Expression of progesterone receptors in bovine *corpus luteum* during pregnancy

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Summary. *The expression of progesterone receptors and matrix ribonucleic acid in 37 corpora lutea in 1–7 month's pregnant cows was investigated. Corpora lutea were obtained from slaughtered animals. Progesterone receptors expression was confirmed using biotin-streptavidin immunohistochemistry. Matrix ribonucleic acid expression was studied using pyronin staining by Unna-Brashe method. The expression of progesterone receptors was the highest in the luteal cells of the one-month pregnancy corpus luteum. It decreased significantly ($p<0.05$) from the third to the fourth month of pregnancy, but there was no significant difference in the expression of progesterone receptors from the first to the second and from the second to the third month of pregnancy. Also, there was no significant difference in the expression of progesterone receptors from the fourth to the fifth month and from the fifth to the sixth month of pregnancy. The expression of matrix ribonucleic acid also decreased during pregnancy, but gradually, with no significant monthly differences. It decreased significantly ($p<0.05$) from the sixth to the seventh month of pregnancy only. Our study shows that both indices – progesterone receptors and matrix ribonucleic acid decrease in the steroidogenic luteal cells during pregnancy and that a strong linear correlation ($r=0.88$) exists between these indices.*

Introduction

The *corpus luteum graviditatis* is a transitive gland producing progesterone an important hormone for the maintenance of pregnancy. Recent investigations have suggested that progesterone influences the functional homeostasis of the *corpus luteum graviditatis* during early pregnancy via inhibition of apoptosis in luteal cells (1, 2). Insufficiency of *corpus luteum graviditatis* to produce progesterone during early pregnancy hinders the development of the embryo and the ability of the embryonic cells to produce τ interferon (3–5).

Corpus luteum is an autocrine gland. Luteal cells produce the progesterone and they are also one of the target cells for this hormone (6, 7). Therefore the functional activity of *corpus luteum* is characterized by the expression of the intracellular progesterone receptors (PR Rec) and also of matrix ribonucleic acid (mRNA), which is involved in the PR Rec responses in steroidogenic cells of the *corpus luteum*. Many cellular actions of progesterone are mediated by nuclear PR Rec (8). The presence of PR Rec and mRNA in luteal cells of monkey and human suggest the possibility of direct action of progesterone on luteal cells and thus a regulation of luteal function (9–11). The correlation between the serum progesterone and the luteal PR Rec levels indicates that progesterone may regulate its receptor over the course of the luteal phase (9). However, the serum progesterone concentration does not describe the functional activity of steroidogenic cells of *corpus luteum graviditatis* objectively, because at approximately the second month of pregnancy the placenta is fully developed and starts to produce progesterone. Therefore the PR Rec and mRNA expression in the steroidogenic cells of *corpus luteum graviditatis* is important as an indicator of the functional activity of *corpus luteum graviditatis*. The PR Rec indicates the ability of steroidogenic cells to bind progesterone, but mRNA expression indicates the synthesis of progesterone in the steroidogenic cells. The PR Rec are saturated with progesterone in the course of time. Thereby PR Rec activity is decreased by progesterone saturation. Then the steroidogenesis declines and results in a decrease also of mRNA expression that gets trough the progesterone synthesis.

It is necessary to find out the changes of morpho-functional activity of the *corpus luteum graviditatis*

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in cow's ovaries. These investigations could help to develop a great problem in cattle's reproduction – early mortality of embryo. Unfortunately, there is lack of such investigations.

The aim of our work was to represent the functional activity of the bovine *corpus luteum graviditatis* steroidogenic cells using immunohistochemical and histochemical methods for the characterization of PR Rec and mRNA expression in bovine *corpus luteum graviditatis* steroidogenic cells at different stages of pregnancy.

Material and methods

Thirty-seven *corpora lutea graviditatis* obtained from the clinical healthy Latvian brown cows' ovaries immediately after slaughter at the different abattoirs in Latvia were studied. Four *corpora lutea graviditatis* were taken from one-month pregnant cows, two were taken from two-month pregnancies, fourteen – from three-month pregnancies, nine – from four-month pregnancies, six – from five-month pregnancies, one was taken from six-month and one from seven-month pregnancy.

