Peroxisome Proliferator-Activated Receptors of Trophoblast Cells in Miscarriage

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

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 152, No. 12, pp. 716-719, December, 2011
Original article submitted August 22, 2011.

Comparative histological study of uterine curettage specimens in missed abortion in women with spontaneous and induced pregnancy and progressing spontaneous pregnancy revealed similar changes in the endometrium and placental villi. Immunohistochemical assay showed enhanced expression of PPARγ and increased proliferation index (Ki-67) in cells of the villous and extravillous trophoblasts in missed abortion.

Key Words: *PPAR; missed abortion; placenta; proliferation; extracorporeal fertilization*

Normal development of pregnancy largely depends on the interaction between the endometrium and trophoblast cells. Disturbances in these processes can result in reproductive losses [9]. Missed abortions constitute 10-20% of all miscarriage cases, of them about 68.6% are first trimester miscarriages. Chromosome abnormalities and autoimmune disorders are the main causes of missed abortions [6].

An important role in the formation of the fetoplacental complex is played by peroxisome proliferator-activated receptors (PPAR) [4,5,11]. PPAR are a group of nuclear receptor proteins that affect transcription factors regulating expression of genes involved into cell division, differentiation, and metabolism [2]. In the placenta, three PPAR isotypes are expressed; PPARγ is primarily expressed on trophoblast cells, which attests to its special role in the differentiation and functioning of the trophoblast [4,5].

Here we performed an immunohistochemical analysis of PPAR expression and proliferation index of trophoblast cells in missed abortion.

MATERIALS AND METHODS

We performed a morphological analysis of uterine curettage samples from patients with missed abortion at gestational age of 6-11 weeks. Group 1 included 28 women (age 23-37 years, mean age 31.7±2.3 years) with missed abortion (MA). Group 2 comprised 32 patients with MA occurring after *in vitro* fertilization (IVF) program; mean age of patients in this group was 35.2 years (27-41 years). The main indications for IVF were tuboperitoneal and endocrine factors. The control group included 34 patients with progressing spontaneous pregnancy comparable by the age and medical history with patients of the study groups and admitted for medical abortion. Preliminary cytogenetic examination of specimens was performed using standard metaphase chromosome analysis; only samples with normal karyotype were included in the analysis.

Histological study was performed on paraffin sections stained with hematoxylin and eosin. Immunohistochemical study was performed routinely using polyclonal antibodies to PPARγ (Abcam; 1:50 dilution; 24 h incubation at 4°C) and rabbit monoclonal antibodies to Ki-67 (Spring Bioscience; clone SP6, ready-to-use, 30-min incubation at room temperature) and a polymer detection system (Spring Bioscience). Preliminary antigen unmasking was performed by boiling in citrate buffer (pH 6.0). Endogenous peroxidase was blocked by incubation with 0.3% hydrogen peroxide for 15 min. Hematoxylin was used for background staining. Expression of PPAR in villous and extravillous trophoblast was evaluated by the percent of immunopositive cells and staining intensity score: weak, moderate, and intensive staining corresponded to 1, 2,

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

0007-4888/12/1526-0768

and 3 points, respectively. Index of proliferation was evaluated by the percent of Ki-67-positive cells. The data were processed using Statistica 6.0 software.

RESULTS

Histological examination of hematoxylin-eosin-stained preparations revealed stereotype changes in all groups. Chorionic villi and fragments of the endometrium with decidual tissue and vessels infiltrated with trophoblast cells and containing large amounts of fibrinoid were seen; these changes attested to intrauterine pregnancy. The villous tree was primarily presented by mesenchymal (embryonic) villi. Elements of embryonic tissues were detected in few cases. In the control group, gestation remodeling of spiral arteries was completed in all cases. In MA, diffuse fibrosis of the stroma and obliteration of villous vessels as well as small calcium depositions near the trophoblast basal membrane in the villous stroma and perivillous fibrinoid depositions were observed. Necrotic foci, hemorrhages, and small aggregates of lymphocytes and plasma cells were seen.

