

# Frequency of Chromosomal Abnormalities in Products of Conception

## *Frequência de anomalias cromossômicas em material de aborto*

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### Abstract

**Purpose** To describe the frequencies of chromosomal abnormalities found in abortion material, and to observe its correlation to maternal age.

**Methods** A retrospective study was conducted based on data obtained from the databank of a medical genetics laboratory in Belo Horizonte, MG, Brazil. A total of 884 results from products of conception analysis were included, 204 of which were analyzed by cytogenetics, and 680 by molecular biology based on quantitative fluorescence polymerase chain reaction (QF-PCR). The frequency of individual chromosomal aberrations and the relationship between the presence of anomalies and maternal age were also evaluated.

**Results** The conventional cytogenetics technique was able to detect 52% of normal and 48% of abnormal results in the analyzed material. Quantitative fluorescence polymerase chain reaction revealed 60% of normal and 40% of abnormal results from the samples evaluated by this method. The presence of trisomy 15 was detected only by cytogenetics, as it was not included in the QF-PCR routine investigation in the laboratory. A significant increase in abnormal results was observed among women aged 35 years or older compared with younger women ( $p = 0.02$ ).

**Conclusion** Chromosomal aberrations are still a major cause of spontaneous abortion, and the conventional cytogenetics technique is efficient for miscarriage material analysis, but molecular methods such as QF-PCR are adequate complementary strategies to detect the major chromosomal anomalies, leading to technical reports with reliable results.

### Keywords

- ▶ spontaneous abortion
- ▶ aneuploidies
- ▶ QF-PCR
- ▶ cytogenetics

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## Resumo

**Objetivos** Descrever a frequência de anomalias cromossômicas encontradas em material de aborto, e observar se estas estão relacionadas com a idade materna.

**Métodos** Foi realizado um estudo retrospectivo no banco de dados de um laboratório de genética médica em Belo Horizonte, MG. O estudo incluiu 204 resultados avaliados por citogenética, e 680 resultados por biologia molecular baseada em reação em ensaio fluorescente da reação em cadeia da polimerase (QF-PCR), totalizando um número de 884 análises. A frequência de diferentes anomalias cromossômicas e a relação entre a presença de anomalias e a idade materna também foi avaliada.

**Resultados** A citogenética convencional foi capaz de detectar 52% de resultados normais e 48% de resultados anormais no material analisado. A QF-PCR revelou 60% de resultados normais e 40% de anormais nas amostras avaliadas por esta técnica. A presença da trissomia 15 foi detectada por citogenética, mas até então não era incluída na investigação por QF-PCR no laboratório. Um aumento significativo na quantidade de resultados anormais foi observado em mulheres com idade de 35 anos ou mais, quando comparado a mulheres mais jovens ( $p = 0,02$ ).

**Conclusão** As aberrações cromossômicas são causas importantes de abortos espontâneos, e o estudo citogenético é eficaz para a análise das amostras de material de aborto, mas as técnicas moleculares, como a QF-PCR, representam métodos complementares adequados para detectar as principais anomalias cromossômicas, possibilitando a liberação de laudos com resultados confiáveis.

## Palavras-chave

- ▶ aborto espontâneo
- ▶ aneuploidias
- ▶ QF-PCR
- ▶ citogenética

## Introduction

Miscarriage is defined by the World Health Organization (WHO) as the premature loss of a fetus before the 20th week of pregnancy, or, if the gestational age is unknown, the loss of an embryo or fetus weighing less than 400 g.<sup>1</sup> Spontaneous pregnancy loss is the most common complication of pregnancy, and occurs in ~12–15% of clinically recognized pregnancies. The chance of a couple experiencing two consecutive losses is of 2 to 4%, but most women who have miscarriages can give birth to a healthy child later in life.<sup>2</sup>

The etiology of abortion is multifactorial, and may involve endocrine, anatomic, immunological, infectious, environmental and genetic factors.<sup>3</sup> Chromosomal abnormalities have been reported in 50% of spontaneously aborted fetuses of clinically recognized pregnancies, and can be divided in two basic groups: numerical and structural anomalies. These can involve one or more autosomal, sex or both chromosomes simultaneously.<sup>4,5</sup>