Luteal tissue was placed in Stefanini fixative (2% formaldehyde, 0.5% picric acid in 0.1-M phosphate buffer, pH 7.2). The fixative was rinsed by tyrode buffer (0.1 M phosphate buffer + saccharose, pH 7.4). After dehydration in an ethanol-xylene series, pieces of luteal tissue were embedded into paraffin. Paraffin sections (about 6–7 μm) were used for the detection of PR Rec (Rabbit Anti-Human Progesterone Receptor, work dilution 1:25, Dako, *Dakopatts*, Denmark) using the biotin-streptavidin complex immunohistochemical method by A. M. Traish and H. H. Wotiz (12). In case of positive reaction, the relevant places are stained brown. As control we used two specimens – one without primary antibodies, and second without secondary antibodies. mRNA was detected by staining with pyronin according to the Unna-Brashe method (13, 14). In our investigation RNA (mRNA) expression in nuclei of luteal cells was examined. Granular structures in the nucleus stained dark pink or red were considered as positive. As the control we used specimens prepared before with ribonuclease (13).

The preparations were analyzed under the microscopes with 400 times of magnification. Avtandilov (15) semiquantitative method was applied for cell counting: the positive cells were counted in randomly selected 6 visual fields and then the average number of positive cells per section was calculated. For cell counting two histological sections were used. For mathematical processing of obtained data Microsoft Excel computer software was used. For evaluation of significance of differences and comparison of the mean values of progesterone receptors and mRNA in various stages of development of pregnancy *corpus luteum* Student T-test was applied. For the evaluation of correlation between both indices (PR Rec and mRNA) linear correlation coefficient (r) using Microsoft Excel computer software was calculated.

Results

Significant changes in the expression of PR Rec and of mRNA in the bovine *corpus luteum graviditatis* were observed during the time of pregnancy (Fig. 1). The expression of PR Rec was highest in steroidogenic

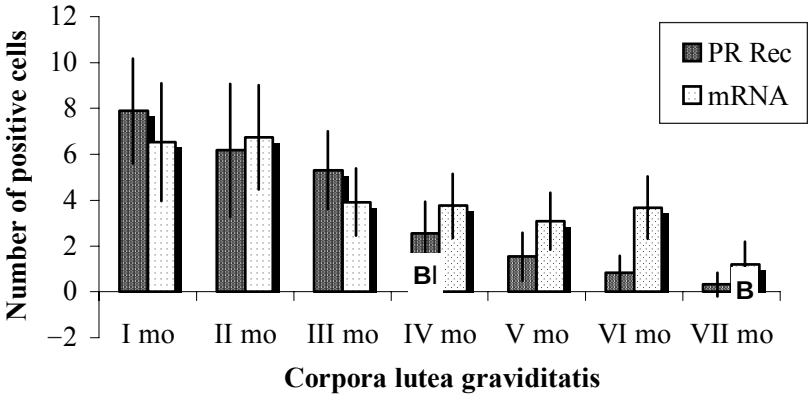

Fig. 1. **The graphic illustration of the expression of progesterone receptors and mRNA in the steroidogenic cells of bovine *corpus luteum graviditatis***

I mo., II mo., III mo., IV mo., V mo., VI mo., VII mo. – *corpora lutea* of respective months of pregnancy; PR Rec – expression of progesterone receptors, mRNA – expression of matrix ribonucleic acid; B – statistically significant decrease of parameter ($p<0.05$).

cells of one-month *corpus luteum graviditatis* (7.9±2.3 positive cells in the visual field, Fig. 1, Fig. 2A). A significant decline of PR Rec expression was observed in the steroidogenic cells of the fourth-month *corpus luteum graviditatis* ($p<0.05$, 2.6±1.4 positive cells in the visual field, Fig. 1, Fig. 2B). Significant changes in the expression of PR Rec were not found in the steroidogenic cells of the second to the third month *corpus luteum graviditatis*. Also, there was no significant difference in the expression of PR Rec from the fourth to the fifth month and from the fifth to the seventh month of pregnancy (Fig. 2C).

