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Cytogenetic analysis of 132 products of conception after spontaneous abortions and fetal death *in utero*

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ARTICLE INFO	ABSTRACT					
Received 15 December 2023 Accepted 16 March 2024	Spontaneous abortion is the most common pregnancy related complication, and more than half of these pregnancy losses are attributed to chromosomal abnormalities					
Keywords: spontaneous abortion, cytogenetic analysis, chromosomal abnormalities.	Cytogenetic analysis of spontaneous abortion produce to chromosoniar abiormatites. Cytogenetic analysis of spontaneous abortion products provides valuable information on the nature and frequency of chromosomal abnormalities in various populations, their origin and the risk of recurrence in a population. Characterization of chromosomal abnormalities in spontaneous miscarriages or fetal deaths using both classical and molecular cytogenetics to discover the incidence and types of chromosomal aberrations. One hundred and thirty-two samples derived from cases of spontaneous abortion and fetal death <i>in utero</i> were sent for analysis to the medical cytogenetics department of Clermont-Ferrand (France) between January 2011 and December 2015. The various specimens were carefully dissected and cultured to produce karyotypes. In case of culture failure or microbial contamination, a Bac en Beads analysis (BoBs [™]) has been performed. All abnormalities detected by caryotype or BoBs [™] were confirmed by Fluorescent <i>In Situ</i> Hybridization Cytogenetic analysis was successfully performed in 89% of cases (117/132). For normal karyotypes, male-female ratio was 0.81. Twenty-five karyotypes involved chromosomal rearrangement, which represents 21.4% of all cases (25/117). Karyotype failed in 41% of all issues (54/132). A BoBs [™] analysis was performed when cultures failed; it was found to be uninformative in 15 cases and revealed 2 chromosomal abnormalities. The average gestational age in aberrant karyotypes was 17.12±8.89 SA, while the average age of mothers was 31.55±6.67 years. The presence of a chromosomal abnormality may explain the cause of the miscarriage, hence, will improve genetic counseling and reproductive planning.					

INTRODUCTION

When considering the probability of success with regards to conception (POC), the results of human reproduction are quite poor. About 78% of all fail to complete [1]. Indeed, the combined data from numerous studies of women attempting to be pregnant revealed a 42% preimplantatory loss rate among clinically proven pregnancies (by the presence of HCG) [2,3]. Furthermore, the overall net fertility for patients aged between 20 and 30 years

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is between 21 and 28% [4], a fairly low level compared to most other mammalian species. Spontaneous abortion (SA) is the most common complication during pregnancy [5,6]. It is defined as the spontaneous loss of a clinically recognized pregnancy before viability (20 weeks of pregnancy) [7]. The majority of spontaneous abortions occur during the first trimester [7,8], but losses continue to occur throughout the second and third trimester of pregnancy, with a slight increase in term mortality [9]. It is estimated that about 15-20% of all clinically recognized pregnancies fail early [10], but the overall prevalence is probably 4-5 times higher [11].

Obstetricians group losses before 20 weeks under the term "abortions" and fetal losses later under the term "fetal death in utero" (FDIU). The causes of these losses are numerous and diverse, but fetal chromosomal abnormalities account for at least half of cases before 12 weeks, and nearly a third of miscarriages in the second trimester [12]. These can be meiosis, mitosis or fertilization errors. These embryonic chromosomal abnormalities are related to the age of the mother, the number of previous miscarriages or other factors [13]. Between 25% and 50% of all women experience one or more SAs during their reproductive life, often due to a chromosomal anomaly of the conceptus [14,15]. Nearly 90% of the women concerned have new pregnancies and do not miscarriage. However, for a small but significant percentage, this is repeated: This is referred to as "repetitive spontaneous abortion" (RSA), defined as two or more clinical pregnancy losses not necessarily consecutive [16]. Thus, cytogenetic analysis of (POCs) is essential to establish the etiology of fetal losses and assess patients at risk of recurrence in future pregnancies by providing valuable information for genetic counseling and reproductive planning [17,18].

The objective of this study was to discover the incidence and types of chromosomal aberrations in an unselected series of spontaneous miscarriages in the French population.

