COMPARATIVE PROTEOMIC AND GENETIC TESTING METHODS IN MISCARRIAGE

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Biopsy is used for the diagnosis when treating miscarriage. However, it does not guarantee that a healthy oocyte will be acquired. The study aimed to identify proteins that are specific for pregnancy development and determine rDNA in the maternal and fetal genomes during embryogenesis. A total of 45 patients took part in the continuous prospective survey. Non-viable pregnancy was terminated in 25 patients. Another five underwent abortion due to teratogenic effects. Artificial abortion was performed in 15 cases (controls). To quantify proteins, tissues of the chorion and/or embryo and the decidua were collected from all the assessed individuals during surgery, along with blood from the cubital vein. DNA was isolated from all samples by the extraction method involving the use of organic solvents. The rDNA copy number in the DNA was determined by non-radioactive quantitative hybridization (NQH), and the chorion proteins were determined by panoramic mass spectrometry. In individuals with frozen pregnancy, decreased levels of some proteins specific for pregnancy, beta-1-glycoproteins (PSG), were revealed. The rDNA content was the same in blood cells and decidual cells of the same woman. Frozen pregnancy is associated with severe imbalance of the rDNA content in the embryonic and maternal genomes. In most cases, there are significantly less rDNA copies in the embryonic genome, than in the maternal genome and genomes of other embryos, the development of which has not been spontaneously interrupted. Thus, determination of specific proteins in chorionic villi and the rDNA copy number in the potential parents' genomes with subsequent rDNA copy number modeling in the embryo can help determine possible causes of infertility in married couples and improve the prenatal diagnosis quality.

Keywords: anembryony, decidua, frozen pregnancy, rDNA, teratogenic effect, IVF, embryo, mass spectrometry

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СРАВНИТЕЛЬНЫЕ ПРОТЕОМНЫЕ И ГЕНЕТИЧЕСКИЕ МЕТОДЫ АНАЛИЗА ПРИ НЕВЫНАШИВАНИИ БЕРЕМЕННОСТИ

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В прегравидарный период для оценки репродуктивного здоровья пациенток используют комплекс мероприятий (биопсию эндометрия, ультразвуковое определение ооцитов, желтого тела, гормонального баланса). Однако они не дают гарантии получения здоровой яйцеклетки. Целью исследования было идентифицировать специфичные для развития беременности белки и определить рДНК в геноме матери и плода в процессе эмбриогенеза. В сплошном проспективном обследовании участвовало 45 пациенток. У 25 пациенток выполнено удаление неразвивающейся беременности. Еще у пяти проведено прерывание беременности из-за тератогенных эффектов. В 15 случаях (контроль) проведен артифициальный аборт. Для определения количества белков у всех обследуемых в процессе операции отбирали ткани хориона и/или эмбриона и децидуальной оболочки, а также кровь из кубитальной вены. Из всех образцов выделяли ДНК методом экстракции органическими растворителями. Число копий рДНК в ДНК определяли методом нерадиоактивной количественной гибридизации NQH, белки в хорионе — с помощью панорамной масс-спектрометрии. При замершей беременности выявлено снижение уровня некоторых специфичных белков беременности — бета-1-гликопротеинов (PSG). Содержание рДНК было одинаково в клетках крови и в клетках децидуальной оболочки одного и того же женского организма. Замершая беременность ассоциирована с выраженным дисбалансом по содержанию рДНК в геноме эмбриона и геноме матери. В большинстве случаев геном эмбриона содержит достоверно меньше копий рДНК, чем геном матери и геномы других эмбрионов, развитие которых не прерывалось самопроизвольно. Таким образом, определение специфических белков в ворсинах хориона и числа копий рДНК в геномах потенциальных родителей с последующим моделированием числа копий рДНК у эмбриона может помочь в определении возможных причин бесплодия у супружеских пар и повысить качество пренатальной диагностики.

Ключевые слова: анэмбриония, децидуальная оболочка, замершая беременность, рДНК, тератогенный эффект, ЭКО, эмбрион, масс-спектрометрия

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According to some data, spontaneous abortion in the first trimester most often results from natural selection, the number of embryos with chromosome abnormalities reaches 60–80% [1]. The rate of structural karyotype abnormalities in patients with recurrent pregnancy loss is 10 times higher than in the population: 2.4%. The standard embryo karyotyping involves testing for the Down syndrome, Patau syndrome, Edwards syndrome, Turner syndrome, Klinefelter syndrome. The patients having a history of two or more spontaneous abortions receive medical genetic counseling including the partners' cytogenetic testing with explanation of the genealogical and cytogenetic findings, assessment of the risk of giving birth to children with developmental abnormalities, clarification of the need for prenatal diagnosis (including testing of the chorion), and the use of donor cells in severe cases. Cytogenetic testing of the embryo, chorion, determination of translocations in parents are necessary in all stillbirth cases.

The chorionic villi sampling for prenatal testing involving collection of a tissue sample from the placenta for karyotyping and detection of specific genetic or biochemical abnormalities in an unborn child is most often used in obstetric practice [2]. According to the transcriptome analysis, 65% (n = 13,074) of all human proteins (n = 20,090) are expressed in the placenta, and 288 of these genes show expression that is increased relative to other tissue types. In contrast to conventional biochemical approaches involving tracing one specific protein or more, proteomics, specifically LC-MS/MS, represents an effective method to detect the altered protein expression, as well as proteins involved in the disease pathogenesis. In recent years, there have been great advances in identification of the differentially regulated proteins, biomarkers, protein modifications and polymorphisms in various human tissues, and the so-called missing proteins by mass spectrometry [3].