The most frequent autosomal anomaly observed in specimens from spontaneous losses is trisomy 16 (thought to be lethal and incompatible with full fetal development), followed by other autosomal aneuploidies and X monosomy.<sup>6,7</sup> As fetal chromosomal abnormalities are largely responsible for the inefficiency of human reproduction and its associated burdens, it is necessary to perform laboratory investigations of the products of conception (POC) using different diagnostic techniques to help to understand the possible causes of miscarriage and to provide adequate assistance for future pregnancies.<sup>8–10</sup>

The analysis of POC has been traditionally performed by cytogenetic karyotyping through the microscope examination of banded chromosomal preparations, detecting numer-

ical and structural alterations. Molecular cytogenetic diagnostic tests are based on studying the fetal karyotype directly at the DNA level, and use DNA extracted from fetal cells, not requiring tissue culture and allowing the analysis of specimens fixed in ethanol, formaldehyde or included in paraffin.<sup>11,12</sup> Some of these techniques are fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA) and quantitative fluorescent polymerase chain reaction (QF-PCR).

All reproductive losses should be investigated by cytogenetics, considering that conventional karyotyping has been the gold standard for the chromosomal investigations of POC. This method allows the detection of structural (translocations, deletions and inversions) as well as numerical chromosomal aberrations. However, it is a laborious, time consuming procedure that can lead to a significant amount of cases with no results, as it depends on human cells in active process of replication. The rate of culture failure is of 10–40%, as POC tissues are frequently macerated, contaminated or fixated in alcohol or formaldehyde.<sup>12,13</sup> Molecular cytogenetic techniques can be used to study POC abnormalities, as they do not require cell culture; however, the results could be limited because only numerical chromosomal alterations can be identified on the analyzed chromosomes.<sup>14</sup>

The genetic studies of POC provide important information for the genetic counseling of couples who experience pregnancy failure, as they help to elucidate the possible causes of fetal losses, indicating if any chromosomal abnormality was responsible for the miscarriage. They can also indirectly suggest if one of the parents could be the carrier of any structural disorder.<sup>12,14</sup>

The aim of this study was to describe the frequencies of chromosomal abnormalities found in abortion material, and to determine if there is a correlation between the presence of aberrations and maternal age.

## Methods

### Study Type and Samples

An observational, retrospective study was conducted to describe the results obtained from miscarriage material analysis performed by a private medical genetics laboratory in Belo Horizonte, MG, Brazil. The laboratory performs tests in miscarriage material received from different regions of Brazil, covering the whole country. The results were obtained from the laboratory databank, and, besides maternal age, no personal information from the patients was included. As the laboratory receives material from different medical facilities with limited information, clinical data such as gestational age at abortion and clinical history of the parents was not available. A total of 884 results from miscarriage material analysis performed between January 2011 and December 2015 was included.

### Sample Analysis

Miscarriage material analysis techniques were performed by professional staff, according to the laboratory routine, using conventional cytogenetics, or QF-PCR.

Conventional cytogenetics was performed in samples containing tissue that had fetal origin, using standard culture, harvesting and staining conditions, by an experienced cytogenetics technician. A total of 204 cytogenetics results were obtained and included in the study. The aberrations and karyotypes were classified according to the International System for Human Cytogenetic Nomenclature 2013 (ISCN 2013).<sup>15</sup>

In case of cell growth failure by the cytogenetics technique, and according to the laboratory demand, the QF-PCR molecular technique was performed, as previously described.<sup>16</sup> For detection of chromosome aberrations, markers for sexual X and Y, and for autosomal 13, 16, 18, 21 and 22 chromosomes were performed by experienced professionals, according to the laboratory routine. A total of 680 QF-PCR results were available. These included the samples with cell growth failure in cytogenetics that were investigated by molecular QF-PCR, and the miscarriage material samples unsuitable for, or in which cytogenetics was not demanded.

### Statistical Analysis

The results were presented as frequency values according to the different technique performed (cytogenetics or molecular biology), and distributed according to the chromosomal alterations found by each method and total sample results. The relation between the frequency of chromosomal anomalies and maternal age was analyzed by logistic regression using the statistical software Minitab 17.3.1 (State College, PA, USA). Significance was set at  $p < 0.05$ .

The study was submitted and approved by the Ethics Committee of the institution under protocol CEP nº 1.346.235, on December 1, 2015.

