BJMG 26 (1), 2023 • 5-10
DOI: 10.2478/bjmg-2023-0002
sciendo

ORIGINAL ARTICLE

POLYPLOIDY PHENOMENON AS A CAUSE OF EARLY MISCARRIAGES IN ABORTION MATERIALS

Yildirim ME[1], Karakus S[2], Kurtulgan HK[1], Ozer L[3], Celik SB[1]

[*]**Corresponding Author:** Malik Ejder Yildirim, Associate Professor. Department of Medical Genetics, Faculty of Medicine, Sivas Cumhuriyet University, 58104 Sivas, Turkey.
Phone: 03462581085, Email: nemalik2002@gmail.com

ABSTRACT

Objectives: Chromosomal abnormalities are an important cause of especially early miscarriages. The aim of this study was to analyze the chromosomal aberrations and determine the frequencies of numerical and structural chromosome abnormalities in spontaneous abortion materials.

Methods: This was a prospective research and ninety two abortion samples obtained from women who had one or more miscarriages were included in the study. Conventional karyotype analysis was performed on each sample to identify possible chromosomal abnormalities.

Results: By karyotype analysis, 11 polyploidy cases, (9 triploids and 2 tetraploids), 8 trisomies (one of which was mosaic), 2 monosomies (monosomy X), 1 isochromosome, 1 Xq deletion, and 4 translocations were detected in abortion materials. Isochromosome and Xq deletion cases were also mosaic. In addition, five polymorphic variants were revealed. We found higher paternal age in polyploidy cases.

Conclusion: The most common anomaly we found in abortion materials was polyploidy. This was followed by aneuploidy (trisomy and monosomy). Polyploidy (triploidy or tetraploidy) emerged as an important cause in cases of spontaneous abortion. Paternal age may be associated with polyploidy especially triploidy.

Key words: Chromosomal abnormality, polyploidy, miscarriage, abortion materials

[1] Department of Medical Genetics, Faculty of Medicine, Sivas Cumhuriyet University, 58104 Sivas, Turkey.

[2] Department of Obstetrics and Gynecology, Faculty of Medicine, Sivas Cumhuriyet University, 58104 Sivas, Turkey.

[3] Department of Medical Genetics, Faculty of Medicine, Yuksek Ihtisas University, Ankara, Turkey.

INTRODUCTION

A miscarriage is defined as the loss of pregnancy before 20 weeks of estimated gestational age [1]. The first definitive ultrasound evidence of intrauterine pregnancy is the observation of the yolk sac, which should appear by 6 weeks after the last menstrual period [2]. About 10 to 20 percent of known pregnancies result in miscarriage. A miscarriage may be an isolated status or it may occur repeatedly and it is generally accepted that 1 out of 5-6 pregnancies results in miscarriage. However, recurrent miscarriages occur in only 1-2% of couples [3]. Most miscarriages occur in the first trimester, especially between 8th and 12th weeks [4].

The etiology of spontaneous abortion is often unclear and, in this context, it may be multifactorial [5]. There may be various causes of abortion, including anatomical, genetic, immunologic, endocrine, and thrombophilic problems [6]. Since a significant number of pregnancies are lost before implantation, they are not clinically recognized [7]. About 50% of first trimester abortions are caused by chromosomal abnormalities such as aneuploidy and can be determined by conventional cytogenetic analysis. It is claimed that 86% of these abnormalities are numerical, 6% are structural abnormalities and 8% of them result from other genetic mechanisms [8]. Chromosomal variations found in material obtained from chorionic villus sampling are considered to be the chromosome structure of the fetus in the majority of cases [9]. Embryonic aneuploidy, which increases notably with advanced maternal age, constitutes a large part of spontaneous abortion [10]. Recurrent aneuploidy/polyploidy is an event largely related to maternal age but in rare cases, couples may experience recurrent aneuploidy/polyploidy as a result of gonadal mosaicism or another maternal age-independent state [11]. On the other hand, paternal age is also a risk factor that may increase the possibility of spontaneous abortion, independent of

certain conditions, such as socio-demographics and maternal age [12].

Trisomy is the presence of an extra copy of a chromosome in the cell nucleus and it is one of the most common chromosomal abnormalities in miscarriages. In a study conducted with FISH method in early miscarriages, 37.3% of the cases were found to have aneuploidy and 7% have polyploidy. Moreover, it was reported that paternal age may be associated with the risk of fetal aneuploidy in this research [13].

