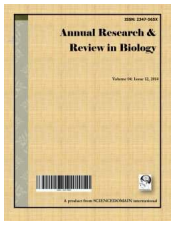

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Development, Degradation and Possible Function of Trabecular Trophoblast in the Course of Placentation of Silver fox *Vulpes fulvus* Desm.

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Authors' contributions

This work was carried out in collaboration between all authors. Author TGZ contributed to design of the study, processed the material, designed and performed statistical analysis, took part in microscopy, data analysis and writing the paper. Author AIZ carried out breeding and selection of wt and domesticated silver foxes, contributed to design of the study concerning two genotypes of foxes, collected the material and discussed the results. Author KMP designed and performed immunohisto-chemistry. Author GIS took part in microscopy and analysis of data. Author IIK took part in design, literature search, data analysis and discussion. Author EVZ contributed to the overall design, literature search, microscopy, analysis of the data, making conclusions and manuscript writing. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Development of trabecular trophoblast cell population in the spongy zone of the endotheliochorial silver fox placenta was investigated with special attention to cytokeratin intracellular arrangement.
Methodology: Cytokeratin immunohistochemistry.
Results: It was shown that keratin filaments contribute to formation of carcass of trabeculae,

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especially in the zone attached to endometrium In the course of placenta development, in the deep part of spongy zone, there occured progressive detachment of the trophoblast cells out of the trabeculae and acquirement of «migratory» phenotype characterized by specific arrangement of the cytokeratin filaments. Another part of differentiated trabecular trophoblast cells undergo progressive destruction of the cytokeratin filaments that results in complete desintegration of cytoplasm. As a result, a zone filled by products of destruction of the trabecular trophoblast cell is formed between trabeculae and labyrinth.

Conclusion: In the silver fox placentation there are several ways of migration of trophoblast cells that play a role in feto-maternal contacts and in histiotrophic nutrition. First, invasion of trophoblast in the depth of uterine glands with partial replacement of glandular epithelium. Second, detachment of the trophoblast cells into the lumen of trabeculae that is accompanied by acquirement of «migratory phenotype» with specific arrangement of cytokeratin filaments. The nutrients that result from degradation of the trabecular trophoblast cell may be uptaken by the labyrinth, i.e. the main site of supply of nutrient and oxigen to embryo.

Keywords: Placenta; carnivores; fox; trophoblast; invasion.

1. INTRODUCTION

Development of the endotheliochorial placenta of Carnivores gives an example of specific invasive behaviour of the trophoblast cells that allow embryo anchoring in the maternal organism without significant modification of the uterine structure. Thus, in Carnivores, the trophoblast promotes a partial degradation of the uterine epithelium and comes in contact with blood vessels without destroying endothelium [1]. Unlike Carnivores, hemochorial placentation in rodents involves complete lysis of the uterine epithelium and a partial degradation and phagocytosis of the endometrial stroma and decidual cells in the sites of embryo implantation. However, the trophoblast induces differentiation of great bulk of maternal decidual cells which together with the giant trophoblast cells form a barrier at the feto-maternal interface [2]. Some trophoblast cells populations invade deeply the uterine wall [3]. In human embryo implantation, trophoblast also destroys uterine epithelium and a part of decidualized endometrium, the rest of it being lined by syncytiotrophoblast interrupted by the sites of chorionic villi anchoring [4,5].

Placenta of many carnivores includes several zones: 1) glandular zone including the intact part of glandular epithelium; 2) junctional zone in which invasive trophoblast cells progressively replace the epithelium; 3) labyrinth or a lamellar part in which syncytiotrophoblast contacts maternal blood vessels thereby providing embryo nutrition and gas exchange [1,6,7,8].

In the recent paper we showed that the trophoblast invading uterine glands in the course of silver fox placentation, progressively replaces the glandular epithelium and forms trabeculae, i.e. folded strata of trophoblast cells attached to the basal membrane [9,10]. In the depth of fetal part of placenta, the trabecular trophoblast cells increase in size, due to their genome multiplication [9]. It should be noted that development of the endotheliochorial carnivore placenta appears to be very complicated. Whereas lamellar zone (labyrinth) formation subjects, most probably, the general laws, the specific functions and behaviour of invasive trophoblast of spongy zone is still poorly understood. In particular, the ways of their differentiation and migration in the fox placenta were the subject of the present paper.