A missing protein is an unconfirmed genetic sequence, for which a protein has not yet been discovered [4]. According to the international Human Proteome Project (HUPO), there are currently 1343 missing proteins without any annotated functions predicted by the bioinformatics analysis or studied experimentally. The missing protein detection complexity can be due to not only their low abundance in many tissues, but also expression confined to several cell types within the human body.

The ribosomal genes that encode ribosomal RNAs (18S, 28S, and 5,8S rRNA) being part of ribosomes, the protein-synthesizing cytoplasmic organelles, are clustered in the ribosomal repeat (rDNA) represented in the genomes of eukaryotes by a large number of copies. The number of rDNA repeats in diploid human genomes varies between 200–711 copies [5]. Clusters of the rDNA tandem repeats of various size that are localized in short arms of five acrocentric chromosome pairs form the nucleolar organiser regions (NORs) of chromosomes. In humans, the rDNA copy number (R parameter) is determined by the combination of five pairs of parental acrocentric chromosomes. All rDNA copies in the cell are differentiated into potentially active and inactive. The number of active rDNA copies that is usually proportional to the number of inactive copies accounts for about 30–40% of the total copy number. The more rDNA copies there are in the genome, the more active copies there are, which are transcribed to ensure the rRNA quantity that is essential for ribosome biogenesis [6].

It has previously been shown that the ribosome biogenesis level, which depends on the number of rDNA copies in the genome, can affect conception and the course of pregnancy. The analysis of the share of non-viable zygotes in the samples of married couples with normal fertility, infertility, and miscarriage has shown that the loss of zygotes in the sample of healthy couples is significantly lower, than in the samples having reproductive problems. Therefore, the zygotic selection based on the dose of active rDNA copies in the genome can be one of the factors determining reproductive problems in some couples. In other words, very low and very high rDNA content in the embryonic genome can potentially hinder normal embryogenesis [7]. Another study has shown that the in vitro fertilization (IVF) procedure success depends on the total rDNA copy number in the woman's genome [8]. Women with the lower rDNA copy number had a lower chance of getting pregnant through IVF. One of the hypotheses explaining this fact assumes that women with the low rDNA copy number transmit the lower number to the embryo, which is insufficient for successful embryogenesis.

Thus, the level and presence or absence of specific proteomes in the chorion must correspond to a certain number of different DNA forms in the extracellular DNA.

The study aimed to identify uncommon proteins and determine rDNA in the maternal and fetal genomes during embryogenesis.

METHODS

The continuous prospective study of patients with miscarriage was conducted in May–July, 2023 at the Family Planning and Reproduction Center. Observations, in which we failed to collect al three biological media within the first 3 h, were excluded. Inclusion criteria: frozen pregnancy of unknown origin; teratogenic effect; abortion by the patient's free will. Exclusion criteria: other obstetric complications.

The study involved 45 patients, who were divided into three groups. A total of 25 patients (group I) were admitted to the hospital due to frozen pregnancy (5–13 weeks). Anembryonic pregnancy was reported in five of them, another 20 had frozen pregnancy of unknown origin. Furthermore, ultrasonography revealed discrepancies between the dead embryos' anthropometric data and gestational age (in early pregnancy there was no heartbeat, and at pregnancy periods over 7 weeks a decrease in the crown-rump length by 3–5 weeks was detected) in these patients. Five patients (group II) underwent pregnancy termination for medical reasons (teratogenic effects) after genetic counseling and the council at 13–21 weeks of pregnancy. The control group (group III) was represented by 15 healthy patients having no problems with their reproductive function, who were through artificial abortion by their own will at 8–11 weeks of pregnancy.

The chorionic and/or embryonic tissue (sample E) and decidual tissue (sample D) were collected from all the surveyed individuals during surgery. Moreover, blood was collected from the women's cubital vein before surgery (sample C).

Proteins of the chorion of patients with frozen pregnancy and patients post artificial abortion were tested by electrospray ionization tandem mass spectrometry (LS-MS/MS) as previously reported [9]. Preparation of chorionic villi samples for further proteomic analysis involved protein extraction using the lysis buffer based on the 2% SDS (sodium dodecyl sulfate), ultrasonic treatment, 1DE-gel concentration procedure for SDS removal [9]. Protein levels in the chorionic villi extracts were determined using bicinchoninic acid as a reference sample [10].

Reduction, alkylation with iodoacetamide, and in-gel tryptic digestion were performed as previously reported [11]. The peptide mixture was analyzed using the Ultimate 3000 nano-flow HPLC system (Dionex, USA) integrated with the Orbitrap Q Exactive HF mass spectrometer (Thermo Scientific, USA) and the Nanospray Flex ion source (Thermo Scientific, USA) [9].

Mass spectra in the ".raw" format were converted into appropriate mgf files using the ProteoWizard MS Convert v. 3.0.6867 software tool (http://proteowizard.sourceforge.net). Files were imported to the SearchGUI platform (v. 3.3.17) [12] and analyzed using the X!Tandem and MS-GF+ search algorithms applied to the SwissProt database (v. 2.22.2022, FASTA format) for the species Homo sapiens. The search was performed in the database of inverted and random amino acid sequences (decoy). The PeptideShaker integrator [13] was used to produce the Excel spreadsheet files with the protein identification results.

To determine the relative content of proteins identified in the chorion, the normalized spectral abundance factor (NSAF) showing high reproducibility was used [14].

DNA was isolated from the samples C, E, D by the extraction method involving the use of organic solvents. The solution containing 0.04 M EDTA, 2% sodium lauryl sarcosylate, and 150 μm/mL RNAse A (Sigma, USA) was added to the samples for 45 min at 37 °C, treated with proteinase K (200 μm/mL, Promega, USA) for 24 h at 37 °C, extracted using the equal volumes of the phenol/chloroform/isoamyl alcohol mixture (25 : 24 : 1), phenol and the chloroform/isoamyl alcohol mixture (24 : 1). DNA was precipitated by adding 1/10 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of ice-cold ethanol. Phenol was stabilized with 8-hydroxyquinoline. DNA was collected by centrifugation at 10,000 G for 15 min at 4 °C, washed with 70% ethanol (v/v), dried, and dissolved in water.