MATERIAL AND METHODS

One hundred and thirty-two products of SA (n=34) and FDIU (n=98) were sent for analysis to the medical cytogenetics department of Clermont-Ferrand (France) between January 2011 and December 2015. The average gestational age at the time of the various samples was 21.62±9.04 (between 7 and 40 weeks gestation). The average maternal age was 30.58±6.53 years (between 16 and 45 years). All women provided written consent. The samples consisted of biopsy specimens from products of curettage (n=74), placenta (n=26), fetal skin fibroblasts (n=21) and chorionic villi (CV) (n=11). All samples were previously analyzed to eliminate non fetal parts and maternal decimal remains. The various samples were carefully dissected and cultured to produce karyotypes according to standard procedures. A minimum of 15 to 20 G-band metaphases were analyzed for each sample.

Karyotypes and chromosomal aberrations were classified and identified in accordance with the current International Standard Nomenclature for Human Cytogenomic (ISCN) [19], a mosaicism was defined as reported by this same nomenclature: the loss of a chromosome must be detected in at least three cells, the gain of a chromosome and/or a structural chromosomal aberration must be present in at least two metaphases. In case of culture failure or microbial contamination, a Bac en Beads analysis (BoBsTM) was performed. This assay is used for the rapid detection of gains and losses of DNA in 75 regions, including aneuploidies of chromosomes 13, 18, 21, X and Y, as well as gains and losses in nine microdeletion syndrome regions that are often associated with genetic disorders. The assay was read using a Luminex 200 analyser, and the fluorescence data were analysed with BOBSOFT_software developed by the assay manufacturer (PerkinElmer, Wallac Oy, Turku, Finland). BoBs[™] test was performed according to the manufacturer's protocol described above [20].

DNA was obtained from different samples without cell culture using the QIAamp DNA Mini Kit (Qiagen, Germany). A sample was defined as "normal disomic" when the fluorescent ratio was approximately 1.0 for all tested loci. When there were deletions or gains, the probe ratios were outside the normal expected ratio range determined by the software for each sample. All abnormalities detected by carvotype or BoBsTM were confirmed by Fluorescent In Situ Hybridization (FISH). Alpha-satellite probes for the centromeric regions (CEP probes for chromosomes X, Y, 13,18 and 21 or locus specific probes were prepared according to manufacturer's instructions (Abbott). Slides were placed in ThermoHybaid (denaturation at 73°C for 2 min followed by hybridization at 37°C overnight), then counterstained with DAPI/Vectashield. Finally, slide epifluorescence was observed under microscope (Axioplane 2 imaging), fitted with camera (CoolCube 1) and analyzed using Metafer (Metafer METASYSTEMS). The samples were studied using the following sequential protocol (Figure 1).



Figure 1. Flow chart of the sequential protocol used in the study

RESULTS

Cytogenetic analysis (CA) was successfully performed in 89% of all cases (117/132). Male karyotypes accounted for 43% of the total number of cases (49/117), while female karyotypes accounted for 57% of all cases (68/117). We counted 44 normal male karyotypes and 54 normal female karyotypes, that is a male-female ratio of 0.81. Twenty-five karyotypes involved chromosomal rearrangement, which represents 21.4% of the full number of cases (25/117). Karyotype failed in 41% of all cases (54/132) due to absence of cell proliferation or microbial contamination. A BoBs[™] analysis was performed when cultures failed, and was found to be uninformative in 15 cases. It revealed 2 chromosomal abnormalities. The average gestational age in aberrant karyotypes was 17.12±8.89 SA, while the average age of mothers was 31.55±6.67 years. Chromosomal abnormalities were identified in Tables 1 and 2.

Structural anomalies (n=9)

Contrary to all expectations, and while usually aneuploidies represent the most common aberrations in POCS, structural changes were the most represented in our study sample, representing 36% (9/25) of the total aberrations. Among them, 5 translocations (2 reciprocal translocations both inherited from the father, and three Robertsonian translocations), two inversions and two deletions (one revealed by BoBsTM).

Triploidy (n=8)

Triploidy is the second most common type of karyotypic abnormalities detected, with 32% of all cases (8/25), including one case in mosaic (69,XXX[8]/46,XX[8]).

Trisomies (n=7)

Trisomies accounted for 28% (7/25) of the total aberrations. All were free and homogeneous. Thus, we found: four trisomies 21, two trisomies 18, of which one was revealed by BoBsTM due to culture failure (Figure 2) and confirmed by FISH, and a sexual trisomy (Klinefelter Syndrome 47,XXY).

Monosomy (n=1)

Only one aberrant karyotype (4%) had gonosomal monosomy (45,X).

