



**Araştırma/Research**

**The effects of transfer day in intracytoplasmic sperm injection (ICSI) pregnancies on first trimester screening test results**

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**ABSTRACT**

**Objects:** To compare first trimester screening (FTS) serum markers free  $\beta$ -human chorionic gonadotropin (free  $\beta$ -hCG and pregnancy associated plasma protein A (PAPP-A) of blastocyst versus cleavage stage embryo transfers after assisted reproductive technologies (ART).

**Methods:** Retrospective examinations of 123 women who conceived with ART were subjected to FTS from January 2017 to December 2017 at Bahçeşehir University Göztepe Medical Park Hospital In Vitro Fertilization (IVF) Unit. The age, gestational week, ultrasound (US) markers and biochemistry markers (PAPP-A, free  $\beta$ -hCG) were collected. Outcomes were regarded as meaningful when the  $p < 0.05$ . Statistical Package For Social Sciences (SPSS) v.17.0 for Windows was preferred to make statistical analysis.

**Results:** PAPP-A amounts were remarkably low in day-3 (cleavage stage embryos) transfer intracytoplasmic sperm injection (ICSI) pregnancies compared to day-5 (blastocyst stage embryos) transfer ICSI pregnancies. Free  $\beta$ -hCG amounts weren't significantly different in two groups ( $p=0.371$ ). NT was unaffected by the transfer day ( $p=0.21$ ). Also CRL was unaffected by the transfer day ( $p=0.693$ ). No differences appeared between 3rd and 5th day embryo transfer according to maternal age ( $p=0.616$ ), weight ( $p=0.693$ ) and gestational age ( $p=0.742$ ) at sampling.

**Conclusion:** Today, the data about the effects of ART over the ingredients of combined FTS for chromosomal analysis are contentious yet. Day-3 ICSI pregnancies had meaningfully low PAPP-A amounts which support the requirement to properly set the combined FTS risks algorithm. These outcomes are likely to be because of changes in the placenta of ART pregnancies.

**Keywords:** Assisted reproduction techniques , blastocyst , cleavage , first trimester screening

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## İlk trimester tarama testi sonuçlarında transfer gününün intrasitoplazmik sperm enjeksiyonu (ICSI) gebeliklerindeki etkileri

### ÖZET

**Amaç:** Blastosist ve klivaj aşaması embriyo transferleri arasında ilk trimester taramasındaki serum belirteçleri olan serbest  $\beta$ - koryonik gonodotropini (serbest  $\beta$ -hCG) ve plazma ilişkili A proteinini (PAPP-A), karşılaştırmak.

**Yöntem:** Ocak 2017-Aralık 2017 tarihleri arasında Bahçeşehir Üniversitesi Göztepe Medical Park Hastanesi IVF Ünitesi'nde ART ile gebe olan 123 kadının retrospektif ilk trimester tarama testi sonuçları tarandı. Yaş, gebelik haftası, ultrason belirteçleri ve biyokimya belirteçleri (PAPP-A, serbest  $\beta$ -hCG) toplandı. Sonuçlar  $p < 0.05$  olduğunda anlamlı görüldü. İstatistiki analiz için Sosyal Bilimler için İstatistik Paketi (SPSS) 17.0 sürümü Windows kullanıldı.

**Bulgular:** PAPP-A düzeyleri klivaj gebeliklerinde blastosist gebeliklerine kıyasla oldukça düşüktü ( $p < 0.05$ ). Serbest  $\beta$ -hCG seviyeleri iki grupta anlamlı olarak farklı değildi ( $p = 0.371$ ). NT transfer gününden etkilenmedi ( $p = 0.21$ ). Ayrıca CRL, transfer gününden etkilenmedi ( $p = 0,693$ ). Örneklemede anne yaşı, ( $p = 0,616$ ) ağırlık ( $p = 0,693$ ) ve gebelik yaşına ( $p = 0.742$ ) göre 3. ve 5. gün embriyo transferi arasında fark saptanmadı.

**Sonuç:** Günümüzde, ART'ın birinci trimester kombine taramanın bileşenleri üzerindeki etkisi üzerine veriler hala tartışmalıdır. Klivaj ICSI gebeliklerinde anlamlı düzeyde düşük PAPP-A seviyeleri ART gebeliklerinin plasantasyonundaki değişikliklere bağlı olabilmektedir.

**Anahtar kelimeler:** Yardımcı üreme teknikleri, blastosist, klivaj, ilk trimester taraması

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## INTRODUCTION

The blastocyst transferences established on an advanced culture structure have been proven efficient to select embryos, which results in an increased implantation ratio besides not effecting pregnancy ratio (1). Increased implantation ratios can let less-transferring but more-qualified embryos at the blastocyst phase, in this way it can be ensured to avoid from multiple pregnancy incidences (2). Time extension of embryo culture to the blastocyst phase for ART presents a number of theoretic benefits for the transference of cleavage-phase embryos. Some of the benefits are: 1) decreasing embryos' being exposed to hyper-induced uterine circumference to minimum level, 2) providing a rather good physiologic synchronicity between embryo layer and the endometrium during embryos transference (3), 3) making the most effective and optimized election of embryos which have the higher implantation capacity, 4) boosting the probability to went through cryopreservation, and 5) decreasing uterine spasms, etc. Nevertheless, there is some particular disadvantages in blastocyst phase. By use of today's methods, several cleavage embryos can't evolve into blastocysts in vitro and cryopreservation of several blastocysts can't be ensured in good way. Hence, there is questions yet over if the transference of blastocyst-phase embryos is useful to entire patients who is not fertile (4-8).

