İKİLİ TARAMA TEST DEĞERLERİ: IVF VE SPONTAN GEBELİKLERDE FARKLI MI?

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ÖZET

Amaç: *IVF gebelik grubu ile spontan gebelik grubunu PAPP-A ve serbest β-hCG açısından karşılaştırmak*

Yöntem: *Eylül 2017 ile Eylül 2018 tarihleri arasında double test screening için başvuran gebelerden, IVF(in vitro fertilization) sonucu gebe kalan 42 tekil gebelik çalışma grubumuzu oluştururken, IVF sonucu gebe kalanların yaş aralığına uyan ve herhangi bir assisted reproductive technologies (ART) kullanmadan spontan yolla gebe kalan 109 tekil gebelik kontrol grubumuzu oluşturdu.*

Bulgular: *Çalışmaya katılan her iki gebeliğin demografik verileri ve gebelik sonuçları benzerdi. PAPP-A (pregnancy ascotiated plasma protein-A) değeri ortalaması kontrol grubunda (2.82 mlU/ml;0.97 MoM) bulunurken çalışma grubunda ise 2.41 mlU/ml,0.94MoM) olarak bulundu. Serbest β-hCG (beta-human chorionic gonadotrophin) değerleri ise kontrol ve çalışma guruplarında sırasıyla (46.44, ng/ml;1.21MoM, 53.79ng/ml;1.39MoM) olarak bulundu. IVF gebelik grubunun PAPP-A ortalaması spontan gebelik grubuna göre daha düşük ve β-hCG ortalamasının da spontan gebelik grubundan daha yüksek olduğu görüldü acak bu değişimler istatiksel olarak anlamlı değildi(p>0.005). İstatiksel olarak anlamlı çıkmasada; trisomy 21 açısından çalışma grubunu kontrol grubundan daha yüksek riskli olarak yorumladık(p>0.05).*

Sonuçlar ve Öneriler: *PAPP-A ve serbest β-hCG değerlerinin ortalamalarının IVF ile spontan gebeliklerde farklı olup bu biyokimyasal parametrelerdeki değişimin trisomy 21 risk hesabına etki etmektedir.IVF yapılan hastalarında ikili tarama testleri daha dikkatli yorumlanmalı ve riskli hastalar perinatoloğa refere edilmelidir.*

Anahtar kelimeler: *β-hCG; PAPP-A; IVF; spontan gebelik*

Double Test Screening Values: are They Different in IVF and Spontaneous Pregnancies?

ABSTRACT

Objective: *To compare the in vitro fertilization (IVF) pregnancy group and spontaneous pregnancy group in terms of pregnancy associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotropin (β-hCG).*

Method: *Among the pregnant women who applied for double test screening between September 2017 and September 2018, 42 singleton pregnancies that were pregnant as a result of IVF constituted our study group where 109 singleton pregnancies whose age range were matching the IVF pregnacies but were pregnant spontaneously without any assisted reproductive technologies (ART) remained in our control group.*

Findings: *The demographic data and pregnancy outcomes of both pregnancies were similar. The mean PAPP-A was found in the control group 2.82 mlU/ml (0.97 MoM), while in the study group 2.41 mlU/ml (0.94 MoM). Free β-hCG values were found similar between groups (46.44 ng/ml, 53.79ng/ml, respectively). The mean PAPP-A of the IVF pregnancy group was lower than the spontaneous pregnancy group, and the mean β-hCG was higher than the spontaneous pregnancy group, but these differences were not statistically significant (p> 0.005). Although not statistically significant; in terms of trisomy 21 we evaluated the study group as higher risk than the control group (p> 0.05).*

Results and recommendations: *The mean of PAPP-A and free β-hCG values are different in IVF and spontaneous pregnancies, and these differences in biochemical parameters may affect the risk of trisomy 21. In patients undergoing IVF, double test screening should be interpreted more carefully and risky patients should be referred to the perinatologist.*

