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Simultaneous administration of human acidic and recombinant less acidic follicle-stimulating hormone for ovarian stimulation improves oocyte and embryo quality, and clinical outcome in patients with repeated IVF failures

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Abstract. – BACKGROUND: Ovarian stimulation is an integral procedure in assisted reproduction treatment. It is achieved by the administration of exogenous gonadotropins to increase follicular recruitment and oocyte yield. Optimization of ovarian stimulation is an essential prerequisite for the success of IVF treatment.

AIM: This study aimed to evaluate the effect of a combined stimulation protocol of human FSH and recombinant FSH, simultaneously administered, on oocyte and embryo quality and clinical outcome.

PATIENTS AND METHODS: In a prospective randomized study 197 infertile patients with a history of previous IVF failures for at least 3-5 attempts, were enrolled for an *in vitro* fertilization treatment. All patients had a standard down-regulation with GnRH analog and were then stimulated with FSH. The patients were matched into three groups: group A (no = 66) received human FSH combined with recombinant FSH in equal doses, simultaneously administered; group B (no = 67) received human FSH alone and group C (no = 64) received recombinant FSH alone.

RESULTS: There were significantly higher pregnancy ($p < 0.04$) and implantation rates ($p < 0.03$) in favor of group A (hFSH/rFSH) compared to groups B (hFSH) and C (rFSH). A significant increase in the proportion of mature metaphase II oocytes ($p < 0.002$) and grade 1 embryos ($p < 0.03$) was observed in group A with respect to group B and C. Significantly higher delivery rate ($p < 0.01$) was achieved in group A compared to groups B and C. No significant differences were observed between groups regarding miscarriage rate and risk of ovarian hyperstimulation syndrome.

CONCLUSIONS: The results show that the combination of human and recombinant FSH for ovarian stimulation may produce a positive effect on follicular development as it improve oocyte quality, embryo development, and ultimately clinical outcome.

Key Words:
Embryo, Human FSH, Oocyte, Ovarian stimulation, Pregnancy, Recombinant FSH.

Introduction

Ovarian stimulation is an integral procedure in assisted reproduction treatment. It is achieved by the administration of exogenous gonadotropins to increase follicular recruitment and oocyte yield. Two follicle-stimulating hormones (FSH) preparations are commercially available for ovarian stimulation: human-derived FSH (hFSH) and recombinant FSH (rFSH) with its higher purity and higher *in-vitro* bioactivity. Some clinical trials have shown that recombinant FSH is highly effective in terms of oocyte yield, embryo quality and dose of FSH needed, with less risk of causing ovarian hyperstimulation syndrome[1-2]. Other studies, however, have demonstrated that the efficacy of recombinant FSH in terms of oocyte and embryo quality is not superior to urinary hFSH. Some authors have argued that the difference between the two types of FSH may be due to the presence of LH activity in hFSH preparations, which has a positive effect on oocyte maturation and embryo quality[3,4], while other investigators have postulated that such differences may reside in the nature of FSH isoform activities[3,5].

A substantial difference exists between human-derived FSH and rFSH in terms of their glycosylation patterns: hFSH contains a higher proportion of acidic isoforms whereas rFSH contains a higher proportion of less acidic isoforms[6,7]. This difference in the glycosylation pattern of FSH is reflect-

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ed in its biological bioactivity, its clearance rate and its biological function[5,8,9]. A growing body of evidence shows that follicular development patterns and oocyte quality are strongly affected by the FSH glycoform range, and that the requirements of the growing follicle may change during its progress through different stages of follicular development[10,11]. In a recent study, using combined sequential stimulation protocol starting with acidic hFSH followed by less acidic rFSH, resulted in a significant improvement of oocyte quality and clinical outcome[12].

In this study, we evaluated the effect of using a combined protocol of both hFSH and rFSH for ovarian stimulation, administered simultaneously, on oocyte maturity, embryo quality and clinical outcome in patients with a history of previous IVF failures.

Patients and Methods

Patient Selection

In a prospective, open, randomized study a total of 197 infertile couples with a history of repeated IVF failures for at least 3-5 attempts, following stimulation with either hFSH or rFSH alone, were enrolled for this study from January 2010 to December 2011 at two IVF Centers. The women aged 28-39 years were included if they fulfilled the following criteria: (1) infertility attributable to tubal factor, male factor or idiopathic infertility; (2) serum hormonal profile (FSH and LH < 12 mIU/ml, E2 < 50 pg/ml and prolactin < 30 ng/ml) within the normal range; (3) regular ovulatory menstrual cycles; (4) presence of normal uterine cavity; and (5) body mass index (BMI) ≥ 20 - ≤ 30 kg/m^2. The patients were excluded if they had previous poor response to gonadotropins, history of severe OHSS, or current polycystic ovarian syndrome or the male partner had azoospermia.

