Expression of Carbohydrate Metabolism Markers in Full-Term Spontaneous and Induced Pregnancy

N. V. Aleksandrova, E. A. Dubova, O. R. Baev, A. I. Shchegolev, and G. T. Sukhikh

Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 2, pp. 68-72, April, 2012
Original article submitted December 23, 2011

Peculiarities of the expression of glucose transporter (GLUT1 and GLUT3) and insulin-like growth factor immunophenotypes in placental villi in full-term physiological pregnancy were studied by immunohistochemical method. In induced pregnancy, changes of different degree in the expression of carbohydrate metabolism markers were detected (most pronounced changes were detected in GLUT3 expression), which was probably associated with higher incidence of obstetrician complications in these patients.

Key Words: *placental villi; insulin-like growth factor; in vitro fertilization; intracytoplasmic sperm injection; glucose transporters GLUT1 and GLUT3*

Adequate functioning of the mother–placenta–fetus system, the key factor of physiological pregnancy, is characterized by high rate of metabolic processes, in particular carbohydrate metabolism. Glucose is the basic source of carbohydrates for the placenta and the fetus and glucose requirement increases during pregnancy [11]. Since fetal tissues are not able to synthesize glucose *de novo* in sufficient amount, the required glucose level is maintained by its transfer from maternal blood across the placenta, where the major barrier function is performed by the syncytiotrophoblast. It was shown that transplacental glucose transport is mediated by transmembrane proteins, specific sodium-independent glucose transporters (GLUT) [10]. GLUT1 and GLUT3 transporters play the major role: GLUT3 primarily regulates trophoblast/placenta functioning and indirectly affects fetal growth, while GLUT1 primarily affects organogenesis in the embryo and further development of the fetus [6]. Transport capacities of the placenta including functioning of the glucose transporter system largely depend on the expression of insulin-like growth factor (IGF_2) [5]. Glucose transport disturbances in the placenta can be the cause of various obstetrician complications including gestational diabetes, fetal hypoxia, and fetal growth restriction [4].

On the other hand, the number of pregnancies initiated using assisted reproductive technologies (ART) progressively grew in the recent years. *In vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are most frequently used. By now, more than 1 mln children were born after ART, but there is still no consensus on the effect of ART on placental and fetal development [12].

Here we compared the expression of glucose transporters (GLUT1 and GLUT3) and IGF_2 in the terminal placental villi during spontaneous and induced singleton pregnancy.

MATERIALS AND METHODS

Placentas from 65 women (mean age 34.0±2.3 years) with full-term singleton pregnancy after ART were examined. Women without heavy extragenital pathology and developmental abnormalities of the fetus were included in the study. Women with singleton pregnancy developing after reduction of one fetus of the twins were excluded.

Research Center for Obstetrics, Gynecology, and Perinatology, Ministry of Health and Social Development of the Russian Federation, Moscow, Russia. ***Address for correspondence:*** N_aleksandrova@oparina4.ru. N. V. Alexandrova

0007-4888/12/1534-0540

The history of infertility varied from 2 to 13 years (mean 5.3±2.6 years). Stimulation of ovulation in ART program was carried out by the long protocol in 38 cases (58%) and by the short one in 27 cases (42%). Traditional IVF was used in 35 patients and ICSI method was used in 30 patients. The prevailing causes of infertility were the tuboperitoneal factor in the IVF group (9 cases, 26%) and combination of male and female factors in ICSI group (16 cases, 54%). The average number of unsuccessful ART cycles was 2.1±1.4.

The control group comprised 27 women with full-term spontaneous singleton pregnancy (mean age 29.9±4.7 years). The mean gestation age at birth in IVF and ICSO groups was insignificantly lower than in the control group (38.2±1.3 and 38.2±2.3 *vs.* 39.1±1.7 weeks, 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).

