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The Effect of Serum and Follicular Fluid Fetuin-B Levels on In Vitro Fertilization Treatment

Serum ve Folikül Sıvısı Fetuin-B Düzeylerinin Tüp Bebek Tedavisi Üzerine Etkisi

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The Effect of Serum and Follicular Fluid Fetuin-B Levels on In Vitro Fertilization Treatment

ABSTRACT

Objective: The aim of this study was to investigate the difference between fetuin-B levels in serum and follicular fluid of patients with unexplained infertility, polycystic ovary syndrome (PCOS) and poor ovarian response undergoing In vitro fertilization (IVF) treatment and to investigate the effect of fetuin-B levels on oocyte quality, embryo number and clinical pregnancy.

Material and Method: In this prospective study, women with unexplained infertility (n=25), women with polycystic ovary syndrome (n=25), and women with poor ovarian response (n=25) who were diagnosed with infertility and started IVF treatment were included. Fetuin-B levels in serum and follicular fluid of the groups were tested using the ELISA method. In addition, the effect of the difference in fetuin-B levels between the groups on reproductive success was investigated.

Results: The differences between the distributions of fetuin-B levels measured in follicular fluid were not statistically significant in all three groups ($p>0.05$). On the other hand, the fetuin-B level measured in serum was statistically significantly higher in the PCOS group than in the bad ovary group ($p<0.05$). According to the results of the Mann-Whitney U test performed as post-hoc, only the differences in serum fetuin-B levels between the PCOS group and the bad ovary group were statistically significant ($p<0.05$).

Conclusion: No statistically significant correlation was found between serum fetuin-B and fetuin-B in follicular fluid in the PCOS group. However, in the unexplained infertility group, there was a statistically significant and negative correlation between the level of fetuin-B in the follicular fluid and the number of biochemical pregnancies as well as the number of clinical pregnancies. In this respect, fetuin-B level may have predictive value in predicting the outcome of in vitro fertilization (IVF) in the unexplained infertility group.

Keywords: Fetuin-B, follicular fluid, in vitro fertilization, PCOS, unexplained infertility.

ÖZET

Amaç: İnfertilite tanısı olan ve tüp bebek tedavisi gören açıklanamayan infertilite, polikistik over sendromu ve kötü over yanıtlı hasta gruplarında serum ve folikül sıvılarındaki fetuin-B düzeyleri arasındaki farkı araştırmak ve fetuin -B düzeyinin oosit kalitesi, embriyo sayısı ve klinik gebelik üzerine etkisini araştırmaktır.

Gereç ve Yöntem: Bu prospektif çalışmaya, infertilite tanılı ve tüp bebek tedavisine başlayan açıklanamayan infertilitesi olan kadınlar (n=25), polikistik over sendromlu kadınlar (n=25) ve kötü over yanıtı olan kadınlar (n=25) dahil edildi. Grupların serum ve foliküler sıvısındaki fetuin-B düzeyleri ELIZA yöntemi kullanılarak test edildi. Ayrıca gruplar arasındaki fetuin-B düzeyi farklılığının üreme başarısına etkisi araştırıldı.

Bulgular: Her üç grupta da folikül sıvısından ölçülen fetuin-B değerinin dağılımları arasındaki farklar istatistiksel olarak anlamlı değildi ($p>0.05$). Öte yandan serumda bakılan fetuin-B düzeyi istatistiksel olarak anlamlı şekilde PCOS grubunda kötü over grubundan daha yüksekti ($p<0.05$). Post Hoc olarak yapılan Mann Whitney U testi sonuçlarına göre, sadece PKOS grubu ile kötü over grubu arasındaki serum fetuin-B düzeyi arasındaki farklar istatistiksel olarak anlamlıydı ($p<0.05$).