The expression of mRNA in the steroidogenic cells of corpus luteum graviditatis decreased gradually, generally without the significant monthly differences, observed for PR Rec (Fig. 1, Fig. 3A, B and C). However a significant decline of mRNA expression in the steroidogenic cells of *corpus luteum graviditatis* was found during the period from the sixth to the seventh month of pregnancy ($p<0.05$; from 3.7±1.4 to 1.1±1.0 positive cells in the visual field) only.

A strong positive correlation ($r=0.88$) was detected between both indices – expressions of PR Rec and mRNA in the steroidogenic cells of *corpus luteum graviditatis* during the pregnancy.

Also structural changes, such as karyopyknosis, eccentric localization of nucleus, cytolysis and vacuolization of cytoplasm were observed in *corpus luteum graviditatis* steroidogenic cells during the time of pregnancy (Fig. 4A, B and C). The size and number of vacuoles varied. The cells changed their shape during the time of pregnancy (Fig. 4B, C). Also, the chromatin of the steroidogenic cells nuclei underwent condensation during pregnancy (Fig. 4B, C).

Discussion

In the first month of pregnancy the steroidogenic cells of the *corpus luteum graviditatis* are very active cells. It is indicated both by progesterone receptor expression in cells and mRNA expression that could be show evidence that cells produce progesterone intensively. Also in the second month of pregnancy the *corpus luteum* cells show great morphofunctional activity. The cells activity of the pregnancy *corpus luteum* in the early stage of pregnancy from the 8th to 20th day is supported by τ interferon secreted by the embryo trophoblast binuclear cells. τ interferon blocks arachidonic acid transfer into $PGF_{2\alpha}$ and stimulates progesterone synthesis in the large luteal cells (16). Approximately on the 21st day the formation of the placenta starts and the effect of τ interferon on the pregnancy *corpus luteum* cells gradually diminishes. That explains the decrease of the luteal cell activity in our investigation at the third month of pregnancy. Undoubtedly, the pituitary hormones – luteinizing hormone (LH) and follicle-stimulating hormone (FSH) play an important role because exactly in the first months of pregnancy LH concentration in blood is high but afterwards it rapidly decreases. The role of LH in the maintenance of the *corpus luteum* activity decreases significantly after completion of the placenta (17). In most animals the placenta formation is complete and starts its functions on about the 45th day of pregnancy (16, 18). Nevertheless, the function of the *corpus luteum* is maintained even until the 200th day of pregnancy (19). The general increase of progesterone concentration causes the decrease of sensitivity of the hypothalamic-pituitary tract hindering the LH release, while the decrease of progesterone produced by ovaries cause the increase of FSH concentration (17). As a result it causes a statistically significant decrease ($p< 0.05$) of morphofunctional activity of *corpus luteum* in our investigation at the fourth months of pregnancy. Our data show, that small luteal cells start prevailing in the parenchyma of the *corpus luteum* tissue in the fourth month of pregnancy the activity, which depends on LH. Our investigation shows the mean number of progesterone receptor and mRNA reactive cells per visual field of a slide in the *corpus luteum* tissue in the fifth and the sixth months of pregnancy an evidence of the decrease of number of the active cells.

The data of our investigation show, that in the fourth month of pregnancy immune cells appeared in great quantity in tissues because the progesterone produced by luteal cells decreased. Progesterone has a peculiarity of suppressing functions of immune cells and the local immunity response (20–22). Infiltration of immune cells introduces the intensification of regressive changes in luteal cells. Cytokines produced by the immune cells decrease even more the synthesis of progesterone stimulated by LH and stimulate the cytokines synthesis in the immune cells (20–22). Cytokines cause the cytotoxicity affect in luteal cells (22), which could be determined in our investigation as a karyopyknosis and cytolysis in the fourth month of pregnancy. Our investigation suggests, that these processes continue to increase with progressing of pregnancy and in the seventh month of pregnancy they include a lot of luteal cells. The *corpus luteum* cells in the seventh month of pregnancy are non-active due to their destruction.