Patients with induced pregnancy more often had obstetrician complications than women with spontaneous pregnancy: threatened abortion (47% *vs.* 42%, respectively; $p<0.05$), bloody vaginal discharge (32% *vs.* 15%, $p<0.05$), fetoplacental insufficiency (23% *vs.* 18%; $p<0.05$), fetal growth restriction (16 and 12%; $p<0.05$), and preeclampcia (11% *vs*. 7%). Moreover, peculiarities of placental location were shown, in particular placenta praevia was observed in 2.4% *vs*. 1.4% in the control group ($p<0.05$).

Macroscopic and microscopic studies of the placentas were performed as described earlier [1]. 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 on histological section stained with hematoxylin and eosin.

Immunohistochemical study was carried out on 3-4-μ paraffin sections using monoclonal mouse antibodies to GLUT1 (dilution 1:100) and polyclonal rabbit antibodies to GLUT3 (dilution 1:50) and IGF_2 (dilution 1:100; all antibodies were purchased from Abcam). Preliminary antigen unmasking was carried out by high-temperature processing of the samples in citrate buffer (pH 6.0) in a Pascal programmed pressure chamber (Dako). For blockade of endogenous peroxidase, the sections were incubated with 0.3% H_2O_2 for 15 min. Incubation with the antibodies was carried out at 4°C for 24 h. We used Dako REAL EnVision detection system (Dako) and Harris hematoxilin (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 data were processed using Statistica 6.0 software.

RESULTS

Histological study of control placenta preparations stained with hematoxylin and eosin showed that the 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 most placentas from 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 lagging from the gestation age) was observed. Moreover, in 9 placentas after IVF (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 in spontaneous and induced pregnancy showed different levels of the expression of glucose transporters in structures of placental villi (Figs. 1 and 2). In placenta preparations from women with physiological pregnancy, the highest expression of GLUT1 and GLUT3 was shown in the syncytiotrophoblast and the lowest expression in endothelial cells of capillaries of terminal villi (Fig. 2*, a, c*).

According to published data [9], GLUT1 expression in the placenta was maximum by the end of pregnancy. GLUT1 is localized on the villous and basal membranes. Its concentration in microvilli 3-fold surpasses that on the basal membrane. This asymmetry of GLUT1 expression reflects its transport activity: the basal membrane acts as the rate-limiting step in transplacental glucose transport [2].

In induced pregnancy, the expression of glucose transporters in the placental tissue somewhat differed from the control values (Fig. 1). In the IVF and ICSI groups, GLUT1 expression surpassed the corresponding values in physiological pregnancy (Fig. 2, *b*). However, this difference in the syncytiotrophoblast was only 1.5% in both cases, while in the capillary endothelial cells it was 1.9% after IVF and 7.4% after ICSI.

The expression of GLUT3 in induced pregnancy was lower than in the control group (Fig. 1). After ICSI, the reaction intensity was minimum in mesenchymal cells and capillary endothelial cells of terminal placental villi (by 13 and 12.2% below the control, respectively; $p<0.05$; Fig. 2, *d*). After IVF, GLUT3 ex-

pression in villous endothelial and mesenchymal cells was below the control values by 9.8 and 2.2%, respectively. In the villous syncytiotrophoblast, GLUT3 expression after IVF was higher than in physiological pregnancy by 3.7% and after ICSI it was lower than in physiological pregnancy by 3.7%.

These changes in glucose transporter activity reflect specific features of carbohydrate metabolism in the placenta. *In vitro* experiments showed that GLUT expression on the basal membrane is inversely proportional to glucose concentration in the medium. This leads to GLUT1 translocation from the membrane to the cytoplasm and its inactivation [8]. It should be noted that glucose uptake remains constant at glucose concentrations <15 mmol/liter and only at higher concentrations (20 and 25 mmol/liter) GLUT1 activity decreases [7].

Experiments on sheep showed that maternal hypoglycemia reduced GLUT1 and did not affect GLUT3 in the placenta even under conditions of long-term hypoxia [3]. According to published reports [13], reduced glucose level at the early terms of placenta formation does not affect placental GLUT1, but in late gestation these changes lead to the an increase of GLUT1.