 Table 1. Conventional cytogenetic results and obstetric history in

 23 spontaneous abortions or foetal deaths with abnormal karyotype

Patients	Maternal age (Years)	Gestationnal age (Weeks)	Obstetric history	Cytogenetics analysis (CA)
1	32	12	Unknown	69,XXX[8]/46,XX[8] unknown
2	36	10	G5P0 (5 SA) (Father with t(7;12)(q32;p13)	46,XX,t(7;12)(q32. p13)pat
3	41	13	G4P2 (1 SA)	47,XY,+21
4	26	9	G5P0 (Father with t(3;6)(p22;q23)	46,XX,t(3,6) (p22;q23)
5	33	16	Unknown	69,XXX
6	29	12	Unknown	69,XXY
7	33	14	Unknown	47,XY,+18
8	22	13	Unknown	45,X
9	33	33	G1P0	69,XXY
10	41	11	Unknown	47,XY,+21
11	Unknown	39	Unknown	47,XYY
12	39	14	Unknown	47,XY,+21
13	24	13	G4P0 (1 SA + 2 TOP)	46,XX,der(1)t(1;9) mat
14	29	26	G1P0	69,XXX
15	45	13	G1P0	47,XX,+21
16	Unknown	12	Unknown	45,XX,der(13;14) (q10;q10)pat
17	24	21	Unknown	69,XXX
18	35	8	G2P1	45,XX,der(13;21) (q10;q10)
19	27	15	G2P1	46,XY,del(3) (q23q26)
20	21	13	Unknown 69,XXX	
21	Unknown	23	Unknown	46,XX,inv(9) (p11q13)
22	Unknown	Unknown	G4P3	69,XXY
23	31	17	G3P2	46,XY,inv(9) (p11q13)

SA: Spontaneous abortion; G: Gravida; P: para ; TOP: termination of pregnancy

Table 2.	BoBs™	studies	in 2	cases	of	spontaneous	abortion	and
foetal death with culture failure								

Patients	Maternal age (Years)	Gestationnnal age (Weeks)	Obstetric history	Cytogenetics analysis (CA)	
1	25	40	Unknown	BOBs XY del17 (FISH confirmation 17p13.3)	
2	27	14	G3P1(1 SA)	BOBs XY;T18 (FISH confirmationTel18p/ Tel18q)	
CA. Crantanaque abortion, C. Cravida, D. nara					

SA: Spontaneous abortion; G: Gravida; P: para



Figure 2. Case whit trisomy 18: BoBs[™] profile with gain in chromosome 18

DISCUSSION

Cytogenetic studies provide valuable genetic information that can be extremely useful for genetic counseling and be a predictor for future pregnancies. The cytogenetic analysis carried out as part of our study provided a result in 89% of the total number of cases. The karyotype was successfully carried out in 59% of all cases. Indeed, and despite the emergence of many molecular cytogenetic techniques, karyotype remains the first-line examination in genetic analysis of pregnancy losses in most laboratories [21]. Recent studies have shown its reliability and effectiveness in highlighting aneuploidies related to spontaneous abortions [18,22,23]. The success rate of the karyotype in our study historically corresponds to the success rates of between 48 and 59% reported by the literature [24,25]. Cytogenetic studies have always been limited, after evacuation of the product of conception retained in utero, by two aspects: contamination of samples by maternal cells and the absence of proliferation of cells in vitro linked to in utero maceration of the conceptus. The majority of our samples were taken after removal from POCs and received in a relatively advanced maceration state, which could explain our high karyotype failure rate (41%). These rates tend to be greatly reduced, by removing CV prior to discharge and treating it within

a few hours, thereby minimizing the risk of microbiological contamination and improving the short-term culture success of the karyotype [18,22].

The male/female ratio among normal karyotypes in this study was 0.81 which is similar to the literature data. Shearer *et al.* [22] had actually observed a ratio sex of 1.0, while this was a constant feature in the study of Eiben *et al.* [26] (male-female ratio sex estimated at 0.71). These findings support the theory that there is a specific disadvantage in the development of early embryonic female conceptus [26,27]. The almost balanced male-female ratio in normal chromosomal pregnancy losses is largely due to the absence of maternal contamination: the fetal parts studied having been thoroughly analysed and dissected. Another theory advanced by later studies is that the excess of normal female karyotypes increases with gestational age [26], which is the case for our study.