Ribosomal gene copy number determination: the number of rDNA copies in DNA was determined by non-radioactive quantitative hybridization (NQH) [5]. To detect human rDNA (GenBank sample No. U13369), the mixture of probes for the rDNA oligo(18S) biotin-CTGTAATGATCCTTCCGCAGGTTCACCTAC and oligo(18S) biotin-TATCGGTCTCGTGCCGGTATTTAGCCTTAG was used. Denatured DNA was applied onto the filter (Optitran BA-S85, GE Healthcare, USA), 4–6 spots per sample. Standard genomic DNA samples (50 ng/ml) with the known rDNA content were applied onto the same filter to construct a calibration curve of signal intensity vs. rDNA copy number. The lambda phage DNA (50 ng/mL) was also applied onto the same filter to control the noise level. Then the filter was vacuum heated at 80 °C for 1.5 h. After the hybridization completion, the membrane filter was treated with the streptavidin-alkaline phosphatase conjugate (Sigma, USA) and placed in a solution of alkaline phosphatase substrates (bromo-chloro-indolyl-phosphate/nitro blue tetrazolium, BCIP/NBT). Then the filter was washed with water, dried in the dark, and scanned. The Imager 6 software allowing one to calculate the integrated intensity of signals from each point was used for rDNA quantification. Signals from all points corresponding to the same sample were summed up to calculate the mean and standard error for each sample.

No sample size calculation principles were used. Statistical analysis methods: descriptive statistics for quantitative variables are presented as the mean, median, and range of values. Pairwise comparison of samples was performed using the Mann–Whitney *U*-test (*p*). This test is best suited for our task due to small size of the samples used in the study. The test can be used to compare two groups, when there are at least three different trait values in each group. The StatPlus2007 software tool (http://www.analystsoft.com/) was used for calculation. The differences were considered significant at $p < 0.05$. The number of rDNA copies in the embryo's genome was used to predict frozen pregnancy in patients. Our results are preliminary and descriptive due to the fact that the sample was small.

RESULTS

Infertility has become a global health problem, with the number of people suffering from this condition growing every year. The IVF procedure holds great promise for infertility treatment. However, the early embryogenesis is complex, since a number of processes take place in this phase, including the transition from mother to zygote. In humans, the early embryonic development can be complicated by genomic errors that occur after fertilization. The nuclear abnormalities found in human embryos, especially those resulting from IVF, are due to DNA damage, aneuploidy, and decreased developmental potential. Transcription and expression of certain genes in the embryo are through a number of changes in the early embryonic development phase [15].

Comparative proteomic analysis

The multicopy genes that encode ribosomal rRNAs (rDNA) determine the ribosome biogenesis and, therefore, protein biosynthesis levels in the body, especially in early embryogenesis [16, 17]. The comparative proteomic analysis can provide new insights into biological pathways underlying the spontaneous abortion pathogenesis. That is why in the first phase of the study we assessed the human chorionic protein profile alterations associated with frozen pregnancy by panoramic mass spectrometry. Among proteins identified, pregnancy-specific glycoproteins were determined (PSG; Table 1). Human PSGs represent a group of molecules that are almost exclusively expressed by placental trophoblasts (chorionic villi) in pregnancy. Ten protein-encoding closely linked human PSG genes (PSG1–PSG9 and PSG11) form a subgroup of the carcinoembryonic antigen (CEA) gene family [18] (https://www.proteinatlas.org/humanproteome/tissue/placenta). CEA is an important tumor marker of colorectal and some other carcinomas [19]. We have also managed to identify all the CEA family subgroup members (Table 1). Furthermore, in individuals with frozen pregnancy, there was a significant decrease in the levels (estimated based on NSAF values) of such glycoproteins, as PSG3 and PSG2; glycoproteins PSG7 and PSG4 were not detected in the chorionic tissue. The decrease in PSG7 levels during fetal development can result in pregnancy loss [20, 21]. Thus, our data showing that low PSG levels are associated with poor pregnancy outcomes are consistent with the results of other authors [18].

Moreover, alpha-L-fucosidase, which plays an important role in cell adhesion during attachment and detachment of fetal membranes, was detected in samples of the chorion after abortion [22]. We also detected the decrease in expression of the reticulon-4 protein (RTN4), which is involved in apoptosis (GO: 0006915), in cases of embryo loss. The RTN4 deficiency can result in such phenotypes, as "abnormal trophoblast layer morphology", "fetal growth restriction", "reduced fetal size", and "embryonic lethality" [23].

In cases of embryo loss, PSG8, PSG7, PSG4 proteins were not determined in samples of the chorion; the pregnancy-specific beta-1-glycoprotein 6 (Q00889, PSG6) levels were low relative to the control (Table 1).