Randomization was performed using a computer-generated random assignment schedule for each patient. Sealed and numbered envelopes were used to conceal the treatment allocation until randomization. The randomization took place after the confirmation of down-regulation and immediately before gonadotropin administration in order to minimize post-randomization withdrawals. All patients were counseled about the nature of the study and gave their written informed consent for their participation to the randomization procedure. Participating patients were registered in our local ethical committee register that approved the study. Only patients that satisfied the inclusion criteria were enrolled in the study to reduce the heterogeneity of the patients and minimization of any confounding variables that may affect the results.

The primary end points were oocyte maturity, embryo quality, and clinical pregnancy and implantation rates. The secondary end points were serum estradiol level and endometrial thickness on the day of hCG administration, fertilization rate, embryo cleavage rate, delivery rate, miscarriage rate and incidence of moderate or severe OHSS. All end points except the incidence of OHSS were analyzed statistically.

Ovarian Stimulation

All patients underwent a standard down-regulation protocol with GnRH analogue hormone (triptorelin) (Decapeptyl, Ipsen, Milan, Italy) 0.1 mg/day sc, starting 1 week before the expected menses (usually on day 21 of their cycle). After down-regulation was achieved (serum estradiol level < 150 pmol/liter) ovarian stimulation was commenced with the administration of gonadotropins, starting on day 3 of the cycle, while triptorelin administration was continued up to and including day 5 of the cycle. The patients were randomized in three groups: group A (n = 66), stimulated with a mixture of both hFSH (Fostimon, IBSA, Geneva, Switzerland) and rFSH (Gonal-F, Merck Serono, Rome, Italy) in equal doses (1:1 IU) administered simultaneously, starting with 150 IU hFSH and 150 IU rFSH from the third day of the cycle; group B (n = 67) received 300 IU of human FSH and group C (n = 64) received 300 IU of recombinant FSH. The FSH dose was established based on the previous protocols used for ovarian stimulation. After 6 days of stimulation the FSH dose was adjusted as necessary according to follicular size and estradiol level. The patients with a poor response to gonadotropin treatment were withdrawn from the study. Patients with excessive response to gonadotropins were counseled about the risk for OHSS and were advised to interrupt the stimulation cycle or to undergo oocyte retrieval with cryopreservation of resultant embryos for replacement in the subsequent cycle.

Final oocyte maturation was triggered by the administration of 10.000 IU of human chorionic gonadotropin (hCG) (Gonasi HP, IBSA, Switherland) when the leading follicle was 18-20 mm and there were at least two follicles of 16-17 mm. Oocyte re-

trieval was performed 36 h after hCG administration and the harvested oocytes were denuded from their cumulus cell immediately after retrieval and were assessed for their maturity. Mature metaphase II oocytes were inseminated by ICSI and the resultant embryos were scored according to established criteria[13,14]. Ultrasound guided embryo transfer took place on day 3 following insemination. The luteal phase was supported with the administration of 50 mg/day of progesterone.

Statistical Analysis

Statistical analysis was performed using the JMP software (version 4.0.4; SAS Corp., Cary, NC, USA). For a desired statistical power of 80% based on an α level of 0.05, confidence intervals (CI) of 95%, and anticipated effective size (Cohen's D) of 0.5 (medium size), the minimum total sample size required according to two-tailed hypothesis was 192 patients – at least 64 evaluable patients per group. The parameters were compared using the two tailed Student's *t*-test for independent data, Fisher's exact test and two by two table between groups where appropriate, setting the significance level at $p \leq 0.05$. The data were also analyzed by use of an analysis of variance (ANOVA) two-way test to analyze continuous variables, including primary and secondary outcome parameters. All analyses were adjusted for age stratum in line with the study design. Correction for multiple comparison analysis was performed using either Bonferroni's or Sidak's adjustment methods.