Keywords: *β-hCG; PAPP-A; IVF; spontaneous pregnancy*

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INTRODUCTION

The horizons of prenatal diagnosis are constantly being expanded and the development of faster, less costly, less harmful methods for both mother and baby is being developed (Pakniat, Bahman & Ansari, 2019). The main purpose of prenatal diagnosis is to minimize diagnostic interventions such as amniocentesis, cordocentesis and corion villi sample (CVS) which can damage mother and fetus. The majority of congenital anomalies associated with chromosomal aberrations are pathologies that cannot be treated. These disorders include a variety of ethical and legal obligations affecting the family and the society in terms of socioeconomic aspects negatively. Congenital defects are seen in 3% of all newborns (Gagnon & Wilson, 2008).

Prenatal screening tests are increasingly used to detect trisomy 21 (Down Syndrome), trisomy 18, neural tube defect (NTD) and congenital cardiac anomalies (Cignini et al, 2016). The developments in ultrasonographic screening and the widespread use of this technology in all parts of the world and the rapid development of biochemical and cytogenetic methods have made it possible to diagnose more fetal chromosomal anomalies (Zhong, Bradshaw, Stanley & Odibo, 2011). One of the most frequently used one among these tests for screening is "Double Test Screening". All data obtained by these methods are evaluated together with a computer program which was previously designed. At this stage, the parameters measured from maternal blood, previously determined multiple of the median (MoM) values which are highly variable according to the location and individual characteristics, becomes important. The value of the MoM of a test indicates how much the measured value deviates from the median value for that test (Cignini et al, 2016).

In the double test screening test performed between 11-14 weeks of gestation, maternal demographic data (maternal age, weight, ethnicity, smoking status, multiple pregnancy and IVF), as well as obstetric ultrasonography (USG) and nuchal translucency (NT) are used and PAPP-A and β-hCG results from the mother blood are also taken (Smith et al, 2002). As a result of the evaluation, the risk of congenital diseases such as NTD and Down Syndrome is calculated. While β-hCG levels increase in maternal blood in pregnancies of fetal trisomy 21, PAPP-A levels decrease in pregnancy, while in fetal trisomy 18 and 13 pregnants, both free β-hCG and PAPP-A levels decrease .

Infertility is defined as the inability of couples to obtain pregnancy despite at least one year of unprotected sexual intercourse (American College of Obstetricians and Gynecologists, 2015). In recent years, thanks to advances in assisted reproductive technologies (ART), most infertile couples have been able to obtain pregnancies resulting in live births. In the recent years, the number of IVF pregnancies that have undergone double test screening has been increased due to the increased success rates of IVF (American College of Obstetricians and Gynecologists, 2015). We compared the β-hCG and PAPP-A values, which are one of the double test screening serum markers of the singleton pregnancies conceived by IVF, with the values of singleton pregnancies obtained via spontenaous method without any ART.

METHOD

In a study conducted in September 2017 to September 2018, Tepecik Training and Research Hospital, a tertiary care center addressing a large population in the region, 42 singleton pregnancies who were admitted to the outpatient clinics for a double test screening constituted the study group where 109 singleton pregnancies, which match the age range of the IVF pregnancies and spontaneously conceived without any ART, constituted the control group.

The Local Ethics Committee approved the study. The universal principles of the Helsinki Declaration were implemented. The patients who met the research criteria and gave birth on the day were recorded consecutively. The database of our perinatology department was used to identify these pregnancies. Bilateral screening test was compared with the test results of the patients in both groups.Our primary inference; Since IVF pregnancies and pregnancies in normal way were compared with the test results during pregnancy and pregnancy results, pregnancies lost during the follow-up period or the ones who could not be reached were excluded. In addition to interviews with patients, computer-based patient records were reviewed for the following information: gravida, parity, body mass index, form of conception (IVF, spontaneous), gestational age at birth, type of delivery, birth weight, APGAR score, neonatal intensive care unit (NICU) need, smoking status, diabetes mellitus, thyroid disease and hypertension presence of maternal systemic disease, whether there is a genetic disease in the family, multiple pregnancies, USG findings, maternal serum PAPP-A and free β-hCG values.