Triploidy is a chromosomal abnormality in which a cell has three copies of each chromosome and this accounts for 1% of conceptions. Most of the affected fetuses are usually lost during the first trimester [14]. Tetraploidy is defined as the presence of four haploid sets of chromosomes in the cell nucleus. Complete tetraploidy is usually fatal, and very few live births with this condition have been reported so far [15].

Besides conventional cytogenetic analysis, more detailed techniques can be used to analyze miscarriage specimens including fluorescence in situ hybridization (FISH) and array comparative genomic hybridization (aCGH) [16]. In this study, we aimed to examine the karyotype of abortion materials and to detect possible numerical and structural chromosome abnormalities from polyploidy and aneuploidies to translocations.

MATERIALS AND METHODS

Sample selection

After a comprehensive anamnesis for each case, ninety two abortion materials were included in this research and all were first trimester miscarriages. Samples were collected at Cumhuriyet University Research Hospital between 2017 and 2021. The study complies with the Helsinki Declaration and it was approved by the Ethics Committee of Cumhuriyet University. Informed consent was obtained from the families. The mean age of the females participating in the study was calculated.

Laboratory analysis

For the examination of abortion materials, separate cultures were prepared by placing the sample into two flasks, one containing a half Chang D and another with Amniomax which were then left to incubate in a CO2 incubator at 37 °C. When there was sufficient growth, 60 µL colchicine were added for every 5mL of culture, for 45 minutes. The growth medium was then removed and a fixative solution was added for 10 minutes at room temperature. This step was repeated twice and then the plates were prepared as usual and stained by G band. Captured cells by a computerized system (Applied Imaging, USA) were analyzed and karyotyped in the metaphase stage. The results were defined according to the International System for Human Cytogenetic Nomenclature (ISCN). A few cases with a family history of translocation or inadequate karyotype analysis were subsequently analyzed with aCGH for confirmation.

Statistical analysis was performed with SPSS program, version 22.0 (SPSS, Chicago, IL, USA). For the evaluation of the data, descriptive statistical criteria such as frequency, mean and standard deviation were chosen. The Independent Sample T test was used to compare the means.

RESULTS

In order to detect chromosomal abnormalities, karyotyping was performed on 92 spontaneous abortion samples and aCGH was applied whenever needed. The averages of age, body mass index, gravidity, parity and miscarriage numbers of females were calculated (Table 1). The mean age of women with miscarriage was 27±4.43. Twelve of these women (13%) had a history of recurrent miscarriage. There was no growth in 23 samples (25%) due to culture failure, while the results were normal in 42 materials (45.7%). A total of 32 various numerical and structural chromosomal abnormalities and chromosomal variations were found in 27 of the cases (29.3%) (Table 2). Triploidy was the most common among them (28%) (Table 3). In terms of numerical chromosomal abnormalities, 11 polyploidy cases (2 tetraploids and 9 triploids) were found as well as 8 trisomies (one of which was mosaic) and 2 sex chromosome aneuploidies (monosomy X) in our study. Trisomies included chromosomes 9, 13, 15 (two cases), 16 (two cases), 18, and chromosome 2 as mosaic. We detected 4 translocations as structural chromosomal abnormalities. Translocations consisted of two t(9;18), one t(1;7) and one t(7;11). On the other hand, the short arm isochromosome of chromosome 6 was found in one of the cases, while another case had a deletion of the long arm of the X chromosome and both abnormalities were mosaic. Among the numerical and structural chromosomal abnormalities, the rate of polyploidy was the highest (40.7%) (Figure 1). No abnormalities were detected in the karyotype analysis of the parents of polyploidy cases.

Table 1. Characteristics of females with spontaneous abortion.

Characteristics	Females
Age	27±4.43
BMI	24.92±3.42
Gravidity	3 (1-8)
Parity	1 (0-5)
Abortion	2 (1-7)

Table 2. Chromosome abnormalities and variations in abortion materials.

92,XXXX	47,XX,+18
92,XXYY,t(1;7)(p13;p13)	47,XY,+9
69,XXY (6 cases)	46,XX,1qh+[53]/47,XX,1qh+,+2[3]/ 46,XX,1qh+,del(X)(q22.1;qter)[1]
69,XXX (3 cases)	
45,X (2 cases)	46,XY[10]/47,XY,+i6(p)[4]
47,XX+13	46.XX.t(9;18)(q21;p11.3)
47,XX,+15,t(7;11)(p13;q25)	46,XY,9qh+
47,XX,+15	46,XX,15ps+
47,XX,+16,der(18)t(9;18)(q22;q21.1)	46,XX,22ps+
47,XX,+16	46,XX,inv(9)(p12q13)

Table 3. Ratio of chromosomal abnormalities and variations.

