Annual Research & Review in Biology
8(6): 1-12, 2015, Article no.ARRB.20235
ISSN: 2347-565X, NLM ID: 101632869

SCIENCEDOMAIN *international*
www.sciencedomain.org

Placenta Development and Ki-67 Nuclear Immunolocalization in Placental Tissues of the Wild Type and Domesticated Silver Fox (*Vulpes fulvus* Desm.)

T. G. Zybina[1*], K. M. Pozharisski[2], G. I. Stein[1], I. I. Kiknadze[3], A. I. Zhelezova[3] and E. V. Zybina[1]

[1]Institute of Cytology RAS, Saint-Petersburg, Russia.
[2]Institute of Radiology and Surgical Technologies, Saint-Petersburg, Russia.
[3]Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia.

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 immunohistochemistry. 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.

Article Information

DOI: 10.9734/ARRB/2015/20235
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Maria De Falco, University Federico II of Naples, Italy.
(2) Jorge Isaac Castro Bedriñana, National University of the Center of Peru, Peru.
Complete Peer review History: http://sciencedomain.org/review-history/12204

Original Research Article

Received 17th July 2015
Accepted 5th October 2015
Published 9th November 2015

ABSTRACT

Aims: To study development of placenta in wild type and domesticated silver fox with special reference to cell cycle progression of the invasive trophoblast cells.
Study Design: Immunohistochemistry with cytokeratin and CD34 primary antibodies to evaluate trophoblast, epithelium and blood vessel arrangement. Semiquantitative evaluation of Ki-67

**Corresponding author: E-mail: zybina@cytspb.rssi.ru, zybina@mail.cytspb.rssi.ru;*

immunolocalization to characterize involvement of the cells in cell cycle during placenta development.
Place and Duration of the Study: Institute of Cytology RAS, St.-Petersburg, Institute of Cytology and Genetics, Novosibirsk, Institute of Radiology and Surgical technologies, Saint-Petersburg, between March 2014 and July 2015.
Methodology: Paraffin-embedded placentae of wild type and domesticated silver fox at the 19-22 day of gestation were sectioned and stained with hematoxylin/eosin and immunostained with standard procedure. Percentage of nuclei of different patterns of Ki-67-immunolocalization was calculated.
Results: Endotheliochorial placenta development in the silver fox *Vulpes fulvus* Desm. includes invasion of endometrial glands by the trophoblast cells. Immunohistochemical Ki-67 reaction demonstrated high incidence of cell cycle progression in the invasive trophoblast cells of spongy zone of placenta at early developmental stages (19 day of gestation) followed by decrease of Ki-67 immunopositivity at the later stages (21-22 days) in both genotypes. Attenuation of Ki-67 expression starts from the loss of labeling of chromatin while labeling of nucleoli persists for a longer time.
Conclusion: Invasive trophoblast cells show high capability of cell cycle progression that attenuate by the time of definitive endotheliochorial placenta development both in wild type and domesticated silver fox placenta.

Keywords: Placenta; trophoblast; carnivores; fox; cell cycle; Ki-67; cytokeratin; CD34.

ABBREVIATIONS

GZ – Glandular Zone
JZ – Junctional Zone
SZ – Spongy Zone
LZ – Lamellar Zone
gd – Gestation Day
wt – Wild Type

1. INTRODUCTION

The endotheliochorial placenta of Carnivores develops by means of penetration of trophoblast cells into the lumen of endometrial glands [1]. Upon the lysis of a part of epithelial lining of endometrium, the trophoblast cells replace the epithelial cells and come in contact to the maternal blood vessels. As a result, the placenta 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 [1,2,3,4,5,6,7,8]. Spongy zone (SZ) composed by trophoblast cells that replaced epithelium, connects the intermediate zone with labyrinth. In some cases it is quite bulky structure [6,7], meantime, its function is not well understood.

It should be mentioned that dynamics of placenta structure formation in carnivores and role of different cell populations are not completely understood; a range of the species characterized in this respect is not wide. Nevertheless, recent investigations [9,10,11,12,13,14] prove that placenta of carnivores is of great interest as a model of studying pathological processes, placental immunity etc. That is why we aimed to study development of placenta structures in a another carnivore, a farmed animal – silver fox – that possess now both a wild type and a domesticated form [15,16]. It was also of interest to compare the two genotypes that differ by their fertility and endocrine status [16,17] that may influence development of reproductive system.

We have previously demonstrated that trophoblast cells of the silver fox, like other animals, undergo genome multiplication [18]. The peculiarities of polyploidization include transition from ordinary mitoses to polyploidizing ones that, in turn, switch to endoreduplication. That is why one of our aims was to study a capability of cell (genome) reproduction in the invasive trophoblast cells in correlation with development of the basic structural units in silver fox placenta.

Ki-67, a protein involved in cell cycle progression is mostly used as a marker of proliferation (see, for example, [19,20]. However, the peculiarities of Ki-67 intranuclear immunolocalization may prove not only involvement in a cell cycle but also supply with the additional information about stages of cell cycle [21,22,23] including possible transition to endoreduplication [24,25]. That is why it seems of interest to estimate the

peculiarities of Ki-67 immunolocalization in the silver fox placenta (wild type and domesticated), in particular, in the invasive trophoblast cells, at the early stages of placenta development.

2. MATERIALS AND METHODS

2.1 Materials

Material of placentae of wild type (wt) and domesticated silver fox at the 19-22 day of gestation was collected at the Experimental Fur Farm of the Institute of Cytology and Genetics SB RAS (Novosibirsk, Russia) where breeding and selection has been carried out for behavioral features of domesticated animals in a period from 1970 to present time [16,17]. Implantation sites of wt foxes with established behavioral phenotypes of domesticated animals were taken for analysis. Placentae of five embryos at each stage of pregnancy, of each genotype were fixed with a mixture of ethanol and glacial acetic acid (3:1). The material was embedded in paraffin using standard procedure.