Pregnancy weeks were calculated on the basis of USG values in the first trimester and on the first day of the last menstrual period. All patients in the study were evaluated according to the detailed USG evaluation, structural fetal malformations, amniotic fluid volume and placental localization before the test. During the usual pregnancy follow-up, all subjects who were screened were informed about the test. Antepartum tests or additional USG were not performed except for obstetric indications.

For double test screening, fetal nuchal translucency (NT) values, serum PAPP-A and free β-hCG values of pregnant women between 11-14 weeks of gestation were used for statistical analysis, while head butt distance (CRL) measurements was used for the USG to determine the gestational week at the time the serum sample was taken.

The biochemical parameters of β-hCG and PAPP-A in venous blood samples taken for double test screening were measured on the IMMULITY 2000 device (Diagnostic Product Comporation, Los Angeles, USA) which is using chemiluminescent assays method in the biochemistry lab of our hospital. NT and CRL measurements were performed with the "General Electric Logic 5 pro, USA 2-5 MHz convex probe" instrument. Prenatal risk calculation and median values of pregnant women combined with data such as NT, nasal bone availability, race, maternal age, weight, gestational week, CRL, diabetes, smoking status in the double test screening application form in perinatology outpatient clinic were evaluated with the package software PRISCA 5.0 (TYPOLOG Software/GmBH, Hamburg, Germany). The results of the risk calculations were

explained to the patients in detail and genetic counseling was provided to the patients with problematic results after had increased risk in double test screening. Of these, 18 were amniocentesis and 14 were CVS. Written informed consent was obtained before the procedure, procedure technique and possible complications. Each fetus underwent anatomical screening prior to amniocentesis. Fetal cardiac activity, place of placenta, amniotic fluid amount, place of intervention were determined. The procedures were performed by two different specialists working with prenatal diagnosis and treatment center under USG guided by free hand technique. Classical CVS and amniocentesis procedures were performed. Adequate material was obtained in all cases for genetic examination. The samples were evaluated for numerical and structural irregularities in chromosomes and sent to the genetic laboratory of our hospital for evaluation.

The diagnoses part of this study relied on the following descriptions: advanced maternal age: 35 years completed; preterm birth: pregnancy resulting in birth before 37 gestational weeks; surmaturity: 15 or more days over expected date of delivery; intra-uterine growth restriction (IUGR): fetal weight less than standard deviation from the normal fetal weight expected according to the gestational week and progressive deviation from the growth curve during at least three weeks of follow-up; low birth weight: fetal weight less than 10 percentiles compared with the normal weight expected according to the gestational week; fetal death: losses after week 20; gestational hypertension: blood pressure ≥140/90 mmHg without proteinuria or other symptoms and findings of preeclampsia; and preeclampsia: blood pressure >140/90 mmHg measured at 6-hour intervals, urine ++ or ≥300 mg protein in 24-hour urine.

Those cases found with a marker of chromosomal anomaly (increased nuchal translucency, anencephaly, gastroschisis, neural tube defect, hyperechogenic cardiac focus, choroid plexus cyst and pyelectasis) in gestational weeks 11-14 during fetal USG screening and those cases presenting a structural fetal anomaly during the USG screening in later weeks were excluded from the study. We

The number of spontaneous pregnancies in our study was 109 while the number of IVF pregnancies was 42. There was no statistically significant difference in terms of the mean age, BMI, average gestational weeks at birth, average baby birth weight, mode of delivery, APGAR scores, and the need for NICU between the pregnant women in study and control groups (p>0.05) (Table 1). IVF and spontaneous pregnancy groups included in the study were similar in their mean gestational weeks, both according to their last menstrual dates and CRL measurements.

the perinatologist informed them. Invasive procedures were performed in 32 cases between 24 and 35 years of age who recorded pregnant women younger than 24 and older than 34 years, those with diabetes, multiparous, gestational diabetes, twin pregnancy, preterm early membrane rupture, ablatio placentae, placentae previa fetal distress, oligohydramnios, body mass index (BMI) >30 and smokers; and we excluded the foregoing cases, considering them to be causes of neonatal and perinatal morbidity. Patients who did not know the last menstrual date or embryo transfer date and those who did not give birth in our hospital were excluded from the study. Patients with significant obstetric or medical complications and unreliable maternal and fetal status information were also excluded from the study.

