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Method of immunohistochemical identification of extravillous cytotrophoblast in the structures of utero-placental bed and myometrium

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Abstract

In this article, we discover and demonstrate the way of immunohistochemical identification of extravillous cytotrophoblast in the structures of the uteroplacental area and myometrium, which can be used to study the uteroplacental form of placental insufficiency, in the morphogenesis of which the key reason is a violation of the gestational changes of the spiral arteries of the uterus.

Key words: utero-placental bed; extravillous cytotrophoblast; placental lactogen; bcl-2 protein.

Introduction. During pregnancy, a placental bed is formed at the site of attachment of the ovum to the uterus, which is called utero-placental bed (UPB), and the myometrium of the entire uterus significantly increases its volume and changes its spatial configuration, gestational changes in the spiral arteries of the UPB and myometrium [1, 2]. A key mechanism in the morphogenesis of the utero-placental site – cytotrophoblastic invasion

(CTI) - is the process of migration of cytotrophoblast (CT) cells from the placenta to the endometrium and myometrium, where they selectively penetrate into the arteries of the uterus and modify them into dilated vascular channels, which provide the establishment of uteroplacental circulation, which affects the course of pregnancy. Invasive or extravillous CT is divided into following types: interstitial (IC), multinucleated giant cells (MGC) and endothelium-replacing cytotrophoblast (EC) [2, 5].

During cytotrophoblast differentiation, receptors that are specific for CT in the villous epithelium are reduced. EC is necessary for remodeling of large radial arteries, its unique properties are the ability to mimic the phenotype of endothelial cells and by their phagocytosis to be embedded inside the walls of the radial arteries for further gestational transformation. MGCs are localized at the extreme limit of the invasive process in the myometrium and are considered a kind of reserve of CTI, which determines the possibility of compensatory-adaptive reactions in the uteroplacental complex and a favorable termination of pregnancy [3].

Material and methods. 64 biopsies of UPB and myometrium obtained during caesarean section were examined. The term of childbirth was 37-40 weeks. The material was fixed in 10% buffered neutral formalin solution for 24 hours, dehydrated in an ascending battery of alcohols and poured into paraffin. Immunohistochemical procedures with primary antibodies against placental lactogen, bcl-2 protein with peroxidase label imaging and diaminobenzidine were performed on histological sections of standard thickness of 5 µm after dewaxing. Cell nucleus were stained with Grott's hematoxylin.

Digital copies of the image were obtained using a Delta Optical EvoUPBon 100 microscope (planachromatic lenses) and an Olympus SP-550UZ digital camera. Digital images were analyzed in a specialized histological computer program ImageJ (1.48v, free license, W. Rasband, National Institute of Health, USA, 2015), in particular, the optical color density of the immunohistochemical concentration of placental lactogen and bcl-2 protein (in the range from "0" to "1") on the basis of logarithmic transformations of brightness (in gradations from "0" to "255"). The arithmetic mean and its error were calculated, the samples were tested for normality of distribUPBon according to the Shapiro-Wilk criterion, comparisons between study groups were performed according to the odd two-sided Student's test (computer program PAST 3.06, free license, O.Hammer, 2015).

Results. We found extravillous cytotrophoblasts in different positions, all of which are to some extent difficult to identify trophoblasts, especially EC. EC cells flatten and it is almost impossible to distinguish it morphologically from the endothelium, which he replaced,

without any special methods. Because the trophoblast belongs to epithelial cells, there has been an attempt to use polyclonal antibodies to cytokeratins. They gave a positive effect for the EC, which had a cubic shape, but in flat (endothelium-like) forms immunohistochemical cytokeratins were mostly not determined. Because trophoblasts produce specific pregnancy proteins (they are found only in trophoblastic cells), methods for determining these proteins have also been tested. The best results were obtained for placental lactogen (IC - $0,318 \pm 0,0026$, EC - $0,112 \pm 0,0024$ optical density units (ODU)). Thus, ex-extravillous CT was verified by placental lactogen in cubic and flat forms of intravascular and interstitial fractions of CT (Fig. 1).