In vivo studies showed that basal expression of GLUT1 increases in pregnant women with diabetes mellitus and decreases under conditions of chronic hypoxia, whereas its level on the membrane of microvilli remains constant. At the same time, basal expression of GLUT1 increases under the effect of IGF_2, placental growth hormone, and hypoxia [2].

Indeed, the major role in the transport of nutrients to the fetus is played by IGF_2, which also controls cell differentiation and metabolism of some hormones (growth hormone, cortisol, insulin, thyroid hormones, and sex hormones). The progeny of *Igf* knockout animals have reduced body weight (by ~60%).

Our previous studies showed that in physiological pregnancy IGF_2 expression in the syncytiotrophoblast is higher than in endothelial cells (Fig. 1, *c*; Fig. 2, *e*). IGF_2 expression in syncytiotrophoblast and villous mesenchymal cells in placentas slightly decreased after IVF (by 3 and 2.9%, respectively) and increased after ICSI (by 2% in comparison with 5.7%; Fig. 2, *f*). The maximum differences in IGF_2 expression were found in capillary endothelial cells of terminal villi: by 20% after ICSI ($p<0.05$); after IVF this parameter corresponded to that in physiological pregnancy (Fig. 1, *c*).

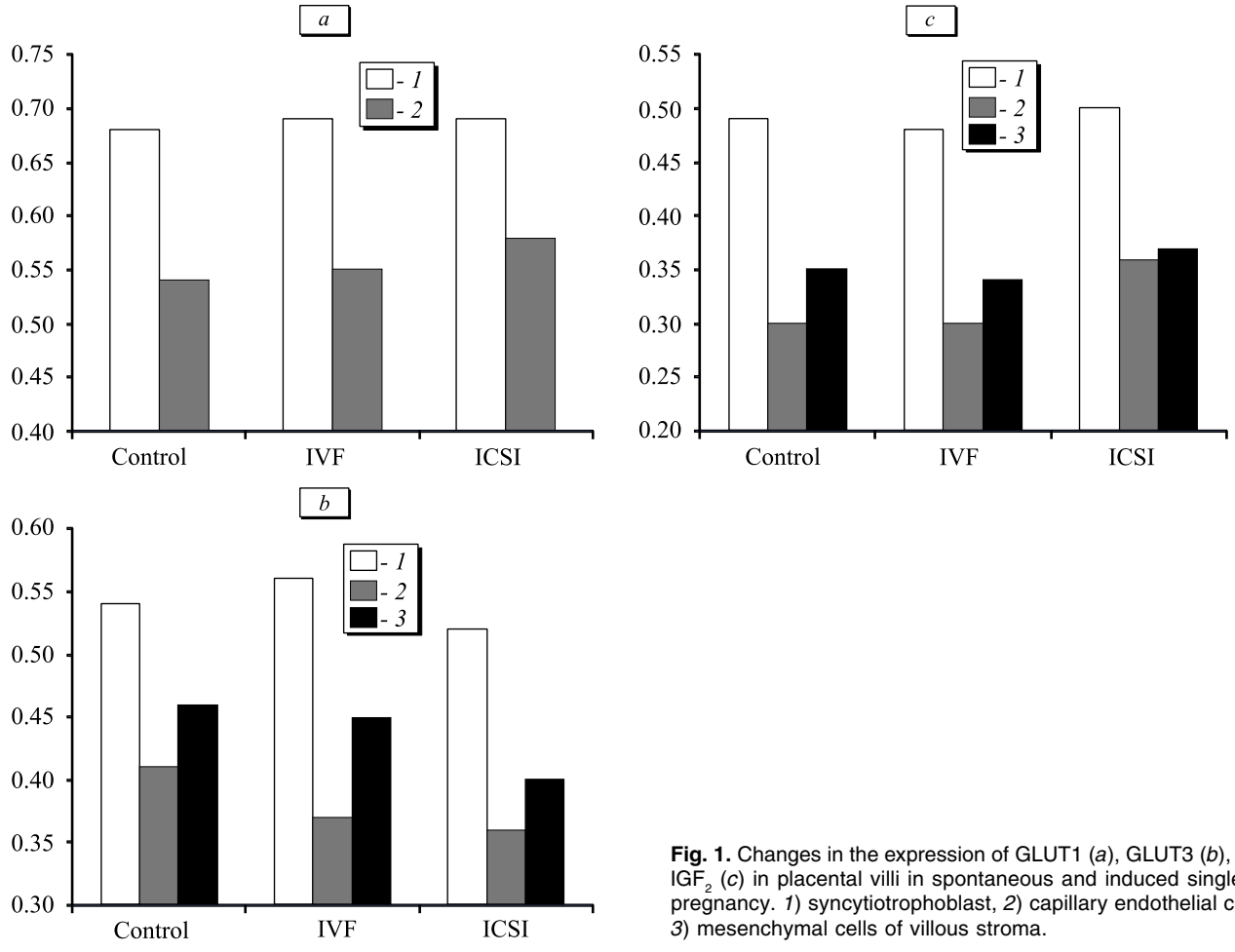

Fig. 1. Changes in the expression of GLUT1 (*a*), GLUT3 (*b*), and IGF_2 (*c*) in placental villi in spontaneous and induced singleton pregnancy. *1*) syncytiotrophoblast, *2*) capillary endothelial cells, *3*) mesenchymal cells of villous stroma.

