Markers of Transmembrane and Energy Exchange in Cells of Terminal Placental Villi in Spontaneous and Induced Pregnancy

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We performed a comparative morphological study of the placentas in spontaneous and induced pregnancy. Immunohistochemical analysis revealed pronounced decrease in the expression of markers of transmembrane (α-SNAP 23, annexin 3) and energy (ferritin light chain, ATP5J) in placental terminal villi.

Key Words: *in vitro fertilization; metabolism; placenta*

Normal functioning of the mother–placenta–fetus system is an essential condition of successful pregnancy development. In infertility of different genesis, assisted reproductive technologies (ART) are used. According to Russian Association of Human Repproduction, more than 15,000 in vitro fertilization (IVF) procedures and 10,000 intracytoplastim sperm injections (ICSI) were performed in 2008. At the same time, the safety and possible complications of ART are still an open question. According to published reports, gestation abnormalities even in singleton induced pregnancy are more often detected than in spontaneous pregnancy [6-8]. Unfortunately, the structural and functional peculiarities of the placenta after ART are poorly studied.

Here we compared the parameters of transmembrane (α-SNAP 23, annexin 3) and energy (ferritin light chain — FLC, STP5J) in placental terminal villi in spontaneous and induced singleton pregnancy.

MATERIALS AND METHODS

We examined 65 women with full-term singleton pregnancy resulting from ART. The mean age of patients was 34.0±2.3 years. Patients with fetal body weight below 2900 g and above 4000 g and patients with severe extragenital pathology in mother and congenital fetal abnormalities were excluded. The history of infertility varied from 2 to 13 years (mean 5.3±2.6 years). In patients with induced pregnancy, secondary infertility predominated (62%). Stimulation of ovulation according to long and short ART protocol was performed in 58 and 42% cases, respectively; 41% patients get pregnant after the first ART cycle. Routine IVF was used in 35 patients and ICSI in 30 patients. The main cause of infertility in the group of IVF was tuboperitoneal pathology (27%) and in the group of ICSI the combination of male and female factors (51%).

The control group comprised 27 women with spontaneous fill-term singleton pregnancy. This group was age-matched with the main group. The main gestation age at birth in IVF and ICSI groups was insignificantly lower than in the control group (38.2±1.3 and 38.2±2.3 weeks *vs*. 39.1±1.7, respectively). The same was noted for birth weight (3060±254 and 3148±301 g *vs*. 3280±352 g, respectively) and placenta weight (485±43 and 502±24 g vs. 548±21 g, respectively).

In the group of patients with induced pregnancy, obstetrical complications occurred more often than in the group with spontaneous pregnancy: threatened

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abortion (47% *vs*. 42%, $p<0.05$), bloody vaginal discharge (32% *vs*. 15%, $p<0.05$), fetoplacental insufficiency (23% *vs*. 18%), and preeclampcia (11% *vs*. 7%). Moreover, peculiarities of placenta attachment were noted: placenta praevia was observed in 2.4% *vs*. 1.4% in the control group ($p<0.05$).

Macroscopic examination of the placenta was performed as described elsewhere [2]. Placenta samples were fixed in 10% neutral formalin. On histological sections stained with hematoxylin and eosin, the maturity of the villous tree and its correspondence to gestation age, the width of intervillous space, the presence and type of compensatory and adaptive processes and pathological changes were evaluated. Immunohistochemical analysis was performed routinely [1] on 3-4-μ paraffin sections using polyclonal rabbit antibodies (Abcam): to SNAP 23 (dilution 1:200), ATP5J (1:250), FLC (1:100), and annexin A3 (1:250). Preliminary antigen unmasking was performed by high-temperature exposure in citrate buffer (pH 6.0) in a Pascal programmed pressure chamber (Dako). Endogenous peroxidase was blocked by incubation with 0.3% hydrogen peroxide for 15 min. Incubation with antibodies was performed at 4°C for 24 h. Dako REAL EnVision detection system (Dako) was used. Harris' hematoxylin was used for background staining. Expression of the specified markers in syncytiotrophoblast and endothelial and mesenchymal cells of placental villi was quantitatively evaluated using an image analysis system on the basis of a Nicon Eclipse 80i microscope with Nis Elements 3.2 software. The quantitative data were processed using Statistica 6.0 software.