Immunohistochemical analysis of PPARγ expression revealed nuclear localization of the reaction product; this parameter depended on the cell type and patient group (Fig. 1, *a*). In the control group, PPARγ expression was seen only in the nuclei of villous (Fig. 2, *a*) and extravillous (Fig. 2, *b*) trophoblast cell. The number of immunopositive cells in the villous cytotrophoblast 10-fold surpassed that in structures of extravillous trophoblast (4.0 *vs*. 0.4%, $p<0.01$) and did not depend on the gestational age (6-11 weeks).

In patients with MA, considerably enhanced expression of PPARγ was observed in the extravillous trophoblast (Fig. 2, *c*), while in cells of villous cytotrophoblast this increase was less pronounced (Fig. 2, *d*). The highest values were found in women with physiological (spontaneous) pregnancy eventuating in missed abortion: the level of PPAR in extravillous trophoblast surpassed the control values by 19.3 times ($p<0.01$) and in the cytotrophoblast by 112.5% ($p<0.01$). In IVF patients with MA, the content of PPARγ in the corresponding structures also surpassed the control values by 17.7 and by 75.8% ($p<0.01$). However, these values were somewhat lower then in the first group: by 17.3% ($p<0.05$) in cytotrophoblast and by 8.6% ($p>0.05$) in extravillous trophoblast cells.

In the control group, proliferation was more pronounced in tissues of the extravillous trophoblast (Fig. 1, *b*), where Ki-67-immunopositive cells constitute 86.1% (vs. 64.7% in villous trophoblast; Fig. 2, *b*). In MA, the intensity of cell proliferation was higher in comparison with the control: by 8.4% in the extravillous trophoblast (Fig. 2, *e*) and by 5.1% in the villous cytotrophoblast (Fig. 2, *f*; $p>0.05$). In IVF patients with MA, parameters of cell proliferation were reduced by 16.2 and 14.1% ($p<0.05$) in the villous and extravillous trophoblast, respectively.

The major functions of the fetoplacental complex are participation in gas exchange, metabolism, and homeostasis maintenance in the intervillous space. A unique feature of PPARγ is that it participates is all these processes.

Experiments on mice showed that PPARγ blockade lead to irreversible arrest of trophoblast differentiation and therefore disturbances in placenta vascularization [1]. Moreover, the degree of cytotrophoblast invasion was inversely proportional to the degree of PPARγ activation and this effect was dose-dependent [8,14]. Inactivation of PPARγ and PPARb/d in female mice led to pregnancy interruption at terms, when the placenta starts to perform its metabolic function. Morphological analysis of the placentas from these mice revealed delayed devel-

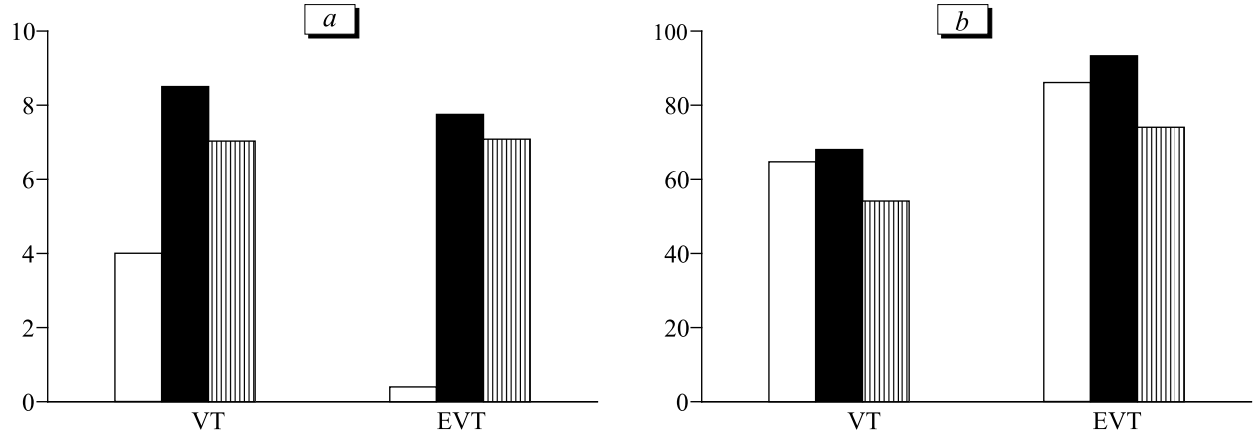

Fig. 1. Expression of PPARγ (*a*) and index of cell proliferation (*b*) for the villous (VT) and extravillous trophoblast (EVT) of placentas in abortion (light bars), MA (dark bars), and MA after IVF (hatched bars). Ordinate: % of immunopositive cells.

