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Genomic and structural abnormalities of the fetus chromosome set depend on the age of pregnant women

Abstract. To study the frequency and spectrum of fetal chromosomal abnormalities in pregnant women, depending on age, a cytogenetic study of 2248 biopsies of the chorion and placenta, cord blood lymphocytes and amniocytes was carried out in 2016-2020. The work used the generally accepted methods of direct preparation of chromosome preparation and methods of cell cultivation. The analysis of the preparations was performed based on GTG, CBG staining method and FISH method, using DNA probes for centromeric regions 13, 18, 21 and X, Y chromosomes. Numerical and structural abnormalities of chromosomes were considered according to the ISCN 2013 system. As a result of the study, 272 (12.1%) cases of genomic and structural abnormalities of the fetus chromosomes of the fetus of pregnant women were registered. Genomic abnormalities were 69.1%, and chromosome structural rearrangements – 30.9%. Among genomic disorders, the frequency of the Down syndrome in the karyotype of the fetus was 58.5%. Frequency of occurrence of fetal chromosomal abnormalities varies significantly depending on the age group of pregnant women. The frequency of fetal chromosomal pathology in pregnant women under 20 years old was 4.4%, at the age from 20 to 34 years old – 7.6%, from 35 to 39 years old – 16.3%, from 40 to 44 years old – 19.8%, after 45 years figure increased significantly – up to 29.6%. In older pregnant women, when compared with pregnant women of the reproductive period from 20 to 34 years, when the highest level of pregnancy rate (54.6%) in the population is observed, there is an increase in the frequency of fetal chromosomal pathology by 2.1; 2.6 and 3.9 times, respectively.

Key words: fetal karyotype, chromosomal pathology, factor of the age of a pregnant woman.

Introduction

Chromosomal pathology is the most important cause of birth defects, stillbirth, infant mortality, and diseases of infants and children. Currently, the prevention, diagnostics, and treatment of these diseases are the main problem of modern medicine.

According to the National Genetic Register of the Republic of Kazakhstan for 2012, the annual number of children with birth defects in the country was 3500-4500. Every year, during genetic screening of pregnant women, 1000-12000 fatal birth defects of the fetus are detected and which of 140-150 chromosomal pathologies are observed. The share of causes of perinatal death from birth defects in the structure were 10-15% and there is no downward trend [1].

Over the past two decades, the maternal age profile in Europe has changed significantly, and the average age of mothers is increasing every year. The age of a woman is associated with the risk of developing certain diseases in her children. Older women have a higher risk of having children with chromosomal abnormalities, such as Down syndrome. If the maternal age is very young, the risk of giving birth to children with chromosomal disorders is low, but there is a high risk of some abnormalities, in particular congenital heart defects, and abdominal wall defects in children [2-4].

The cause of chromosomal abnormalities is quantitative and structural changes in chromosomes. During cell division, quantitative changes can occur in the wrong distribution of chromosomes. Many hypotheses of incorrect chromosome distribution (seasonality, ethnicity, age of mother and father, delayed or late fertilization, birth order, family history, mother's medication, bad habits, hormonal or non-hormonal contraception, fluuridins, viral diseases in women) have been tested. In most cases, these hypotheses were not confirmed, but the genetic

predisposition to disease was not excluded. However, in most cases, the nonseparation of chromosomes in humans is formed by chance, so to some extent it can be assumed that they have a genetic basis [5].

The biological factor that affects the increased risk of incorrect chromosome proliferation is maternal age, but the mechanism of this phenomenon is not clear. The risk of giving birth to a child with a chromosomal disease characterized by aneuploidy increases sharply with age, especially after 35 years. After 45 years, every 5th pregnancy ends with the birth of a child with chromosomal disease. Especially pronounced age dependence is manifested by trisomy 21 (Down syndrome). For aneuploidy of sex chromosomes, the age of the parents does not play a significant role or has a minor impact. Up to 45 years, the frequency of spontaneous abortions increases by 3 times or more with age. This phenomenon can be explained by the fact that spot abortion is often caused by chromosomal diseases, the frequency of which depends on age (up to 40-45%) [6-9]. In addition, it was shown that the probability of giving birth to a child with Down syndrome at the age of 30 by the mother is about 1:2500, at the age of 31-34 — 1:1200, at the age of 35-39 — 1:200 [10].

