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# Evaluation of First-Trimester Screening Biomarkers in Spontaneous (Natural) and In Vitro Fertilization Pregnancies

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**Abstract**

**Objectives:** The present study was designed to evaluate and compare the screening markers of spontaneous and in vitro fertilization (IVF) pregnancies in individuals who underwent combined screening in the first trimester of pregnancy.

**Materials and Methods:** In this retrospective study, we examined the difference between natural and assisted-reproductive technology (ART) pregnancies in 2252 mothers from January 2011 to October 2019. In both groups, the first trimester screening parameters including NT, free beta-human chorionic gonadotropin (β-hCG), and PAPP-A were measured.

**Results:** According to the results of the present study, BMI was higher in the group that became pregnant by IVF compared to the normal pregnancy group ($P<0.001$). Additionally, in patients using IVF, the history of maternal disease was less frequent compared to the other group ($P=0.003$). Most cases of twin fetuses, dichorionic twin pregnancy, and fetal abnormality were seen in the IVF group. Although the β-hCG and free PAPP-A levels were high in the IVF group, no significant difference was observed. The group with spontaneous pregnancy had a higher penetration rate of nuchal translucency <95th percentile compared to the IVF group ($P<0.001$). The results of comparing the first trimester test showed that in the IVF group, 81.3% of patients were at low risk and 6.6% were at high risk, while in the normal pregnancy group, 78.9% of patients were at low risk and 7.0% of them were at low risk, indicating that the difference was not significant.

**Conclusions:** Overall, although the risk of aneuploidy in IVF pregnancies was not higher compared to normal pregnancies, in IVF pregnancies, NI is associated with a high risk of fetal abnormalities. Therefore, NT sonography can help to diagnose fetal anomalies especially aneuploidy.

**Keywords:** IVF, first-trimester screening, free β-HCG, PAPP-A, NT

## Introduction

The use of ultrasound markers and maternal serum samples during pregnancy are two important methods for screening along with other maternity care worldwide. Maternal age, nuchal translucency (NT), free beta-human chorionic gonadotropin (β-hCG) level, and maternal serum level of PAPP-A in the first trimester of pregnancy are the main tools in diagnosing Down syndrome and other trisomies (1). Based on the results of studies, the rate of false-positive results of this method are reported to be about 5% and approximately 90% of trisomy 21 cases can be detected by this method.

The assisted-reproductive technologies (ART) annually lead to a large number of pregnancies. The age of the women who undergo assisted reproductive treatment for their pregnancies is higher than the average age. Therefore, the risk of Down syndrome and other aneuploidies is higher in them (2, 3). Based on various research works, there is an association between pregnancies achieved by ART and alteration in the first trimester screening biomarkers, which influences the assessment of the risk of Down syndrome (4-6). The application of an efficient screening test with a low rate of false-positive results is needed in these patients. It has been shown that the combination of such parameters as NT, mother's age, β -hCG, and PAPP-A in first-trimester screening helps identify almost 85%–90% of trisomy 21 cases and other aneuploidies, with a screen positive rate of 5%–6% (1,7). The reduced maternal serum level of PAPP-A and normal or higher β-hCG levels in vitro fertilization (IVF) pregnancies are the most consistent results in these research works, which was found by a new meta-analysis in this regard (2,6,8). However, findings of some other studies showed that there is not any difference between IVF and natural pregnancies in terms of these biomarkers (9,10). The inconsistency among the research findings is apparently due to some confounding factors, including adverse obstetric outcomes, maternal age, and non-homogeneous patient populations. Hence, the present research attempted to examine the probable impact of ART on first-trimester screening for aneuploidy through controlling fetal NT and PAPP-A levels and the maternal serum concentration of free β-HCG.

## Patients and Methods

The population of this study included pregnant women in the first trimester of pregnancy who became pregnant

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**Key Messages**

- There was no difference in the risk of aneuploidy between normal and IVF pregnancies.
- In IVF pregnancies, NT levels were associated with a high risk for fetal abnormalities.
- There was no difference in biochemical markers of aneuploidy between normal and IVF pregnancies.

spontaneously and those who used assisted reproductive techniques. Assisted reproductive techniques included ovulation induction (OI), intrauterine insemination (IUI), and IVF. The mentioned samples who referred to a sub-specialized center in Ahvaz from January 2011 to October 2019 were examined

All patients referred during this period were included in the study. Participants who were dissatisfied with the study and cases whose information was incomplete were excluded from the study. Totally, 2252 mothers were evaluated, including 1879 spontaneous and 373 IVF pregnancies.

