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ORIGINAL ARTICLE

Karyotype analysis of products of conception in patients with recurrent pregnancy loss

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ABSTRACT

Objective: The purpose of this study is to discuss the results of products of conception (POC) karyotype analysis in cases with recurrent pregnancy loss (RPL).

Material and Methods: The data of the cases evaluated with the diagnosis of RPL were retrospectively obtained and the results were examined (n=485). Among them, 135 cases with karyotype analysis were included in the study. Maternal age, gestational week, and karyotype analysis results were recorded (n=129). Cases between 6 and 14 weeks of age were included in the study. Maternal age, gestational week, and karyotype analysis results of cases with RPL between 6 and 14 weeks of gestation were recorded as data. Genetic analysis of POC was made with conventional cytogenetic techniques.

Results: One hundred and thirty-five cases diagnosed with RPL were included in the study. Mean maternal age was 34.43±5.41 years and mean gestational age was 8.36±1.58 weeks. Abnormal karyotype was detected in 40 cases (40/129, 31%). Karyotype analysis was normal in 89 cases and among these, two fetuses had 46, XX, 9qh+ polymorphism. In cases with abnormal karyotype, maternal age was found to be more advanced compared to euploid karyotypes (35.97±5.31 vs 33.57±5.31, p=0.0188). Again, in male fetuses (17/35), when compared to females (23/83), significantly more abnormal karyotypes were detected (p=0.048).

Conclusion: The frequency of abnormal karyotype in RPL cases was 31%, and the most common abnormality was autosomomal trisomies (62.5%). Trisomy 22 was the most common of the trisomies (24%). We also emphasize that the frequency of abnormal karyotype increases with advanced maternal age, according to our results.

Keywords: Genetics, karyotype analysis, recurrent pregnancy loss.

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INTRODUCTION

Habitual abortion was defined as the spontaneous loss of two or more episodes of miscarriage before 20–24 weeks of gestation.[1]

Reproductive age couples encounter recurrent pregnancy loss (RPL) between 1% and 5% and the incidence depends on the definition and the population studied.[2–4]

Pregnancy must be proven by β-HCG test or histopathological methods and pregnancy losses do not have to be consecutive to define RPL. The probability of abnormal diagnostic tests in patients with two pregnancy losses is almost equal to the probability of abnormal results in tests initiated at three losses. Therefore, diagnostic investigation begins when two abortions occur.[5]

In studies conducted to determine the etiological cause in RPL, a maximum of 50% can be found, that is, most of them are idiopathic. Known causes are genetic, anatomical, immunological, endocrine, thrombophilic, and infectious. Although different results were obtained in various studies, the generally accepted frequencies are "antiphospholipid syndrome" 15%, anatomical causes 10–12%, prenatal genetic causes 2–5% and other identifiable causes 10% (endocrin imbalances, thrombophilic disorders, diabetes mellitus, hypothyroidism, and polycystic ovary syndrome).[2,6–8]

However, the probable cause can be identified nearly only in half of RPL cases with American Society of Reproductive Medicine (ASRM) RPL workup (analysis of the parental karyotype, maternal lupus anticoagulant, anticardiolipin antibodies, anti-β2 glycoprotein 1, evaluation of uterine anatomy, thyroid function, prolactin, and hemoglobin AI c) and the rate drops to 18% by adding the cytogenetic testing of the products of conception (POC) in the evaluation, according to Marquard et al.'s[2] study.[9] Popescu et al.[6] reported that the addition of the 24-chromosome microarray (CMA) of the POC increases the detection rate of the cause of RPL to >90%. At the same time, POC analysis is useful in determining which couples should be offered "pre-implantation genetic testing for aneuploidies" in RPL cases.[10]

In this study, it is aimed to discuss the results of POC karyotype analysis in cases with RPL.

METHODS

This study was carried out in the genetics and obstetrics and gynecology departments of Şişli Memorial Hospital. The data of the cases evaluated with the diagnosis of RPL were retrospectively obtained and the results were examined (n=485). Multiple pregnancies, cases with fetal anomaly and RPLs with known causes were excluded from the study (n=350). Among them, 135 cases with karyotype analysis were included in the study. Six cases whose karyotype analysis was not successful were excluded from the study.

Maternal age, gestational week, and karyotype analysis results were recorded (n=129). Cases between 6 and 14 weeks of age were included in the study. Maternal age, gestational week, and karyotype analysis results of cases of RPL cases between 6 and 14 weeks of gestation were recorded as data. Genetic analysis of POC was made with conventional cytogenetic techniques. Cell culture and chromosomal analysis were used to detect karyotypes.

