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Assessment of follicular and serum VEGF and IGF-1 in ICSI patients: hMG *vs* rFSH

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Summary

Purpose: To investigate the effect of recombinant follicular stimulating hormone (r-FSH) and human menopausal gonadotropin (hMG) on follicular microenvironment via assessment of follicular and serum vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1) levels in intracytoplasmic sperm injection (ICSI) cycles. *Materials and Methods:* Designed as a prospective cohort study. Twenty-five patients underwent controlled ovarian hyperstimulation (COH) with r-FSH and 20 patients underwent with hMG. *Results:* Both groups were comparable regarding the women's mean age and body mass index (BMI). The amount of VEGF (pg/ml) in serum and follicular fluid in the group I and II were comparable (275 ± 135.3 *vs* 330.7 ± 190.0; $p > 0.05$ and 2,081.1 ± 1095.1 *vs* 1,971.1 ± 975.6; $p > 0.05$, respectively). The amount of IGF-1 (ng/ml) in serum and follicular fluid in the group I and II were also comparable (225.3 ± 69.3 *vs* 204.1 ± 56.3, $p > 0.05$ and 176.1 ± 67.2 *vs* 185.8 ± 48.7, $p > 0.05$, respectively). Pregnancy rates were also comparable between groups. *Conclusions:* The hMG and r-FSH in COH produced comparable follicular microenvironment regarding follicular VEGF and IGF-1.

Key words: Recombinant FSH; hMG; Follicular fluid; VEGF; IGF-1.

Introduction

Vascular endothelial growth factor (VEGF) is released by the theca and granulosa cells and plays a basic and crucial function during the follicular growth and development via regulation of angiogenic processes in the ovary [1]. It has been shown that VEGF secretion, which is induced by gonadotropins, results the formation of adequate vascular structures in the thecal cell layer of the follicle [2, 3]. Adequate follicular vascularity indicates good levels of intrafollicular oxygen and consecutively good oocyte quality [4].

Insulin-like growth factor (IGF-1) is also released by the theca and granulosa cells and it also plays an important role in follicular development, dominant follicular growth, steroidogenesis, inhibin secretion, oocyte maturation, and follicular atresia [5]. IGF-1 enhances impacts of gonadotropins and coordinates the functions of the theca and granulosa cells [6].

Gonadotropins are routinely used agents to stimulate follicles in the controlled ovarian hyperstimulation (COH) in in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles. The human menopausal gonadotropin (hMG) and recently recombinant follicle-stimulating hormone (r-FSH) are widely used in COH. Several studies compared the performance of hMG with r-FSH regarding clinical pregnancy rates [7-9]. To the present authors' best knowledge, there is limited data about the comparing the effect of these agents on the follicle microenvironment.

Aim of this study was to compare the effects of the hMG and r-FSH on follicular microenvironment with assessing follicular and serum VEGF and IGF-1.

Materials and Methods

The study was designed as a prospective cohort study. It was conducted at Hacettepe University IVF Center. The patients who admitted to our IVF center for ICSI and embryo transfer (ET) during September 2008 and May 2009 were evaluated for eligibility to the study. Inclusion criteria were: [1] female age >18 and <40 years, [2] body mass index (BMI) between 18-29 kg/m^2 [3] patients having normal menstrual period [4], fresh cycles [5], ejaculatory sperm used for ICSI.

Patients having any endocrine disease (hyperprolactinemia, hypothyroid and hyperthyroid disease, and Cushing disease), polycystic ovary syndrome (PCOS) and severe endometriosis, those with ovarian cyst or endometrioma, patients having hypogonadotropic hypogonadism, and those with a poor response in the previous IVF cycles were not included into the study.

Forty-five patients met the inclusion criteria and approved to participate in the study. All patients underwent controlled ovarian hyperstimulation consisting of luteal-long leuprolide acetate with or without oral contraceptive pre-treatment. When desensitization was achieved, as evidenced by plasma E2 levels of ≤ 50 pg/ml, the absence of ovarian follicles and endometrial thickness ≤ 6 mm on transvaginal ultrasound examination [10], the authors divided these patients into two groups according to the numbers which were randomly generated by software. Group I patients (n=25) underwent s.c. injection of gonadotropin (re-

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Table 1. — *The baseline characteristics of Group I and Group II.*

Variable	Group I (r-FSH)	Group II (hMG)	*p* values
No. of patients	25	20	
Female age (yrs)	28.9 ± 4.3	31.1± 5.1	NS
Male age (yrs)	32.4 ± 5.3	34.9 ± 5.1	NS
Body mass index (kg/m^2)	25.38 ± 4.7	23.68± 2.6	NS
Duration of infertility (m)	84.9 ± 59.0	60.9 ±41.1	NS

NS: Non-significant

combinant FSH (rFSH) preparation. Group II patients (n=20) underwent i.m. injection of gonadotropin hMG preparation. The starting dose of gonadotropins was determined based on the age of the female, antral follicle count at baseline transvaginal ultrasonography, BMI, and previous ovarian response, if available.

