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The Use of Atomic Force Microscopy in Comprehensive Assessment of the "Mother—Placenta—Fetus" System in Obstetric and Endocrine Pathology during Pregnancy

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Atomic force microscopy is not very popular in practical health care, therefore, its potential is not studied enough, for example, in obstetrics when studying the "mother—placenta—fetus" system. Our study summarizes the possibilities of using atomic force microscopy for detection of various circulatory disorders and vascular changes at the microscopic level in the uterus (endometrium and myometrium), placenta, and umbilical cord in the main variants of obstetric and endocrine pathology. For instance, in the case of endocrine pathologies, changes in the form of stasis, sludge, diapedesis, ischemia, destruction and separation of endotheliocytes in villous blood vessels were found in the mother. The oxygen content in erythrocytes also naturally decreased in pathologies; poikilo- and anisocytosis were observed.

Key Words: *scanning microscopy; obstetrics; placenta; uterus*

Modern methods for studying biological materials can be conditionally divided into two main groups: chemical and microscopic [7]. The capabilities of scanning electron microscopy (SEM) make it possible to combine them together [4] and to study the microscopic structure of tissues with transition to the molecular level. SEM is used in both scientific and practical biology and medicine [2]: in clinical and laboratory hematology, immunology, microbiology (bacteriology, mycology, and virology), cytology, molecular biology, and diagnostic genetics. SEM also allows studying human reproductive functions under normal or pathological conditions [1]. This method allows not only visualizing cell elements at high magnifications, but also studying their local composition [8]. In addition, 3D images of the tissue provide better insight into structural interrelationships of its elements [6]. The possibility of studying native material makes it possible to study the components of the "mother—placenta—fetus" system at a different structural level in the pathology of pregnancy [3,5].

Here we studied the possibilities of using SEM (with elemental analysis) in the study of the "mother—placenta—fetus" system: the uterus (endometrium and myometrium), blood erythrocytes, placenta, and umbilical cord in different types of obstetric and endocrine pathologies.

MATERIALS AND METHODS

The studies were carried out at the Department of Pathology of the Belgorod State National Research

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University. We examined pregnant women (age 25-35 years) with thyroid diseases: hypothyroidism (postoperative, autoimmune thyroiditis; *n*=15), hyperthyroidism (diffuse toxic goiter (DTG) at the stage of compensation; *n*=10), and euthyroid state (thyroid hypertrophy of the 1st and 2nd degree, mixed goiter; *n*=17). The study also included patients with type 1 diabetes mellitus (DM1; *n*=10), gestational diabetes (GDM; *n*=10), and pregnant women with moderate preeclampsia (*n*=10). The comparison group consisted of pregnant women (*n*=5) without endocrinopathies and obstetric pathologies. Childbirth in all examined women was carried out via cesarean section at gestation week 38-40. During the study, general clinical, radioimmunological, and instrumental research methods were used. Clinical data about the patients were obtained by studying the outpatient records of pregnant women, childbirth records, and medical histories. Common blood and urine tests and biochemical blood tests were performed, thyroid function was assessed by the level of thyroid-stimulating hormone, total and free T4 and T3; antibodies to thyroperoxidase and thyroglobulin were assayed using a kit of reagents for radioimmunoassay of the thyroid group.

To determine tissue changes, fragments of the placenta, umbilical cord, and uterus (myometrium and endometrium) were taken. Macroscopic description of the placenta included its shape, size, and weight, the state of the amnion, pathological changes in the placental tissue, the state of the decidua and umbilical cord (attachment site, number of vessels). The samples were studied without additional processing under a Quanta 600 FEG scanning electron microscope integrated with an X-ray microanalysis detector for determining oxygen content in tissues from EPAX (method sensitivity is 0.1-0.3%).

We also examined erythrocytes of maternal venous blood taken from the basilic vein of the upper limb prior to childbirth. Depending on their size, erythrocyte were distributed into 3 groups: microcytes (<6.4 μm), normocytes (6.5-8.9 μm), and macrocytes (9.0-10.9 μm). When describing morphological characteristics, the erythrocyte population was divided into the main groups: discocytes; transitional forms (capable of reverse transformation): ellipses, discocytes with a crest, flat disks, discocytes with outgrowth, discocytes with multiple outgrowths, mulberry-shaped erythrocytes; prehemolytic forms (with irreversible changes): domed, spherical, "deflated ball"; degenerative forms.