Regarding this issue, it is important to obtain accurate information about the risk of birth of children with congenital malformations associated with maternal age, which is necessary to assess the impact of increasing maternal age on public health, in particular the need for medical and genetic counseling, prenatal screening and prenatal diagnosis. However, most of the data was obtained from studies conducted in European countries that are part of the EUROCAT (European Population Network for Epidemiological surveillance of congenital abnormalities) system, which covers about a third of births in Europe or America. In the Republic of Kazakhstan, such a study makes it possible to conduct a statistical analysis of the data obtained due to long-term monitoring of birth defects [11].

The purpose of the study was to assess the dependence of genomic and structural disorders in the set of chromosomes of fetuses on the age of pregnant women.

Materials and methods

To determine the frequency of occurrence of chromosomal pathology in the fetus of pregnant women 2016-2020 molecular cytogenetic analysis of the karyotypes of the fetus of pregnant women of the risk group was carried out in LLP "Center for Molecular Medicine" in Almaty. By age, pregnant women were divided into the following groups: under 20 years, 20-34 years, 35-39 years, 40-44 years, and 45 years and older [10]. The material of chorion and placenta biopsy of 2248 pregnant women, lymphocytes, and amniocytes of umbilical cord blood were taken for molecular cytogenetic examination. The age of pregnant women was between 17 and 48 years. During molecular cytogenetic analysis 20-25 metaphase cells of each fetus were examined using a light microscope (Axioscope 40, Zeiss, Germany).

Fetal material was obtained by transabdominal puncture under the supervision of ultrasound examination without anesthesia, in compliance with the rules of asepsis and antiseptics. Chorionbiopsia or placentobiopsia, amniocentesis, and cordocentesis methods were performed depending on the timing of invasive prenatal diagnostics [12].

Chromosomal preparations were developed according to the method of direct analysis from the chorion and placenta cones of the fetus, which were taken by the majority, after the umbilical cord blood lifocytes and amniocytes were grown in a nutrient medium under a thermostat for 72 hours, and the preparations were also colored according to GTG, CBG methods [12-15]. The study depends on the time of cultivation after obtaining the working material, the duration of preparation, and analysis. The analysis of metaphase cells was carried out according to ISCN 2013 (International system for Human Cytogenetic Nomenclature – international system of human cytogenetic nomenclature) [16]. 20-25 metaphase cells of each fetus were examined using a light microscope (Axioscope 40, Zeiss, Germany).

The analysis of amniocyte metaphase cells was performed by Fluorescent In Situ Hybridization (FISH). The FISH method is based on the basic property of two complementary DNA chains of any size, which are separated when heated in the corresponding buffer and reconnected when the solution cools, due to the property of decomposition and regeneration of hydrogen bonds between complementary nitrogenous bases [17; 18].

In our research work, centromere probes (chromosomal numertors) were used. Chromosomal Enumerator Probe (CEP): fully or partially specific to tandem alpha and beta satellite duplicates located mainly in the centromere and centromere heterochromatin parts of chromosomes. During the study, 50 cells of each fetus were examined. Special probes were used for 13,18,21, and centromeres of the DNA sequences of X and Y chromosomes. In principle, they give 100% accurate results in the detection of aneuploidia, because each chromosome

is colored in its own unique color. They are hybridized in both homologues of chromosomes and are also used only in metaphase chromosomes [19; 20].

Results and discussion

The study examined the distribution of pregnant women by age groups who underwent molecular cytogenetic studies between 2016 and 2020. The results obtained are shown in Table 1.

According to Table 1, the largest proportion of pregnant women, as expected, was observed in Group 2, aged 20-34 years, with a frequency of 54.6%. The original data was compared with EUROSTAT data for 2000-2004 [21]. As can be seen from the table, it is observed that the age composition of pregnant women, according to EUROSTAT, is generally at a close level, 54.6% and 76.0%, respectively. According to our data, it is necessary to note an increase in the frequency of pregnant women in groups older than 35 years. In general, there is a 2.2 – frequency increase, especially at the age of 40-44, this figure increased by 6.0 times.

In the period from 2016 to 2020, a total of 2,248 cases of fetal karyotype of pregnant women were studied, which underwent cytogenetic analysis of the molecule, including 272 cases of chromosomal pathology (12.1%).

The total number and frequency of disorders in the age-dependent fetal chromosomal set of pregnant women are shown in Table 2.