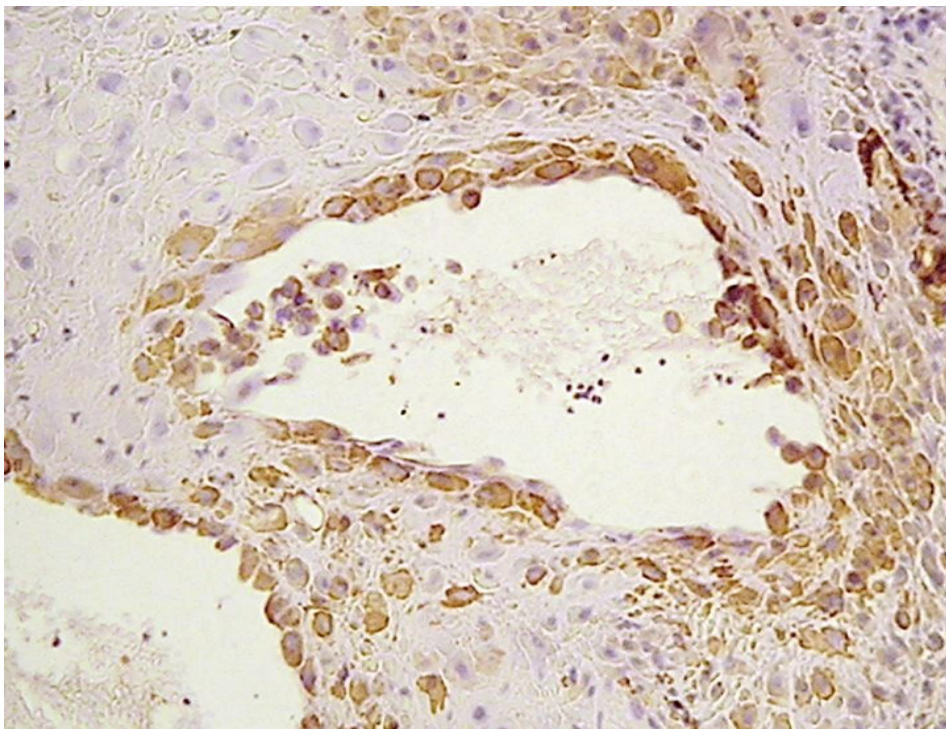


Figure 1 - Immunoexpression of placental lactogen in invasive cytotrophoblast cells of the utero-placental bed at 39 - 40 weeks of pregnancy.

In our opinion, the detection of placental lactogen can be recommended as a way to identify endothelium-replacing CT.

We obtained reliable results when using a method that was not planned for the verification of endothelium-replacing CT (Fig. 2).

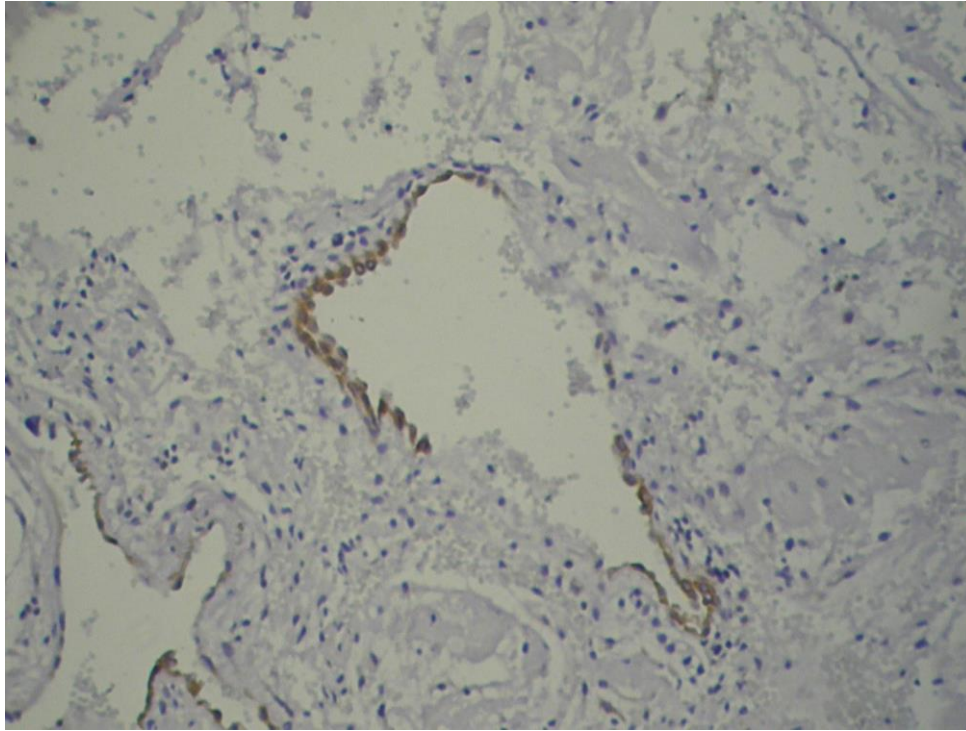


Figure 2 - Immunoexpression of bcl-2 protein in invasive cytotrophoblast cells of the utero-placental bed at 39 - 40 weeks of pregnancy.

In particular, on serial sections of UPB it was noted that the anti-apoptotic protein bcl-2 reliably labeled endothelium-replacing CT, as well as placental lactogen. The extravillous CT, which did not reach the endothelium of blood vessels UPB, was either negative for bcl-2, or weakly positive (IC - $0,111 \pm 0,0014$, EC - $0,189 \pm 0,0018$ ODU).

Conclusions. In our opinion, the most reliable and specific ways to identify endothelium-replacing invasive cytotrophoblast in the vessels of the utero-placental bed is immunohistochemical determination of placental lactogen and bcl-2 protein. These methods can be useful for morphological study of the utero-placental form of chronic placental insufficiency, the key reason of which is the violation of gestational changes of the spiral arteries of the uterus.

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