2. MATERIALS AND METHODS

2.1 Materials

Breeding of silver foxes was carried out at the Experimental Fur Farm of the Institute of Cytology and Genetics SB RAS (Novosibirsk, Russia). The wild type silver foxes females mated with males, then the females were kept in the open cages under the roof raised above the ground floor. Each pregnant female was kept in its individual cage. The foxes were kept in a condition of the natural photoperiod, i.e. at the natural lighting corresponding to 18 hours of daily light in February when mating took place. Feeding of foxes was carried out according the special ration adopted in fur farms. According the ration, the amount of meat feed is 50-60% of the total caloric intake being 500 kkal daily; dairy milk food being 5%, grain - 35-40%, vegetables rich in vitamins - 3%, yeast - 5%, fish oil — 2%. The veterinary inspection was carried out daily, different special investigation were performed if

necessary, for example, blood tests from a vein in the hind paw. Animals were sacrificed by electric current (380v) at different stages of pregnancy, i.e. at 18th (No9624-9628), 19th (N0156-160), 20th (No14364-14368), 21st (No4164-4168) and 22nd (No4060-4064) day of pregnancy, i.e. 5 females at each developmental stage. Sacrification of the pregnant females by electric current (380v) allowed by the Bioethic commission of the Institute of Cytology and Genetics SB RAS was made in accordance with the rules developed by the Commission for Bioethics of the State Scientific Center of Russia «Institute of Biomedical Problems», Moskow. Justification numbers of animals previously approved by the Bioethics committee are indicated in parentheses (see above). 2-6 embryos were found and collected in each pregnant female. The number of embryos corresponded to the number of implantation sites, the data agreed with the literature data on silver fox wild type embryogenesis in which embryo lethality is mainly accounted for preimplantation embryo death [11,12].

Implantation sites were fixed with a mixture of ethanol and glacial acetic acid (3:1). The material was embedded in paraffin using standard procedure.

2.2 Methods

The paraffin-embedded material was sectioned into 3 μm thick sections. The slides were deparafinized in xylene (20 min), then in the decreasing concentrations of ethanol aqueous solution - 100% (15 min), 96% (5 min) and and 70% (5 min). The deparaffinized sections were rinsed in 0.6% Tris buffer (pH 7.6) and incubated with bromelin (Biotest) for 15 min at 37℃. Endogenous peroxidase activity was quenched by the 15 min incubation with 3% hydrogen peroxide. Non-specific antibody binding was blocked by incubation for 30 min in rabbit serum diluted 1:20 in 0.6% Tris buffer, 1.5% bovine serum albumin (BSA), pH 7.6. Then these sections were incubated with the primary antibodies Cytokeratin pan (DAKO, cat. N MO82101) diluted 1:300 in 0.6% Tris buffer, 1.5% BSA (pH 7.6), then for 30 min with biotynilated rabbit antimouse antibodies (1:400), and with Streptavidin-peroxidase (1:400). Each step of procedure was followed by three rinses in Tris buffer for 5 min each, with the exception that the primary antibody was applied immediately after the rabbit serum blocking. 3'-3- diaminobenzidine was used as a substrate for peroxidase. Then the slides were rinsed in the bidistilled water, counterstained with hematoxylin and embedded into Canada balm. The slides were examined at the inverted microscope Axiovert 200M with objective lenses 10x/0.30, 20x/0.5 and 40x/0.75. The photos were taken with color CCD camera Leica DFC 420, format 2592x1944.

3. RESULTS

In the fox placenta, the trophoblast trabeculae penetrating the lumen of uterine glands, run radially out of the lumen of the uterine horn toward the allantois (Fig. 1). The trabecular trophoblast cells show tight attachment to each other and to the basal membrane (Figs. 1, 2a). It is reflected in the cytokeratin arrangement. The latter is distributed throughout the cytoplasm though the most dark immunostaining is seen at the cell periphery where it goes over the contours of the tightly attached cells. In this case, cytokeratin filaments may serve a carcass supporting the structure of trabeculae.

