VASCULAR ENDOTHELIAL GROWTH FACTOR, NERVE GROWTH FACTOR RECEPTOR p75, PROTEIN GENE PRODUCT 9.5, TUMOR NECROSIS FACTOR–α AND APOPTOSIS IN THE COW ENDOMETRIUM IN POST PARTUM PERIOD

I. Sematovica[1], M. Pilmane[2], A. Jemeljanovs[1]

[1]*Research Institute of Biotechnology and Veterinary Medicine „Sigra", Latvia University of Agriculture*
[2]*Institute of Anatomy and Anthropology, Riga Stradins University*

ABSTRACT. Vascular endothelial growth factor, nerve growth factor receptor p75, protein gene product 9.5, tumor necrosis factor-α and apoptosis in the cow endometrium in post partum period. *Biopsy samples from cows uterus were taken in winter 2004/2005 on the research and training farm "Vecauce". Histological investigations were performed at the Institute of Anatomy and Anthropology of Riga Stradins University. Nine cows were biopsied twice – in the first and fifth week after parturition. The aim of the work was to investigate vascular endothelial growth factor (VEGF), nerve growth factor receptors p75 (NGFR p75), protein gene product 9.5 (PGP 9.5), tumor necrosis factor α (TNF–α), and apoptosis in the cow endometrium in post partum period.*

The results showed a significant increase of VEGF, NGFR p75, TNF–α amount, and the number of apoptotic cells in the cow endometrium between the first and the fifth week after parturition ($p<0.05$). VEGF expression in the cow endometrium was found in the fifth week after parturition, mainly localized in blood vessels. A close positive correlation ($r=0.72$; $p<0.001$) was found between VEGF and TNF–α, as well as between VEGF and NGFR p75 ($r=0.64$; $p<0.05$) expression in the cow endometrium at that time of post partum period. NGFR p75 were localized in the nerves around endometrial glands, blood vessels and under epithelium. A mild positive correlation ($r=0.51$; $p<0.05$) was found between the expression of NGFR p75 and TNF–α in that period. The amount of TNF–α showed a mild positive correlation ($r=0.59$; $p<0.05$) with a number of apoptotic cells in the cow endometrium in post partum period, as well as a significant mild positive correlation ($r=0.59$; $p<0.05$) was found between the amount of apoptotic cells and expression of VEGF in the cow endometrium. PGP 9.5 was found in nerves of the walls of endometrial glands, under epithelium and in the walls of blood vessels. A mild positive correlation was found in the cow endometrium between PGP 9.5 and NGFR p75 expression from the first to the fifth week after parturition. In conclusion, the increase of NGFR p75, VEGF and TNF–α expression in the cow endometrium from the first up to the fifth week after parturition seems to correlate with the ischaemia of tissue increased by inflammatory action. The latter also seems to stimulate the apoptosis and proliferation of nerve fibres in the cow endometrium.

Keywords: *cow endometrium, post partum, vascular endothelial growth factor, nerve growth factor receptors p75, protein gene product 9.5, tumor necrosis factor–α*

Introduction

The optimum function of the reproductive system is essential for maximum productivity in dairy cows, because lactating occurs only after successful insemination, fertilization and gestation period. Remarkable morphological and physiological changes occur in the uterus tissue, blood vessels, and nerves during the reproductive cycle and gestation (Hickey, Fraser, 2003; Zoubina *et al*., 1998). All the time new methods and approaches are used for investigation of cow uterus morphology and physiology in post partum period.

Vascular endothelial growth factor (VEGF) is an important signalprotein, cytokine that is involved in the formation of embryonic, as well as in adult organism angiogenesis from the existing vascular tissues (Wei *et al*., 2004; Wang *et al*., 2003, Halder *et al*., 2000; Guidi *et al*., 1998). VEGF is a heparin binding protein, and it has many isoforms in the organism (Ribeiro *et al*., 2006; Halder *et al*., 2000). VEGF producers are mast cells, which produce also neutrophile and eosinophile haemotactic factors, as well as fibroblast growth factor. Activity of VEGF is mainly tended to the endothelial cells of blood vessels, and its influence on other types of cells like monocyte/macrophage stimulation is established. There is observed ability of VEGF to stimulate mitogenesis of endothelial cells, and migration of cells. It also enhances the microvascular permeability *in vitro* that is why VEGF is sometimes referred to as a vascular permeability factor (Halder *et al*., 2000).

