

ORIGINAL ARTICLE

OPEN ACCESS

Scientific Basis of Cytogenetic Analysis of Miscarriage Material in Invitro Fertilisation/Embryo Transfer Patients

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Introduction: This study was planned to determine the relationship between the karyotype of either partner of IVF-ET couples who experienced abortion before the 20th gestational week and the cytogenetic findings in product of conception (POC).

Materials and Methods: 192 patients who had a miscarriage before 20 weeks of gestation after IVF-ET were included in the study. Cytogenetic analysis was performed using fluorescent in situ hybridization method from chorionic villi in POC. The karyotypes of each parent were compared with the cytogenetic findings obtained from the POC.

Results: Cytogenetic anomaly was detected in the POC in 92 (75.4%) of 122 patients who had first abortion, while cytogenetic anomaly was found in only 28(40%) of the 70 patients who had recurrent abortion. Karyotype anomalies were detected in 19 parents, 10 females and 9 males. Cytogenetic anomaly was detected in the product of conception of 7 out of 10 cases (70%) with maternal cytogenetic anomaly. Cytogenetic anomaly was detected only in 3 miscarriage materials of 9 cases with paternal cytogenetic anomaly (33.3%). In other words, karyotype anomaly was detected in the POC of 10 of 19 parents whose karyotypes were impaired (52.6%). Five of 10 women with abnormal karyotype had a history of miscarriage before. Likewise, 4 of 9 men with abnormal karyotype previously had a miscarriage.

Conclusion: In the presence of a female partner with abnormal karyotype, the risk of cytogenetic anomaly in POC is higher than male partner with karyotype anomaly.

Keywords: Chromosomal aberrations, invitro fertilisation/embryo transfer, miscarriage, product of conception

Introduction

Approximately 15% of the first trimester natural cycle pregnancies result in spontaneous abortion. Chromosomal aberration in recurrent miscarriage materials was found in 29-60% (1). Interestingly most of the numeric aberrations occur de novo rather than being inherited (2). In Invitro Fertilisation/Embryo Transfer (IVF-ET)

cycles, the first trimester spontaneous abortion rates are slightly higher than the natural cycles (3). This difference may be due to the fact that the patient group in IVF-ET cycles are different in terms of age, hormonal and genetic features compared to natural cycles. In addition, subjecting the patients in this group to further research before IVF-ET treatment may be

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Received: Dec 21, 2021 **Accepted:** Jan 13, 2022

Published: March 9, 2021

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The Ulutas Medical Journal © 2022



another reason for the difference in rates. Although, in most miscarriages, aneuploidies occurring due to de novo mutations take the first place (4), we do not know clearly the factors affecting the encounter with genetic anomaly in the product of conception (POC). Having the first abortion of the patient is the most important factor that increases the risk of aneuploidy. Although the first pregnancy results in abortion with cytogenetic increases the risk of abortion in subsequent pregnancies, it does not increase the risk of genetic anomaly in the same amount (1). Interestingly, the number of chromosomal anomaly detection decreases in the POC as the number of abortions increases. In line with this, the miscarriage rate increases with the number of previous spontaneous miscarriages. On the contrary, the frequency of finding an abnormal cytogenetic finding in POC significantly decreases and that of normal karyotypes significantly increases with the number of previous miscarriages (1). This data suggests that recurrent abortion cases are due to structural and hormonal factors related to reproductive organs rather than genetic factors. The presence of chromosomal anomaly in one of the partners leads to an approximately 5% anomaly in the POC. In recurrent miscarriages, at least one of the couples may have a 4.7% chromosomal defect (5). These rates are mostly valid for abortion occurring in natural cycles. Although the IVF-ET cycles are thought to have similar miscarriages rates with natural cycles, the link between the karyotype in either partner and the karyotype in the POC is not clear. This study was planned to investigate the relationship between the karyotype of either partner of IVF-ET who experienced abortion before the 20th gestational week and cytogenetic findings in POC.