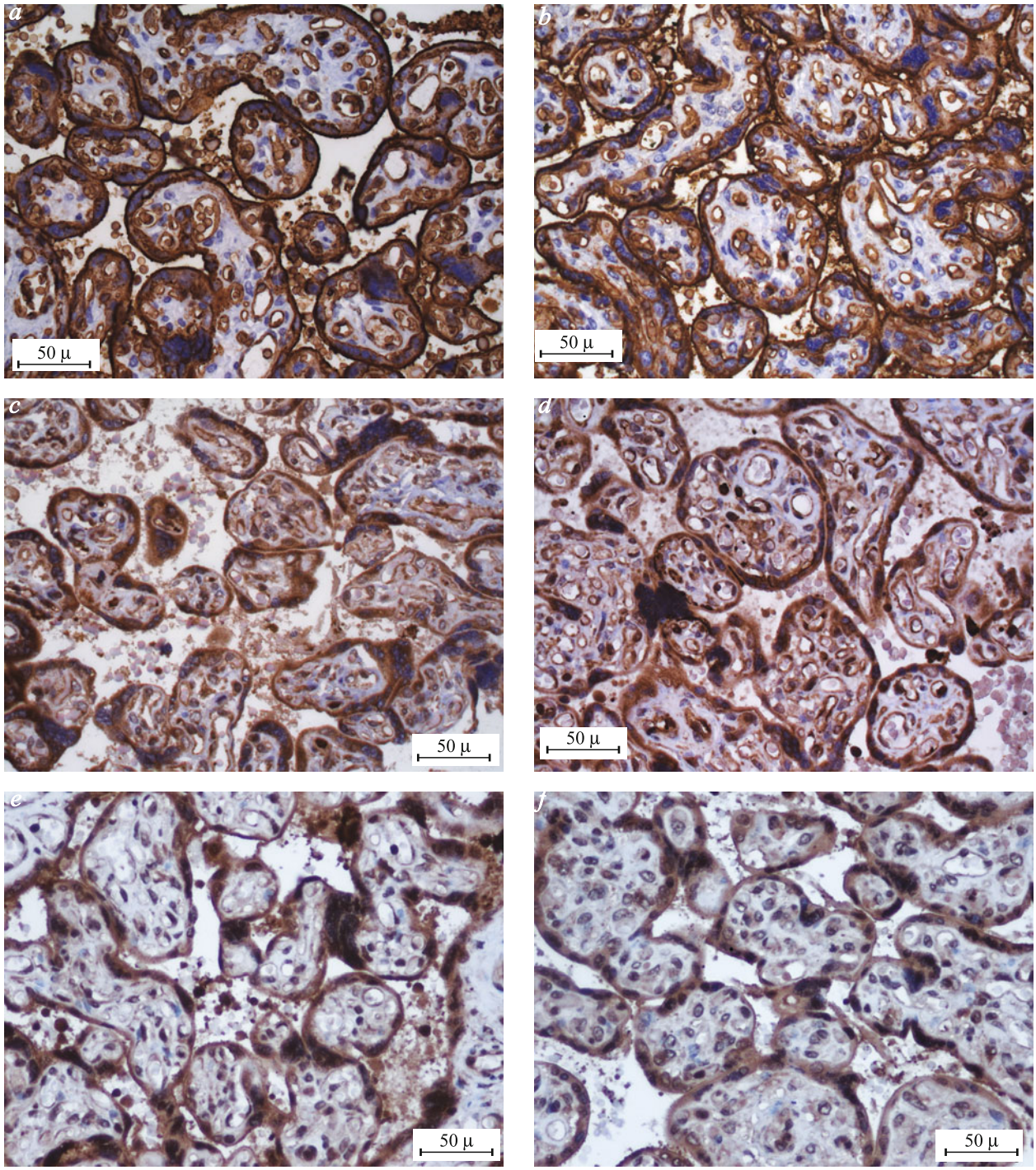

Fig. 2. Expression of carbohydrate metabolism markers in placental villi in spontaneous (*a, c, e*) and induced (*b, d, f*) pregnancy. *a*, *b*) GLUT1; *c, d*) GLUT3; *e, f*) IGF_2. Immunoperoxidase staining.

Thus, immunohistochemical analysis revealed specific features of the expression of glucose transporter immunophenotypes (GLUT1 and GLUT3) in structures of placental villi in full-term physiological pregnancy (maximum expression in the syncytiotrophoblast and minimum in capillary endothelial cells of terminal villi), which corresponds to their functions. In induced pregnancy, differences in the expression of carbohydrate metabolism markers of different degree were revealed (GLUT3 demonstrated maximum changes), which was associated with higher frequency of obstetrician complications and can be determined by the effect of ART on the forming fetoplacental complex.

REFERENCES

1. E. A. Dubova, K. A. Pavlov, and E. I. Borovkova, *Byull. Eksp. Biol. Med*., **151**, No. 2, 218-223 (2011).
2. M. U. Baumann, S. Deborde, and N. P. Illsley, *Endocrine*, **19**, No. 1, 13-22 (2002).