Furthermore, in cases of frozen pregnancy, we revealed the decreased levels of such proteins, as coactosin-like protein (*COTL1*), protein canopy homolog 2 (*CNPY2*), sideroflexin-3 (*SFXN3*), prohibitin-2 (PHB2), and hyaluronan and proteoglycan link protein 1 (HAPLN1), in the chorionic villi compared to the control samples of the chorion. For example, HAPLN1 is essential for production of the cartilaginous proteoglycan

Table 1. Pregnancy-specific glycoproteins (PSG) detected in human chorionic villi by panoramic mass spectrometry (LC-MS/MS)

##	Protein ID in the *UniProt database*	Gene	Protein	Biological process	NSAF value	
					Artificial abortion	Frozen pregnancy
1	Q00887	*PSG9*	Pregnancy-specific beta-1-glycoprotein 9	immune system process (GO:0002376)	0.0099	0.0051
2	Q9UQ74	*PSG8*	Pregnancy-specific beta-1-glycoprotein 8	immune system process (GO:0002376)	0.0053	undefined
3	Q13046	*PSG7*	Pregnancy-specific beta-1-glycoprotein 7	female pregnancy (GO:0022414)	0.0054	undefined
4	Q00889	*PSG6*	Pregnancy-specific beta-1-glycoprotein 6	immune system process (GO:0002376); female pregnancy (GO:0022414)	0.0051	0.0058
5	Q15238	*PSG5*	Pregnancy-specific beta-1-glycoprotein 5	cell adhesion (GO:0007155); female pregnancy (GO:0022414)	0.0129	0.0094
6	Q00888	*PSG4*	Pregnancy-specific beta-1-glycoprotein 4	immune system process (GO:0002376); female pregnancy (GO:0022414)	0.0159	undefined
7	Q16557	*PSG3*	Pregnancy-specific beta-1-glycoprotein 3	immune system process (GO:0002376); female pregnancy (GO:0022414)	0.0132	0.0079
8	P11465	*PSG2*	Pregnancy-specific beta-1-glycoprotein 2	cell adhesion (GO:0007155); female pregnancy (GO:0022414)	0.0146	0.0087
9	Q9UQ72	*PSG11*	Pregnancy-specific beta-1-glycoprotein 11	cell adhesion (GO:0007155); female pregnancy (GO:0022414)	0.0108	0.0089
10	P11464	*PSG1*	Pregnancy-specific beta-1-glycoprotein 1	immune system process (GO:0002376); female pregnancy (GO:0022414)	0.0072	0.0064

Note: NSAF — normalized spectral abundance factor reflecting protein content.

aggregates having a broad spectrum of biological functions. The lack of HAPLN1 results in perinatal mortality associated with severe chondrodysplasia [24] and heart malformations [25].

Thus, comparative proteomic analysis allowed us to determine the low abundance proteins specific for pregnancy development that are characterized by considerable alteration of their content (decrease and/or lack) during early embryogenesis in cases of spontaneous abortion.

Genetic testing

As is known, the body's level of protein biosynthesis is determined by ribosome biogenesis, the multicopy genes encoding ribosomal rRNA (rDNA), which represents one of the characteristics of adaptive capacity in humans. In this regard, in the next phase we determined rDNA in the maternal and fetal genomes during embryogenesis. Table 2 presents experimental

Table 2. Descriptive statistics for R parameter

Tissue type	Parameters	Group I (n = 25)	Group II (n = 5)	Group III (n = 15)
Maternal peripheral blood	Mean	417 ± 88	373 ± 183	425 ± 78
	Interval	322–629	191–713	314–612
	Median	398	442	412
Embryonic tissue	Mean	373 ± 183	363 ± 82	468 ±134
	Interval	191–713	242–436	295–723
	Median	261	401	478
Decidua	Mean	405 ± 92	434 ± 36	422 ± 70
	Interval	204–595	379–473	318–580
	Median	405	445	409

Note: the data are presented as the median (min–max); descriptive statistics for quantitative variables are presented as the mean and standard deviation — M (SD); sample C — blood from the patient's cubital vein; sample E — embryonic tissue; sample D — decidual tissue.