2.2 Slide Processing and Immuno-histochemistry

A part of paraffin sections were stained with Boemer hematoxylin/eosin, another part undergone immunohistochemical reactions using primary antibodies the Cytokeratin pan (DAKO, cat. N MO82101), Ki-67 (DAKO, cat. N IS626) and CD34 (DAKO, cat. N M716529). The deparaffinized sections were incubated with bromelin for 15 min at 37°C. 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, then these sections were incubated with the primary antibodies 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), 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.

2.3 Microscopy

The slides were examined at the inverted microscope Axiovert 200M with objective lenses 10x/0.30, 20x/0.50, 40x/0.75. The photos were taken with color CCD camera Leica DFC 420, format 2592x1944.

2.4 Statistics

Percentage with standard error of each type of Ki-67 intranuclear immunolocalization was calculated using the formaula $SEp = \sqrt{[p * (1 - p) / n]}$. The margins of error were determined using the confidence level 95%.

3. RESULTS

3.1 Placenta Development

The endotheliochorial placenta of silver fox at the 19-22 gestation day (gd) is developed via cytotrophoblast invasion into the lumen of the superficial part of endometrial glands lined by one-layer columnar epithelium. In the process of trophoblast invasion a part of the epithelium is lysed and replaced progressively by the trophoblast. As a result, the trophoblast forms a system of folded trabeculae penetrating into the depth of the glands (Fig. 1). Trabeculae are separated from each other by layers of the endometrial stroma with the maternal blood vessels (detected by CD34 immunostaining) growing into the embryonic part of placenta toward to invading trophoblast (Figs. 1A-D). In some sites rows of trabecular folds are interspersed by hemophagic areolae lined both by glandular epithelium (from the side of endometrium) and trophoblast cells from the side of fetal part of placenta (data not shown). Beginning from the 21st gd lamellar part of placenta is formed by lamellae of syncytiotrophoblast that surround thin layers of the endometrial stroma with the maternal capillaries whereas from outside it faces pieces of mesenchyme with the fetal capillaries. Beginning from this stage, the basic zones of Carnivore placenta are formed in the silver fox placenta (Figs. 1B, 2E): 1) glandular zone (GZ) representing a part of the superficial zones of endometrial glands that did not undergo the trophoblast invasion; 2) spongy zone (SZ) that consists of large, probably polyploid trophoblast cells forming trabeculae; 3) the above mentioned lamellar zone (LZ) or labyrinth that represents interhemal barrier that ensures embryonic nutrition and gas exchange. Both wt and domesticated fox characterized by the same characteristics of placenta development.

3.2 Formation of the Spongy Zone

Silver fox placenta shows intensive cytokeratin immunostaining both in invasive trophoblast and in the glandular epithelium. The latter differs from

the trophoblast by the clear-cut columnar monolayer in which the cells have approximately the same size and shape; cytokeratin is distributed throughout cytoplasm with especially intensive staining of cytoplasm periphery repeating cell contours (Fig. 1A). High mitotic activity is a characteristic of epithelium. The trophoblast cells of the spongy zone invading endometrial glands, are also cytokeratin-positive with the most intensive staining of cytoplasm periphery. However, in distinct from epithelium, a variety of size and shape of cells is a characteristic of this cell population (Fig. 1A). The oval cells attached to the basal membrane prevail, meantime area of their contact with the basal membrane is more narrow than in epithelial cells, making trabeculae similar to bunches of grapes. From the zone of contact of epithelium and trophoblast toward the chorioallantois the size of trophoblast cell nuclei enlarges gradually that, is, probably accounted for by the polyploidization of trophoblast cells [18]. Simultaneously, at the border with endometrium a great number of mitoses is observed that attenuate at the deeper part of SZ. At the border of trophoblast and epithelium the latter forms overgrowths that disturbs a clear-cut monolayer cell arrangement. In these regions large, goblet-like, most probably, trophoblast cells appear over the epithelium and penetrate between them. Probably, this is a way of trophoblast invasion of endometrium.

The trophoblast cells, as distancing from the epithelium, undergo degradation of the cytokeratin cytoskeleton (Fig. 1A) that suggests that the trophoblast of the spongy zone undergo apoptosis at the final step of their lifespan. All of the above characteristics applies to both the wild type and domesticated animals.

3.3 Immunolocalization of Ki-67

An intensive Ki-67 immunostaining is observed in the trophoblast of the spongy zone as well as in the proliferative areas of the lamellar zone at the 19^{th}gd (Fig. 2A). In the superficial part of glandular epithelium lower incidence of Ki-67-positivity is evident (Fig. 2A), meantime their deep part shows Ki-67-negative epithelium during the whole period studied (Fig. 2A). The growth of the spongy zone at the 19-22 gd is achieved by high mitotic activity as well as polyploidization [18]. The intensive growth of spongy zone trophoblast cell population is connected with high expression of Ki-67 both in the mitotically active zone and in the deep part of SZ in which large, probably polyploid cells do not show mitoses suggesting switch to endoreduplication.

In the lamellar zone that acquire its definitive structure by the 22^{nd} gd, intensive immunolabeling of Ki-67 is observed mostly in the cytotrophoblast (Fig. 2E) that is, most probably, a source of revenue of syncytiotrophoblast, the latter is always Ki-67-negative.