The decrease of PR Rec and mRNA expression in pregnancy is bound with luteal regression like in the *corpus luteum periodicum*. The first signs of *corpus luteum graviditatis* regression we had found by mic-

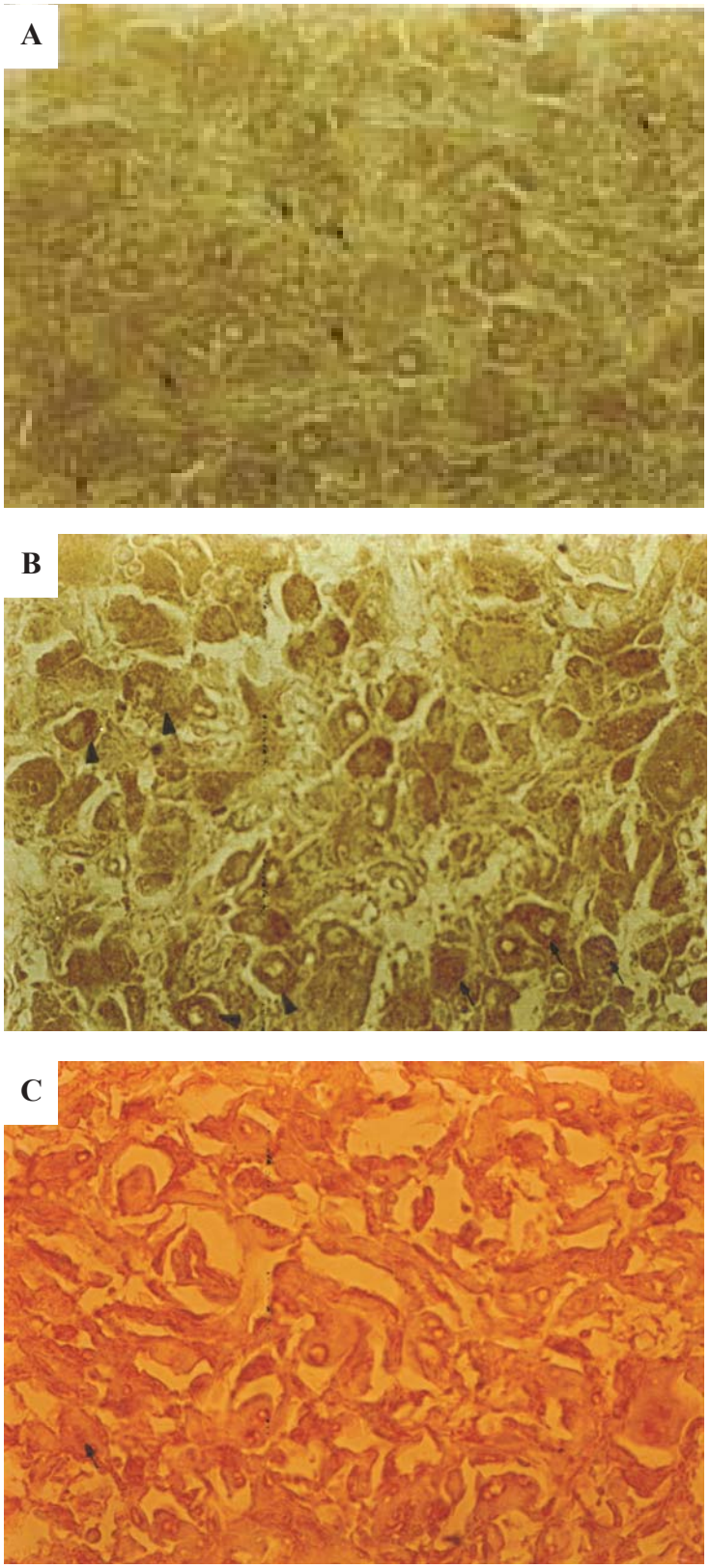

Fig. 2. **Light microphotographs of progesterone receptor expression in cells of bovine *corpus luteum graviditatis;* background stained by hematoxylin, scale bar 20 μm**

A) Nuclei and cytoplasm of 1-month *corpus luteum graviditatis* show a great activity of PR Rec. Arrows indicates localization of progesterone receptors in nucleuses of luteal cells; B) Nuclei and cytoplasm of 4-month *corpus luteum graviditatis* show a middle activity of PR Rec. The PR Rec localize in the cytoplasm here and there (grosses arrows) and in nucleuses (small arrows); C) Only few nuclei of cells in 7-month *corpus luteum graviditatis* contain PR Rec. Arrows indicate localization of progesterone receptors.

