The effect of intrafollicular IGF 1 and IGFBP 3 on IVF outcome in patients using different gonadotropins: a prospective study

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Abstract

Aim To investigate follicular fluid IGF 1, IGFBP 3 and their effects on IVF outcome in patients using different gonadotropins.

Materials and methods This prospective study was performed among 92 IVF patients who were categorized according to IVF pregnancy outcome. Two groups were formed as Group 1 : pregnant, Group 2: non pregnant.

Results Follicular IGF 1 and IGFBP 3 levels were not different between the two groups. There were negative correlations between follicular IGF 1 and age, gonadotropin dose, embryo numbers in all patients and group 2, but there were no correlations between IGF1 and these parameters in group 1. Although IGFBP 3 levels were not different in patients using rec FSH or HMG, IGF 1 were higher in patients treated by rec FSH compared to patient using HMG. When the groups using rec FSH or HMG were analyzed according to pregnancy rate, there were no differences in IGF 1 levels.

Conclusion Follicular IGF 1, IGFBP 3 do not predict clinical IVF outcome regardless of the different gonadotropin preparations.

Keywords IVF · IGF1 · IGFBP 3 · Follicular fluid · recFSH · HMG

Introduction

The insulin like growth factor (IGF) system is composed of two ligands (IGF 1 and 2), two receptors and insulin like growth factor binding proteins (IGFBP). The involvement of the IGF system as intraovarian regulators of folliculogenesis has been extensively studied. With regard to the IGF system, IGF 1 has no effect on primordial follicle development, but both IGF 1 and IGF 2 stimulate growth of secondary follicles. In antral follicles, these IGFs stimulate granulosa cell and steroidogenesis in most mammals. According to some authors, the increased IGF1 concentrations can modify the regulatory system of ovarian folliculogenesis to enable: (1) the activation and development of more preantral follicles, (2) the maintenance of larger pools of small antral follicles, (3) the recruitment of more follicles within the cohort of developing follicles, and (4) the selection of two or more dominant follicles within a follicular wave [1, 2].

The bioavailability of IGFs is regulated by a family of intrafollicular expressed IGF binding proteins (IGFBPs) which bind IGF 1 and 2 with high affinity. The IGFBPs (IGFBP 1-6) are present in all biological fluids. IGFBP 3 is the most predominant IGFBP in serum. The concentration of IGFBP 3 is positively regulated by Growth Hormone (GH) and IGF 1. The IGFBPs have four essential functions in the regulation of IGF activities: (1) they act as transport proteins in plasma, (2) they prolong the half-lives of IGFs by regulating their metabolic clearance, (3) they provide a mean of tissue and cell type-specific targeting, and (4) they directly modulate interaction of the IGFs with their receptors and thereby indirectly control their bioreactivity [1, 2].

Apparently, the IGF system prepares the selected dominant follicle for the decreasing availability of FSH

Capsule Follicular IGF 1, IGFBP 3 levels do not predict clinical IVF outcome regardless of the different gonadotropin preparations.

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and increasing availability of LH. Treatment of ewes with GnRH increased expression of mRNA for IGF 1, IGF 2, and IGFR 2 in antral follicles, and decreased expression of mRNA for IGFR 1 [3]. These data stress the roles that endogenous FSH and LH play in regulating IGF ligand and receptor gene expression in the sheep follicle.

The IGFBPs are inhibitory to gonadotrophin-induced follicular growth and differentiation and inhibit the actions of IGFs at the level of target cells [4]. Thus, changes in intrafollicular IGFBPs lead to changes in IGF bioavailability and the up or down regulation of gonadotropin actions on follicular cells.

The ovulation control exerted by IGF 1 is evidenced by the fact that in induced assisted reproduction technology (ART) cycles, follicles with higher IGF 1 concentrations require a lower number of FSH ampoules and a shorter induction time [5].