Structurally VEGF belongs to a cysteine group containing growth factors like platelet–derived growth factor (PDGF) family. All VEGF members act on the surfaces of cells through two tyrosine kinase family receptors: VEGFR1 – tyrosine kinase (Flt-1) and VEGFR2 – kinase-insert domain-containing receptor (KDR/Flk-1) (Halder *et al*., 2000). VEGF production can be induced in the cells which have not received enough oxygen, and then these cells produce transcription factor, namely ~ hypoxia inducible factor (HIF). Among other functions (modulating of erythropoesis)

HIF stimulates VEGF production (Ankoma–Sey *et al*., 2000). VEGF is a potent angiogenetic factor implicated in many pathological processes. It is increasingly released after TNF–α irritation, and it is a response to inflammatory processes in tissue (Scott *et al*., 1998). It is proved that VEGF expression, localisation and intensity in endometrium depend on the phase of reproductive cycle in woman and the cow (Wijayagunawardane *et al*., 2005; Wei *et al*., 2004; Zhang *et al*., 1998). However, action of the VEGF in proliferation processes in endometrium and pathological process, for example adenomyoma, is not comprehended yet (Ota, Tanaka, 2003).

Peptides of the nerve growth factors (NGF) provide maintenance, survival and differentiation of the neurons in tissue. They prevent a retrograde degeneration of neurons and apoptotic processes in the damaged cholinergic neurons (Wilcox *et al*., 2004). NGF is implicated in neuroimmune reactions and inflammatory processes, as well as it is mentioned as one of the inductive factors in apoptotic processes (Vaidyanathan *et al*., 1998). NGF belongs to the family of neurotrophic factors, that connects to a low affinity nerve growth factor receptors (LNGFR), named also p75 neurotrophic receptor. Another known NGF receptor group is high affinity receptors, named also tirosinkinase A (Track–A). Proteins of both of these groups stimulate alimentation, survival and differentiation of the neurons (Wilcox *et al*., 2004; Davidson *et al*., 2003). An increased count of the histamine containing mast cells was established in inflammatory processes in the skin, as well as enhanced expression of NGFR (Liang *et al.*, 1998). Coexpression of NGF together with the molecules involved in angiogenetic processes, and protein tirosinkinase A (p-TrkA) expression in endothelial cells indicates to their proangiogenetic role in case of formation of ovarian cancer. In their turn p-85 polymerase of polyadenozin-di-phosphate-ribose (p85–PARP), like a marker of apoptosis, and p-TrkA expression in cytoplasm (probably representation of non-glycolisated receptors) determine a favourable outcome, while activation of p-TrkA correlates with an ill-affected prognosis in case of a progressive stage of serous carcinoma (Davidson *et al*., 2003).

Programmed cells death (PCD) is genetically determined, biochemically specific kind of cells death, that starts with specific signals and activation of nonlysosomal endogen endonucleases resulting in defragmentation of the cell DNS, thereby releasing an organism from olden, needless and damaged cells (Goyeneche, Telleria, 2005).

Protein gene product 9.5 constitutes 1–5% of the total soluble proteins in the vertebrate neurons and neuroendocrine cells (Piccinini *et al*., 1996). There were observed alteration of the nerve fibres displaying PGP 9.5 dependency on different biological processes in the uterus tissue (Tingaker *et al*., 2006).

Only a few investigations related to the cow uterus morphology were performed in Latvia (Антане, 1990; Емельянова, 1974). Not sufficient awareness still persists concerning endometrium changes, distribution of growth stimulating factors and apoptosis in the cow endometrium in post partum period.

The objective of the present study was to investigate vascular endothelial growth factor, nerve growth factor receptors p75, protein gene product 9.5, tumor necrosis factor α, and apoptosis in the cow endometrium in post partum period, as well as to find out interactions of the mentioned substances, receptors and processes.

Materials and methods

Biopsy samples from cows uterus were taken in winter 2004/2005 on the research and training farm "Vecauce", with original biopsy instrument (Denmark, „*Kruuse"*, kat. nr: 141700k).