RESULTS

Histological study of control placenta preparations stained with hematoxylin and eosin showed that the

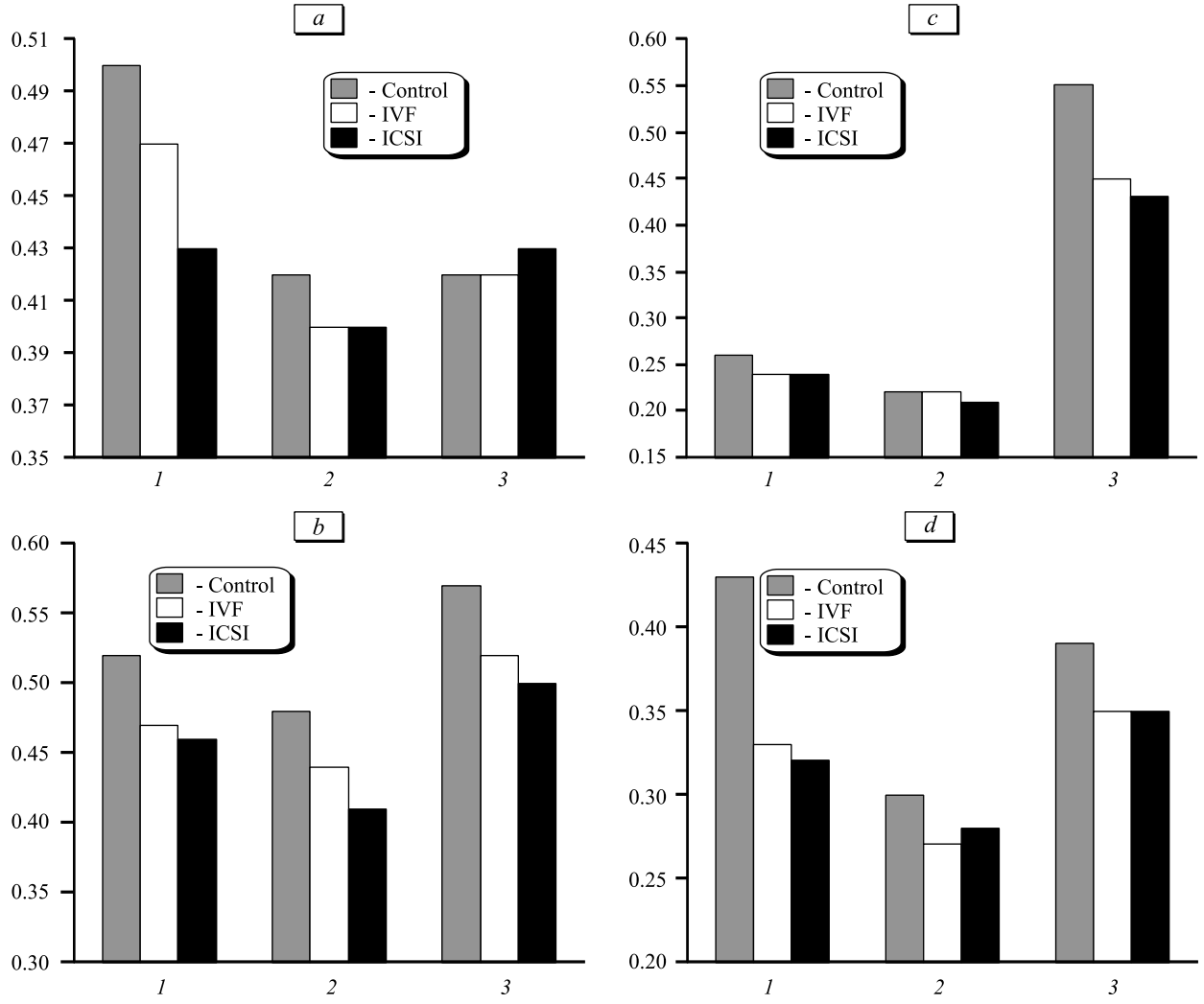

Fig. 1. Changes in the expression of SNAP 23 (*a*), FLC (*b*), ATP5J (*c*), and annexin 3 (*d*) in terminal villi of the placentas in spontaneous and induced singleton pregnancy. *1*) syncytiotrophoblast; *2*) capillary endothelial cells; *3*): mesenchymal stromal cells of villi.

villous tree was primarily presented by terminal villi and to a lesser extent by mature intermediate villi. The structure of the placentas generally corresponded to gestation age. The compensatory and adaptive processes (syncytial knots and syncytiocapillary membranes) were moderately expressed. Fibrinoid depositions (primarily around the truncal villi) and small calcinosis foci were seen near the basal plate.