FTS unites the mother age connected risks with the measurements of biochemical markers (free  $\beta$ -hCG and PAPP-A) and fetal nuchal translucency (NT) to estimate Down Syndrome (DS) (trisomy21), Edwards Syndrome (ES) (trisomy18) and Patau Syndrome (PS) (trisomy13) risks.

This test uses the motherhood blood amounts of free  $\beta$ -hCG and PAPP-A and the measurements of the NT and crown rump length (CRL) by US method in the period of 11+3rd and 13+6th weeks pregnancy (9). The motherhood serum amounts of PAPP-A and free

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$\beta$ -hCG are stated as multiples of the median (MoM).DS threat increases if the rate of the free  $\beta$ -hCG MoM to PAPP-A MoM increases, however reduced amounts of each markers are related with a higher threat for ES and PS.

The combine FTS has 95 percent perception ratio for DS (9), with 5 percent monitor-positive ratio (10). An increased threat, or monitor-positive combine FTS outcome shows that the defined danger based on the US and biochemistry is higher than 1 in 300. Plenty of monitor-positives are not affected from aneuploidy,for this reason they may be incorrect-positives (9).

The perception ratio can be made higher up to 95 percent, decreasing the incorrect-positive ratio to lesser than 3 percent with the inclusion of extra US markers (nasal bone, ductus venosus or tricuspid Doppler) (11,12). The scanning test elements must be set by use of several factors which can change US or biochemical markers for improving it's correctness. Some of the factors are: pregnancy and motherhood features such as pregnancy age, mother weight, ethnicity, smoke dependence, diabetes mellitus and pregnancy way (11-15).

Today, 1 to 4 percent of infants born in entire advanced countries became pregnant thanks to ART (16,17).

### **Material and Methods**

This retrospective research was confirmed within the Ethics Committee of Göztepe Medicalpark Hosp., Istanbul, Turkey (2017/13019).123 singleton pregnancies (SP) conceived by ART, who admitted combine FTS of DS from January 2017 to December 2017 in IVF center.

FTS was applied after the Fetal Medicine Foundation (FMF) principles at 11+0 to 13+6th week of pregnancy, subsequently acquiring inscribed permission.The US survey and the

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motherhood blood collection for the biochemical test were fulfilled on the same date. Merely SP were comprised in the research. Exception criteria were multiple pregnancies, SP consequence of embryo declining (inherent or stimulated), and pregnancies with structural fetal disabilities or any chromosomal deformities. Fresh embryos were included. The calculated marker grades were stated as MoM for pregnancy age after setting for mother weight.

### **Statistical analysis**

SPSS v.17.0 for Windows was preferred to make statistical analysis. The Shapiro-Wilk testing was preferred to determine the accuracy of data regulation. Meaning of differentiations of each group was defined with the use of the unparametric Mann-Whitney U-testing. Differentiations in serum levels and fetal NT were evaluated with the Kruskal-Wallis testing. Meaning was accepted at  $p < 0.05$ .

### **Results**

First chart summarizes motherhood features and pregnancy age at case exemplifications. No differences appeared between 3rd and 5th day embryo transference according to motherhood age ( $p=0.616$ ) and weight ( $p=0.693$ ). It wasn't seen any differentiation of pregnancy age in exemplification in both groups. ( $p=0.742$ ). Chart 2 shows ultrasound markers and biochemistry markers of the cases. However, day-3 transfer patients presented meaningfully low PAPP-A MoM data than day 5 transfer patients ( $p=0.031$ ). There weren't significant differentiation of free  $\beta$ -hCG amounts in both groups ( $p=0.371$ ). NT was unaffected by the transference day ( $p=0.21$ ). Also CRL was unaffected by the transference day ( $p=0.693$ ).

