ANALELE UNIVERSITĂȚII "DUNĂREA DE JOS" GALAȚI
MEDICINĂ
FASCICULA XVII, no. I, 2011

ORIGINAL STUDY

THE VASCULOGENESIS OF THE FETAL OVARY – MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

Mihaela Niculescu[1], Liliana Novac[1], Garofița Olivia Mateescu[1], Stănescu Relu Mihail[1], Simona Neamțu[1], Anastasia Papachristu[2]

[1]University of Medicine and Pharmacy, Craiova, Romania
[2]Department of Surgery, University Hospital, Atena, Greece

ela07071@yahoo.com

ABSTRACT

The ovarian function is dependent on an intense vascular morphology which includes cyclic phenomena of angiogenesis, angioregression and angiolisis. The vascular morphogenesis goes through the mechanisms of the vasculogenesis and is associated with the differentiation and the ovarian maturation. The literature information concerning the morphological and immunohistochemical characteristics of the ovarian vasculogenesis is quite scarce, that is why we considered this study to be of great importance. The aim of this study is to establish a correlation between vasculogenesis and the differentiation of the ovarian structures with the redefining the morphofunctional unit.

KEYWORDS*: fetal ovary, fetal vasculogenesis, immunohistochemistry, morphofunctional unit.*

1.Introduction

The formation of the blood vessels at the embryo, fetus and then adult involves complex provesses of differentiation, regression and functional remodeling. Angiogenesis is defined as the formation of new blood vessels from preexisting ones. [1]. It is obvious now that angiogenesis plays a key role during folliculogenesis and the formation of the corpora lutea. [2, 3] It is now clearly evident that angiogenesis play a key role in the ovary during folliculogenesis and corpus luteum formation. Angiogenesis is preceeded by vasculogenesis [4]. Vasculogenesis involves the development of the vascular system in the embryo and is caused by in situ differentiation of endothelial progenitors or angioblasts. During development, the expansion capacity of the vasculogenesis process implies the mesenchymal-derived angioblastes should be able to unite in the primitive mesenchyme. The primitive mesenchyme produces VEGF – Vascular Endothelial Growth Factor which induces the formation of hemangioblasts, precursor cells that are common for the blood vessels and the blood cells. [5,6] The first blood isles appear during the third week of

development in the mesoderm that surround the vitelious wall. It is well known that the hemangioblasts located in the centre of the blood isles are responsabile for the creation of the hematopoietics cells, while the peripherical ones differentiate in angioblasts. The angioblasts proliferate and, under the influence of the VEGF they transform into endothelial cells. [7, 8]. These are unified under the action of the same factor, they tuneliasate and transform into primitive blood vessels.

2. Materials and methods

The material used in this study consisted in ovaries (9) with the age between 12 to 24 weeks antepartum, that were obtained through necropsies and therapeutical abortions. This was preceded by the obtaining of an informed consent from every patient and the protocol was aproved by the Ethic Commission of the U.M.F. Craiova. The material used was processed through the classic method of parafine inclusion. The series sections that were formed were usual coloured with hematoxiline-eosine. The immunohistochemical processing was done on serial sections using the classical method ABC (Avidine- Biotin Complex)/ Horse Radish Peroxidase (HRP). A cocktail of primary monoclonal antibodies produced in mice against the human antigenes CD31 (clona JC 70 A, IgG1 Kappa, Dako), CD34 (clona QBEnd 10, IG1 Kappa, Dako) and Von Willebrand factor (clona F8/F6, Ig G1 Kappa, Dako) was used and also, the anti-human monoclonal mouse antibody VEFF (clona VG1, IgG1 Kappa, Dako). The dilution of the primary antibodies was 1:50, while the incubation was made during the night at a temperature of – 4 ^{0} C. The antigenic recovery was established with the help of the microwave heat (HIER) for 12 minutes, 800 W, with pH 6 citrate for the cocktail and Tris-EDTA pH9 in the VEGF case.

The secondary antibody Goat Anti-Mouse (GAM) was used at a dilution of 1: 100 for two hours and then it was amplified with the Avidine- Biotine Complex for a hour. The reaction was developed with diaminobenzidine (DAB) for ten minutes and it was followed by a contracolouring with hematoxilin. In order to verify the immunoreaction, it was used both the external negative control (by leaving out the primar antibody) and the external positive control (sections of renal parenchimous for CD31, CD34, VEGF and from the palatine tonsils level for the VIII Von Willebrand factor).

The acquisition of the images was made from the research microscope Nikon with the help of a video camera Sony through an acquisition board Matrox- Compet and a Pentium-MMX / 233Mhz system.

3. Results

The microscopic study of the histological preparations examined at 12 weeks ovaries, outlined many neoformation capillari vessels, especially immature, difusely spreaded among the ovocites and the primordially folliculis and creating a homogeneous, monomorph aspect. (figure 1).