Case	Number	%
Tetraploidy	2	6.3
Triploidy	9	28
Trisomy	8	25
Monosomy X	2	6.3
Translocation	4	12.5
Isochromosome	1	3.1
X deletion	1	3.1
Heterochromatin +	2	6.3
Satellite +	2	6.3
İnversion 9	1	3.1
Total	32	100

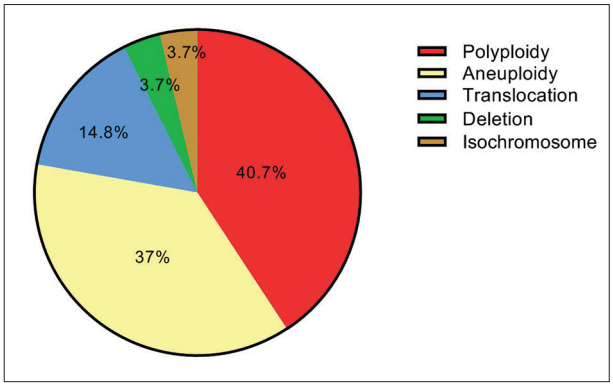

Figure 1. The distribution of cases in chromosomal abnormalities.

We found 5 chromosomal variations (two heterochromatin and two satellite increments and inv9) in abortion materials. Although there was no significant difference in terms of maternal age, we determined a higher paternal age in polyploidy cases than in abortions with normal karyotype. (31.2±6.4 versus 34.6±4.8 and p=0.002).

DISCUSSION

Although various problems from immune dysregulation to thrombophilia have been shown to play a role in the etiology of miscarriages, it is stated that most of the abortions are caused by genetic abnormalities [17]. Various chromosomal changes are particularly predominant in cases of abortion. Most chromosomal disorders are sporadic and have little risk of recurrence, but their analysis is important for couples and has a prognostic value in terms of the next pregnancy [18]. Chromosomal problems in the form of aneuploidy (trisomy-monosomy) and polyploidy (triploidy-tetraploidy) are determining factors in at least 50% of first trimester abortions, 25% of second, and 11% of third trimester miscarriages [19]. Cytogenetic analysis of conception products (POCs) can be done to identify the genetic cause of miscarriages, to predict recurrence risk, and to provide important information for genetic counseling and reproductive planning. This procedure, however, has some limitations due to a risk of culture failure, maternal cell contamination, suboptimal chromosome preparation etc. [20]. It is important to carry out cytogenetic analysis in terms of Preimplantation Genetic Screening (PGS) in IVF clinics in order to suggest suitable management and/or treatment approaches in recurrent miscarriage [21]. Conventional G-banding karyotyping is used as a gold standard procedure to determine chromosomal aneuploidy and some other variations [22].

Along with polyploidy, aneuploidy (trisomy or monosomy) was also prominent as a significant cause of spontaneous abortions in our study. In addition to chromosomal abnormalities (triploidy, tetraploidy, trisomy, monosomy, translocation, etc.), we found some chromosomal variations (heterochromatin and satellite increments, inversion 9) in abortion materials. The relationship of these variations with miscarriage is is not clear.

Polyploidy is a fatal cytogenetic anomaly that often results in pregnancy loss. [23]. Russo et al. stated that most chromosomal abnormalities in early spontaneous abortion are aneuploidies [24], while Brancati et al. claimed that triploidy is the most common chromosome abnormality in first trimester spontaneous abortions [25]. On the other hand, in a study by Carson et al., triploidy was declared as the third most common cause of pregnancy loss [26]. Polyploidy cases can be found at a high rate in spontaneous abortions, and they are expected to be lethal with multiple congenital defects. Triploidy is a numerical chromosomal aberration characterized by the presence of an extra haploid chromosome set and may occur in approximately 17% of spontaneous abortions in first trimester [27]. A third chromosome set in this condition may originate either from the mother or the father at the time of fertilization. Triploid pregnancies often result in early miscarriage or infants die within the first days of life. Some cases who have a mosaic form may survive with various problems. On the other hand, Dória et al reported that approximately 1,3% of first trimester miscarriages are true tetraploid [28]. Tetraploidy is very rare condition in live-born infants and it is usually detected in mosaic form with various anomalies [29]. Complete tetraploidy can often be found in spontaneous abortions [30].