Data were entered into a database containing demographic information, ultrasound findings, and biochemical test results which were examined and specific hazards were identified.

Ultrasound specialists certified by the Fetal Medical Foundation evaluated fetal NT in the first trimester of pregnancy using the abdominal ultrasound methods like the maximum vertical distance between the skin and subcutaneous tissues of the back of the neck in a sagittal section of the fetus in the neutral position. Screening for chromosomal abnormalities was done using three methods: maternal age, fetal NT, and concentrations of β-HCG and PAPP-A.

For the assessment of aneuploidy (trisomy 21, trisomy 18, and trisomy 13), we used amniocentesis and chorionic villus sampling and subsequent karyotyping or cell-free DNA test.

### Statistical Analysis

Data analysis was performed using GraphPad Prism version 7. Descriptive statistics were used to describe the demographic characteristics, which included maternal age, body weight, gestational age, and BMI (mean ± standard deviation). Mean, SD, and percentile were also used to describe the MoM (multiple of median) screening markers of the first trimester (NT, β-hCG, and PAPP-A). *T*-test and Mann-Whitney U test were used to express the monthly mean differences between spontaneous and IVF pregnancies. The relationship between variables in the first-trimester screening test was performed using bivariate Pearson correlation test and linear regression analysis. $P<0.05$ was considered statistically significant.

## Results

According to the results of the study, there was no significant difference between the mean age of the IVF pregnancy group and the normal pregnancy group ($P>0.05$). The mean weight of patients in the IVF pregnancy group was lower than the other group but BMI was higher ($P<0.001$) (Table 1).

### Clinical Characteristics

The frequency of cases with a history of maternal disease among IVF group was statistically less than those with normal pregnancy ($P=0.003$).

In contrast, the prevalence of twin fetuses, dichorionic twins, and fetal abnormalities was higher in the IVF group (Table 2).

### First Trimester Screening Biomarkers in Spontaneous and IVF Pregnancies

The mean β-hCG and free PAPP.A levels in the IVF pregnancy group were higher but not significantly. In the normal pregnancy group, the penetration rate of NT was less than 95th percentile compared to the IVF group ($P<0.001$) (Table 3).

**Table 2.** Clinical Characteristics of the Participants in the Two Groups

| Variables | Spontaneous Pregnancy | IVF Pregnancy | *P* Value |
|---|---|---|---|
| History of maternal disease | | | 0.003 |
| Yes | 238 (22.1%) | 15 (11.1%) | |
| No | 837 (77.9%) | 121 (89.9%) | |
| *P* value | <0.0001 | <0.0001 | |
| Fetus | | | <0.001 |
| Single | 1862 (99.1%) | 354 (94.9%) | |
| Twin | 17 (0.9%) | 19 (5.1%) | |
| *P* value | <0.0001 | <0.0001 | |
| Chorionicity | | | 0.010 |
| Monochorionic | 9 (52.9%) | 2 (10.5%) | |
| Dichorionic | 8 (47.1%) | 17 (89.5%) | |
| *P* value | 0.157 | 0.001 | |
| History of fetal trisomy[a] | | | 0.101 |
| Yes | 25 (2.3%) | 0 (0%) | |
| No | 1059 (97.7%) | 136 (100%) | |
| *P* value | <0.0001 | - | |
| Anomaly in sonography[b] | | | <0.001 |
| Yes | 2 (0.1%) | 15 (3.3%) | |
| No | 537 (99.9%) | 439 (96.7%) | |
| *P* value | <0.0001 | <0.0001 | |

SD: Standard deviation, BMI: Body mass index, IVF: In vitro fertilization.
[a] Trisomy 21, trisomy 18, or trisomy 13.
[b] Any structural anomaly reported in the first-trimester ultrasound.

**Table 1.** Demographic Characteristics of the Participants in Two Groups (Mean ± SD)

| Variables | Spontaneous Pregnancy | IVF Pregnancy | *P* Value |
|---|---|---|---|
| Number | 1879 | 373 | |
| Age | 30.13 ± 4.86 | 30.17 ± 5.53 | 0.885 |
| BMI | 26.17 ± 4.43 | 27.18 ± 4.28 | <0.001 |
| Gravidity | 1.91 ± 1.07 | 1.50 ± 0.87 | <0.001 |

BMI, Body mass index; IVF, In vitro fertilization.