Moving to deeper part of trabeculae, there occur changes in arrangement of trophoblast cells as well as cytokeratin distribution in them. A part of trophoblast cells detaches out of trabeculae into the lumen separating trophoblast cell layers (and represents, in fact, the former lumen of uterine glands). The cells undergo several types of changes. A part of cells undergo the features of cells capable of migration: they acquire round or polar shape, having a sprout on one side, the latter sometimes reminds pseudopodia. The distribution of cytokeratin in these cells also changes: they acquire a clear-cut dark perinuclear cytokeratin ring. In some cases, especially in the round cells the whole cytoplasm show dark cytokeratin immunostaining (Figs. 2c, e, f). In other cases, the dark immunostaining is observed mostly perinuclearly and at the cell periphery; the rest of cytoplasm, especially in their sprouts, shows less intensive staining (Figs. 2b, c, f).

Another type of trabecular trophoblast transformation represents a progressive decrease of intensity of the cytokeratin immunostaining (in particular, at the cell periphery) that results in complete degradation of cytokeratin filaments and their disappearance (Figs. 2a-d). The cytoplasmic degradation starts before the cells separate from trabeculae; the process goes on at different distances from the border with the endometrium but more often it is observed in the depth of trabeculae. Here the

process is massive, it covers vast area in the deepest part of trabeculae (Figs. 1, 2b). The nuclei devoid of cytoplasm appear to be large, probably highly polyploid, as well as the small ones. The latter may arise via fragmentation of the polyploid ones — the process characteristic of mammalian trophoblast cells [2] including silver fox [9]. The subdivision of large nuclei into 2-4 small ones starts inside different parts of trabeculae (Fig. 2a) but more often it occurs in their deep parts (Fig. 2b). Thus, in the course of differentiation of trabecular trophoblast, not only genome multiplication but also depolyploidization take place.

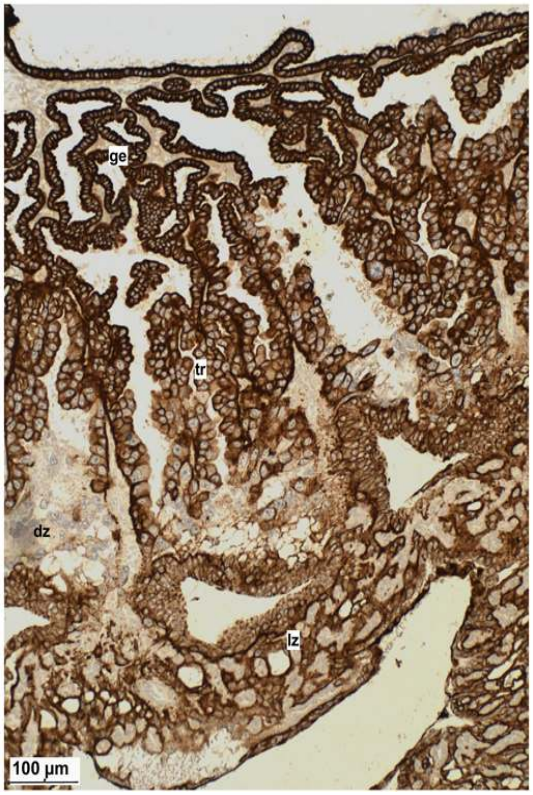

Fig. 1. Silver fox placenta at the 21st day of pregnancy. Trophoblast trabeculae (tr) attach the uterine glandular epithelium (ge). The trophoblast cells in the deep part of placenta show progressive increase in size. The lumen of the uterine glands are countinuous with the folds of trabecules. In the deep part of the fetal part of placenta a vast zone of destruction of trophoblast cells (dz) is observed, it borders the area of labyrinth (lamellar zone, lz) formation. Cytokeratin immunostaining

As a result of above processes, between trabeculae and the zone of labyrinth formation (lamellar zone), a zone filled by weakly cytokeratin-positive extracellular matrix is formed. In this «zone of destruction» nuclei devoid of cytoplasm are scattered - both single and in a form of agglomerations (Figs. 1, 2b, 3). Besides, some amount of trophoblast cells of migratory phenotype are observed in this zone — they show round or polar shape, i.e. having pointy processes or pseudopodia. It suggests that detachment of trophoblast cells from trabeculae and their degradation is a step of their differentiation program. It cannot be ruled out that it is one of the ways of histiotroph nutrition of embryo in silver fox. The nutrients arisen as a result of trabecular trophoblast cell degradation may be uptaken by the labyrinth (syncytiotrophoblast) that is being formed beneath the «destruction zone»; the latter, in its definitive function, is the main site of supply of nutrient and oxigen to embryo.