Biopsy samples were taken from previosly gravid uterus corn, in which a calve was located, from ventrally side of the dorsal wall of the uterus, intercarruncular places. Each animal was prepared for the procedure of endometrium biopsy sampling by washing and disinfection (70^0 *spiritus aethylici*) of external part of genital organs. A catheter was inserted into the uterus through the vagina to remove the endometrium samples by gently grasp of tissue with forceps being careful not to crush it. Specimens were snipped free at the base. Endometrium samples were inserted in labelled containers with 10% formalin solution, pH 7.5 (Humason, 1967) and histological investigations were performed at the Institute of Anatomy and Anthropology of Riga Stradins University. Nine cows were biopsied twice – in the first and the fifth week of post partum period.

TUNEL method was used for detection of apoptosis in the cow endometrium in post partum period (Negoescu *et al*., 1998) and In Situ Cell Death Detection, POD., Roche Diagnostics and DAB substrate vector was used. Deparafinised tissue samples (xylol 2 x 4 min., 99^0 *spiritus aethylici* 2 x 2 min., 95^0 *spiritus aethylici* 2 x 2 min. and 70^0 *spiritus aethylici* 2 x 2 min.) were rinsed (with water 7–10 min.) and iserted in PBS (phosphat buffer) (pH7.5) for 10 min., then slides were inserted in 50 ml PBS solution with 500 ml of 30% hydrogen peroxide for 30 min. in vibrator, to blocate the endogen nucleasis. Then tissue samples were washed with PBS (3 x 5 min.), and inserted in 0.2 M boric acid (pH 7.0), and placed into microwave (700W) for 10 min.for fixation of antigen, cooled to room tempetarure, rinsed with PBS. After that the slides were kept in refrigerator in 0.1% BSA (bovine serum albumin) solution with PBS for 10 min., and then slides were incubated in TUNEL mix (Tdt – mix of T-end deoxynucleotidiltransferases and dioxygeninlabeled nucleotyds) for 1h at $+37^0$C . Then slides were rinsed with PBS 1:10, and then they were coloured 30 min. in $+37^0$C with POD (sheep antifluoriscence antibody with horseradish peroxydasis binded Fab fragment). Then the slides were washed with PBS and covered with DAB (diaminobenzidin hromogen) for 7 min., and rinsed with running water for 5 min.. Finally haematoxylin and eosin staining was performed for each endometrium sample, and

slides were processed with polystyrene and covered with a cover-slip.

Immunohistochemically detected substances: vascular endothelial growth factor (mouse monoclonal VEGF, clone VG1, code Nr. M 7273 working dilution 1:50, DakoCytomation, Denmark), nerve growth factor receptors p75 (mouse monoclonal NGFR p75, code Nr. M3507, working dilution 1:150, DakoCytomation, Denmark), protein gene product 9.5 (rabbit polyclonal PGP 9.5, code Nr.Z5116, working dilution 1:1600, DakoCytomation, Denmark), tumor necrosis factor alpha (rabbit polyclonal TNF-α, code Nr. Ab6671, working dilution, 1:100, Abcam, England; Hsu *et al*., 1981). All working dilutions were prepared in accordance with the instructions of producers from above mentioned reagents.

Sections were deparafinised in xylene, and kept in ethanol from 99.7% up to 100%, rinsed with PBS solution pH 7.4 (10 min.), and inserted 4% citrate buffer solution, and placed in microwave for 20 min. (microwaving in 4% hydrochloric acid buffersolution of natrium). After cooling and rinsing with PBS tissue samples were covered with 150 µl 3% hydrogen peroxide (10 min.). Rinsing with PBS and subjecting to primary antibody (30 µl) were carried out (exposition 2h), as well as LSAB + LINK (linked streptavidin antibody) (30 min.) and LSAB + KIT (streptavidin connected with enzyme peroxidase) for 25 min., and DAB for 10 min. Finally, hematoxilline staining was carried out (Aughey, Frye, 2001).

For the data statistical processing the following methods were used: Student's t-test, Wilkinson's test, and statistical correlation analyses (Arhipova, Bāliņa, 2003).

Results and discussion

The above mentioned biological substances, receptors and a number of apoptotic cells were observed in the cow endometrium in the first and the fifth week after parturition (table 1). VEGF expression in the cow endometrium was found only in the fifth week after parturition, it was localized in blood vessels and under epithelium. Post partum period is characterized by a tissue repairing processes in the uterus as a response to hypoxic stimuli during the wound healing. It is reported that then an enhanced production of angiogenic factors, such as VEGF take place (Ankoma–Sey *et al*., 2000). Moreover, in the first weeks after parturition resumption of ovarian cyclicity occurs, and VEGF dose-dependently stimulates the release of prostaglandin E2, prostaglandin F2α, and endothelin-1, and that correlates with ovarian function (Wijayagunawardane *et al*., 2005). A close statistically significant positive correlation ($r=0.72$; $p<0.001$) was found between VEGF and TNF–α, as well as VEGF and NGFr p75 ($r=0.64$; $p<0.05$) expression in the cow endometrium at that time of post partum period.