Statistical analysis

The statistical analysis of the data obtained from our study was performed with the SPSS (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. USA). Descriptive statistics are presented as mean ± standard deviation for normally distributed numerical data, as median (min-max) for non-normally distributed numerical data, and as number and percentage for categorical data. The Shapiro Wilk's test was used to investigate the suitability of the data for normal distribution. For situations involving two groups, the Mann-Whitney U-test was used to compare the groups that did not conform to normal distribution. In determining the direction and magnitude of the correlation between parameters, we calculated Spearman's correlation coefficients for the variables that did not present normal distribution. The multiple effects of the relationships between variables were analyzed by the Linear Regression Analysis. Yate's (Continuity Correction) Chi-Square and Fisher's Exact Chi-Square analyses were used in the analysis of the cross tables. The 21.0 SPSS for Windows program was used in the application of the analyses. P<0.05 was accepted as the threshold for statistical significance.

FINDINGS

Table 1 Comparison of demographic characteristics and PAPP/fß-hCG of working groups

	Spontaneous (109)	IVF(42)	p*
Maternal age (years)	30.87 ± 4.79 min:27.00 max: 35.00	31.40 ± 4.51 min:27.75 max: 35.25	0.516
Gestational age (weeks)	12.30 ± 0.70 12.30 (11.60 - 13.00)	12.12 ± 0.60 12.20 (11.58 - 12.50)	0.158
CRL	12.53 ± 0.74 12.40 (12.00 - 13.10)	12.25 ± 0.65 12.30 (11.90 - 12.60)	0.163
PAPP-A	2.82 ± 1.53 2.62 (1.74 - 3.58)	2.41 ± 1.35 2.37 (1.13 - 3.38)	0.151
PAPP-A MoM	0.97 ± 0.47 0.90 (0.63 - 1.19)	0.94 ± 0.53 0.86 (0.57 - 1.12)	0.636
fß-Hcg	46.44 ± 35.07 37.00 (23.75 - 54.30)	53.79 ± 38.69 40.05 (28.73 - 64.28)	0.200
fß-hCG MoM	1.21 ± 0.87 1.00 (0.66 - 1.45)	1.39 ± 0.96 1.09 (0.75 - 1.69)	0.255

* Mann Whitney U Test

Values are given as mean ± standard deviation, n (%) or median (range). A P value of <0.05 was considered as statistically significant. Abbreviation: Spontaneous, spontaneous pregnancies; IVF, in vitro fertilization; CRL, Crown-rump length; PAPP-A, pregnancy associated plasma protein-A; β-hCG , beta-human chorionic gonadotropin; MoM; multiple of the median

The mean PAPP-A values of the spontaneous pregnancy group (0.97 Mom) were higher than that of the IVF pregnancy group (0.94 MoM), while free β-hCG averages (1.21 MoM and 1.39 MoM, respectively) were lower but not statistically significant in both results (p>0.05) (Table 1). We also compared the relationship between the CRL measurement weeks of both groups and the PAPP-A and β-hCG levels measured in the serum of the pregnant women. In our analysis, we found that there was an increase in the PAPP-A level in both groups as CRL week increased, yet this increase was statistically insignificant in both groups (p>0.005) (Table 2). When we made the same comparison for free β-hCG, we found that as the CRL week increased, there was a decrease in the free β-hCG level. While this decrease was significant in the control group (p<0.05), it was statistically insignificant in the study group (p>0.05) (Table 2).