4. DISCUSSION

Placentation in Mammalia is characterized by trophoblast cell invasion into endometrium and partial lysis of its cells that results in establishing contacts of embryo with blood circulatory system that is necessary for its nutrition and gas exchange. Thus, in the rodent placenta, the primary and secondary giant trophoblast cells lyse completely the endometrial epithelium in the site of its contact with embryo, a part of endometrial stroma also is lysed and phagocytosed [2,13,14]. Endoreduplication allows them to combine the fast growth of the cell population with implementation of their specific functions — establishing contact of maternal organism and embryo and supplying it with blood, as well as development of a barrier between semiallogenic tissues of mother and fetus [2,13,14]. In the hemochorial human placenta, the trophoblast also performs invasion into endometrium, complete lysis of epithelium in the sites of embryo implantation and partial lysis of its stroma, that results in contact of trophoblast with maternal blood circulatory system [4,5]. In the course of differentiation the highly invasive cells of extravillous trophoblast also undergo several rounds of endoreduplication before their invasion in the depth of endometrial stroma and myometrium [13,15,16] though their level of ploidy is much lower than in rodents and rarely exceeds 16c.

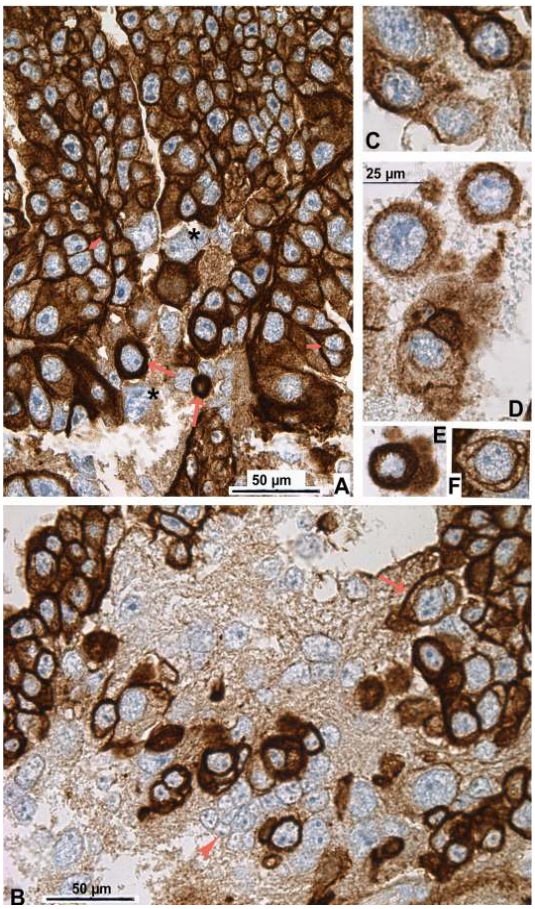

Fig. 2. A portion of the fetal part of placenta remote from the boundary with the endometrium (21st day of pregnancy). A - the majority of trophoblast cells show tight attachments to each other; an intense cytokeratin positivity localizes along the cell contours; a few cells detach from trabeculae (arrows, see also Fig. 3); other cells show decrease of cytokeratin immunostaining (*); the largest cells undergo subdivision into 2 or more nuclei (small arrows); B — the destruction zone; the cytokeratin-positive cells detached from trabeculae show round or polar shape with pointy sprouts; note the predominantly perinuclear immunostaining; the trophoblast cells$_T$ lie among the weakly cytokeratin-positive extracellular matrix. Some cells lose cytokeratin and become devoid of cytoplasm, the latter often form agglomerations (arrowheads); the largest cells undergo subdivision (small arrows) into 2 or more nuclei (A, B); C — the cells at the different stages of destruction; d-f — a clear-cut perinuclear cytokeratin localization in the cells migrating through the destruction zone