Intranuclear distribution of Ki-67 in silver fox is characterized by some peculiarities as comparaed to the other mammalian species. The most intensive is observed in nucleoli (Figs. 2A-D), besides, rather heavy staining is seen in the condensed chromatin; a weaker staining is observed in the rest of chromatin and caryoplasm. A significant heterogeneity was observed in the patterns of Ki-67 immunolocalization among the SZ trophoblast cell nuclei. By the Ki-67 immunolocalization, there observed several types of nuclei: 1) high immunostaining of nucleoli and chromatin; 2) deep staining of nucleoli and weakly stained or immunonegative chromatin; 3) weak staining of nucleoli and chromatin; 4) completely immunonegative nuclei.

Beginning from 19^{th} day gd progressive decrease of staining was observed in the trophoblast of SZ and areolae in a direction from epithelium to chorioallantois (Figs. 2A-E). It was accompanied by increase of nuclear size accounted for by, most probably, polyploidization. Nevertheless, just in the near-epithelial regions of GZ, there were some amount of small (low-ploid) weakly stained nuclei. And vice versa, in the depth of SZ, there were a number of large, probably highly polyploid trophoblast cell nuclei with deeply stained nucleoli and chromatin. It cannot be ruled out that it reflects differentiation of GZ trophoblast cells into a range of functionally different cell populations with various capability of cell reproduction. Between 19^{th} to 22^{nd} gd progressive attenuation of Ki-67 immunopositivity is observed (Figs. 2A-E) which is the most prominent in the deep area of SZ (Figs. 2B-E). As a result, by the 22^{nd} gd the nuclei of third and fourth type are mostly observed. The nuclei of the second type are also encountered but the nucleolus also shows much weaker immunostaining than at the previous stages of pregnancy.

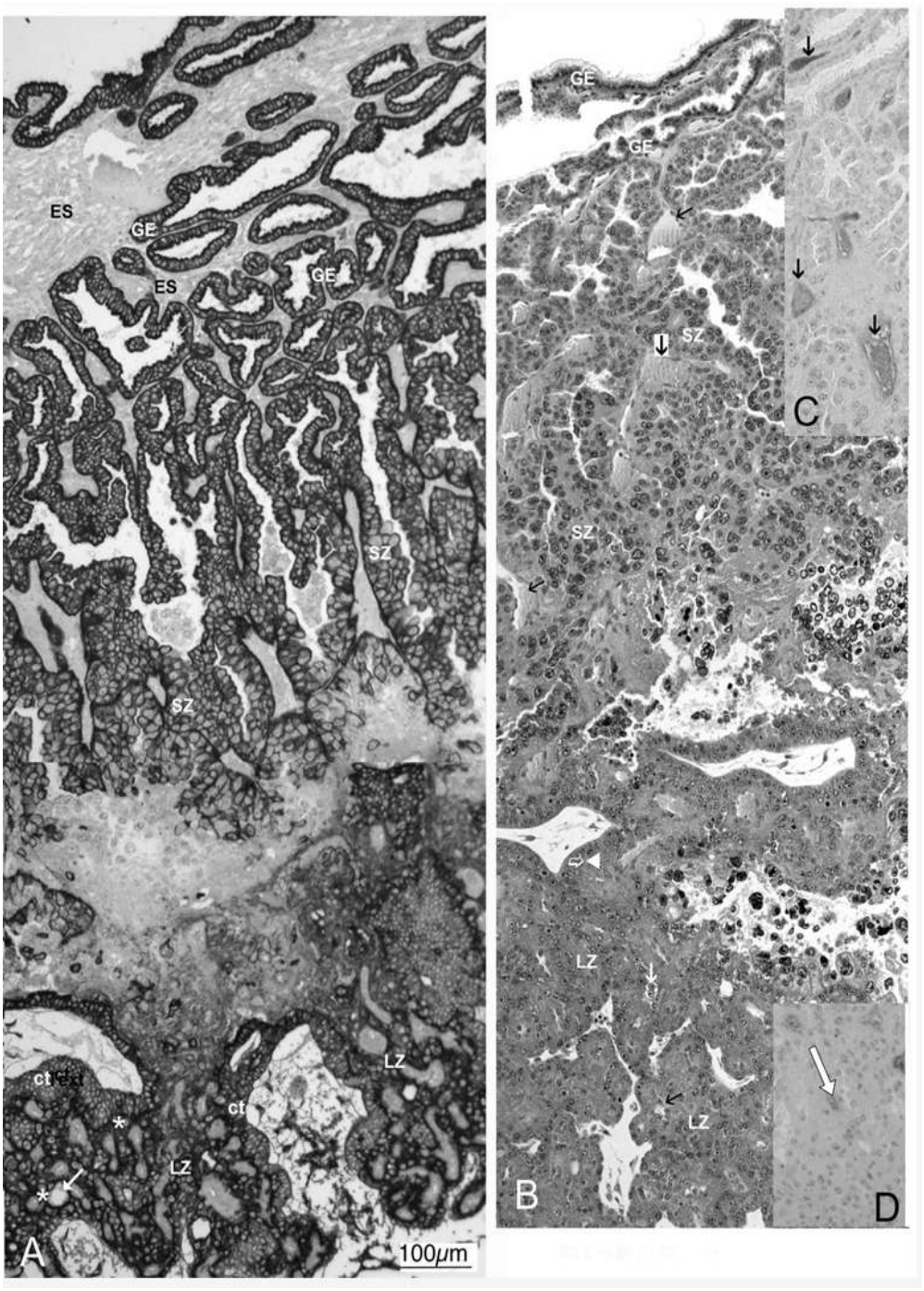

Fig. 1. Silver fox placenta at the 22nd gd. A, B — trophoblast trabeculae of the growing spongy zone (SZ) of placenta penetrate into the lumen of endometrial glands (EG); maternal vessels (arrows) with surrounding endometrial stroma (ES) penetrate into the fetal part of placenta; in the depth of the fetal part of placenta lamellar zone (LZ) is formed in which syncytiotrophoblast (*) surrounds maternal blood vessels (arrows). A — immunolabeling with Cytokeratin Pan; B — hematoxylin/eosin. C, D — CD34 immunolabeling of blood vessels in endometrium in junctional zone (C) and labyrinth (D)

