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Comparison of Biochemical Markers Between Spontaneous Pregnancies and Frozen Embryo Transfer Cycles

Spontan Gebelikler İle Dondurulmuş Çözülmüş Embryo Transferi İle Elde Edilen Gebeliklerde Biyokimyasal Belirteçlerin Karşılaştırılması

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ABSTRACT

Aim: To investigate the difference between spontaneous pregnancy and frozen embryo transfer (FET) cycles in terms of age, BMI, PAPP-A, and free β-HCG.

Material and Method: This cross-sectional retrospective study was done on 160 participants who attended the outpatient clinic of our hospital from 2020 January to 2020 September. The participants were divided into two groups: group 1 (Spontaneous pregnancy) and group 2 (FET Cycle). All cases underwent ultrasound assessment at 11-14 weeks to measure pregnancy-associated plasma protein-A (PAPP-A) concentrations and free beta-human chorionic gonadotrophin (β-HCG).

Results: The mean age of the participants was 28.7 years (±4.5). The mean BMI of the participants was 24.3(±2.8), mean PAPP-A was 3.34(±2.3), and mean free β-HCG was 43.6(±28.36). There was a statistically significant difference between the two groups in age (P=0.000). There was no statistically significant difference between the two groups in BMI (P=0.7). The free β-HCG did not show a statistically significant difference between the two groups (P=0.2). The two groups showed a statistically significant difference in PAPP-A levels (P=0.008). There was a negative moderate correlation between age and PAPP-A (-0.21, p-value =0.007) but there was no correlation between age and free β-HCG (-0.045, p-value =0.5), between BMI and PAPP-A (-0.14, p-value =0.07) and free β-HCG (-0.1, p-value =0.1).

Conclusion: The type of pregnancy (spontaneous pregnancy and FET Cycle) did not show any significant difference in BMI and free β-HCG, but FET Cycle reduced the PAPP-A levels. The participants undergoing FET Cycle were older than those with spontaneous pregnancy. The older participants had lower PAPP-A levels. IVF pregnancies, including FET Cycle, decreased the PAPP-A levels associated with pregnancy-related complications development, nictitating the appropriate adjustment of the combined screening for IVF conceptions at first trimester.

Keywords: Spontaneous Pregnancy, Frozen Embryo Transfer (FET) Cycle, PAPP-A, free β-HCG levels

ÖZET

AMAÇ: Spontan gebelikler ile dondurulmuş çözülmüş embriyo transferi (DÇET) ile elde edilen gebeliklerde yaş, vücut kitle indeksi, pregnancy-associated plasma protein-A (PAPP-A) and serbest beta-human chorionic gonadotrophin (β-HCG) düzeylerinin karşılaştırılması amaçlanmıştır.

MATERYAL METOD: Bu retrospektif çalışma Ocak 2020 ile Eylül 2020 tarihleri arasında kliniğimizde takip edilen 160 gebeyi kapsamaktadır. Hastalar spontan gebelikler (Grup 1) ve DÇET ile elde edilen gebelikler (Grup 2) olarak iki gruba ayrılmıştır. Tüm hastalara 11-14. gebelik haftaları arasında ultrason değerlendirmesi yapılmış ve serumda PAPP-A ve serbest β-HCG düzeyleri ölçülmüştür.

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BULGULAR: Hastaların ortalama yaşı 28.7(±4.5), vücut kitle indeksi (VKİ) 24.3(±2.8), PAPP-A düzeyi 3.34(±2.3) ve serbest β-HCG 43.6(±28.36) olarak bulunmuştur. Yaş ortalamaları açısından iki grup arasında anlamlı farklılık bulunmuştur (P=0.000). VKİ ve serbest β-HCG açısından iki grup arasında anlamlı fark bulunmamıştır. (P=0.7;P=0.2). PAPP-A değerleri DÇET ile elde edilen gebeliklerde anlamlı olarak düşük bulunmuştur (P=0.008). Yaş ve PAPP-A arasında negatif ılımlı korelasyon vardır (-0.21, p-value =0.007). Ancak yaş ve serbest β-HCG arasında korelasyon bulunamamıştır (-0.045, p-value =0.5). VKİ ile PAPP-A (-0.14, p-value =0.07) ve serbest β-HCG arasında da korelasyon saptanmamıştır (-0.1, p-value =0.1).