The data of the present work demonstrate that in the silver fox placentation there are several ways of migration of trophoblast cells that play a role in feto-maternal contacts and in histiotroph nutrition (Fig. 3). First, invasion of trophoblast in the depth of uterine glands with partial replacement of glandular epithelium. Second, detachment of the trophoblast cells into the lumen of trabeculae, that is accompanied by acquirement of «migratory phenotype» — round or polar shape with specific arrangement of cytokeratin filaments. Perinuclear, ring-like keratin arrangement instead of a widespread, ramified network was demonstrated in the human epithelial tumor cells under the influence of sphingosylphosphorylcholine [17,18]. This reorganization led to increase their capability of migration due to the elasticity of the cells and was accompanied by keratin phosphorylation at K8(S431) and K18 (S52) [17,18]. Third, movement of «migratory» trophoblast cells inside the zone of destruction filled by products of degradation — between trabeculae and labyrinth.

In the beginning of trabecular trophoblast differentiation, the cytokeratin filaments, being a part of desmosomes or hemidesmosomes, ensure contacts between cells and the basal membrane, thereby ensuring integrity of placenta. In the differentiated trophoblast cells arrangement of the intermediate filaments changes. Detachment from trabeculae and acquirement «migratory» phenotype may be a kind of epithelial-mesenchyme transition. It may be due to the plasticity with which cells change their behavior and phenotype in response to cell intrinsic and extrinsic cues is an essential feature of normal physiology [19]. Keratin intermediate filaments rearrangement may play a noticeable role in this transition because they are known to play various roles in cell type–specific functions, such as adhesion, migration, and metabolism This multistep process keeps the cytoskeleton in motion, facilitating rapid and protein biosynthesis–independent network remodeling while maintaining an intact network [20].

Disruption of cytokeratin filaments that precede destruction of cytoplasm is probably a manifestation of apoptosis. Breakdown of cytokeratin filaments as a part of caspase cascade was demonstrated in the course of apoptosis of syncytiotrophoblast of human chorionic villi as a normal physiological process [21,22]. The apoptosis cascade plays a crucial role in cytotrophoblast differentiation into syncytiotrophoblast and controls the turnover of

trophoblast in the human chorionic villi. In the course of pregnancy the syncytiotrophoblast undergo aging by apoptosis, the grown old regions detach from syncytium into the intervillous space filled by maternal blood. Interestingly, the apoptosis starts as early as in the villous cytotrophoblast cells and initiate their fusion with syncytium [21] thereby stimulating renewal of the syncytial layer. The initiation of the caspase cascade comprise induction of apoptosis by activation of initiator caspases. During the execution stage, the execution caspases are activated leading to the destruction of cytoskeletal and nuclear proteins. This step includes, in particular, cleavage of intermediate filaments (cytokeratin) as well as actin microfilaments [22,23]. The final stages include DNA degradation and lead to formation of apoptotic bodies that desquamate into the intervillous space.