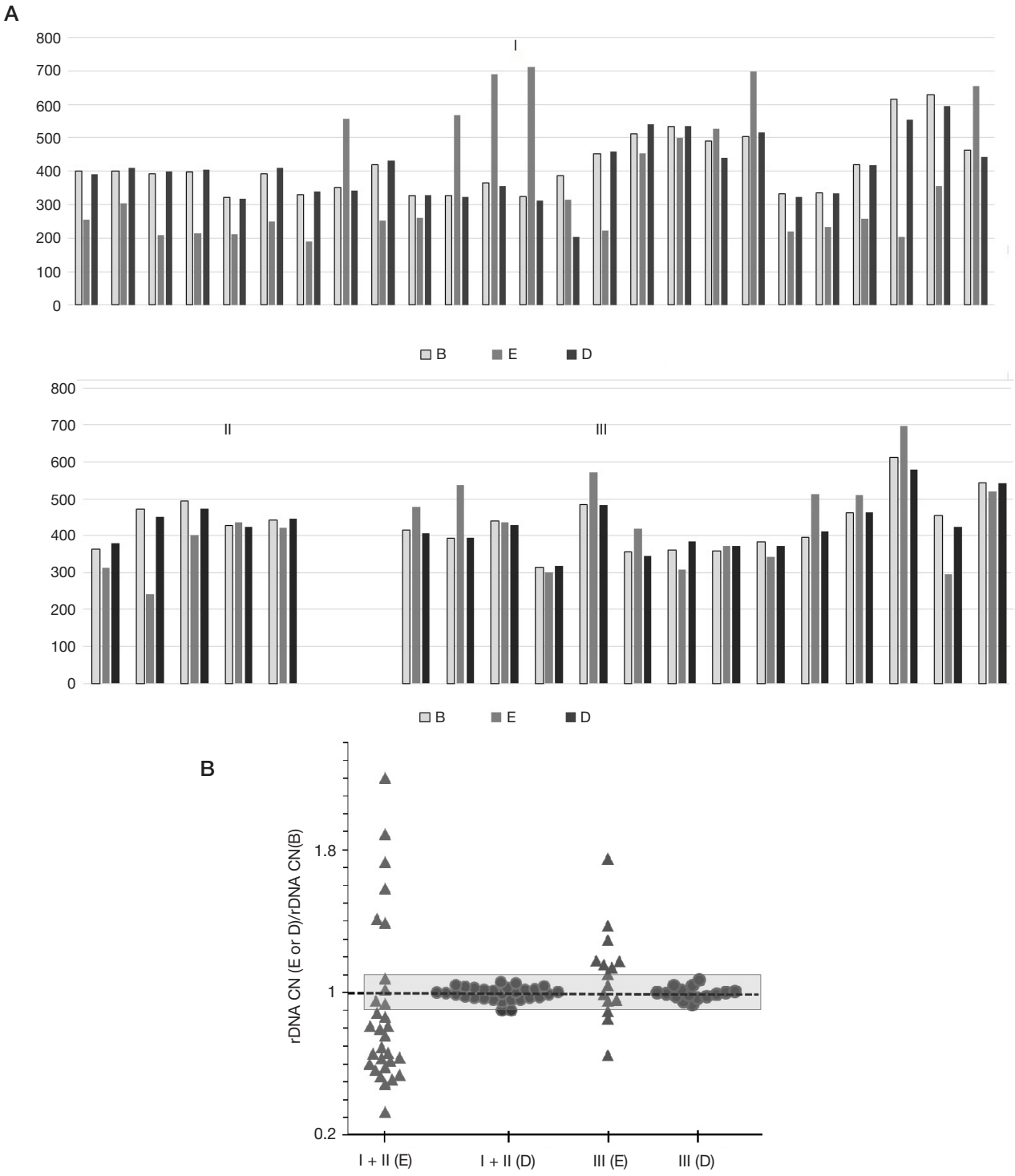

Fig. rDNA copy number in DNA isolated from three types of cells in groups I–III. **A**. The mean copy number in the DNA sample (n = 3) and the measurement error are provided. **B**. Alteration of rDNA copy number in embryonic and decidual tissues relative to rDNA levels in blood leukocytes

data that reflect the number of rDNA copies (R parameters) in DNA of blood leukocytes, decidua and the embryos' DNA.

Group I (Figure) — frozen pregnancy (No. 1–25) or suspected (visually) lack of the embryo (No. 20–25). Samples of the DNA isolated from blood cells and D tissue of the same women showed no differences in rDNA content ($p > 0.05$). This fact confirms the earlier reported data on equal rDNA levels in different tissues within the same body [26].

Embryonic genomes (tissue E) were dramatically different from maternal genomes (tissues C and D) based on the rDNA copy number. It is interesting to note that the differences were reported for five cases (No. 20–25), when the embryo could not be visually detected. It is likely that the cell division arrest occurred in early pregnancy. Among the maternal cells, there are most likely also cells from another organism. It is possible that the rDNA content in these cells is even lower.

As for the ratio of rDNA content in the embryo's genome and maternal cells, all embryos in group I split into two subgroups. In subgroup Ia (n = 20), the R (E) parameter was 1.4–3.3 times lower (mean — 1.7 times; $p < 0.001$), than R (C or D). In subgroup Ib (n = 5), the R (E) parameter was 1.7–2 times higher (mean — 1.8 times; p = 0.02), than R (C). Thus, the embryo development arrest is associated with either too low, or too high rDNA content compared to the woman's genome.

Group II — termination of pregnancy due to medical reasons (encephalocele, signs of congenital limb deformity, congenital CNS abnormality acrania, Edwards syndrome, and fusion of the pulmonary artery to aorta in the fetus). In this group, genomes of 4 embryos comprised more rDNA copies, than in group Ia (p = 0.01). The differences between groups IIC and IIE were non-significant. Only one embryonic DNA sample had lower rDNA content compared to the woman's leukocyte DNA.

As for groups with healthy and frozen pregnancy, the most significant results were obtained for the embryos only (Fig. 1), and the threshold number of rDNA repeats was 322. In our study, lower rDNA content values were associated with the embryos' death.

The analysis of the data reported allows us to draw the following conclusions

In cases of embryo loss, there are severe abnormalities of protein composition and the genes involved in pregnancy development in tissues of the chorion. Thus, low PSG levels were associated with poor pregnancy outcomes. In particular, in cases of frozen pregnancy no pregnancy-specific beta-1-glycoprotein 7 (PSG7), the decrease in the levels of which during fetal development can result in pregnancy loss, was found in the chorion.

We also detected downregulation of the reticulon-4 protein (RTN4) involved in apoptosis (GO: 0006915) in cases of embryo loss. The RTN4 deficiency can result in such phenotypes, as "abnormal trophoblast layer morphology", "fetal growth restriction", "reduced fetal size", and "embryonic lethality" [23].

The rDNA content is the same in blood cells and cells of the decidua of the same female body.

The threshold values of rDNA repeats in embryonic tissues (322) were determined, after the decrease of which pregnancy was terminated.