3. U. G. Das, J. He, R. A. Ehrhardt, *et al., Am. J. Physiol*. *Regul. Intern. Physiol.*, **279**, No. 6, R2252-R2261 (2000).
4. G. Desoye, M. Gauster, and C. Wadsack, *Am. J. Clin. Nutr.* **94**, No. 6, Suppl., 1896S-1902S (2011).
5. A. L. Fowden, J. W. Ward, F. P. Wooding, *et al., J. Physiol.*, **572**, Pt. 1, 5-15 (2006).
6. A. Ganguly, R. A. McKnight, S. Raychaudhuri, *et al., Am. J. Physiol. Endocrinol. Metab.*, **292**, No. 5, E1241-1255 (2007).
7. T. Hahn, S. Barth, U. Weiss, *et al., FASEB J.*, **12**, No. 12, 1221-1231 (1998).
8. T. Hahn, D. Hahn, A. Blaschitz, *et al., Diabetologia*, **43**, No. 2, 173-180 (2000).
9. T. Jansson, M. Wennergren, and N. P. Illsley, *J. Clin. Endocrinol. Metab.* **77**, No. 6, 1554-1562 (1993).
10. H. G. Joost and B. Thorens, *Mol. Membr. Biol.*, **18**, No. 4, 247-256 (2001).
11. H. Khan, K. T. Kusakabe, S. Wakitani, *et al., J. Vet. Med. Sci*., **73**, No. 9, 1177-1183 (2011).
12. A. Sazonova, K. Källen, A. Thurin-Kjellberg, *et al., Hum. Reprod*., **26**, No. 10, 2878-2886 (2011).
13. E. Sciullo, G. Cardellini, M. Baroni, *et al., Ann. First. Supersanita.*, **33**, No. 3, 361-365 (1997).