Dynamics of Myocytes of Different Types in Rat Myometrium during Pregnancy and Early Postpartum Involution

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Light and electron microscopy and morphometry of rat myometrium revealed 5 morphological types of myometrial smooth muscle cells. Quantitative evaluation of all cytotypes was performed in nonpregnant animals, during normal pregnancy, and during the early postpartum period. Transformation of some cells with nonvacuolated cytoplasm and clear nuclei (type 1) into type 3 cells (with small vacuoles in the cytoplasm), type 4 cells (in a state of balloon degeneration), and type 5 cells (apoptotic bodies) was observed during gestation and early postpartum period.

Key Words: *uterus; myocytes; cytotypes; dynamics*

Uterine myocytes are a heterogeneous and variable cell population [5], which seems to reflect the degree of their functional differentiation. Integration of myocytes in the myometrium and their different reactions to changing conditions determining their function explain the high plasticity of the myometrium, which manifests most brightly during pregnancy and postpartum period [8].

For this reason, the uterus in mammals is a natural model for studies of structural changes during normal development of anabolic and catabolic processes. All morphological changes in this organ during pregnancy and after labor are unique due to their high intensity.

We studied the dynamics of counts of myocytes of different types in rat myometrium during pregnancy and early postpartum involution of the uterus.

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MATERIALS AND METHODS

The study was carried out on 55 adult female Wistar rats (160-180 g). The uteri were examined on days 10 and 21 of pregnancy and during the first 5 days after delivery, when its fulminant involution was in progress. The uteri of nonpregnant animals served as the control. Pregnancy term was determined starting from the day of spermatozoa detection in vaginal smears. The animals were sacrificed by decapitation under ether narcosis. The placental zone of the uterus (participating in the formation of the placenta) and the interplacental zone located between the placentas of the neighboring fetuses were collected for the analysis. Material for histological study was fixed in 10% aqueous solution of formalin, dehydrated in ascending alcohols, and embedded in paraffin. The sections (5-7 μ) were stained by the method of van Gieson. Specimens for electron microscopy were fixed in 1% OsO_4 in phosphate buffer (pH 7.4), dehydrated in alcohols, and embedded in epon. Ultrathin sections were contrasted with uranyl acetate and lead citrate [4] and examined under a transmission electron microscope at ×4000.

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In order to objectively evaluate changes in myocytes during pregnancy and involution, a morphometrical analysis of their structural organization was carried out. Since the structure and function of myocytes were different during all the studied periods and taking into consideration published data [1], we distinguished 5 types of myocytes by the following signs: proportion of diffuse and compact chromatin, shape and topography of the nuclei, degree of cytoplasm vacuolation, and cell size.

The percentage of all above-mentioned myocyte types was evaluated on histological sections at a final magnification of 630. A total of 50-70 visual fields (65-120 cells examined per field) were analyzed for each period of observation. Volume density of blood vessels in the myometrium was evaluated at the same magnification using an open test square system. At least 70 visual fields were analyzed for each period of observation. Myocytes in the placental and interplacental zones of the uterus were examined.

The differences between the means were considered significant at $p<0.05$ according to Student's t test [2].

Changes in the studied morphometric parameters in both examined uterine areas (placental and interplacental) were similar, and therefore we present the data for the myometrium without specification of the area.

RESULTS

Myocytes of different structure, the majority of them spindle-shaped, were detected in the myometrium of nonpregnant animals. Cells with nonvacuolated cytoplasm and clear nuclei stretched along the long axis and containing diffuse chromatin (type 1 cells) predominated. There were also type 2 myocytes with dark flat nuclei containing compact chromatin. Presumably, they represented the pool of so-called silent myocytes. The content of myocytes of other types was negligible in control animals (Table 1). Sometimes myocytes irrespective of the nuclear status contained numerous clear vacuole-like structures in the cytoplasm (type 3). The presence of these vacuoles indicated the development of hydropic degeneration in the myocytes, which could be caused by hypoxia, disorders of water metabolism in the cell, or malnutrition [6]. Type 4 cells were characterized by small size, sickle-shaped hyperchromatic nuclei shifted to the periphery, and clear cytoplasm resembling a solitary intracellular vacuole. The morphology of these myocytes corresponded to balloon degeneration status, when the cells were transformed into a huge vacuole filled with fluid. Type 5 cells were round, small, had fragmented or hyperchromatic nuclei lined with a rim of clear cytoplasm. From functional viewpoint they presumably represented one of apoptosis stages: apoptotic bodies. The presence of modified myocytes in the group of nonpregnant animals is in line with a previous report about their constant regeneration in the myometrium [8]. However, at the histological level not a single mitotic division was detected, which could indicate regeneration of the cell pool. Presumably, they were realized by extremely rare mitosis episodes or, most likely, by hypertrophy of types 1 or 2 cells.

