

Urinary follicle-stimulating hormone (FSH) is more effective than recombinant FSH in older women in a controlled randomized study

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Objective: The following study was conducted to determine which FSH, recombinant or urinary, works better in older women.

Design: We conducted a controlled randomized study in a single university IVF center.

Setting: University IVF center.

Patient(s): Women (N = 257) over 39 years old undergoing IVF.

Intervention(s): The patients were randomized into two study groups at their first IVF cycle: 121 patients were treated with recombinant FSH, and 120 patients were treated with urinary FSH. Both groups were suppressed with a long GnRH analog protocol.

Main Outcome Measure(s): Days of stimulation, E₂ at the day of hCG, total amount of FSH administered, number of oocytes collected, amount of FSH per oocyte, and number of embryos obtained.

Result(s): Patients treated with urinary FSH required a significantly lower total amount of FSH, and a lower amount of FSH per oocyte than women treated with recombinant FSH. The other measures evaluated did not show any statistically significant differences.

Conclusion(s): Our study showed that urinary FSH performed better in older women than recombinant FSH when associated with the long protocol. (Fertil Steril® 2006;85:1398–403. ©2006 by American Society for Reproductive Medicine.)

Key Words: Urinary FSH, recombinant FSH, IVF outcome, controlled ovarian hyperstimulation

The first pregnancy resulting from IVF was obtained from a natural (unstimulated) cycle (1), but since then several protocols of controlled ovarian hyperstimulation (COH) have been used. Different drugs, such as clomiphene citrate, have been used to obtain superovulation to harvest a larger number of oocytes, as well as human menopausal gonadotropins, urinary FSH (uFSH), and highly purified FSH other than the GnRH analogs for pituitary desensitization (2–4).

The use of GnRH analog plus gonadotropins for COH has gained widespread popularity, because better results have been achieved using this treatment in terms of number of oocytes collected, number of embryos obtained, and pregnancy rate (5). Recently recombinant gonadotropins have

been introduced for the treatment of infertility and several authors have claimed that recombinant FSH (rFSH) had better results in COH in terms of pregnancy rate, oocyte quality and severe ovarian hyperstimulation syndrome (OHSS) rate compared with uFSH (6–12).

However, from the time that rFSH was introduced in 1996, many studies have been performed to compare urinary and recombinant FSH, but no univocal results have been reached (6–24). Furthermore, several meta-analyses have been published with contradicting conclusions (25, 26). These different results may be due to different patient selection, protocol of pituitary suppression, route of administration, dose of gonadotropin, or study design.

The number of childbearing women in their advanced reproductive age (over 39 years old) is increasing; they are a challenge for clinicians, because these women are potentially low responders to controlled ovarian hyperstimulation and have a poor reproductive outcome. It has been reported that in COH for IVF the frequency of poor responder women,

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estimated to be 10% in the general population, is significantly higher in patients who are 40 years or older (27, 28).

Despite the large number of papers published on COH protocols comparing rFSH to uFSH, there is a lack of data on women with a reduced ovarian reserve due to the women's age or other coexisting problems. To our knowledge, only two studies have reported data on poor responder women (29, 30). These two papers reported data on small samples of young patients (less than 37 years) who were poor responders in a previous IVF cycle; both studies showed that rFSH worked better than uFSH in terms of FSH amounts used, and the pregnancy rates were similar.

In order to evaluate the effectiveness of urinary and recombinant gonadotropins in women in their late reproductive age, we performed a controlled study comparing uFSH and rFSH in patients older than 39 years undergoing their first IVF cycle.

MATERIALS AND METHODS

Patient Selection

All patients older than 39 years referred to the IVF program of Department of Obstetrics and Gynecology, Policlinico Umberto I Hospital, Università "La Sapienza," Rome, Italy, to undergo their IVF first cycle from January 2001 to December 2004 were eligible for the study. The trial was designed according to the Consolidated Standards of Reporting Trials guidelines.

