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Exploring the association of male-factor related infertility with first-trimester anomalies, aneuploidy, and biochemical markers in infertile patients undergoing ART

Mahvash Zargar, Batool Asarzadeh, Mojgan Barati

Department of Obstetrics and Gynecology Fertility, Infertility and Perinatology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Correspondence

Dr. Batool Asarzadeh, Department of Obstetrics and Gynecology; Fertility, Infertility and Perinatology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. phone: 9861333615, fax: 9861333614, email address: b.assarzadeh@ajums.ac.ir

Abstract

Background: A higher risk of birth defects in assisted reproductive technologies (ART) pregnancies compared to natural pregnancies has been previously reported.

Objective: This retrospective study aimed to explore the association of male-factor related infertility with first-trimester anomalies, aneuploidy, and biochemical markers in infertile patients undergoing ART.

Method: The complete medical records of infertile patients referred to the infertility and prenatal centers in Ahvaz, Iran during the past five years were assessed, which included ART-induced pregnancies due to male factors (n=124) and or other infertility causes (n=176).

Results: The most common causes of infertility were respectively the male factor (41.3%), ovulation disorders (26%), and unexplained factors (13.3%). A significant correlation was found between the infertility causes and the history underlying systemic diseases and medication history ($p<0.05$). The serum levels of MoM β-hCG and PAPP-A were significantly lower in the male factor-related infertilities than non-male factor-related infertilities ($p=0.0001$). The rate of fetal and placental anomalies was significantly higher in male factor-related infertilities than non-male factor-related infertilities ($p=0.03$). However, according to the results of multivariable logistic regression, association between the male factor-related infertility and fetal/placental anomalies was not independent of the effects of low levels of MoM β-hCG and PAPP-A.

Conclusion: Causes of infertility, particularly male factor, together with low levels of MoM β-hCG and PAPP-A, might be associated with the high risk of fetal and placental anomalies in ART pregnancies. However, further large-scale multi-center and prospective investigations are needed to reach more accurate conclusion.

Key words: Infertility, Assisted reproductive technology, Male, Fetal Anomalies, Placental, beta-human chorionic gonadotropin, Pregnancy-Associated Plasma Protein-A

What does this study add to the clinical work?

To date, contradictory results have been reported by a few studies regarding the association between the congenital anomalies resulting from ART treatments with the male factors-related infertility. Our study is one of the rare studies indicating that the male causes of infertility as well as low levels of MoM β-hCG and PAPP-A might be associated with the high risk of fetal and placental anomalies in ART pregnancies.

Acronyms

ART: Assisted reproductive technology, ICSI: intracytoplasmic sperm injection, IVF: in vitro fertilization, fβ-hCG: free beta-human chorionic gonadotropin, PAPP-A: pregnancy-associated plasma protein-A, NT: nuchal translucency, BMI: body mass index, MoM: Multiples of the Median for free β-hCG, PAPP-A

Introduction

Infertility affects at least 180 million couples worldwide[1]. There are many possible causes of infertility, which are classified as male factors, female factors, a combination of male and female factors, or unexplained causes[2]. Also, environmental and lifestyle risk factors such as environmental pollution, obesity, smoking, excessive alcohol intake, and psychological stress have been associated with infertility[2].

A great deal of evidence has indicated a high risk of birth defects such as congenital anomalies in infants conceived by intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF), compared to the babies born from the natural pregnancy[3,4]. In this regard, the results of the first trimester screening using biochemical markers [e.g., free beta-human chorionic gonadotropin (fβ-hCG), pregnancy-associated plasma protein-A (PAPP-A)] and the measurement of the fetal nuchal translucency (NT) showed several variations in the levels of these factors caused by ART in comparison with the natural pregnancies[5,6].

The health and lifestyle of parents play important roles in the increase of congenital malformations in couples who use ART[7]. Age, obesity, metabolic disease, diabetes, and hypertension in mothers are associated with congenital malformations in ICSI/IVF pregnancies[8-11]. Congenital anomalies such as Down syndrome and trisomy cause many economic, social, and cultural problems for couples. Thus, the early detection of anomalies in the first trimester of pregnancy and timely termination of pregnancy can prevent these complications[12].

Despite several studies on ART-induced pregnancies and birth defects, there is relatively little information about the association between the causes of infertility and ART related birth defects. Accordingly, this study evaluated the results of the anomaly scan and the aneuploidy screening tests in the first-trimester of pregnancy in infertile cases based on infertility-related female/male factors. Aim of this study was exploring the association of male-factor related infertility with first-trimester anomalies, aneuploidy, and biochemical markers in infertile patients undergoing ART.