The frequency of chromosomal abnormalities was only 21.4%, which is lower than literature data [12,28,29]. However, the proportions of chromosomal aberrations among the different spontaneous studies on miscarriages have always been extremely varied. Table 3 compares the results of recent studies on success rates, failure rates and frequency of abnormalities.

Study	Samples (n)	Tissue	Success rate, %	Abnormality rate, %
Doria <i>et al.</i> , 2009 [12]	332	FT	74,6	36,6
Shearer <i>et al.</i> , 2011 [22]	3418	CV	90	55
Jenderny, 2014 [27]	534	CV	73	61
Wang <i>et al.</i> , 2014 [30]	5457	CV or FT	75	45
Soler <i>et al</i> ., 2017 [18]	1119	CV	90,3	70,3

Table 3. Success rate of POCs karyotype in recent studies

CV: chorionic villi; FT: fetal tissue

The difference between the frequencies of chromosomal aberrations revealed by conventional cytogenetics in POCs in the literature can be associated with many factors, essentially: the characteristics of the studied sample (gestational age, maternal age) and the protocol used by the laboratory [18]. Our results must, however, be analysed with particular attention, taking into account that samples of products of miscarriage with cytogenetic aim are rarely carried out before 12 SA, it is, therefore, possible that this is due to the fact that gestational age in our series was high (21,62±9,04 weeks), whereas, usually, most cytogenetic studies are performed on AS occurring during the first trimester of pregnancy [12,13,23,31]. However, the genetic factor in the losses of pregnancies in the second and third trimesters is no longer the first order of the causes of these failures. Indeed, a negative correlation was observed between the frequency of chromosomal abnormalities and the gestational age of the products of conception by Pylyp et al. [23]: their study revealed that in the 11th week of gestation, only 38.6% of all POCs had chromosomal abnormalities (compared to 46.7% to 55.5% in week 4-10). This decrease in the frequency of chromosomally abnormal POCs at week 11 of gestation may be mainly due to the end of a major fraction of abnormal chromosomal pregnancies at that time, as well as a decrease in the risk of confined placental mosaics in cases where fetal fibroblasts have been studied [23]. Abnormal karyotypes are,

therefore, less frequently found as pregnancy progresses, with most non-viable abnormal pregnancies having already ended in miscarriage. During the second trimester of pregnancy, infections become the primary cause of pregnancy loss. Gaillard *et al.* [32] studied 462 pregnancy losses in the second quarter, 78.6% of which had recently occurred because their maceration was not extensive, ascending infections accounted for 85% of these abortions.

Numerical anomalies were a large part of the anomalies identified in our study sample. The average maternal age was higher in the sample with abnormalities. It is well known that maternal age is an important risk factor, although strongly correlated with parity and reproductive history, the literature clearly shows a strong and independent effect of maternal age on the risk of spontaneous abortion [5]. The trisomies came in the first position of the aneuploidies observed, trisomies 21 were the most common (n=4/7). This aneuploidy is described as one of the most common in preimplantation embryos at the blastocyst stage and in POCs [33-35]. All cases of trisomy 21 (n=4) were from mothers over the age of 39 (41, 41, 39 and 45 years, respectively). Many studies have endorsed this trend [26,36,37], demonstrating that non-viable and viable trisomies showed a significantly increased trend with maternal age, particularly trisomy 21 [13]. In contrast, the trisomies 18 (n=2) observed in our study came from young mothers (27 and 33 years, respectively). This finding is supported by the study by Soler et al. [18] which showed no correlation between trisomies 18 and advanced maternal age. It also confirmed the elimination of most trisomies 18 early during pregnancy, which is the case in our study.

Many studies have shown that the risk of monosomy X does not also appear to be related to maternal age [38,39]. We observed only one case of monosomy in our study (45,X) which did not deviate from this observation (maternal age=22 years). Elimination of this monosomy also occurred very early in pregnancy (13 SA), which is the case for 99% of all POCs with monosomy X [40]. Monosomy X is more likely to be derived from the meiotic error of the father than the mother [41], which could probably explain why the frequency of monosomy X is not associated with maternal age [13].

Only one sexual trisomy was revealed by our cytogenetic analysis: 47,XXY (Klinefelter's Syndrome). Karyotype 47,XXY also appears to be associated with advanced maternal age [26,42,43], but the age of the mother in our study was unknown, thus we cannot provide support for this theory or contradict it. On the other hand, this FDIU occurred very late in pregnancy (33 weeks of gestation), which is not an exceptional fact given that sexual trisomies are part of viable trisomies, but the majority of conceptions 47,XXY do not survive to term [44].