Table 2 Evaluation of the relationship between CRL levels and PAPP-A (MOM)/fß-hCG (MOM) of groups

			PAPP-A	PAPP-A MoM	fß-hCG	fß-hCG MoM
CRL	Spontaneous (109)	r	*0.272;*	*-0.264;*	*-0.293;*	-0.074;
		p	*0.004*	*0.005*	*0.002*	0.447
	IVF (n=42)	r	0.280;	-0.115;	-0.080;	0.102;
		p	0.073	0.470	0.613	0.522

* Spearman Correlation Test, r = correlation coefficient

While the control group presented a moderate increase in the PAPP-A level measured in the pregnant serum with an increase in patient age, the study group had a very slight decrease in the PAPP-A level measured in the pregnant serum as gestational age increased. However, both results were statistically insignificant (p > 0.05) . There was a moderate increase in β-hCG levels with age in the control group. The study group also presented an increase, but this increase was much less than the control group. However, in general, these two results were statistically insignificant (p>0.05).

We evaluated the biochemical and combined risk ratios of our study groups found with double test screening by grouping them based on 1/300 and 1/1000 cutoff values (Table 4). The reason for our preference of these cutoff values is that, as part of our clinical practice, we evaluate patients whose risk is higher than 1/300 based on their week and with a patient-specific invasive diagnostic method to obtain a definitive diagnosis for trisomy. We monitor patients above 1/1000 risk without additional screening and diagnostic testing. We evaluate the patients that present a risk between 1/300 and 1/1000 with non-invasive prenatal diagnostic tests (NIPT) (American College of Obstetricians and Gynecologists, 2015). The number of pregnancies with a greater risk than 1/300 in the IVF pregnancy group was found to have a higher percentage than the spontaneous pregnancy group, in terms of both the combined risk and biochemical risk calculation. Regarding the pregnancies with less than 1/1000 risk ratio, we found that the number of pregnancies in the spontaneous pregnancy group were higher than those in the IVF pregnancy group in terms of both biochemical and combined risk calculations. The details of these patients are shown in Table 3.

Table 3 Comparisons of categorically transformed risk levels (biochemical and combined) of working groups

		Group		p
		Spontaneous	IVF	
Biochemical	>1/1000	40 (%36.7)	18 (%42.9)	0.610*
	<1/1000	69 (%63.3)	24 (%57.1)	
Combined	>1/1000	18 (%16.5)	10 (%23.8)	0.424*
	<1/1000	91 (%83.5)	32 (%76.2)	
Biochemical	>1/300	22 (%20.2)	10 (%23.8)	0.790*
	<1/300	87 (%79.8)	32 (%76.2)	
Combined	>1/300	6 (%5.5)	6 (%14.3)	0.094**
	<1/300	103 (%94.5)	36 (%85.7)	

* Yate's (Continuity Correction) test
** Fisher's Exact test

In the spontaneous pregnancy group, PAPP-A and free β-hCG variables, which are thought to affect the biochemical risk, were found to have a significant effect on biochemical risk when examined together (F=42.781; p<0.001). The established linear regression model is as follows: Biochemical Risk = 3597.502 + (3078.167 * PAPP-A MoM) – (2502.192 * free β-hCG MoM). Similarly, in the IVF pregnancy group, PAPP-A and free β-hCG variables, which are thought to have an effect on the biochemical risk, were examined together and both were found to have a significant effect on the biochemical risk (F=15.502; p<0.001). The established linear regression model is as follows: Biochemical Risk = 2851.534 + (3094.213 * PAPP-A MoM) – (2012.871 * free β-hCG MoM). Again, in this study, it was revealed that PAPP-A and free β-hCG changes had a significant effect on the combined risk in both the study group and the control group (Table 4).

