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Placenta-Associated Plasma Protein and Free Human Chorionic Gonadotropin Levels in Day 3 Versus Day 5 Transfer

3. gün ve 5. gün embryo transferinde plasenta ilişkili plasma protein ve serbest insan koryonik gonadotropoin seviyelerinin karşılaştırılması

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ÖZ

Amaç: Bu çalışma blastokist evresi embriyo transferi ile klivaj evresi embriyo transferinde plasenta ilişkili plasma protein A (PAPP-A) ile serbest insan koryonik gonadotropin B (B-hCG) arasında farklılık olup olmadığını araştırmak amacıyla yürütülmüştür.

Gereç ve yöntemler: Bu retrospektif çalışmaya toplam 449 kadın dahil edilerek iki çalışma grubu oluşturuldu. Grup 1: İntrasitoplazmik sperm enjeksiyonu yapılarak klivaj evresi (2. veya 3. gün) taze embriyo transferi sonrası gebe kalan ve 11-14. gebelik haftasında birinci trimester fetal anöploidi biyokimyasal markerlarına bakılan 275 kadından oluşmaktadır. Grup 2: İntrasitoplazmik sperm enjeksiyonu yapılarak blastokist evresi (5. gün) taze embriyo transferi sonrası gebe kalan ve 11-14. gebelik haftasında birinci trimester fetal anöploidi biyokimyasal markerlarına bakılan 174 kadın çalışma kapsamına alınmıştır. Kadınların demografik özellikleri, infertilite sebebi, infertilite süresi, protokol rejimi, total gonadotropin dozu, follikül ve toplanılan oosit sayısı, endometrial kalınlık, funsus embriyo arası mesafe kaydedildi ve PAPP-A ile serbest B-hCG düzeyleri ölçüldü.

Bulgular: PAPP-A ve serbest B-hCG düzeyleri, protokol rejimi, endometrial kalınlık ve fundus-embryo arası mesafe de istatistiksel olarak anlamlı fark bulunamamıştır. Azalmış over reservi ve total gonadotropin dozu grup 1 de ($p < 0.05$) ve follikül sayısı, toplanan oosit sayısı ve MII sayısı anlamlı olarak grup 2 de yüksek bulunmuştur ($p < 0.05$).

Sonuç: 3. gün ve 5. gün taze embriyo transferi arasında PAPP-A ve serbest B-hCG düzeylerinde fark gözlenmemiştir.

Anahtar kelimeler: PAPP-A, serbest B- hCG, 3. gün ve 5. gün taze embriyo transferi

ABSTRACT

Aim: This study was conducted to investigate placenta-associated plasma protein (PAPP-A) and free human chorionic gonadotropin (B-hCG) levels after blastocyst transfer versus cleavage-stage embryo transfer.

Materials and method: A total of 449 women were included in this rerospective sudy. The study consisted of two groups: Group 1: pregnant women conceived by intracytoplasmic sperm injection (ICSI) procedures after fresh embryo transfer at the cleavage stage (day 2 or day 3) and had first trimester fetal aneuploidy biochemical markers performed at 11-14th gestational week (n: 275). Group 2: pregnant women conceived by ICSI procedures after fresh embryo transfer at the blastocyst stage (day 5) and had first trimester fetal aneuploidy biochemical markers performed at 11-14th gestational week (n:174). Demographic characteristics, causes of infertility, duration of infertility, stimulation protocol regimens, total gonadotropin doses, number of follicles and oocytes retrieval, endometrial thickness, fundus –embryo distance were recorded and placenta-associated plasma protein (PAPP-A) and free human chorionic gonadotropin (B-hCG) levels were measured.

Results: We found no significant differences in PAPP-A and free B-hCG levels, stimulation protocols regimens and endometrial thickness and fundus-embryo distance. Diminished ovarian reserve and total gonadotropin doses were significiantly higher in group 1 ($p < 0.05$). Number of follicles, number of oocytes retrieval and MII oocytes were significiantly higher in group 2 ($p < 0.05$).