Triploidy constitutes between 1% and 3% of aberrations encountered in recognized pregnancies, 99% of which occur during the first or second trimester [45]. The frequency of 32% (n=8/25) is greater in this study than in other important previous reports [22,26,46]. Triploidy often has the origin of double fertilizations (dispermia, digynia), it occurs most often in young women [26,36,37]. All triploidy observed in our study came from mothers under the age of 32.

Structural rearrangements were found in 32% (n=9) of all abnormal cases, which is significantly higher than literature data (4% for Jenderny et al. [27], 4.9% for Gardner et al. [15], 5.6% for Soler et al. [18], 7.6 for Doria et al. [12]). Among these, 7 were balanced (including 2 reciprocal translocations, 3 Robertsonian translocations and 2 inversions) and 2 were unbalanced (2 deletions). Reciprocal translocations are the most commonly encountered structural chromosomal rearrangements in humans, with an estimated incidence of 1/712 in newborns [47]. Among the balanced abnormalities, we observed 2 reciprocal translocations, both hereditary (paternal translocations), and mothers suffering from repetitive spontaneous abortions (G5P0 and G4P0). Usually, RSA can occur, as in our study, when one of the parents carries a balanced anomaly: reciprocal translocation, Robertsonian translocation or inversion [48]. The abnormal segregation of balanced meiosis translocations increases not only the risk of RSA but also the risk of the birth of a disabled child [49], hence the interest of diagnosing couples suffering from RSA. This would provide appropriate genetic advice, including prenatal and preimplantatory diagnosis [50].

The two inversions encountered in our study were identical: 46,XY,inv(9)(p11q13) and 46,XX,inv(9)(p11q13). This inversion is a chromosomal alteration that occurs frequently in humans, with an incidence in the general population of 1-3% [51]. Its clinical impact is widely discussed, with some studies considering it as a normal variant, others associating it with numerous pathologies such as infertility and reproductive failure [52]. Xie et al. [53] found this inversion in 31 fetuses of 1865 miscarriage products analyzed and in 67 adults of 2988 with infertility, making it a recurrent aberration. The carriers of pericentric chromosomal inversions are phentotypically normal, but when pairing homologous chromosomes during gametogenesis, a single inversion cycle will form during the first meiosis. The exchange of homologous chromosomes in the inversion circle will produce four gametes, one of which is normal, the other inverted, and the other two partially duplicated or suppressed. These imbalanced gametes can cause miscarriage, infertility and reproductive failure [53]. In our study, this is most likely an anomaly inherited from one of the parents, but unfortunately, we did not have any additional information to support this possibility.

Cytogenetic analysis of our sample also revealed 2 deletions: del(17)(p13.3) and del(3)(q23q26). Deletion 17p13.3 was detected by BoBsTM after cell culture failure and confirmed by FISH. It corresponds to Miller Dieker Syndrome (MDS). These structural abnormalities account for about 6% of the abnormalities found in POCs [54], and with reason, MDS is characterized by a defect in brain development (type 1 lissencephalitis), which is caused by incomplete neuronal migration. This microdeleterious syndrome is also characterized by distinctive facial features and other birth defects that induce pregnancy failure [55]. It is important to stress that structural rearrangements are of particular importance for couples and their future offspring. Their identification can be very useful for the genetic counseling of other parents at risk of unbalanced offspring [27]. In this study, 54 spontaneous miscarriages did not grow in vitro. We used BoBsTM to improve our success rate in detecting clinically relevant chromosomal aberrations. BoBsTM analysis was not informative in only 6 cases and detected chromosomal aberration in 2 spontaneous miscarriages. This technique offered a very interesting alternative to conventional karyotype, despite its limitations in the detection of polyploidy (triploidy and tetraploidia) [56,57], and is, in addition, a more informative technique than the rapid analyses FISH and QF-PCR, which only screen for frequent aneuploidy [56].

The study limitations: The number of participating patients in the study was relatively low due to limited access to samples, the absence of maternal consent, advanced maceration in some cases and maternal contaminations are among the major challenges in cytogenetic studies of conception products. Consequently, a larger cohort could provide more comprehensive and precise information. The two molecular techniques used, Prenatal BoBs[™] and FISH, remain targeted methods that only detect gains and losses of genetic information but do not identify balanced micro-rearrangements, including balanced translocations and inversions. This limitation could introduce potential distortion into the results. Additionally, chromosomal abnormalities not covered by the probes used may go unnoticed during the analysis.