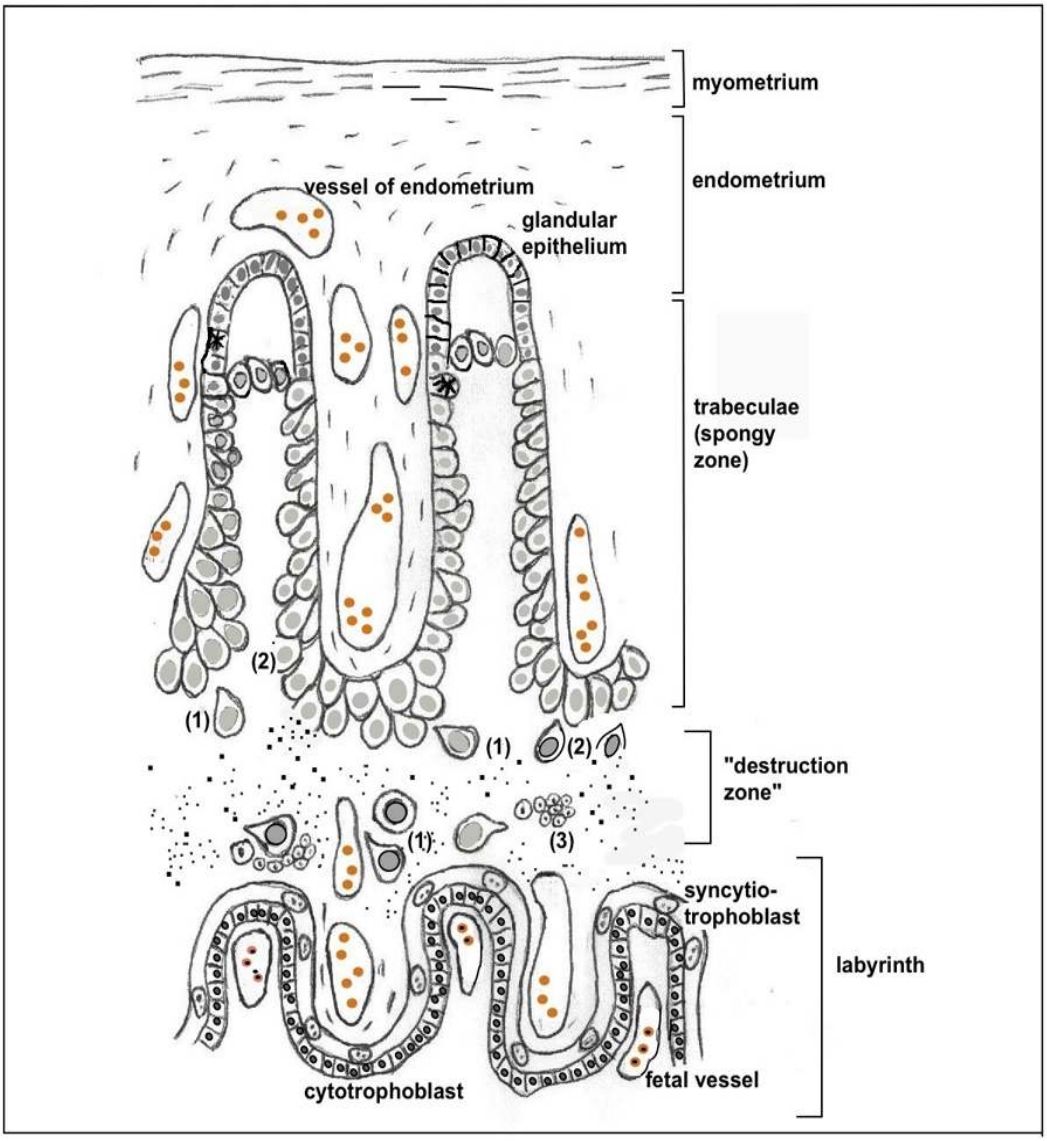

Fig. 3. Scheme of developing placenta of silver fox (20-22 day of pregnancy). Upon moving to deep part of trabecules, trophoblast cells polyploidize and then detach from trabecules (1) or undergo destruction of cytoplasm (2). In the destruction zone formed between trabecules and labyrinth there are trophoblast cells migrating through the extracellular matrix as well as nuclei devoid of cytoplasm — single or agglomerated (3)

It seems to be important that the massive desintegration of trabecular trophoblast cells in the fox placenta takes place in the deep parts of trabeculae, it coincides with cessation of cell cycle progression [10].

The possibility of nutrition of embryo via destruction of trophoblast cells is similar to holocrine secretion. In this case the cells accumulate the necessary substances and then undergo cytoplasm disruption, its content being uptaken by other structures [24,25]. There are some data in literature that holocrine secretion may involve apoptosis [26]. Thus, in the chicken uropygial gland, the secretory and degenerative cells of the luminal epithelium were TUNEL-immunopositive. Besides, these cell leaved cell cycle judging by PCNA-immunonegativity.

In distinct from rodents, the population of giant trophoblast cells in the endotheliochorial placenta of fox does not border the fetal part of placenta but is formed in the depth of it. It cannot be ruled out that it is accounted for by another mechanism of the histiotroph nutrition of embryo in this species. The trabecular trophoblast cells accumulate the nutrients, probably, at the account of lysis of epithelial cells as well as via their own synthetic processes that occur in parallel with fast cell growth accomplished by polyploidization. Then a part of the highly polyploid cells are destroyed thereby forming the zone rich in nutrient; another part of cells detach from trabeculae and move inside this zone; the nutrients are probably uptaken by syncytiotrophoblast of the lamellar zone. Thus, in distinguish from rodents, the histiotroph nutrition of embryo in the fox placenta seems to be realized by by destruction of highly polyploid trophoblast cells rather than by phagocytosis of endometrial tissues.

