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ORIGINAL ARTICLE

Sequential protocol with urinary-FSH/recombinant-FSH versus standard protocol with recombinant-FSH in women of advanced age undergoing IVF

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Abstract

A stimulation protocol mimicking the physiological pattern of FSH release may improve IVF outcome in women of advanced age. Urinary-FSH delivers a wider range of isoforms including the most acidic produced during the early follicular phase when oestradiol level is low, a common condition in women of advanced reproductive age. We hypothesized that a stimulation protocol using urinary-FSH during the early follicular phase and then shifting toward recombinant-FSH may improve oocyte quality and pregnancy rate in 35–40 years old patients in IVF program. A retrospective study was performed: after a standard down-regulation with GnRH-analogue, 115 women underwent stimulation with urinary-FSH for 6 days according to a step-down approach and then shifting to recombinant-FSH (group A), 115 women underwent a stimulation protocol with only recombinant-FSH (group B). Days of stimulation were lower in group A than in group B, a higher proportion of MII oocytes and of grade 1 embryos, higher implantation rate and pregnancy rate were observed in group A versus group B. We conclude that a sequential protocol using urinary-FSH in the early days of stimulation and subsequently recombinant-FSH may improve the IVF outcome in patients of advanced reproductive age.

Keywords

Advanced reproductive age, ART, FSH, infertility, oocyte quality

History

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Introduction

FSH, the pivotal hormone in folliculogenesis, has a complex structure with two proteic subunits, alpha and beta, carrying 4 sites of N-glycosylation. Each of the four sugar chains may exert a variable level of branching (0–4 branches), each branch being typically closed with a sialic acid residue that stabilizes the structure. Inasmuch, a higher degree of glycosylation implies a larger amount of sialic acid per molecule and a more acidic point of isoelectricity of the protein. Thus, FSH molecules are available as different isoforms according to their acidity and a higher acidity/sialic acid content is associated with a longer half-life *in vivo* [1].

The relative and absolute FSH isoform concentration varies throughout the ovarian cycle [2] as a response to feedback mechanism of oestradiol and inhibin from the dominant follicle [3]. Acidic FSH isoforms with longer half-life are produced during the Luteal-follicular transition and the early follicular phase when the oestradiol serum level is low; less acidic FSH isoforms are produced during the mid-cycle when the oestradiol level is high [1,4]. Such shift towards the less acidic FSH molecules in the mid-cycle and in the preovulatory phases may be a relevant regulating mechanism of FSH action during the final steps of follicular maturation [5]. Thereafter, the occurrence of short-living FSH molecules at the time of ovulation may make more rapid the clearance of FSH from the blood [6] when shifting to the luteal phase.

The available commercial gonadotrophin drug products are derived either from human urine purification (uFSH) or from recombinant technology (rFSH): uFSH delivers a wider range of isoforms including the most acidic ones, whereas rFSH carries a rodent-type glycosylation that results into a lower level of sialilation and a global shift toward less acidic isoforms [7,5]. Many trials compared the effectiveness of rFSH and uFSH during *in vitro* fertilization (IVF) cycles, but no conclusive results are available. Some authors reported that rFSH administration is associated with a higher number of retrieved oocytes [8–10] and a lower total dose of FSH [8,9,11,12], whereas opposite findings have been reported by others [1,13,14]. However, in all reports the number of embryos and the pregnancy rate were not significantly different [9,15].

It is well known that 35–40 years old patients exhibit worst response to ovulation induction because female age is the main factor that affects oocyte quality and gonadotropins set [16]. More recent studies comparing rFSH versus uFSH provided evidences in support of the hypothesis that uFSH may carry some added benefits in patients of advanced reproductive age [4,13,17]. This encourages efforts aimed at personalising the stimulation protocol according to the characteristic of the patients. It has been already

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shown that in patients with good prognosis stimulation protocols mimicking the physiological pattern of FSH release result in improved treatment outcome [14,18]. Such studies have been performed using a sequential schedule with an uFSH administered during the early follicular phase to shift toward a rFSH in the second part of the stimulation cycle. We hypothesised that follicle maturation and oocyte quality may be improved in 35–40 years old women undergoing IVF cycles using a sequential stimulation protocol starting with uFSH and then shifting to rFSH, i.e. mimicking the physiological cycle. Accordingly, in the present study a sequential protocol using uFSH and rFSH was retrospectively compared with protocol using only rFSH in women aged 35–40 years undergoing IVF.