Table 4 Assessment of PAPP (MOM) / fß-hCG (MOM) relationships with groups' risk levels

			PAPP-A	PAPP-A MOM	fß-hCG	fß-hCG MOM
Biochemical	Spontaneous	r	*0.377*	*0.397*	*-0.611*	*-0.684*
		p	*<0.001*	*<0.001*	*<0.001*	*<0.001*
	IVF	r	*0.419*	*0.545*	*-0.610*	*-0.632*
		p	*0.006*	*<0.001*	*<0.001*	*<0.001*
Combined	Spontaneous	r	*0.347*	*0.326*	*-0.595*	*-0.642*
		p	*<0.001*	*0.001*	*<0.001*	*<0.001*
	IVF	r	*0.353*	*0.423*	*-0.654*	*-0.671*
		p	*0.022*	*0.005*	*<0.001*	*<0.001*

Spearman Correlation Test, r = correlation coefficients

DISCUSSION

Today, double test screening is a very commonly employed screening method for pregnancy follow-up, applied in gestational weeks 11-13 and used in determining the risk of aneuploidy and triploidy by combining the PAPP-A (secreted from trophoblasts) and free β-hCG (synthesized by syncytiotrophoblasts) with maternal age and NT (Gagnon & Wilson, 2008; Pakniat et al, 2019). Recent studies conducted with free β-hCG and PAPP-A, which are double test screening parameters, provide us with many data suggesting that these parameters can be used to predict gestational prognosis (American College of Obstetricians and Gynecologists, 2015; Ong et al, 2000; Wapner et al, 2003).

As prenatal screening test, maternal age alone has a sensitivity of 30%, this is 60-70% for triple-test screening and 90% for double-test screening (Oxvig, Sand, Kristensen, Gleich & Sottrup-Jensen, 1993). In high-risk pregnancies, it has been demonstrated that 40-70% of babies with Down syndrome can be detected with NT alone (Brambati, Lanzani & Tului, 1990). This is considered to be one of the superior aspects of double-test screening over triple-test screening. The importance of the NT value found with USG in the calculation of risk probability is also emphasized here. As with any screening test, these prenatal screening tests have a certain rate of false positivity. Double test screening is also said to be highly sensitive for multiple pregnancies and false positivity is reported to be slightly higher in IVF pregnancies (Overgaard et al, 2000). As with all screening tests, prenatal screening tests report the possibility of risk, indicate high-risk pregnancies in terms of congenital abnormalities and ensure the performance of further tests needed for definitive diagnosis.

PAPP-A and free β-hCG are present at certain levels in maternal blood according to certain gestational

weeks. When these biochemical parameters are examined, it hCG level measured from the maternal serum gradually decreases, while the PAPP-A levels increase. In the first trimester, the PAPP-A level in the maternal blood increases exponentially with a doubling time of 3-4 days, which continues throughout pregnancy until delivery. This increase is reported to be slower in infants with trisomy than in normal infants (Boldt et al, 2006; Haaning et al, 1996; Kristensen et al, 1994). In our study, there was a negative correlation between gestational weeks and β-hCG levels, while there was a positive correlation between PAPP-A values and gestational week. Studies have found that PAPP-A levels are lower than normal and β-hCG levels are higher in infants with abnormalities (Boldt et al, 2006; Haaning et al, 1996; Kristensen et al, 1994).