Table 1 – Distribution of pregnant women by age group

Group	Age of pregnant women	Number of pregnant women	Separation frequency, %	
			Self-study	On EUROSTAT [3]
1	<	45	2.0	3.9
2	20-34	1227	54.6	76.0
3	35-39	540	24.0	17.0
4	40-44	409	18.2	3.0
5	45 >	27	1.2	0.1
	Total	2248	100	100

Table 2 – Frequency of fetal chromosomal disorders in pregnant women depending on their age

Group	Age of pregnant women	Number of pregnant women	In fetal chromosomal set disorders	
			No.	% of chromosomal abnormalities
1	<	45	2	4.4
2	20-34	1227	93	7.6
3	35-39	540	88	16.3
4	40-44	409	81	19.8
5	45 >	27	8	29.6
	Total	2248	272	12.1

In different age groups of pregnant women, the frequency of chromosomal pathology of the fetus has significantly changed. As can be seen in Table 2, the frequency of chromosomal disorders in the fetal karyotype of pregnant women aged less than 20 years and 20-34 years was 4.4% and 7.6%, respectively. There was a significant increase in this indicator in the risk groups. Fetal karyotype disorders increased to 16.3% at the age of 35-39 years, 19.8% at the age of 40-44 years, and 29.6% at the age of 45 years and older.

Compared to the reproductive period (20-34 years), over 35 years, where the highest

level of pregnancy is observed in the population (54.6%), there was an increase in the frequency of chromosomal pathology of the fetus by 2.1; 2.6 and 3.9 times, respectively.

According to the International Center for birth Defects Surveillance and Research (ICBDSR) in Europe and Asia, the share of mothers over 35 years of age in European countries increased from 10.9% in 1993 to 18.8% in 2004. In South Korea, the percentage of older pregnant women has increased more than 2 times over a 10-year period: from 6.2% in 1999 to 15.4% in 2009 [22]. In addition, the age of mothers is associated with an increased risk of chromosomal abnormalities, and this applies primarily to chromosomal disorders such as trisomy of chromosomes 13, 18, and 21.

Thus, the age of a pregnant woman over 35 years of age is considered as an indisputable risk factor for the development of aneuploidy, which is explained by the fact that maternal age is directly related to the frequency of meiotic nondivision of chromosomes during oogenesis.

For some types of chromosomal defects, a number of studies have shown a link with maternal age. For example, mothers under the age of 19 have a lower risk of chromosomal trisomy in the fetus, but a higher risk of some birth defects, in particular anencephaly, gastroschis [23]. For women over 35 years of age, a number of studies show an increased risk of giving birth to children with neural tube defects, facial openings [24].

The spectrum and frequency of fetal genomic and chromosomal structural disorders are shown in Tables 3 and 4.

As shown in Table 3, the frequency of occurrence of complete and mosaic forms of autosomal chromosomes 13 (Patau syndrome), 18 (Edwards syndrome), 21 (Down syndrome), and quantitative changes in the X-and Y – chromosomes in the human population in the capriotype of live-born children is very high. The indicators that attract attention are the frequency of complete and Mosaic forms of trisomy of chromosome 21 (58.5%) as well as the frequency of monosomic (Shereshevsky-Turner syndrome) and polysomic (Klinefelter syndrome) forms of the X-chromosome of the sex was13.8% and 12.8%. The frequency of polyploid forms was 5.35%.

The spectrum and frequency of structural disorders of fetal chromosomes are summarized in Table 4.

Table 3 – Spectrum and frequency of fetal genomic disorders

Genomic abnormalities		Fetal karyotype	Number of fetus	Total percentage %
Trisomy 13		47,XY,+13	4	2.1
Trisomy 18		47,XY,+18	6	3.2
		47,XX,+18		
Trisomy 21	complete	47,XY,+21	110	58.5
		47,XX,+21		
	mosaicism	47,XY,+21/46,XY		
		47,XX,+21/46,XX		
X-chromosome monosomies	complete	45,X0	26	13.8
	mosaicism	45,X0/46,XX		
X-chromosome polysomies	complete	47,XXY	24	12.8
	mosaicism	47,XXY/46,XY		
Triploidy		69,XXX	2	1.1
Tetraploidy	complete	92,XXXX	8	4.25
	mosaicism	46,XY/92,XXУУ		
		46,XX/92,XXXX		
A marker chromosome (mar)	complete	47,XY,+mar	8	4.25
		47,XX,+mar		
	mosaicism	46,XX/47,XX,+mar (70%/30%)		
Total			188	100