**Chart 1 motherhood features and pregnancy age at case exemplifications**

| Parameters  | Cleavage stage n=40 | Blastocyst n=83 | P value |
|---|---------------------|-----------------|---------|
| Motherhood age<br>Average±std.deviation                           | 32,6±4,09           | 32,18±4,44      | 0.616   |
| Motherhoodweight in exemplification (kg)<br>Average±std.deviation | 64,90±10,82         | 65,19±12,82     | 0.693   |
| Pregnancy age in exemplification (week)<br>Average±std.deviation  | 12,2±0,67           | 12,32±0,64      | 0.742   |

**Chart 2: Ultrasound markers and biochemistry markers of the cases**

| Parameters  | Cleavage stage n=40 | Blastocyst n=83 | P value |
|---|---------------------|-----------------|---------|
| <b>Free B-hCG levels (IU/L)median±std.deviation</b> | 41,19±28,91         | 48,33±34,51     | 0.371   |
| <b>PAPP-A levels(IU/L) median±std.deviation</b>     | 3,06±2,02           | 3,92±2,94       | 0.031   |
| <b>NT median±std.deviation</b>                      | 1,55±0,35           | 1,47±0,37       | 0.21    |
| <b>CRL median±std.deviation</b>                     | 58,06±7,43          | 58,75±7,96      | 0.693   |

## Discussion

Serum proteins are synthesized by placenta (PAPP-A, free  $\beta$ -hCG, hCG and unconjugated estriol (uE3) and by the fetus (alpha fetoprotein (AFP)). Motherhood serum amount of AFP in second trimester (ST) is produced by malformed alteration of the fetal liver in DS embryos

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(18), the alterations in the amounts of placenta-provided proteins weren't took into account. Recent researches have shown that the synthesis of placenta-produced proteins in DS and normal placentae has differences from cyto- to syncytiotrophoblast that is disrupted in DS placenta, pursued by a changing of the synthesis of placenta-produced proteins (19). The free  $\beta$ -hCG has been described as the preferable calculation value in scanning DS, in spite of the fact that it has not been verified in any researches which have been carried out till today (20,21). Free  $\beta$ -hCG is necessary for the maintaining of pregnancy. This serum marker first gets higher till 10th week of pregnancy and after gets lower toward term (22,23). In FT of pregnancy, increased amounts of free  $\beta$ -hCG are related to DS, and in the ST related to bad obstetric result (24,25,26). The differentiation between the two amounts of  $\beta$ -hCG is related to the effects of unfavorable pregnancies and advancing GA (26).

The amount of free  $\beta$ -hCG is low when FT IVF is compared with usual pregnancy (27), whereas in the ST free  $\beta$ -hCG is high in IVF pregnancy (28-30).

The low amount of PAPP-A observed in DS pregnancy indicates a common crisis of the insulin-like development component axis in DS pregnancy (31,32). Because IGFs are included in trophoblast incursion, alterations in the bioavailability of these hormones may be associated with the higher ratio of pregnancy problems depended on DS pregnancy. Low motherhood serum amounts of PAPP-A in DS pregnancy is not particular, because diminished amounts of PAPP-A are observed in pregnancies of pre-eclampsia and intrauterine growing delay (18,19).

Form of impregnation, culture environment and cultural circumstances are different ways to treat fertility that may have an effect on the embryos, implantation and untimely pregnancies (33). Low PAPP-A has been notified without looking if the reason of being infertile was because of the male, female, or both. The pathophysiological ground for this decrease in

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PAPP-A amounts following conceived pregnancies with assistance could still not found out. Problems relating to a placenta are the most possible explanations about the noticed biochemical changings. Each of PAPP-A and free  $\beta$ -hCG is came into being in the placenta. High ST free  $\beta$ -hCG and low FT PAPP-A concentrations which are defined in usual conceived pregnancies with assistance are coherent with biochemical marker values stated at early pregnancy terms. A late development in placenta caused by the infertility (34) is because of the medicines which are taken to stimulate ovary (35). Laboratory based manipulations, or each of the factors (in this examination) may be reasonable causes for these changings. Being infertile itself or its level may be straight connected with these outcames. Actually, Ranta et al. (34) observed that PAPP-A amounts were meaningfully decreased in natural conception pregnancies after some while to pregnancy more than two years compared with these conceived after a shorter while. This shows that infertility itself could be responsible for these biochemical changes. With the interpretation of the datas declared in singleton pregnancies obtained by ART, the higher the numbers of extra damaging factors in ART, the greater the retardation of development duration in the placenta and the higher the decrease of PAPP-A amounts.

### **Conclusion**

Consequently, some researches have put forward that low FT PAPP-A concentrations may be the sign of hazard of pregnancy problems (38), in view of the increased perinatal morbidity observed in ART pregnancy (39). Nevertheless, Amor et al. (37), and Bender et al. (40), when complicated pregnancies were excluded, still observed meaningful decreases in PAPP-A amounts in ART pregnancies compared with natural conception pregnancies. Thus IVF pregnancies show PAPP-A decreases even in the lack of pregnancy complications.

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Mentioned differentiations in the FT serum marker amounts may be accountable for increased incorrect positive outcomes causing to growing needless sprawling processes or supplementary requiremet for further usage of cell-free fetal DNA test.The usage of ovarian stimulation in a controlled way preceding IVF is advised to be the basic cause for these changings of biomarkers in ART pregnancy.

**Ethics Committee Approval:** Ethics Commitee of Göztepe Medical Park Hospitatal/İstanbul approved the study.

**Informed Consent:** Informed consent was not received on account of the retrospective content of the study.

**Conflict of interest:** Writers was not notified any conflict of interest.

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