The data were statistically processed in Microsoft Excel 2010 and Statistica 10.0 (StatSoft, Inc.), the significance of differences of relative and mean values was determined using the Student's *t* test at $p<0.05$.

RESULTS

Examination of the umbilical cord under a scanning microscope at ×50-100 allows evaluating the entire section of the organ and revealing changes in the lumens and walls of blood vessels, their edema and exudative-leukocytic reactions, and the state of Wharton's jelly.

At higher magnification, circulatory disorders were presented by stasis, sludge, diapedesis (hypothyroidism, GDM, DM1, and DTG), and fibrin formation (DM1 and preeclampsia) (Fig. 1, *a*).

The distance between the endotheliocyte folds significantly ($p<0.05$) increased in the following order: 2.9±0.3 μm in euthyroidism, 3.1±0.3 μm in GDM, 3.9±0.4 μm in hypothyroidism, 4.3±0.8 μm in preeclampsia, 4.5±1.5 μm in DTG, and 4.6±1.4 μm in DM1 (*vs* 2.0±0.5 μm in the control). The formation of folds on the luminal surface of endothelial cells and structural abnormalities of contacts between the endothelial cells were also found.

Fragments of the villous chorion with the maternal and fetal surfaces within 3-5 cotyledons were examined (×50-250). Mosaic changes in the fragments

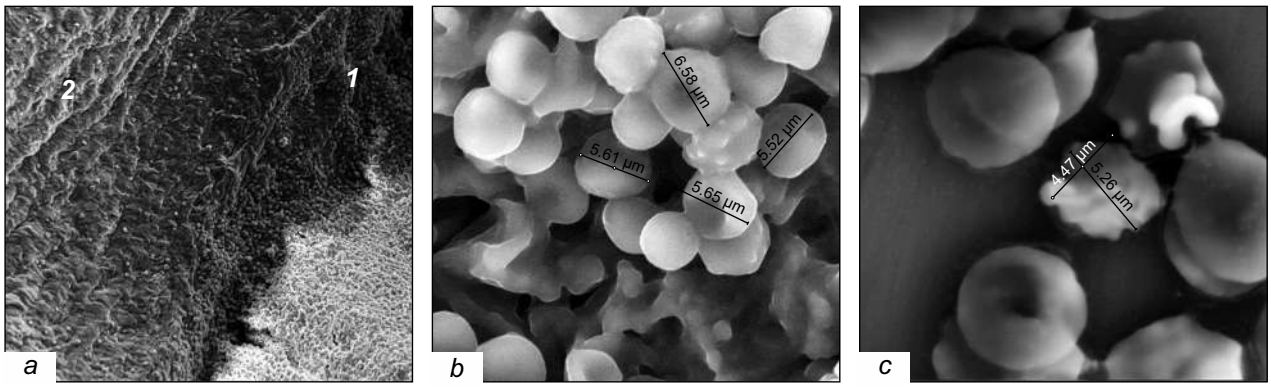

Fig. 1. Plethora and blood stasis in the umbilical vein in preeclampsia (*a*); erythrocytes in IVS of the placenta: changes in the cell shape (*b*); erythrocytes in the maternal venous blood (*c*). SEM, ×600 (*a*), ×4000 (*b*), ×12,000 (*c*). *1*) Elemental composition in the erythrocytes of the umbilical cord vessels, 2) erythrocytes in the IVS.

of the maternal and fetal surfaces in obstetric and endocrine pathology were revealed; they manifested in circulatory disorders such as uneven blood filling, focal hemorrhages, and thrombosis in the intervillous spaces, villous stroma and lumen of blood vessels, sclerosis of the stroma of the stem and intermediate villi, single or, in some cases, multiple petrification in the stroma of villi of different orders, which are more pronounced in case of their combination (Fig. 2).

In endocrine pathology and preeclampsia, in contrast to the control, infarctions in the placenta and thrombus formation in the intervillous space (IVS) of the placenta were. Deposition of calcium salts in IVS was typical of preeclampsia and DM1; in hypothyroidism, the tissue was ischemic and sclerosed. In all types of the studied pathology, the order of branching of villi decreased, deficiency of terminal villi and formation of afunctional zones by mature intermediate chorionic villi were seen. The walls of the vessels of the stem and "anchoring" villi were often thickened and sclerosed, and their lumen was narrowed. The proportion of unchanged vessels in the stem villi significantly decreased in the following order: 35.6±3.7 μm in patients with grade 1-2 thyroid hypertrophy and mixed goiter, 32.1±2.6 μm in GDM, 12.6±1.9 μm in postoperative hypothyroidism and autoimmune thyroiditis, 11.2±1.4 μm in DM1, 10.3±1.6 μm in DTG, and 9.3±1.5 μm in preeclampsia (*vs* 28.0±2.4 μm in the control). The tendency towards a decrease in the proportion of unchanged vessels in the same sequence was also observed in terminal compartments.