Materials and Methods

This retrospective analysis assessed infertile women of miscarriage after IVF-ET occurring between January 2015 and December 2019. The

participants gave informed consent and the study had been performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000. Ethical approval was obtained from the Ethics Committee of Erciyes University Medical Faculty (Approval date and issue number; 2020-439). All participants were treated according to a standard antagonist protocol with individually dosed recombinant FSH(rFSH) starting on day 2–3 of the menstrual cycle. Gonadotrophin-releasing hormone antagonist cetrorelix acetate (Cetrotide, Merck, Germany) was started on the 5th or 6th day of stimulation. When at least three follicles reached 17 mm in diameter, maturation of follicles was induced with recombinant hCG (Ovitrelle, Merck-Serono, 250 mg, Modugno, BA, Italy). Oocyte collection was performed 35–36 hours after hCG application. Ovarian follicles were aspirated using a single-lumen, 17-gauge needle (Cook Medical, Bloomington, IN, USA) guided by transvaginal ultrasonography (TV-USG). Pregnancy was evaluated in all patients with serum β -HCG following menstruation delay. Patients with a positive β -HCG result underwent TV-USG and clinical pregnancy was considered with the presence of a fetal heart beat. Weeks of gestation were made according to the embryo transfer date and confirmed with USG. The diagnosis of miscarriage was established via gynecological examination and transvaginal ultrasonography. A retrospective cohort of 192 infertile women undergoing IVF-ET treatment and exposed to abortion before the first 20 weeks of pregnancy were included the study. The following were determined as the basic inclusion criteria for the study; (i) those with a history of abortion in their personal or family history, (ii) advanced age patients who underwent IVF / ET, (iii) those with a history of previous anomalies or stillbirths, and (iv) couples request this evaluation. The cases excluded from the study are; (i) the couple does not want this test, (ii) cases with obvious

gynecological and hormonal causes that may lead to abortion, (iii) cases bringing abortion material from outside the hospital. The causes of abortion included complete, incomplete, missed, and recurrent miscarriages. Distribution of abortion types according to weeks is shown in Table 1. 122 had abortion for the first time, while 70 had 2 or more abortion history.

The patients were divided into three groups according to the gestational weeks of abortion. The number of patients with abortion between 5-7 weeks was 45, 121 between 8-12 weeks and 26 between 13-19 weeks. Similarly, in cases with recurrent miscarriages, they were divided into 3 groups according to weeks of miscarriage. The number of patients with recurrent miscarriage between 5-7 weeks was 15, 49 between 8-12 weeks and 6 between 13-19 weeks. Cytogenetic analysis of the chorionic villi in product of conception (POC) following miscarriage was performed using a comparative genomic hybridization (CGH) analysis with fluorescence in situ hybridization (FISH). More than 20 cells in metaphase were examined and reported. Peripheral blood karyotype analysis of each partner was also examined. Parental cytogenetic findings were compared with cytogenetic findings obtained from fetal tissue. The correlations between the karyotypes of parents and the karyotypes obtained from product of conception were evaluated.

Statistical Evaluation

Statistical analyzes were performed with NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package. In addition to descriptive statistical methods (mean, standard deviation, median, interquartile range) in the evaluation of the data, the independent t-test was used for comparison of the paired groups of the variables with normal distribution, and Mann-Whitney U test for the pairwise comparison of the groups with non-normally distributed variables. Chi-square and Fisher exact test were used in the comparison

of qualitative data. The results were evaluated at the significance level of $p < 0.05$.

Results

The Distribution of abortion types according to gestational weeks are shown in Table-1 and the demographic characteristics of all cases are shown in Table-2. The ages of the participants were between 20-44. The fetal materials cytogenetically examined were between 5 and 19 weeks on average. Of the 192 IVF-ET patients who had a miscarriage, 122 had their first miscarriage (group 1) while 70 had a history of 2 or more miscarriages (group 2).