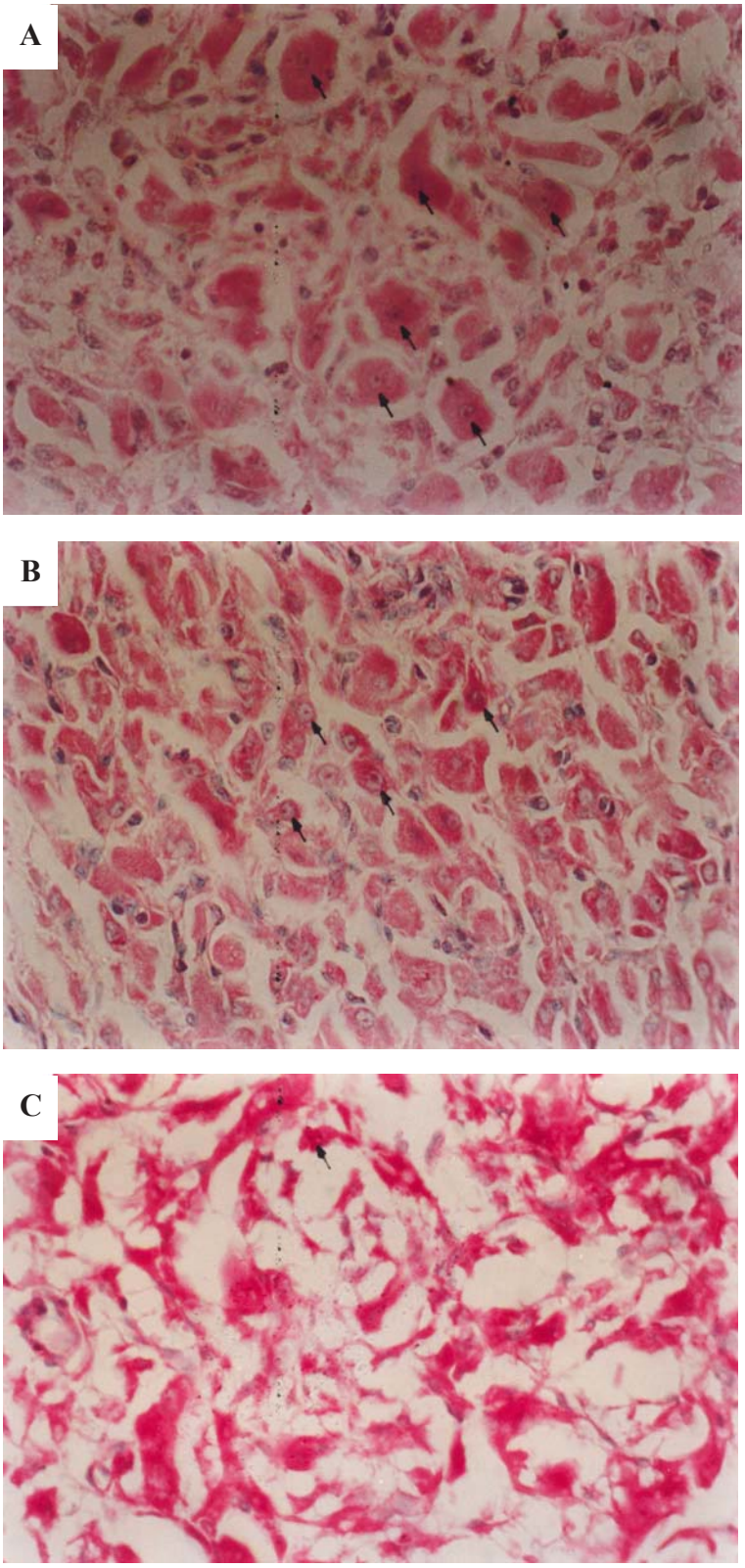

Fig. 3. **Light microphotographs of mRNA expression in cells of bovine *corpus luteum graviditatis* (stained by Unna-Brashe), scale bar 20 μm**