Table 4 – Spectrum and frequency of structural disorders of fetal chromosomes

Structural chromosome abnormalities		Fetal karyotype	Number of fetus	Total percentage, %
the derivative chromosome 18, translocation between chromosome 18 and marker chromosome		46,XX,der(18)t (18:mar)(q11,1;q11,1)	6	7.2
Ring chromosome 18		46,XY,r(18)(p11.21q21.2)	3	3.6
translocation between chromosome 13 and chromosome 14		45,XY,t(13;14)	8	9.5
Trisomy 21	translocation	46,XY,t(14;21)(q11,1;q11,1)	28	33.3
		46,XX,t(14;21)(q11,1;q11,1)		
Chromosome 9 inversion		46,XY,inv(9)	31	36.9
Deletion of the short arm of the X chromosome		46,X,del(Xp)	8	9.5
Total			84	100

According to Table 4, the following forms of transformations were found in relation to structural changes in chromosomes: detection, inversion, translocation. The most common type of chromosomal aberrations was the translocation form of chromosome 21-10.3% and the inversion of chromosomes 9 – 11.4%.

Thus, as a result of the study of the spectrum and frequency of fetal karyotype disorders, quantitative abnormalities – 69.1% and various structural abnormalities of chromosome – 30.9%.

According to numerous literature sources, among chromosomal pathologies, the frequency of occurrence of DS has a large percentage. If we compare the frequency of occurrence of children born with DS in pregnant women younger than 35 years and older than 35 years, we can see a special Data (Table 5).

Table 5 – Frequency of births and fetuses with the karyotype of Down syndrome in women under 35 and over 35 years of age

Country	Year	Under 35 years old		Over 35 years old	
		No.	total percentage, %	No.	total percentage, %
USA, [25]	2005	353	59.5	240	40.5
Czech Republic, [26]	2005	219	87.3	32	12.7
India, [27]	2006	64	92.7	5	7.3
Independent research	2020	56	40.6	82	59.4

Table 5 shows the results of foreign researchers on the age factor of pregnant women in the United States, the Czech Republic, and India on demographic indicators. In the United States, the frequency of children born with DS in terms of age factor is the highest, the reasons for which are the most common births among the countries of this country over the age of 35. Due to this, chromosomal abnormalities occur equally between the ages of 35 and older [25]. In the Czech Republic, the frequency of births with DS in relation to the age factor is very low, the reason is that childbirth at the age of more than 35 years is extremely rare, in addition, pregnant women are often examined for the age factor in prenatal diagnostics [26]. In India, the vast majority of mothers account for the birth of children under the age of 35. Childbirth in mothers over 35 years of age is very rare, in this country, women have already married at a younger age, and the birth rate has already formed. For these reasons, a large frequency (92.7%) of children born with DS occurs in mothers under 35 years of age [27].

According to our data, the frequency of fetuses with karyotype in DS was 18.8% higher in pregnant women over 35 years of age than in pregnant women under 35 years of age compared to previous results. As shown above (Table 1), there is a high frequency

of occurrence of pregnant women in groups older than 35 years. We can say that the reason for this result is due to social circumstances, when parents postpone the possibility of giving birth. At the same time, although low income is an indirect determinant, developmental disabilities are more common in countries with insufficient families and resources [28].

According to our data, the incidence of fetuses with DC karyotype in pregnant women over 35 years of age is 18.8% (1.5 times) higher than in pregnant women under 35 years of age. This is due to the high incidence of pregnancies in pregnant women over the age of 35, as shown above (Table 1). It can be said that the transition of childbearing to adulthood is possible due to the specific social conditions of parents.

Conclusion

Molecular cytogenetic studies have shown that the frequency of chromosomal abnormalities in the fetus increases with age in pregnant women. Genomic and structural abnormalities were observed in 12.1% of fetal karyotypes. Among them, the quantitative changes were 69.1%, and various chromosomal abnormalities were 30.9%. The incidence of fetuses with the karyotype of Down syndrome was 58.5%.

Pregnancy in women over 35 years of age is considered a risk factor for fetal development. In addition, there is a large proportion of chromosomal abnormalities among the fetuses of pregnant women under the age of 35.

According to the World Health Organization, adult motherhood increases the risk of chromosomal abnormalities, including Down syndrome, and young motherhood increases the risk of some congenital defects [28].

Therefore, the risk factor of age during pregnancy should not be considered the sole cause of chromosomal abnormalities in the fetus. In this regard, it is important that an invasive prenatal diagnosis be performed in all pregnant women. Prenatal diagnosis of hereditary diseases and congenital malformations are important preventive measures that can significantly reduce the risk of miscarriage and stillbirth. As a result, it provides a great future for the genetic health of the human race.

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