Table 1. Abortion types according to gestational weeks in total miscarriage

Week of Miscarriage	No	Complete Abortion	Incomplete Abortion	Missed Abortion
5-7	45	18(40%)	18(40%)	9(20%)
8-12	121	37(30.5%)	57(47.1%)	27(22.3%)
13-19	26	6(23.07%)	12(46.1%)	8(30.7%)
Total	192	61(31.7%)	87(45.3%)	44(22.9%)

*Data are presented as percentage

When Group-1 and Group2 were compared, no difference was observed between the 2 groups as for body mass index, maternal age, progesterone level, endometrial thickness, total oocyte count, consanguineous marriage, thrombophilia and smoking. Estradiol values on the day of hCG of the first miscarriage group were higher than the recurrent miscarriage group ($p=0.035$). In recurrent miscarriage, the risk was 2,29 (0,91-5,86) for consanguineous marriage, 2,28 (1,02-5,14) for thrombophilia and 1.92 (1,02-3,65) for smoking.

While abnormal karyotype was found in 22 of 30 patients (73.33%) who experienced first miscarriage between 5-7 weeks, 65 of 85 cases (76.47%) who experienced first miscarriage between 8-12 weeks and 5 of 7 cases (71.42%) who experienced first miscarriage between 13-19 weeks had karyotype anomalies. While karyotype anomaly was found in the POC of 92 out of 122 cases (75.4%) with the first abortion

(Table-3), no pathology was detected in the remaining 30 cases (24.59%). While karyotype anomaly was detected in the POC of 28 of 70 (40%) with recurrent miscarriage (Table-4), no pathology was detected in 42 (60%). Karyotype analysis was performed on 192 couples with a miscarriage history. Karyotype anomalies were detected in 19 parents, 10 females and 9 males.

Table-2. Demographic characteristics of the first and recurrent miscarriage groups

Variables	(Group-1) First Miscarriage (n=122)	(Group-2) Recurrent Miscarriage (n=70)	p
Age (years)	34.9±5.5	35.4±1.3	0.748 [*]
Body Mass Index (kg/m ²)	26.2±4.6	27.4±3.4	0.058 [*]
Estradiol on the hCG-day (pg/ml)	3643±336	3546±245	0.035 [*]
Progesterone Level (ng/ml)	0.95±0.7	0.91±1.4	0.846 [‡]
Endometrial Thickness (mm)	10.5±1.9	9.87±3.4	0.101 [*]
Total Oocyte Count	11.3±9.1	10.2±7.1	0.489 [‡]
Consanguineous Marriage	9 (%7.3)	11 (%15.7)	0.115 ⁺
Trombophilia	13 (%10.6)	15 (%21.4)	0.068 ⁺
Smoking	71 (%58.2)	51 (%72.8)	0.061 ⁺

*Independent t-test, ‡Mann Whitney U test, +Chi-square test

Cytogenetic anomaly was detected in the product of conception of 7 out of 10 (70%) with cytogenetic anomaly. Cytogenetic anomaly was detected only in 3 miscarriage materials of 9 cases with paternal cytogenetic anomaly (33.3%). Statistical comparison between the gender and cytological finding is not possible because the obtained karyotypes are quite different from each other. In the presence of different genetic analysis reports, only the number of abnormal cases can be compared between groups. While the risk of cytogenetic anomalies in the POC was 70% in cases with karyotype anomaly in

the expectant mother, the risk of cytogenetic anomaly in the POC of the cases with karyotype anomaly in the male partner was approximately 33%. The difference was not significant (p=0.25). But the risk of cytogenetic anomalies in the POC was 4,67 (0,67-22,37) times higher in women with karyotype anomaly than male partners with karyotype anomaly.