DISCUSSION

Frozen pregnancy is associated with severe imbalance of rDNA content in the embryonic and maternal genomes. In most cases, the embryo's genome comprises significantly less rDNA copies, than the maternal genome and genomes of other embryos, the development of which has not been not spontaneously interrupted. Very low rDNA content in the genome is likely to be associated with the low number of ribosomes, which is incapable of ensuring the protein synthesis level appropriate for the development of a particular embryo. It has been previously shown that the low rDNA copy number in the human genome (less than 300 copies) is associated with the lower life expectancy and dementia in the elderly [7, 27]. Cystic fibrosis, a monogenic disorder caused by the CFTR gene mutation, is associated with the larger rDNA copy number in the affected individual's genome. Schizophrenia, being a multifactorial disorder, is also associated with the increased rDNA content in the affected individual's genome [27, 28]. It is likely that low amounts of rDNA in the genome do not allow the genome with genetic abnormalities to be realized, and embryogenesis is interrupted at an early stage. Only five chromosomes in the human genome contain the rDNA encoding ribosomal RNA, of which the ribosome is assembled with the help of certain proteins. The ribosome provides molecular machinery for the synthesis of all proteins in our body [17, 29]. The very high rDNA content in the embryonic genome compared to maternal one also negatively affects embryogenesis. There are two possible explanations for this fact. First, high ribosome biogenesis levels resulting from the larger number of rDNA copies in the embryo's genome require a large amount of nutrients from the mother's body. If the maternal genome contains a low number of rDNA copies, it will not be able to fulfill the needs of the embryo. Second, genomes with the larger number of rDNA copies can contain mutations that block embryogenesis at the later stages, but allow for early embryo development. Genomes with low rDNA amounts and this genetic abnormality are rejected in early embryogenesis.

CONCLUSIONS

Determination of the *PSG8* (pregnancy-specific beta-1-glycoprotein 8), *PSG7* (putative pregnancy-specific beta-1-glycoprotein 7), *PSG6* (pregnancy-specific beta-1-glycoprotein 6), and *PSG4* (pregnancy-specific beta-1-glycoprotein 4) content be useful for prenatal prediction of pregnancy course. In most cases (16 surveyed individuals out of 20), frozen pregnancy is associated with the low rDNA content in the embryo's DNA relative to the control group (normal pregnancy). The low R parameter value shows that the cells are unable to ensure the ribosome biogenesis level appropriate for embryogenesis. Determination of specific proteins in the chorionic villi and rDNA copy number in the potential parents' genomes with subsequent modeling of the rDNA copy number in the embryo can help determine the cause of infertility in married couples and predict the ongoing pregnancy course.

References

1. Sidelnikova VM. Privychnaya poterya beremennosti. M.: Triada-H, 2002; 32 s. Russian.
2. Tarrade A, Lai Kuen R, Malassiné A, et al. Characterization of human villous and extravillous trophoblasts isolated from first trimester placenta. Lab Invest. 2001; 81 (9): 1199–211. Available from: https://doi.org/10.1038/labinvest.3780334.
3. Elguoshy A, Magdeldin S, Xu B, et al. Why are they missing? Bioinformatics characterization of missing human proteins. J Proteomics. 2016; 149: 7–14. Available from: https://doi.org/10.1016/j.jprot.2016.08.005.
4. Zhou L, Wong L, Wen Bin Goh W. Understanding missing proteins: a functional perspective. Drug Discov Today. 2018; 23 (3): 644–51. Available from: https://doi.org/10.1016/j.drudis.2017.11.011.
5. Malinovskaya E, Ershova E, Golimbet V, et al. Copy number of human ribosomal genes with aging: unchanged mean, but narrowed range and decreased variance in elderly group. Front Genet. 2018; 9: 306. Available from: https://doi.org/10.3389/fgene.2018.00306.
6. Lyapunova NA, Veiko NN. Ribosomnye geny v genome cheloveka: identifikaciya chetyrekh frakcij, ih lokalizaciya v yadryshke i metafaznyh hromosomah. Genetika. 2010; 46 (9): 1205–9.
7. Porohovnik LN, Egolina NA, Kosyakova NV, i dr. Zigoticheskij i embrional'nyj otbor po genomnoj doze aktivnyh ribosomnyh genov kak odin iz vozmozhnyh faktorov snizhennoj plodovitosti supruzheskih par. Medicinskaya genetika. 2012; 11 (6): 31–4. Russian.
8. Veiko N, Ershova E, Porokhovnik L, et al. Ribosomal, telomere, and mitochondrial repeat copy number variations in female genomes during ovarian stimulation and the prediction of in vitro fertilization outcome: a pilot study. Front Biosci (Schol Ed). 2023; 15 (3): 9. Available from: https://doi.org/10.31083/j.fbs1503009.
9. Shkrigunov T, Pogodin P, Zgoda V, et. al. Protocol for Increasing the Sensitivity of MS-Based Protein Detection in Human Chorionic Villi. Curr Issues Mol Biol. 2022; 44 (5): 2069–88. Available from:

https://doi.org/10.3390/cimb44050140.
10. Smith P, Krohn R, Hermanson G, et al. Measurement of Protein Using Bicinchoninic Acid Anal Biochem. 1985; 150 (1): 76–85. Available from: https://doi.org/10.1016/0003-2697(85)90442-7.
11. Shevchenko A, Tomas H, Havlis J, et al. In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat Protoc. 2006; 1 (6): 2856–60. Available from: https://doi.org/10.1038/nprot.2006.468.
12. Vaudel M, Barsnes H, Berven F, et al. SearchGUI: An open-source graphical user interface for simultaneous OMSSA and X!Tandem searches. Proteomics. 2011; 11 (5): 996–9. Available from: https://doi.org/10.1002/pmic.201000595.
13. Vaudel M, Burkhart J, Zahedi R, et al. PeptideShaker enables reanalysis of MS-derived proteomics data sets. Nat Biotechnol. 2015; 33 (1): 22–4. Available from: https://doi.org/10.1038/nbt.3109.
14. Neilson K, Keighley T, Pascovici D, et al. Label-free quantitative shotgun proteomics using normalized spectral abundance factors. Methods Mol Biol. 2013; 1002: 205–22. Available from: https://doi.org/10.1007/978-1-62703-360-2_17.
15. Godini R, Fallahi H. Dynamics changes in the transcription factors during early human embryonic development. J Cell Physiol. 2019; 234 (5): 6489–502. Available from: https://doi.org/10.1002/jcp.27386.
16. Porohovnik LN, Veiko NN, Ershova ES, i dr. Kopijnost' ribosomnoj DNK (rDNK) v genomah zhenshchin kak faktor uspeshnosti EKO i nalichiya oslozhnenij beremennosti. Medicinskaya genetika. 2019; 18 (11): 14–25. Available from: https://doi.org/10.25557/2073-7998.2019.11.14-25. Russian.
17. Liao C, Pang N, Liu Z, et al. Transient inhibition of rDNA transcription in donor cells improves ribosome biogenesis and preimplantation development of embryos derived from somatic cell nuclear transfer. FASEB J. 2020; 34 (6): 8283–95. Available from: https://doi.org/10.1096/fj.202000025RR.
18. Hammarström S. The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. Semin Cancer Biol. 1999; 9 (2): 67–81. Available from: https://doi.org/10.1006/scbi.1998.0119.
19. An Online Catalog of Human Genes and Genetic Disorders: PREGNANCY-SPECIFIC BETA-1-GLYCOPROTEIN 11; PSG11. Available from: https://omim.org/entry/176401.
20. Su X, Zhang J, Yang W, et al. Identification of the Prognosis-Related lncRNAs and Genes in Gastric Cancer. Front Genet. 2020; 11: 22. Available from: https://doi.org/10.3389/fgene.2020.00027.
21. Khan W, Hammarström S. Identification of a new carcinoembryonic antigen (CEA) family member in human fetal liver--cloning and sequence determination of pregnancy-specific glycoprotein 7. Biochem Biophys Res Commun. 1990; 168 (1): 214–25. Available from: https://doi.org/10.1016/0006-291x(90)91696-p.
22. Jamioł M, Wawrzykowski J, Mojsym W, et al. Activity of selected glycosidases and availability of their substrates in bovine placenta during pregnancy and parturition with and without retained foetal membranes. Reprod Domest Anim. 2020; 55 (9): 1093–102. Available from: https://doi.org/10.1111/rda.13747.
23. Sui L, An L, Tan K, et al. Dynamic Proteomic Profiles of In Vivo- and In Vitro-Produced Mouse Postimplantation Extraembryonic Tissues and Placentas. Biol Reprod. 2014; 91 (155): 1–16. Available from: https://doi.org/10.1095/biolreprod.114.124248.
24. Watanabe N, Kato T, Fujita A, et al. Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. Nat Cell Biol. 1999; 1 (3): 136–43. Available from: https://doi.org/10.1038/11056.
25. Wirrig E, Snarr B, Chintalapudi M, et al. Cartilage link protein 1 (Crtl1), an extracellular matrix component playing an important role in heart development. Dev Biol. 2007; 310 (2): 291–303. Available from: https://doi.org/10.1016/j.ydbio.2007.07.041.
26. Veiko N, Ershova S, Veiko R, et al. Mild cognitive impairment is associated with low copy number of ribosomal genes in the genomes of elderly people. Front Genet. 2022; 13: 967448. Available from: https://doi.org/10.3389/fgene.2022.967448.
27. Ershova E, Malinovskaya E, Golimbet V, et al. Copy number variations of satellite III (1q12) and ribosomal repeats in health and schizophrenia. Schizophr Res. 2020; 223: 199–212. Available from: https://doi.org/10.1016/j.schres.2020.07.022.
28. Kondrateva E, Ershova E, Nikolaeva E, i dr. Rezul'taty izucheniya kompleksa ribosomnyh genov cheloveka pri mukoviscidoze. Pul'monologiya. 2023; 33 (1): 7–16. Available from: https://doi.org/10.18093/0869-0189-2023-33-1-7-16.
29. Sun Y, Hu X, Qiu D, et al. rDNA Transcription in Developmental Diseases and Stem Cells. Stem Cell Rev Rep. 2023; 19 (4): 839–52. Available from: https://doi.org/10.1007/s12015-023-10504-6.