Sonuç: PKOS grubunda serum fetuin-B ve folikül sıvısındaki fetuin-B arasında istatistiksel olarak anlamlı bir ilişki bulunamadı. Ancak açıklanamayan infertilite grubunda folikül sıvısındaki fetuin-B seviyesi ile biyokimyasal gebelik ve aynı zamanda klinik gebelik sayısı arasında istatistiksel olarak anlamlı ve negatif yönde ilişki bulundu. Fetuin-B düzeyi bu bakımdan açıklanamayan infertilite grubunda in vitro fertilizasyon (IVF) sürecinin sonuçlarını tahmin etmede prediktif değere sahip olabilir.

Anahtar Sözcükler: Açıklanamayan kısırlık, Fetuin-B, foliküler sıvı, PKOS, tüp bebek tedavisi.

Introduction

Infertility is the inability of a couple to conceive despite 12 months of regular unprotected intercourse under the age of 35 and 6 months of regular unprotected intercourse over the age of 35 (1). In 85 per cent of infertile couples, a cause can be found, while in about 15 percent no cause can be found. The patient group within this 15% is defined as unexplained infertility (2). Polycystic ovary syndrome, one of the common causes of infertility, is a metabolic disease with heterogeneous metabolic processes such as insulin resistance, with a prevalence of approximately 4-8% in reproductive age (1). Approximately 40% of patients with polycystic ovary syndrome (PCOS) present with infertility.

Poor ovarian response is when the ovaries do not respond or respond poorly. In recent years, especially pregnancies postponed for economic and social reasons or the increase in the childbearing age have increased the poor ovarian reserve (3). Ovarian reserve and oocyte quality are especially important in expectant mothers who want to conceive in their late 30s and early 40s and in patients undergoing in vitro fertilization (IVF) (4). Oocytes are surrounded by a layer of extracellular matrix called zona pellucida (ZP). Studies in the literature have reported that the thickness of the ZP, which is one of the morphological characteristics of oocytes, is effective on fertilization (5). In a recent study, it was found that serum fetuin-B level was associated with fertilization rate in IVF and may be a predictive marker of fertilization in IVF treatment (6)

Fetuin-B is a serum protein produced from the liver that is required for fertilization. Fertilization triggers proteolytic cleavage of the glycoproteins of the zona pellucida, resulting in a hardened state of the zona pellucida (7). Hardening of the zona pellucida prevents further sperm binding, further sperm penetration and protects the embryo before implantation. Ovastacin mediates hardening of the zona pellucida (8).

Fetuin-B is a potent ovastacin inhibitor that prevents early zona pellucida hardening. Thus, fetuin-B prevents premature zona pellucida hardening before fertilization and thus keeps the oocytes in a fertilizable state (7). Studies have shown that female mice lacking fetuin-B are infertile because their oocytes have prematurely hardened zona pellucida (9). The condition of the zona pellucida is essential for the success of in vitro fertilization in both humans and animals. Although the important effects of Fetuin-B protein on fertilization are known, the underlying mechanisms have not yet been fully elucidated.

This study aimed to investigate whether fetuin-B molecule levels in serum and follicular fluid of patients presenting with infertility differ between patients with PCOS, unexplained infertile patients, and infertile patients with poor ovarian response, and to investigate the effect on fertility.

Material and Method

The study included 75 infertile women admitted to the IVF center of a university hospital between November 2021 and November 2022. The Hitit University Faculty of Medicine Clinical Research Ethics Committee authorized this study according to the Declaration of Helsinki (approval number: 409). The study included patients who completed written informed consent forms after receiving comprehensive oral and written information. This study was conducted in accordance with the Declaration of Helsinki.

A total of 75 patients were divided into three groups. Infertile patients with PCOS (n=25), unexplained infertile patients (n=25), and patients with poor ovarian response (n=25) were included in the study. Unexplained infertility was diagnosed in women who met the infertility criteria and could not find the cause of their infertility. Patients' oligo-anovulation status, polycystic ovarian morphology (PCOM) on transvaginal ultrasonography and Ferriman-Gallwey scoring, which are clinical indications for hirsutism, were used for the diagnosis of PCOS. Bologna Criteria were used for indication of poor ovarian response. Bologna Diagnostic Criteria: age ≥40 years or presence of other risk factors for poor ovarian response, previously ≤3 oocytes with conventional stimulation, presence of abnormal ovarian reserve test (antral follicle count <5-7 or AMH<0.5-1.1 N-ng/ml).