It should be noted that the above mentioned peculiarities of trophoblast trabecules in the silver fox placenta serve for their function of a barrier at the feto-maternal interface. The trabecular trophoblast cells that border with endometrial epithelium undergo polyploidization like the trophoblast cells in rodents [2], human [15] and ruminants [27,28,29]. Their endoreduplication, i.e. cell growth without nuclear envelope disappearance in mitoses, may minimize mutagenic effect of phagocytosed allogenic trophoblast and endometrial cells in the course of their contacts [7]. In distinct from the hemochorial placenta, the trophoblast cells contacting maternal tissues are mitotically active. It may be accounted for the fact that invasion of these trophoblast cells is restricted by lysis of epithelium: they do not penetrate beyond the basement membrane and do not contact tightly the maternal tissues. Similar regularity was revealed in the epitheliochorial placenta of ruminants: the uterine epithelium stays intact, despite its direct contact with trophoblast. The latter case gives an example of even greater limitation of invasion than in carnivore placenta. In this case, the barrier function of trophoblast also involves polyploidization that may, like in other Mammalia, protect trophoblast cells from some damage from maternal organism. However, they reach genome multiplication via polyploidizing (reduced) mitoses that is possible, probably, due to the minimized trophoblast invasion of endometrium.

5. CONCLUSIONS

In the silver fox placentation there are several ways of migration of trophoblast cells that play a role in feto-maternal contacts and in histotrophic nutrition. First, invasion of trophoblast in the depth of uterine glands with partial replacement of glandular epithelium. Second, detachment of the trophoblast cells into the lumen of trabeculae that is accompanied by acquirement of «migratory phenotype» with specific arrangement of cytokeratin filaments. The nutrients resulting from degradation of trabecular trophoblast cells may be uptaken by the labyrinth, i.e. the main site of supply of nutrient and oxigen to embryo.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Leiser R, Koob B. Development and characteristics of placentation in a carnivore, the domestic cat. J Exper Zool. 1993;266:642-656.
2. Zybina TG, Zybina EV. Cell reproduction

and genome multiplication in the proliferative and invasive trophoblast cell populations of mammalian placenta. Cell Biol Intern. 2005;29:1071-1083.
DOI: 10.1016/j.cellbi.10.015