NGFR p75 were localized around the endometrial glands, blood vessels, and under epithelium. The amount of them was more expressed in the fifth week in comparison with the first week after parturition ($p<0.05$). It can be explained by the ability of NGF to be involved in neuroimmune interactions, tissue inflammation, and repairing processes (Vaidyanathan *et al*., 1998).

A mild positive correlation ($r=0.51$; $p<0.05$) was found between the expression of NGFR p75 and TNF–α, as well as a significant mild correlation between NGFR p75 and the amount of PGP 9.5 ($r=0.49$; $p<0.05$) in the cow endometrium in that period. NGFR p75 is reported as one of the inducer of apoptosis (Vaidyanathan et al., 1998), but PGP 9.5 is a highly expressed component in vertebrate neuronal system (Piccinini *et al*., 1996), that indicates to the possible amount of neuropeptides in the neuronal structures of endometrium in the investigated cows. Possibly both these substances are connected, but it is difficult to make a decision which of them has a primary role in stimulation.

The amount of cells expressed TNF–α in the cow endometrium was increased significantly from the first to the fifth week after parturition ($p<0.001$). There was a mild positive correlation between the amount of TNF–α, and the ($r=0.59$; $p<0.05$) number of apoptotic cells in the cow endometrium in post partum period. That could be explained by the fact that TNF–α is a physiological factor inducing apoptosis (Goyeneche, Telleria, 2005).

PGP 9.5 was found under epithelium of the endometrium, around walls of blood vessels, and around walls of endometrial glands. A mild positive correlation was found between PGP 9.5 and NGFR p75 expression in the cow endometrium from the first to the fifth week after parturition. PGP 9.5 is a cytosolic protein belonging to the ubiquitin C-terminal hydrolyses subclass, high pronounced in the neuronal structures (Piccinini *et al*., 1996). That is why alterations of it may occur in case of interactions in different biological processes, also in endometrium. Remarkable changes of the uterus: size, structure of tissue, innervation, number of glands and blood vessels, as well as amount and activity of immuncompetent cells occur depending on the reproductive cycle and physiological condition (Tingaker *et al*., 2006) that could also change the expression of PGP 9.5.

A significant increase of apoptotic cell number was observed in the cow endometrium from the first to the fifth week after parturition ($p<0.05$), as well as a mild positive correlation between the number of apoptotic cells and TNF–α ($r=0.59$; $p<0.05$) at that time. An interesting finding was a significant mild correlation between the expression of VEGF and the number of apoptotic cells ($r=0.59$; $p<0.05$). Possibly it is connected with more intensive metabolic processes relevant to postischaemic vascularisation of the structure of uterus tissue when regenerates the endometrium in post partum period, and cyclicity of ovarian function occurs.

Table 1.Vascular endothelial growth factor (VEGF), nerve growth factor receptors p75 (NGFR), protein gene product 9.5 (PGP 9.5), tumor necrosis factor α (TNF–α) and apoptosis in the cow endometrium in 1st and 5th week after parturition

Sample	Week	VEGF	NGFR p75	PGP 9.5	TNF-a	Apoptosis; %
Tapa/F2	1	–	–	++	–	34.5±3.20
Tapa/1	5	–	++	+++	+	28.8±6.15
Esma/F3	1	–	–	+	–	25.1±3.15
Esma/7	5	–	–	+	+	63.2±1.50
Dakota/F4	1	–	++	++	–	……..
Dakota/3	5	+	++	+	+++	…….
Tērvete/F5	1	–	+	–	–	30.2±5.50
Tērvete/2	5	–	+	+	++	12.8±0.85
Vaida/F6	1	–	+	+	–	14.0±0.3
Vaida/6	5	–	–	+	+	62.5±7.8
Okence/F7	1	–	–	+	++	……..
Okence/8	5	+	++	+	+++	74.0±14.35
Elba/F8	1	–	+	+	+	……..
Elba/9	5	+	+++	++	+++	……..
Akāce/F9	1	–	–	+	++	……..
Akāce/5	5	+	+++	+	+	……..
Loda/F10	1	–	–	+	–	21.6±28.85
Loda/4	5	–	+++	+	++	23.9±4.00

