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Rapid Detection of Aneuploidies in Spontaneous Aborted Fetal Samples by Quantitative Fluorescence-PCR: A Descriptive Study

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ABSTRACT

Chromosomal aneuploidies are the most chromosomal abnormalities at birth due to maternal meiosis I errors. Pregnancies with autosomal chromosomal aneuploidies that survive are namely trisomies 13 (Patau syndrome), 18 (Edward syndrome), and 21 (Down syndrome), account for 89% of chromosome abnormalities. Quantitative fluorescent polymerase chain reaction (QF-PCR) which amplifies specific DNA sequences called short tandem repeats (STRs), by using fluorescently labeled primers is a rapid technique for prenatal diagnosis of common aneuploidies. In this study, DNA extraction was performed from 100 samples isolated from muscle tissue of aborted fetuses. The analysis was performed by multiplex QF-PCR using a panel of 25 STRs markers for chromosomes X, Y, 13, 18, and 21. Our results showed that 20% of abortions were due to aneuploidy. 53% of mothers who had abortions were aged 26-35 years old and 32% of them were aged 36-45 years old. The analysis of muscle samples of aborted fetuses indicated that 20 samples showed chromosomal aneuploidy. Of the abnormal cases, 10 cases (~50 %) showed trisomy 21 followed by trisomy 18 (7 cases, ~35%), Klinefelter syndrome (2 cases, ~10 %), and showed trisomy X (1 case, ~5 %). Our results indicated that the *D21S1414* marker showed the highest rate of heterozygosity in the study population. Besides some limitations of this study such as sample size, these results suggest that one of the causes of these abortions could be maternal age. We concluded that QF-PCR could be a rapid and reliable method to screen prenatal chromosomal aneuploidy and allow appropriate counseling.

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Introduction

Clinical cytogenetics is an indispensable tool for prenatal diagnosis of chromosomal abnormalities including aneuploidies and unbalanced structural rearrangements (Shaffer and Bui, 2007). Chromosomal aneuploidies are the most chromosomal abnormalities at birth due to maternal meiosis I errors (Nagaoka *et al.*, 2012). Pregnancies with autosomal chromosomal aneuploidies that survive are namely trisomies 13 (Patau syndrome), 18 (Edward syndrome), and 21 (Down syndrome), account for 89% of chromosome abnormalities (Mann and Ogilvie, 2012). Trisomy 16 is the most frequent autosomal aneuploidy (more than 7% of the cases) that is lethal and incompatible with fetal development followed by other autosomal and sex aneuploidies (Choi *et al.*, 2014; Teles *et al.*, 2017). Studies have shown that spontaneous abortions due to aneuploidies have approximately 35% incidence (Hassold *et al.*, 1996; Nagaoka *et al.*, 2012). These abnormalities are detected normally by full karyotype analysis with 2 to 3 weeks on average (Waters and Waters, 1999; Badenas *et al.*, 2010). Conventional cytogenetic tests such as

karyotyping and fluorescence in situ hybridization (FISH) have some problems including contaminations and culture failures and their expensiveness (Diego-Alvarez *et al.*, 2005). Recently, some alternative methods including array comparative genomic hybridization (array-CGH), quantitative fluorescent PCR (QF-PCR), and multiplex ligation-dependent probe amplification (MLPA) have been introduced and developed as rapid aneuploidy tests (RATs) that reduce the time and the workload (Comas *et al.*, 2010).

QF-PCR is a PCR-based method that amplifies specific DNA sequences called short tandem repeats (STRs) known as polymorphic markers, using fluorescently labeled primers (Nicolini *et al.*, 2004). This method has some advantages compared to conventional cytogenetic methods, including the requirement of a small sample, high throughput at low costs, automation of the procedure, and rapid turnaround time (Langlois and Duncan, 2011). However, the main disadvantage of this technique is its inability to detect structural chromosome abnormalities (Hulten *et al.*, 2003).