Method

Study design

Data related to our retrospective study were ob-

tained from the medical records belonging to the patients who referred to the infertility and prenatal centers of Ahvaz, IRAN during the past five years and was approved by Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran with Ethical Code: IR.AJUMS.REC.1398.663.

Patient selection criteria

This study included 326 ART-induced pregnancies due to male factors and other infertility causes. But couples who chose the IVF method for gender selection and those with incomplete documentation were excluded from the main analysis.

Methods and Variables

All available characteristics of patients including demographics (age, body mass index [BMI]), gravidity, parity, medical and obstetric histories, NT ultrasound, PAPP-A, free β-hCG, results of aneuploidy screening test (cell-free DNA test), and scan anomalies were extracted from their medical records. All ART-induced pregnancies were classified into two groups based on infertility-related female/male factors, including male factor-related infertilities and non-male factor-related infertilities. The results of cell-free DNA screening test were evaluated as negative/low to high-risk and or unidentified populations to confirm high-risk pregnant women. Moreover, the results of genetic amniocentesis were extracted and evaluated. Eventually, the results of aneuploidy screenings, fetal and/or placental anomalies, and biochemical tests in ART-induced pregnancies were compared between two groups.

Outcomes

Distribution of fetal and placental anomalies based on various causes of infertility were the main outcomes. Also, changes in biochemical markers based on various causes of infertility were the secondary outcomes.

Statistical analysis

The quantitative variables were compared between two studied groups using a t-test, Mann-Whitney test based on their normality status. The qualitative variables were evaluated by Chi-square test. Moreover, univariate and multivariate logistic regression analyses were used to assessing the association between fetal and placental anomalies with other variables. $P < 0.05$ is considered as statistically significant and the data were analyzed by SPSS version 26 (SPSS Inc., Chicago, Ill., USA).

Results

From a total of 326 cases, only 300 patients aged 18-49 years (31.33 ± 5.19) with a history of infertility and ART were included into the study and 26 cases were excluded from analysis due to ineligibility (Figure 1). All eligible cases were evaluated and divided into two groups according to the cause of infertility, including male factor related infertilities (n= 124) and non-male factor-related infertilities (n= 176). Moreover, male factor related infertilities were also classified into five subgroups based on male causes of infertility, including oligozoospermia plus asthenozoospermia [n=40 (32.3%)], sperm morphology defects or Teratospermia [n=34 (27.4%)], varicocele [n=31 (25%)], primary testicular defects [n=13 (10.5%)], and normospermia with functional defects [n=6 (4.8%)].

Demographics and Obstetric history

There was no significant difference between the two groups in terms of the mean age, height, weight, BMI, and obstetric history [i.e., gravity, parity, abortion, and duration of infertility] ($P>0.05$). Also, these demographic and obstetric characteristics did not differ between infertile groups due to various causes ($P>0.05$). But the systemic underlying diseases [i.e., thalassemia minor, hypertension, asthma, hypothy-

roidism, diabetes, kidney disease, epilepsy, and musculoskeletal disorders] were significantly more prevalent in non-male factor-related infertilities (68.5 %), compared to male factor group (31.5 %, P: 0.0001, Figures 2). History of the use of specific medicines and systemic diseases were significantly correlated with the causes of infertility (p= 0.0001, Table1). Most patients with a history of systemic underlying diseases and/or those who had used special drugs had infertility caused by the ovulation disorders (37%), male factor (31.5%) and unexplained factors (13%), respectively.

Hypothyroidism (50 [31.4 %]), thalassemia minor (28 [17.6 %]) and diabetes (24 [15.1%]) were the most prevalent underlying diseases in patients, respectively. Levothyroxine was also the most common medicine used by patients (97 [60 %]). (Table1).

Polyhydramnios was detected in two cases, one in the male factor group and the other in non-male factor related infertility group. Also, one case of pyelectasis was identified in the male factor group. No significant correlation was found between the polyhydramnios and/or pyelectasis and the causes of infertility (p>0.05). (Table1).

The most common causes of infertility were respectively the male factor (41.3%), ovulation disorders (26%), unexplained factors (13.3%), uterine factors (9.7 %), obstruction and adhesion tube injuries (8%), and decrease in ovarian reserves (1.7%), respectively (Figure 3, Table 2).

Association of NT, free β-hCG, and PAPP-A with various causes of infertility

No significant difference in NT results has been found between the male and non-male factor-related infertilities (p=0.14, Table 1). The mean levels of free β-hCG and MoM values (Multiples of the Median) for PAPP-A were lower among the male factor-related infertilities than non-male factor-related infertilities, but not significantly so (p=0.07). Only the mean levels of free PAPP-A and MoM β-hCG were significantly different between the two groups (p=0.0001). (Table1).