The study was reviewed and approved by the institutional review board. Of the 298 eligible patients undergoing IVF during the study period, 257 agreed to participate. The patients were randomized by means of a computer-generated randomization number sequence at the time that their cycle was scheduled. All patients undergoing IVF and participating in the study gave their informed consent. All patients were nulliparous who underwent a standard infertility evaluation, and none of the patients eligible for the study showed FSH >10 IU/mL or E₂ >60 ng/mL on cycle day 3.

Women with polycystic ovaries were excluded from the study, because these women often respond unpredictably, with an increased risk of hyperstimulation or a low response and bad-quality oocytes. All basal FSH assays were done in the same laboratory using the same RIA kit assay (OCFF07-FSH RIAGnost; CIS Bio-International, Milan, Italy).

Patients were randomly allocated to two study groups: group A: 129 patients treated with rFSH (Gonal-F; Serono, Rome Italy); and group B: 128 patients treated with purified uFSH (Fostimon; AMSA, Rome, Italy).

Procedure

Patients of both groups were treated with a long pituitary suppression protocol using GnRH analog buserelin, 0.4 mg subcutaneous daily, on days 22 to 24 of their previous cycle. Ovarian suppression was assessed by daily hormonal profiles

of E₂ and ultrasound (US) scan of the ovaries every day. Suppression was confirmed when E₂ reached the level of <30 pg/mL and no follicles with a dimension of ≥12 mm mean diameter were visible on US examination. When suppression was confirmed by E₂ and US examinations, 300 IU rFSH or uFSH was commenced on the second day of the menstrual cycle in the long protocol.

From the seventh day of stimulation in both groups, daily monitoring of follicle size by US was performed, and plasma levels of E₂ was measured. From this stage, the dose of FSH was adjusted dependent on the individual response of each patient. The criteria used for triggering ovulation with 10,000 IU hCG (Gonasi HP 5000; AMSA) IM were plasma E₂ between 1,000 and 4,500 pg/mL and at least four follicles >16 mm mean diameter (two perpendicular measurements). The cycle was canceled in case of poor ovarian response, with less than three follicles observed on the ninth day, or in case of ovarian hyperstimulation syndrome with E₂ >4,500 pg/mL.

Oocyte retrieval was performed under US guidance by the transvaginal route on day 0, 36 h after the injection of hCG, and oocytes were observed 18 h after insemination for their pronuclei and 44 hours after insemination for embryo development.

The embryos obtained were categorized on day 3 into three categories, depending on their morphologic appearance. Grade A had a number of 6–8 or more equal and regular blastomeres without the presence of cytoplasm fragments; Grade B had fewer than 6–8 unequal blastomeres with or without cytoplasmic fragments; Grade C were fragmented (more than 50%) embryos (31).

Embryos were transferred about 72 h after insemination using the Wallace embryo transfer catheter (H. G. Wallace, Hythe, Kent, UK). The policy of our clinic is to transfer no more than three embryos (preferably of the best quality). All transfer procedures were performed by the same physician to avoid interoperator variability. All pregnancies were confirmed by a rising titer of serum β-hCG 12 days after embryo transfer and US demonstration of the gestation sac 4 weeks after the transfer. Biochemical pregnancies alone have not been included.

The same luteal phase support was used in both groups: 50 mg IM daily progesterone (Prontogest; AMSA) from the day of replacement.