Name, surname, age, weight, height, BMI, menstrual cycle, number of menstrual days, duration of unprotected sexual intercourse, acne status, and previous pregnancies were recorded. Hirsutism

was assessed using the modified Ferriman Gallwey scoring method. The results of a baseline ultrasound performed routinely throughout the evaluation were recorded. In addition, routinely requested basal hormones (FSH, LH, E2, Prolactin and AMH) were recorded. Chronic diseases, drug use, smoking and alcohol use were questioned.

The exclusion criteria for patients are as follows: women with a body mass index >30 kg/m^2, the patient did not consent to the study, alcohol use in the last 6 months, smoking more than 20 cigarettes a day, heavy exercise up to 1 month before the procedure, known chronic disease, chronic drug use.

Patient Blood Serum and Follicular Fluid Collection Since there were 3 different infertility groups in our study, the most appropriate ovarian stimulation protocol was applied to each patient individually. Oocyte collection was standardised and performed at 35 hours (between 10:00 and 12:00) after hCG treatment when sufficient follicular development was observed on serial transvaginal ultrasonography. Five cc of the remaining follicular fluid was placed in a dry tube and the oocytes were separated from the follicular fluid and stored at -20 °C. Approximately 8 cc of patient blood was placed in a 10 cc empty dry tube at the same time as routine blood tests were performed during the patient's hospitalization. Within 15 minutes, the blood sample was centrifuged at 4000 rpm for 10 minutes. The supernatants obtained were kept in Eppendorf test tube (1.5 ml, FIRADMED, polypropylene) at -20°C. The collected samples were stored at -20°C for 3 months. After the number of patients was completed, the samples were delivered to the laboratory according to the cold chain rule.

Fetuin-B levels were determined using the Enzyme Linked Immunosorbent Assay Fetuin- B Kit. After washing with Human brand, Combi Wash model washing device and reading with Next Level brand. Pregnancy and Oocyte Quality Monitoring

The embryologist noted the number of oocytes, their morphologic parameters (MII, PNII, etc.) and the number of embryos after oocyte collection. On day 14 after embryo transfer, patients were checked for hCG. hCG positive patients were monitored until fetal heartbeat was detected. The patient was considered clinically pregnant when a fetal heartbeat was detected. The study took approximately five months to complete.

Statistical analysis

In the analysis of the data, nominal and ordinal parameters, which are categorical variables, were defined by frequency analysis, and measurement data were defined by mean and standard deviation values. The chi-square test was used in the difference analysis of nominal and ordinal data. Before the difference analysis, the Kolmogorov-Smirnov Test was performed for normality analysis of the measurement data. One-way ANOVA test was used in the difference analysis of the parameters that fit the normal distribution between groups, and the Kruskal-Wallis test was used in the difference analysis of the parameters that did not fit the normal distribution. Spearman's rho analysis was used for correlational screening analysis. ROC analysis was performed for the prognostic value of Fetuin-B level. All analyses were performed in SPSS 17.0 for Windows package program with 95% confidence interval. We used the G*Power programme to calculate the sample size. We estimate that a sample size of 75 women (25 per treatment group) would have 90% power to compare serum and follicular fetuin-B levels in PCOS, unexplained infertility and poor ovarian response patient groups using an effect size of 0.25.

Results

Age and body mass index (BMI) were higher in the group with poor ovarian response, height in the unexplained group and weight in the PCOS group. The differences in age, height, weight and BMI between the patient groups were not statistically significant (*p>0.05*). Table I shows descriptive statistics.