On the other hand, comparison of different factors based on six causes of infertility showed no significant association between the various causes of infertility and NT, MoM PAPP-A, and free β-hCG (p>0.05). However, MoM β-hCG was at its highest level in the group with reduced ovarian reserves but at its lowest level in those with male factor-related infertility and/or ovulation disorders (p=0.0001). Moreover, the level of free PAPP-A was at its highest level in the group with reduced ovarian reserves and tubular injuries, obstruction, adhesions but at its lowest level in those with male factor-related infertility (p=0.0001). (Table 2).

In another aspect, these variables were compared based on five male factors of infertility; the results indicated that there was no significant difference in the mean levels of NT, free β-hCG, MoM β-hCG, PAPP-A, and MoM PAPP-A between different male causes of infertility (p>0.05, Table 3).

The length of infertility in two infertility groups, i.e., the decreased ovarian reserves and/or unexplained factors, was significantly longer than other groups (p= 0.0001, Table 2). Whereas, no significant association was found between the male causes of infertility and length of infertility (P=0.08, Table 3).

Association of fetal and placental anomalies with various causes of infertility

A total of 12 cases were diagnosed with fetal and placental abnormalities in ultrasound, including 11 fetal abnormalities and one placental anomaly (Twin-to-twin transfusion syndrome) (Table 4). A significant correlation has been found between the causes of infertility and fetal anomalies (p=0.03), as 75 % of anomalies were found in the male factor-related infertility group. Nonetheless, according to the results of multivariable logistic regression, association between the male factor-related infertility and

Table 1: Demographic and clinical characteristics of the evaluated groups

Variables	Male factor-related nfertility (N=124)	Non-Male factor-related infertility (N=176)	P-value
Age (mean±SD)	31.44±4.55	32.55±5.74	0.28
Weight (mean±SD)	71.11±11.47	69.33±8.1	0.92
Height (mean±SD)	164.22±5.47	161.83±4.69	0.56
BMI (mean±SD)	26.62±3.71	26.45±2.37	0.06
Gravidity (N, frequency)	51 (41.1 %)	87 (49.4 %)	0.1
Parity (N, frequency)	17 (13.7 %)	31 (17.6 %)	0.4
Length of Infertility, years (mean±SD)	4.73± 0.96	4.8± 0.5	0.26
History of underlying systemic disease (N, frequency)	39 (31.5 %)	120 (68.2 %)	0.0001
History of the use of special medicines (N, frequency)	40 (32.2 %)	122 (69.3 %)	0.0001
Abortion (N, frequency)	47 (37.1 %)	81 (46 %)	0.2
NT results (N, frequency):			0.14
Low Risk	92 (74.2%)	146 (83%)	
Intermediate Risk	31 (25%)	28 (16%)	
High Risk	1 (0.8%)	2 (1%)	
Free β-hCG, IU/L (mean±SD)	34.84 ± 10.87	37.3 ± 11.6	0.07
MoM β-hCG (mean±SD)	1.03± 0.34	1.21±0.4	0.0001***
PAPP-A (mean±SD)	2.05 ± 0.46	3.01 ± 0.84	0.0001***
MoM PAPP-A (mean±SD)	0.97± 0.32	1.06±0.43	0.07
Down Syndrome (N, frequency)	0	0	-
Trisomy 13 (N, frequency)	1	0	0.24
Trisomy 18 (N, frequency)	0 (0.0%)	0 (0.0%)	0.41
Cell Free DNA (N, frequency):			0.47
Low Risk	57 (46 %)	75 (42.6 %)	
Intermediate Risk	0 (0.0%)	1 (0.57 %)	
High Risk	1 (0.8 %)	0 (0%)	
Polyhydramnios	1 (0.8 %)	1 (0.57 %)	>0.9
Pyelectasis	1 (0.8 %)	0	0.24
Anomaly scan (N, frequency)	9 (7.3 %)	3 (1.7 %)	0.03*

BMI: body mass index, NT: nuchal translucency, PAPP-A: Pregnancy-associated plasma protein-A, MoM: multiples of the median for free β-hCG and PAPP-A

fetal/placental anomalies was not independent of the effects of low levels of free PAPP-A and MoM β-hCG (p >0.05, Table 5). This implies that male-factor-related infertility was not an independent risk factor for inducing fetal/paired abnormalities, but it may increase the risk of fetal/paired abnormalities in cooperation with other factors like low levels of free PAPP-A and MoM β-hCG.