This study aimed to detect the 13, 18, 21, X, and Y chromosome aneuploidies of spontaneous miscarriages that occurred in an Iranian population.

Materials and Methods

The data for this study were derived from the analysis of tissue samples isolated from aborted fetuses before the 16th week, that were supplied from several welfare centers and medical genetic laboratories in Tehran and Golestan provinces. The criteria for cytogenetic examination were the following: termination of pregnancy, fetal loss, and a history of miscarriage, in participated women. A total of 100 samples were collected from muscle tissue of aborted fetuses of women that most of them had at least one abortion in the past.

DNA extraction

After digesting the samples isolated from the muscle tissue of aborted fetuses, DNA was extracted using the salting-out method. Nucleic acids were then eluted in a final volume of 25 µl of distilled water.

Markers used

In this study, using multiplex PCR, a total of 25 STR markers were amplified to detect the copy numbers of chromosomes 13, 18, 21, X, and Y (Table 1). The *amelogenin* (*AMXY*) and *SRY* markers were used to determine the fetus's sex (Cirigliano *et al.*, 2001; Atef *et al.*, 2011).

Table 1. Markers selected for QF-PCR detection of chromosome aneuploidies

	Marker	Chromosomal location	Product size		Marker	Chromosomal location	Product size
1	D13S797	13q33.2	115-155	14	D21S1442	21q11.11	136-174
2	D13S325	13q14.11	125-190	15	IFNAR	21q22.1	350-402
3	D13S252	13q12.2	405-455	16	SRY	Yp11.31	214
4	D13S634	13q21.33	220-248	17	DXS7132	Xq12	115-150
5	D13S258	13q21.33	250-320	18	Y/X B 3	Yp11.2, Xq21.31	117 (Y)-124 (X)-(110-135)
6	D18S390	18q22.3	210-250	19	DYS437	Yq11.21	160-200
7	D18S391	18p11.31	160-200	20	Dx HPRT	Xq26.3	145-185
8	D18S535	18q21.3	275-325	21	DXS6803	Xq21.31	315-344
9	GATA178F11	18p11.32	370-430	22	*AMXY*	X p22.2, Y p11.2	110 (X),116 (Y)-(105-120)
10	D21S1414	21q21.1	350-430	23	DXS981	Xq13.1	330-365
11	D21S1809	21q22.2	260-284	24	DX-TATC13.35	Xp21.2	245-271
12	D21S1446	21q22.3	285-328	25	7X 4	7q34, Xq13.3	215 (7)-238 (X)-(210-240)
13	D21S1411	21q22.3	346-460	-	-	-	-

Multiplex QF-PCR

Using the KBC-Aneuquick-VII kit, samples were tested to assess the aneuploidies of the above-mentioned chromosomes. PCR reaction was performed in a total volume of 25 µL containing 10-20 ng of DNA and 20 µL of 1X reaction master mix. The mixture was pre-heated at 95 °C for 5 minutes, subsequently 30 cycles of 1 min at 95 °C, 90 seconds at 63 °C, and 90 seconds at 72 °C with a final extension step at 72 °C for 10 minutes. Electropherogram peaks were evaluated on the ABI3130xl instrument (Applied Biosystems, USA) and analyzed using GeneMarker v.195. Each PCR fragment peak corresponds to an STR and each peak uniquely

represents an allele (one of maternal and one of paternal origin). A normal heterozygote sample generates two peaks, while trisomy generates two or three peaks. The ratio of the picks leads to the diagnosis.

Statistical analysis

Data were analyzed for potential associations between spontaneous abortion and the presence of fetal chromosomal abnormalities using a Chi-squared test. Data were analyzed by SPSS ver.16 software. Data were expressed as mean ± SD and all tests were repeated three times. A *P-value* less than 0.05 was considered significant.