Table I. Distribution of Age, Height, Weight and BMI Values of Patient Groups and Results of Difference Analysis

Mean±SD	**Unexplained infertility (n=25)**	**PCOS (n=25)**	**Poor ovarian response (n=25)**	*p value**
Age(year)	30.96±4.57	29.84±4.34	32.84±5.05	*0.078*
Height (cm)	162.64±5.45	162.44±5.97	159.56±4.77	*0.087*
Weight (kg)	64.08±7.25	67.72±10.20	64.84±6.43	*0.349*
BMI (kg/m^2)	24.14±2.60	25.64±3.53	25.66±2.86	*0.134*

*One-way ANOVA Test, *p<0.05* was considered statistically significant.

The total number of oocytes, M2 oocytes, G6 oocytes, PN2 oocytes and day 2 embryos were statistically significantly higher in the PCOS group (*p<0.05*). The difference in transfer data between the groups was not statistically significant (*p>0.05*) (Table II).

Table II. In Vitro Fertilization Parameters of Groups and Results of Difference Analysis

Mean±SD	Unexplained infertility (n=25)	PCOS (n=25)	Poor ovarian response (n=25)	*p value*
Total oocyte count	5.68±3.47	**10.12±4.92**	3.80±3.14	0.000*
M2 oocyte count	5.28±3.63	**9.76±4.64**	3.48±3.16	*0.000***
G6 oocyte count	3.84±3.21	**6.36±4.36**	1.56±2.14	*0.000**
PN2 oocyte count	3.44±2.45	**5.08±3.99**	1.68±1.44	*0.000**
Day 2 embryo counts	3.00±1.89	**3.96±3.75**	1.64±1.41	0.013*
Number of patients undergoing embryo transfer	1.00±0.65	0.72±0.61	0.84±0.69	*0.316**

*Kruskal Wallis Test, **One-way ANOVA, *p<0.05* was considered statistically significant.

The differences between the distributions of fetuin-B levels measured in follicular fluid were not statistically significant in all three groups (*p>0.05*). On the other hand, the fetuin-B level measured in serum was statistically significantly higher in the PCOS group (*p<0.05*). According to the results of the Mann-Whitney U test performed as post hoc, the differences in serum fetuin-B levels were statistically significant only between PCOS and the bad ovary (*p<0.05*). The differences in serum fetuin-B between the unexplained infertility group and the bad ovary group or between the unexplained infertility group and the PCOS groups were not statistically significant (*p>0.05*) (Table III).

Table III. Distribution of Fetuin B Values of Patient Groups and Results of Difference Analysis

Mean±SD	Unexplained infertility (n=25)	PCOS (n=25)	**Poor ovarian response (n=25)**	*p value*
Fetuin-B Follicul* (pg/mL)	240.50±59.60	270.72±85.80	301.15±117.04	0.067*
Fetuin-B Serum* (pg/mL)	184.22±93.47	**221.81±182.64**	136.51±60.67	0.035**

*One Way ANOVA Test, ** Kruskal Wallis Test, SD: Standard Deviation, *x1/500000.

There were statistically significant and positive correlations between PCOS group and menstrual cycle duration (*r=0.675; p<0.01*), menstrual pattern (*r=0.762; p<0.01*), Ferriman-Gallwey score (*r=0.818; p<0.01*), AMH (*r=0.655; p<0.01*). Among infertility outcomes, there were statistically significant and positive correlations between PCOS and total oocytes (*r=0.549; p<0.01*), M2 (*r=0.564; p<0.01*), G6 (*r=0.414; p<0.01*) and pN2 (*r=0.344; p<0.01*) (Table IV).