A) There is observed a manifest activity of mRNA in cells of 1-month *corpus luteum graviditatis*. Arrows points localization of mRNA in nucleuses of luteal cells; B) Cells of 4-month *corpus luteum graviditatis* present a middle activity of mRNA. Arrows points localization of mRNA in nucleuses of luteal cells; C) Cells of 7-month *corpus luteum graviditatis* present a low activity of mRNA. Arrows point localization of mRNA in nucleuses of luteal cells.

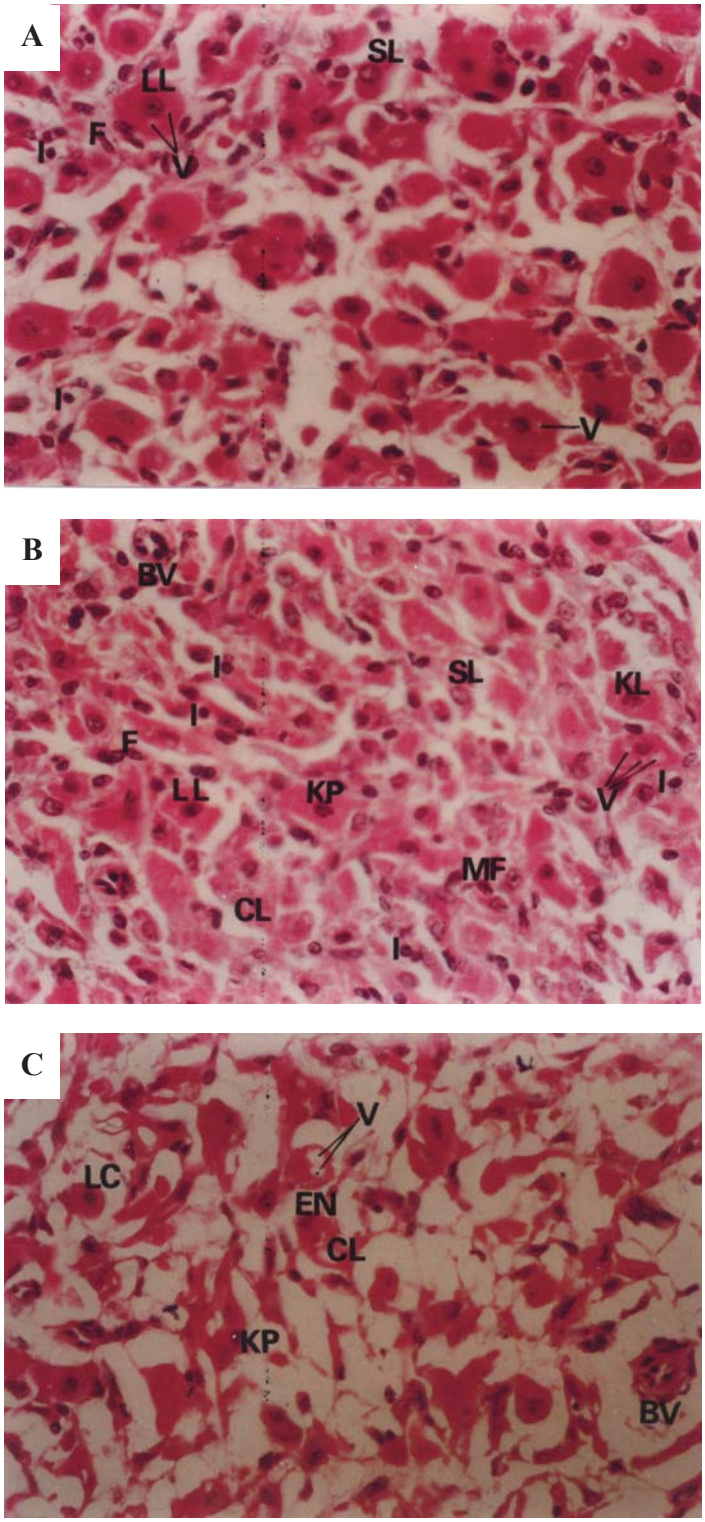

Fig. 4. **Light microphotographs of hematoxylin-eosin stained cells of bovine *corpus luteum graviditatis*, scale bar 20 μm**

