Villos Cytotrophoblast Turnover. Implications In Forensic Ortology And Pathology Of Gestation

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Abstract: The authors proposed themselves to evaluate the phenotype changes of the structural elements forming placenta villi based on the concept of "cellular turnover". The research has been carried out on first and third trimester of gestation placentas. Based on their personal observations they considered that trophoblast cells maintain its homeostasis by means of proliferation, differentiation and apoptosis, contributing to the formation of syncytiotrophoblast layer during placenta evolution. In pathology, trophoblast proliferation is accelerated by processes accompanied by hypoxia.

Key Words: cellular turnover, cytotrophoblast, phenotype changes, hypoxia

The elaboration of the turn-over general model belongs to March and Simon (1958) and it was largely applied in the study of human resources sociology. The concept of cell turn-over was nominated lately to describe the renewal biologic process of old cells with another new generation. The term "trophoblast cells turnover" was intensely studied and mediatized by Huppertz et al (1998, 1999, 2004).

In the present work we proposed ourselves to evaluate the placenta villous cytotrophoblast turnover by analyzing the microanatomy of the proliferation and apoptosis processes undergone by those cells and their effects.

Materials and methods
The study was achieved on paraffin embedded placenta fragments during the third trimester of gestation – 15 cases. A reference group of 8 placentas was formed of placentas during the first trimester of gestation. The serried sections were stained with Hematoxiline Eosine and Giemsa. The microanatomic imagery was achieved by digital acquisition.

Results
The structural characteristics of human placenta villosities during differentiation and genesis dynamics evaluated by traditional microanatomic methods imposed us to group our personal observations into two categories: A. The micro anatomic analysis of cytotrophoblast relations to the elements inside placenta villosity; B. The micro anatomic analysis of genesis, evolution and relations of syncytial buds and knots.

The micro anatomic analysis of villous cytotrophoblast relations
The examination of the serried sections through placenta fragments during the first trimester

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of gestation, allowed us to identify the “growing buds” at the periphery of the mesenchyme placenta villosities (Fig. 1 A). In some areas we noticed that these growing buds have pedicles that somehow detach in the spaces between villosities (Fig. 1 B). The evaluation of the relations between the trophoblast cells layer (cytotrophoblast) with the subjacent mesenchyme and the superjacent syncytial layer (syncytiotrophoblast) was performed by analyzing with the x40 objective the sections stained with Hematoxiline Eosine and Giemsa. The mesenchyme villosities sectioned under variable angles, contain at its periphery two classic cell layers: an intern layer, cellular (cytotrophoblast) and an external layer, syncytial (syncytiotrophoblast) (Fig. 1 B, C). One can easily notice the existence inside the intern layer of two cellular populations: non differentiated trophoblast cells with proliferative abilities and highly differentiated trophoblast cells with syncytial fusion properties. Equally there are visible numerous apoptotic nuclei inside trophoblast cells (Fig. 1 C, F, G).

Inside the syncytial layer (syncytiotrophoblast) we observed numerous hyper chromatic nuclei with a heterogeneous structure, distributed around placenta villi inside a mass of fibrinoid substance (Fig. 1 C, G). The syncytial layer may be interrupted by isles of proliferated trophoblast cells that contain apoptotic nuclei (Fig. 1 F, G).

**The micro anatomic analysis of syncytial knots’ genesis, evolution and relations**

On the serried sections through third trimester human placenta we identified isles of proliferated trophoblast cells, syncytial conglomerates of apoptotic nuclei and we described the stereo topography of highly differentiated trophoblast cells and their relations to the central villous mesenchyme crossed by sinusoid blood capillaries. When examining with the x40 objective the longitudinal, oblique and transverse sections through terminal villi, we noted its structural heterogeneity due to the reduction of villous mesenchyme, the existence of an eosine fibrinoid band at its periphery, that contains apoptotic nuclei and/or syncytial knots (Fig. 2 A-C). In some regions the nuclei conglomerates burst into the spaces between villi and form syncytial sprouts (Fig. 3 A-E). Highly differentiated trophoblast cells have been observed when examining with the x40 and x63 objectives near the fibrinoid layer around villi and in contiguity relations with sinusoid blood capillaries (Fig. 2 C). In the sectors with fibrinoid substance deposited inside villi creating the appearance of “fibrinoid necrosis”, we spotted conglomerates of apoptotic nuclei (Fig. 4).

**Discussions**

During the last years, the phenotype changes of the human placenta structural elements remained in our attention due to different problems concerning their genesis, evolution and signification during forensic ortology and pathology of gestation, such as: fetal membranes – amnion and chorionic lamina [Dragoi et al, 2009 a]; trophoblast membrane [Dragoi et al, 2009 b]; epigenetic induction in embryo hemi allograft by cytotrophoblast-like stem cells [Dragoi et al, 2010 a] signification in time and space of the fibrinoid substance in the genesis and evolution of human placenta [Dragoi et al, 2010 b]; the organization placenta mesenchyme around vessels [Dragoi et al, 2010 c]; the structural heterogeneity of chorial villi phenotype determind by angiogenesis [Dragoi et al, 2011].

The micro anatomic homeostatic equilibrium of placenta bio system was investigated by many researchers. The trophoblast stem cells are actually implemented in the knowledge and evolution of human placenta. During the development of human placenta, the mononuclear trophoblast cells are resident of anchored or floating chorionic villi.

