Structural Manifestations of Mechanisms of Myometrium Involution after Repeated Pregnancies in Mice

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Myocyte hypertrophy and proliferation in the myometrium were observed in mice during the first pregnancy and involution of the myometrium was completed by day 10 postpartum. Parameters of all structures returned to values observed in intact uterus. During the third pregnancy, the processes of myometrium hypertrophy were similar to those during the first pregnancy, but the intensity of proliferation was higher against the background of poorer vascularization, which led to destruction of some myocytes. Postpartum involution of the myometrium in these mice was not completed by day 10 after delivery. We observed hypertrophy and proliferation of some myocytes followed by their destruction, decrease in vessel number, and many-fold decrease in collagen content in the myometrial interstitium. The number of fetuses in the litter decreased after multiple pregnancies.

Key Words: *multiple pregnancies; involution of the myometrium; mice; morphology*

The risk of complications during delivery and after it (poor uterine contraction strength, uterine atony, *etc*.) increases with increasing the number of pregnancies. The muscular layer of the uterus undergoes considerable changes during pregnancy and after delivery [3,4]. The most important events are the increase in myometrium weight during pregnancy and its involution in the postpartum period, when its weight returns to the initial value; the latter process is considerably less studied. It was shown that myometrium growth during the first pregnancy is determined by its hypertrophy, while postpartum involution is realized via autophagocytosis, apoptosis, clasmocytosis, and necrosis [3,4]. However, the peculiarities of myometrium hypertrophy in repeated pregnancies and its postpartum involution as well as possible mechanisms of reparation of lost structures are poorly studied.

Here we studied structural reorganization of mouse myometrium in the second half of the third pregnancy and during the early and delayed postpartum periods of uterine involution.

MATERIALS AND METHODS

The study was performed on 2-month-old C57Bl/6 mice weighing 20-22 g (nursery of the Institute of Cytology and Genetics, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk).

Group 1 (control) comprised intact non-pregnant mice (n=5). Group 2 included pregnant mice (n=30, 5 animals per term); the myometrium was examined during pregnancy and after delivery. The myometrium of group 3 (n=30, 5 animals per term) mice was examined before and after the third physiological delivery (multiparous mice). In mice of groups 2 and 3, the myometrium samples were obtained on days 10 and 20 of gestation and on days 1, 3, 5, and 10 after physiological delivery. The term of gestation was counted from the day when vaginal plug was detected. The mice were decapitated under ether narcosis. An interplacental fragment of the uterus (between the placentas of adjacent fetuses) was taken for histological analysis. The samples were fixed in 10% neutral for-

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malin, dehydrated in ascending alcohols, and embedded in paraffin. Sections (5-7 μ) were prepared on a HM 355S rotation microtome (Microm; Carl Zeiss), stained with Mayer's hematoxylin and eosin and after van Gieson, and examined under an AxioStar Plus light microscope (Carl Zeiss). The preparations were morphometried at ×400 using a closed test system consisting of 25 squares with an area of 1600 μ^2. We measured volume densities (Vv) of myocyte nuclei and cytoplasm, myometrium and its vessels, collagen fibers in the myometrium stroma, and myocytes in the state of vacuolar (type 3) and balloon (type 4) degeneration [4]. Moreover, numerical densities (Nai) of myocytes, their nuclei, and perpendicular and tangential profiles of blood vessels were evaluated. The coefficient of myometrium vascularization was calculated as the ratio of volume density of the myometrium to the volume of vessels in it (*i.e.* the amount of muscle tissue in relative units per relative unit of vascular lumen); the mean sizes of myocytes and their nuclei were also calculated and expressed in relative units. The number of fetuses in the litter was determined. The significance of differences between the means was evaluated using Student *t* test, the differences were significant at $p<0.05$.