In all groups, changes in villi with predominance of intermediate-type villi and a sharp decrease in the number of branches were found. Thus, the length of intermediate-type villi in patients with euthyroid state was 460±36 μm, with hypothyroidism 690±48 μm, with DTG 720±55 μm, with GDM 420±42 μm, with DM1 730±95 μm, and with preeclampsia 710±101 μm (*vs* 300±30 μm in the control). In addition, structural abnormalities of microvilli up to their desquamation were found.

The content of erythrocytes in the vessels of the villous chorion significantly decreased to 76.0±1.5% in euthyroid patients, to 67.0±1.9% in hypothyroidism, to 65.0±1.3% in DTG, to 70.0±4.5% in GDM, to 60.0±3.8% in DM1, and to 63.0±3.3% in preeclampsia (*vs* 85.0±1.6% in the control). In case of combination of maternal endocrinopathy (DM1 and DTG) with

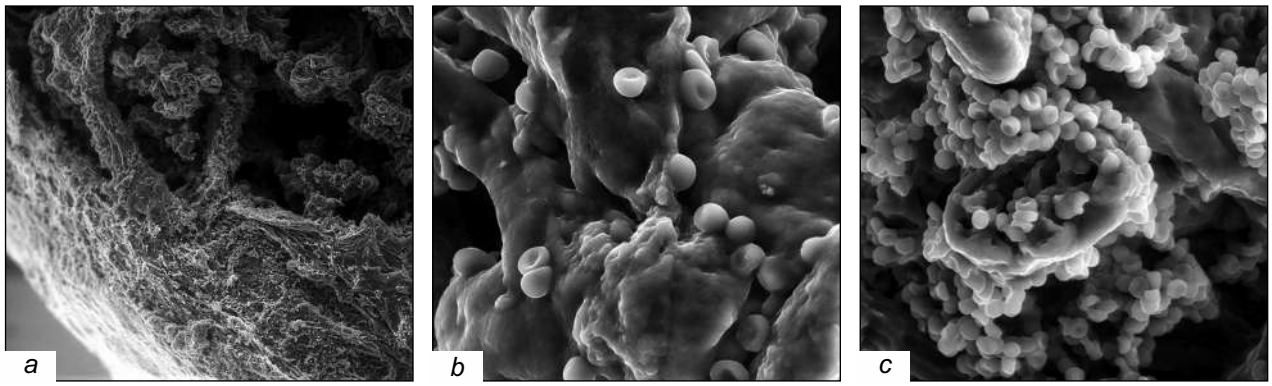

Fig. 2. Fragments of the villous chorion in the control group (*a, b*) and in a patient with preeclampsia (*c*). SEM, ×200 (*a*), ×4000 (*b*), ×2000 (*c*). *b* is a fragment of *a* at higher magnification.

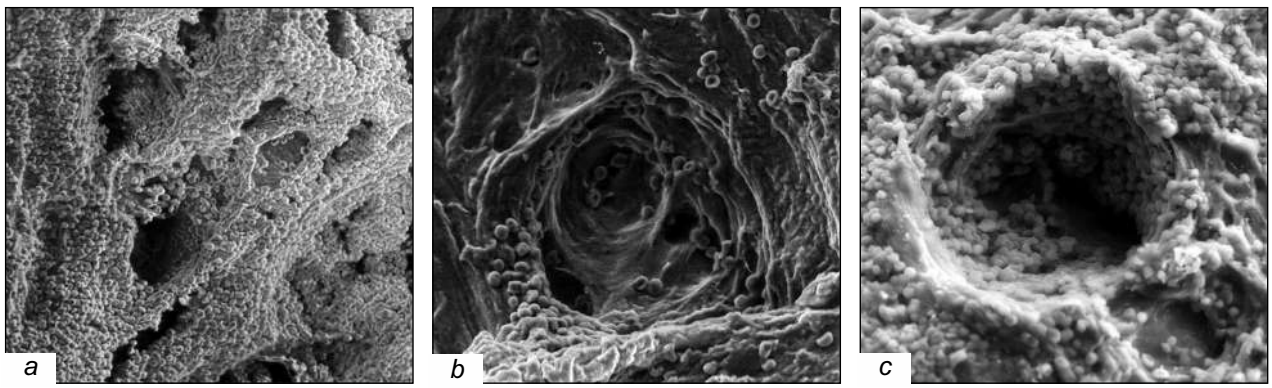

Fig. 3. Fragments of the uterine tissue from a woman of the control group (*a*, *b*) and a patient with DTG (*c*): endometrium (*a*), myometrium (*b*, *c*) (spiral arteries). SEM, ×1000 (*a*), ×2000 (*b*), ×1500 (*c*).