Inside anchored villi, the trophoblast cells proliferate and invade the endometrium. That migrating cell population is known as “extra villous trophoblast” and plays an important role in the remodeling of endometrium and spiral blood vessels [Aplin, 1991].

During placenta formation, the existence of equilibrium between proliferation, differentiation and apoptosis ensures the homeostasis of trophoblast cells and consequently of normal functions of placenta villi. Numerous studies reported a low apoptotic rate during the first trimester of gestation and many limitations of cytotrophoblast phenotype changes followed by an increase of those processes and the ability to transform trophoblast cells into syncytium [Smith et al, 1997; Gruslin et al, 2001].
Figure 1. Placenta during the first trimester of gestation. Phenotype changes inside mesenchyme villosities. 1. Mesenchyme villosities; 2. Pedicle of the syncytial sprout; 3. Syncytial sprout; 4. Cytotrophoblast layer; 5. Syncytiotrophoblast layer; 6. Trophoblast membrane; 7. Cytotrophoblast proliferation; 8. Highly differentiated trophoblast cell; 9. Low differentiated trophoblast cell; 10. Fibrinoid substance inside syncytiotrophoblast layer; 11. Double layer trophoblast. Paraffin inclusion. Hematoxiline Eosine stain. x 70 (A,D); x140(C); x 280 (B,H,G); x 441 (E).
Figure 2. Placenta during the third trimester of gestation. The location and microanatomic relations of the syncytiotrophoblast conglomerates. 1. Sinusoid blood capillaries; 2. Syncytiotrophoblast; 3. Fibrinoid substance inside the syncytiotrophoblast layer; 4. Syncytiotrophoblast conglomerates; 5. Trophoblast cells nuclei; 6. Apoptotic nuclei in the syncytiotrophoblast layer; 7. Inter villous bridges. Paraffin inclusion. Hematoxiline Eosine stain. x 280 (A,B); x441 (C,D,E).
Figure 3. Placenta during the third trimester of gestation. The location and micro anatomic relations of the syncytial sprouts conglomerates. 1. Sinusoid blood capillaries; 2. Syncytial sprout; 3. Fibrinoid substance inside the syncytiotrophoblast layer; 4. Syncytial conglomerates; 5. Trophoblast cells nuclei; 6. Apoptotic nuclei in the syncytiotrophoblast layer; 7. Inter villous bridges. Paraffin inclusion. Hematoxiline Eosine stain. x 280 (A,B); x441 (C,D,E).
Figure 4. Fibrinoid substance topography around and inside the villosity and its relations to the villous nuclear conglomerates. 1. Floating terminal villosity; 2. Syncytial conglomerate; 3. Syncytial sprout; 4. Trophoblast cells; 5. Sinusoid blood capillaries; 6. Fibrinoid necrosis inside the villosity; 7. Inter villous bridges with nuclear conglomerates; 8. Fibrinoid inter villous bridges. Paraffin inclusion. Hematoxilin Eosine stain. x 140 (C); x 280 (A,D); x 441(B).
After analyzing our observations and studying functional and structural anatomic data from literature, we can conclude that trophoblast cells inside floating villi have the capacity to fuse to form the syncytiotrophoblast layer. We consider that this layer undergoes a continuous renewal by its capacity to form “syncytial knots”.

This dynamic process known as “trophoblast turn-over” is necessary for the formation of syncytiotrophoblast layer that is incapable of regeneration.

During placenta development it is established that apoptosis is associated with trophoblast cells turn-over giving syncytiotrophoblast the possibility to renew itself (Huppertz et al, 1999). It has been accepted that apoptosis is initiated inside villous trophoblast thus facilitating the fusion inside syncytiotrophoblast layer before the extrusion of apoptotic nuclei as “syncytial knots” [Huppertz et al, 1998]. The equilibrium between trophoblast differentiation/fusion and the elimination of old material is maintained in time: 2-4 days for non differentiated cells fusion and 3-4 weeks for the nuclei syncytial fusion and their extrusion [Huppertz and Kingdom, 2004].

The increase of villous cytotrophoblast is remarkable during hypoxia: maternal anemia [Kosanke et al, 1998], mother’s arterial hypertension [Wigglesworth, 1962], preeclampsia [Fox, 1964, 1997] and gestation at high altitude [Mayhew et al, 1990]. The importance of hypoxia for trophoblast proliferation was probed on tissue cultures [Fox, 1964; Castellucci et al, 1990]. Those facts prove the role of oxygen as a regulator of syncytiat proliferation and fusion. During the evaluation of medical legal cases of abortion or pregnancy one can take into consideration the phenotype changes generated by cytotrophoblast turn-over in order to correctly classify the case from biologic and medical point of view.

Conclusions
The concept of “trophoblast cells turnover” opened the path for understanding the phenotype changes inside placenta villi during the last trimester of gestation.

The presence of conglomerates of apoptotic nuclei at the terminal villi periphery, represents a marker for the evolution of trophoblast cells resident around the villous sinusoid capillaries during the last trimester of gestation.

The fibrinoid present around the villities ensures the location for apoptotic nuclei of cytotrophoblast and gives the micro anatomic aspect of syncytiotrophoblast.

The beginning of the phenotype changes undergone by trophoblast cells is determined by apoptosis correlated with the proliferation, differentiation, syncytial fusion and the degeneration of these cells, all these processes being disturbed by hypoxia.

References