Chromosomal abnormalities

The results of nuchal translucency test indicated that seven patients from the non-male factor-related infertile group with the mean NT thickness of 1.84 and five patients from the male factor-related infertile group with the mean NT of 1.76 were suspected of having Down syndrome. Also, one case in the male factor group was suspected of having Patau syndrome or trisomy 13. (NT: 1.50). Subsequently, these patients were more inspected through amniocentesis as confirmation test; only one case of trisomy 13 was confirmed in the male factor-related infertile group.

Table 2. Comparison of different factors by causes of infertility.

Cause of IVF	Frequency (%)	NT (mm)	Mean± SD Free β-hCG	MoM β-hCG	PAPP-A	MoM PAPP-A	Length of infertility
Male factor	124 (41.3)	1.65± 0.35	34.84± 26.9	**1.03± 0.34**	**2.05± 0.46**	0.97± 0.32	4.73± 0.96
Decreased ovarian reserves	5 (1.7)	1.7± 0.36	41± 10.3	1.72± 0.22	**4.16± 0.87**	1.32± 0.45	5± 0.83
Ovulation disorders	78 (26)	1.65± 0.35	36.95± 12.12	**1.01± 0.31**	2.86± 0.57	0.99± 0.4	3.85± 0.26
Tubular injuries, obstruction, adhesions	24 (8)	1.67± 0.33	37.66± 8.5	1.49± 0.22	**4.07± 1.08**	1.17± 0.47	4.55± 0.71
Uterine factors	29 (9.7)	1.6± 0.36	36± 9.61	1.25± 0.25	2.92± 0.54	1.14± 0.43	3.92± 0.47
Unexplained factors	40 (13.3)	1.59± 0.36	38± 14.05	1.35± 0.26	2.58± 0.64	1.05± 0.43	6.67± 0.7
P-Value		0.95	0.48	0.0001	0.0001	0.06	0.0001

NT: nuchal translucency, PAPP-A: Pregnancy-associated plasma protein-A, MoM: multiples of the median for free β-hCG and PAPP-A

Discussion

Diagnosing the cause of infertility is the first step of investigation and treatment for infertile couples. According to recent reports, infertility due to the "male factor" is responsible for most infertility cases [13-15], which was also confirmed by our findings. The male factor-related infertility (41.3%) was the most prevalent causes of infertility among our patients underwent ART, followed by ovulation disorders (26%) and unexplained factors (13.3%).

Most of the fetal and placental abnormalities detected in our study population belonged to infertility caused by male factors. To date, contradictory results have been reported in this regard. Kissin et al. did not find any significant association between the congenital anomalies resulting from ART or ICSI treatments with the male factors-related infertility. They reported that male-induced infertility could not be the main cause of the increased risk of autism due to ART[16]. By contrast, Sandin et al.'s prospective cohort study on more than 2.5 million infants showed the significant role of male factor-related infertility in developing neurodevelopmental disorders (autism and mental retardation)[17]. Our results indicated a probable association between the male factor-related infertility and high risk of developing fetal and placental anomalies. However, its association was not independent of the low levels of β-hCG and PAPP-A factors, implying that these risk factors may together affect the fetal growth.

The present findings showed that the serum levels of β-hCG and PAPP-A were lower in the male factor-related infertilities than non-male factor-related in-

Table 3. Comparison of different factors by Male factors related to infertility.

Male factors related to infertility	Frequency	NT (mm) (%)	Free	MoM β-hCG	PAPP-A β-hCG	MoM PAPP-A	Length of infertility
Oligozoospermia & Asthenozoospermia	40 (32.3)	1.65±0.36	34.79±27.7	1.1±0.9	2.0±0.56	1.1±0.4	4.4±1.3
Varicocele	31 (25)	1.66±0.35	36.65±27.9	1±0.27	1.5±0.3	0.9±0.35	5.1±0.9
Sperm morphology defects (Teratospermia)	34 (27.4)	1.65±0.37	34.98±25.8	1.1±0.17	2.2±0.77	1±0.34	4.6±1
Primary testicular defects	13 (10.5)	1.64±0.47	32.11±30.2	0.92±0.21	1.9±0.42	0.8±0.31	5±1.2
Normospermia with functional defects	6 (4.8)	1.65±0.51	31±22.5	0.7±0.15	1.5±0.26	0.8±0.23	5.3±0.5
P-Value		0.9	0.98	0.77	0.74	0.1	0.08