### Relationship Between First Trimester Screening Biomarkers and Clinical Characteristics

In screen-positive cases, the mean Free β-hCG level was significantly higher compared to screen-negative cases ($P=0.016$). The mean PAPP-A level was higher in single pregnancy than in twin pregnancy ($P=0.071$). However, the mean PAPP-A level in cases with a positive history of fetal trisomy was lower than in cases with a negative history of fetal trisomy. Table 4 shows the association between NT and independent variables. NT less than 95th percentile was more likely among cases with a history of maternal disease ($P=0.009$) and single fetus ($P<0.001$) (Table 4).

### Distribution of the Risk for Aneuploidy

In the IVF group, 81.3% of patients were at low risk and 6.6% were at high risk. Additionally, in the group with normal pregnancy, 78.9% were at low risk and 7% were at high risk, which was not a significant difference ($P=0.609$) (Table 5).

### Demographic Characteristics According to Risk Factor

The distribution of independent variables and covariates in three risk categories is presented in Table 6. It was shown that mean age, mean gravidity, and mean BMI were statistically lower among low-risk groups of cases ($P<0.001$). Moreover, the rate of anomaly was statistically high in the high-risk group ($P<0.001$) (Table 6).

**Table 3.** First Trimester Screening Biomarker in Spontaneous and IVF Pregnancies

| Variables | None IVF | IVF | *P* Value |
|---|---|---|---|
| Free β-hCG | 1.19 ± 0.89 | 1.21 ± 0.69 | 0.822 |
| PAPP-A | 1.18 ± 0.68 | 1.31 ± 0.93 | 0.060 |
| NT | | | |
| (>95$^{th}$ centile) | 102 (5.4%) | 61 (16.4%) | <0.001 |
| (<95$^{th}$ centile) | 1777 (94.6%) | 312 (83.6%) | |

SD: Standard deviation, BMI: Body mass index, IVF: In vitro fertilization, NT: nuchal translucency.

### First Trimester Screening Biomarkers according to Risk Factor

The rate of fetal malformations was higher in the high-risk group than in the low-risk and moderate groups. There was no difference between the three groups in terms of maternal disease and history of fetal trisomy in previous pregnancies (Table 7).

## Discussion

The findings of the present research indicated a significantly higher BMI in the IVF pregnancy group compared to the natural pregnancy group ($P<0.001$). As found by Rittenberg et al, the live birth rate using ART was lower in women having a high BMI than women who had a normal BMI (11). Various other studies, which evaluated the effect of BMI on ART results, supported this finding (12-14). Mortality, genital diseases, infertility, and amenorrhea are significantly related to obesity. Moreover, the probability of experiencing menopausal disorders in obese women is three times higher compared to women with normal weight. The impact of obesity on the quality of eggs, embryo growth, implantation rate, and number of mature eggs has been reported in various studies. The success rate of assisted reproductive techniques is lower in obese women compared to women with normal BMI, as shown by different research works. It can be justified by the relationship between obesity and lower estradiol levels and the lower number of fertilized oocytes (15-17).

In this study, the birth rate of twins was significantly higher in the IVF pregnancy group compared to the natural pregnancy group ($P=0.003$). A total of 582 normal pregnancy cases and 2414 ART pregnancy cases were studied by Levi Setti et al, and it was found that twin and triplet birth rates were significantly higher in the ART pregnancy group compared to the normal pregnancy group (18). Approximately 77% of triplet and higher-order births (25% IVF birth and 52% non-IVF birth) and 40% of twin births (19% IVF birth and 21% non-IVF birth) are estimated to be accounted for assisted birth (19,

