

*Materials of Conferences*

**WHAT IS THE QUESTION? HOW RAT  
UTERINE CERVICAL SPHINCTER  
LEYOMYOCYTES CHANGE  
IN PREGNANCY AND CHILDBIRTH?**

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Cervical sphincter morphological examination using optical, phase-contrast and scanning electron microscopy shows a number of reactive changes among different leyomyocytes populations. Bright hypertrophied myocytes and small myocytes were found in the inner myometrium layer before and during the vaginal birth. The number of special purpose organelles is reduced. Also, the development of granular endoplasmic reticulum is enhanced in the smooth muscle cell cytoplasm. There are two processes which occurred synchronously during the childbirth: first is – intercellular substance synthesis, second is – the myocyte cytoplasm dispose.

**Introduction.** The mammal uterus is a unique organ, which adapted to significant morphological and functional changes [5, 6]. Well known, the main functions of the uterus are carried out through the myometrium myocytes activities [3, 4], those fact is still poorly known. The cervix sphincter structure and functioning are the most controversial facts [2, 6]. First, the cervix is a barrier to ascending infection [4], second – it provides the fetus retention in the uterus during pregnancy, at last, during childbirth, it's a biomechanical basis of cervical dilation [1, 5, 6].

**The purpose** of our research is to investigate the cervix sphincter muscle cells reactive changes during late pregnancy and natural delivery childbirth.

**Challenges of achieving** are the following:

1. To study the lower uterine segment structural changes during late pregnancy and childbirth;
2. To determine the myocyte morphological features arising in “maturation” of the cervix during the childbirth.

**Material and methods.** We have used 25 rats, in accordance with the “Ethical guidelines of the experiments using experimental animals”. The object of experiment was been the adult rats uterus during natural delivery childbirth. We investigated 20 and 21 days of pregnancy uteruses first, and uteruses during birth after. Intact uteruses were used as an experimental control. We used the methods of optical microscopy, phase contrast microscopy and electron microscopy. For optical microscopy, the material was fixed in buffered formalin, after it

was prepared in a vacuum Leica ASP300 smart tissue processor. Than material was poured with paraffin “Histomix” Bio Optica. Frontal, sagittal and transverse sections were prepared on a rotary microtome thickness of 6 microns. Finished sections were stained with hematoxylin and eosin. We used monoclonal antibodies to smooth muscle actin in IHC (Immunohistochemistry). The antibodies typing were performed with using DACO antibodies in IHC research. To perform phase contrast microscopy and scan electron microscopy the material was fixed in 2,5% glutaraldehyde 0,1 M phosphate buffered pH 7,4. Then the material was placed in 1% solution of osmium tetroxide. After this the material was washed with phosphate buffer solution and dehydrated in alcohols of increasing concentration and embedded in Epon-araldite mixture. The preparations were contrasted with uranyl acetate and lead citrate, and then we prepared semi-thin sections were cut 1–2 micron thick and ultra-thin sections of 200–500 nm. The materials were investigated and photographed with an electron microscope JEOL JEM-1400 PLUS.

**Results and discussion.** We found that at birth the rat cervix wall undergoes softening and thickening in contrast to the body and the uterine horns, which are contrary thinner. The changes in uterus were caused by reactive changes of muscle tissues and extracellular substances.

The size differences in myocyte population were detected during pregnancy and reached a maximum at childbirth. Myocyte size increased during pregnancy. The myocytes with  $29,48 \pm 4,23$  microns length and  $9,69 \pm 2,74$  microns wide were dominated during late pregnancy. The lower uterine segment myometrium myocytes have spindle-shaped form with  $19,79 \pm 4,62$  microns length and  $3,75 \pm 1,27$  microns wide. This fact indicates small leyomyocytes presence in cervix sphincter. These cells are regarded as poorly differentiated in literature.

Via electron microscopy there were defined dark and light smooth myocytes, which were integrated into a single system in mature nulliparous rat's cervix. These cells were contacted with each other by no specialized simple contacts, desmosomes and slit contacts.

The number of contacts is increased during pregnancy. At birth the diametric opposite reaction is observed in sphincter myometrium inner layer. The myocytes are isolated and rounded, cell separation occurs (Fig. 1). The cell membrane forms folds – cytoplasmic protrusions (clasmatosis), followed by cell cytoplasm separation – clasmacytosis (Fig. 2). The number of special purpose organelles reduces. The granular endoplasmic reticulum is changed. Myocyte protein-synthesizing apparatus

is presented with numerous ribosomes, collected in the outlet, which composed clasmatosis.

There is an euchromatin predominance with 1–2 nucleoli presence in light cell nuclei. Predomi-

nant population by the end of pregnancy and child-birth are bright myocytes. This fact means the myocytes phenotypic transformation to secretory type. At birth cell death is not observed.