Materials and methods

This retrospective study was based on 230 women included in an ICSI program at the IVF Unit of the Second University of Naples. All the women were 35–40 years old, showed regular menstrual cycles, serum hormonal profile within the normal range (FSH and LH <10 IU/ml, E2 <50 pg/ml, prolactin <30 ng/ml, Inhibin B >7 pg/mL), normal karyotype, normal uterine cavity as diagnosed at ultrasonographic and hysteroscopy examination. The patients were excluded if they had previous poor response to gonadotropins (<3 oocytes previous pick-up), previous history of severe OHSS, current polycystic ovarian syndrome, previous two or more abortions, endometriosis stage III and IV, ≥2 previous IVF cycles, severe OAT.

All the patients underwent a standard down-regulation with GnRH analogue hormone at a dose of 0.1 mg/day (Triptroline, Decapeptyl, Ipsen, Milan, Italy) until oestradiol levels ≤40 ng/mL and no follicle >7 mm; then two groups were analyzed according to the stimulation protocol:

- group A (115 patients): a sequential stimulation protocol starting with uFSH (Fostimon, IBSA, Switzerland) for 6 days according to a step-down approach (225 IU for 4 days and 150 IU for the last two days) and then shifting to rFSH at the standard dosage of 150 IU, subsequently personalized according to the hormonal and ultrasonographic assessment;
- group B (115 patients): a standard protocol with rFSH (Gonal-F; Serono, Rome, Italy), at a daily dose of 225 IU for 4 days and 150 IU for the last two days to be titrated thereafter according to the hormonal and ultrasonographic assessment.

Ovulation induction was monitored by vaginal ultrasound and hormonal assessment every second–third day. When at least three follicles had reached a diameter of 18 mm, a single s.c. bolus of 10.000 IU of hCG (Gonasi HP 5000; IBSA, Rome, Italy) was administered. Transvaginal follicular aspiration was performed 34–36 h after hCG administration. The fluid from the main follicle from each ovary was collected and stored for research purposes. According to the Italian law regulation (law 40/2004) on IVF prohibiting formation of more than three embryos per cycle, up to three oocytes were inseminated.

The experimental study was conducted in accordance with principles of the Helsinki Declaration of 1975, using routine clinical practice procedures usually performed during IVF cycles; such procedures did not involve additional risks to the patients and all the medical decisions concerning individual patients were not affected by the study. Moreover, written consent was obtained from the patients for the collection and analysis of data. Therefore, the study did not require permission from the ethics committee.

Oocytes were cultured in Petri dishes in IVF-20 (Vitrolife, Göteborg, Sweden) at 37 °C in a humidified 5% carbon dioxide/95% air environment. The semen was processed with 80% Percoll (Sigma Chemical Company, St. Louis, MO) discontinuous gradient centrifugation at 800 *g* for 15 minutes. ICSI procedures have been described in detail previously [19,20] and was performed 4–6 hours after oocyte retrieval. After IVF, the resulting embryos were cultured in IVF-20 at 37 °C under 5% carbon dioxide in air. Embryos were graded by morphological analysis: grade 1, blastomeres with <10% extracellular fragmentation; grade 2, 10–20% extracellular fragmentation; grade 3, 20–30% extracellular fragmentation; grade 4, >30% blastomeric fragmentation. The luteal phase was supported with the administration of 33 mg/day of natural progesterone starting from pick-up day and than 50 mg/day from embryo transfer day. Serum levels of hCG were measured 14 days after embryo transfer and, if positive, were obtained every 3–6 days until an intrauterine gestational sac was demonstrated by US examination.