**Table 1** Normal and abnormal results observed by cytogenetics and molecular biology techniques

	Cytogenetics (n = 204)	Molecular biology (n = 680)	Total (n = 884)
Normal	106 (52%)	410 (60%)	516 (58%)
Abnormal	98 (48%)	270 (40%)	368 (42%)

## Results

A total of 884 results from miscarriage material samples was included in the study. **Table 1** shows the frequency of normal and abnormal results obtained by conventional cytogenetics and molecular biology QF-PCR technique. From the total sample of 884, 368 (42%) cases of chromosome abnormalities were detected, while 516 (58%) cases had no detected alterations. Cytogenetics was able to identify 52% of normal results (106 out of 204 tested), and QF-PCR, 60% (410 out of 680 tested). Cytogenetics showed 48% (98 out of 204 tested) of abnormal results, and molecular biology detected 40% (270 out of 680 tested) of abnormal cases, considering the chromosomes analyzed by this technique.

The chromosomal abnormalities identified by each method are demonstrated in **Table 2**.

Cytogenetics results showed 18.4% X monosomy, 13.3% trisomy 16, and 10.2% trisomy 22. It also revealed that 8.2% of the abnormalities were trisomy 15. Molecular biology results were 15.9% for X monosomy, 18.9% for trisomy 16, and 19.7% for trisomy 22. Chromosome 15 was not evaluated by QF-PCR in this study (**Table 2**).

Considering all the samples, trisomy was the most common chromosome aberration, accounting for 63% (232 out of 368) of the abnormalities. The most frequent was trisomy 16 (17.4%), followed by trisomy 22 (17.1%). Monosomy accounted for 16.6% (61 out of 368) of the anomalies, and polyploidies, for 18.8% (69 out of 368) (**Table 2** and **Table 3**).

The relationship between aneuploidy and maternal age is shown in **Fig. 1**. In the young maternal age group ( $n = 452$ ), 62.2% (281) had normal results, while abnormalities were found in 171 cases (37.8%). For the advanced maternal age group ( $n = 432$ ), 235 (54.4%) normal results were observed, compared with 197 (45.6%) abnormal findings. This difference was considered statistically significant ( $p = 0.02$ ) (**Fig. 1**).

## Discussion

The analysis of chromosomal abnormalities in POC is useful to determine the possible causes of miscarriage, and to provide information and counseling for couples regarding future pregnancies.

This retrospective study showed the presence of aneuploidies in 42% of all the analyzed samples. When the different techniques were considered, the results were similar (40% by QF-PCR and 48% by cytogenetics). A recent study by Coelho et al<sup>17</sup> demonstrated the presence of aneuploidies in 54.6% of miscarriage material from Brazilian patients, analyzed by

**Table 2** Frequency of chromosomal anomalies

Anomaly	Cytogenetics n (%)	Molecular biology n (%)	Total n (%)
X monosomy	18 (18.4%)	43 (15.9%)	61 (16.6%)
Trisomy 2	1 (1%)	NA	1 (0.3%)
Trisomy 4	2(2%)	NA	2 (0.5%)
Trisomy 7	1 (1%)	NA	1 (0.3%)
Trisomy 8	3 (3.1%)	NA	3 (0.8%)
Trisomy 10	3 (3.1%)	NA	3 (0.8%)
Trisomy 13	4 (4.1%)	25 (9.3%)	29 (7.9%)
Trisomy 14	2 (2%)	NA	2 (0.5%)
Trisomy 15	8 (8.2%)	NA	8 (2.2%)
Trisomy 16	13 (13.3%)	51 (18.9%)	64 (17.4%)
Trisomy 17	2 (2%)	NA	2 (0.5%)
Trisomy 18	5 (5.1%)	16 (5.9%)	21 (5.7%)
Trisomy 20	1 (1%)	NA	1 (0.3%)
Trisomy 21	9 (9.2%)	23 (8.5%)	32 (8.7%)
Trisomy 22	10 (10.2%)	53 (19.7%)	63 (17.1%)
47 XXY	1 (1%)	NA	1 (0.3%)
Triploidy	9 (9.2%)	56 (20.7%)	65 (17.7%)
Trisomies 16 and 22	0	2 (0.7%)	2 (0.5%)
Trisomies 16 and 21	0	1 (0.4%)	1 (0.3%)
Other aneuploidies	6 (6.1%)	NA	6 (1.6%)
Total	98 (100%)	270 (100%)	368 (100%)