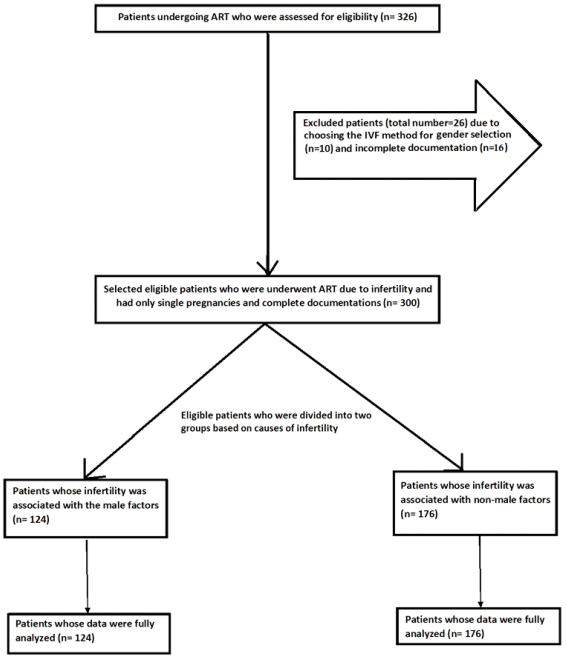

Figure 1. Flowchart of patients' selection.

fertility, and this difference was particularly significant for free PAPP-A and MoM β-hCG. Previously, Anckaert et al. reported that PAPP-A levels decreased in infertile women after treatment with ART, particularly among male factors-related infertility, compared to the spontaneous pregnancies. In addition, the serum free β-hCG levels at 11-14 weeks of gestation were significantly increased in ART pregnancies due to non-male infertility factors compared to other causes of infertility[18].

Amor et al., indicated that the decreased levels of PAPP-A in ART pregnancy were not associated with the etiology of infertility, and the elevated levels of βhCG were significantly associated with non-male factor-related infertility (5), which were in contrast to our results. Such an inconsistency in the results of various studies may be due to differences in ART methods and/or patients' racial/genetic differences, which warrants further large-scale investigations through multi-center clinical trials.

The present results showed that the rates of systemic underlying diseases and medication history were higher in infertilities related to non-male factors, which are consistent with data from recent studies that supported such an association between systemic diseases and the causes of infertility[19-21]. The present study showed no significant association between the cause of infertility and a series of demographic and clinical variables. By contrast, previous finding demonstrated that the risk of birth defects in the infertile women under ART treatment was higher among younger women (≤ 29); but its risk in children born from the fertile population was higher

Table 4. Distribution of fetal and placental anomalies based on various causes of infertility.

Fetal anomalies	Frequency	Cause of infertility	NT	MoM β-hCG	MoM PAPP-A
Microcephaly	1	Male Factor	1.5	0.24	0.6
Anencephaly	2	Male Factor	1.56	0.58± 0.24	0.65± 0.07
Twin-to-twin transfusion syndrome	1	Male Factor	0	0.7	0.3
Hydrops fetalis	1	Male Factor	1.2	0	0
Congenital heart defects	1	Male Factor	1.6	3.3	0.4
Unilateral renal agenesis	1	Male Factor	1.3	1.2	0.4
Hydronephrosis	1	Ovulation disorders	0	0.5	0.4
Duodenal Atresia	1	Male Factor	1.7	0.4	0.7
Macrocephaly	1	Unexplained factors	2.1	0.6	0.5
Hydronephrosis	1	Male Factor	1.4	1.1	0.5
Absence of finger	1	Ovulation disorders	2.6	1.2	0.5

NT: nuchal translucency, PAPP-A: Pregnancy-associated plasma protein-A, MoM: multiples of the median for free β-hCG and PAPP-A

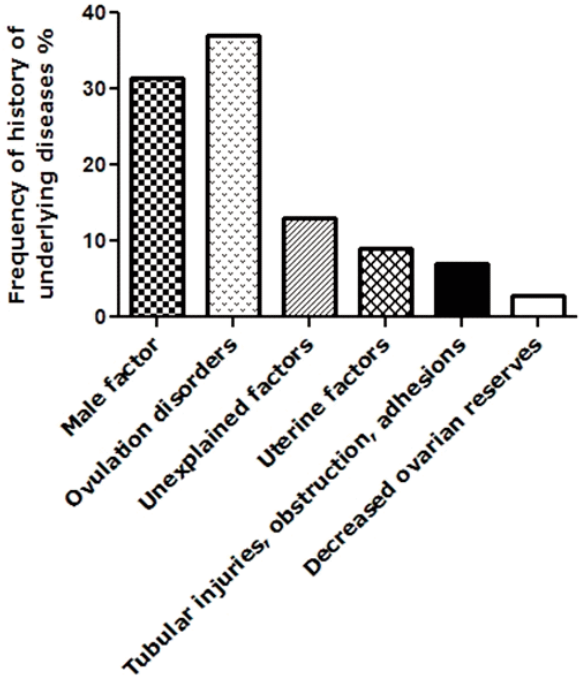

Figure 2. Comparison of the frequency of underlying systemic diseases based on the different causes of infertility

among mothers with older maternal age[22]. Additionally, Davies et al. found that maternal age more than 35 years was associated with a lower risk of congenital defects following ART[20].