3. Soares MJ, Chacraborty D, Karim Rumi MA, Konno T, Renaud SJ. Rat placentation: an experimental model for investigation the hemochorial maternal-fetal interface. Placenta. 2012;33(4):233-243.
4. Benirschke K, Kaufmann P. Pathology of the human placenta. 5th ed. Anonymous Springer, NY; 1995.
5. Benirschke K, Kaufmann P, Baergen RN. Pathology of the human placenta. 3rd ed. Anonymous Springer, NY; 2006.
6. Enders AC, Carter AM. What can comparative studies of placental structure tell us? — A review. Placenta. 2004; 25(Suppl. A), Trophoblast Res 18:S3-S9.
7. Miglino MA, Ambrosio CE, dos Santos Martins D, Wenceslau CV, Pfarrer C, Leiser R. The carnivore pregnancy: The development of the embryo and fetal membranes. Theriogenology. 2006;66: 1699-1702.
DOI: 10.1016/j.theriogenology/2006.02.027
8. Furukawa S, Kuroda Y, Sugiyama A. A comparison of the histological structure of the placenta in experimental animals. J Toxicol Pathol. 2014;27:11-18.
9. Zybina TG, Zybina EV, Kiknadze II, Zhelezova AI. Polyploidization in the trophoblast and uterine glandular epitheium of the endotheliochorial placenta of silver fox (*Vulpes fulvus Desm.*) as revealed by the DNA content. Placenta. 2001;22:490-498.
DOI: 10.1053/plac.2001.0675
10. Zybina TG, Pozharisski KM, Stein GI, Kiknadze II, Zhelezova AI, Zybina EV. Placenta development and Ki-67 nuclear immunolocalization in placental tissues of the wild type and domesticated silver fox (*Vulpes fulvus* Desm.). Ann Res Rev Biol. 2015;8:1-12.
11. Belyaev DK, Trut LN, Ruvinsky AO. Genetically determined lethality and possibilities of eliminating its effect in foxes. Genetics. 1973;9(9):71-82. Russian.
12. Belyaev DK. Destabilizing selection as a factor of domestication. J. Hered. 1979;70: 301-308.
13. Zybina T, Zybina E. Cell cycle modification in trophoblast cell population in the course of placenta formation. In: Zgela V, editor. DNA replication and related cellular processes. Rijeka: InTech. 2011;258. Available:http://www.intechopen.com/articles/show/title/cell-cycle-modification-in-trophoblast-cell-populations-in-the-course-of-placenta-formation
14. Zybina TG, Zybina EV. Genome variation in the trophoblast cell lifespan: Diploidy, polyteny, depolytenization genome variation. World J Med Genet. 2014;4(4):77-93.
DOI: 10.5496/wjmg.v4.i4.77
15. Zybina TG, Kaufmann P, Frank HG, Freed J, Kadyrov M, Biesterfeld S. Genome multiplication of extravillous trophoblast cells in human placenta in the course of differentiation and invasion into endometrium and myometrium. I. Dynamics of polyploidization. Tsitologiya 2002;44:1058-1067.
16. Zybina TG, Kaufmann P, Frank HG, Freed J, Kadyrov M, Biesterfeld S. Genome multiplication of extravillous trophoblast cells in human placenta in the course of differentiation and invasion into endometrium and myometrium. II. Mechanisms of polyploidization. Tsitologiya. 2004;46:640-648.
17. Beil M, Micoulet A, von Wichert G, Paschke S, Walther P, Omary MB, et al. Sphingosylphosphorylcholine regulates keratin network architecture and visco-elastic properties of human cancer cells. Nat Cell Biol. 2003;5:803-811.
18. Busch T, Armacki M, Eiseler T, Joodi G, Temme C, Jansen J, et al. Keratin 8 phosphorilation regulates keratin reorganization and migration of epithelial tumor cells. J Cell Science. 2011;125: 2148-2159.
DOI: 10.1242/jcs.080127
19. Muthuswamy SK, Xue B. Cell polarity as a regulator of cancer cell behavior plasticity. Annu Rev Cell Dev Biol. 2012;28:599–625. DOI:10.1146/annurev-cellbio-092910-154244
20. Windoffer R, Beil M, Magin TM, Leube RE. Cytoskeleton in motion: The dynamics of keratin immediate filaments in epithelia. J Cell Biol. 2011;194:669-78.
21. Huppertz B, Frank HG, Kingdom JCP, Reister F, Kaufmann P. Villous cytotrophoblast regulation of the synsytial apoptotic cascade in the human placenta. Histochem Cell Biol. 1998;110:495-508.
22. Huppertz B, Kaufmann P. The apoptosis

cascade in human villous trophoblast. Trophoblast Res. 1999;13:215-242.
23. Miehe U, Kadyrov M, Neumaier-Vagner P, Bartz C, Rath W, Huppertz B. Expression of the actin stress fiber-associated protein CLP36 in the human placenta. Histochem Cell Biol. 2006;126:465-471.
24. Jung Y, Tam J, Jalian HR, Anderson RR, Evans CL. Longitudinal, 3D *in vivo* imaging of sebaceous glands by coherent anti-Stokes Raman scattering microscopy – normal function and response to cryotherapy. J Invest Dermatol. 2014;135: 39-44.
DOI: 10.1038/jid.2014.293
25. Zara FJ, Pereira GRR, Sant'Anna BS. Morphological changes in the seminal receptacle during ovarian development in the speckled swimming crab *Arenaeus cribrarius.* Biol Bull. 2014;227:19-32.
26. Liman N, Alan E. The process of apoptosis in a holocrine gland as shown by the avian uropygial gland. Anat Rec. 2013;296:504-520.
27. Klisch K, Hecht W, Pfarrer C, Schuler G, Hoffmann B, Leiser R. DNA content and ploidy level of bovine placentomal trophoblast giant cells. Placenta.1999;20: 451-458.
28. Klisch K, Thomsen PD, Dantzer V, Leiser R. Genome multiplication is a generalised phenomenon in placentomal and interplacentomal trophoblast giant cells in cattle. Reprod Fertil Dev. 2004;16:301-6.
29. Klisch K, Bevilacqua E, Olivera LV. Mitotic polyploidization in trophoblast giant cells of the alpaca. Cell Tissue Organs. 2005;181: 103-108.

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