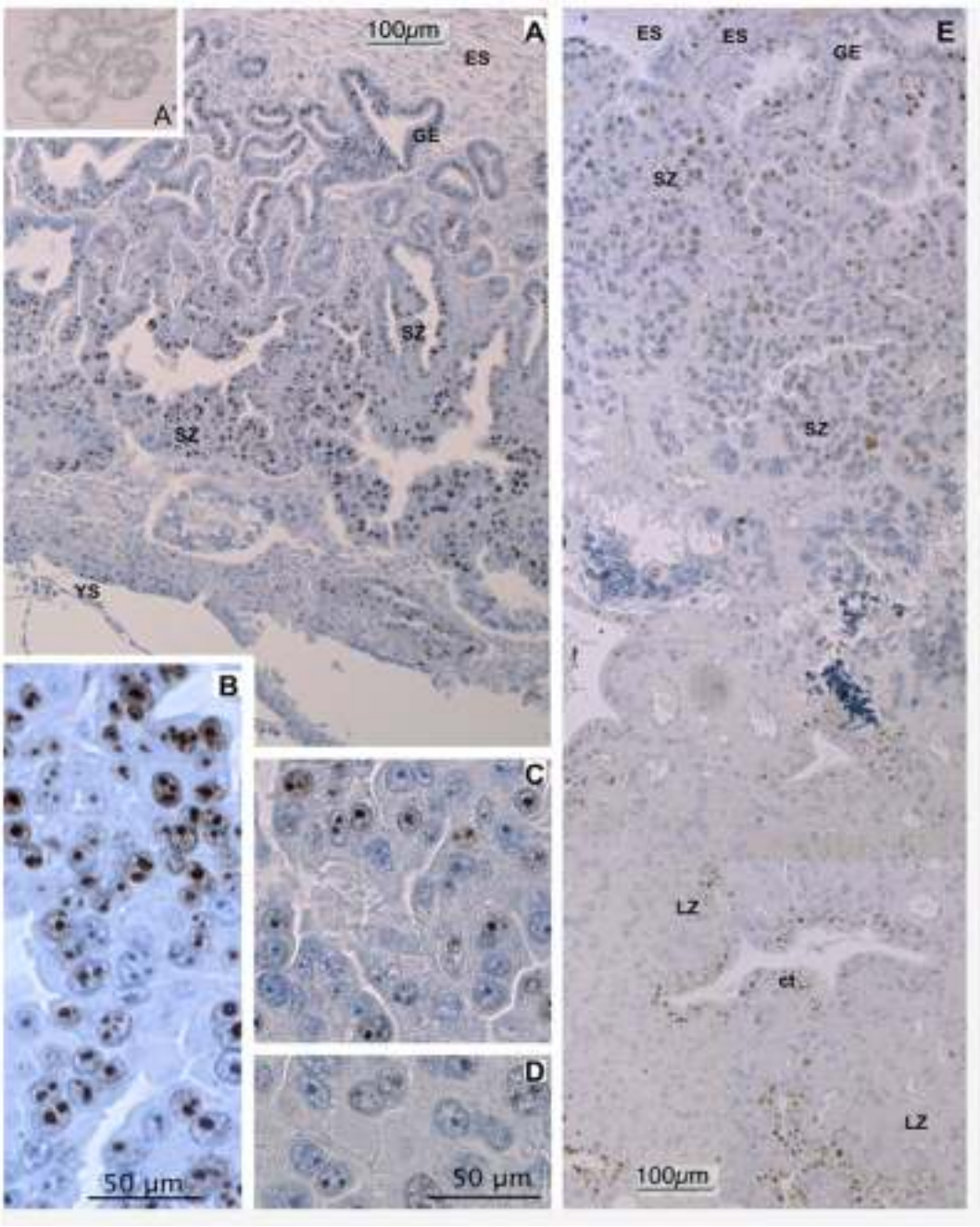

Fig. 2. Ki-67 immunolabeling in the silver fox placenta at the 19th (A, B) and 22nd (C-E) gd in the glandular epithelium (GE), cytotrophoblast of sponge zone (SZ) and lamellar zone (LZ); endometrial stroma (ES) is immunonegative. A' — lack of Ki-67 immunolabeling in the glandular epithelium (GE) in the depth of endometrium. B — in transition to the depth (from top to bottom) of spongy zone (SZ) Ki-67 expression decreases while it persists in many nucleoli. At the 22nd gd Ki-67 immunostaining of SZ trophoblast cells is weak, or absent (C-E), it is restricted mainly by nucleoli - both near endometrium (C) and in the depth of SZ (D) whereas cytotrophoblast (ct) of LZ (E) is strongly Ki-67-immunopositive. YS — yolk sac. Brown — Ki-67 labeling, light blue — hematoxylin counterstaining