Results

Of the197 studied patients, 192 underwent oocyte retrieval, 65 patients in Group A, 65 in Group B and 62 in Group C. Five patients were cancelled because of excessive ovarian response leading to high risk for OHSS (one woman in Group A, two women Group B, and two in Group C). No patients had poor ovarian response to gonadotropin treatment. The three groups were comparable regarding demographic data and stimulation outcome (Table I). There was no significant difference observed among the three groups regarding the mean number of oocytes retrieved per patient. With respect to oocyte maturation, a statistically higher proportion ($p < 0.002$) of MII oocytes was observed in favor of Group A compared to Group B and C (62.2%, 44.3%, 43.6% respectively). Statistically significantly lower proportions of MI oocytes ($p < 0.03$) (27.3%, 36.2%, 37.3% respectively) and immature GV oocytes ($p < 0.02$) (10.5%, 19.5%, 19.1% respectively) were found in favor of Group A compared to Group B and C. Significant differences ($p < 0.003$) were also found in favor of Group A with respect to Group B and C in terms of Grade I embryos ($p < 0.03$) (61.5%, 41.1%, 38.3% respectively) and Grade II embryos ($p < 0.05$) (26.7%, 42.7%, 43.5% respectively), whereas Grade III and Grade IV embryos were similar (Table II). As depicted in Table III, although the mean number of transferred embryos was similar, significantly higher ($p < 0.003$) implantation rate (20.7%, 9.2%, 8.3% respectively) and pregnancy rate ($p < 0.04$) (41.5%, 18.5%, 17.7% respectively) were observed in favor of groups A compared to Group B and C. Delivery rate was also significantly higher ($p < 0.01$) (43%, 18.4%, 16.1% respectively) in favor of Group A compared to group B and C, whereas miscarriage rate was comparable between groups. No significant differences were observed between groups B and C in terms of oocyte maturity, embryo quality, pregnancy and implantation and delivery rates.

Table I. Demographic data and stimulation outcome.

	Group A uFSH/rFSH	Group B uFSH	Group C rFSH)	*p* value
Patients (n)	66	67	64	
Mean age (ys) ± SD	35.4 ± 3.23	34.6 ± 3.31	35.2 ± 3.74	0.169
Mean BMI ± SD	24.6 ± 1.7	25.1 ± 1.9	24.9 ± 2.1	0.289
Mean number of failed IVF attempts ± SD	3.7 ± 0.65	3.6 ± 0.68	3.7 ± 0.66	0.752
Duration of stimulation (days)	12.7 ± 1.8	13.4 ± 1.5	13.7 ± 1.4	0.871
Estradiol level on HCG day (pg/ml)	2040 ± 580	1975 ± 658	1966 ± 699	0.877
Endometrial thickness on HCG day (mm)	10.8 ± 2.1	10.6 ± 2.4	10.2 ± 2.1	0.957

hFSH: human-derived follicle-stimulating hormone; rFSH: recombinant follicle-stimulating hormone; No statistically significant differences observed between groups.

Table II. Demographic data and stimulation outcome.

	Group A uFSH/rFSH	Group B uFSH	Group C rFSH)	*p* value
N° of patients underwent egg retrieval	65	65	62	0.365
Mean number of retrieved oocytes ± SD	7.8 ± 1.1	7.5 ± 1.5	7.1 ± 1.3	0.87
Mature ocytes (MII) %	62.2[a]	44.3	43.6	0.002
Mature oocytes (MI) %	27.3[b]	36.2	37.3	0.03
Immature oocytes (GV) %	10.5[b]	19.5	19.1	0.02
Mean number of inseminated oocytes ± SD	4.4 ± 0.7	4.2 ± 0.7	4.3 ± 0.7	0.84
Fertilization rate %	75.1	74.4	74.5	0.94
Embryo cleavage rate %	78.4	75.3	77.6	0.94
Grade I embryos %	61.5[c]	41.1	38.3	0.03
Grade II embryos %	26.7[d]	42.7	43.5	0.05
Grade III embryos %	9.3	13.5	15.7	0.24
Grade IV embryos %	2.5	2.7	2.5	1.00

[a]Statistically significantly higher proportion of mature metaphase II oocyte in group A compared to group B or group C; [b]Statistically significantly lower proportion of mature metaphase I and immature GV oocytes in group A with respect to group B and C; [c]Statistically significantly higher proportion of Grade 1 embryos in group A compared to group B or group C; [d]Statistically significantly lower proportion of Grade II embryos in group A with respect to group B and C. No statistically significantly differences observed between groups regarding Grade III and IV embryos.

Discussion

Optimization of ovarian stimulation is essential for the success of IVF treatment. Although improved results and important innovations have occurred in ART, pregnancy rate per retrieved oocyte remains far too low. A major limiting factor in ART success rate is oocyte quality. In stimulated cycles the achievement of oocytes with the proper maturation remains a difficult issue[10,15,16]. Of note, the oocyte acquires its nuclear and cytoplasmic competence during folliculogenesis and consequently controlled ovarian hyperstimulation or collection of immature oocytes for *in vitro* maturation perturb this process which might result in reduced developmental competence of oocytes. Exogenous ovarian stimulation increases oocyte yield but may compromise the developmental competence of the oocytes in stimulated cycle[17].

Oocyte growth and maturation are directly regulated by intraovarian factors: steroids, cytokines and other growth factors, and the follicular level of estradiol may be the most important factor for supporting cytoplasmic growth and maturation. Gonadotropin stimulation results in a modified steroid profile, thus, altering the microenvironment of the developing follicles and their enclosed oocytes[18]. There is some evidence that estradiol appears to play a key role in oocyte growth and maturation[19-21]. It has been reported that estradiol exerts a beneficial effect on cytoplasmic maturation via a non-genomic calcium-mediated mechanism, which contributes to oocyte capacitation for fertilization

Table III. Clinical outcome.