preeclampsia, their number did not exceed 53.0±2.3%. The mean size of erythrocytes in the villous tree was slightly lower than in IVS. Among abnormal erythrocytes, elongated cells shaped as irregular ovals were often observed, echinocytes and degenerative forms were less common (Fig. 1, *b*). Some blood cells were connected to each other and to endotheliocytes by cytoplasmic bridges and fibrin filaments (mostly in DM1 and preeclampsia); in DM1 and DTG, the plasma membranes of cells were changed.

In patients with pathology of the thyroid gland, hypothyroidism, DTG, DM1, and preeclampsia, spiral arteries of the myometrium had abnormal shape (Fig. 3). In patients of the experimental groups, endotheliocytes were flattened and thinned, the height of endothelial cells was 3.0±0.2 μm in hypothyroidism, 2.5±0.3 μm in DTG, 2.3±0.4 μm in the euthyroid state, 3.3±0.2 μm in DM1, 2.5±0.3 μm in GDM, 2.6±0.4 μm in preeclampsia (*vs* 3.45±0.70 μm in the control; $p<0.05$).

In the endometrium and myometrium, significant changes in the shape and structure of erythrocytes and their diapedesis were noted. Thrombi as well as filaments and sheets of fibrin, which are more common in DM1, thyroid disease and preeclampsia, were detected.

In umbilical cord vessels (arteries and vein), vessels of the villi, IVS, endometrium, and spiral arteries of the uterus, the blood oxygen content was significantly reduced in the following order: euthyroidism, GDM, hypothyroidism, preeclampsia, DTG, and DM1. However, in different pathologies, oxygen content did not differ significantly between these groups. Thus, this parameter in the spiral arteries was 27.86±2.06% in hypothyroidism, 25.11±2.03% in DTG, 24.10±1.12% with euthyroid state, 23.10±2.01% with DM1, 23.08±1.60% with GDM and 21.06±2.50% with preeclampsia (*vs* 34.59±2.31% in the control).

In maternal venous blood in all types of endocrine and obstetric pathologies, the content of normocytes was reduced in comparison with macrocytes and especially with microcytes. Thus, in euthyroidism, hypo-, and hyperthyroidism, the relative content of microcytes was 19.70±2.01, 13.25±1.08, and 12.06±1.07%, respectively, in GDM and DM1, they constitute 19.51±2.03 and 12.45±2.50%, respectively, and in preeclampsia 20.35±2.90% (*vs* 16.30±2.03% in the control; $p<0.05$). The content of macrocytes was 11.50±1.34% in hypothyroidism, 17.45±1.01% in DTG, 18.30±1.24% in the euthyroid state, 17.50±1.25% in DM1, 20.30±1.34% in GDM, and 18.60±1.10% in preeclampsia (*vs* 15.60±1.08% in the control) ($p<0.05$).

The decrease in the number of discocytes led to a significant ($p<0.05$) increase in the content of transitional forms, which was indicative for DTG (19.0±1.5%), DM1 (21.3±2.4%), and preeclampsia (18.5±1.3%) (*vs* 11±2% in control). The relative content of pre-hemolytic forms in these groups was 4.5±0.8, 5.8±0.9, and 6.2±1.1%, respectively (*vs* 0.2±0.1% in the control), and the content of degenerative forms was 5.4±0.8, 6.2±0.9, and 6.5±1.0%, respectively (*vs* 0.2±0.1% in the control) ($p<0.05$; Fig. 1, *c*).

Due to great depth of view, scanning electron microscopy allows studying the surfaces of tissues without complex preparation procedures. Examination of easily available placental tissue and uterine tissue samples by SEM makes it possible to create an extensive database of typical changes for a particular pathology in the "mother—placenta—fetus" system, which will open up new opportunities for rapid diagnostics of the structural state and biochemical parameters of the uterine and placental tissue for fairly accurate preliminary identification of pathological changes.

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