CONCLUSION

Our study showed that the cytogenetic aspects of spontaneous miscarriage have not really changed. The presence of a chromosomal abnormality may explain the cause of the miscarriage, improve genetic counseling and reproductive planning. Cytogenetics is a valuable tool for genetic counseling and prediction of the outcome of future pregnancies, but should not be considered only as a set of techniques useful in medical practice, but as part of human genetics that contribute to research as diverse as the inactivation of X, the significance of chromosomal polymorphisms, the segregation of abnormalities, the dynamics of their evolution in vitro, the behaviour of chromosomes to mitosis and meiosis and the mechanisms involved, telomere abnormalities etc. In addition, the combination of different cytogenetic techniques now offers a diverse range of options, opening the way to a wide range of developments, including the study of human development and genome variations.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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REFERENCES

- 1. Roberts CJ, Lowe CR. Where have all the conceptions gone? Lancet. 1975;1:498-9.
- Edmonds DK, Lindsay K, Miller JF, Williamson E, Wood PJ. Early embryonic mortality in women. *Fertil Steril*. 1983;38:447-53.
- Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield R E, et al. Incidence of early loss of pregnancy. N Engl J Med. 1988;319:189-94.
- 4. Short RV. When a conception fails to become a pregnancy. *Ciba Foundation Symposium*. 1978;(64):377-94.
- Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. *BMJ*. 2000;320(7251):1708-12.
- 6. Wang X, Chen C, Wang L, Chen D, Guang W, French J. Conception, early pregnancy loss, and time to clinical pregnancy: a populationbased prospective study. *Fertil Steril*. 2003;79(3):577-84.
- Jauniaux E, Farquharson RG, Christiansen OB, Exalto N. Evidencebased guidelines for the investigation and medical treatment of recurrent miscarriage. *Hum Reprod.* 2006;21(9):2216-22
- 8. Goldhaber MK, Fireman BH. The fetal life table revisited: spontaneous abortion rates in three Kaiser Permanente cohorts. *Epidemiology*. 1991;2(1):33-9.
- 9. Leridon H. Human fertility: *The basic components*. University of Chicago Press London; 1978.
- 10. Gardner RJ, Sutherland GR, Shaffer LG. *Chromosome abnormalities* detected at prenatal diagnosis. Chromosome Abnormalities and Genetic Counseling. Oxford University Press; 2011:439-85.
- 11. Rai R, Regan L. Recurrent miscarriage. *Lancet*. 2006;368(9535): 601-11.
- 12. Dória S, Carvalho F, Ramalho C, Lima V, Francisco T, Machado AP, et al. An efficient protocol for the detection of chromosomal abnormalities in spontaneous miscarriages or foetal deaths. *Eur J Obstet Gynecol Reprod Biol.* 2009;147(2):144-50.
- Ozawa N, Ogawa K, Sasaki A, Mitsui M, Wada S, Sago H. Maternal age, history of miscarriage, and embryonic/fetal size are associated with cytogenetic results of spontaneous early miscarriages. J Assist Reprod Genet. 2019;36(4):749-57.
- 14. Stirrat GM. Recurrent miscarriage. Lancet. 1990;336(8716):673-5.
- Greenwold N. Collection of villous tissue under ultrasound guidance to improve the cytogenetic study of early pregnancy failure. *Hum Reprod.* 2002;17(2):452-6.
- 16. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss: a committee opinion. *Fertil Steril.* 2020;113(3):533-5.
- Bianco K, Caughey AB, Shaffer BL, Davis R, Norton ME. History of miscarriage and increased incidence of fetal aneuploidy in subsequent pregnancy. *Obstet Gynecol.* 2006;107(5):1098-102.
- Soler A, Morales C, Mademont-Soler I, Margarit E, Borrell A, Borobio V, et al. Overview of chromosome abnormalities in first trimester miscarriages: A series of 1,011 consecutive chorionic villi sample karyotypes. *Cytogenet Genome Res.* 2017;152(2):81-89.
- Shaffer LG, McGowan-Jordan J, Schmidt M. ISCN 2013 an international system for human cytogenetic nomenclature. Basel: Karger Verlag AG; 2013.
- Lakovschek IC, Streubel B, Ulm B. Natural outcome of trisomy 13, trisomy 18, and triploidy after prenatal diagnosis. *Am J Med Genet A*. 2011;155A(11):2626-33.
- Haoud K, Mellali S. Current data on the use of cytogenetic techniques in the study of spontaneous abortions. *Adv Biores*. 2019;10(6): 24-30.