**Table 4.** The Association of Free β-hCG and PAPP.A with Independent Variables

| | | PAPP.A | Free β-hCG | NT (>95th Percentile) | NT (<95th Percentile) |
|---|---|---|---|---|---|
| History of maternal disease | No | 1.21 ± 0.72 | 1.19 ± 0.86 | 86 (9%) | 872 (91%) |
| | Yes | 1.16 ± 0.70 | 1.21 ± 0.90 | 10 (4%) | 243 (96%) |
| | *P* value | 0.269 | 0.836 | 0.009 | |
| Fetus | Single | 1.19 ± 0.72 | 1.19 ± 0.88 | 153 (6.9%) | 2063 (93.1%) |
| | Twin | 1.41 ± 0.60 | 1.20 ± 0.70 | 10 (27.8%) | 26 (72.2%) |
| | *P* value | 0.071 | 0.962 | <0.0001 | |
| History of fetal trisomy[a] | No | 1.22 ± 0.68 | 1.26 ± 0.97 | 93 (7.8%) | 1102 (92.2%) |
| | Yes | 0.87 ± 0.58 | 1.02 ± 0.72 | 3 (12%) | 22 (88%) |
| | *P* value | 0.001 | 0.130 | 0.438 | |
| Anomaly in sonography[b] | No | 1.31 ± 0.7 | 0.86 ± 0.39 | 26 (4.8%) | 519 (95.2%) |
| | Yes | 1.45 ± 0.56 | 1.35 ± 0.76 | 11 (6.1%) | 169 (93.9%) |
| | *P* value | 0.582 | 0.016 | 0.479 | |

[a] Trisomy 21, trisomy 18, or trisomy 13.

[b] Any structural anomaly reported in the first-trimester ultrasound.

**Table 5.** Distribution of the Risk for Aneuploidy

| Category | Spontaneous Pregnancy | IVF Pregnancy | *P* Value |
|---|---|---|---|
| Low | 840 (78.9%) | 74 (81.3%) | 0.609 |
| Intermediate | 150 (14.1%) | 11 (12.1%) | |
| High | 74 (7.0%) | 6 (6.6%) | |
| *P* value | <0.0001 | <0.0001 | |

**Table 6.** The Association of Risk and Independent Variables

| Parameter | Low | Intermediate | High |
|---|---|---|---|
| Age | 29.79 ± 4.32 | 33.66 ± 5.20[a] | 33.61 ± 4.45[a] |
| BMI | 25.86 ± 4.28 | 27.36 ± 4.25[a] | 27.55 ± 4.53[a] |
| Gravidity | 1.97 ± 1.14 | 2.49 ± 1.41[a] | 2.43 ± 1.24[a] |

[a] Significant difference with Low risk group.

20). According to the findings of recent studies, maternal age, young oocyte age, number of high-quality embryos transferred, treatment period, obesity, and higher-quality of embryos transferred are associated with a higher possibility of twin birth after IVF (21-24).

In our study, the rates of congenital abnormalities and dichorionic twins were higher in the IVF group compared to the natural pregnancy group. Dizygotic (DZ) twins are dichorionic (DC), while monozygotic (MZ) twins might be DC or monochorionic (MC). Regardless of some deficiencies resulting from intrauterine crowding such as deformities in foot, skull asymmetry, and hip dislocation, the abnormality rate in singletons is similar to that in DZ twins, while the abnormality rate in MZ twins is 2-3 times higher. In addition, mortality rates among MZ twins are higher. These higher risks have been indicated to be limited to MC MZ twins, while DC MZ and DZ twins show similar results (25-27).

Due to double-embryo transfer, most of ART twin pairs are DZ. However, MZ twinning from embryo splitting is concerned in ART twin conceptions too. More recent studies have reported higher MZ twinning rates (1%–5%) among ART conceptions compared to the rates seen in normal conceptions (0.4%) (28-30). The age of infertile couples, the main cause of infertility, the medicines taken for induced ovulation or for maintaining the pregnancy in the first months, as well as the procedure-related factors, such as the delayed fertilization, the embryo freezing, and defrosting, and the polyspermic fertilization potential are among the factors that increase the risk of birth abnormalities (31).

It is theoretically logical to assume that IVF directly influences the first-trimester screening, and it is especially affected by the presence of multiple corpora lutea and hormonal treatments. Hence, evident serum marker alterations can be expected during the first trimester. These changes are caused by this treatment (32). However, IVF and natural conceptions showed no significant differences in terms of the PAPP-A levels in the first trimester in the present study. According to previous studies, ART protocols did not influence PAPP-A serum levels in the first trimester (10,32). Following IVF, some impacts on serum markers were found in the first trimester, i.e., reduced PAPP-A level (9,33,34). Decreased PAPP-A levels indicate a decrease in insulin growth factor in Down syndrome pregnancy (35,36).

Given the involvement of IGFs in trophoblast invasion, it is possible to associate the alterations in the bioavailability of these hormones with the higher prevalence of pregnancy complications related to Downs' syndrome conceptions. The reduced maternal serum level of PAPP-A in Down's syndrome conceptions is not specific since low PAPP-A levels are also observed in pre-eclampsia and intrauterine growth retardation pregnancies (37,38).