RESULTS

In primigravida mice, the relative content of myocytes in the myometrium increased by the end of pregnancy (day 20) by 20% from the corresponding parameter in intact female mice (Table 1), the relative content of collagen fibers decreased before delivery by almost 40%. The concentration of collagen fibers in the myometrial interstitium increased starting from day 3 postpartum and by day 10 it approximated the level observed in intact mice. The volume and numerical densities of vessels in the middle of pregnancy (day 10) did not differ from those in intact uterus, but by the end of pregnancy (day 20) the volume density of vessels increased by 48%, while their numerical density remained unchanged and corresponded to that in intact uterus, which attested to blood congestion in the myometrium at this term of pregnancy (Tables 1, 2).

In tertigravida mice, the relative contents of myocytes in the myometrium in the middle (day 10) and by the end (day 20) of pregnancy were similar to those in primigravida mice at the same terms, while the relative content of collagen fibers in the myometrium interstitium was higher by 29% and 80%, respectively (Table 1). In the postpartum period, the relative number of myocytes gradually increased (by 22% on day 10) compared day 1 postpartum, while the relative content of myocytes in primigravida mice by day 10 decreased by 12%. On the contrary, the relative content of collagen in myometrial interstitium of multigravida mice by day 10 postpartum decreased by 6 times (Table 1). It can be hypothesized that collagen elimination was associated with its capture by macrophages and fibroblasts [2] or by secretion of protease by these cells. Extracellular lysis of collagen due to secretion of catepsin B by myocytes [2] or its release from myometrial smooth-muscle cells during their necrosis is also possible. In the middle of the third pregnancy (day 10), the volume density of blood vessels in the myometrium was by 4.5 times lower (Table 1), while their numerical density was by 2 times lower (Table 2) than in intact mice. Before delivery (day 20), the volume density of blood vessels in multigravida mice increased by more than 56% compared to day 10 of pregnancy, while their numerical density remained unchanged, which attested to blood congestion in the myometrium (Tables 1 and 2). However, the volume density of blood vessels on day 20 of pregnancy was lower by 4 times than in intact and primigravida mice (Table 1), while their numerical density was lower by 2 times (Table 2). The worst blood supply to the myometrium was observed in the middle of pregnancy, because the amount of muscle tissue per myometrium volume unit was maximum during this period (Fig. 1). These findings also attest to blood congestion in the myometrium before the third delivery. Before delivery and during the postpartum period, the volume and numerical densities of blood vessels started to increase, but these parameters attained the values observed in intact animals only by day 10 postpartum (Tables 1, 2).

The relative content of the myometrium on day 10 after the third delivery increased by 25% compared to the corresponding parameter before delivery (day 20 of pregnancy) and by 40% compared to that in intact animals (Table 1) against the background of considerably impaired blood supply compared to the control, which was seen from the vascularization coefficient (Fig. 1), especially between the first and fifth days postpartum: 6.5, 5.1, and 6.3 in intact mice, at the end of the first pregnancy, and on day 10 postpartum, respectively. However, the vascularization coefficient before the third delivery and on days 5 and 10 postpartum was 18.8, 16.2, and only 10.8, respectively, *i.e.* structural provision of myometrium trophicity was somewhat improved, but remained by 40% lower than in intact animals and primiparous mice (Fig. 1). Under these conditions, the increase in the relative content of the myometrium in the muscular layer cannot be determined by myocyte hypertrophy alone. Indeed, in primigravida mice the number of myocytes 1 day before delivery increased 2-fold, but on day 10 postpartum this parameter returned to the level observed in intact mice. A similar regulation was found for the number of myocyte nuclei in the myometrium (Table

TABLE 1. Results of Morphometrical Measurement of Volume Density (Vv) of Myometrial Structures in Primiparous and Muliparous Mice (*M*±*m*)

