The human placenta and aneuploidy — trisomies 21, 18, 13

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The purpose of this research was to investigation the morphological features of human placenta with karyotyped trisomy 21, 18 and 13 chromosomes. The study included 50 placenta of fetuses in miscarriages pregnancy at 19–20 weeks, among them 10 placentas obtained as a result of induced abortions due to karyotyped trisomy 21 of the fetuses; 10 placentas obtained as a result of induced abortions due to karyotyped trisomy 18 of the fetuses; 10 placentas obtained as a result of induced abortions due to karyotyped trisomy 13 in the fetuses; 20 placentas obtained as a result of spontaneous miscarriages without any congenital defects and abnormal karyotype of the fetuses. As a result of the investigation, in placentas with karyotyped trisomies, a violation of villi branching was noted. With karyotyped trisomy 21, inhibition of angiogenesis and sclerosis of villi with proliferation of fibroblasts were revealed. In cells of chorionic villi with karyotyped trisomy 18, increased apoptosis was noted. In placentas with karyotyped trisomy 13 hydropic changes in villus stroma and inhibition of angiogenesis were noted.

Keywords: trisomy, placenta, immunohistochemical analysis, angiogenesis, apoptosis.

Introduction

Proper placental development and function are central to the health of both the mother and the fetus during pregnancy. A critical component of healthy placental function is the proper development of its vascular network [1]. The process of angiogenesis — the formation of new blood vessels from preexisting ones — is a hallmark of tissue repair, expansion, and remodeling in physiological processes, such as wound healing, ovulation, and embryo development, and in various pathologies including cancer, atherosclerosis, and chronic inflammation. Therefore, different *in vivo*, *ex vivo*, and *in vitro* bioassays and techniques have been developed to investigate the specific stages of the angiogenesis [2].

According to the literature, placental ultrasound in cases of trisomy 21 shows fetal vascular malperfusion and impaired differentiation of the villous chorion [3]. At the same time, the volume of the placenta with trisomy 21 does not significantly differ from the volume of the placenta of fetuses with a normal karyotype [4]. In addition, trisomy 21 shows a tendency to form an absolutely short umbilical cord [3]. In placentas with trisomy 18 and 13 a decrease in the vascularization index and blood flow was revealed [5].

The frequency of aneuploidy including miscarriages for medical reasons, stillbirths and newborns is: trisomy 21 — 35.6 per 10,000 births, trisomy 18 — 4.08 per 10,000 births, trisomy 13 — 1.68 per 10,000 births [6–8]. In addition, according to J. M. Jackson et al. (2014) the rate of birth of a child with trisomy 21 in women of fertile age up to 35 years is 7.3–7.4 per 10,000 live births and increases to 31.4–33.9 per 10,000 live births in women over 35 years [9]. From a clinical point of view, the importance of this condition is due to the high risk of complications in childbirth, which are observed in both mosaic and complete chromosomal abnormalities. The most common complications include fetoplacental insufficiency, antenatal developmental delay, and fetal death [10; 11]. Usually, aneuploidy is suspected in the fetus during prenatal ultrasound examination. If a pregnant woman has a high risk for chromosomal abnormalities in the fetus (individual risk of 1/100 or higher) in the first trimester of pregnancy and/or if congenital abnormalities (malformations) are detected in the fetus in the first, II and III trimesters of pregnancy, the obstetrician-gynecologist directs her to the medical genetics center for counseling and establishing or confirming the prenatal diagnosis using invasive methods of examination [12; 13]. The decision to terminate pregnancy among women who received a positive diagnosis of fetal aneuploidy during the prenatal period varies between 86% and 97% [13]. Moreover, a common outcome of pregnancy is miscarriage, with most studies reporting 12% to 15% loss among recognised pregnancies by 20 weeks of gestation, and there is no possibility of karyotyping [14]. It necessitates the identification of morphological markers of aneuploidy in the placenta.

The purpose of investigation was to study the features of human placenta angiogenesis in karyotyped trisomies 21, 18, and 13.