Results

A total of 100 cases were included in this study. About 68% of enrolled mothers had a consanguineous marriage. About 60% of mothers had one abortion, 20% had two abortions and 13% of them have experienced three abortions in the past (Table 2). Also, Table 2 shows that the distribution of gestational ages of the participated mothers was as 15-18 years (15%), 25-36 years (53%), and 36-45 years (32%). Our statistical analyses showed that 53% of the abortions occurred in mothers aged 25 to 36. About 70% of participated women had no child, 20% had one child and 10% of them had two children. About 70% of the aborted fetuses were up to 10 weeks.

In 20% of cases (N= 20), aneuploidy was detected. The aneuploidies included trisomy 21 (N= 10, 50 %), trisomy 18 (N=7, 35 %), Klinefelter syndrome (N= 2, 10 %), and trisomy X (N= 1, 5 %) (Table 2).

The gender determination of all samples was performed by PCR amplification of *Amelogenin* and *SRY* markers. Samples retrieved from trisomy 18 and/or 21 showed either a triallelic (ratio 1:1:1) or diallelic (ratio 2:1) pattern with chromosome 18 and/or 21-specific markers, respectively (Fig. 1). The samples with Klinefelter syndrome showed the 1:2 pattern for *AMXY* marker, 2:1 pattern for *Y/XB3* marker, 1:1 for *DX-TATC13.35*, *DXS6803*, *DXS7132*, and *Dx HPRT* markers, and sample retrieved from trisomy X showed 1:1:1 pattern for *DXS6803*, *DXS7132*, *DXS981*, and *Dx HPRT* markers and did not show any signal for *SRY* marker (Fig. 1).

Table 2. Demographic data of mothers and aborted fetuses included in this study.

Sex of aborted fetuses	**Percent**
Male	54
Female	46
Number of past abortions in mothers	
No abortion	7
One abortion	60
Two abortions	20
Three abortions	13
Mother age (Year)	
15-18	15
25-36	53
36-45	32
Number of children	
0	70
1	20
2	10
Age of aborted fetuses (Week)	
Up to 10	70
10 to 20	20
20 to 36	10
Abortion type	
Normal abortion	80
Abortion due to chromosomal abnormalities	20
Types of aneuploidy	**Number (%)**
Trisomy 21	10 (50 %)
Trisomy 18	7 (35 %)
Klinefelter syndrome (XXY)	2 (10 %)
Trisomy X	1 (5 %)

Discussion

The analysis of chromosomal abnormalities helps us to determine the possible causes of miscarriages or abortions (Goud *et al.*, 2009; van den Berg *et al.*, 2012). It has been reported that 50% to 60% of abortions have chromosomal abnormality causes and recurrent abortions because chromosomal abnormalities are 2% to 8% worldwide (Pal *et al.*, 2018).

This study aims to detect some common chromosomal aneuploidies in spontaneously aborted fetuses in an Iranian population. In this study, the first and the most important factor for selecting cases was the occurrence of abortion (regardless of consanguinity). It is necessary to mention that in some parts of Iran, especially in the Golestan province, consanguineous marriages are common. Nevertheless, our results showed that although a high percentage of participated mothers (93%) had a history of miscarriage, about 80% of miscarriages were not due to chromosomal abnormalities.