The volume of the myometrium increased significantly during normal pregnancy, particularly in the placental area, which was due to myocyte enlargement. By day 21 of pregnancy, the myometrium was maximally hypertrophic.

Analysis of changes in the presentation of myocytes of different types in the myometrium suggested that by day 10 of pregnancy some inert myocytes (type 2) were activated (type 1), as a

TABLE 1. Proportion (%) of Myocytes of Different Types in the Myometrium of Nonpregnant Rats (Control), during Pregnancy and Postpartum Involution of the Uterus

Status	Myocyte morphotype				
	1	2	3	4	5
Control	84.0±0.7	12.2±0.7	0.3±0.1	2.4±0.3	1.1±0.2
Pregnancy day 10	88.5±0.5*	7.0±0.4*	1.6±0.2*	2.3±0.3	0.5±0.1*
Pregnancy day 21	70.2±1.1*	6.9±0.5*	15.4±0.9*	6.6±0.6*	0.9±0.2*
Day 1 postpartum	66.4±1.4*	8.2±0.6*	12.3±0.9*	7.6±0.7*	5.5±0.7*
Day 2 postpartum	65.4±1.3*	7.0±0.5**	14.3±0.9*	9.1±0.8*	4.2±0.6*
Day 3 postpartum	71.2±1.4*	7.4±0.5*	12.7±0.8*	6.4±0.9*	2.3±0.4
Day 4 postpartum	80.6±1.3*	5.7±0.4**	10.2±0.8*	2.6±0.4*	0.8±0.2**
Day 5 postpartum	58.8±1.4*	9.1±0.6***	17.9±0.7*	9.5±0.8*	4.7±0.6*

Note. *$p<0.001$, **$p<0.01$, ***$p<0.05$ compared to the control.

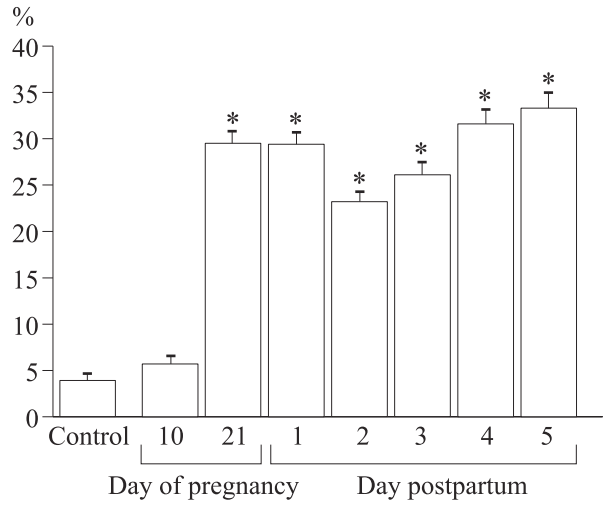

Fig. 1. Summary percentage of types 3, 4, and 5 myocytes in the myometrium of rats during pregnancy and early postpartum period. *p<0.001 compared to the control (myometrium of nonpregnant rats).