Table IV. Spearman's Rho Correlation Analysis Results for the Relationship between PCOS and Variables with Significant Differences

Group	**r**	**p**
Age	*-0.211*	*<0.01*
Menstrual cycle duration	*0.675*	*<0.01*
Menstrual pattern	*0.762*	*<0.01*
FGW Score	*0.818*	*<0.01*
AMH (ng/ml)	*0.655*	*<0.01*
Total oocyte count	*0.549*	*<0.01*
M2 oocyte count	*0.564*	*<0.01*
G6 oocyte count	*0.414*	*<0.01*
PN2 oocyte count	*0.344*	*<0.01*
Day 2 embryo count	*0.201*	*0.084*

In the unexplained infertility group, there was a statistically significant and negative correlation between the number of biochemical pregnancies and follicular fetuin-B level (*r=-0.562; p<0.01*) and between serum fetuin-B level and number of clinical pregnancies (*r=-0.409; p<0.05*) (Table V).

Figure I. ROC Analysis Results for the Diagnostic Value of Fetuin-B Serum Levels on PCOS

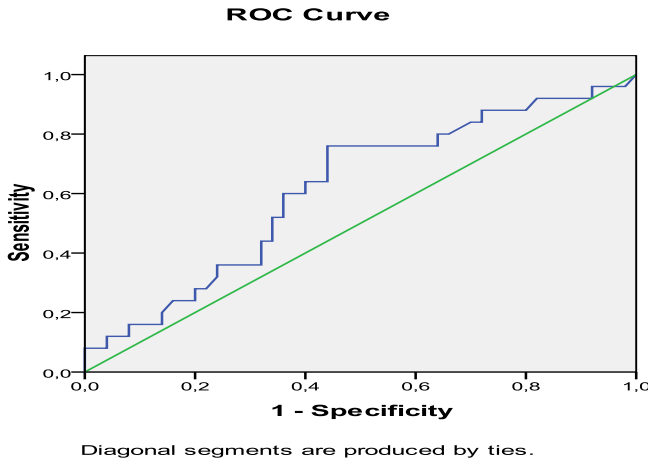

Table V. The Relationship Between Follicular Fluid Fetuin-B and Serum Fetuin-B Levels and Biochemical Pregnancy, Clinical Pregnancy, and Oocyte Quality in Unexplained Infertility Group

		Correlation coefficient	*p*
Number of biochemical pregnancy	Fetuin-B (Follicul)	-0.562**	*0.003*
	Fetuin-B (Serum)	-0.111	*0.597*
Number of clinical pregnancies	Fetuin-B (Follicul)	-0.205	*0.327*
	Fetuin-B (Serum)	-0.409*	*0.042*
Total oocyte count	Fetuin-B (Follicul)	-0.296	*0.151*
	Fetuin-B (Serum)	-0.050	*0.811*
M2 oocyte count	Fetuin-B (Follicul)	-0.221	*0.288*
	Fetuin-B (Serum)	-0.125	*0.551*
Day 2 embryo count	Fetuin-B (Follicul)	-0.162	*0.438*
	Fetuin-B (Serum)	-0.217	*0.297*
PN2 oocyte count	Fetuin-B (Follicul)	-0.246	*0.237*
	Fetuin-B (Serum)	-0.335	*0.101*
G6 oocyte count	Fetuin-B (Follicul)	-0.031	*0.881*
	Fetuin-B (Serum)	-0.281	*0.174*
Number of patients undergoing embryo transfer	Fetuin-B (Follicul)	-0.018	*0.934*
	Fetuin-B (Serum)	-0.026	0.901

**p<0.05 **p <0.01*

In the PCOS group, no statistically significant correlation was found between follicular fluid fetuin-B and serum fetuin-B levels and biochemical pregnancy number, clinical pregnancy number and oocyte quality (*p>0.05*) (Table VI).