Conclusion: No difference was observed in PAPP-A and free B-hCG levels between the 3rd and the 5th day fresh transfer.

Keywords: PAPP-A, free B- hCG, 3rd -5th day fresh embryo transfer.

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INTRODUCTION

The increase in pregnancies with assisted reproductive technologies in recent years have led to more emphasis on healthy infant. Therefore, it has become more important whether there is a difference in fetal aneuploidy markers in assisted reproductive technologies pregnancies compared to spontaneously conceived pregnancies. Numerical changes in chromosomes are defined as aneuploidy, and half of the chromosomal abnormalities seen in 0.4% of births are trisomy 21, 15% are trisomy 18 and 5% are trisomy 13 (1). Maternal age is a very important factor, especially in trisomy 21, usually those who conceive with assisted reproductive technologies are older than those who conceive spontaneously; because of that, the risk of chromosomal anomaly is higher in ART pregnancies. In addition, in pregnancies after intra cytoplasmic sperm injection (ICSI), chromosomal aberrations have been shown to increase (2). Placenta-associated plasma protein (PAPP-A) and free human chorionic gonadotropin (B-hCG) are biochemical markers used in first trimester fetal aneuploidy screening. While performing risk analysis for first trimester fetal aneuploidy screening, risk analysis should be performed by considering personal factors such as maternal weight, ethnicity, fetal sex, insulin-dependent diabetes, smoking, use of assisted reproductive technologies that may affect maternal serum biochemical markers (3). Compared to natural pregnancies, the level of PAPP-A decreases in pregnancies with assisted reproductive technologies, while the level of free B-hCG increases (4). However, Orlandi F et al. reported that, there was no change in the free B-hCG level (5). Embryo transfer can be performed at cleavage stage, which refers denoting the 2nd or 3rd day after the oocyte retrieval; or at the "blastocyst stage" on the 5th or 6th day (6). There is no clear data in the literature on which embryonic developmental period for transfer is best (7). With the development of embryo culture techniques, the day of embryo transfer changes in favor of the blastocyst (8). The embryo in the blastocyst stage has a better endometrial implantation rate than in the cleavage stage (9), poor perinatal outcomes such as low birth weight, small for gestational age were seen less frequently in blastocyst transfer in previous study (10).

Conditions such as hormonal treatments used for oocyte development and endometrial receptivity or embryo culture period may have an effect on first trimester biochemical markers. The aim of our study is to investigate PAPP-A and B-hCG levels after blastocyst transfer versus cleavage-stage embryo transfer.

MATERIALS AND METHOD

This study was carried out with a total of 449 women, who applied to in vitro fertilization clinics of Etlik Zübeyde Hanım Women's Health Training and Research Hospital between 2007-2021. The present study was approved by the Ethics committee of the Etlik Zübeyde Hanım Women's Health Training and Research Hospital, Ankara, Turkey (Clinical study 16.02.2022/2022/22). The study consisted of two groups:

Group 1: pregnant women conceived by intracytoplasmic sperm injection (ICSI) procedures after fresh embryo transfer at the cleavage stage (day 2 or day 3) and had first trimester fetal aneuploidy biochemical markers performed at 11-14th gestational week (n: 275).

Group 2: pregnant conceived by ICSI procedures after fresh embryo transfer at the blastocyst stage (day 5) and had first trimester fetal aneuploidy biochemical markers performed at 11-14th gestational week (n:174). More than one embryo transfers, vanishing-twin pregnancies, multiple gestations, smoking and history of chronic disease, congenital anomalies were excluded.