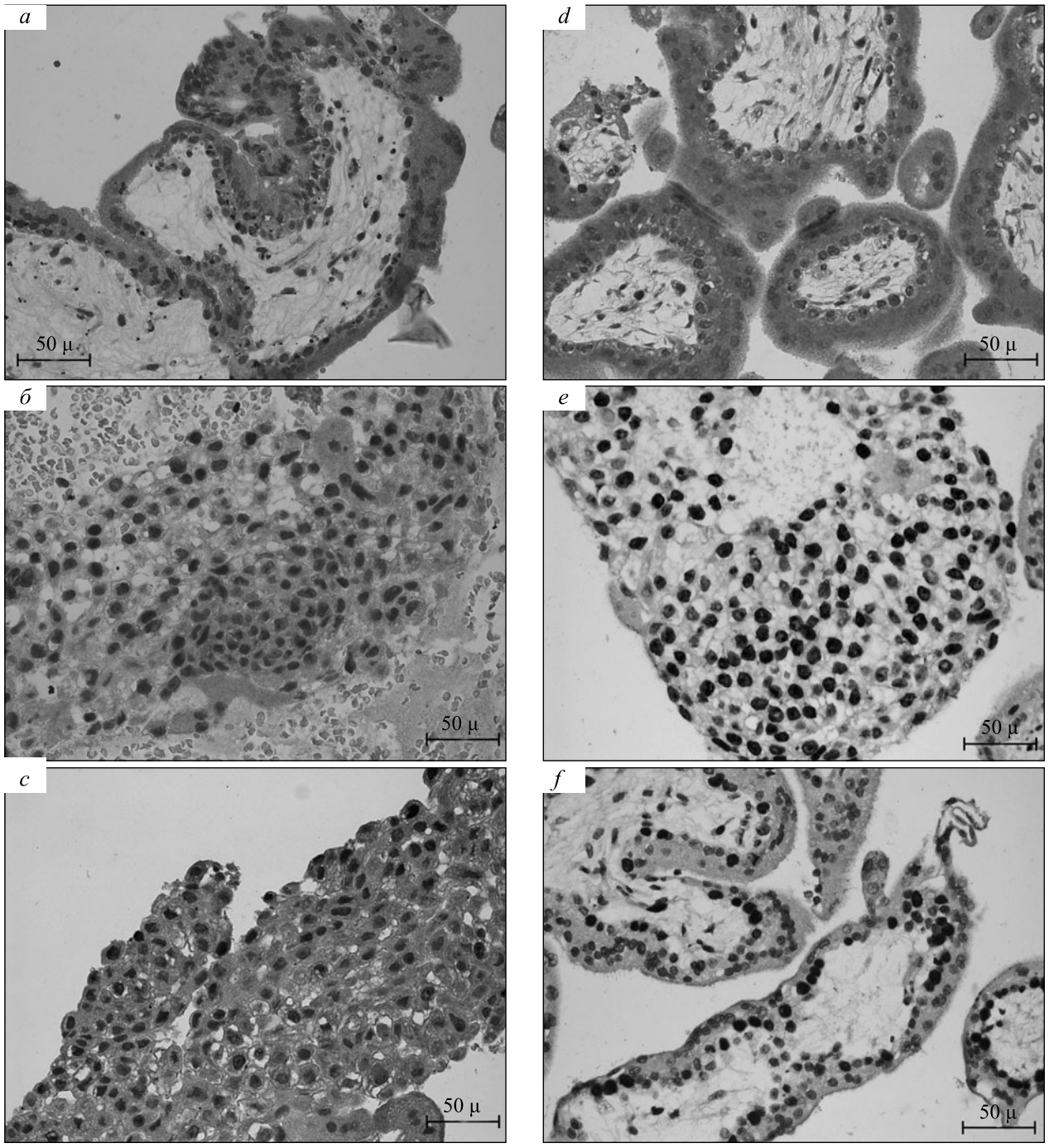

Fig. 2. Expression of PPARγ (*a-d*) and Ki-67 (*e*, *f*) in cells of extravillous trophoblast (*b*, *c*, *e*) and cytotrophoblast of placental villi (*a*, *d*, *f*). Immunoperoxidase staining, ×400.

opment of the trophoblast and the heart of the fetus [4]. In turn, stimulation of PPARγ blocks trophoblast invasion and disturbs differentiation processes in the villous trophoblast [12].

These results generally agree with published data on positive expression of PPARγ in cells of the villous and extravillous trophoblast of human placenta at early terms of gestation [10,15]. At latter terms, PPARγ was detected in anchoring villi and in the amnion and chorion [7,13]. During the third trimester, PPARγ was detected in syncytiotrophoblast [14]. It should be noted that the expression of PPARγ remains stable throughout the pregnancy period and only slightly decreased before delivery [3].

Thus, the considerable increase in PPAR expression detected by immunohistochemical methods re-

flects an imbalance between proliferation and differentiation, which disturbes the formation of the fetus-mother complex. At the same time, the absence of reliable data on proliferation of trophoblast cells in MA in women with spontaneous and induced pregnancy attests to the absence of specific disturbances in implantation and placentation after application of assisted reproductive technologies.

REFERENCES