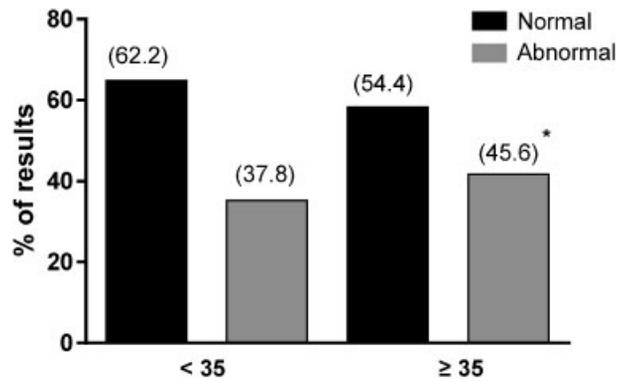
Abbreviation: NA, Not analyzed by QF-PCR.

QF-PCR. Jenderny<sup>15</sup> reported a frequency of 61% abnormal results in POC analyzed by cytogenetics and QF-PCR in the German population. Other studies found similar (48%) or lower frequencies (36%) of total chromosomal aberrations.<sup>18,19</sup>

The present study revealed that the main chromosomal abnormality detected in abortion material was trisomy, followed by triploidy and monosomy X. These results corroborate with other studies that demonstrated similar results, showing that trisomies, especially those involving chromosomes 16 and 22, are implicated in spontaneous abortion.<sup>17,20-25</sup>

**Table 3** Groups of chromosomal anomalies

Anomaly	Total n (%)
Trisomy	232 (63%)
Polyploidy	69 (18.8%)
X monosomy	61 (16.6%)
Others	6 (1.6%)
Total	368 (all)



**Fig. 1** Maternal age and chromosomal abnormality. Numbers between parentheses show the percentage of the results. The rates of abnormal results were significantly higher for the advanced maternal age group (n = 432) when compared to the younger maternal age group (n = 452) (p = 0.02).

An important point noted in the present study was that trisomy 15 was found in 8.2% of the samples analyzed by conventional cytogenetics. Similar values were observed by Coelho et al<sup>17</sup> (14.1%), Moraes et al<sup>20</sup> (9%), Subramaniam et al<sup>21</sup> (13.5%), and Romero et al<sup>25</sup> (7.7%), suggesting that trisomy 15 is recurrent in POC samples. Until the present moment, chromosome 15 markers have not been included in routine QF-PCR analysis of abortion material at the laboratory. However, the results showed that a marker for chromosome 15 should be included in the molecular analysis, improving the quality of the released technical reports.

Conventional cytogenetics technique remains highly recommended for spontaneous miscarriage analysis. However, this method has certain disadvantages, such as a long laboratory cycle, labor intensity and culture failure, especially when the tissues obtained from patients are not well preserved.<sup>17,26</sup>

The QF-PCR technique is regarded as a highly accurate, low cost and rapid diagnostic method to facilitate the detection of clinically relevant chromosome aberrations. However, some limitations of the technique are the difficulty to offer a correct diagnosis for mosaicism, small deletions, translocations and duplications.<sup>15,17</sup>

Since our laboratory miscarriage material is received from different medical facilities, the QF-PCR method plays an important role as a reliable method for the detection of aneuploidies, when culture fails or when the karyotype is not possible due to inadequate material preservation.

Many reports have suggested that advanced maternal age is an important factor related to chromosomal aneuploidies.<sup>22</sup> Our study showed a significant increase in the rate of aneuploidy in the advanced maternal age group when compared with the young maternal age group (p = 0.02). Bastos et al<sup>6</sup> and Jia et al<sup>22</sup> also suggest a maternal age-related increase in chromosomal anomalies. Hormonal changes during the aging process, as decreased production of progesterone can lead to increased rates of miscarriage in women older than 35 years.<sup>27</sup>

In conclusion, chromosomal aberrations are still a major cause of miscarriage, and the conventional cytogenetics study

is highly recommended, as it can detect different types of chromosomal abnormalities. Molecular biology techniques, such as QF-PCR, are important complementary methods that can be effective to detect the main chromosomal anomalies, and may be used in combination with cytogenetics to allow the release of technical reports with reliable results.

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