Table 1: Frequency of detected chromosomal abnormalities

Abnormality	Cases (n)	Frequency (%)
Monosomy X	6	15
Trisomy 3	1	2.5
Trisomy 7	1	2.5
Trisomy 8	2	5
Trisomy 9	1	2.5
Trisomy 12	3	7.5
Trisomy 13	1	2.5
Trisomy 14	1	2.5
Trisomy 15	3	7.5
Trisomy 16	2	5
Trisomy 20	1	2.5
Trisomy 21	1	2.5
Trisomy 22	6	15
Undefined trisomy (47,XX + mar)	2	5
Double trisomy	2 (48,XX,+2,+4 and 48,XXX,+20)	5
Triploidy	6	15
Tetraploidy	1 (50% mosaic)	2.5
Total	40	100

Statistical analysis was performed using Statistical Package for the Social Sciences Version 24 (SPSS Inc., Chicago, IL, USA). Descriptive statistical methods (mean, standard deviation, and percentiles) were used to evaluate the data. Kolmogorov–Smirnov test was performed to determine whether or not parameters are normally distributed. Student's t-test was performed to compare parameters among the groups. The Chi-square statistic with Yates correction was used for testing relationships between categorical variables. P<0.05 was considered statistically significant.

RESULTS

One hundred and thirty-five cases diagnosed with RPL were included in the study. Mean maternal age was 34.43±5.41 years and mean gestational age was 8.36±1.58 weeks.

It was determined that karyotype analysis could not yield results in 6 cases (4.4%) (maternal cell contamination in two cases and bacterial contamination in four cases). Abnormal karyotype was detected in 40 cases (40/129, 31%) (Table 1). Karyotype analysis was normal in 89 cases and among these, two fetuses had 46,XX,9qh+ polymorphism.

In cases with abnormal karyotype, maternal age was found to be more advanced compared to euploid karyotypes (35.97±5.31 vs. 33.57±5.31, p=0.0188). Again, in male fetuses (17/35), when compared to females (23/83), significantly more abnormal karyotypes were detected (p=0.048).

DISCUSSION

Two or more consecutive pregnancy losses are considered as RPL and occur approximately in 3% of couples.[1] Uterine abnormalities, autoimmune factors, endocrin imbalances, thrombophilic disorders, parental chromosomal abnormalities, antiphospholipid antibodies, diabetes mellitus, hypothyroidism, and polycystic ovary syndrome are the most common causes of RPL.[2,7,8]

Thrombophilia tests as Factor V Leiden, Prothrombin, Methylene tetra hydrofolate reductase, deficiencies of protein S, C, and Antithrombin are not recommended routinely in patients with RPL by ASRM and European Society of Human Reproduction and Embryology.[1]

Aneuploidy rates vary between 26.7 and 64.7% in primary RPL and 32–81.3% in secondary RPL and these rates differ according to the countries where the study was conducted.[8] The rate of aneuploidy in RPL cases has been reported to be approximately 55% detected by CMA in the literature.[1] The results of karyotype analysis of POC in RPL cases differ between studies.

Abnormal karyotypes were detected in 47.4% of RPL cases in Nikitina et al.'s[8] study. The most common chromosomal abnormality was "autosomal trisomy" (40.8%) followed by triploidy (14.6%) and autosomal monosomy (13.1%), double trisomy fruquency was 5.2%. In another study, Nikitina et al.[11] reported the rate of abnormal karyotype in RPL cases as 46.6%. In Nikitina et al.'s[11] study, mean maternal age was 28.9±6.1 years, average gestational age was 9.6±2.7 weeks. The mean maternal age was 34.43±5.41 years and the mean gestational age was 8.36±1.58 weeks in our study. Most common chromosomal abnormalities in RPL patients were reported as trisomy 16 (19.4%), trisomy 22 (14.9%), trisomy 21 (13.4%), and monosomy X (10.4%) by Popescu et al.[6] Frequency of trizomy 16, trisomy 22, trisomy 21, and monosomy X were detected as 5%, 15%, 2.5%, and 15%, respectively, in the current study, which is different than Popescu et al.'s[6] results. Sheng et al.[12] obtained abnormal results in 57.52% of RPL cases. The rates of detected abnormal findings were reported as: autosomal trisomy (64%), monosomy X (10%), autosomal monosomy (1%), and triploidy (5%).[12] The leading abnormality was reported as trisomies (71.8%) in Marquard et al.'s[2] study; most common was trisomy 16 followed by trisomy 15 and trisomy 22, but the study was conducted in women >35 years of age and the RPL was defined as the loss of three or more clinical pregnancies.