The β-hCG is known as one of the biochemical markers in the first-trimester screening. Despite the increase or reduction of β-hCG MoM levels in ART conceptions in comparison with control groups reported by some research works (5,6,39,40), most studies, as well as the present study, did not show any difference between ART and natural conceptions in terms of β-hCG levels (2,4,9,41). The inconsistency among the findings is due to small sample sizes, the heterogeneous research populations, and different levels of β-hCG at different pregnancy weeks (4,41). For a long time, free β-hCG has been utilized as a chemical marker in early pregnancy,

**Table 7.** The Association of Risk and Independent Variables

| Parameter | | Low | Intermediate | High | *P* Value |
|---|---|---|---|---|---|
| History of maternal disease | No | 721 (80.2%) | 120 (13.3%) | 58 (6.5%) | 0.248 |
| | Yes | 191 (75.4%) | 41 (16.2%) | 21 (8.3%) | |
| | *P* value | <0.0001 | <0.0001 | <0.0001 | |
| History of fetal Trisomy[a] | No | 900 (79.4%) | 157 (13.8%) | 77 (6.8%) | 0.288 |
| | Yes | 14 (66.7%) | 4 (19%) | 3 (14.3%) | |
| | *P* value | <0.0001 | <0.0001 | <0.0001 | |
| Anomaly in sonography[b] | No | 444 (96.3%) | 63 (82.9%) | 29 (72.5%) | <0.001 |
| | Yes | 17 (3.7%) | 13 (17.1%) | 11 (27.5%) | |
| | *P* value | <0.0001 | <0.0001 | 0.004 | |

[a] Trisomy 21, trisomy 18, or trisomy 13.

[b] Any structural anomaly reported in the first-trimester ultrasound.

and the free β-subunit has been identified as having a higher predictive value for Down's syndrome. However, not all studies have confirmed this claim (42,43). Free β-hCG, a vital marker for pregnancy maintenance, is generated by trophoblast. It is assumed that the hCG level in early pregnancy is representative of the mass of syncytial trophoblast (44). For the purpose of screening Down's syndrome, measurement of free β-hCG is done at 8–14 weeks of pregnancy. The high free β-hCG levels in the first trimester are related to Down's syndrome, and they are associated with poor obstetric outcomes in the second trimester (38). The free β-hCG level is lower in the first trimester in IVF pregnancies compared to natural conceptions. However, the level of free β-hCG in the second trimester is higher in IVF conceptions (44,45).

The single ultrasound marker in the first trimester combined screening procedure is NT. As indicated by the current research, NT thickness is different in ART conception and natural conception. These findings are in contrast to the findings of other research concerning the thickness of NT in ART pregnancies. Previous studies did not report any significant difference in the NT in ICSI, IVF conceptions, or ovulation induction (10,32). According to a study conducted in Italy, which consisted of 32 IVF and 42 ICSI conceptions, there was not any difference in NT (9). In a cohort study conducted in Israel, with a smaller sample size, a non-significant rise was found in NT measurements (46,47). Nevertheless, the finding of the present study is consistent with a previous study indicating increased NT in IUI conceptions compared to natural conceptions (48). The basic pathophysiology for higher NT thickness is not still clear. The mechanisms reported for this include changed the composition of the extracellular matrix of the nuchal skin, cardiac failure, and delayed or abnormal growth of the lymphatic system (49-51). There is an association between increased NT and syndromal, chromosomal, and structural anomalies (52,53).

## Conclusions

To sum up, there was no significant difference in β-hCG and PAPP.A levels between the two groups with IVF and spontaneous pregnancies, but there was a significant difference in NT measurements. On the other hand, there are significant differences between IVF pregnancies and spontaneous pregnancies. It can be concluded that the risk of aneuploidy in IVF pregnancies is not higher compared to normal pregnancies, However, In IVF pregnancy, NT was associated with a high risk for fetal anomalies. Therefore, NT sonography can help to diagnose fetal anomalies, especially aneuploidy.

### Authors' Contribution

Study concept and design: MB and MZ; analysis and interpretation of data: MB, MZ, and BA; drafting of the manuscript: BA; critical revision of the manuscript for important intellectual content: MB and MZ.

### Conflict of Interests

Authors have no conflict of interests.

### Ethical Issues

This study was approved by the Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (ethics code: IR.AJUMS.REC.1398.780).

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