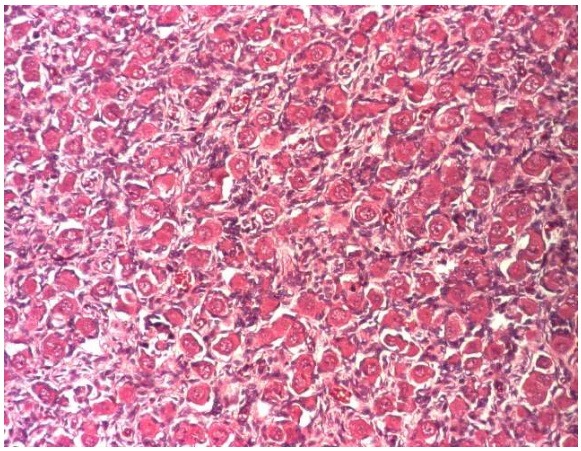

Figure 1. *Fetal ovary 12 S numerous outbreaks of stromal vasculogenesis Ob. 10 Col HE*

At 23-24 weeks ovaries it can be identified the presence of an extended vascular network in the stroma. You can observe mature vessels of arterial and capillar type, hiperplasics, expanded with a turgescent endothelium and obvious pericites. A weak intrafollicular vascular differentiation can be seen at the level of the follicular structures (figure 2).

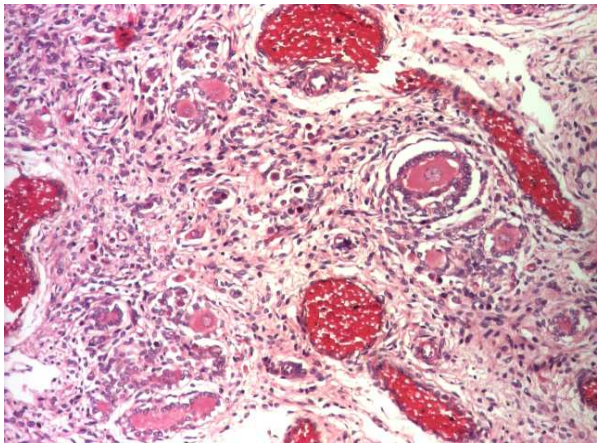

Figure 2. *Fetal ovary 24 s outbreaks of peri vasculogenesis and intrafollicular Ob. 10 Col HE*

The immunohistochemical study of the vasculogenesis process created by a panel of antibodies outlines a positive, intense immunomarking on the capillar endothelial cells which are many especially at the fetal ovary of 12-13 weeks old. In addition to this, it can be observed a positive immunomark on conglomerates of endothelial cells or isolated endothelial cells, separated by microvessels that are adjacent to the primordial folliculi. That is why the VEGF is positive on the capillar endothelial, outlining the immature vessels which present a basal, thin and discontinously membrane in the 12 weeks fetal ovary’s stroma. Also, the VEGF is negative at the primordial foliculli level (figures 3,4). On the doubled coloured sections: CD31/FVIII and CD34/FVIII can be seen frequently interfollicular, immature vessels. At the 23-24 weeks old ovaries, it can be identified a weakly positive immunomarking on the capillar adjacent to the primordial foliculli. It is also outlined immunohistochimically the development of a perifollicular capillar network (figures 5,6).

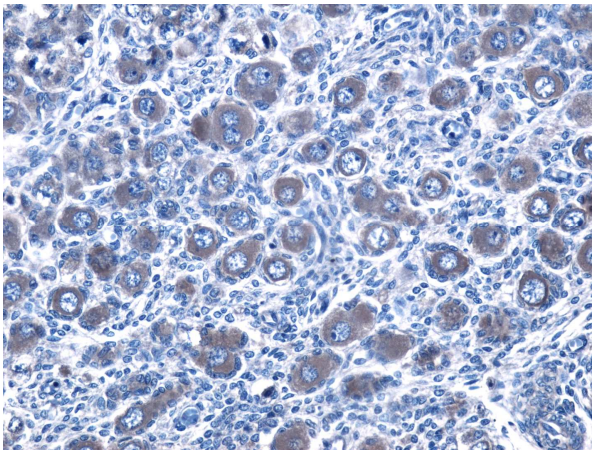

Figure 3. *Fetal ovary 12 s VEGF slightly positive in primordially folliculi Ob.10*