Research problem

1) Study the morphological features of the placenta in trisomy 21, 18 and 13.
2) Evaluate the results of immunohistochemical reactions using bFGF, CD31, and FasL antibodies in placentas in both karyotyped trisomies 21, 18, and 13, and without aneuploidy.
3) Evaluate the relationship between the degree of expression of bFGF, CD31, and FasL and the presence of trisomies 21, 18, and 13.

Material and methods

According to the data of the St. Petersburg Medical Genetics Diagnostic Center, the number of pathological karyotypes detected before 22 weeks of pregnancy in St. Petersburg in 2017 amounted to 153 cases (table 1).

The study included 50 placentas for miscarriages at 19–20 weeks gestation, which made up four groups:

group 1 — 10 placentas obtained as a result of induced abortions due to karyotyped trisomy 21 in the fetus;

group 2 — 10 placentas obtained as a result of induced abortions due to karyotyped trisomy 18 in the fetus;

group 3 — 10 placentas obtained as a result of induced abortions due to karyotyped trisomy 13 in the fetus;

group 4 (control group) — 20 placentas obtained as a result of spontaneous miscarriages without any congenital defects and abnormal karyotype of the fetuses.

Table 1. **Trisomies detected in St. Petersburg in 2017 after invasive prenatal diagnosis before 22 weeks of pregnancy**

Trisomy	n	%
Trisomy 21	99	64.7
Trisomy 18	37	24.2
Trisomy 13	13	8.5
Other trisomies	4	2.6
Summary	153	100

Study of the histological structure of the placenta was carried out with a standard method.

Immunohistochemical (IHC) reactions were performed in the Laboratory of Pathomorphology (chief — Karev V. E.) of the Pediatric Research and Clinical Center for Infectious Diseases.

IHC using monoclonal mouse antibodies bFGF (Santa Cruz Biotechnology, 1:50), CD31 (Dako, 1:100), FasL (Diagnostic BioSystems, 1:75) was performed in accordance with the manufacturer's recommendations contained in the Protocol provided in the accompanying documents for the reagents.

A semi-quantitative assessment of the results of the IHC was carried out using a computer analysis system for microscopic images consisting of a Zeiss Axio Imager.A2 microscope, an Axiocam 506 color video camera, an Intel Pentium 4-based personal computer, and the ImageJ program.

Statistical analysis

AtteStat (version 13.1) was used for statistical processing of the received data. In the case of comparing two groups, the comparison was carried out by the student's criterion or the Mann-Whitney criterion. To test the null hypothesis about the independence of the distribution of discrete features, the χ^2 criterion was used. To test the null hypothesis of equality of fractions, we used the procedure of comparing fractions with approximation by χ^2 statistics. The null hypotheses of the tests were rejected if the probability of error of the first kind was less than 0.05.

Results

Morphological study of the material of groups with trisomies revealed a violation of branching of the villi with a predominance of immature intermediate villi (Fig. 1). In placentas with trisomy 21, the villi were hypovascularized, and increased fibroblast proliferation was detected in the stroma (Fig. 1A).

In placental villi with trisomy 13, there were pronounced hydropic changes with hypovascularization (Fig. 1C). At the same time, in the placenta of the control group, branching and vascularization of the villi were not disturbed (Fig. 1D).