In the group of women with induced pregnancy, the maturity of the villous tree in most cases corresponded to gestation age. In 11 cases (31.5%) in IVF group and in 9 cases (30%) in ICSI group, delayed maturation (by 2-3 weeks below the gestation age) was observed. Moreover, in 9 placentas after ЭКО (25.7%) and in 5 placentas after ICSI (16.6%), uteroplacental circulation disturbances were detected; they were presented by small infarction foci in the villi and small thrombi of the intervillous space and were primarily located in the paracentral and marginal zones. More abundant peri- and intervillous fibrinoid depositions were seen in 15% cases.

Immunohistochemical analysis revealed differences in the parameters of transmembrane and energy exchange in structural components of terminal placental villi in spontaneous and induced pregnancies (Fig. 1).

The choice of the spectrum of the studied proteins was based on their role in the formation of the fetoplacental complex. An important role in vesicle fusion with the plasmalemma during syncytiotrophoblast formation is played by soluble proteins: NSF (N-ethylmaleimide-sensitive factor) exhibiting ATPase activity and proteins of the SNAP family (soluble NSF attachment proteins). The proteins acting as SNAP receptors (SNARE) are localized in both cytoplasmic membrane (t-SNARE) and membrane of vesicles (v-SNARE). ATP5J, a mitochondrial respiratory chain protein, is a F5 subunit of ATP-synthase. Annexin 3 is a Ca-dependent protein bound to phospholipids and participating in the formation of cell membranes. Moreover, annexin 3 exhibits anticoagulant (due to blockade of phospholipase A2) and angiogenic (via stimulation of the synthesis of vascular endothelial growth factor) properties [9]. FLC, the main iron-containing protein, protects from free radicals and stimulates cell growth and differentiation [4].

In preparations of the placenta obtained after physiological pregnancy, the highest expression of membrane SNAP-23 protein and annexin 3 were seen in syncytiotrophoblast, while FLC and ATP5J in mesenchymal cells. The lowest expression of the studied protein was observed in endothelial cells of terminal villus capillaries. These differences in the expression in different cells are probably determined intracellular localization of these proteins and specialization of placental cells.

In the placentas obtained after induced pregnancies resulting from IVF or ICSI, the expression of the studied markers was less intensive than in placentas obtained after physiological pregnancies. The lowest reaction was detected in the syncytiotrophoblast for annexin 3: they were below the corresponding control levels by 23.3% (IVF) and 25.6% (ICSI). In capillary endothelial cells and mesenchymal cells of terminal villi in placentas obtained after IVF and ICSI, the decrease in annexin 3 expression in comparison with spontaneous pregnancy was statistically insignificant (10 *vs.* 10.3%; 6.7 *vs.* 10.3%, respectively, $p>0.05$; Fig. 2, *a*, *b*).

The maximum changes in ATP5J expression from the control values were detected in mesenchymal cells of terminal villi (Fig. 2, *c, d*): by 18.2% after IVF ($p<0.05$) and by 21.8% after ICSI ($p<0.05$). ATP5J is localized in mitochondria and therefore plays an important role in cell energy supply [10]. Immunohistochemical study showed that its expression in endothelial cells of placental villus capillaries after IVF corresponded to normal level; after ICSI this parameter did not significantly differ from the control.

At the same time, FLC expression in endothelial cells of villous capillaries was reduced (Fig. 2, *e, f*): by 8.3% after IVF ($p<0.05$) and by 14.6% after ICSI ($p<0.05$). In syncytiotrophoblast cells and mesenchymal cells, the level of ferritin after IVF was below the control by 9.6 and 8.8% ($p<0.05$), respectively and after ICSI by 11.5 and 12.3% ($p<0.5$).

Experimental studies showed that reduced expression of FLC is associated with inhibition of cell proliferation. Moreover, the latter become more susceptible to oxidative stress and liable to apoptosis [3]. In our study, FLC expression in syncytiotrophoblast and mesenchymal and endothelial cells was similar in induced in spontaneous pregnancy, which attests to retained protective properties of the placenta and the absence of ART (IVF or ICSI) effects.

We observed only minor changes from physiological parameters in terminal villi for SNAP 23 (Fig. 2, *g*, *h*). This stability of SNAP 23 level attests to high stability of transmembrane exchange in structures of placental terminal villi. Indeed, cytosol proteins of the SNAP family including several proteins with different molecular weight (SNAP 25 and SNAP 23 with molecular weights of 25 and 23 kDa, respectively) participate in pinocytosis and transport between organelles [5]. Its level was significantly below the control by 14% ($p<0.05$) only in syncytiotrophoblast of placental villi is samples obtained after ICSI.