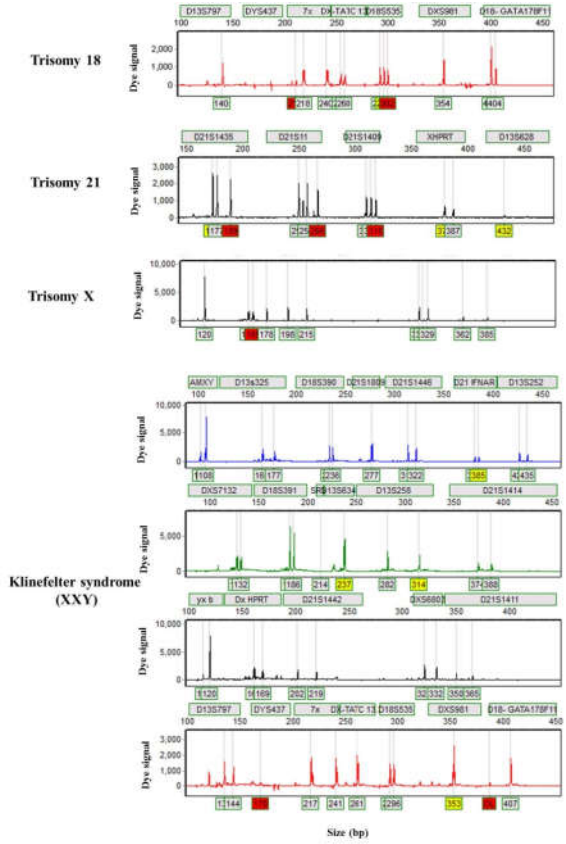

Fig. 1. Multiplex QF-PCR assays with STR markers in DNA samples of aborted fetuses: In the trisomy 18 sample, D18S535 shows a triallelic pattern with a dosage ratio of 1:1:1, and GATA178F11 shows a 2:1 pattern. The sample with trisomy 21, D21S1435, D21S11, and D21S1409 show a triallelic pattern with a dosage ratio of 1:1:1. In the trisomy X sample, DxHPRT and DXS6803 show a triallelic pattern with a dosage ratio of 1:1:1. In the sample with Klinefelter syndrome (XXY), AMXY shows a 1:2 pattern, DXS7132 shows diallelic with a dosage ratio of 1:1, Y/XB3 shows a trisomic pattern with a dosage ratio of 2:1, DxHPRT shows a diallelic pattern with a dosage ratio of 1:1, DXS6803 shows a diallelic pattern with a dosage ratio of 1:1, 7X marker shows a diallelic pattern with a dosage ratio of 1:1, and DX-TATC13.35 shows a diallelic pattern with a dosage ratio of 1:1.

There are several methods to detect chromosomal abnormalities (numerical or structural). Traditional karyotyping using growing cells derived from amniotic or chronic villus samples is costly, time-consuming (2-3 weeks), and labor-intensive (Comas *et al.*, 2010). The interphase FISH assay could not determine the origin of the sample, and the result would be a normal female; therefore, it is sufficient to distinguish between polyploid, monoploid, or diploid cells (Mann *et al.*, 2004; Zhang *et al.*, 2018). Also, the FISH assay could not detect the meiotic origin of aneuploidy (Diego-Alvarez *et al.*, 2005). Comparative genomic hybridization (CGH) is a time-consuming (one week) and costly assay that is not suitable for detecting chromosomal rearrangements, uniparental disomy, maternal contamination, or meiotic origin of aneuploidy (Mann *et al.*, 2004). Apart from its limitations including its inability to detect the balanced structural chromosomal aberrations, QF-PCR has exclusive advantages determining the fetal and/or parental origin of samples, and results can be obtained in 24-48h; a very low amount of DNA is needed as starting material (Diego-Alvarez *et al.*, 2005; Ahangari *et al.*, 2016).

In this retrospective study, 60% of participants have had one abortion, 20% had two and 13% of them had three abortion experiences so far. QF-PCR analysis of 100 miscarriage samples revealed the presence of aneuploidies in 20% of the cases. Coelho *et al.* (2016) reported 54.6% (Coelho *et al.*, 2016), Wu et al. (2016) reported 35.8% (Wu *et al.*, 2016), Shearer *et al.* (2011) reported 48% (Shearer *et al.*, 2011), Zou *et al.* (2008) reported 36.1% (Zou *et al.*, 2008), Lebedev *et al.* (2003) reported 53.3% (Lebedev *et al*., 2003), and Gug *et al.* (2019) reported that 47.2% of samples showed aneuploidy (Gug *et al.*, 2019). Several reasons may account for these discrepancies including the number of patients, gestational ages, and also ethnicity (Khoshnood *et al.*, 2000; Chitayat *et al.*, 2007; Saadi *et al.*, 2010; Bernatowicz *et al.*, 2019).