Demographic characteristics (maternal and paternal ages, body mass index (BMI), gravida), causes of infertility, duration of infertility, stimulation protocol regimens, total gonadotropin doses, number of follicles and oocytes retrieval, endometrial thickness were recorded and placenta-associated plasma protein (PAPP-A) and free human chorionic gonadotropin (B-hCG) levels were measured. Free B-hCG and PAPP-A were analyzed by enzyme-linked immunosorbent assays (ELISAs). The free B-hCG assay has a detectable range from 0.5 to1350 mIU/ml. The inter- and intra-assay coefficient of variation (CV) were < %10 at concentrations greater than 3,9 mIU/ml. The PAPP-A assay has a detectable range from 1-5000 ng/m. The inter- and intra-assay coefficient of variation (CV) were < %8 at concentrations greater than 10 ng/ml.

Statistical Analysis

Statistical analyzes were performed using a package program SPSS (IBM SPSS Statistics 24). Frequency tables and descriptive statistics were used to interpret the findings and parametric methods for suitable measurement values for normal distribution. In accordance with parametric methods, the "Independent Sample-t" test method was used to compare the measurement values of two independent groups. Non-parametric methods were used for the measurement values that did not conform to the normal distribution. In accordance with non-parametric methods, the

"Mann-Whitney U" test (Z-table value) method was used to compare the measurement values of two independent groups and Pearson-χ2 cross tables were prefered for analyzing two qualitative variables.

RESULTS

Demographic and obstetric characteristics of pregnant women are given in Table 1.

Table 1. Comparison of demographic characteristics

Variables	3.day (n=275)		5.day (n=174)		Satistical analysis*
	$\bar{X} \pm S.S.$	Median [Min-Max]	$\bar{X} \pm S.S.$	Median [Min-Max]	P value
Gravida	0,52±,98	0,0 [0,0-7,0]	0,54±0,12	0,0 [0,0-8,0]	Z=-0,370 p=0,711
Living	0,11±0,43	0,0 [0,0-3,0]	0,11±0,38	0,0 [0,0-3,0]	Z=-0,569 p=0,570
Abortus	0,27±0,67	0,0 [0,0-4,0]	0,32±0,93	0,0 [0,0-8,0]	Z=-0,186 p=0,853
Maternal age (year)	30,18±4,69	30,0 [19,0-44,0]	28,98±4,24	29,0 [18,0-40,0]	Z=-2,525 **p=0,012**
Height (cm)	159,01±5,71	159,0 [146,0-179,0]	158,69±6,02	158,0 [140,0-174,0]	Z=-0,554 p=0,580
Weight (kg)	67,11±13,27	64,5 [42,0-117,0]	66,67±12,26	67,5 [41,0-108,0]	Z=-0,138 p=0,890
BMI(kg/m²)	26,52±5,11	25,8 [15,7-42,7]	26,37±4,71	26,3 [16,8-40,0]	Z=-0,063 p=0,949
Paternal age (year)	33,47±5,14	33,0 [25,0-51,0]	32,46±4,85	32,0 [23,0-51,0]	Z=-2,013 **p=0,044**

* "Mann-Whitney U" test (Z-table value) statistics were used to compare the measurement values of two independent groups that did not have a normal distribution.

We found no significant differences in gravida, living child, abortion, height, weight and BMI between the two groups (p>0.05). Maternal and paternal age were significantly higher in group 1 than in group 2 (p<0.05).

There was no significant difference in PAPP-A and free B-hCG levels between the two groups (p >0.05) (Table 2).

Table 2. Comparison of placenta-associated plasma protein (PAPP-A) and free human chorionic gonadotropin (B-hCG) levels

Variables	3.day (n=275)		5.day (n=174)		Satistical analysis*
	$\bar{X} \pm S.S.$	Median [Min-Max]	$\bar{X} \pm S.S.$	Median [Min-Max]	P value
PAPP-A	1,03±0,68	0,9 [0,1-4,8]	0,99±0,64	0,8 [0,2-3,1]	Z=-1,077 p=0,281
Free B-hCG	1,27±0,78	1,1 [0,2-5,5]	1,22±0,89	1,0 [0,3-7,4]	Z=-1,463 p=0,144

* "Mann-Whitney U" test (Z-table value) statistics were used to compare the measurement values of two independent groups that did not have a normal distribution

We found no significant differences in male factor, tubal factor, unexplained infertility, stimulation protocols, hCG and GnRH ana og doses between the groups (p>0.05) (Table 3).