Legends: VEGF – vascular endothelial growth factor; NGFR p75 – receptor p75 of nerve growth factors; PGP 9.5 – protein gene product 9.5; TNF–α – tumor necrosis factor-α ;
– – lack of cells containing VEGF, NGFR p75, PGP 9.5, TNF–α,
+ – small number of cells containing VEGF , NGFR p75, PGP 9.5, TNF–α,
++ – moderate number of cells containing VEGF , NGFR p75, PGP 9.5, TNF–α ;
+++ – numerous cells containing VEGF , NGFR p75, PGP 9.5, TNF–α ;
++++ – significant number of cells containing VEGF , NGFR p75, PGP 9.5, TNF–α .

Conclusions

1. Increase of NGFR p75, VEGF and TNF–α expression in the cow endometrium from the first to the fifth week after parturition ($p<0.05$) seems to correlate to the ischaemia of tissue raised by inflammatory action. The last one event also seems to stimulate the apoptosis and proliferation of nerve fibers in the cow's endometrium.
2. A close positive correlation ($r=0.72$; $p<0.001$) was found between VEGF and TNF–α, as a possible reaction to ischaemia caused by inflammatory processes, as well as between VEGF and NGFR p75 ($r=0.64$; $p<0.05$) expression in the cow endometrium at the time of post partum period as evidence that the mentioned substances are implicated in the programmed cells death processes.
3. NGFR p75 were localized in the nerves around the endometrial glands, blood vessels and under epithelium. VEGF expression in the cow endometrium was found in the fifth week after parturition. A mild positive correlation ($r=0.51$; $p<0.05$) was found between the expression of NGFR p75 and TNF–α in that period, probably, caused by the stimulation of nerve fibers proliferation. A mild positive correlation ($p<0.05$; $r=0.49$) was found in the cow endometrium between PGP 9.5 and NGFR p75 expression from the first to the fifth week after parturition that show evidence of intensive stimulation of neuronal structures.

References

Ankoma–Sey V.M.D., Wang Y., Dai Z. 2000. Hypoxic stimulation of vascular endothelial growth factor expression in activated rat hepatic stellate cells. – Hepatology, 31 (1), 141–148.

Arhipova, I., Bāliņa S. 2003. Statistika Ekonomikā. Risinājumi ar SPSS un Mikrosoft Exel. Rīga: Datorzinību centrs. 352 pp.

Aughey, E., Frye F.L. 2001. Comparative veterinary histology with clinical correlates. Manson Pub.Ltd., London, 14–127.

Davidson B., Reich R., Lazarovici P., Nesland J.M., Skrede M., Risberg B., Tropé C.G., Florenes V.A. 2003. Expression and activation of the nerve growth factor receptor TrkA in serous ovarian carcinoma. – Clinical cancer research, 9, 2248–2259.

Goyeneche, A.A., Telleria, C. M. 2005. Exogenous estradiol enhances apoptosis in regressing post-partum rat corpora lutea possibly mediated by prolactin. – Reproductive Biology and Endocrinology, 3, 40.

Guidi A.J., Abu-Jawdeh G., Tognazzi K., Dvorak H.F., Brown L.F. 1998. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in endometrial carcinoma. Cancer, 78 (3), 454–460.

Halder J.B., Zhao X., Soker S., Paria B.C., Kagsbrun M., Das S.K., Dey S.K. 2000. Differential expression of VEGF isoforms and $VEGF_{164}$–specific receptor neuropilin-1 in the mouse uterus suggests a role for $VEGF_{164}$ in vascular permeability and angiogenesis during implantation. – Genesis, 26 (3), 213–224.

Hickey M., Fraser I. 2003. Human uterine vascular structures in normal and diseased states. – Microscopy research and technique, 60 (4), 377–389.

Hsu, S.M, Raine, L. Fanger, H. 1981. The use of antiavidin antibody and biotin-streptavidin peroxydase complex in immunoperoksidase technics. – American journal of clinical pathology, 75, 816 pp.

Humason, G.L. 1967. Animal tissue techniques. Second edition. W.H.Freeman and Company, San Francisco and London, 569 pp.