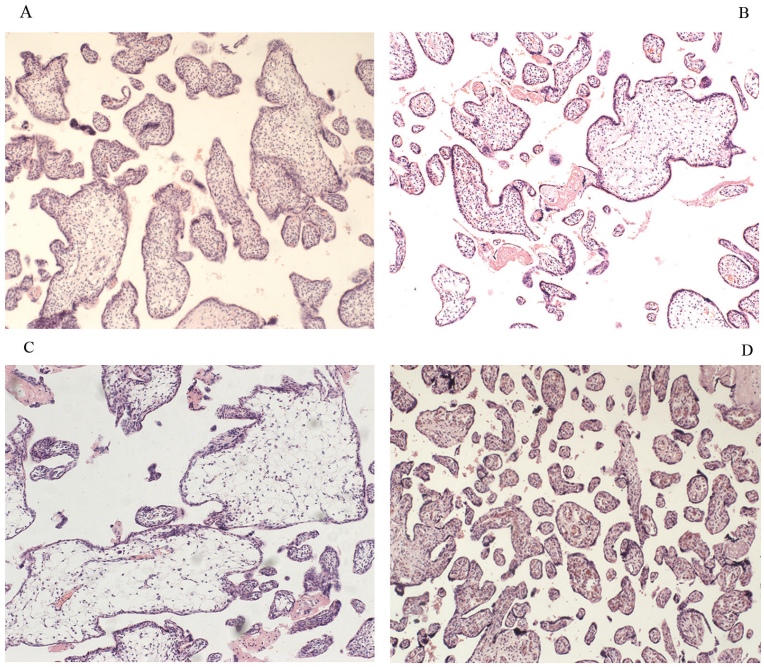

Fig. 1. Morphological features of placentas: A — placenta with trisomy 21; B — placenta with trisomy 18; C — placenta with trisomy 13; D — placenta of control group; H&E, magnification × 100

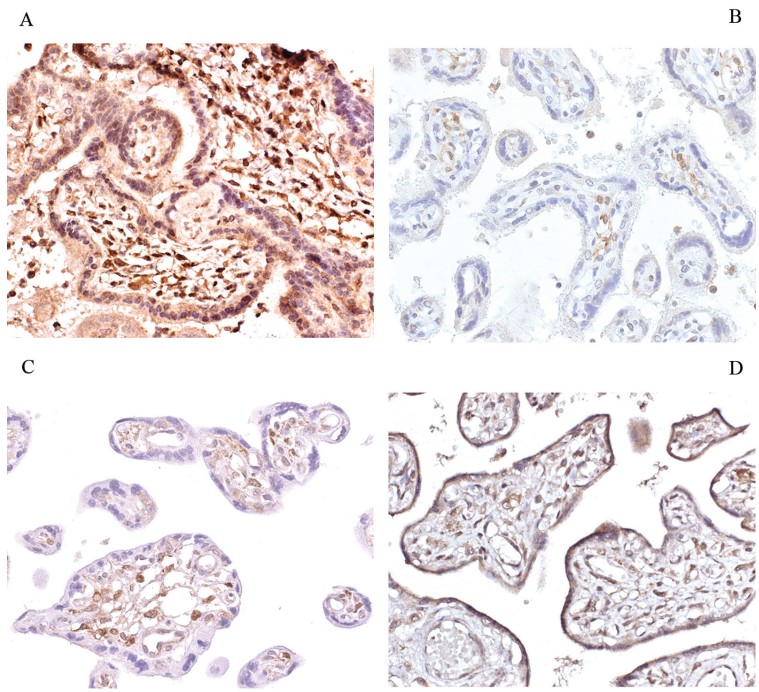

Fig. 2. Expression of bFGF in placenta's cells: A — placenta with trisomy 21; B — placenta with trisomy 18; C — placenta with trisomy 13; D — placenta of control group; immunohistochemical study, magnification × 400