1. Y. Barak, D. Liao, W. He, *et al*., *Proc. Natl. Acad. Sci. USA*., **99**, No. 1, 303-308 (2002).
2. S. J. Bensinger and P. Tontonoz, *Nature*, Vol. **454**, pp. 470-477 (2008).
3. L. R. Dunn-Albanese, W. E. Ackerman, Y. Xie, *et al., Am. J. Obstet. Gynecol.,* **190**, No. 3, 809-816 (2004).
4. T. Fournier, K. Handschuh, V. Tsatsaris, and D. Evain-Brion, *Placenta*, **28**, Suppl. A, S76-S81 (2007).
5. T. Fournier, V. Tsatsaris, K. Handschuh, and D. Evain-Brion, *Placenta*, **28**, Nos. 2-3, S65-S76 (2007).
6. T. C. Li, M. Makris, M. Tomsu, *et al., Human Reprod. Update,* **8**, No. 5, 463-481 (2002).
7. K. W. Marvin, R. L. Eykholt, J. A. Keelan, *et al., Placenta*, **21**, No. 4, 436-440 (2000).
8. L. Pavan, A. Tarrade, A. Hermouet, *et al., Carcinogenesis*, **24**, No. 8, 1325-1336 (2003).
9. R. Rai and L. Regan, *Lancet*, **368**, 601-611 (2006).
10. V. A. Rodie, A. Young, F. Jordan, *et al*., *J. Soc. Gynecol. Invest.,* **12**, No. 5, 320-329 (2005).
11. W. T. Schaiff, Y. Barak, and Y. Sadovsky, *Mol. Cell. Endocrinol.*, **249**, Nos. 1-2, 10-15 (2006).
12. W. T. Schaiff, M. G. Carlson, S. D. Smith, *et al., J. Clin. Endocrinol. Metab.* **85**, No. 10, 3874-3881 (2000).
13. A. Tarrade, K. Schoonjans, J. Guibourdenche, *et al., Endocrinology*, **142**, No. 10, 4504-4514 (2001).
14. A. Tarrade, K. Schoonjans, L. Pavan, *et al., J. Clin. Endocrinol. Metab.* **86**, No. 10, 5017-5024 (2001).
15. Q. Wang, H. Fujii, and G. T. Knipp, *Placenta*, **23**, Nos. 8-9, 661-671 (2002).