Several associations between response to ovarian stimulation and abnormalities in IGF 1 and IGFBP 3 concentrations have been discussed, but there was no consensus on this subject. Serum IGFBP 3 has been shown to decrease during stimulation in normal control subjects and may be a factor predictive of IVF outcome [6–9].

We have studied follicular fluid parameters and matched IVF outcomes to identify the relationship among the ovarian IGF/IGFBP system, oocyte quality, and subsequent embryo development and predicting pregnancy.

Materials methods

A prospective study was performed in a total of 100 infertile Turkish women who required professional help in the Infertility Department of Zekai Tahir Burak Women's Health Research and Education Hospital. This study was approved by the Ethics Committee and Institutional Review Board of Dr Zekai Tahir Burak Women's Health Research and Education Hospital. Written informed consent was obtained from all volunteers.

The Ethical Principles for Medical Research Involving Human Subjects enunciated in the 18th World Medical Association Declaration of Helsinki were applied throughout our research.

A total of 100 primary infertile women aged ≤ 42 years with FSH levels ≤ 15 mIU/ml on day 3 undergoing their first cycle of fresh, nondonor ART at our department were included in the study. The patients with ovulation disorders were excluded.

Five patients with blood contaminated follicular samples and 3 patients whose cycle treatments were cancelled were excluded from the study.

Gonadotropin stimulation for IVF, oocyte retrieval

Leuprolide acetate (Lucrin daily; Abbott Cedex, Istanbul) therapy was started on menstrual cycle d 21 to induce pituitary down-regulation and was initiated at a dose of 1.0 mg sc daily until pituitary down-regulation was established. The initial gonadotropin dose used for ovarian stimulation was individualized according to the patient's age, baseline serum FSH concentrations on day 3, body mass index and previous response to ovarian stimulation. The starting regimen was fixed for the first 4 days (150–450 IU rec FSH or HMG/day) and thereafter the dose of gonadotropin was adjusted according to the individual ovarian response. Serial E2 levels and two-dimensional follicle measurements by transvaginal ultrasonography (LOGIC 200 PRO, GENERAL ELECTRIC, Korea) were performed until at least two dominant follicles reached dimensions of 18 mm or greater in diameter. Human chorionic gonadotropin (Pregnyl, Organon, Netherland, 10,000 IU, im) was administered and oocytes were retrieved 36 h after HCG injection and then IVF or ICSI were applied. Luteal phase support was routinely given as progesterone in the form of Crinone 8% gel (Serono, Istanbul) 90 mg daily for 14 days, when a pregnancy test was performed. Pregnancy was diagnosed by the ultrasonographic demonstration of heartbeat in an intrauterine gestational sac.

Follicular samples collection

During oocyte retrieval, the contents of the dominant follicle were collected into sterile plastic tubes (Sarstedt, Numbrecht, Germany) by electric suction, with care taken avoiding contamination with blood. The samples contaminated with blood were discarded. None of the samples included flush medium. Each fluid sample was centrifuged at 2500g for 15 min to separate out the cellular contents and debris, and each supernatant was transferred to sterile polypropylene tubes. All collected fluid samples were frozen at -20°C and assayed on the same day.

Assay methods

Biochemical and endocrine tests were carried out in the laboratories of Zekai Tahir Burak Women's Health Research and Education Hospital. The results were obtained through the two-site immunoradiometric assay (IRMA). Human IGF 1 and IGFBP 3 levels in follicular fluid were measured using an OBI and DSL kit.

Patient selection

Group 1 consisted of 45 patients who were pregnant after IVF treatment and group 2 consisted of 47 patients who weren't pregnant after IVF treatment. Since ovulatory disorders can indirectly effect insulin, IGF and IGFBP levels, these group of patients were excluded.