Table-3. Abnormal and normal karyotype distribution in product of conception (POC) in the first miscarriage

Week of Miscarriage	Number of First Miscarriage	Abnormal Karyotype in POC	Normal Karyotype in POC
5-7	30	22 (73.33%)	8 (26.66%)
8-12	85	65 (76.47%)	20 (23.52%)
13-19	7	5 (71.42%)	2 (28.57%)
Total	122	92 (75.4%)	30 (24.59%)

*Data are presented as percentage

Karyotype anomaly was detected in the POC of 10 of 19 parents whose karyotypes were impaired (52.6%). On the other hand, karyotype anomalies detected in the either partner and karyotype findings in their POC did not overlap in the most of cases. Five of 10 women with abnormal karyotype had a history of miscarriage before. Likewise, 4 of 9 men with abnormal karyotype had a miscarriage. The cytogenetic findings were found in the miscarriage material of the parents with abnormal karyotype (Table-5 and Table-6). Twenty of 192 patients had a consanguineous marriage and only 8 of those had chromosomal aberration from the POC.

Table-4. Abnormal and normal karyotype distribution in product of conception (POC) in recurrent miscarriage

Week of Miscarriage	Number of Recurrent Miscarriage	Abnormal Karyotype in POC (%)	Normal Karyotype in POC (%)
5-7	15	6 (40%)	9 (60%)
8-12	49	21 (42.8%)	28 (57.1%)
13-19	6	1 (16.6%)	5 (83.3%)
Total	70	28 (40%)	42 (60%)

*Data are presented as percentage

Table-5. The cytogenetic findings in miscarriage material of the female partner with abnormal karyotype

Abnormal Maternal Karyotype	Karyotype in Product of Conception
45,X[3]/46,XX[27]	1th baby :47,XX,+ 16 2nd baby:46,XY
46,XX,inv(9)(p12q13)	46,XY,inv(9)(p12q13)
46,XX,inv(10)(p11.2q26.1)	46,XX,inv(10)(p11.2q26.1)
46,XX,inv(9)(p11q12)	45,X,inv(9)(p11q21)
45,X[1]/47,XXX[1]/46,XX[30]	Trisomy21
45,X[10]/46,XX[40]	46,XX
46,XX,inv(9)(p12q13)	46,XY
46,XX,inv(9)(p12q13)	47,XXY
45,X[5]/48,XXXX[1]/ 46,XX[94]	48,XY,inv(9)(p12q13) de novo,+16,+22
46,XX,13p-	46,X,+13

Discussion

Approximately 10% of women in reproductive period experienced cytogenetic anomaly in the product of conception (POC) (6). Most of the aneuploid pregnancies either die in utero or result in miscarriage (3). If genetic evaluation is performed in POC for first time, chromosomal anomaly can be detected in more than half of the cases (7). In cases where normal karyotype was found in the first miscarriage material, an increased risk of chromosomally normal POC is observed in the following pregnancy (8).

The most common trisomy is trisomy 16 in POC (9). Also, trisomy 21, 18, and 13 occur in some live births (10). In the study, we did a retrospective analysis to examine the frequency of chromosomal abnormalities in products of conception from patients with first and recurrent miscarriages in relation to the parental karyotype anomalies. We studied 122 pregnancies with a history of first miscarriage and 70 pregnancies with 2 or more consecutive first-trimester abortions. The frequencies of abnormal/normal embryonic karyotypes first and recurrent miscarriages (RM) were studied.