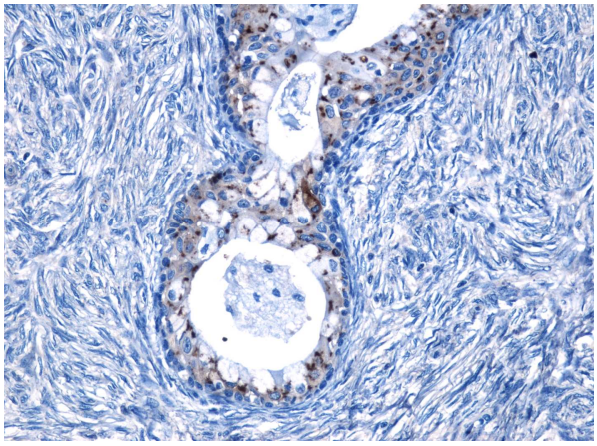

Figure 4. *Fetal ovary 24 s VEGF positive in the active primary folliculi Ob.20*

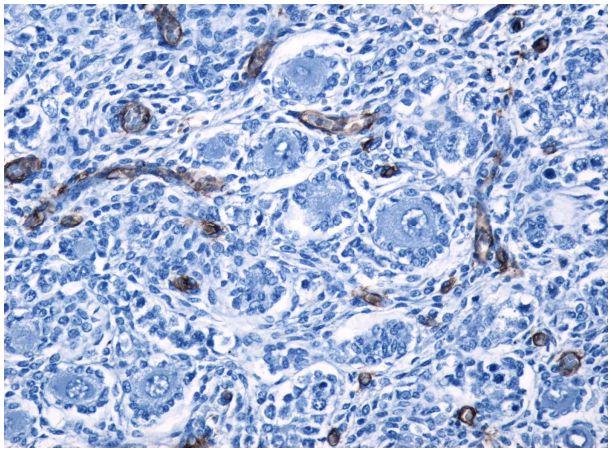

Figure 5. *Fetal ovary 12 s interfollicular immature vessels CD31 – CD34 Ob. 20*

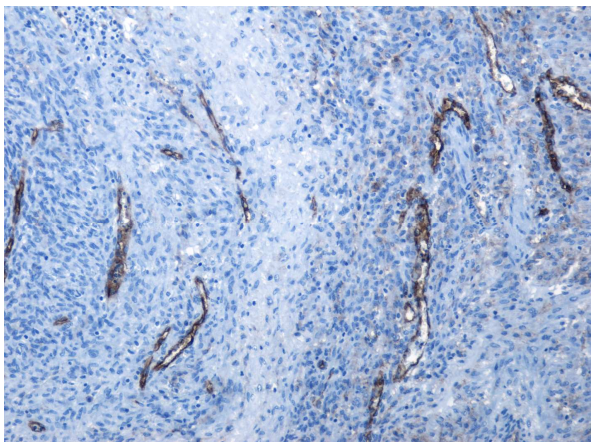

Figure 6. *Fetal ovary 24 s mature vessels in the ovarian hilum CD 31-CD34 Ob. 10*

4. Discussion

The vasculogenesis process starts with the hemongioblasts appearence in the extraembrionary mesoderm surrounding the vitellious sack in the third week of development. Their formation is induced by the vascular endothelial growth factor (VEGF) which is produced by the surrounding mesodermic cells [11]. Under the influence of the vascular endothelial growth factor, the peripheric hemangioblasts differentiate in angioblasts, the predecessesors of the blood vessels. The maturation from the embrionary stem cells in the endothelial cells was outlined in vitro in a model of the angiogenesis[12]. The existing studies have demonstrated that during the vasculogenesis, the vascular endothelial growth factor (VEGF) acts through two types of receptors (VEGF-R1 and VEGF-R2), interposed by the Flk 1. The interaction between the endothelial cells and the mural cells (pericites and the smooth muscular cells) is essential for the vascular development. The endothelial cells are formed by the mesodermal cells of the Flk 1, while the mural cells are believed to arise from the mesoderm, the neural crests or epicardial cell and migrate in order to contribute to the formation of the vascular wall. [13,14]. The formation of the endothelial tubes is dependent on cadherina 5 and CD31 which interacts with the actine filaments from the mural cells. [15]

The ovarian vasculogenesis is characterized by the appearence of primary immature networks of ectasied capillars disociated by ovogonies. These capillar networks develop in the primitive mesenchyme which produces VEGF. The ovarian vasculogenesis in the early fetal period is located in the ovarian stroma of a mesenchymal origin [16,17]. Afterwards, the appearence and the differentiation of the primordial folliculiconducts to the perifollicular vasculogenesis. During the differentiation and the ovarian maturation, the vasculogenesis will take place intrafollicular. The vasculogenesis is rapidly replaced by angiogenesis which develops around the ovarian folliculis through differentiation and maturation [18, 19]. It creates a homogenous aspect of the new-born ovary and while the organ maturates, the vasculogenesis becomes more intense at the selected foliculli level to become dominant and capable of ovulation. Due to the fact all the processes of ovarian remodelling take place with the stromal and follicular participation it is necessary to redefine the ovarian morphofunctional unit. This is not only formed from the ovarian folliculi but is also realised by a morphological trepide consisting in: follicul, stroma and the adjacent vessels [20].