Statistical analysis

The data were analyzed with SPSS for Windows 11.5 package program. Shapiro-Wilk test was used to test the normality of distribution for continuous variables and data were expressed as mean±standard deviation. Whereas, means were compared among groups by One Way ANOVA, otherwise, comparison of medians was performed by Mann–Whitney *U* test or Kruskal-Wallis test according to the number of independent groups. If a significant overall difference was found in One Way ANOVA or Kruskal Wallis test, post hoc Tukey or multiple comparison tests were performed to identify any significant differences among individual groups. The degree of association between continuous variables was calculated by Spearman's "rho" correlation coefficient. Area Under Curve (AUC) and 95% confidence intervals for both IGF-1 and IGF-BP 3 determination of pregnancy was evaluated by ROC analysis. A *p* value of <0.05 was considered statistically significant.

Results

The diagnostic accuracy of follicular fluid levels of IGF-1 and IGFBP-3 to predict pregnancy, we evaluated by a receiver operating characteristic (ROC) curve. The area under ROC, were statistically insignificant ($p>0,05$) in predicting pregnancy when the study group was analyzed according to hormonal treatment (HMG or rec FSH). Since that, we could not find any discriminating cut-off point for follicular fluid levels of IGF-1 and IGFBP-3. So, we analyzed our data according to pregnancy outcome.

The clinical and laboratory characteristics of patients from the two groups are presented in the Table 1.

According to IVF indications: Group 1; 16 patients with unexplained infertility (35,6%), 3 patients with multiple factor (6,7%), 22 patients with male factor infertility (48,9%) and 4 patients with tubal factor infertility (8,9%). Group 2; 12 patients with unexplained infertility (25,5%), 4 patients with multiple factor (8,5%), 23 patients with male factor infertility (48,9%) and 8 patients with tubal factor infertility (17%). There were no significant differences respect to IVF indications between the two groups ($p=0,59$).

There were no differences regarding COH protocols (rec FSH vs HMG), duration of stimulation, gonadotropin dose, peak E2, retrieved oocytes, mature oocytes, fertilized oocytes and embryo numbers between the two groups (Table 1).

Follicular IGF 1 and IGFBP 3 levels were not significantly different between the groups ($p=0,48$; $p=0,85$ respectively) (Table 1).

Table 1 Demographic, clinical and laboratory IVF parameters of Group 1 vs. Group 2 $p<0,05$ is significant

	Group 1 (pregnant) (n: 45)	Group 2 (not pregnant) (n:47)	<i>p</i>
	Mean±SD	Mean±SD	
Age (years)	30.8±5,25	32.6±5,38	0.97
Duration of stimulation (day)	9,91±1,90	10,27±2,27	0.40
Gonadotropin use			0.38
Rec FSH n (%)	17 (37,8%)	22 (46,8%)	
HMG n (%)	28 (62,2%)	25 (53,2%)	
Gonadotropin dose			0.46
Rec FSH (IU)	2141,22±888,41	2515,53±1485,75	
HMG (IU)	791,96±198,26	1472,00±917,34	
Peak E2	2900,73±1378,08	2744,02±1394,07	0.44
IGF 1 (ng/ml)	152,77±68,19	144,34±70,09	0.48
IGFBP 3 (ng/ml)	3241,51±700,94	3267,24±612,55	0.85
Oocyte numbers	8,40±3,13	8,08±3,93	0.65
M II oocyte numbers	7,22±3,25	7,04±3,86	0.81
PN numbers	4,37±1,81	4,19±2,92	0.36
Embryo numbers	3,13±0,91	2,74±1,40	0.21

Table 2 IGF 1 and IGFBP 3 levels according to the gonadotropin use in all patient $p < 0,05$ is significant

	recFSH(n:39) (pregnant+notpregnant)	HMG(n:53) (pregnant+notpregnant)	p (rec FSH vs HMG)
IGF1 (ng/ml)	175,71±65,31	128,42 ±65,02	0.001
IGFBP3 (ng/ml)	3386,43±624,58	3157,68±663,53	0.11