Table VI. The Relationship Between Follicular Fluid Fetuin-B and Serum Fetuin-B Levels and Biochemical Pregnancy, Clinical Pregnancy, and Oocyte Quality in PCOS Group

		Correlation oefficient	*p*
Number of biochemical pregnancies	Fetuin-B (Follicul)	0.305	*0.138*
	Fetuin-B (Serum)	0.236	*0.257*
Number of clinical pregnancies	Fetuin-B (Follicul)	-	*>0.05*
	Fetuin-B (Serum)	-0.020	*0.923*
Total oocyte count	Fetuin-B (Follicul)	-0.059	*0.781*
	Fetuin-B (Serum)	0.047	*0.822*
M2 oocyte count	Fetuin-B (Follicul)	-0.086	*0.684*
	Fetuin-B (Serum)	0.083	*0.693*
Day 2 embryo count	Fetuin-B (Follicul)	-0.001	*0.996*
	Fetuin-B (Serum)	-0.280	*0.175*
PN2 oocyte count	Fetuin-B (Follicul)	0.012	*0.956*
	Fetuin-B (Serum)	-0.063	*0.765*
G6 oocyte count	Fetuin-B (Follicul)	0.197	*0.345*
	Fetuin-B (Serum)	0.043	*0.839*
Number of patients undergoing embryo transfer	Fetuin-B (Follicul)	0.111	*0.599*
	Fetuin-B (Serum)	-0.203	*0.330*

No statistically significant correlation was found between follicular fluid fetuin-B and serum fetuin-B levels and biochemical pregnancy number, clinical pregnancy number and oocyte quality in the poor ovarian response group (*p>0.05*) (Table VII).

Table VII. The Relationship Between Follicular Fluid Fetuin-B and Serum Fetuin-B Levels and Biochemical Pregnancy, Clinical Pregnancy and Oocyte Quality in Patients with Poor Ovarian Response

		Correlation coefficient	*p*
Number of biochemical pregnancies	Fetuin-B (Follicul)	0.184	*0.378*
	Fetuin-B (Serum)	0.020	*0.923*
Number of clinical pregnancies	Fetuin-B (Follicul)	0.184	*0.378*
	Fetuin-B (Serum)	0.020	*0.923*
Total oocyte count	Fetuin-B (Follicul)	-0.382	*0.059*
	Fetuin-B (Serum)	0.143	*0.495*
M2 oocyte count	Fetuin-B (Follicul)	-0.338	*0.099*
	Fetuin-B (Serum)	0.137	*0.515*
Day 2 embryo count	Fetuin-B (Follicul)	-0.130	*0.536*
	Fetuin-B (Serum)	0.156	*0.456*
PN2 oocyte count	Fetuin-B (Follicul)	-0.167	*0.424*
	Fetuin-B (Serum)	0.182	*0.385*
G6 oocyte count	Fetuin-B (Follicul)	-0.024	*0.910*
	Fetuin-B (Serum)	0.000	*0.999*
Number of patients undergoing embryo transfer	Fetuin-B (Follicul)	0.011	*0.957*
	Fetuin-B (Serum)	0.140	*0.504*

There was a statistically significant and negative correlation between fetuin-B level in follicular fluid and total oocytes (*r=-0.286; p<0.05*) and M2 (*r=-0.264; p<0.05*) in all patients (Table VIII).

Although the difference in serum fetuin-B level was significant between the PCOS group and other groups, the diagnostic value of serum fetuin-B level on PCOS was not statistically significant (AUC: 0.616; *p>0.05*) (Figure I).

Table VIII. Relationship Between Follicular Fluid Fetuin-B and Serum Fetuin-B Levels and Biochemical Pregnancy, Clinical Pregnancy and Oocyte Quality in All Patients

		Correlation coefficient	*p*
Number of biochemical pregnancies	Fetuin-B (Follicul)	-0.094	*0.422*
	Fetuin-B (Serum)	0.074	*0.526*
Number of clinical pregnancies	Fetuin-B (Follicul)	-0.003	*0.977*
	Fetuin-B (Serum)	-0.148	*0.206*
Total oocyte count	Fetuin-B (Follicul)	-0.286	*0.013*
	Fetuin-B (Serum)	0.148	*0.204*
M2 oocyte count	Fetuin-B (Follicul)	-0.264	*0.022*
	Fetuin-B (Serum)	0.129	*0.271*
Day 2 embryo count	Fetuin-B (Follicul)	-0.154	*0.189*
	Fetuin-B (Serum)	-0.046	*0.698*
PN2 oocyte count	Fetuin-B (Follicul)	-0.191	*0.102*
	Fetuin-B (Serum)	0.022	*0.854*
G6 oocyte count	Fetuin-B (Follicul)	-0.034	*0.769*
	Fetuin-B (Serum)	0.063	*0.591*
Number of patients undergoing embryo transfer	Fetuin-B (Follicul)	-0.024	*0.838*
	Fetuin-B (Serum)	-0.029	*0.807*