The most common male causes of infertility in our study population were respectively oligozoospermia plus asthenozoospermia (32.3%), sperm morphology defects or Teratospermia (27.4%), varicocele (25%), primary testicular defects (10.5%), and normospermia with functional defects (4.8%). Öztekin et al (2019) evaluated abnormal sperm parameters in 406 infertility cases and reported that asthenozoospermia was the most common cause of male infertility (16.7%) and most severe varicocele cases were in this group[23]. Our findings confirmed Öztekin et al.'s results. However, no significant association was found between different male causes of infertility and the levels of NT, free β-hCG, MoM β-hCG, PAPP-A, and MoM PAPP-A in our study population. This point was one of our novel findings. The most common identified causes of reduced sperm motility or asthenozoospermia are sperm dysfunction, prolonged sexual abstinence, varicocele, genital tract infections, genetic factors, and deleterious lifestyle [24]. Male infertility related to varicocele has been reported as high as 42.7% in Iran[25]; the high concentrations of adrenal cortical hormones in refluxing blood may lead to arteriolar vasoconstriction and testicular hypoxia, which in turn damage the seminiferous epithelium [26].

The present study is one of rare studies that comprehensively investigated first-trimester anomalies, aneuploidy, and biochemical markers in infertile patients undergoing ART based on infertility-related female/male factors. However, this study has some limitations. First, its retrospective nature makes the results prone to bias and confounding. Second, the

Table 5**.** Association of fetal and placental anomalies with male factor-related infertility by considering other probable related factors.

Variables	Univariate logistic regression			Multivariable logistic regression		
	B	OR (95% CI)	P Value	B	OR (95% CI)	P Value
History of underlying systemic disease	0.6	1.81 (0.53_6)	0.34	_	_	_
MoM PAPP-A	-4.22	67.86 (13.48_341.48)	0.0001	0.47	1.6 (0.0001)	1
PAPP-A	-7.27	1435 (119.95_1717.93)	0.0001	-21.57	0.0001 (0.0001)	0.99
Free βhCG	-6.6	715 (91.23_5603.66)	0.0001	-21.05	0.0001 (0.0001)	0.99
MoM βhCG	-7.27	1435 (119.95_1717.93)	0.0001	-0.5	0.61 (0.021_17.9)	0.77
Male factor-related infertility	1.5	4.5 (1.19_17.03)	0.02	0.02	1.02 (0.0001)	1

NT: nuchal translucency, PAPP-A: Pregnancy-associated plasma protein-A, MoM: multiples of the median for free β-hCG and PAPP-A

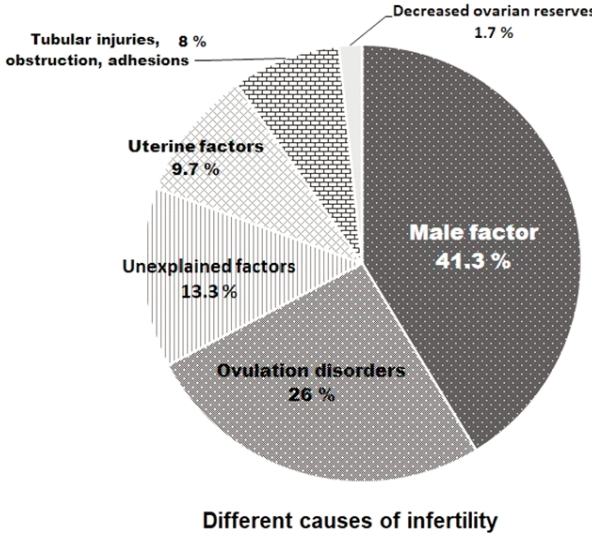

Figure 3. Frequency of different causes of infertility in the study population.

small sample size of patients with fetal abnormalities limits coming to a definitive conclusion. Furthermore, the single-center nature of study limits generalizing the results.