In the present study, abnormal karyotype was detected in 40 out of 129 cases, the rate of abnormal karyotype was 31% (Table 1). Trisomy 16, which is usually the most frequently detected in the literature, was the third most common trisomy in our study.[2,6] The fact that our abnormal karyotype rate is less than that stated in the literature can be explained by the differences in the geographic location of the study, the technique used in the POC analysis, and the age group in which the study was performed. Autosomal trisomies are the most common aneuploidies which occur in 60% of abnormal cases.[1] In line with the literature, the most common karyotype abnormality was autosomal trisomies with a 62.5% frequency in our study. We detected trisomy 22 as the most common trisomy in our study, the frequency among trisomies was 25% and 15% in total. Trisomy 22 was detected as the second common trisomy in Popescu et al.'s[6] and as the third common trisomy in Marquard et al.'s[2] study. From these findings, it can be concluded that trisomy 22 is seen quite frequently in RPL cases. In addition, we detected double trisomy in two cases (5%) similarly to Nikitina et al's[8] study which reported the frequency of double trisomy as 5.2%.

It has been reported that pregnancy loss rates increase with increasing maternal age and this rate rises to 33.2% between the ages of 40 and 44. It is known that as maternal age increases, errors in the meiosis stage also increase leading to aneuploidy.[13] In women with RPL, the most commonly observed chromosomal anomaly type changes with maternal age; non-viable autosomal trisomies are seen more common in advanced maternal age while unbalanced structural anomalies are detected more common in younger women (29 vs. 4.9%).[14] In our study, supporting the literature, in cases with abnormal karyotype, maternal age was found to be more advanced (35.97±5.31 vs. 33.57±5.31, p=0.0188).

Significantly more abnormal karyotypes were detected in male fetuses (17/35) compared to females (23/83) in our study (p=0.048). However, no information could be found in the literature about this subject. We propose that this issue should be investigated in future studies.

Chromosome 9 heterochromatic variants ([9qh+, 9cenh+, 9ph+, 9qh-, inv 9] [p11q13]) are detected in 1.5% of general population during routine cytogenetic analysis.[15] These are polymorphic variations in the length of the centromeric heterochromatin on the long arms of chromosome 9.[16] Heteromorphisms of chromosome 9 involving the pericentromeric region are considered normal population variants.[17] Hong et al.[16] stated that chromosomal polymorphic variations did not affect ivf results. Kosyakova et al.[15] stated that there was no evidence that infertility could be linked to this condition in their study where they evaluated heteromorphic variants of the nineth chromosome. However, in a 1997 study, Kumar R reported that the 9qh+ heteromorphism might be associated with RPL, only in the Arab population.[18] 46,XX,9qh+, a polymorphic variant of the heterochromatin region of chromosome 9, was detected in two patients in the current study. These two cases were accepted as normal karyotype in line with the literature.

Limitations of our study are the small sample size and the use of conventional karyotype testing since in POC genetic analysis, CMA technology has a higher success rate than the classical G-banding technique; 86% versus 75%.[1] CMA technique can achieve a successful read in over 86% cases while conventional cytogenetic reaches about 75% culture success.

However, CMA's weaknesses are that it cannot detect balanced translocations and low-level mosaicism.[1] G-banding karyotyping can also be preferred because it can detect numerical anomalies and has a lower cost.[19]

CONCLUSION

The frequency of abnormal karyotype in RPL cases was 31%, and the most common abnormality was autosomomal trisomies (62.5%). Trisomy 22 was the most common of the trisomies (24%). We also emphasize that the frequency of abnormal karyotype increases with advanced maternal age, according to our results.

Statement

Ethics Committee Approval: The Şişli Memorial Hospital Ethics Committee granted approval for this study (date: 04.03.2022, number: 001).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – FŞ, YÖ, RA; Design – YÖ, FŞ, RA; Supervision – RA, FŞ, KÖ; Data Collection and/or Processing – YÖ, RA; Analysis and/or Interpretation – FŞ, RA, KÖ; Literature Search – KÖ, FŞ, AS; Writing – FŞ, KÖ, RA; Critical Reviews – RA.

Conflict of Interest: The authors have no conflict of interest to declare.

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