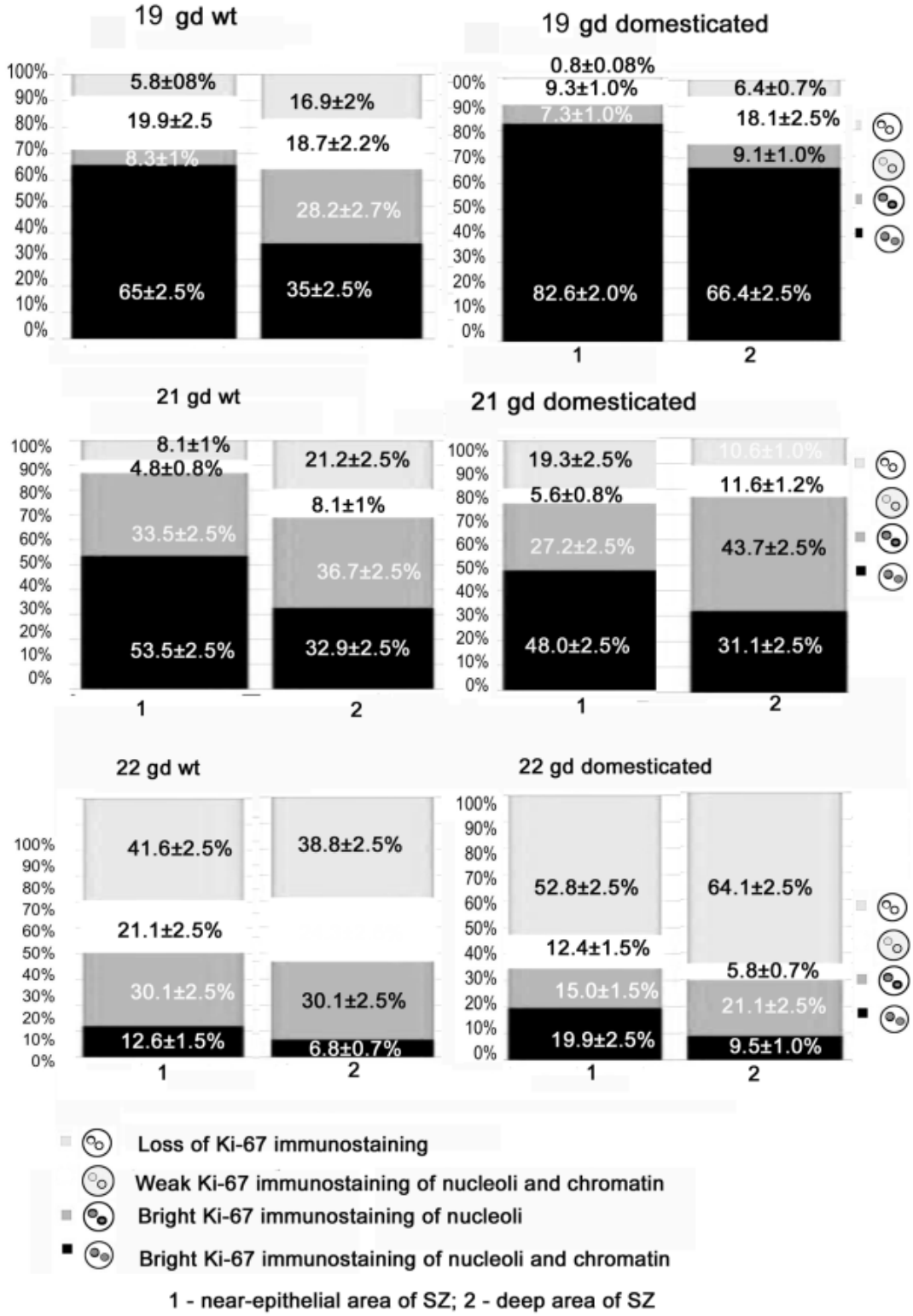

Fig. 3. Diagram showing change in proportions (P<0.05) of nuclei with different types of Ki-67 immunolocalization in SZ trophoblast at the 19-22 gd in placentae of wt and domesticated silver foxes

Fig. 3 above shows the percentage of nuclei of different patterns of Ki-67 immunostaining in the placentae of both genotypes in the near-epithelial and deep areas of SZ at the different developmental stages. According to these data, at the 19th gd a high level of cell cycle progression is observed (both in wt and domesticated foxes). In wt, in the near-epithelial area, 65.6% nuclei are characterized by active immunolabeling of nucleoli and chromatin, 8.3% were with predominantly stained nucleoli, 19.9% were weakly stained and 5% - unstained.

In the depth of SZ, percentage of nuclei with deeply stained nucleoli and chromatin decreased to 36.1%, the other passing to classes of predominantly labeled nucleoli (28.2%), weakly stained (18.7%) and unstained (16.9%) nuclei. It should be mentioned that at this stage placenta of domesticated foxes show higher percentage of deeply stained nucleoli and chromatin, and in this genotype there were not a noticeable difference between near-epithelial and deep areas of SZ in Ki-67 expression. By 21st gd placentae of both genotypes show decrease of the rate of deeply stained nucleoli and chromatin, especially in the deep area of SZ. Decrease of intensiveness of labeling is, most probably, due to the loss of labeling in the chromatin, while maintaining it in the nucleolus (33.5% in wt). In the deep part of SZ, no significant changes of Ki-67 immunostaining was observed as compared to the previous stage excepting some increase of percentage of unlabeled nuclei. Placentae of domesticated foxes did not show a significant difference in Ki-67 expression as compared to wt foxes.

At the 22nd gd, the overwhelming majority of unstained and weakly stained nuclei indicates a tendency of total attenuation of replicative processes in the SZ trophoblast cells. Meantime, high enough percentage of nuclei in which nucleolar Ki-67 labeling persists (30.1%) suggests maintaining of the replicative potential in this cell population.