In conclusion, our findings indicated that the causes of infertility, particularly male factor, in collaboration with the low levels of free PAPP-A and MoM β-hCG may increase the risk of developing fetal and placental anomalies following ART treatment. Accordingly, healthy lifestyle factors, e.g., body weight, diet, male reproductive health, etc. are recommended to be considered for preventing or reducing the male infertility problems. Nevertheless, further large-scale multi-center and prospective researches are needed to precisely identify various factors affecting congenital defects following ART.

References

1. Committee on Practice Bulletins—Obstetrics. Gestational diabetes mellitus. Obstet Gynecol. 2013;122(2 pt 1):406-416.
2. Farina A, Eklund E, Bernabini D, et al. (2016). A First-Trimester Biomarker Panel for Predicting the Development of Gestational Diabetes. Reproductive Sciences, 1933719116675057.
3. Savvidou M, Nelson SM, Makgoba M, Messow CM, Sattar N & Nicolaides K . First-trimester prediction of gestational diabetes mellitus: examining the potential of combining maternal characteristics and laboratory measures. Diabetes 2010; 59(12):3017-22.
4. Syngelaki A, Visser GH, Krithinakis K, Wright A, & Nicolaides KH. First trimester screening for gestational diabetes mellitus by maternal factors and markers of inflammation. Metabolism 2016; 65(3):131-7.
5. Eleftheriades M, Papastefanou I, Lambrinoudaki I et al. Elevated placental growth factor concentrations at 11–14weeks of gestation to predict gestational diabetes mellitus. Metabolism 2014; 63(11): 1419-1425.
6. https://www.nice.org.uk/
7. Rani PR & BeGuM J. Screening and Diagnosis of Gestational Diabetes Mellitus, Where Do We Stand. JCDR 2016, 10(4):QE01.
8. Mishra S, Rao CR & Shetty A. Trends in the Diagnosis of Gestational Diabetes Mellitus. Scientifica 2016.
9. International Association of Diabetes and Pregnancy Study Groups Consensus Panel. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes care 2010; 33(3):676-682.
10. Sacks DA, Hadden DR, Maresh M et al. Frequency of gestational diabetes mellitus at collaborating centers based on IADPSG consensus panel–recommended criteria. Diabetes car 2012; 35(3):526-8.
11. Kane SC, Da Silva Costa F & Brennecke S. First trimester biomarkers in the prediction of later

pregnancy complications. BioMed research international, 2014.
12. Brink HS, Van Der Lely AJ & Van Der Linden J. The potential role of biomarkers in predicting gestational diabetes. Endocrine Connections 2016; 5(5): R26-R34.
13. Hassiakos D, Eleftheriades M, Papastefanou I et al. (2016). Increased maternal serum interleukin-6 concentrations at 11 to 14 weeks of gestation in low risk pregnancies complicated with gestational diabetes mellitus: Development of a prediction model. Hormone and Metabolic Research, 48(01), 35-41.
14. Papastefanou I, Eleftheriades M, Kappou D et al. Maternal serum osteocalcin at 11–14 weeks of gestation in gestational diabetes mellitus. European journal of clinical investigation 2015; 45(10):1025-1031.
15. Chang SC & Vivian Yang WC (2013). Hyperglycemia induces altered expressions of angiogenesis associated molecules in the trophoblast. Evidence-Based Complementary and Alternative Medicine.
16. Tsiakkas A, Duvdevani N, Wright A, Wright D & Nicolaides KH. Serum placental growth factor in the three trimesters of pregnancy: effects of maternal characteristics and medical history. Ultrasound in Obstetrics & Gynecology 2015; 45(5): 591-8.
17. Wright D, Syngelaki A, Bradbury I, Akolekar R & Nicolaides KH. First-trimester screening for trisomies 21, 18 and 13 by ultrasound and biochemical testing. Fetal diagnosis and therapy 2014;35(2):118-126.
18. Lamain-de Ruiter M, Kwee A, Naaktgeboren CA et al. External validation of prognostic models to predict risk of gestational diabetes mellitus in one Dutch cohort: prospective multicentre cohort study. Bmj 2016; 354:i4338.
19. Syngelaki A, Kotecha R, Pastides A, Wright A & Nicolaides KH. First-trimester biochemical markers of placentation in screening for gestational diabetes mellitus. Metabolism 2015; 64(11):1485-9.
20. Ong CY, Lao TT, Spencer K & Nicolaides KH. Maternal serum level of placental growth factor in diabetic pregnancies. Journal of reproductive medicine,Chicago 2004; 49:477-480.
21. Mosimann B, Amylidi S, Risch L et al. First-Trimester Placental Growth Factor in Screening for Gestational Diabetes. Fetal diagnosis and therapy 2015;39(4): 287-291.
22. Griffin M, Seed PT, Duckworth S et al. Prediction of delivering a small for gestational age infant and adverse perinatal outcome in women with suspected pre-eclampsia. Ultrasound in obstetrics & gynecology 2017.
23. Leach L. Placental vascular dysfunction in diabetic pregnancies: intimations of fetal cardiovascular disease?. Microcirculation 2014; 18(4): 263-9.
24. Weel IC, Baergen RN, Romão-Veiga M et al. Association between placental lesions, cytokines and angiogenic factors in pregnant women with preeclampsia. PLoS One2016; 11(6): e0157584.
25. Pietro L, Daher S, Rudge MVC et al. Vascular endothelial growth factor (VEGF) and VEGF-receptor expression in placenta of hyperglycemic pregnant women. Placenta 2010; 31(9) : 770-780.
26. Abell SK, De Courten B, Boyle JA & Teede HJ. Inflammatory and other biomarkers: role in pathophysiology and prediction of gestational diabetes mellitus. International journal of molecular sciences 2015; 16(6): 13442-13473.
27. Ogden CL, Carroll MD, Fryar CD & Flegal KM. Prevalence of obesity among adults and youth: United States, 2011–2014. NCHS data brief 2015; 219(219):1-8.