There were no significant correlations between follicular IGFBP 3 concentrations and age, duration of stimulation, gonadotropin dose, peak E2, retrieved oocytes, mature oocytes, fertilized oocytes, embryo numbers in all patients and when Group 1 and Group 2 were analyzed separately. There were no significant correlations between follicular IGF 1 concentrations and duration of stimulation, peak E2, retrieved oocytes, mature oocytes, fertilized oocytes in all patients and when Group 1 and Group 2 were analyzed separately. There were negative correlations between follicular IGF 1 concentrations and age, gonadotropin dose, embryo numbers in all patients (age: $r: -0,369$; $p=0,001$; gonadotropin dose: $r: -0,286$; $p=0,006$; embryo numbers: $r: -0,21$; $p=0,043$) and group 2 ($r: -0,481$; $p=0,001$; $r: -0,349$; $p=0,016$; $r: -0,373$; $p=0,01$), but there were no correlations between IGF 1 and these parameters in Group 1.

When the pregnancy outcome of the Group 1 has been analyzed, there were 6 patients with abortion, 7 patients with intrauterine exitus and 22 healthy, term delivery. When these patients with different pregnancy outcome were compared with regard to follicular fluid levels of IGF1 and IGFBP-3 there was no statistically significant difference ($p=0,39$, $p=0,21$ respectively).

There was no difference in follicular IGFBP 3 levels between the groups treated by rec FSH or HMG ($p=0,11$) either. However, the follicular IGF 1 levels in patients receiving rec FSH or HMG were $175,71 \pm 65,31$ ng/ml and $128 \pm 65,02$ ng/ml respectively. This difference was statistically significant ($p=0,001$) (Table 2). When the groups using rec FSH or HMG were analyzed according to being pregnant or not, there was no difference in IGF 1 level. ($p=0,49$; $p=0,10$ respectively) (Table 3).

Discussion

Various prognostic factors were required in order to determine the suitable patient therapy plan and to evaluate the success of therapy. With the increased understanding of

the physiology of ovarian folliculogenesis and steroidogenesis it is now understood that the ovarian microenvironment is very important for normal physiology. Although there are many local factors affecting the ovarian physiology, the importance of IGF has been better understood in recent studies. It has been proven that during the ART cycles ovulation is controlled by IGF 1. Previous studies have reported that IGFBP3 has an inhibitor effect on the activity of IGF1 [5, 6, 10]. Since ovulatory disorders can indirectly effect insulin, IGF and IGFBP levels, these group of patients were excluded in our study.

Urman et al. reported that the gonadotropin stimulation time and dose were lower in patients with high ratios of IGF-1 in the follicular fluid [11]. Again, in the study conducted by Nardo et al., a correlation between the IGFBP 3 and ART success was investigated and showed that patients with a high level of IGFBP-3 in the follicular fluid needed a higher FSH application [12]. On the contrary we did not determine any correlations between the follicular IGFBP 3 levels and induction periods and gonadotropin doses. Parallel to these two studies we have also noted a decline in gonadotropin need as the follicle IGF 1 levels increase in all patients and Group 2.

Nardo et al. used 3 different gonadotropins (alfa follitropin n:8 patients, beta follitropin n:6 patients, urofollitropin n: 6 patients) to investigate whether follicular IGF 1 and IGFBP 3 didnot differ between the tree different gonadotropin groups [12]. We did not determine a difference in the follicular IGFBP 3 levels in our patients receiving rec FSH and HMG but determined higher IGF 1 levels in patients receiving rec FSH compared to patients using HMG. However there was no significant difference in IGF 1 level when the groups using rec FSH or HMG were analyzed according to state of pregnancy.