Discussion

The aim of this study was to investigate the relationship between the difference in serum and follicular fetuin-B levels and biochemical pregnancy number, clinical pregnancy number and oocyte quality in unexplained infertile patients, patients with polycystic ovary syndrome (PCOS) and patients with poor ovarian response during in vitro fertilization treatment. In the study, 25 samples were taken from each patient group and fetuin-B levels were obtained from the follicular fluid and blood obtained after ovulation induction.

IVF methods are increasing and improving every day. Studies and clinical applications range from oocyte quality to embryo transfer process and are planned to increase the efficiency of IVF (10). In order for the IVF process to be successful, the number of oocytes must be sufficient, the culture and spermatozoa obtained must be appropriate, the transfer process must be safe and post-implantation care is required. IVF procedures that are carried out successfully within this process have a very high success rate today (11, 12).

Fetuin-B is a pluripotent peptide hormone secreted by various tissues such as liver and adipose tissue. Fetuin-B, an inhibitor of papain-like cysteine proteinases, is an important inhibitor of ovastacin, a metalloproteinase (9, 13). In addition, studies on fetuin-B in serum and follicle fluid in the IVF process and oocyte quality and pregnancy process are becoming more prominent. Although limited today, the expansion of studies in this field and the demonstration of the predictive value of fetuin-B levels in unexplained infertility, PCOS and poor ovarian conditions may provide important clues for further studies and clinical applications.

The type of infertility is one of the important variables in both the occurrence and the process of pregnancy. In primary infertility, genetic and biological causes are more prominent, while in secondary infertility, it is possible to state that subsequent causes are also effective (14). In our study, the type of infertility (primary-secondary), biochemical pregnancy and clinical pregnancy rates were similar in all three groups and the differences between the groups were not statistically significant. In this respect, it is possible to state that the type of infertility has no effect on the relationships between the parameters examined in the study and on the outcomes of the IVF process. Again, the fact that the biochemical and clinical pregnancy rates were similar for the three groups and the difference was not statistically significant indicates that the effect of infertility type (unexplained, PCOS and bad ovary) was not significant.

Undoubtedly, oocyte quality is one of the most important parameters for pregnancy success in both normal pregnancies and IVF processes (15). Although it is not possible to link all parameters to oocyte quality until the last stage of pregnancy, it has been reported in many studies that low oocyte quality has a significant effect both in normal pregnancy and IVF process. In our study, total oocyte count, M2 oocyte count, G6 oocyte count, PN2 oocyte count and day 2 embryo count values, which are indicators of oocyte quality, were all higher in the PCOS group. From this point of view, it can be expected that the

IVF process was higher in the PCOS group than in the unexplained and bad ovary group. However, the fact that the difference in both biochemical pregnancy and clinical pregnancy rates between the groups was not statistically significant indicates that oocyte quality cannot be directly associated with pregnancy outcome and other predictive values are needed.

In the IVF process, many predictive markers are being studied to predict pregnancy success. Follicle and serum fetuin-B levels may have important value in this regard (7). However, there are not enough studies in the literature showing the difference between fetuin-B and IVF and our groups. The studies were mainly concerned with fertilization rates.

In a study investigating the success of serum and follicular fluid fetuin-B levels in predicting IVF outcomes, serum and follicular fluid fetuin-B levels were positively correlated (r=0.675, p<0.01). Serum and follicular fetuin-B levels were also lower in women with low fertility rates than in women with normal fertility rates [(6.09±1.31) µg/mL vs. (7.13±1.47) µg/mL, t=3.050, p<0.05; (5.13±0.96) µg/mL vs. (6.22±1.33) µg/mL, t=3.755, *p<0.01*] (16).