Литература

1. Сидельникова В. М. Привычная потеря беременности. М.: Триада-Х, 2002; 32 с.
2. Tarrade A, Lai Kuen R, Malassiné A, et al. Characterization of human villous and extravillous trophoblasts isolated from first trimester placenta. Lab Invest. 2001; 81 (9): 1199–211. Available from: https://doi.org/10.1038/labinvest.3780334.
3. Elguoshy A, Magdeldin S, Xu B, et al. Why are they missing? Bioinformatics characterization of missing human proteins. J Proteomics. 2016; 149: 7–14. Available from: https://doi.org/10.1016/j.jprot.2016.08.005.
4. Zhou L, Wong L, Wen Bin Goh W. Understanding missing proteins: a functional perspective. Drug Discov Today. 2018; 23 (3): 644–51. Available from: https://doi.org/10.1016/j.drudis.2017.11.011.
5. Malinovskaya E, Ershova E, Golimbet V, et al. Copy number of human ribosomal genes with aging: unchanged mean, but narrowed range and decreased variance in elderly group. Front Genet. 2018; 9: 306. Available from: https://doi.org/10.3389/fgene.2018.00306.
6. Ляпунова Н. А., Вейко Н. Н. Рибосомные гены в геноме человека: идентификация четырех фракций, их локализация в ядрышке и метафазных хромосомах. Генетика. 2010; 46 (9): 1205–9.
7. Пороховник Л. Н., Еголина Н. А., Косякова Н. В., и др. Зиготический и эмбриональный отбор по геномной дозе активных рибосомных генов как один из возможных факторов сниженной плодовитости супружеских пар. Медицинская генетика. 2012; 11 (6): 31–4.
8. Veiko N, Ershova E, Porokhovnik L, et al. Ribosomal, telomere, and mitochondrial repeat copy number variations in female genomes during ovarian stimulation and the prediction of in vitro fertilization outcome: a pilot study. Front Biosci (Schol Ed). 2023; 15 (3): 9. Available from: https://doi.org/10.31083/j.fbs1503009.
9. Shkrigunov T, Pogodin P, Zgoda V, et. al. Protocol for Increasing the Sensitivity of MS-Based Protein Detection in Human Chorionic Villi. Curr Issues Mol Biol. 2022; 44 (5): 2069–88. Available from: https://doi.org/10.3390/cimb44050140.
10. Smith P, Krohn R, Hermanson G, et al. Measurement of Protein Using Bicinchoninic Acid Anal Biochem. 1985; 150 (1): 76–85. Available from: https://doi.org/10.1016/0003-2697(85)90442-7.
11. Shevchenko A, Tomas H, Havlis J, et al. In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat Protoc. 2006; 1 (6): 2856–60. Available from: https://doi.org/10.1038/nprot.2006.468.
12. Vaudel M, Barsnes H, Berven F, et al. SearchGUI: An open-source graphical user interface for simultaneous OMSSA and X!Tandem searches. Proteomics. 2011; 11 (5): 996–9. Available from: https://doi.org/10.1002/pmic.201000595.
13. Vaudel M, Burkhart J, Zahedi R, et al. PeptideShaker enables reanalysis of MS-derived proteomics data sets. Nat Biotechnol. 2015; 33 (1): 22–4. Available from: https://doi.org/10.1038/nbt.3109.
14. Neilson K, Keighley T, Pascovici D, et al. Label-free quantitative shotgun proteomics using normalized spectral abundance factors. Methods Mol Biol. 2013; 1002: 205–22. Available from: https://doi.org/10.1007/978-1-62703-360-2_17.
15. Godini R, Fallahi H. Dynamics changes in the transcription factors during early human embryonic development. J Cell Physiol. 2019; 234 (5): 6489–502. Available from: https://doi.org/10.1002/jcp.27386.
16. Пороховник Л. Н., Вейко Н. Н., Ершова Е. С., и др. Копийность рибосомной ДНК (рДНК) в геномах женщин как фактор успешности ЭКО и наличия осложнений беременности. Медицинская генетика. 2019; 18 (11): 14–25. Available from:

https://doi.org/10.25557/2073-7998.2019.11.14-25.
17. Liao C, Pang N, Liu Z, et al. Transient inhibition of rDNA transcription in donor cells improves ribosome biogenesis and preimplantation development of embryos derived from somatic cell nuclear transfer. FASEB J. 2020; 34 (6): 8283–95. Available from: https://doi.org/10.1096/fj.202000025RR.
18. Hammarström S. The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. Semin Cancer Biol. 1999; 9 (2): 67–81. Available from: https://doi.org/10.1006/scbi.1998.0119.
19. An Online Catalog of Human Genes and Genetic Disorders: PREGNANCY-SPECIFIC BETA-1-GLYCOPROTEIN 11; PSG11. Available from: https://omim.org/entry/176401.
20. Su X, Zhang J, Yang W, et al. Identification of the Prognosis-Related lncRNAs and Genes in Gastric Cancer. Front Genet. 2020; 11: 22. Available from: https://doi.org/10.3389/fgene.2020.00027.
21. Khan W, Hammarström S. Identification of a new carcinoembryonic antigen (CEA) family member in human fetal liver--cloning and sequence determination of pregnancy-specific glycoprotein 7. Biochem Biophys Res Commun. 1990; 168 (1): 214–25. Available from: https://doi.org/10.1016/0006-291x(90)91696-p.
22. Jamioł M, Wawrzykowski J, Mojsym W, et al. Activity of selected glycosidases and availability of their substrates in bovine placenta during pregnancy and parturition with and without retained foetal membranes. Reprod Domest Anim. 2020; 55 (9): 1093–102. Available from: https://doi.org/10.1111/rda.13747.
23. Sui L, An L, Tan K, et al. Dynamic Proteomic Profiles of In Vivo- and In Vitro-Produced Mouse Postimplantation Extraembryonic Tissues and Placentas. Biol Reprod. 2014; 91 (155): 1–16. Available from: https://doi.org/10.1095/biolreprod.114.124248.
24. Watanabe N, Kato T, Fujita A, et al. Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. Nat Cell Biol. 1999; 1 (3): 136–43. Available from: https://doi.org/10.1038/11056.
25. Wirrig E, Snarr B, Chintalapudi M, et al. Cartilage link protein 1 (Crtl1), an extracellular matrix component playing an important role in heart development. Dev Biol. 2007; 310 (2): 291–303. Available from: https://doi.org/10.1016/j.ydbio.2007.07.041.
26. Veiko N, Ershova S, Veiko R, et al. Mild cognitive impairment is associated with low copy number of ribosomal genes in the genomes of elderly people. Front Genet. 2022; 13: 967448. Available from: https://doi.org/10.3389/fgene.2022.967448.
27. Ershova E, Malinovskaya E, Golimbet V, et al. Copy number variations of satellite III (1q12) and ribosomal repeats in health and schizophrenia. Schizophr Res. 2020; 223: 199–212. Available from: https://doi.org/10.1016/j.schres.2020.07.022.
28. Кондратьева Е., Ершова Е., Николаева Е., и др. Результаты изучения комплекса рибосомных генов человека при муковисцидозе. Пульмонология. 2023; 33 (1): 7–16. Available from: https://doi.org/10.18093/0869-0189-2023-33-1-7-16.
29. Sun Y, Hu X, Qiu D, et al. rDNA Transcription in Developmental Diseases and Stem Cells. Stem Cell Rev Rep. 2023; 19 (4): 839–52. Available from: https://doi.org/10.1007/s12015-023-10504-6.