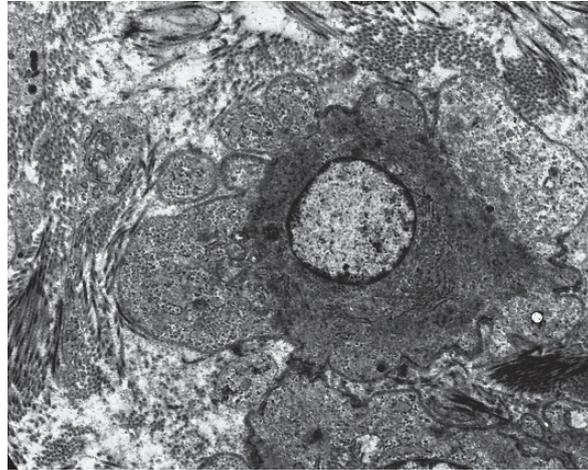


Fig. 1

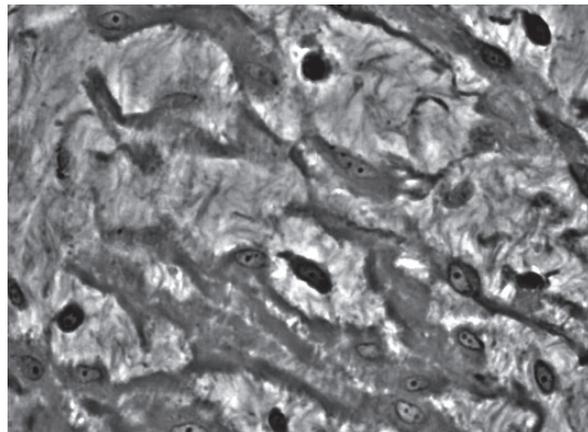


Fig. 2

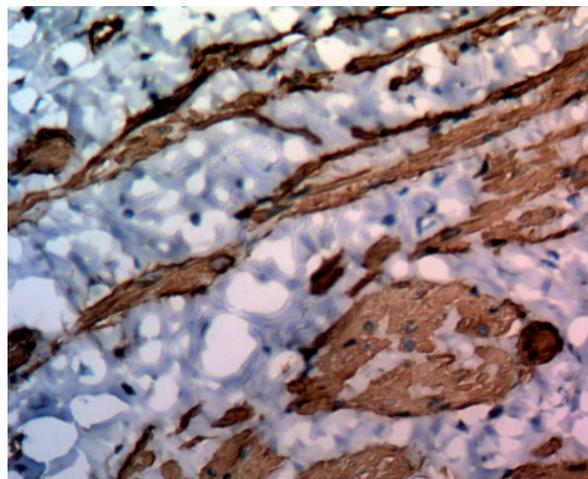


Fig. 3

We can identify leiomyocytes and interstitial matrix increase after using monoclonal antibodies to smooth muscle actin (Fig. 3). The intercellular substance changes in childbirth are one of the most important morphological aspects of cervix "maturation".

**Conclusion.** We found that during late pregnancy and vaginal birth in myometrium inner layer bright and hypertrophied small myocytes were dominated. Reactive changes in sphincter cervical cells during late pregnancy and childbirth can be regarded as a variant of differentiation and cell specialization. Clasmacytosis phenomenon, detectable during childbirth contributes to changing the qualitative and quantitative composition of the intercellular substance. It prepares the cervix for childbirth and also myocytes cytoplasm utilization during postmature involution.

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#### INFLUENCE OF SELENIT OF SODIUM ON PHYSICAL EFFICIENCY OF RATS IN THE CONDITIONS OF INTENSIVE PHYSICAL ACTIVITIES

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The intensive physical activities (PA), having accompanied the sports activities, are usually

being led to the fatigue and sharp decline further development in performance. So, it is expected, that the sodium selenite use, the antioxidant properties of which have already been shown by us earlier at IA (Kornyakova V.V., Konvay V.D., 2013), can be increased the intensive physical activities (PA).

The PA have already been modeled on the male albino rats, by the forced swimming test method with the 10% load of the body weight (e.g. Kornyakova V.V., and et.al., 2007). Then, the rats' swimming has been conducted in the 60 cm deep special pool, and with the 28–30°C water temperature in it. Thus, the three main groups have already been examined: the first one – to the optimal mode PA (OA), having swum with the load every other day during the five weeks (e.g. 35 days) of the experiment, the second one – with the PA (IA), intensive regime, having swam with the load during the first three weeks (e.g. 21 days) of the experiment in a day, and the last two weeks (e.g. 14 days) – every day, the third one (IA + C) – swam by the IA scheme, in the last week of the experiment personally received orally sodium selenite at the 30 mg/kg body weight before their swimming. They, moreover, have measured the rats' swimming time, jumping numbers, then they have recorded the electrocardiogram (ECG) at the end of the experiment.

In addition, it has been found, that rats, having subjected to the forced swimming with the load in the IA + C mode, have been characterized by the increased PA, and the cardiovascular system adaptivity to the PA, in comparison with the IA group animals. Thus, the rats' swimming time of the IA + C is practically higher up to 116,9% (e.g.  $P = 0,001$ ), and the jumping number – by 110,0% (e.g.  $P = 0,009$ ), in comparison with the IA group animals. It has been experienced the indicator decline of the rats' stress index of the IA + C group for by 30,2%, in comparison with the IA group animals (e.g.  $P = 0,02$ ) in the heart rate analysis (e.g. by R.M. Baevsky). So, all these indices in the IA + C group rats have not statistically and significantly been differed from the similar parameters in the OA group animals.

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