A) Good visible cells' lines round nuclei with good visible chromatin in cells of 1-month old *corpus luteum graviditatis*. The large luteal cells predominate. LL – large luteal cells, SL – small luteal cells, I – immune cell, F – fibroblast, V – vacuoles; B) The small luteal cells predominate, dark cytoplasm, dark and blurry nuclei, blurry cells' cytoplasm in cells of 4-month *corpus luteum graviditatis*. SL – small luteal cells, LL – large luteal cells, V – vacuoles, BV – blood vessel, MF – macrophage, I – immune cell, F – fibroblast, KP – karyopyknosis, KL – karyolysis, CL – cytolysis; C) Vacuolization, of cells' cytoplasm, karyopyknosis and cytolysis in the tissue of 7-month *corpus luteum graviditatis*. LC –luteal cells, V – vacuoles, BV – blood vessel, KP – karyopyknosis, CL – cytolysis; EN – eccentric localization of nucleus.

roscopy at second month of pregnancy. These investigations agree with the data of H. Gase et al (23), who indicated the first signs of *corpus luteum graviditatis* regression at the end of the first month of pregnancy. R. Tamane et al (24) reported that the expression of mRNA in the steroidogenic cells of the *corpus luteum periodicum* during regression was greater than in those of the cells of *corpus luteum graviditatis* at seventh month's pregnancy. These data, reported by us, are consistent with those of D. M. Duffy et al (11).

Conclusion

In conclusion, the morphofunctional activity of steroidogenic cells in *corpus luteum graviditatis* continues to the seventh month of pregnancy in spite of increasing functionality of placenta. The decrease of both PR Rec and mRNA expression in luteal cells of *corpus luteum graviditatis* during pregnancy points to the gradual decrease of progesterone production by steroidogenic cells of the *corpus luteum graviditatis*. These declines correlate with regressive structural changes in the *corpus luteum graviditatis*, indicating the increasing role of the placenta in the maintenance of pregnancy.

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Veršingų karvių geltonkūnių progesterono receptorių tyrimas

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Raktažodžiai: progesterono receptoriai, matrikso ribonukleino rūgštis, geltonkūniai.

Santrauka. Buvo tiriami veršingų karvių (nuo 1 iki 7 mėnesio) 37 geltonkūniai siekiant nustatyti juose progesterono receptorius bei matrikso ribonukleino rūgštį.

Progesterono receptoriams nustatyti naudota biotino-streptavidino imunohistochemija. Matrikso ribonukleino rūgščiai nustatyti naudotas dažymas pironinu pagal Unna-Brashe metodiką.

Daugiausia progesterono receptorių aptikome vieno mėnesio veršingumo karvių geltonkūnio luteocituose. Jų skaičius žymiai sumažėjo ($p<0,05$) nuo trečiojo iki ketvirtojo veršingumo mėnesio. Reikšmingo skirtumo tarp pirmojo antrojo, antrojo trečiojo, ketvirtojo penktojo ir penktojo šeštojo veršingumo mėnesių nenustatyta.

Matrikso ribonukleino rūgšties taip pat mažėjo veršingumo laikotarpiu, bet palaipsniui, o gerokai sumažėjo ($p<0,05$) tik šeštąjį septintąjį mėnesiais.

Šio tyrimo duomenimis, veršingų karvių luteocituose mažėja progesterono receptorių ir matrikso ribonukleino rūgšties, tarp kurių pastebėta reikšminga linijinė koreliacija ($r=0,88$).

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