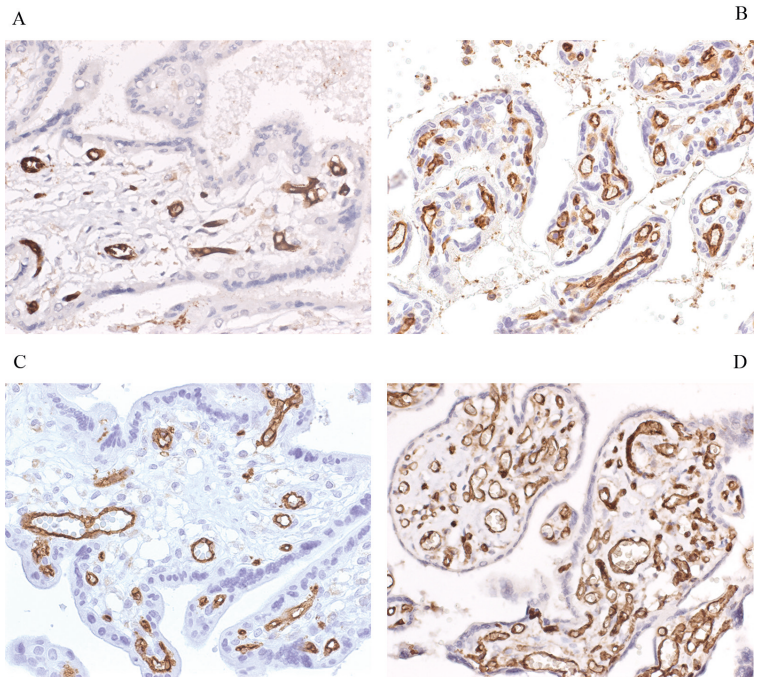

Fig. 3. Expression of CD31 in placenta's cells: A — placenta with trisomy 21; B — placenta with trisomy 18; C — placenta with trisomy 13; D — placenta of control group; immunohistochemical study, magnification × 400

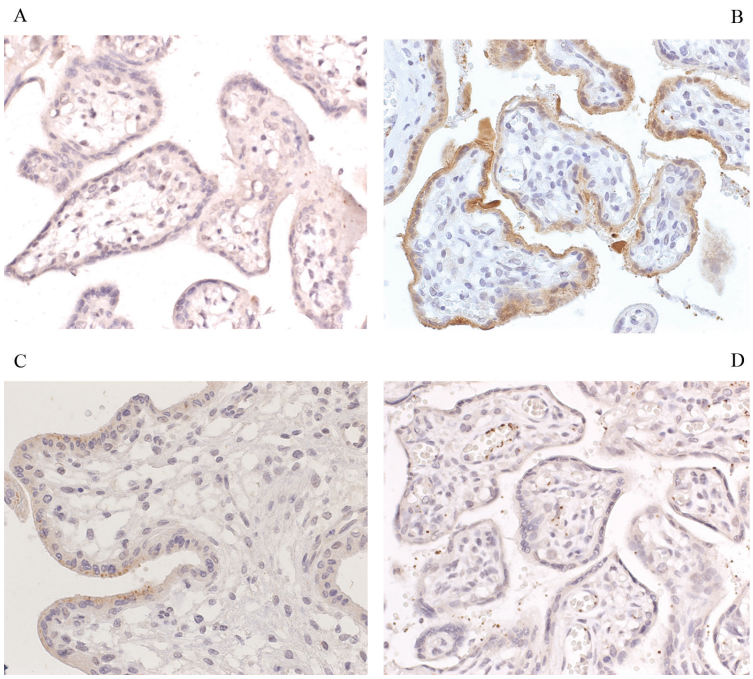

Fig. 4. Expression of FasL in placenta's cells: A — placenta with trisomy 21; B — placenta with trisomy 18; C — placenta with trisomy 13; D — placenta of control group; immunohistochemical study, magnification × 400