5. Conclusions

The ovarian vasculogenesis from the early fetal period (12-14 weeks), identified through morphological and immunohistochemical methods is intense at the hilius and the stroma level. Subsequently, during the ovarian differentiation, the vasculogenesis takes place perifollicular. The ovarian maturation implies the development of a intrafollicular vascular network. The differentiation

of the blood vessels interposed and controlled by the VEGF produced by the surrounding stromal cells imposes the redefining of the ovarian morphofunctional unit. The morphofunctional unit consists in the folliculi and the adjacent stroma which represents the decisive factor of the ovarian folliculi cyclemorphosis.

Acknowledgement. Written consent was obtained from the patient for publication of study.

References

1. C. Gargett, g. Weston and Paw Rogers Mechanisms and regulations of endometrial angiogenesis , Cambridge University Press, Reproductive Review, 2002 ; 10: 45-61
2. Augustin HG, Vascular morphogenesis in the ovary. Bailliers Best Pract Res Clin Obstet Gynaecol. 2000; 14:867-882
3. Augustin HG Development of the vascular system of the corpus luteum. In Morphogenesis of Endothelium , 2000, 237-254
4. Abulafia O, Sherer DM. Angiogenesis of the ovary Am J Obstet Gynecol. 2000; 182:240-246
5. Ferrara N. The role of VEGF in the regulation of physiological and pathological angiogenesis EXS, 2005; 94:209-231
6.Ferrara N., **Davis-Smyth T.** The biology of vascular endothelial growth factor. Endocrine Rev. 1997; 18:4-25
7. Ferrara N. Role of vascular endothelial growth factor in regulation of physiological angiogenesis Am J Physiol Cell Physiol 2001, 280:C 1358-1366
8. Ferrara N. vascular endothelial growth factor and the regulation of angiogenesis, rec Prog Horm Res, 2000 ; 55: 15-35
9. Ann Hoeben, Bart Landuyt, Martin S. Highley, Hans Wildiers, Allan T. Van Oosterom And Ernst A. De Bruijn, Vascular Endothelial Growth Factor and Angiogenesis, Pharmacol Rev , 2004; 56: 549-580
10. J. Langmann, Embryologie médicale, Masson, 1994; 75-83; 233-237
11. Yancopoulos Gd, Davis S., Gale Nw, Rudge Js, Wiegand Sj, Holash J, Vascular- specific growth factors and blood vessel formation, Nature 407, 2000:242-248
12. Hirashima M, Kataoka H, Nishikawa S, Matsuyoshi N, Nishikawa Si Maturation of embryonic cells into endothelial cell in an in vitro model of vasculogenesis Blood, 1999; 93: 1253-1263
13. Jun Yamashita, Hiroshi Itoh, Masanori Hirashima, Minetaro Ogawa, Satomi Nishikawa, Takami Yurugi, Makoto Naito, Kazuwa Nakao & Shin-Ichi Nishikawa Flk1-positive cells derived from embryonic stem cells serve as vascular progenitors, Nature, 2000; 408 : 92-96
14. Duval M , Bedard- Goulet S, Delisle C., Gratton Jp., Vascular endothelial growth factor-dependent down-regulation of Flk-1/KDR involves Cbl-mediated ubiquitination. Consequences on nitric oxide production from endothelial cells. J Biol Chem. 2003; May 30; 278(22):20091-7.
15. Matsumura T, Wolff K, Petzelbauer P : Endothelial cell tube formation depends on cadherin 5 and CD 31 interactions with filamentous actin. J. Immunol 1997; 58: 3408-3416
16. Carmeliet P., Mackman N., Moons L., Gressnes P., Van Vlaenderen I., Demunck H., Kasper M., Breier G., Evrard P., Muller M., Risau W., Edgington T, And Collen D. Role of tissue factor in embryonic blood vesel development Nature, 1996; 383:73-75
17. Mihaela Niculescu, Mărgăritescu C., Cristiana Simionescu, Stănescu M.R., Mîndrilă I., Embrio-fetal ovary vasculogenesis: immunohistochimic and morphologic study, La 17-eme Session des Journees Medicales Balkaniques, Craiova; 2007, 6
18. Ferrara N, Gerber Hp The role of vascular endothelial growth factor in angiogenesis Acta Haematol. 2001; 106(4): 148-156
19. Carmeliet P Mechanisms of angiogenesis and arteriogenesis Nat Med 2000, 6: 389-395
20. Mihaela Niculescu Contribution to the ovary morphological study, PhD Thesis, 1998, 149-150