Thus, we revealed some differences in the expression of the studied factors in the placentas from women with induced pregnancy. Significant decrease of the parameters was demonstrated for annexin 3

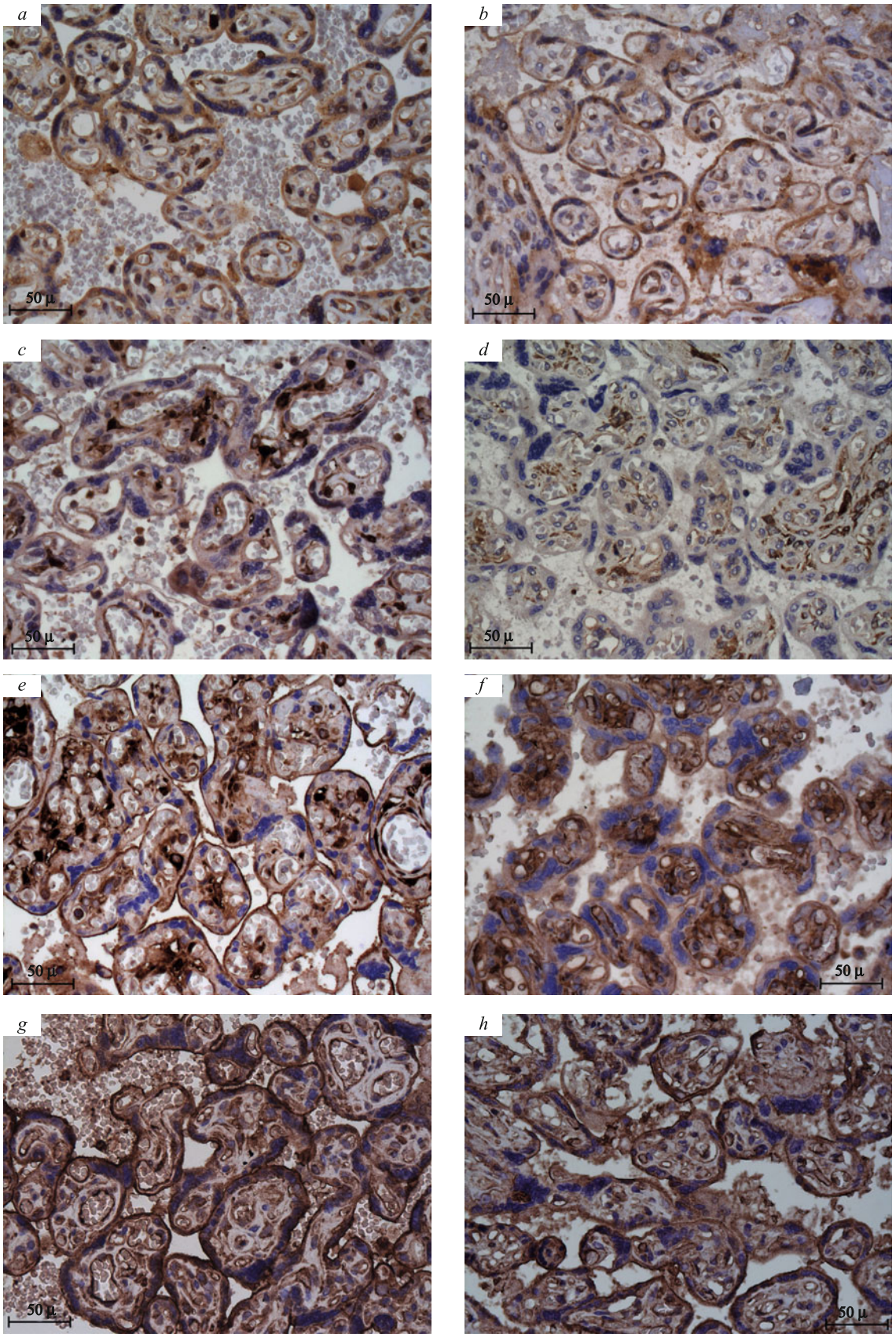

Fig. 2. Expression of markers of transmembrane and energy exchange in cells of placental terminal villi in spontaneous (*a*, *c*, *e*, *g*) and induced (*b*, *d*, *f*, *h*) pregnancy. Immunoperoxidase staining. *a, b)* annexin 3; *c, d*) ATP5J; *e*, *f*) FLC; *g*, *h*) SNAP 23.

and ATP5J, markers of transmembrane and energy exchange. These differences can be determined by peculiarities of the formation and development of pregnancy in women of this group related to initial reproductive disorders, higher incidence of gestation complications, and the effects of ART.

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