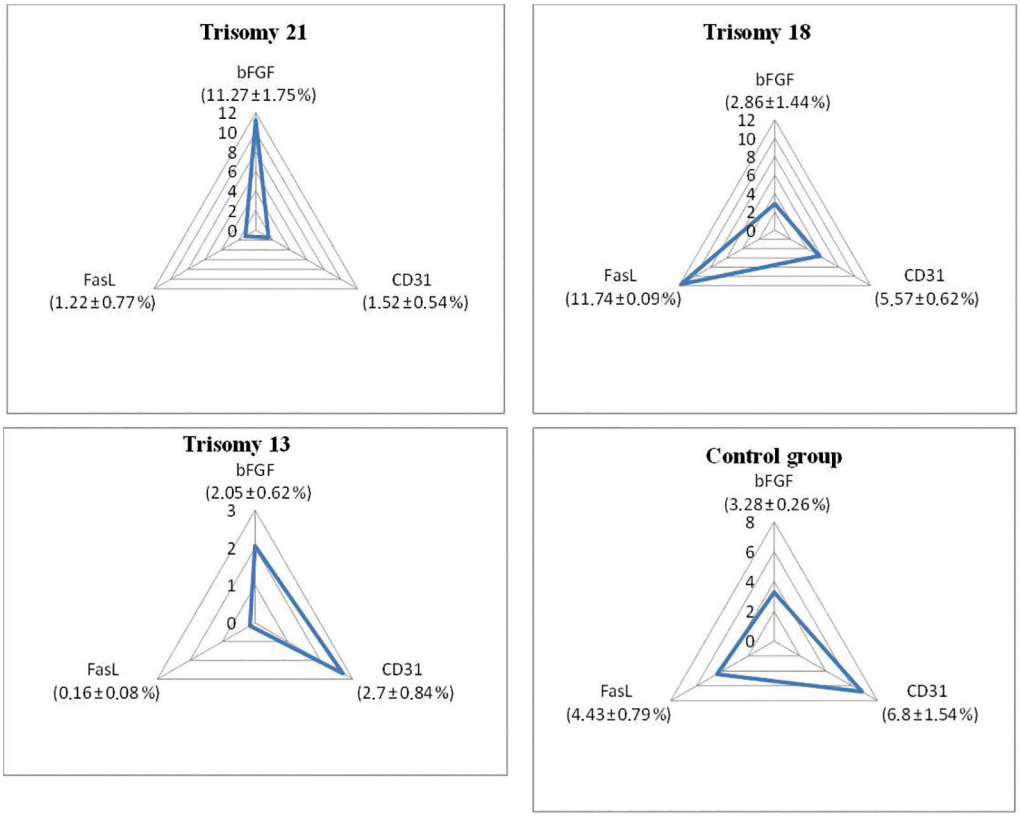

Fig. 5. Relative expression area (%) of signal molecules as a percentage of the studied groups

In the immunohistochemical study of placentas, bFGF expression was observed in villus stroma cells — fibroblasts and macrophages, as well as in endotheliocytes (Fig. 2). The expression of CD31 was detected in endothelial cells of vessels of the villi (Fig. 3). FasL expression was present primarily in syncytiotrophoblasts and endotheliocytes (Fig. 4).

Summary results for the relative expression area of signal molecules are shown in Figure 5.

There was a statistically significant increase in bFGF expression in placentas with trisomy 21 — the relative area of bFGF expression in placentas of the first group was 11.27 ± 1.75 %, and in placentas of the control group — 3.28 ± 0.26 % ($p < 0.01$). The relative area of CD31 expression in placentas of the first group was significantly lower than in the control group: 1.52 ± 0.54 % and 6.80 ± 1.54 %, respectively ($p < 0.05$). A similar pattern was observed when comparing data on the relative area of FasL expression: in placentas with trisomy 21, it was statistically significantly lower than in the control group and amounted to 1.22 ± 0.77 % and 4.43 ± 0.79 %, respectively ($p < 0.05$) (Fig. 5).

In trisomy 18, there was a statistically significant increase in FasL expression in placentas: the relative area of FasL expression in placentas of the second group was 11.74 ± 0.09 %, and in placentas of the control group — 4.43 ± 0.79 % ($p < 0.01$) (Fig. 5).

In placentas with trisomy 13, the relative expression area of CD31 and FasL was significantly lower than in the control group. Thus, the relative expression area of CD31 in placentas with trisomy 13 was 2.7 ± 0.84 %, in the control group — 6.80 ± 1.54 % ($p < 0.05$), and the relative expression area of FasL — 0.16 ± 0.08 % and 4.43 ± 0.79 %, respectively ($p < 0.01$) (Fig. 5).

Discussion

In the course of our study, we found a violation of branching and hypovascularization of villi with increased proliferation of fibroblasts in placentas of fetuses with trisomy 21 [15]. These data correlate with the results of the Doppler metric performed in the study by E. Corry et al. (2016), where there was a reduction of the vascular bed and impaired perfusion in the placenta in trisomy 21 [3].

Increased apoptosis was observed in the material with karyotyped trisomy of chromosome 18. In placentas with karyotyped trisomy 13, hydropic changes in the villus stroma and angiogenesis inhibition were observed.

As a result of this work, we can say that we have studied data on the immunohistochemical features of the human placenta in aneuploidy — trisomies 21, 18 and 13.

Conclusion

The features of the morphogenesis of the placenta with aneuploidy suggest this pathology in cases if karyotyping is impossible.