The study which included a total of 4,265 spontaneous pregnancies and 49 IVF pregnancies found that both β-hCG levels and PAPP-A levels in the spontaneous pregnancy group were lower than in the IVF pregnancy group (Marko, 2003). In another study that included 300 single IVF pregnancies and 700 single spontaneous pregnancies in total, PAPP-A was found to be significantly lower in the IVF pregnancy group than in the spontaneous pregnancy group. In this study, β-hCG levels were significantly higher in the IVF group. In another study that reported low PAPP-A and high β-hCG levels, this difference was not considered to be statistically significant (Robabeh, Maryam, Donya & Hamed, 2017). In a study by Engels et al., β-hCG was found to be significantly higher in the IVF group, but PAPP-A remained at the same rates (Engels et al, 2013). In the study conducted by Cavoretto et al., β-hCG was found to be the same in IVF and spontaneous pregnancy groups, yet PAPP-A was significantly lower in the IVF Group (Cavoretto et al, 2017). Savasi et al. found PAPP-A levels in the IVF group to be low and β-hCG levels statistically to be significantly higher (Savasi et al, 2015). There is no common conclusion reported by the studies on this subject in the literature. Regarding mean values, although we found PAPP-A to be lower in the IVF pregnancy group than in the spontaneous pregnancy group and β-hCG to be higher, these differences were statistically insignificant. When we compared the relationship between gestational weeks, PAPP-A and β-hCG levels according to CRL measurement, we found that there was a moderate increase in PAPP-A levels as CRL weeks increased in both groups. In our opinion, this was because while PAPP-A doubles every 3-4 days in the first trimester, it continues to increase throughout pregnancy until delivery, albeit at a lower rate (Smith, Bischof, Hughes & Klopper, 1979). However, although this increase was significant in the control group, it was insignificant in the study group. When we compared the free β-hCG values and the gestational weeks according to CRL measurement, it was observed that the β-hCG rate tended to decrease as the CRL week progressed in both the study group and the control group. We interpreted this to be caused by the fact that that β-hCG in the maternal serum peaks from the gestational week 8 to 10 after fertilization, starts to decrease from week 10 to 12 and decreases to the lowest level in the gestational week 16. However, although this decrease was significant in the control group, it was insignificant in the study group.

We found that there was a moderate increase in the PAPP-A level with age in the control group. In the IVF is observed that with the progression of pregnancy, the β-pregnancy group, however, we found that there was a minimal decrease in the PAPP-A level as the age of conception increased. We observed that while there was a moderate increase in β-hCG levels in the control group with age, there was an increase in the study group as well, but this increase was much less than in the spontaneous pregnancy group. Yet neither result was statistically significant. Given the very limited number of studies on this subject in the literature, the relationship between age and double-test screening parameters can be evaluated and discussed in more detail by conducting further multi-centered studies with higher numbers of samples.

In our study group, the percentage of pregnant women with high risk in terms of both biochemical and combined risk was higher than in the control group. In terms of risk, the percentage of pregnant women in the safe area was higher in the control group than in the study group. In our opinion, the reason for this was because we found that the β-hCG level in our study group to be higher than that of the control group and the PAPP-A level was lower than that of the control group. Studies have shown that double-test screening PAPP-A MoM mean value of pregnant women with Trisomy 21 was approximately 0.4 MoM lower than normal pregnancies and serum β-hCG mean value was about 2.0 MoM higher. As the study group's PAPP-A mean was low and the β-hCG mean was high, and these parameters had a significant impact on the risk calculation, the biochemical and combined risk ratios of IVF pregnant women changed in favor of the risky side.

Our contributions to the literature:

The present study has certain merits regarding its possible results as well. Given the fact that this study included those individuals in the IVF pregnancy group to whom max. one embryo was transferred as part of embryo transfer processes as it decreased the likelihood of a co-twin in early gestational weeks and missed pregnancy, and taking into account that patients with NT >2.5 mm were excluded, the present study is an important contribution to the literature. Although our study has a retrospective design, we can say that we compared a homogeneous situation. Thanks to a previous meticulous matching procedure applied to patients' age (24-34) and BMI (<30 kg/m), the distribution was equal in both groups. We analyzed all the samples with the same instrument throughout the study period and for the entire study group. We thought that using a well-configured program and evaluation of the parameters measured by the USG by the same experts of perinatology with the required level of experience and precision might result in a more accurate calculation of the risk. Moreover, we excluded many obstetric and medical conditions that could potentially affect the parameters investigated, which increased the quality of the present study. Besides the biochemical markers in the double-test screening, maternal characteristics and obstetric history are important as well. These factors are less likely to affect the outcome of the study, given the fact that pre-eclamptic and diabetic pregnancies were excluded from the study.

There were some limitations to consider in our study. Firstly, although we excluded many conditions, biochemical markers in double-test screening can still be affected by some individual pregnancy-related conditions. Secondly, this study was conducted in a single institution.

The results of this study can be enormously helpful in evaluating double-test screening results in treated pregnancies. Our findings as part of this study will be further strengthened with the support of other multi-centered studies.

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