Lee et al. identified single autosomal trisomy (71%) as the most common genetic defect in miscarriage. In this study, certain rates of polyploidy (16.1%), multiple aneuploidy (9.7%) and monosomy X (3.2%) were present. The rate of genetic abnormalities was also high in this research (49.2%) [31]. Similarly, Gug et al. found autosomal trisomies as the most common chromosomal abnormality in miscarriages (56.4%). In this study, the rate of polyploidy was 16.2%, monosomy 16.2%, and structural abnormalities 11.5% [32]. However, Lathi et al. state that the most common numerical chromosome problem identified in first trimester spontaneous abortions is monosomy X, followed by trisomy 16 and other chromosomal disorders, such as triploidy and tetraploidy are responsible for about 8% of miscarriages with numeric chromosomal defects [33]. Oliveira et al. suggested that a case of repeated triploid pregnancies in the same woman, from different fathers strongly point to recurrent triploidies with maternal origin, and therefore genetic predisposition should be considered [34]. We detected a high rate of polyploidy in spontaneous abortion materials. A polyploid case has three or more times the haploid number of chromosomes and it is not clear why polyploidy occurs in pregnancy. Polyploidy does not appear to be a condition related to maternal age [35]. However, high paternal age may be associated with the occurrence of polyploidy, particularly triploidy. A possible dyspermia may be the source of an extra set of paternal chromosomes. The prevalence of paternal (diandric) triploidy varies between 20% and 85% in various studies and the vast majority of them are lost in the first trimester of pregnancy [36]. Most triploidies may have paternal origin, and diandric triploidy can often be diagnosed as partial hydatidiform moles with abnormal placentas characterized by trophoblastic proliferation [37]. Although diandric triploids are often caused by dispermy, data regarding the effect of paternal age on the incidence of triploidy are still lacking [38]. We encountered a higher average paternal age in polyploidy cases than in abortions with normal karyotype. Most of the polyploidies (81.8%) were triploidy in our study (Figure 2). However, this issue is not clear due to the relatively small number of cases in our study. Therefore, the possible cause of polyploidy or triploidy should be investigated in larger groups including many abortion samples and thus more triploidy cases.

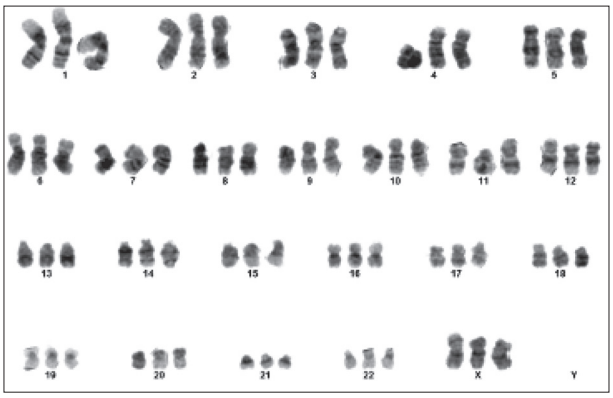

Figure 2. Karyotype image for a triploidy case (69,XXX).

In conclusion, we investigated karyotype abnormalities in spontaneous abortion materials in this study and detected various structural and numerical chromosomal anomalies and some variations. Routine studies and analyses to be carried out in this manner are important for subsequent pregnancies. The most common abnormality we observed was polyploidy (triploidy and tetraploidy). It was followed by trisomy, translocation and monosomy. In this context, we can say that polyploidy is involved in the etiology of a significant portion of miscarriage cases. In addition, more comprehensive study groups should be screened to evaluate clearly, whether paternal age contributes to polyploidies, especially triploidies.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES

1. Wesselink AK, Wise LA, Hatch EE, Mikkelsen EM, Savitz DA, Kirwa K, et al. A Prospective Cohort Study of Seasonal Variation in Spontaneous Abortion. Epidemiology. 2022; 33(3): 441-448.
2. Schmidt-Hansen M, Cameron S, Lord J, Hasler E. Initiation of abortion before there is definitive ultrasound evidence of intrauterine pregnancy: A systematic review with meta-analyses. Acta Obstet Gynecol Scand. 2020; 99(4): 451-458.
3. Woolner AMF, Raja EA, Bhattacharya S, Danielian P, Bhattacharya S. Inherited susceptibility to miscarriage: a nested case-control study of 31,565 women from an intergenerational cohort. Am J Obstet Gynecol. 2020; 222: 168.e1-168.e8.
4. Rolnik DL, Carvalho MH, Catelani AL, Pinto AP, Lira JB, Kusagari NK, et al. [Cytogenetic analysis of miscarriage material from spontaneous abortion]. Rev Assoc Med Bras. 2010; 56(6): 681-683.
5. Kim JW, Lyu SW, Sung SR, Park JE, Cha DH, Yoon TK, et al. Molecular analysis of miscarriage products using multiplex ligation-dependent probe amplification (MLPA): alternative to conventional karyotype analysis. Arch Gynecol Obstet. 2015; 291(2): 347–354.
6. Carp H, Toder V, Aviram A, Daniely M, Mashiach S, Barkai G. Karyotype of the abortus in recurrent miscarriage. Fertil Steril. 2001; 75(4): 678-682.
7. Pinar MH, Gibbins K, He M, Kostadinov S, Silver R. Early Pregnancy Losses: Review of Nomenclature, Histopathology, and Possible Etiologies. Fetal Pediatr Pathol. 2018; 37(3): 191-209.
8. Colley E, Hamilton S, Smith P, Morgan NV, Coomarasamy A, Allen S. Potential genetic causes of miscarriage in euploid pregnancies: a systematic review. Hum Reprod Update. 2019; 25(4): 452-472.
9. Ljunger E, Cnattingius S, Lundin C, Annerén G. Chromosomal anomalies in first-trimester miscarriages. Acta Obstet Gynecol Scand. 2005; 84(11): 1103-1107.
10. Du Y, Chen L, Lin J, Zhu J, Zhang N, Qiu X, et al. Chromosomal karyotype in chorionic villi of recurrent spontaneous abortion patients. Biosci Trends. 2018; 12(1): 32-39.
11. Robinson WP, McFadden DE, Stephenson MD. The origin of abnormalities in recurrent aneuploidy/polyploidy. Am J Hum Genet. 2001; 69(6): 1245-1254.
12. Nguyen BT, Chang EJ, Bendikson KA. Advanced paternal age and the risk of spontaneous abortion: an analysis of the combined 2011-2013 and 2013-2015 National Survey of Family Growth. Am J Obstet Gynecol. 2019; 221(5): 476.e1-476.e7.
13. Jia CW, Wang L, Lan YL, Song R, Zhou LY, Yu L, et al. Aneuploidy in Early Miscarriage and its Related Factors. Chin Med J (Engl). 2015; 128(20): 2772-2776.
14. Toufaily MH, Roberts DJ, Westgate MN, Holmes LB. Triploidy: Variation of Phenotype.
Am J Clin Pathol. 2016; 145(1): 86-95.
15. Wada Y, Kakiuchi S, Mizuguchi K, Nakamura T, Ito Y, Sago H, et al. A female newborn having mosaicism with near-tetraploidy and trisomy 18. Am J Med Genet A. 2016; 170A(5): 1262-1267.
16. Shah MS, Cinnioglu C, Maisenbacher M, Comstock I, Kort J, Lathi RB. Comparison of cytogenetics and molecular karyotyping for chromosome testing of miscarriage specimens. Fertil Steril. 2017; 107(4): 1028-1033.
17. Magnus MC, Hockey RL, Håberg SE, Mishra GD. Prepregnancy lifestyle characteristics and risk of miscarriage: the Australian Longitudinal Study on Women's Health. BMC Pregnancy Childbirth. 2022; 22(1): 169.
18. Bastos R, Ramalho C, Dória S. [Prevalence of chromosomal abnormalities in spontaneous abortions or fetal deaths]. Acta Med Port. 2014; 27(1) :42-48.
19. Babu R, Van Dyke DL, Bhattacharya S, Dev VG, Liu M, Kwon M, et al. A rapid and reliable chromosome analysis method for products of conception using interphase nuclei. Mol Genet Genomic Med. 2018; 6(3): 370-381.
20. Pylyp LY, Spynenko LO, Verhoglyad NV, Mishenko AO, Mykytenko DO, Zukin VD. Chromosomal abnormalities in products of conception of first-trimester miscarriages detected by conventional cytogenetic analysis: a review of 1000 cases. J Assist Reprod Genet. 2018; 35(2): 265–271.
21. Turki RF, Assidi M, Banni HA, Zahed HA, Karim S, Schulten H, et al. Associations of recurrent miscarriages with chromosomal abnormalities, thrombophilia allelic polymorphisms and/or consanguinity in Saudi Arabia. BMC Med Genet. 2016; 17(Suppl 1): 69.
22. Shen J, Wu W, Gao C, Ochin H, Qu D, Xie J, et al. Chromosomal copy number analysis on chorionic villus samples from early spontaneous miscarriages by high throughput genetic technology. Mol Cytogenet. 2016; 9: 7.