The data analyzed were: total dose of FSH administered, total number of days of stimulation, serum oestradiol levels on the day of hCG administration, number of oocytes and MII oocytes retrieved, embryo quality, fertilization rate, implantation rate, pregnancy rate, cancellation rate.

Data were analyzed using the Statistical Package for the Social Scienze (SPSS Release 16.0.2; SPSS Inc., Chicago, IL). Statistical analysis was performed using an unpaired *t*-test for independent data and χ^2-test, setting the significance level at $p \leq 0.05$.

Results

Ten cycles were not completed because of either low or excessive ovarian response leading to coasting: 6 (5.2%) cycles in group A and 4 cycles (3.4%) in group B. The remaining 220 patients underwent oocyte retrieval, 109 patients in group A and 111 in group B. Group A and B were similar concerning age and causes of infertility as shown in Table 1. There was no significant difference between the two groups regarding oestradiol level, number of cancelled cycles, IU of FSH administrated and mean number of oocytes retrieved per patient, but a significant lower days of stimulation in group A versus group B was shown (Table 2). A statistically higher proportion of MII oocytes (83.2% versus 77.7%, $p < 0.01$), higher implantation rate (13.9% versus 8.3%, $p < 0.05$) and pregnancy rate (33.9% versus 20.7%, $p < 0.05$) in group A compared to group B (Table 3) was observed. Moreover, higher percentage of grade 1 embryos and

Table 1. Baseline clinical characteristics.

	Group A ($n = 115$)	Group B ($n = 115$)	*p*
Age (years)	38.2 ± 1.4	38.3 ± 1.3	n.s.
Primary infertility (%)	74/115 (64.3%)	82/115 (71.3%)	n.s.
Female factor (%)	42/115 (36.5%)	33/115 (28.7%)	n.s.
Male factor (%)	52/115 (45.2%)	62/115 (53.9%)	n.s.
Unexplained infertility (%)	3/115 (2.6%)	4/115 (3.5%)	n.s.
Mixed factor (%)	18/115 (15.7%)	16/115 (13.9%)	n.s.

n.s., not significant

Table 2. Stimulation data.

	Group A ($n = 115$)	Group B ($n = 115$)	*p*
Days of stimulation	11.2 ± 2	12.1 ± 2.1	<0.01
Oestradiol peak (pg/ml)	1067 ± 387	957 ± 441	n.s.
FSH (IU/ml)	1820 ± 347	1720 ± 273	n.s.
Cancelled cycles (%)	6/115 (5.2%)	4/115 (3.4%)	n.s.
No. oocytes retrieved	6.9 ± 3.6	6.3 ± 4	n.s.

n.s., not significant

Table 3. Oocytes quality, embryo characteristics and clinical outcome.

	Group A ($n = 109$)	Group B ($n = 111$)	p
No. of oocytes M2 (%)	625/751 (83.2%)	545/701 (77.7%)	<0.01
No. of oocytes M1 (%)	88/751 (11.7%)	105/701 (15%)	n.s.
No. of GV (%)	38/751 (5.1%)	51/701 (7.3%)	n.s.
Embryos Grade 1 (%)	151/301 (50.1%)	116/299 (38.8%)	<0.01
Embryos Grade 2 (%)	105/301 (34.9%)	112/299 (37.5%)	n.s.
Embryos Grade 3 (%)	36/301 (12%)	50/299 (16.7%)	n.s.
Embryos Grade 4 (%)	9/301 (3%)	21/299 (7%)	<0.05
Fertilization rate (%)	301/306 (98.4%)	299/307 (97.4%)	n.s.
Pregnancy rate (%)	37/109 (33.9%)	23/111 (20.7%)	<0.05
Implantation rate (%)	42/301 (13.9%)	25/299 (8.3%)	<0.05

n.s., not significant

lower percentage of grade 4 embryos (3% versus 7%, $p < 0.05$) were found in group A compared to group B (50.1% versus 38.8%, $p < 0.01$) (Table 3).