Ovarian response was monitored with frequent serum E2 measurements and transvaginal ultrasonography, as described previously [11]. The criterion for hCG administration was the presence of three or more follicles exceeding 17 mm in diameter.

Oocyte retrieval was carried out under local anesthesia using vaginal ultrasound– guided puncture of follicles 36 hours after hCG administration. Following exposure to 20 IU/ml hyaluronidase in order to clean the cumulus cells, oocyte assessment was carried out. Blood serum was simultaneously collected from the patients who have follicular fluids without blood mixed and at least one morphologically normal oocyte. Follicular fluid and blood serum were centrifuged at 3,000 rpm for 15 minutes and kept at – 80ºC until the analysis.

VEGF and IGF-1 Assay

The levels of VEGF and IGF-1 in serum and follicular fluid were studied at the same time in the clinical biochemistry laboratory. Amount of VEGF and IGF-1 in serum and follicular fluid were evaluated using enzyme immunoassay (ELISA) kits (human VEGF ELISA Kit and IGF-1 ELISA Kit). Minimum measurement sensitivity was > 5 pg/dl for VEGF and > 4.9 ng/dl for IGF-1.

Standard procedures were carried out for gamete-embryo handling and cleavage-stage ET was performed under abdominal ultrasonography guidance in all cases using a soft catheter. The luteal phase was supported by daily vaginal progesterone suppositories starting one day after oocyte pick-up.

Clinical pregnancy was defined as the presence of an intrauterine gestational sac by transvaginal ultrasonography.

The statistical analyses were performed using Statistics Package for Social Sciences version 17.0 (SPSS). The chi-squared and Fisher exact tests were used to analyze nominal variables in the form of frequency tables. Normally distributed (Kolmogorov-Smirnov test) parametric variables were tested by Student *t*-test. Non-normally distributed metric variables were analyzed by Mann-Whitney U test. Pearson or Spearman correlation was used to investigate correlation. All *p* values of < 0.05 were considered statistically significant. Values were expressed as mean ± SD, unless stated otherwise. Institutional review board of our university approved the study protocol.

Results

Both groups were comparable regarding the women's mean age and BMI (Table 1). COH responses of the patients, embryologic and pregnancy outcomes and the amount of VEGF and IGF-1 in serum and follicular fluid are given in Tables 1-2.

Table 2. — *The controlled ovarian hyperstimulation response, the embryological data, and pregnancy outcome of Group I and Group II.*

Variable	Group I (r-FSH)	Group II (hMG)	*p* values
Duration of stimulation (d)	9.6 ± 1.5	9.2 ± 1.3	NS
Total dose of FSH used (IU)	4950 ± 1540.6	4680 ±1456.2	NS
No. of antral follicle count	14.6 ± 6.9	16.5 ± 4.2	NS
No. of follicles >17 mm in diameter at hCG administration	3.6 ± 2.3	4.5 ± 2.1	NS
E2 level on the day of hCG administration (pg/ml)	3476 ± 2041.5	3175 ± 1404.4	NS
No. of oocyte-cumulus complexes	14.0 ± 5.8	12.5 ± 5.1	NS
No. of metaphase II oocytes	11.5 ± 5.5	10.6 ± 5.0	NS
No. of 2 pronucleated oocytes	8.8 ± 4.8	8.4 ± 4.0	NS
No. of grade 1 embryos at the day 3	0.52 ± 1.0	0.35 ± 0.6	NS
No. of grade 2 embryos at day 3	2.4 ± 1.0	2.3 ± 0.8	NS
No. of embryos at day 3	2.7 ± 0.7	2.7 ± 0.5	NS
No. of transferred grade 1 embryos	0.28 ± 0.6	0.5 ± 0.8	NS
No. of transferred grade 2a embryos	0.12 ± 0.4	0.11 ± 0.3	NS
No. of transferred grade 2b embryos	1.4 ± 1.2	1.0 ± 0.9	NS
No. of transferred grade 2ab embryos	0.8 ± 1.2	1.4 ± 0.9	NS
Clinical pregnancy/embryo transfer (%)	40	45	NS
Serum VEGF (pg/ml)	275 ± 135.3	330.7 ± 190.0	NS
Follicular VEGF (pg/ml)	2081.1 ± 1095.1	1971.1±975.6	NS
Serum IGF-1 (ng/ml)	225.3 ± 69.3	204.1 ± 56.3	NS
Follicular IGF-1 (ng/ml)	176.1 ± 67.2	185.8 ± 48.7	NS

NS: Non significant; VEGF: Vascular endothelial growth factor; IGF-1: Insulin like growth factor-1.