	Group A hFSH/rFSH	Group B hFSH	Group C rFSH)	*p* value
Patients underwent embryo transfer	65	65	62	
Mean number of embryos per patient ± SD	2.6 ± 0.5[c]	2.5 ± 0.5	2.5 ± 0.6	0.91
Clinical pregnancies (%)	27 (41.5)[a]	12 (18.5)	11 (17.7)	0.04
Implantation rate %	20.7[b]	9.2	8.3	0.03
Delivery rate %	43.0	18.4	16.1	0.01
Miscarriage rate/pregnancy %	3 (11.5)[c]	2 (16.6)	1 (18.2)	0.64

[a]Statistically significantly higher pregnancy rate in hFSH/rFSH group compared to hFSH or rFSH groups; [b]Statistically significantly higher implantation rate in hFSH/rFSH group compared to hFSH or rFSH groups; [c]No statistically significant differences between groups.

and early post-fertilization development[22,23]. Moreover, profound suppression of LH has been shown to be associated with a reduced cohort of embryos and a reduced estradiol/oocyte ratio[24,25], and some authors have suggested that when using recombinant FSH only, it may be of clinical benefit to add LH in the late follicular phase or to further reduce the dose of GnRH analogue[26,27].

Recently, some Authors have assumed that oocyte quality and competence could be affected by FSH isoform range. Several studies have documented that a significant change in FSH heterogeneity occurs during certain physiologic conditions, including puberty and the menstrual cycle. Acidic FSH isoforms prevail during the luteal-follicular phase transition when the estradiol level is low, whereas less acidic FSH isoforms are produced during the mid-cycle and preovulatory phases when the estradiol level is high. This shift may be an important mechanism in regulating the intensity of FSH stimulus during the final steps of follicular maturation[9]. Additionally, it has been reported that the antral follicle threshold dose and the maximal tolerated dose are highly variable with respect to different sources of FSH and appear to be related to the combination of isoforms. Less acidic rFSH isoform fractions induce antral follicles' development *in vitro* at lower doses than pituitary FSH, while higher doses of the less acidic fraction, in conjunction with a longer time period of follicle culturing, result in a more detrimental effect on embryo production than higher acidic fractions do[9,10]. Mixing the acidic and less acidic FSH isoforms has resulted in remarkably improved follicle structure definition, clarity of somatic cell organization and normal appearance of the cumulus-oocyte complex (COC) compared to the use of acidic or less acidic fractions alone. A combination of both acidic and less acidic isoform fractions in unfractionated FSH may provide an appropriate balance for cell differentiation, as well as providing protection against the detrimental effects of overdosing[10].

In view of these concerns we attempted to use a protocol of combined acidic hFSH and less acidic rFSH, in equal doses, administered from the beginning of ovarian stimulation in patients with a history of previous IVF failures following stimulation with either hFSH or rFSH alone. Our results show significantly higher proportions of mature oocytes, Grade 1 embryos in patient stimulated with combined hFSH/rFSH protocol compared to those stimulated with hFSH or rFSH alone, although the number of retrieved oocytes was similar between groups. Also, pregnancy, implantation and delivery rates are significantly improved in combined hFSH/rFSH group with respect to hFSH and rFSH groups. This could be partially explained by the fact that combining acidic hFSH with less acidic rFSH may provide appropriate environment for follicular growth and improve oocyte maturation competence. Indeed, detection of a competent oocyte with the simple microscopic observations is quiet difficult and it could only be determined by the quality of produced embryos. In addition, the production of a competent oocyte to undergo fertilization and embryonic development following gonadotropins administration remains a critical issue in assisted reproductive treatment. During folliculogenesis as the oocyte acquires its maturation competence, the FSH glycosylation range has a pronounced effect on the follicular development and thus the oocytes[10].

On the other hand, combined protocol of human derived FSH (HMG or highly purified urinary FSH) and recombinant FSH, with the aim to improve oocyte quality, are used for ovarian stimulation, but they are administered at different time or even at different period of stimulation: starting with HMG for the first days and lasting with rFSH or vice versa. Previous study[28] showed that adding HMG, on day 5 of stimulation, to rFSH improve oocyte quality in some women, but in this case HMG was used as a source of LH rather than the type of isoforms content.

Conclusions

Our findings indicate that the combination between acidic and less acidic FSH for ovarian stimulation may have a positive effect on follicular development and oocytes by improving oocyte quality, embryo development, and ultimately clinical outcome in women with a history of previous IVF failures.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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