Discussion

Our data show that in 35–40 years old women undergoing IVF, a sequential protocol using uFSH in the early days of stimulation and subsequently rFSH leads to better reproductive outcomes than a standard protocol using rFSH.

Previous studies compared the efficacy of rFSH and uFSH in good prognosis patients undergoing *in vitro* fertilization cycles, but no clear conclusion was reached. The higher purity and *in vitro* bioactivity of rFSH was supposed to account for a lower FSH total dose [8,9,11,12] and a higher number of oocytes retrieved [2,19] in some studies. In contrast, the higher *in vivo* biopotency of the uFSH was the possible reason for its better performance in other studies. However, even if in all these studies the number of embryos and the pregnancy rates were not significantly different [8,11], it is reasonable to consider the better standardised rFSH as the first choice treatment for good-responder women. On the other side, female age is known to be the main factor that affects oocyte quality [16] and 35–40 years old patients are known to show a poorer prognosis. However, recent studies showed that patients aged 35–39 exhibited a better response to uFSH than to rFSH [1,14].

We designed a sequential protocol to replicate the quantitative (step-down regimen) and qualitative (acidic to least acidic shift) pattern of FSH release in non-stimulated cycles and to mimic the natural cycle. This approach was assumed to be particularly suitable for patients of advanced reproductive age that suffer from decreased oocyte quality and have already been shown to be better responder to uFSH (13).

The threshold stimulation values of follicles are higher for highly glycosylated isoforms than for less glycosylated isoforms [21]; therefore, the highly glycosylated isoforms are more selective in the induction of follicular growth. Therefore, these isoforms are likely to be released during the early follicular phase in the natural cycle, i.e. they could be better suitable to drive the selection process resulting into a narrower cohort of more competent follicles. Moreover, in pre- and post-menopausal age the dominant FSH-released isoforms are highly glycosylated, suggesting a specific need for those isoforms in such period of the reproductive life. In summary, there is a role for a physiology-mimicking approach based on the use of highly glycosylated FSH molecules in advanced age patients undergoing IVF.

Indeed, our results although retrospective suggest that the sequential protocol can be used as a specific procedure in those women undergoing IVF that need an improved oocytes quality. In particular, the target patients seem to be women in advanced reproductive age that would otherwise require an increasing number of ampoules and more days of stimulation in a standard rFSH protocol. The use of a sequential protocol in 35–40 years old patients has relevant benefits when compared to conventional rFSH protocol: less stimulation days which may also improve patients' compliance to treatment, improved oocytes and embryo quality, lower cost due to the decreased number of rFSH ampoules. In conclusion, a sequential protocol using uFSH in the early days of stimulation and subsequently rFSH may result in an improvement of clinical reproductive outcome in patients of advanced reproductive age.

Declaration of interest

The authors have no financial affiliation (e.g. employment, direct payments, stock holdings, retainers, consultantship, patent-licensing arrangements, or honoraria) or involvement with any commercial organization with direct financial interest in the subject or material discussed in this manuscript. The authors have no financial interest in any aspect of the work and did not receive any financial support. No support in the form of financial aid, grants, or equipment was obtained for this study. Any other potential conflict of interest also is disclosed.

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Notice of Correction

The version of this article published online ahead of print on 20 Jun 2014 contained an error in the author list. The author list ''Colacurci Nicola, Caprio Francesca, La Verde Eugenio, Trotta Carlo, Ianniello Raffaele, Mele Daniela, and De Franciscis Pasquale'' should have read ''Nicola Colacurci, Francesca Caprio , Eugenio La Verde, Carlo Trotta, Raffaele Ianniello, Daniela Mele, and Pasquale De Franciscis.'' Each author was mistakenly listed with their surnames first, followed by their given names. The error has been corrected for this version.