- 22. Shearer BM, Thorland EC, Carlson AW, Jalal SM, Ketterling RP. Reflex fluorescent in situ hybridization testing for unsuccessful product of conception cultures: a retrospective analysis of 5555 samples attempted by conventional cytogenetics and fluorescent *in situ* hybridization. *Genet Med.* 2011;13(6):545-52.
- 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):265-71.
- 24. Kajii T, Ferrier A, Niikawa N, Takahara H, Ohama K, Avirachan S. Anatomic and chromosomal anomalies in 639 spontaneous abortuses. *Hum Genet.* 1980;55(1):87-98.
- 25. Hassold T. Chromosome abnormalities in human reproductive wastage. *Trends Genet.* 1986;2:105-10.
- 26. Eiben B, Bartels I, Bähr-Porsch S, Borgmann S, Gatz G, Gellert G, et al. Cytogenetic analysis of 750 spontaneous abortions with the direct-preparation method of chorionic villi and its implications for studying genetic causes of pregnancy wastage. *Am J Hum Genet*. 1990;47(4):656-63.
- 27. Jenderny J. Chromosome aberrations in a large series of spontaneous miscarriages in the German population and review of the literature. *Mol Cytogenet*. 2014;7:38.
- Petracchi F, Colaci DS, Igarzabal L, Gadow E. Cytogenetic analysis of first trimester pregnancy loss. *Int J Gynaecol Obstet*. 2009;104(3): 243-4.
- 29. Kroon B, Harrison K, Martin N, Wong B, Yazdani A. Miscarriage karyotype and its relationship with maternal body mass index, age, and mode of conception. *Fertil Steril.* 2011;95(5):1827-9.
- Wang BT, Chong TP, Boyar FZ, Kopita KA, Ross LP, El-Naggar MM, et al. Abnormalities in spontaneous abortions detected by G-banding and chromosomal microarray analysis (CMA) at a national reference laboratory. *Mol Cytogenet*. 2014;7:33.
- Cheng HH, Ou CY, Tsai CC, Chang SD, Hsiao PY, Lan KC, et al. Chromosome distribution of early miscarriages with present or absent embryos: female predominance. J Assist Reprod Genet. 2014; 31(8):1059-64.
- 32. Gaillard DA, Paradis P, Lallemand AV, Vernet VM, Carquin JS, Chippaux CG, et al. Spontaneous abortions during the second trimester of gestation. *Arch Pathol Lab Med.* 1994;117(10):1022-6.
- Nagaishi M, Yamamoto T, Iinuma K, Shimomura K, Berend SA, Knops J. Chromosome abnormalities identified in 347 spontaneous abortions collected in Japan. J Obstet Gynaecol Res. 2004;30(3): 237-41.
- 34. Fragouli E, Alfarawati S, Spath K, Jaroudi S, Sarasa J, Enciso M, Wells D. The origin and impact of embryonic aneuploidy. *Hum Genet.* 2013;132(9):1001-13.
- 35. Rodriguez-Purata J, Lee J, Whitehouse M, Moschini RM, Knopman J, Duke M, et al. Embryo selection versus natural selection: how do outcomes of comprehensive chromosome screening of blastocysts compare with the analysis of products of conception from early pregnancy loss (dilation and curettage) among an assisted reproductive technology population? *Fertil Steril.* 2015;104(6): 1460-66.
- 36. Grande M, Borrell A, Garcia-Posada R, Borobio V, Muñoz M, Creus M, et al. The effect of maternal age on chromosomal anomaly rate and spectrum in recurrent miscarriage. *Hum Reprod.* 2012;27(10): 3109-17.
- 37. Hardy K, Hardy PJ, Jacobs PA, Lewallen K, Hassold TJ. Temporal changes in chromosome abnormalities in human spontaneous abortions: Results of 40 years of analysis. *Am J Med Genet A*. 2016; 170(10):2671-80.
- Hassold T, Arnovitz K, Jacobs PA, May K, Robinson D. The parental origin of the missing or additional chromosome in 45,X and 47,XXX females. *Birth Defects Orig Artic Ser*. 1990;26(4):297-304.
- Haoud K, Mellali S, Gouas L, Tchirkov A, Vago P, Moulessehoul S. Prevalence of aneuploidies in products of spontaneous abortion: interest of FISH and MLPA. *Morphologie*. 2014; 98(320):40-6.
- 40. Simpson JL. Genetics. In: *CREOG basic science monograph in obstetrics and gynecology council on resident education in obstetrics and gynecology*. Washington DC; 1986.