Statistical Analysis

All statistical analyses were performed using the SPSS statistical package (SPSS, Chicago, IL). The amount of FSH used and rate of FSH/oocyte were the primary outcomes, and secondary outcomes were days of stimulation, E₂ at the day of hCG, number of oocytes collected, number of embryos obtained, number of embryos transferred, pregnancy rate, implantation rate, and abortion rate. The primary and sec-

TABLE 1**Demographic data of patients treated with recombinant FSH (rFSH) or urinary FSH (uFSH).**

	rFSH	uFSH	P
Age (y)	40.9 ± 1.6 (39–43)	41.3 ± 1.3 (39–43)	NS
Body mass index (kg/m ²)	25.4 ± 2.6	24.7 ± 2.4	NS
Duration of sterility (yrs)	4.1 ± 1.1	3.9 ± 1.4	NS
Cause of sterility			
Male factor	24.7%	26.1%	NS
Tubal factor	35.6%	34.8%	NS
Endometriosis	18.5%	16.9%	NS
Idiopathic	16.4%	15.3%	NS
Other	4.8%	6.9%	NS

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secondary outcomes were chosen on the basis of our previous experience (32) and literature data (28–30) on these patients for expected pregnancy rate, implantation rate, fertilization rate, number of oocytes harvested, etc.

Mann Whitney U test and Student *t* test were used for continuous variables (days of stimulation, E₂ at the day of hCG, amount of FSH administered, number of oocytes collected, number of embryos obtained, and number of embryos transferred), and Fisher exact test was used for dichotomous variables (clinical pregnancy rate per cycle started, implantation rate, and abortion rate).

RESULTS

The demographic data of patient groups are reported in Table 1. No statistically significant differences were found

for patients' age, body mass index, time of infertility, causes of infertility, etc.

The cycles were canceled in 11 cases owing to poor ovarian response observed from the beginning of monitoring in five cases in group A and six in group B and in 5 cases because of ovarian hyperstimulation syndrome, 2 in group A and 3 in group B. A total of 121 cycles were analyzed for rFSH and a total of 120 cycles for uFSH.

Table 2 describes the results of the comparison between rFSH and uFSH. Patients treated with rFSH used statistically significantly more units of FSH than patients treated with uFSH (*P* < .001). Days of stimulation, E₂ levels at the HCG day, and number of oocytes retrieved did not show statistically significant differences. No relevant differences were observed among the range of ages for FSH requirement (data

TABLE 2**Results of comparison between recombinant FSH (rFSH) and urinary FSH (uFSH) in women >39 years old.**

Parameters	rFSH	uFSH	P
No. of cycles	121	120	
Basal FSH (IU/mL)	7.8 ± 1.1	7.4 ± 1.3	NS
Basal E ₂ (pg/mL)	41.3 ± 9.7	42.4 ± 8.8	NS
Days of stimulation	14.0 ± 1.5	13.3 ± 2.5	NS
Amount of FSH used (IU)	5,533 ± 2,398	3,213 ± 1,527	<.001
E ₂ at hCG day (pg/mL)	2,444 ± 1,117	2,265 ± 915	NS
Oocytes retrieved	6.8 ± 3.2	6.2 ± 2.8	NS
FSH used/oocyte (IU/oocyte)	1,146 ± 946	608 ± 357	<.01
Total embryos transferred	278	240	NS
No. of embryos transferred	2.3 ± 1.3	2.0 ± 1.2	NS
Pregnancy rate	17.3%	19.2%	NS
Implantation rate	8.6%	10.4%	NS
Abortion rate	14.3%	17.4%	NS

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not shown). No patients failed to make the second cutoff after they had made the first. At the beginning of monitoring, on day 7 of cycle, no appreciable differences were observed between the two treatment groups. The amount of FSH per oocyte retrieved was significantly higher in women treated with rFSH than in women treated with uFSH ($P < .01$). The other parameters evaluated—number of embryos transferred, pregnancy rate, implantation rate, and abortion rate—were not shown to be statistically significant.

The study showed a power of 0.82 both for the units of gonadotropins used and for the ratio oocyte/unit of FSH used. The values of P were corrected, by dividing its value by the number of comparisons performed.