Table-6. The cytogenetic findings in miscarriage material of the male partner with abnormal karyotype

Abnormal Paternal Karyotype	Karyotype in Product of Conception
46,XY,t(11;22)(q24;q12)	46,XY
47,XXY(2)/46,XY(50)	46,XY
46,XY,t(9;15)(q32;p11.1)	46,XX,der(15)t(15;17)
46,XY,16qh+	46,XX
46,XY,16qh+	47,XX,+21
47,XY,+15	47,XY,+13
46,XY,inv(9)(p11q12)	46,XX
46,XY,inv(9)(p11q12)	46,XX
46,XY,inv(2)(p25q37)	46,XY

Chromosomal analysis was performed on POC using a FISH technique. 122 of the 192 IVF-ET patients had their first miscarriage while 70 had a history of 2 or more miscarriages. About 70% of first trimester miscarriage cyto-genetic anomaly can be detected in the POC (1). In this study, the incidence of cytogenetic anomalies in POC of cases who had abortion in the first 12 weeks of pregnancy was 75.4%. This rate is 5% higher than the literature data. Since all of the miscarriages in our series occurred after IVF-ET, this difference may dependent on the personal characteristics of participants. Because most of the individuals in IVF-ET cases are older than natural cycles and have a group of patients who are more likely to undergo genetic research. It has been reported that chromosomal anomaly detection rate is about 5% in at least one of the partners who had miscarriage. In recurrent miscarriages the rate of a cytogenetic problem is high in at least one of the partners (5). In our study, cytogenetic anomalies were detected in 19 people, including ten female and 9 male. This rate was compatible with the literature (5). In our study, structural chromosomal abnormality

in the POC was the most common findings in women with abnormal karyotype. On the other hand, numerical chromosome defects in the POC were the most common finding in male partner with abnormal cytogenetic findings. Compared with the recurrent miscarriage, the prevalence of chromosomal abnormality was significantly higher in cases experiencing the first miscarriage. Likewise, the frequency of normal embryonic karyotypes in miscarriage materials significantly increased in male partner with abnormal karyotype compared to female partners with abnormal cytogenetic findings. While the risk of cytogenetic anomalies in the POC was 70% in cases with karyotype anomaly in the expectant mother, the risk of cytogenetic anomaly in the POC of the cases with karyotype anomaly in the male partner was approximately 33%. The difference was statistically insignificant but the risk of cytogenetic anomalies in the POC was found 4,67 times higher in women with karyotype anomaly.

In the light of these findings, we can argue that karyotype problems in the mother are reflected more frequently in the fetus than the problems in the father. Although it is not possible to interpret the reason clearly, we can argue that the presence of a healthy oocyte can correct the negative effects of the cytogenetically abnormal sperm to some extent. However, it is obvious that this hypothesis needs to be investigated in detail.

Although there were similar karyotypes in some cases, cytogenetic anomalies belonging to parents and anomalies detected in the POC were independent of each other. For example, karyotype was 47,XX+21 in miscarriage material in which the mother had 46, XX and the father had 46,XY,16qh+ karyotype. Numerical chromosome anomalies in parents are also detected in POC of most miscarriages cases. Interestingly, structural chromosomal defects

sometimes appear as numerical chromosome defects in the POC. For example, in the POC of the mother with 46,XX,inv(9) (p11q13) anomaly, 47,XXY karyotype was detected. These data suggest that simple structural chromosomal anomalies in parents may remain the same as they are transferred to fetuses, or they may return to more serious numerical anomalies. In the light of all these data, it would not be wrong to suggest that individuals with first or recurrent miscarriage history should be analysed cytogenically. Thus, we can learn the probability of a more serious karyotype anomaly in the next pregnancy of the couple with a structural chromosome anomaly in the first POC. By giving preimplantation genetic diagnosis counseling to these cases, we can help them give birth to healthy babies.

In the first 19 weeks of pregnancy, the rate of abnormal karyotype in the miscarriage material was 75.4%. When subgroup analysis was made according to gestational weeks, rates of abnormal karyotype encounter were found to be similar. In other words, the rate of abnormal karyotype detection in POC of women who had abortion between the first 5-7 weeks of pregnancy was similar to the cases between 13-19 weeks (73.33% vs. 71.42%).