Table 3. Comparison of reproductive outcomes

Variables	3.day		5.day		Satistical analysis*
	n	%	n	%	P value
Male factor					
no	164	60,5	93	54,7	χ^2=1,451
yes	107	93,5	77	45,3	p=0,228
Tubal factor					
no	252	93,0	157	92,4	χ^2=0,063
yes	19	7,0	13	7,6	p=0,802
Diminished ovarian reserve					
yes	51	32,3	17	20,0	χ^2=4,135
no	107	67,7	68	80,0	**p=0,042**
Unexplained infertility					
no	189	69,7	111	65,3	χ^2=0,950
yes	82	30,3	59	34,7	p=0,330
Stimülation protocol**					
Micro-dose flare up	7	1,8	-	-	
OC	92	23,8	65	27,0	
Long luteal	95	24,6	67	27,8	
Antagonist	163	42,2	101	41,9	
Hypo-Hypo	3	0,8	1	0,4	χ^2=10,612
Femara	3	0,8	-	-	p=0,101
Luteal E2	23	6,0	7	2,9	
hCG and GnRH doses for trigger	170	62,7	116	69,0	
6500 IU HCG	76	28,0	32	19,0	
13000 IU HCG	1	0,4	-	-	
5.000 IU HCG	3	1,2	2	1,2	
10.000 IU HCG	9	3,3	14	8,4	χ^2=10,906
Lucrın 20	5	1,8	2	1,2	p=0,091
Lucrın 20 + 1.500 IU HCG	7	2,6	2	1,2	
Lucrın 20 + 6500 IU HCG					

*"Pearson-χ2" were used to examine the relationships between two qualitative variables. **More than one answer was given to the question and the percentages were determined according to the total number of samples.

There was no significant difference in treatment cycles, duration of infertility, 10-14 mm follicle number on trigger day, endometrial thickness on trigger day, endometrial thickness on opu day, endometrial thickness on ET day and fundus – embryo distance between the groups (p>0.05). There was a significant difference in duration of ovulation induction and total doses were used between the two groups (p<0.05). Duration of ovulation induction and total doses were significantly higher in group 1 than in group 2 (p<0.05). We found significant differences in ≥17 mm follicle number on trigger day, 15-17 mm follicle number on trigger day, number of total oocytes retrieval and number of MII oocytes between the groups (p<0.05) (Table 4).

Table 4. Comparison of cycle outcomes

Variables	3.day (n=275)		5.day (n=174)		Satistical analysis*
	$\bar{X} \pm S.S.$	Median [Min-Max]	$\bar{X} \pm S.S.$	Median [Min-Max]	P value
Treatment cycles	1,66±0,98	1,0 [1,0-6,0]	1,48±0,80	1,0 [1,0-5,0]	Z=-1,766 p=0,077
Duration of infertility (month)	56,56±42,92	48,0 [3,0-288,0]	62,11±43,94	48,0 [2,0-228,0]	Z=-1,044 p=0,297
Duration of ovulation induction (day)	10,07±1,63	10,0 [6,0-18,0]	9,67±1,52	10,0 [6,0-15,0]	Z=-2,584 **p=0,010**
Total doses were used	2261,25±864,28	2025,0 [675,0-4725,0]	1938,60±661,41	1800,0 [600,0-4500,0]	Z=-3,485 **p=0,000**
≥17 mm follicle number on trigger day	2,90±2,31	2,0 [0,0-15,0]	4,08±2,75	4,0 [0,0-12,0]	Z=-4,389 **p=0,000**
15-17 mm follicle number on trigger day	3,35±2,46	3,0 [0,0-16,0]	4,39±2,77	4,0 [0,0-16,0]	Z=-4,423 **p=0,000**
10-14 mm follicle number on trigger day	5,77±4,26	5,0 [0,0-25,0]	6,53±4,54	6,0 [0,0-25,0]	Z=-1,522 p=0,128
Endometrial thickness on trigger day	10,14±1,98	9,9 [5,2-16,1]	10,54±2,05	10,4 [6,4-17,0]	Z=-1,742 p=0,081
Endometrial thickness on opu day	10,11±2,33	10,0 [4,3-18,8]	10,02±2,20	10,0 [3,6-16,0]	Z=-0,200 p=0,841
Number of total oocytes retrieval	10,42±6,12	9,0 [2,0-43,0]	13,63±6,20	13,0 [2,0-33,0]	Z=-5,941 **p=0,000**
Number of MII oocytes	7,89±4,71	7,0 [1,0-32,0]	10,65±4,93	10,0 [0,0-30,0]	Z=-6,327 **p=0,000**
Endometrial thickness on ET day	10,39±2,33	10,1 [5,1-19,3]	10,60±2,19	10,4 [6,7-17,0]	Z=-1,349 p=0,177
Fundus – embryo distance	9,02±3,83	8,6 [0,3-25,0]	9,32±4,07	9,0 [0,1-22,2]	t=-0,670 p=0,503