No statistically significant difference was found between the groups in terms of the amount of gonadotropin used, the total number of the follicles ≥17 mm in size at the day of hCG administration, the number of oocyte cumulus complexes retrieved, the metaphase 2 oocyte number, the 2 pronuclear oocyte number, the number of grade 1 embryos existing at the third day, the total number of grade 2 embryos at the third day, the number of the transferred grade 1 embryos, the numbers of the transferred grades 2a and 2b embryos, and pregnancy rates (Table 2).

The amount of VEGF (pg/ml) in serum and follicular fluid in groups I and II were comparable (275 ± 135.3 vs 330.7 ± 190.0; $p > 0.05$ and 2081.1 ± 1095.1 *vs* 1971.1 ± 975.6; $p > 0.05$, respectively, Table 2). The amount of IGF-1 (ng/ml) in serum and follicular fluid in the group I and II were also comparable (225.3 ± 69.3 *vs* 204.1 ± 56.3, $p > 0.05$ and 176.1 ± 67.2 *vs* 185.8 ± 48.7, $p > 0.05$, respectively, Table 2).

Pregnant patients had lower follicular VEGF (pg/ml) levels when compared to non-pregnant patients (160.8 ± 747.8 *vs* 2669.1 ± 692.0, $p < 0.05$, Table 3). The serum VEGF levels were positively correlated with the number of oocyte retrieved, the number of M2 oocyte, and the number of 2PN oocyte (Table 4).

Table 3. — *Ovarian responses, the VEGF, and IGF-1 levels in the serum and follicular fluid of the clinically pregnant and non-pregnant patients.*

Variable	Pregnant (n=19)	Non-pregnant (n=26)	*p* values
COH with r-FSH	40%	60%	NS
COH with hMG	45%	55%	NS
No. of oocyte-cumulus complexes	14.8 ± 5.7	12.3 ± 5.1	NS
No. of metaphase II oocytes	10.53 ± 3.7	7.27 ± 4.4	<0.05
No. of embryos transferred	2.95 ± 0.8	2.62 ± 0.2	NS
Serum VEGF (pg/ml)	353.4 ± 193.5	260.6 ± 125.1	NS
Follicular VEGF (pg/ml)	1160.8 ± 747.8	2669.1 ± 692.0	<0.05
Serum IGF-1 (ng/ml)	214.3 ± 64.0	217.0 ± 65.3	NS
Follicular IGF-1 (ng/ml)	195.8 ± 66.9	169.1 ± 66.9	NS

NS: Non-significant; COH: Controlled ovarian hyperstimulation;
VEGF: Vascular endothelia growth factor; IGF-1: Insulin like growth factor-1
r-FSH: Recombinant follicle stimulating hormone;
hMG: Human menopausal gonadotropin.

Table 4. — *Correlation of the VEGF and IGF-1 levels in the serum and follicular fluid with the ovary and embryologic responses (cross table).*

Variable	Serum VEGF	FF VEGF	Serum IGF-1	FF IGF-1
No. of follicles at the day of hCG	NS	NS	NS	NS
No. of oocyte-cumulus complexes	$r = 0.33$ (<0.05*)	NS	NS	NS
No. of metaphase II oocytes	$r = 0.38$ (<0.05*)	NS	NS	NS
No. of 2 pronuclear oocytes	$r = 0.42$ (<0.05*)	NS	NS	NS
No. of day 3 embryos	NS	NS	NS	NS

r = Pearson correlation coefficient; *Correlation is significantly positive;
FF: Follicular fluid: VEGF: Vascular endothelial growth factor;
IGF-1: Insulin like growth factor-1; hCG: Human chorionic gonadotropin.

Discussion

In the present study, the authors noted that the amount of VEGF (pg/ml) in serum and follicular fluid in the group I and II were comparable (275 ± 135.3 *vs* 330.7 ± 190.0; $p > 0.05$ and 2,081.1 ± 1,095.1 vs 1,971.1 ± 975.6; $p > 0.05$, respectively, Table 2). The amount of IGF-1 (ng/ml) in serum and follicular fluid in the group I and II were also comparable (225.3 ± 69.3 *vs* 204.1 ± 56.3, $p > 0.05$ and 176.1 ± 67.2 *vs* 185.8 ± 48.7, $p > 0.05$, respectively, Table 2). In other words, hMG and r-FSH for COH in ICSI cycles did not produced different follicular and serum VEGF and IGF-1. The clinical pregnancy rates were comparable between hMG and r-FSH groups.