The overall rate of abnormal karyotypes in the POC was 75.4 % (92/122) in cases experiencing the first miscarriage, and the rate of abnormal cytogenetic findings associated with abnormal chromosomal findings in either partner was 52.63% (10/19). Although there was a karyotype anomaly in the parents, no chromosomal anomaly was detected in nine POC (47.4%). We can interpret this data as follows: If any of the parents have karyotype anomalies, the risk of cytogenetic anomaly is observed in approximately half of the babies, while the other half does not have any risk. In the first 20 weeks of pregnancy, 120 of 192 cases with miscarriage history had karyotype anomaly in POC (62.5%). While only 10 of 120 cases

had karyotype anomaly in one of the parents (8.3%), the remaining 110 parents had normal karyotype (91.7%). Miscariages occurring in IVF-ET cycles are 3.3% more likely to have karyotype anomalies in one of the partners than natural cycles. For this reason, there is no need to perform a karyotype analysis in the parents of the cases with chromosomal anomaly in POC.

In the present study the risk of karyotype anomaly was found to be lower in patients with recurrent miscarriage (RM) compared to those who first experienced abortion (40% vs. 75.4%). Consistent with our findings, literature data also show the presence of normal karyotypes in RM cases (11,12). While abnormal karyotype was found in the POC of 28 of 70 cases with recurrent abortion history (40%), 42 cases were reported as normal karyotype (60%). The frequency of detection of chromosomal anomaly in the POC may be decreasing, since the etiology of most RM cases is due to the presence of antiphospholipid antibody, endocrine pathologies or uterine anomaly (13,14). Interestingly, abnormal karyotype rates in POC occurred in 13-19 weeks were reduced (40-16.6%) compared to recurrent miscarriages in 5-7 weeks. The rate of detection of karyotype anomalies in cases experiencing the first miscarriage at the 7th gestational week and between 8-12th weeks were found similar. In other words, the occurrence of miscarriage in the first 7 weeks of gestation or between 8-12 weeks does not change the rate of occurrence of cytogenetic anomaly in the POC. For this reason, in all first trimester miscarriage, the physicians must decide whether to perform a cytogenetic analysis according to the patient's history and their clinical experience. On the other hand, since there is a low risk of karyotype anomaly in advanced pregnancy weeks in RM cases, performing karyotype analysis does not provide any extra benefit. Carp et al (2) reported patients with parental chromosomal rearrangements do not have a significantly

lower livebirth rate than those in the patients without chromosomal aberrations. In view of the our results and literature data we can propose that parental karyotyping might not be a good predictor of the outcome of subsequent pregnancies. However, in order to make a clear conclusion, cytogenetic examination should be performed on all subsequent POC.

Although karyotyping is part of management of spontaneous or recurrent miscarriage our results are indicating that parental karyotyping is of limited value in diagnosing the underlying cause. The aneuploidy rate in POC following IVF-ET was found to be approximately 5% more than natural spontaneous miscarriages (70% vs. 75.4%). This difference may be related to the nature of IVF-ET cycles. Miscarriage occurring in IVF-ET cycles are 3.3% more likely to have karyotype anomalies in one of the partners than natural cycles (5% vs. 8.3%).

The frequency of normal embryonic karyotype in miscarriage materials significantly increased in male partners with abnormal karyotype compared to female partners with abnormal cytogenetic findings. Since karyotyping in POC is an indirect predictor of fetal aneuploidy its predictive value is limited. Finally we do not recommend routine karyotyping in POC of women undergoing IVF-ET cycles. Our study was conducted with remarkably higher number of patients. However, there is a need for multicenter studies that will determine the prognosis of patients in the long term.

Conflict of Interest

The authors report no conflict of interest.

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How to cite?

Yurci A, Genc S, Gungor ND. Scientific Basis of Cytogenetic Analysis of Miscarriage Material in Invitro Fertilisation/Embryo Transfer Patients. Ulutas Med J. 2022;8(1):37-44

DOI: 10.5455/umj.20211227090519