Attention is drawn to the fact that in the domesticated foxes at this stage the overwhelming majority are the unstained nuclei, the share of nuclei with labeled nucleoli and weak staining is noticeably lower as compare to wt.

4. DISCUSSION

In the silver fox, like in other carnivores, the trophoblast shows more shallow invasion of endometrium as compared to well-studied recently hemochorial placenta of human and rodent [26,27,28,29,30,31,32,33]. In the fox placenta, like in cat, dog, mink and other carnivores, trophoblast cells invade the lumen of endometrium and replaces only a part of epithelium; its distal part is retained during the whole period of pregnancy [1,34]. Besides, according to our study, in the fox placenta an area of epithelium that borders trophoblast retain its proliferative activity thereby, probably, ensuring partial regeneration of the epithelium. In the case of hemochorial placenta, in distinct from carnivore placenta, the sites of placentation are characterized by a local complete lysis of the epithelial lining and its replacement by cyto- or syncytiotrophoblast [26,29,35], moreover, numerous trophoblast cells massively migrate into endometrial stroma and uteroplacental vessels; all this, probably ensures more close contact of trophoblast cells with semiallogenic maternal tissues and blood.

As we demonstrated recently, the trophoblast cells in the fox placenta undergo polyploidization up to 64c-128c [18]. According to the data of the present paper, this period of embryo development is accompanied by high expression of Ki-67 cells that confirms cell cycle progression in the trophoblast cells. In direction from trophoblast-epithelial border to the depth of SZ, increase of size of trophoblast cell nuclei takes place, most probably, via genome multiplication characteristic of fox trophoblast. The largest cells are found in the depth of SZ and hemophagic areolae; the nuclei with signs of non-classic polyteny are encountered here [18]. By contrast, mitotic activity is absent here, that suggests that genome multiplication is achieved via endoreduplication. Ki-67- immunopositivity of the large, probably highly endopolyploid nuclei suggests that replication cycles persists in these cells.

Characteristic of Ki-67 immunostaining indicates some peculiarities of cell cycles progression in the SZ trophoblast cells in silver fox. It has been known that peculiar intranuclear Ki-67 localization is characteristic of different phases of cell cycle. At the early G1-phase Ki-67 is detected mainly in numerous foci of centromere and telomere DNA localization scattered throughout nucleoplasm [22]. At the later G1, S and G2-phase Ki-67 is found mainly inside the well-formed nucleolus as well as in the foci of condensed chromatin [21,36]; and at the transition to mitosis the protein associates mitotic

chromosome arms [22,37,38,23]. In a shortened cell cycle of culture of embryonic stem cells, in late S-phase Ki-67 is restricted by nucleolar localization [39]. In the regenerating liver, at the early G1-phase Ki-67 is scattered throughout nucleoplasm in a form of numerous small granules; at the late G1 and early S-phase Ki-67 is detected as a few large nucleolus-like structures whereas later at the S phase Ki-67 immunolocalization is restricted by nucleoli only [40]. In this case, at the G2-phase Ki-67-positive nucleoli form a united bright discontinuous structure in which prophase chromosome-like entities may be seen [40]. Therefore, Ki-67 cyclic relocalization may coincide with change in the cycle of chromosome condensation that take place during the cell cycle phases. According to the data presented here, a peculiar of fox placenta is more clear-cut intensive immunolabeling of nucleoli found in the overwhelming majority of the trophoblast and epithelial cells as compared to the cells of other mammalian species. This feature seems to be due to species characteristics of the genome the fox. Because of this, cell cycle-specific changes of Ki-67 may be less pronounced in the placental tissues, in particular, in SZ trophoblast cells. At the same time, changes in Ki-67 immunolocalization is connected, most probably, with attenuation of cell cycle progression that may correlate with cell differentiation that takes place in parallel with their polyploidization during placenta development [18]. In this connection attention is drawn to the fact that attenuation of Ki-67 expression starts with the loss of labeling of the condensed chromatin while maintaining it in nucleoli. As preferential nucleolar Ki-67 localization is connected with S-phase, it is another confirmation of endoreduplication, i.e. specific cell cycle in which cell lose mitotic transformations [41]. Besides, it indicates prolongation of the cell cycle as it was found in the giant trophoblast cell of mouse and rat [42,43]. In this connection it may be suggested that endoreduplication have an advantage in cell cycle progression that allows cells to undergo additive rounds of DNA replication at the stage when cell cycles attenuate in a great majority of the cells.

It should be mentioned that the period of endoreduplication cycles in the trophoblast in the course of embryonic development varies between mammalian species. In the secondary giant trophoblast cells in rat placenta the endoreduplication cycles continue during the most of pregnancy – till 18th gd, whole length of pregnancy being 22 days [44,45]. In the low-ploid (mostly 2c-32c) trophoblast cells of the juctional zone of rat and field vole placenta, replication cycles attenuate by the second third of pregnancy, when a part of these cells migrate extensively in the depth of endometrium. This cell population first undergo transition from mitotic cycles to reduced polypoloidizing mitoses, then – to endoreduplication [45,46] in parallel with differentiation into glycogen cells, spongiotrophoblast as well as invasive endovascular and interstitial trophoblast cells [29,31,32,45,46,47]. With the beginning of invasion, their replication cycles stop which precedes the passage of 1-2 cycles endoreduplication. In human placenta, extravillous trophoblast show similar tendency, i.e. transition from mitotic cycles to endoreduplication (1-2 cycles) with subsequent cessation of DNA replication [24,25,46]. According to the data of the present work, in fox placenta a similar sequence of events is observed: mitotic activity is changed by endoreduplication followed by cessation of DNA replication.