References

1. Pereira R. D., De Long N. E., Wang R. C., Yazdi F. T., Holloway A. C., Raha S. Angiogenesis in the placenta: the role of reactive oxygen species signaling. *Biomed. Res. Int.*, 2015, vol. 2015, Article ID 814543.
2. Nowak-Sliwinska P., Alitalo K., Allen E., Anisimov A. et al. Consensus guidelines for the use and interpretation of angiogenesis assays. *Angiogenesis*, 2018, vol. 21, no. 3, pp. 425–532.
3. Corry E., Mone F., Segurado R., Downey P., McParland P., McAuliffe F. M., Mooney E. E. Placental disease and abnormal umbilical artery Doppler waveforms in trisomy 21 pregnancy: A case-control study. *Placenta*, 2016, vol. 47, pp. 24–28.
4. Wegrzyn P., Faro C., Falcon O., Peralta C. F., Nicolaides K. H. Placental volume measured by three-dimensional ultrasound at 11 to 13 + 6 weeks of gestation: relation to chromosomal defects. *Ultrasound. Obstet. Gynecol.*, 2005, vol. 26, no. 1, pp. 28–32.
5. Rizzo G., Capponi A., Cavicchioni O., Vendola M., Arduini D. Placental vascularization measured by three–dimensional power Doppler ultrasound at 11 to 13 + 6 weeks' gestation in normal and aneuploid fetuses. *Ultrasound. Obstet. Gynecol.*, 2007, vol. 30, no. 3, pp. 259–262.
6. Dashe J. S. Aneuploidy screening in pregnancy. *Obstet. Gynecol.*, 2016, vol. 128, no. 1, pp. 181–194.
7. Goel N., Morris J. K., Tucker D., de Walle H. E. K. et al. Trisomy 13 and 18 — prevalence and mortality — a multi–registry population based analysis. *Am. J. Med. Genet. A.,* 2019, vol. 179, no. 12, pp. 2382–2392.
8. Kluckow E., Halliday J., Poulton A., Lindquist A. et al. Association between timing of diagnosis of trisomy 21, 18, and 13 and maternal socio-economic status in Victoria, Australia: A population-based cohort study from 2015 to 2016. *Prenat. Diagn.*, 2019, vol. 39, no. 13, pp. 1254–1261.
9. Jackson J. M., Crider K. S., Cragan J. D., Rasmussen S. A., Olney R. S. Frequency of prenatal cytogenetic diagnosis and pregnancy outcomes by maternal race–ethnicity, and the effect on the prevalence of trisomy 21, Metropolitan Atlanta, 1996–2005. *Am. J. Med. Genet. A.,* 2014, vol. 164A, no. 1, pp. 70–76.
10. Robinson W. P., Peñaherrera M. S., Jiang R., Avila L., Sloan J., McFadden D. E., Langlois S., von Dadelszen P. Assessing the role of placental trisomy in preeclampsia and intrauterine growth restriction. *Prenat. Diagn.*, 2010, vol. 30, no. 1, pp. 1–8.

11. Toutain J., Horovitz J., Saura R. Comment on "Confined placental mosaicism at chorionic villous sampling: risk factors and pregnancy outcome". *Prenat. Diagn.*, 2013, vol. 33, no. 3, pp. 301–302.
12. Alldred S. K., Deeks J. J., Guo B., Neilson J. P. [et al.] Second trimester serum tests for Down's Syndrome screening. *Cochrane Database Syst Rev.*, 2012, no. 6, pp. CD009925.
13. Badeau M., Lindsay C., Blais J., Nshimyumukiza L. Genomics-based non-invasive prenatal testing for detection of fetal chromosomal aneuploidy in pregnant women. *Cochrane Database Syst. Rev.*, 2017, no. 11, pp. CD011767.
14. Wilcox A. J., Morken N.-H., Weinberg C. R., Håberg S. E. Role of maternal age and pregnancy history in risk of miscarriage: prospective register based study. *BMJ*, 2019, no. 364, p. l869.
15. Kolobov A. V., Pinchukova A. Z., Karev V. E. Morphological features of human placenta with karyotyped trisomy 21 (Down's syndrome). *Clinical pathophysiology*, 2018, vol. 24, no. 2, pp. 42–45. (In Russian)

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