In our study, the *D21S1414* marker showed the highest rate of heterozygosity in our population. Cirigliano *et al.* (2001) used this marker for their studies and showed a high rate of heterozygosity. Due to its location on the long arm of chromosome 21 and flanking the Down syndrome critical region, it shows a high reliability in the diagnosis of trisomy 21 (Cirigliano *et al.*, 2001). In another study, Aleyasin *et al.* assessed and showed the diagnostic value of some markers including *D21S1414* for the rapid detection of Down syndrome in the Iranian population (Aleyasin *et al.*, 2004).

It is thought that about 20% of human eggs and 9% of human sperms show aneuploidy (Lee and Kiessling, 2017). Several lines of evidence have emphasized the complex association between advanced maternal age and chromosomal abnormalities in the fetuses (Allen *et al.*, 2009). For example, recent studies support the association of loss of cohesin protein and its contribution to maternal age-related aneuploidy (Sherman *et al.*, 2005; Hunt and Hassold, 2010; Zhang *et al.*, 2017). Studies on humans and rodents suggest an age-related increase in the number of sperm-bearing chromosomal breaks due to environmental damages, genomic instabilities, genetic and epigenetic factors, but aging decreases the effectiveness of antioxidant-related mechanisms (Sloter *et al.*, 2004).

In our study, more than 30% of mothers have advanced age (≥35 years old). Although its etiology is multifunctional (Nagaoka *et al.*, 2012), it has been reported that hormonal changes during the aging process could be a factor responsible for miscarriage in women older than 35 years (Xu *et al.*, 2011).

Finally, our results showed 20% of abortions were due to aneuploidy. 53% of mothers who had abortions were aged 26-35 years old and 32% of them were aged 36-45 years old.

It is clear that this study has some limitations. Increasing the sample size and investigating other chromosomal abnormalities including chromosome 16 can lead to the strengthening of this study and more accurate results.

Based on our evidence, employing the QF-PCR method requires investments in infrastructure, equipment, and lab staff training. However, its advantages, including reducing the need for conventional cytogenetic techniques and reducing parental anxiety are the factors helping the expansion of this method.

Conflicts of Interest

The authors declare no conflict of interests.

Ethical Statement

This study was approved by the University Ethics Committee (IR.UMZ.REC.1397.092) following the ethical standards of the responsible committee on human experiments. All patients agreed to participate in this study and the written informed consent is following the principles laid down in the Helsinki II declaration obtained from all of them.

References

Ahangari N, Doosti M, Ahangari E, Baradarn Rafiee N, Ghayoor Karimiani E. 2016. Prenatal diagnosis of fetal aneuploidies using QF-PCR in 333 cases. *Mol Med J* 2(1): 38-42.

Aleyasin A, Ghazanfari M, Ganji SM, Jahanshad F. 2004. Application of molecular DNA markers (STRs) in molecular diagnosis of down syndrome in Iran. *J Sci I R Iran* 15(2): 103-108.

Allen EG, Freeman SB, Druschel C, Hobbs CA, O'Leary LA, Romitti PA, Royle MH, Torfs CP, Sherman SL. 2009. Maternal age and risk for trisomy 21 assessed by the origin of chromosome nondisjunction: a report from the Atlanta and National Down Syndrome Projects. *Hum Genet* 125(1): 41-52.

Atef SH, Hafez SS, Mahmoud NH, Helmy SM. 2011. Prenatal diagnosis of fetal aneuploidies using QF-PCR: the egyptian study. *J Prenat Med* 5(4): 83-89.

Badenas C, Rodríguez-Revenga L, Morales C, Mediano C, Plaja A, Pérez-Iribarne MM, Soler A, Clusellas N, Borrell A, Sánchez MA. 2010. Assessment of QF-PCR as the first approach in prenatal diagnosis. *J Mol Diagn* 12(6): 828-834.