Function of the trophoblast cells of SZ in the fox placenta is poorly understood as yet. It is known that these cells including trophoblast cells of hemophagic areolae in the placenta of the dog, hyena, mink, take part in histiotroph and hemotroph nutrition at the initial stages of placenta development [3,4] that indicates the functional similarity of the trophoblast cells of SZ in carnivores with the giant trophoblast cells in rodents (Zybina, Zybina, 2005). An expression of prostaglandins and their receptors was demonstrated both in the superficial area of uterine glandular epithelium and in the trophoblast of intermediate zone including, judging by the presented data, the spongy zone of canine placenta [13,14]. It suggests that trophoblast cells of the intermediate zone are components of physiological barrier [48] between semiallogenic maternal and embryonic tissues of placenta. All above mentioned data indicate that the highly polyploid trophoblast cells of SZ in the silver fox placenta may be analogous of invasive cells of other orders of mammals.

A comparative study showed similar changes of patterns of cell cycle progression in the wild and domesticated foxes in the course of placenta development. Simultaneously, exit from the cell cycle of the majority of SZ trophoblast cells by 22nd gd and the lack of nucleolar persistance of nucleolar immunostaining at this stage in the

domesticated foxes may involve disturbations of replication cycles and result in some functional changes of placenta and embryo development.

5. CONCLUSION

Invasive trophoblast cells show high capability of cell cycle that attenuates progressively by the time of definitive endotheliochorial placenta development both in wild type and domesticated silver fox placenta. Attenuation of Ki-67 expression begins from the loss of binding chromatin while persisting in nucleoli that correlates with the way of trophoblast cell genome multiplication.

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. Pfarrer C, Winter H, Leiser R, Dantzer V. The development of the endotheliochorial mink placenta light microscopy and scanning electron microscopical morphometry of maternal vascular casts. Anat. J. Embryol (Berl.). 1999;199:63-74
3. 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. DOI: 10.1016/j.placenta.2004.01.011
4. Enders AC, Carter AM. Comparative placentation: Some Interesting Modifications for Histotrophic Nutrition – A Review Placenta. 2006;27(Suppl. A, Trophoblast Res 20):S11-S16.
   DOI: 10.1016/j.placenta.2005.10.013
5. 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
6. Aralla M, Groppetti D, Caldarini L, Cremonesi F, Arrighi S. Morphological evaluation of the placenta and fetal membranes during canine pregnancy from early implantation to term. Res Veterinary Sci. 2013;95:15-22.
   DOI: 10.1016/j.rvsc.2013.02.003
7. 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. Ain R, Canham LN, Soares MJ. Gestation stage-dependent intrauterine trophoblast cell invasion in the rat and mouse: novel endocrine phenotype and regulation. Devel Dynam 2003;260:176-190.
8. Favaron P, Morini J, Mess A, Miglino MA, Ambrosio CE. Placentation and fetal membrane development in the South american coati, *Nasua nasua* (Mammalia, Carnivora, Procyonidae). Reprod Biol Endocrinol. 2014;12:57 (online).
   DOI: 10.1186/1477-7827-12-57
9. Fernandez PE, Barbeito CG, Portiansky EL, Gimeno EJ. Intermediate filament protein expression and sugar moieties in normal canine placenta. Histol Histopathol. 2000;15:1-6.
10. Fernandez PE, Diessler ME, Pachame A., HH Ortega, Gimeno EJ, Portiansky EL, Barbeito CG. Reprod Dom Anim. 2014;49: 263-269. DOI: 10.1111/rda.12265
11. Sandowal C, Fisher PJ, Schlafer DH. Characterization of trophoblast cell population by lectin histochemistry in canine placenta during development. J Reprod Fert Suppl. 2001;57:199-206.
12. Jones CJ, Aplin JD, Renfree MB. The fetomaternal interface shows vascular hypoglycosylation in the tammar wallaby Macropus eugenii: Comparison with a range of non-mammalian and eutherian placentae. Placenta. 2013;34:879-84.
    DOI: 10.1016/j.placenta.2013.07.004
13. Kowalewski MP, Bceriklisoy, Pfarrer K, Aslan S, Kindahl H, Kükükaslan I, Hoffman B. Canine placenta: Basourse of prepartal prostaglandin during normal and antiprogestin-induced parturition. Reproduction. 2010;139:655-664,
    DOI: 10.1530/REP-09-0140
14. Gram A, Büchler U, Boos A, Hoffman B, Kowalewski M. Biosynthesis and degradation of canine placental prostaglandins: Prepartum changes in expression and function of prostaglandins F2alpha-synthase (PGFs, AKR1C3) and 15-hydroxyprostaglandin dehydrogenase (HPDG). Biol Reprod. 2013;89:1-12.
    DOI: 10.1095/biolreprod.113.109918