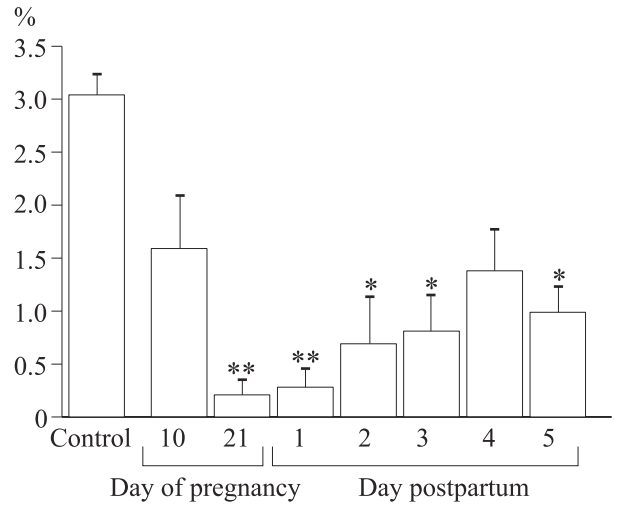

Fig. 2. Volume density of vessels in the myometrium of rats during pregnancy and early postpartum period. *p<0.05, **p<0.01 compared to the control (myometrium of nonpregnant rats).

result of which the percentage of type 1 cells increased (p<0.001). By day 21 of pregnancy, the percentage of type 1 cells decreased significantly in comparison with the control group (p<0.001), while the percentage of types 3, 4, and 5 cells increased. The increase of the percentage of myocytes not characteristic of the myometrium of control animals was mainly due to appearance of numerous myocytes with vacuolated cytoplasm (type 3), particularly in its peripheral zones (obviously preparation to clasmatosis), because fragments of these formations were observed in the interstitium. The percentage of these cells increased during pregnancy (p<0.001) during both periods of observation. Presumably, an appreciable part of active myocytes were subjected to vacuolation and transformed into type 3 cells during pregnancy. Signs of clasmatosis in many myometrial myocytes shortly before delivery were seen under an electron microscope. Separation of myocyte fragments (parts of peripheral cytoplasm) into the adjacent interstitium was observed. Separated material was characterized by homogenous structure and low electron density, which was in line with previous data [3]. Vacuoles in the interstitium were well discernible in the myometrium by the moment of labor even under a light microscope. These accumulations of vacuoles formed presumably as a result of active separation of myocyte cytoplasm fragments into the interstitium in hypoxia.

A sort of a paradox was detected at the final stage of pregnancy. By delivery, the uterus gained maximum weight due to myometrial hypertrophy, among other things, as it was to be ready to labor. Nonetheless, a large-scale transition of myocytes into nonfunctional status (presumably, degeneration) and overall death of myocytes by the apoptosis mechanism and loss of cytoplasm fragments by the clasmatosis mechanism were observed in the myometrium.

Study of the dynamics of proportions of myocyte types showed that from day 21 of pregnancy until day 1 postpartum the total percentage of cells in various modified conditions (types 3, 4, and 5) changed negligibly (Fig. 1); their percentage was just redistributed within this group. The percentage of vacuolated myocytes (type 3) decreased (p<0.001), while that of types 4 and 5 increased significantly (Table 1). Presumably, certain limitations for myocyte elimination mechanisms were removed directly after delivery: the greater part of vacuolated myocytes (type 3) were transformed into type 4 cells with structural changes similar to the balloon degeneration phenomena or subjected to apoptosis and accumulated in tissues as apoptotic bodies (type 5).

A relationship between changes in the percentage of vacuolated myocytes (type 3) and degree of myometrium vascularization was revealed. Myometrial hypertrophy during pregnancy was paralleled by an appreciable reduction of volume density of blood capillaries on day 21 of pregnancy (p<0.001; Fig. 2). Hence, the tissues were inevitably exposed to ischemia and malnutrition, which was seen from intensive vacuolation of myocytes. Cytoplasmic protrusion of cells as a reaction to deficient oxygen supply to tissues has been described for the intestinal epitheliocytes [7] and uterine myocytes after labor [6]. Rejection of the cytoplasm fragments leads to reduction of cell volume, but helps to retain cell viability.

Hence, processes apparently similar to vacuolar degeneration develop in some myocytes of the myometrium during the early postpartum involution of the uterus. They are characterized by condensation of the nucleus, modification of its shape, disappearance of the nucleoli, and cytoplasm vacuolation. However, these processes does not eventuate in necrobiosis. These morphological changes are often paralleled by myocyte elimination by apoptosis. The sources for replenishment of the myocyte pool with every subsequent delivery or pregnancy (if any) remain unknown, which is of extreme importance in uterine inertia in repeated pregnancies, particularly in multigravidae.

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