Bernatowicz K, Zimowski J, Łaczmańska I, Piotrowski K, Kashyap A, Bednarska-Makaruk M, Sąsiadek M, Gronwald J. 2019. Clinical utility of MLPA and QF-PCR techniques in the genetic testing of miscarriages. *Russ J Genet* 55(10):1259-1265.

Chitayat D, Langlois S, Wilson RD. 2007. Prenatal screening for fetal aneuploidy in singleton pregnancies. *J Obstet Gynaecol Can* 33(7): 736-750.

Choi TY, Lee HM, Park WK, Jeong SY, Moon HS. 2014. Spontaneous abortion and recurrent miscarriage: a comparison of cytogenetic diagnosis in 250 cases. *Obstet Gynecol Sci* 57 (6): 518-525.

Cirigliano V, Lewin P, Szpiro-Tapies S, Fuster C, Adinolfi M. 2001. Assessment of new markers for the rapid detection of aneuploidies by quantitative fluorescent PCR

(QF-PCR). *Ann Hum Genet* 65(5): 421-427.
Coelho, F, Marques, F, Gonçalves, M, Almeida VCO, Mateo ECC, Ferreira ACS. 2016. Detection of aneuploidies in spontaneous abortions by quantitative fluorescent PCR with short tandem repeat markers: a retrospective study. *Genet Mol Res* 15(3): gmr.15038617.

Comas C, Echevarria M, Carrera M, Serra B. 2010. Rapid aneuploidy testing versus traditional karyotyping in amniocentesis for certain referral indications. *J Matern Fetal Neonatal Med* 23(9): 949-955.

Diego-Alvarez D, Garcia-Hoyos M, Trujillo MJ, Gonzalez-Gonzalez, C, Rodriguez de Alba M, Ayuso C, Ramos-Corrales C, Lorda-Sanchez I. 2005. Application of quantitative fluorescent PCR with short tandem repeat markers to the study of aneuploidies in spontaneous miscarriages. *Hum Reprod* 20(5): 1235-1243.

Goud TM, Harassi SMA, Salmani KKA, Al Busaidy SM, Rajab A. 2009. Cytogenetic studies in couples with recurrent miscarriage in the Sultanate of Oman. *Reprod BioMed Online* 18(3): 424-429.

Gug C., Raţiu A, Navolan D, Drăgan I, Groza IM, Păpurică M, Vaida MA, Mozoş I, Jurcă MC. 2019. Incidence and spectrum of chromosome abnormalities in miscarriage samples: a retrospective study of 330 cases. *Cytogenet Genome Res* 158:171-183.

Hassold T, Abruzzo M, Adkins K, Griffin D, Merill M, Millie E, Saker D, Shen J, Zaragoza M. 1996. Human aneuploidy: incidence, origin, and etiology. *Environ Mol Mutagen* 28:167-175.

Hulten MA, Dhanjal S, Pertl B. 2003. Rapid and simple prenatal diagnosis of common chromosome disorders: advantages and disadvantages of the molecular methods FISH and QF–PCR. *Reprod* 126(3): 279-297.

Hunt P, Hassold T. 2010. Female meiosis: coming unglued with age. *Curr Biol* 20(17): R699-R702.

Khoshnood B, Pryde P, Wall S, Singh J, Mittendorf R, Lee KS. 2000. Ethnic differences in the impact of advanced maternal age on birth prevalence of down syndrome. *Am J Public Health* 90(11): 1778-1781.

Langlois S, Duncan A. 2011. Use of a DNA Method, QF-PCR, in the prenatal diagnosis of fetal aneuploidies. *J Obstet Gynaecol Can* 33(9): 955-960.

Lebedev IN, Ostroverkhova NV, Nikitina TV, Sukhanova NN, Nazarenko SA. 2003. Molecular cytogenetic characteristics of chromosome imbalance in spontaneous human abortion cells with low proliferative activity in vitro. *Russ J Genet* 39(8): 934-943.

Lee A, Kiessling AA. 2017. Early human embryos are naturally aneuploidy-can that be corrected? *J Assist Reprod Genet* 34(1): 15-21.