23. Gug C, Burada F, Ioana M, Riza AL, Moldovan M, Mozos I, et al. Polyploidy in First and Second Trimester Pregnancies in Romania - a Retrospective Study. Clin Lab. 2020; 66(4). doi: 10.7754/Clin.Lab.2019.190649
24. Russo R, Sessa AM, Fumo R, Gaeta S. Chromosomal anomalies in early spontaneous abortions: interphase FISH analysis on 855 FFPE first trimester abortions. Prenat Diagn. 2016; 36(2): 186-191.
25. Brancati F, Mingarelli R, Dallapiccola B. Recurrent triploidy of maternal origin. Eur J Hum Genet. 2003; 11(12): 972-974.
26. Carson JC, Hoffner L, Conlin L, Parks WT, Fisher RA, Spinner N, et al. Diploid/triploid mixoploidy: A consequence of asymmetric zygotic segregation of parental genomes. Am J Med Genet A. 2018; 176(12): 2720-2732.
27. Kolarski M, Ahmetovic B, Beres M, Topic R, Nikic V, Kavecan I, et al. Genetic Counseling and Prenatal Diagnosis of Triploidy During the Second Trimester of Pregnancy. Med Arch. 2017; 71(2): 144-147.
28. Dória S, Lima V, Carvalho B, Moreira ML, Sousa M, Barros A, et al. Application of touch FISH in the study of mosaic tetraploidy and maternal cell contamination in pregnancy losses. J Assist Reprod Genet. 2010; 27(11): 657-662.
29. Soler A, Badenas C, Margarit E, Madrigal I, Muñoz M, Borobio V, et al. A 92, XXXY Miscarriage Consecutive to a Digynic Triploid Pregnancy. Cytogenet Genome Res. 2016; 149(4): 258-261.
30. Stefanova I, Jenderny J, Kaminsky E, Mannhardt A, Meinecke P, Grozdanova L, et al. Mosaic and complete tetraploidy in live-born infants: two new patients and review of the literature. Clin Dysmorphol. 2010; 19(3): 123-127.
31. Lee JM, Shin SY, Kim GW, Kim WJ, Wie JH, Hong S, et al. Optimizing the Diagnostic Strategy to Identify Genetic Abnormalities in Miscarriage. Mol Diagn Ther. 2021; 25(3): 351-359.
32. Gug C, Rațiu A, Navolan D, Drăgan I, Groza IM, Păpurică M, et al. Incidence and Spectrum of Chromosome Abnormalities in Miscarriage Samples: A Retrospective Study of 330 Cases. Cytogenet Genome Res. 2019; 158(4): 171-183.
33. Lathi RB, Gray Hazard FK, Heerema-McKenney A, Taylor J, Chueh JT. First trimester miscarriage evaluation. Semin Reprod Med. 2011; 29(6): 463-9.
34. Oliveira AF, Torrão MM, Nogueira R, Ferreira M. Recurrent fetal triploidy: is there a genetic cause? BMJ Case Rep. 2021; 14(3): e239843.
35. Xu Q, Chan Y, Feng Y, Zhu B, Yang B, Zhu S, et al. Factors associated with fetal karyotype in spontaneous abortion: a case-case study. BMC Pregnancy Childbirth. 2022; 22(1): 320. doi: 10.1186/s12884-022-04491-8.
36. Massalska D, Ozdarska K, Roszkowski T, Bijok J, Kucińska-Chahwan A, Panek GM, et al. Distribution of diandric and digynic triploidy depending on gestational age. J Assist Reprod Genet. 2021; 38(9): 2391-2395.
37. Zaragoza MV, Surti U, Redline RW, Millie E, Chakravarti A, Hassold TJ. Parental origin and phenotype of triploidy in spontaneous abortions: predominance of diandry and association with the partial hydatidiform mole. Am J Hum Genet. 2000; 66(6): 1807-20.
38. Wiener-Megnazi Z, Auslender R, Dirnfeld M. Advanced paternal age and reproductive outcome. Asian J Androl. 2012; 14(1): 69-76.