SONUÇ: Doğal ve DÇET sonrası elde edilen gebeliklerde VKİ ve serbest β-HCG değerleri açısından fark yoktur. DÇET ile elde edilen gebeliklerde PAPP-A düzeylerinin düşüklüğü bu grubun yaş ortalamasının yüksekliği ile ilişkilendirilebilir. Bu nedenle DÇET siklusu ile elde edilen gebeliklerde etkilenmiş PAPP-A düzeyleri nedeniyle gerekli ayarlamalar yapılıp ikili test sonuçları yorumlanmalıdır.

Anahtar kelimeler: dondurulmuş çözülmüş embriyo transferi, spontan gebelik, PAPP-A, serbest β-HCG

INTRODUCTION

Since proof of Trounson and Mohr (1983) regarding the survival of frozen embryos and creation of pregnancies, several patients have used this technology for pregnancy in case of fresh transfer failure or the pregnancies after successful IVF (1). Certainly, some patients must be subjected to planned-cycle FET due to the irregularity or absence of cycles. Besides, natural cycles can be used for regularly ovulating patients (2). However, it has been found that ICSI/IVF pregnancies may confront with adverse obstetric outcomes more than the natural conceptions (3).

Despite the typical performance of IVF treatment for infertile patients with a very small chance of natural conception, it has been reported that spontaneous conception after IVF cessation has been found in 29% of the couples during 6 years after treatment completion (4) while there is a very low likelihood of spontaneous conception in any specified IVF cycle (5-7).

Biochemical markers are the serum proteins which are synthesized by the placenta (unconjugated estriol [uE3], hCG, free β-HCG, and PAPP-A) (8). Today, PAPP-A is one of the two maternal serum markers which is applied used for screening between the 11th and 14th gestational weeks, and is produced by the decidua basalis and placental syncytiotrophoblast. PAPP-A causes bioavailability of the IGF-1, intervening in the trophoblast input and modulating transport of the amino acid and glucose in the placenta (9,10). PAPP-A in the first trimester is the most important serum marker for fetal Down syndrome, in integrated and combined screening (11). PAPP-A levels are reduced along with an abnormal placental function composing the basis for the screening of fetal Down's syndrome at the first trimester (12-15). The main role of free β-HCG, produced by the syncytiotrophoblast, is to protect progesterone secretion and the corpus luteum in the first trimester (9, 11).

Women whose pregnancies are achieved using the assisted reproduction techniques (ART) are at higher risk of Down syndrome and other aneuploidies and older than the average age (16).

Maternal serum levels of PAPP-A and free β-HCG are affected by parity, maternal weight, maternal cigarette smoking, and fetal gender (17). Studies suggest that the PAPP-A level is reduced in pregnancies caused by *in vitro* fertilization (IVF) (18,19). IVF pregnancies may decrease PAPP-A or increase free β-HCG values of the largest explored group (18-20).

In some studies, a relationship is found between the unexplained increase and reduced maternal serum markers and the obstetric complications (21, 22). There is no known pathophysiological reason for the reduction of PAPP-A values following the ART pregnancies as compared to natural pregnancies. The most probable reason for the biochemical changes observed is the placental problem (8).

The present study investigated the difference between spontaneous pregnancy and FET Cycle in terms of age, BMI, PAPP-A, and free β-HCG levels.