Monteleone *et al.* [4] investigated the correlation of the follicular VEGF with the grade of perifollicular vascularity. They noted that the follicular VEGF levels to be significantly correlated with grade of perifollicular vascularity. They also reported that the higher VEGF levels were associated with higher numbers of the grade 1 embryos and oocytes and pregnancy rates. It was hypothesized that increased VEGF level is an adaptation for the oocytes to create a better vascular structure and a better micro- environment to form. The authors pointed out that oocyte viability of VEGF might be a marker of the fertilization and embryo quality and it also might be an indicator of the follicular microenvironment due to formation of a perifollicular capillary network. The present study supported their findings. The present authors noted that there was a positive correlation between the numbers of oocyte retrieved, the number of metaphase 2 oocyte, the number of pronuclear oocyte, and serum levels of VEGF. However, this was in contradiction with the study by Manaul *et al.* [12]. In their study, no correlation was found between the amounts of VEGF in serum or in follicular fluid with the number of oocyte retrieved and the number of follicles at the day of hCG.

Tokuyama *et al.* [13] evaluated the follicular VEGF concentrations in three COH groups; Group 1: hMG cycles (n=19), Group 2: clomiphene citrate cycles (n=10), Group 3: natural cycles (n=9). They reported that the Group 1 showed lower VEGF concentrations in follicular fluid than Group 2 or Group 3. Excluding high responders from Group 1, no difference was found among these three groups. When they reclassified groups, they noted that the group of highest number of oocytes harvested showed lowest VEGF concentrations in follicular fluid.

Choi *et al.* [14] compared the effects of three different COH protocols (urinary FSH (u-FSH) only, r-FSH only and r-FSH + hMG) on the follicular IGF-1 and IGFBP-4 (insulin-like growth factor binding protein-4). They noted that follicular IGF-1 levels were comparable between groups (IGF-I (ng/ml), 128 ± 12.6 *vs* 120 ± 8.9 *vs* 123 ± 13.8). Of interest, they reported that the follicular IGFBP-4 and pregnancy-associated plasma protein (PAPP-A) levels in r-FSH group were significantly higher than those of other groups ($p < 0.05$). Oosterhuis *et al.* [15] investigated the follicular IGF-1 concentrations in patients who underwent COH with hMG (n=23) and highly purified urinary FSH (n=47) for IVF. They noted that there was no significant difference between the groups received hMG and uFSH in terms of the rates of follicular IGF-1 (ng/ml, 116±92.7 *vs* 119±51.1, $p > 0.05$) and pregnancy.

When the present authors classified all patients in this study as pregnant and non-pregnant groups, the amount of follicular VEGF was found to be lower in the pregnant group than in the non-pregnant group (Table 3). However, the serum VEGF, follicular and serum IGF-1 levels were comparable between the pregnant and non-pregnant groups. No significant difference was observed between pregnant and non-pregnant groups in terms of the number and quality of the oocytes and embryos. Of interest, the number of metaphase 2 oocytes was to be higher in the non-pregnant group ($p < 0.05$). This was consistent with the literature. Numerous studies compared amount

of VEGF in follicular fluid following the ovulation induction carried out with rFSH in the pregnant and non-pregnant groups. These studies reported amount of VEGF to be lower in the pregnant group. Whereas, no significant difference was reported in terms of the number and quality of the oocytes and number of embryo transfers [16, 17].

In the present study, the serum VEGF levels were positively correlated with the number of oocyte retrieved, the number of M2 oocyte, and the number of 2PN oocyte (Table 3). However, the authors failed to find an association between IGF-1 and the oocyte number, the metaphase 2 oocyte number, the pronuclear oocyst number, the follicle number, and the grade 1 embryo number. This result was consisted with the literature. Dorn *et al.* [18] did not establish a correlation between the amount of follicular IGF-1 and pregnancy rate.

There is also no consensus in the literature on the effect of suppression protocols (GnRH agonist *vs* GnRH antagonist) on the VEGF levels in follicles. Asimakopoulos *et al.* [19] conducted a study and they noted that the VEGF concentrations in supernatants from cultures with cetrorelix (2,315.1 ± 1,565.5 pg/ml) were moderately, but significantly lower than in controls (2,604.3 ± 1,907.1 pg/ml) or cultures with leuprolide acetate (2,558.8 ± 1,403.1 pg/ml). Therefore, in the present study, the authors preferred to use GnRH analog (leuprolide acetate) only.

It is known that the VEGF levels in preovulatory follicles are ten times higher than those in serum [20]. Several studies report similar results [16, 17]. The present study supported this finding (Table 2).

In conclusion, hMG and r-FSH produced comparable follicular microenvironment regarding VEGF and IGF-1 in patients undergoing ICSI cycles.

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