DISCUSSION

In the literature there are several reports of trials comparing rFSH and urinary gonadotropins, but this remains a controversial issue and gonadotropin should be preferred for IVF patients owing to the inconclusive data of these studies (6–24). Even the use of meta-analysis could not give a clear answer; two recently published meta-analyses comparing these gonadotropin preparations showed contradictory results: Daya (25) showed that rFSH worked better than uFSH in terms of pregnancy rate, and van Wely et al. (26) showed the opposite. These differences may be due to the heterogeneity of patients included in the analysis, their age, type of GnRH analog suppressions, FSH doses, etc.

In our study, to overcome this bias we selected a group of patients with homogeneous age and clinical characteristics, treated with the same protocol of GnRH analog suppression—the long protocol with daily subcutaneous busserelin, because in a previous study we observed that the long protocol worked better than the short one in older women (32)—and the same starting FSH dose in both groups.

Furthermore we chose the total FSH amount and the ratio amount of FSH/oocyte as the primary outcomes, because the small differences expected in pregnancy rate and implantation rate between the groups of older or poor responder women, as observed in most published studies, would require a much higher number of patients to reach statistical significance. It is worth emphasizing that pregnancy rate and implantation rate did not show any statistically significant differences, as with most of the trials on this issue.

In our study, we observed that older women treated with uFSH used smaller amounts of gonadotropins during ovarian stimulation compared to the rFSH group, obtaining similar number of oocytes with statistically significant differences in the ratio of FSH administered for number of oocytes. In light of these results uFSH seems to be more effective in women older than 39 years compared to rFSH, at least when the long protocol is used. Previously, only two papers have compared uFSH and rFSH in poor responder patients (29, 30), both showing that rFSH performed better than uFSH for the number of oocytes retrieved and the amount of gonadotropins used. However,

these studies included young women, the sample sizes were very small, and one was not a controlled study (30).

In the previous studies claiming a better performance for recombinant gonadotropins, it has been suggested that rFSH containing more relatively basic isoforms (33) allows a better interaction between FSH and its receptor, because basic isoforms are known to have a higher receptor affinity than acidic isoforms in an *in vitro* study (34). However, other authors showed that uFSH has a longer half-life with a decreased clearance rate due to the higher moieties of sialic acid (35). On the other hand it has been reported that rFSH bioactivity tends to be higher than uFSH, whereas the immunoreactivities display an opposite trend (36). Moreover, during a normal menstrual cycle more FSH basic isoforms, with lower sialic acid moieties, are produced in the preovulatory phase, when higher estrogen levels are present (37).

Our study showed that in women with an advanced reproductive age the uFSH performed better than rFSH in terms of total ampoules used per cycle and ampoules needed per oocyte; these results may be explained by the fact that in urinary formulation there are several isoforms of the FSH protein present with relatively higher acidic isoforms which show a decreased clearance rate and are produced when lower estrogen levels are present, such as in the early follicular and late luteal phase when primordial follicles are recruited.

Women in their late reproductive age show a shorter menstrual cycle due to a reduced follicular phase; this has been shown to depend on an earlier start of follicular growth, defined as “advanced growth,” (38–40). Therefore in older patients it has been demonstrated that the available cohort of antral follicles starting the growth in each cycle is significantly smaller than in younger women (39). In older women, the recruitment of a larger follicle cohort is the primary problem in order to achieve a higher number of oocytes, and the use of uFSH could help in the follicle recruitment.

The reduction of the amount of FSH used in the COH also led to a reduction in the cost of the IVF cycle and of the babies born; also considering the differences in cost of the two preparations—0.22 euro (\$0.27) per IU for uFSH versus 0.59 euro (\$0.70) per IU for rFSH—there is a significant saving for the public health system, although this was not an issue in our study.

In conclusion, our study shows that uFSH should be preferred in older women undergoing COH because it allows the use of smaller amounts of gonadotropins with similar results for the number of oocytes retrieved, embryos obtained, and pregnancy rate with a more favorable cost-effective ratio than rFSH. Further studies are needed to confirm these data and to establish the best protocol for different groups of patients.

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