Mann K, Donaghue C, Fox SP, Docherty Z, Mackie Ogilvie C. 2004. Strategies for the rapid prenatal diagnosis of chromosome aneuploidy. *Eur J Hum Genet* 12:907-915.
Mann K, Ogilvie CM. 2012. QF-PCR: Application, overview and review of the literature. *Prenat Diagn* 32: 309-314.

Nagaoka SI, Hassold TJ, Hunt PA. 2012. Human aneuploidy: mechanisms and new insights into an age-old problem. *Nat Rev Genet* 13: 493-504.

Nicolini U, Lalatta F, Natacci F, Curcio C, Bui TH. 2004. The introduction of QF-PCR in prenatal diagnosis of fetal aneuploidies: time for reconsideration. *Hum Reprod Update* 10(6): 541-548.

Pal AK, Ambulkar PS, Waghmare JE, Wankhede V, Shende MR, Tarnekar AM. 2018. Chromosomal aberrations in couples with pregnancy loss: a retrospective study. *J Hum Reprod Sci* 11: 247-253.

Saadi A, Kushtgi P, Gopinath PM, Satyamoorthy K. 2010. Quantitative fluorescence polymerase chain reaction (QF-PCR) for prenatal diagnosis of chromosomal aneuploidies. *Int J Hum Genet* 10(1-3): 121-129.

Shaffer LG, Bui TH. 2007. Molecular cytogenetic and rapid aneuploidy detection methods in prenatal diagnosis. *Am J Med Genet C Semin Med Genet* 145C: 87-98.

Shearer BM, Thorland EC, Carlson AW, Jalal SM, Ketterling RP. 2011. 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* 13(6): 545-452.

Sherman SL, Freeman SB, Allen EG, Lamb NE. 2005. Risk factors for nondisjunction of trisomy 21. *Cytogenet Genome Res* 111:273-280.

Sloter E, Nath J, Eskenaz B, Wyrobek AJ. 2004. Effects of male age on the frequencies of germinal and heritable chromosomal abnormalities in humans and rodents. *Fertil Steril* 81(4): 925-943.

Teles TMA, Paula CMMd, Ramos MG, Coxir SA, Penna MLF. 2017. Frequency of chromosomal abnormalities in products of conception. *Rev Bras Ginecol Obstet* 39 (3): 110-114.

van den Berg MMJ, van Maarle MC, van Wely M, Goddijn M. 2012. Genetics of early miscarriage. *Biochim Biophys Acta* 1822: 1951-1959.

Waters JJ, Waters KS. 1999. Trends in Cytogenetic prenatal diagnosis in the UK: results from UKNEQAS external audit, 1987-1998. *Prenat Diagn* (19): 1023-1026.

Wu Z, Liu N, Zhao Z, Kong X. 2016. Detection of chromosome aneuploidies in spontaneous abortion villus samples by quantitative fluorescence PCR. *Chi J Med Genet* 33(2):227-230.

Xu YW, Peng YT, Wang B, Zeng YH, Zhuang GL, Zhou CQ. 2011. High follicle-stimulating hormone increases aneuploidy in human oocytes matured in vitro. *Fertil Steril* 95(1): 99-104.

Zhang XH, Qiu LQ, Ye YH, Xu J. 2017. Chromosomal abnormalities: subgroup analysis by maternal age and perinatal features in Zhejiang province of China, 2011-2015. *Ital J Pediatr* 43(1): 47.

Zhang T, Sun Y, Chen Z, Li T. 2018. Traditional and molecular chromosomal abnormality analysis of products of conception in spontaneous and recurrent miscarriage. *BJOG* 125(4):414-420.

Zou G, Zhang J, Li XW, He L, He G, Duan T. 2008. Quantitative fluorescent polymerase chain reaction to detect chromosomal anomalies in spontaneous abortion. *Int J Gynaecol Obstet* 103(3): 237-240.