* "Independent Sample-t" test (t-table value) statistics were used to compare the measurement values of two independent groups with normal distribution. "Mann-Whitney U" test (Z-table value) statistics were used to compare the measurement values of two independent groups that did not have normal distribution.

DISCUSSION

This is the first study investigating PAPP-A and free B-hCG levels after blastocyst transfer versus cleavage-stage embryo transfer. PAAP-A value was lower in ART cycles compared with spontaneously conceived pregnancies. Although free B-hCG levels seemed not to be significiantly altered (11-13), some studies revealed increased B- hCG levels (14, 15). This situation causes false positive first trimester aneuploidy screening and increased chorion villus sampling (CVS) and amniocentesis rates in ART pregnancies.

Although the literature is not conclusive, in previous studies, poor perinatal outcomes such as low birth weight, small for gestational age were seen less frequently in 5th day than 3rd day transfer with higher endometrial implantation (9, 10). Unlike previous studies, considering differences between the 3rd and the 5th day transfer, present study was designed to investigate whether there is a biochemical fetal aneuploidy difference between the 3rd and the 5th day fresh transfer to contribute to reduce invasive procedures (CVS and amniocentesis).

In this study, we didn't find any significant differences in PAPP-A and free B- hCG levels between the two groups. In the present study advanced maternal age and diminished ovarian reserve were more common on the 3rd day transfer. When we reviewed the literature, number of oocytes were decreased gradually and more rapidly after age 37 years (16), and only % 3 of the oocyte pool remains at the age of 40 (17). In accordance with the literature, the duration of ovulation induction and increased gonadotropin doses use were more common in group 1. Patient with DOR are expected to respond poorly to controlled ovarian stimulation (COS), with needs high-dose gonadotropin regimen. The reason for using high-dose gonadotropin is the increased pregnancy rate that supported with some data (18, 19). However, increased doses of gonadotropins may not always be associated

with better outcomes, there are some concerns in the literature that high doses of gonadotropin can adversely affect oocyte quality (20). Although in mild stimulation, numbers of oocytes retrieval were less but their quality were found to be better (20). It has been stated that the use of high-dose FSH may increase aneuploidy by premature predivision of chromatids (21).

In the present study, we found significant differences in ≥ 17 mm and 15-17 mm follicle numbers on trigger day, number of total oocytes retrieval and number of MII oocytes. As we mentioned above, age is an important parameter in the ovarian reserve, normal ovarian reserve correlates with increased follicle and oocytes retrieval counts. Young age and not using high-dose gonadotropin may be manifested by increased number of MII oocytes.

In conclusion, we didn't find any significant differences in PAPP-A and free B- hCG levels between the 3rd and the 5th day fresh transfer, our results are needed to be validated by further studies.

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