Liang, Y., Marcusson, J. A., Jacobi, H.H., Haak-frendscho, M., Johansson, O. 1998. Histamine containing mast cells and their relationship to NGFr-immunoreactive nerves in prurigo nodularis: a reappraisal. – Journal of cutaneous pathology, 25 (4), 189

Negoescu, A., Guillermet, Ch., Lorimer, Ph., Robert, C., Lantuejoul, S., Brambilla, E., Labat-moleur, F. 1998. TUNEL apoptotic cell detection in archived paraffin-embledded tissues. – Biochemica, 3, 36–41.

Ota H., Tanaka T. 2003. Stromal vascularisation in the endometrium during adenomyosis. – Microscopy research and technique, 60 (4), 445–449.

Piccinini, M., Merighi, A., Bruno, R., Cascio, P., Curto, M., Mioletti, S. 1996. Affinity purification and characterization of protein gene product 9.5 (PGP 9.5) from retina. – Biochemestry Journal, 6, 318, 711.

Ribeiro L.A., Bacci M.L., Seren E., Tamanini C., Forni M. 2006. Characterization and differential expression of vascular endothelial growth factor isoforms and receptors in swine *corpus luteum* throughout estrus cycle. – Molecular reproduction and development, 74 (2), 163–171.

Scott P.A.E., Gleadle J.M., Bicknell R., Harris A.L. 1998. Role of the hypoxia sensing system, acidity and reproductive hormones in the variability of vascular endothelial growth factor induction in human breast carcinoma cell lines. – International journal of cancer, 75 (5), 706–712.

Tingaker, B.K., Johansson, O., Cluff, A.H., Ekman-Ordeberg, G. 2006. Unaltered innervation of the human cervix uteri in contrast to the corpus during pregnancy and labor as revealed by PGP 9.5 immunohistochemistry. – European Journal Obstetric Gynecology Reproduction Biology, 125 (1), 66–71.

Vaidyanathan S., Krishnan K.R., Mansour P., Soni B.M., McDicken I. 1998. p^{75} nerve growth factor receptor in the vesical urothelium of patients with neuropathic bladder: an immunohistochemical study. – Spinal cord, 36 (8), 541–547.

Wang H., Li Q., Lin H., Yu X., Qian D., Dai J., Duan E., Zhu C. 2003. Expression of vascular endothelial growth factor and its receptors in the rhesus monkey (*Macaca mulata*) endometrium and placenta during early pregnancy. – Molecular reproduction and development, 65 (2), 123–131.

Wei P., Chen X. Song X., Han C., Liu Y. 2004. VEGF, bFGF, and their receptors in the endometrium of rhesus monkey during menstrual cycle and early pregnancy. – Molecular reproduction and development, 68 (4), 456–462.

Wijayagunawardane M.P.B., Kodithuwakku S.P., Yamamoto D., Miamoto A. 2005. Vascular endothelial growth system in the cow oviduct: a possible involment in the regulation of oviductal motility and embryo transport. – *Molecular reproduction and developmen,* 72 (4), 511–520.

Wilcox B.J., Applegate M.D., Portera-Cailliau C., koliatsos V.E. 2004. Nerve growth factor prevents apoptotic cell death in injured central cholinergic neurons.– The journal of comparative neurology, 359 (4),573–585.

Zhang L., Scott P.A.E., Turley H., Leek R., Lewis C.E., Gatter K.C., Harris A.L., Mackenzie I.Z., Rees M.C. P., Bicknell R. 1998. Validation of anti-vascular endothelial growth factor (anti-VEGF) antibodies for immunohistochemical localization of VEGF in tissue sections: expression of VEGF in the human endometrium. – The journal of pathology, 185 (4), 402–408.

Zoubina, E.V., Fan, Q., Smith, P.G. 1998. Variations in uterine innervation during the estrus cycle in rat. – The Journal of Comparative Neurology, 397 (4), 561–571.

Антане, В.В. 1990. Зависимость суперовуляции и качества ембрионов коров-доноров от состояния их гормонального статуса, ендометрия и показателей обмена веществ. *Диссертация на соискание ученой степени кандидата ветеринарных наук.* Елгава, 139 стр.

Емельянова, М. 1974. Гистоморфологические и гистохимические изменения основных структур эндометрия после отёла. *Диссертация на соискание ученои степени кандидата биологических наук.* Сигулда, 194 стр.