancy rate when it was possible to make the selection from a larger cohort of embryos available for placement (13).

In our study, patients between 37 and 41 years of age ended with a higher number of available oocytes and embryos of good quality in cycles treated with H.P. FSH compared to cycles treated with hMG. This is associated with a better prognosis for IVF outcome. Taking into consideration only patients who completed the two regimens of treatment, this improved prognosis was reflected in the higher pregnancy rate obtained in H.P. FSH-treated cycles. However, for accurate statistical analysis, we added the pregnancies obtained during the first cycle in each group, and these data were analyzed as in a parallel randomized study. Using this method, the pregnancy rates obtained were not statistically significant in the two groups.

One might consider the cross-over study design as a factor of overestimation for one or the other treatment (25). In our study, this could be ruled out as pregnancy outcome was not the main point of comparison. The end-points of the study were to compare the parameters that could be directly affected by such treatment, such as the duration of stimulation, the number of ovocytes recovered, and the embryo quality in relation to LH activity. Indeed, Te Velde and Looman (26) recently ruled out the claims made by Khan *et al.* (25) and concluded in favor of a cross-over design for infertility studies. One of the limitations of our study may be the number of cycles included and further investigations may be necessary to confirm our results. However, in women aged 37–41 years, with normal basal Day 3 FSH levels, normal ovulation, and a BMI <28 undergoing IVF treatment, the use of H.P. FSH for ovarian stimulation may be the best choice to induce a good ovarian response.

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