In a study comparing 78 cycles with a low fertilization rate with 104 cycles with a high fertilization rate, it was found that fetuin-B levels in both serum (5.81 ± 1.53 vs. 7.19 ± 1.42, *p<0.001*) and follicular fluid (5.06 ± 1.29 vs. 6.16 ± 1.52, *p<0.001*) were lower in the group with a low fertilization rate than in the group with a high fertilization rate. However, serum fetuin-B levels were not associated with preimplantation embryo development or clinical pregnancy (6).

In our study, although the differences between follicle fetuin-B levels were not statistically significant, serum fetuin-B levels were statistically significant and the highest value was in the PCOS group, followed by the unexplained infertility group and the poor ovarian response group, respectively.

According to the results of correlation analysis, there were statistically significant and negative correlations between the number of biochemical pregnancies and follicle fetuin-B levels and between serum fetuin-B levels and clinical pregnancy in the unexplained infertility group. In other words, in the unexplained group, follicle fetuin-B levels had predictive value for biochemical pregnancy rate and serum fetuin-B levels had predictive value for clinical pregnancy rate. In PCOS and poor ovarian groups, no statistically significant correlation was found between fetuin-B follicle and serum levels and biochemical pregnancy rate, clinical pregnancy rate and oocyte quality in PCOS group.

These results indicate that fetuin-B levels do not have a significant diagnostic value in PCOS and bad ovary groups, but can be used as an important parameter to indicate biochemical pregnancy and clinical pregnancy levels in the unexplained group. According to the results obtained in the study, oocyte quality was lower in the unexplained group and in the bad ovary group compared to the PCOS group. In this respect, the results of the study indicate that fetuin-B level may have a significant role in predicting biochemical pregnancy and clinical pregnancy rates even in the case of low oocyte quality in the unexplained infertility group. This makes the study valuable in terms of guiding further research and studies and clinical applications.

One of the limitations of our study is that since there were 3 different infertility groups, the most appropriate ovarian stimulation protocol was applied to each patient individually. Not applying the same standardised protocol to all groups is an important limitation of the study. Another limitation is that the study was conducted in a single centre. However, these limitations do not reduce the value of the study and constitute a source for further research. In this respect, our study may be a source for research on the estimation of biochemical pregnancy and clinical pregnancy rates, especially in the unexplained infertility group.

Conclusion

According to the results obtained in the study, there was no significant difference between the biochemical pregnancy and clinical pregnancy rates indicating IVF results and infertility types in the unexplained infertility, PCOS, and poor ovarian groups. In this respect, the findings obtained are consistent with the literature, but there is no clear finding on which group will have better results in terms of predicting IVF outcomes. Therefore, parameters that may have

predictive value such as fetuin-B are needed. This shows the originality of the study and its contribution to the literature. Although there are many studies showing the relationship between oocyte quality and pregnancy rate, at the last stage, it can be stated that there is no harmony and completeness in the studies on infertility in terms of the groups in our study. Therefore, more studies are needed. However, the results of our study can guide further research and studies. Although there are some indicators that measure success at intermediate stages in the IVF process, in general, biochemical pregnancy rates and clinical pregnancy rates can be shown as important indicators of a pregnancy achievement process. When these two rates are considered as outputs of the IVF process, oocyte quality is also an important factor in showing the success of the process. In conclusion, no statistically significant correlation was found between serum fetuin-B and fetuin-B in follicular fluid and PCOS. However, in the unexplained infertility group, there was a statistically significant and negative correlation between the number of biochemical pregnancies and follicular fetuin-B and between serum fetuin-B and clinical pregnancy. Fetuin-B level may thus have predictive value in predicting outcomes of the IVF process in unexplained pregnancies.

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