28. Kuźmicki, Mariusz et al. "The IL-6/IL-6R/sgp130 system and Th17 associated cytokines in patients with gestational diabetes." Endokrynologia Polska 65.3 (2014): 169-175.
29. Pantham P, Aye ILH & Powell TL. Inflammation in maternal obesity and gestational diabetes mellitus. Placenta 2015;36(7): 709-715.
30. Bao W, Baecker A, Song Y, Kiely M, Liu S & Zhang C. Adipokine levels during the first or early second trimester of pregnancy and subsequent risk of gestational diabetes mellitus: a systematic review. Metabolism 2015; 64(6): 756-764.
31. Morisset AS, Dubé MC, Cote JA, Robitaille J, Weisnagel S & Tchernof A. Circulating interleukin-6 concentrations during and after gestational diabetes mellitus. Acta obstetricia et gynecologica Scandinavica 2011; 90(5): 524-530.
32. Nergiz S, Altınkaya ÖS, Küçük M et al. Circulating galanin and IL-6 concentrations in gestational diabetes mellitus. Gynecological Endocrinology 2014;30(3):236-240.
33. Hossein-nezhad A, Hossein-nezhad A, Zh M, Kh M, Rahmani M, & Larijani B. "Osteocalcin and cross laps status among women with gestational diabetes mellitus during pregnancy." Journal of Diabetes and Metabolic Disorders 9 2010: 7
34. Kanazawa I. Diabetes mellitus and osteoporosis. The regulation of glucose metabolism by bone. Clinical calcium 2012;22(9): 1375-1382.
35. Kanazawa I. Osteocalcin as a hormone regulating glucose metabolism. World journal of diabetes 2015; 6(18): 1345-1354.
36. Zanatta LC, Boguszewski CL, Borba VZ & Kulak CA. Osteocalcin, energy and glucose metabolism. Arquivos Brasileiros de Endocrinologia & Metabologia 2014; 58(5): 444-451.
37. Ducy P. The role of osteocalcin in the endocrine cross-talk between bone remodelling and energy metabolism. Diabetologia 2011; 54(6): 1291.
38. Winhofer Y, Handisurya A, Tura A et al. Osteocalcin is related to enhanced insulin secretion in gestational diabetes mellitus. Diabetes care 2010; 33(1): 139-143.
39. Saucedo R, Rico G, Vega G et al. Osteocalcin, under-carboxylated osteocalcin and osteopontin are not associated with gestational diabetes mellitus but are inversely associated with leptin in non-diabetic women. Journal of endocrinological investigation 2015; 38(5): 519-526.
40. Telejko B, Kalejta K, Kuzmicki M et al. (2015). The association of bone turnover markers with pro-and anti-inflammatory adipokines in patients with gestational diabetes. Annals of Agricultural and Environmental Medicine 2015; 22(2).
41. Tabatabaei N, Giguère Y, Forest J C, Rodd CJ, Kremer R & Weiler HA. Osteocalcin is higher across pregnancy in Caucasian women with gestational diabetes mellitus. Canadian journal of diabetes 2014; 38(5): 307-313.
42. Lohse N, Marseille E & Kahn JG. Development of a model to assess the cost-effectiveness of gestational diabetes mellitus screening and lifestyle change for the prevention of type 2 diabetes mellitus. International Journal of Gynecology & Obstetrics 2011;115: S20-S25.

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