15. Belyaev DK. Domesticated animals. Sci J. 1969;5:47-52.
16. Belyaev DK. Destabilizing selection as a factor of domestication. J Hered. 1979; 301-308.
17. Trut L, Oskina J, Kharlamova A. Animal evolution during domestication: the domesticated fox as a model. Bioessays. 2009;31:349-360.
DOI: 10.1002/bies.200800070
18. 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
19. Polley MC, Samuel, Leung SCY, McShane LM, Dongxia Gao, Hugh JC, Mastropasqua MG etc. An international Ki67 reproducibility study. J Nat Cancer Inst. 2013;105:1897-1906.
DOI: 10.1093/jnci/djt306
20. Zhu X, Yong H, Zhang L, Huang Y, Zheng J, Liu C, Xiu D, Zhang P. Pure alpha-fetoprotein-producing neuroendocrine carcinoma of the pancreas: A case report. BMC Gastroenterol. 2015;12:15-16.
DOI: 10.1186/s12876-015-0246-x
21. Kill R. Localization of the Ki-67 antigen within the nucleolus. Evidence for a fibrillarin-deficient region of the dense fibrillary component. J. Cell Sci. 1996;109: 1253-1263.
22. Bridger JM, KillI R, Lichter P. Association of pKi-67 with satellite DNA of the human genome in early G1 cells. Chromosome Res. 1998;6:13-64.
23. Booth DG, Takagi M, Sanchez-Pulido L, Petfalski E, Varglu G, Samejima K, et al. Ki-67 is a PP1-interacting protein that organises the mitotic chromosome periphery. eLife. 2014;3:e01641.
DOI: 10.7554/eLife.01641
24. Zybina TG, Kaufmann P, Frank H-G, 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. 2002;44: 1058-1067.
25. 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. 2002;44: 1058-1067.
26. Benirschke K, Kaufmann P, Baergen RN. Pathology of the human placenta. 5th ed. Anonymous Springer, NY; 2006.
27. Adamson SL, Lu Y, Whiteley KJ, Holmyard D, Hemberger M, Pfarrer C, Cross J Interaction between trophoblast cells and the maternal and fetal citculation in the mouse placenta. Devel Biol. 2002;250: 373.
28. Hu D, Cross JC. Development and function of trophoblast giant cells in the rodent placenta. Int J Dev Biol. 2010;54:343-354.
DOI: 10.1387/ijdb.082768dh
29. Rai A, Cross JC. Development of the hemochorial maternal vascular spaces in the placenta through endothelial and vasculogenic mimicry. Devel Biol. 2014; 387:131-141.
DOI: 10.1016/ydbio.2014.01.015
30. Ain R, Canham LN, Soares MJ. Gestation stage-dependent intrauterine trophoblast cell invasion in the rat and mouse: Novel endocrine phenotype and regulation. Devel Dynam. 2003;260:176-190.
31. Caluwaerts S, Vercruysse L, Luyten C, Pijnenborg R. Endovascular trophoblast invasion and associated structural changes in uterine spiral arteries of the pregnant rats. Placenta. 2005;26:574-584.
32. Vercruysse L, Caluwaerts S, Luyten C., Pijnenborg R. Interstitial trophoblast invasion in the decidua and mesometrial triangle during the last third of pregnancy in the rat. Placenta. 2006;27:23-33.
33. Konno T, Rempel LA, Arroyo A., Soares M. Pregnancy in the brown Norway rat: a model for investigating the genetics of placentation. Biol Reprod. 2007;76: 709-718.
34. Benircshke K. Comparative placentation. Domestic dog; 2007. Accessed 11 July 2015.
Available:http://placentation.ucsd.edu/homefs.html
35. Abrahamson P, Zorn T. Implantation and decidualization in rodents. J Exper Zool. 1993;266:603-628.
36. Verheijen R, Kuijpers HJ, Schlingemann RO, Boehmer AL, Van Driel R, Brakenhoff GJ, Ramaekers FC. Ki-67 detects a nuclear matrix-associated proliferation-related antigen. I. Intracellular localization

during interphase. J Cell Science. 1989a; 92:123-130.
37. Verheijen R, Kuijpers HJ, Van Driel R, Beck JL, Van Dierendonck JH, Brakenhoff GJ, Ramaekers FC. Ki-67 detects a nuclear matrix-associated proliferation-related antigen. II. Localization in mitotic cells and association with chromosomes. J Cell Science. 1989B;92:531-540.
38. Suurmeijer AJH, Boon ME. Pretreatment in a high-pressure microwave processor for MIB-1 immunostaining of cytological smears and paraffin tissue sections to visualize the various phases of the mitotic cycle. J. Histochem. Cytochem. 1999;47: 1015-1020
39. Becker K, Chule PN, Therrien JA, Lian JB, Stein J, Van Wijnen AJ, et al. Renewal of human embryonic stem cells is supported by shortened G1 cell cycle phase. J Cell Physiol. 2006;209:383-393.
40. Vorotelyak EA, Delone GV, Rippa AL, Uryvaeva IV. Immunohistochemical detection of the proliferation-associated nuclear pKi-67 in the hepatocyte cell cycle. Tsitologiya. 2014;56:648-649. Russian.
41. 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-for
42. Andreeva LF. DNA synthesis and cell population kinetics study in the giant cells and cellular trophoblast of placenta. In: Cell Cycle and nucleic acid metabolism in the course of cell differentiation. Moskow: Nauka; 1964. Russian.
43. Zavarzin AA. DNA Synthesis and Kinetics of Cell Populations in the Mammalian Onthogenesis. Leningrad: Nauka; 1967. Russian.
44. Zybina EV, Zybina TG. Polytene chromosomes in mammalian cells. 1996; 165:53-119.
45. 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
46. Zybina T, Zybina E. Genome variation in the trophoblast cell lifespan: diploidy, polyteny, depolytenization, genome segregation. 2014;4:77-93.
DOI: 10.5496/wjmg.v4.i4.77
47. Coan PM, Conroy N, Burton GJ, Ferguson-Smith AC. Origin and characteristics of glycogen cells in the developing murine placenta. Devel. Dynam. 2006;235: 3280-3294.
48. Bevilacqua E, Hoshida MS, Amarante-Paffaro A, Albieri-Borges A, Gomes SZ. Trophoblast phagocytic program: roles in different placental system. Int J Dev Biol. 2010;54:495-505.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/12204