MATERIAL AND METHOD

This cross-sectional retrospective study was done on 160 participants who attended the outpatient clinic of our hospital from 2017 to 2019. The participants were divided into two groups: group 1 (Spontaneous pregnancy) and group 2 (FET Cycle). Those who had multiple pregnancies and who had chronic diseases were excluded from the study. The informed consent was received from all of them. Ethics approval was obtained from the institutional review board. All procedures which were conducted in studies including human participants conformed to the ethical standards of the national or institutional research committee and the 1964 Helsinki Declaration and its later amendments or other ethical standards. All cases were subjected to the ultrasound assessment at 11-14 weeks to measure concentrations of PAPP-A and free β-HCG.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA) was used to perform statistics. After examining the normality of the variables with the Kolmogorov-Smirnov test, due to the abnormality of the variables, the Mann-Whitney test was used to examine the significant differences between each of the studied variables. Due to a normal distribution of the BMI variable, a t-test was used. Table 3 uses the Spearman correlation test to examine

the relationship between age and BMI with the placental peptides (PAPP-A and free β-HCG).

RESULTS

This study sample consists of 160 participants (80 patients (50%) in group 1 (Spontaneous pregnancy) and 80 (50%) in group 2 (FET Cycle) with a mean age of 28.7 years (±4.5). Table 1 shows the clinical and demographic features of the patients, including age, BMI, PAPP-A, and F-BHCG:

Table 1. Descriptive Statistics of variables

Variable	Mean	Sd	Min	Max
Age	28.7	4.5	18	39
BMI	24.3	2.8	18.4	32.6
PAPP-A	3.34	2.3	0.17	19.3
free β-HCG	43.6	28.36	10.18	189.8

Table 1 shows that the mean age of the participants is 28.7 years (±4.5). The mean BMI of the participants is 24.3(±2.8), mean PAPP-A is 3.34(±2.3), and mean free β-HCG is 43.6(±28.36).

Table 2 shows the variables of age, BMI, PAPP-A, free β-HCG in Group 1 and Group 2 :

Table 2. Variables of age, BMI, PAPP-A, free β-HCG in Group 1 and Group 2

Variable	Mean (Sd)		P-Value
	Spontaneous pregnancy (80)	FET Cycle (80)	
Age	26 (3.7)	31.5(3.6)	0.000
BMI*	24.4(2.5)	24.3(3.1)	0.7
PAPP-A	3.8(2.8)	2.8(1.6)	0.008
free β-HCG	45.2(27.8)	42.1(28.9)	0.2
*Independent Sample T-test			

Table 2 shows that the mean age of Group 1 (Spontaneous pregnancy) is 26 (3.7), and the mean age of Group 2 (FET Cycle) is 31.5(3.6). The mean BMI of Group 1 is 24.4(2.5), and the mean BMI of Group 2 is 24.3(3.1). The mean PAPP-A of group 1 is 3.8(2.8), and the mean PAPP-A of group 2 is 2.8(1.6). The mean free β-HCG of Group 1 is 45.2(27.8), and the mean free β-HCG of Group 2 is 42.1(28.9). Results show a statistically significant difference between the two groups in age (P=0.000). The two groups did not show any statistically significant difference in BMI (P=0.7). The free β-HCG did not show a statistically significant difference between the two groups (P=0.2). There was a statistically significant difference between the two groups in PAPP-A levels (P=0.008).

Table 3 shows the Non-parametric Correlations between age and two parameters (PAPP-A and free β-HCG). Also, correlations between BMI and two parameters (PAPP-A and free β-HCG) are shown in Table 3.

Table 3. Nonparametric Correlations

Variable		PAPP-A	free β-HCG
Age			
	Correlation Coefficient	-0.21	-0.045
	Sig.	0.007	0.5
BMI			
	Correlation Coefficient	-0.14	-0.1
	Sig.	0.07	0.1

Table 3 shows that there is a negative moderate significant correlation between age and PAPP-A (-0.21, p-value =0.007) but the correlation between age and free β-HCG (-0.045, p-value =0.5), BMI and PAPP-A (-0.14, p-value =0.07) and BMI and free β-HCG (-0.1, p-value =0.1) are insignificant correlations.