- Segawa T, Kuroda T, Kato K, Kuroda M, Omi K, Miyauchi O, et al. Cytogenetic analysis of the retained products of conception after missed abortion following blastocyst transfer: a retrospective, largescale, single-centre study. *Reprod Biomed Online*. 2017;34(2):203-10.
- 42. Ohno M, Maeda T, Matsunobu A. A cytogenetic study of spontaneous abortions with direct analysis of chorionic villi. *Obstet Gynecol.* 1991;77(3):394-8.
- 43. Sánchez JM, Franzi L, Collia F, De Díaz SL, Panal M, Dubner M. Cytogenetic study of spontaneous abortions by transabdominal villus sampling and direct analysis of villi. *Prenat Diagn*. 1999; 19(7):601-3.
- Ljunger E, Cnattingius S, Lundin C, Annerén G. Chromosomal anomalies in first-trimester miscarriages. Acta Obstet Gynecol Scand. 2005;84(11):1103-7.
- 45. Gardner RJM, Sutherland GR. Down syndrome, other full aneuploidies, and polyploidy. In: McKinlay Garner RJ, Sutherland GR (eds). *Chromosome abnormalities and genetic counseling*. New York: Oxford University Press; 2011:249-63.
- 46. Menasha J, Levy B, Hirschhorn K, Kardon NB. Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. *Genet Med*. 2005;7(4):251-63.
- Nielsen J, Wohlert M. Sex chromosome abnormalities found among 34, 910 newborn children: results from a 13-year incidence study in Arhus, Denmark. *Birth Defects Orig Artic Ser.* 1990;26(4):209-23.
- Boué A, Gallano P. A collaborative study of the segregation of inherited chromosome structural rearrangements in 1356 prenatal diagnoses. *Prenat Diagn.* 1984;4:45-67.
- Puig M, Casillas S, Villatoro S, Cáceres M. Human inversions and their functional consequences. *Brief Funct Genomics*. 2015;14(5): 369-79.

- Elhady GM, Kholeif S, Nazmy N. Chromosomal Aberrations in 224 Couples with Recurrent Pregnancy Loss. J Hum Reprod Sci. 2020; 13(4):340-8.
- 51. Dana M, Stoian V. Association of pericentric inversion of chromosome 9 and infertility in romanian population. *Maedica* (Bucur). 2012;7(1):25-9.
- 52. Muthuvel A, Ravindran M, Chander A, Subbian C. Pericentric inversion of chromosome 9 causing infertility and subsequent successful *in vitro* fertilization. *Niger Med J.* 2016;57:142-4.
- Xie X, Li F, Tan W, Tang J. Analysis of the clinical features of pericentric inversion of chromosome 9. J Int Med Res. 2020;48(9): 300060520957820.
- 54. Paxton CN, Brothman AR, Geiersbach KB. Rapid aneusomy detection in products of conception using the KaryoLite[™] BACs-on-Beads[™] assay. *Prenat Diagn.* 2013;33(1):25-31.
- Mellali S, Haoud K, Gouas L, Khaled MB, Vago P, Moulessehoul S. Prenatal BoBsTM in the cytogenetic analysis of products of spontaneous miscarriage. S Afr Med J. 2015;105(10):870-3.
- 56. Vialard F, Simoni G, Aboura A, De Toffol S, Molina Gomes D, Marcato L, et al. Prenatal BACs-on-Beads: A new technology for rapid detection of aneuploidies and microdeletions in prenatal diagnosis. *Prenat Diagn*. 2011;31(5):500-8.
- 57. Vialard F, Simoni G, Gomes DM, Abourra A, De Toffol S, Bru F, et al. Prenatal BACs-on-Beads[™]: The prospective experience of five prenatal diagnosis laboratories. *Prenat Diagn*. 2012;32(4):329-35.