Choi et al. investigated the correlation between the IVF results and the stimulation agents (whether gonadotropin agonist or antagonist) chosen by determining follicular IGF 1, 2 IGFBP 3, 4 and PAPP-A levels. Follicular IGF 2 and IGFBP 4 concentrations and the ratio of IGF 1/IGFBP 4

Table 3 IGF 1 and IGFBP 3 levels according to the gonadotropin use in Group 1 and Group 2 $p < 0,05$ is significant

Gonadotropin use		Group 1 (pregnant)	Group 2 (notpregnant)	p
Rec FSH (n:39)	IGF 1 ng/ml	168,37±54,41	181,39±73,39	0.49
	IGFBP 3 ng/ml	3294,14±681,59	3457,75±582,85	0.51
HMG (n:53)	IGF 1 ng/ml	143,30±74,67	111,75±48,37	0.10
	IGFBP 3 ng/ml	3209,55±722,89	3099,60±599,49	0.53

were significantly different when the cycles using GnRHa long protocols and those using GnRHant and multiple-dose flexible protocols were compared, which may indicate a difference of follicular microenvironment between the two protocols. However, they reported that the difference of the microenvironment does not appear to result in a difference in clinical outcomes [13]. Ulug et al. compared the levels of follicular IGF 1, Inhibin A, Inhibin B, and epidermal growth factor in IVF patients using gonadotropin analogue and antagonists. They reported that follicular IGF 1 levels were the same in the analogue and antagonist cycles [14]. In order to homogenize our study we only included patients receiving gonadotropin analogues different from Choi and Ulug study's.

Although Nardo et al. have reported a correlation between follicular IGF 1, IGFBP 3 levels and oocyte maturation, we have not detected any correlations between IGF 1, IGFBP 3 levels and number of retrieved oocytes, mature oocytes and fertilized oocytes [12]. We have only determined an inverse correlation between IGF 1 and embryo numbers in all the patients and the non-pregnant group. However we did not determine a correlation between IGF 1 and embryo numbers in the pregnant group. There was also no correlation between IGFBP 3 and embryo numbers.

Unlike our findings, Schoyer saw that IGF 1 and IGFBP 3 levels were in a dynamic equilibrium during stimulation in PCOS women undergoing IVF only in the group of PCOS women and male factor control group. He also found that the immature oocyte rate was higher in PCOS women whose IGF 1 was low on HCG day. He reported that pregnancy increased in the PCOS group with a high IGFBP 3 on HCG day and that it increased in the control group who had a low IGFBP 3 [15].

Cunha Filhos et al. reported that IVF was more successful in patients with a low level of IGFBP-3 in the follicular fluid, and there was no correlation between IGF 1 and pregnancy [16]. According to another report by Dorn et al., there was a meaningful relationship between serum IGF 1, IGFBP 3 levels and pregnancy and no correlation between follicular IGF 1, IGFBP 3 and pregnancy [17]. In the study of Choi et al. mentioned earlier, it was reported that after studying follicular IGF1, 2, IGFBP 3, 4 and PAPP-A in 2 groups of patients with different protocols, the difference in the follicular environment did not affect the IVF outcomes (pregnancy rate) [13]. Ulug et al. did not see a correlation between pregnancy and follicular IGF 1 [14]. Like Cunha, Dorn, Choi and Ulug's studies we also couldn't find any correlation between follicular fluid IGF1, IGFBP 3 and pregnancy.

Different results are still reported on this topic. Studies investigating IGF in different patient groups, different therapy protocols and IVF results are ongoing. Keeping in

mind the different studies and results, our study's aim was to investigate if there were correlations between IGF 1 and IGFBP 3 levels in follicular fluid and IVF with different gonadotropin therapy. Although IGF 1, IGFBP 3 are effective mediators in folliculogenesis and steroidogenesis, we saw that there was no correlation with IVF success and follicle IGF 1, IGFBP 3 levels.

As a conclusion, the controversy on this topic rests on evidence that has studied varying IVF indications in different patient groups treated with several treatment protocols. In order to better understand the importance of these mediators that have a local ovarian effect, more comprehensive studies with more homogenous and larger patient groups are necessary.

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