DISCUSSION

The present study aimed to investigate the difference between Spontaneous Pregnancy and FET Cycle in terms of age, BMI, PAPP-A, and free β-HCG to see the relationship between age, BMI, PAPP-A and free β-HCG and the type of pregnancy. The findings showed a statistically significant difference between the two groups in age (P=0.000) and a statistically significant difference between the two groups in PAPP-A levels but the two groups did not show a statistically significant difference in BMI and free β-HCG. A moderate negative correlation was found between age and PAPP-A levels.

The participants who underwent FET Cycle were older than those with spontaneous pregnancy. The PAPP-A levels in the participants with spontaneous pregnancy were higher than those with FET Cycle. The older participants had lower PAPP-A levels.

Güngör et al. (8) examined the different effects of transfer type of embryos on free β-HCG and PAPP-A levels and found that PAPP-A concentration in pregnancies conceived either with fresh or FET was not reduced significantly, which is not consistent with our study results and no significant reduction of free β-HCG concentration, which is in line with our study results.

Our study results are consistent with the study by Bellver et al. (23), who found decreased PAPP-A in the pregnancies with frozen embryos with hormone therapy without affecting the FPR. The FPR was not affected by the higher maternal serum free β-HCG levels in oocyte donation, and maternal age in natural pregnancies was significantly lower than in assisted reproduction (including frozen embryos), which is in line with our study results.

Cavoretto P et al. (24) found that free β-HCG concentration in ART pregnancies including blastocyst transfer, with or without cryopreservation, is higher than that in the spontaneous conceptions, while there is no significant difference in PAPP-A, which is not consistent with our study results.

Our study results are in line with the results of Anckaert et al. (16), who found that women whose pregnancies are achieved using the assisted reproduction techniques (ART) are older than the average (16) but not consistent with their results that median values of PAPP-A MOM of frozen cycles pregnancies are equal to those of spontaneous pregnancies.

Liao et al. (18) showed that PAPP-A level is reduced in *in vitro* fertilization (IVF) pregnancies, which is consistent with our study results, but they found that IVF pregnancies may lead to an increase of free β-HCG values of the largest explored group, which does not support our study findings.

Most studies have found the effect of the conception method on the nuchal translucency measurement, but there were some variations as compared to the spontaneous conception in analyzing the serum markers of PAPP-A and free β-HCG (16, 25, 26).

Bender et al. (27) found that both IVF and ICSI groups significantly reduced PAPP-A and significantly increased β-HCG compared to spontaneous conceptions, while our study found that IVF and spontaneous conceptions were not different in terms of β-HCG but found that IVF and spontaneous conceptions were different in terms of PAPP-A.

Our study results are consistent with the findings of Güzel et al. (3), who found similar NT and β-HCG levels and slightly lower PAPP-A in the IVF pregnancies as compared to the spontaneous conception and also found no differences between the pregnancies following frozen and fresh ET cycles in these parameters. Amor et al. (28) found that levels of maternal PAPP-A in the frozen ET cycles are lower, without stimulation of the ovaries, which is in line with our study results. Matilainen et al. (29) found that PAPP-A was not reduced with FET transfer, which is not in line with our study results.

CONCLUSION

The two pregnancy groups (spontaneous pregnancy and FET cycle) did not show any significant difference in BMI and free β-HCG, but age and PAPP-A levels were significantly different in two groups. The participants undergoing FET Cycle were older than those with spontaneous pregnancy. The older participants had lower PAPP-A levels. These patients should undergo an effective screening test with a low FPR based on the findings. IVF pregnancies, including FET Cycle, decreased the PAPP-A levels associated with the pregnancy-related complications development showing that the risk algorithm of combined first-trimester screening should be appropriately adjusted for IVF conceptions.

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