Object	Control	Pregnancy term				Postpartum							
		day 10		day 20		day 1		day 3		day 5		day 10	
		group 2	group 3	group 2	group 3	group 2	group 3	group 2	group 3	group 2	group 3	group 2	group 3
Myocytes of the myometrium	62.7±1.2	68.6±0.9*	64.8±0.8^{+}	75.5±1.1	70.1±1.3^{+}	71.2±0.9	72.4±0.7	68.2±1.2	80.3±1.1^{+}	65.4±0.9	86.3±1.2^{+}	63.0±1.1	88.2±0.9^{*+}
Type 3 myocytes	1.3±0.2	6.8±0.8*	4.0±0.6^{*+}	11.9±0.7	10.2±0.8	9.4±0.7	16.0±0.9^{+}	7.2±0.8	28.3±1.2^{+}	5.2±0.7	25.7±1.1^{+}	3.2±0.3*	20.9±1.1^{*+}
Type 4 myocytes	1.2±0.3	2.6±0.3	3.1±0.5	3.2±0.5	4.7±0.6	6.5±0.8	6.0±0.8	5.0±0.5	9.9±0.8^{+}	7.7±0.7	4.0±0.4^{+}	2.8±0.4	6.5±0.4^{*+}
Myocyte nuclei	20.7±0.4	24.3±0.9*	20.1±0.8^{+}	25.8±0.9	25.4±0.9	21.4±0.8	30.4±0.7^{+}	20.9±0.5	35.2±1.0^{+}	19.3±0.8	45.4±1.0^{+}	19.1±0.3	47.8±0.7^{*+}
Myocyte cytoplasm	42.0±0.9	41.2±1.0	38.0±1.1	49.8±1.1	51.8±0.9	46.9±0.9	46.2±1.1	45.1±0.3	44.9±0.9	44.7±0.4	44.2±0.9	42.3±0.4	42.6±0.9
Blood vessels of the myometrium	9.5±1.2	9.8±1.0	2.06±0.30^{*+}	14.6±1.4	3.2±0.5^{+}	12.4±1.4	3.9±0.6^{+}	10.8±1.1	4.9±0.8^{+}	10.6±1.0	5.1±0.7^{+}	9.9±0.9	8.1±0.8
Collagen fibers of myometrial stroma	27.3±1.1	25.5±1.0	32.8±1.0^{*+}	15.3±1.1	28.5±1.1^{+}	18.0±1.1	24.0±0.8^{+}	21.2±1.1	16.3±1.2^{+}	22.8±1.0	10.3±1.1^{+}	27.9±0.9	4.5±1.2^{*+}

Note. Here and in Table 2: $p<0.05$ *compared to the control, $^{+}$between groups 2 and 3.

TABLE 2. Results of Morphometrical Measurement of Numerical Density (Nai) of Myometrial Structures and Parameters of Myocyte in Primiparous and Muliparous Mice (*M*±*m*)

Object	Control	Pregnancy term				Postpartum							
		day 10		day 20		day 1		day 3		day 5		day 10	
		group 2	group 3	group 2	group 3	group 2	group 3	group 2	group 3	group 2	group 3	group 2	group 3
Myocyte nuclei (Nai)	28.1±1.0	38.1±1.7*	46.1±1.2*+	78.7±1.0	69.5±1.5+	32.4±1.5	66.4±1.0+	31.8±0.69	94.5±1.0+	30.1±1.3	119.0±1.5+	28.0±0.8	127.1±1.4*+
Nucleus volume (V)	0.7±0.02	0.6±0.02	0.4±0.01*+	0.3±0.01	0.3±0.02	0.6±0.01	0.4±0.02+	0.6±0.02	0.3±0.02+	0.6±0.01	0.3±0.01+	0.6±0.01	0.3±0.02*+
Myocytes (Nai)	33.4±1.0	44.8±1.7*	75.9±1.0*+	84.0±1.0	55.3±1.0+	42.6±1.6	85.1±0.9+	40.1±1.3	102.2±1.8+	35.8±1.2	131.4±1.5+	34.9±0.3	139.1±1.4*+
Myocyte volume (V)	1.8±0.02	1.5±0.02*	0.8±0.01*+	0.8±0.01	1.2±0.02+	1.6±0.01	0.8±0.02+	1.7±0.02	0.7±0.02+	1.8±0.01	0.6±0.02+	1.8±0.01	0.6±0.02*+
Blood vessels of the myometrium (Nai)	1.4±0.1	1.4±0.1	0.7±0.1*+	1.5±0.1	0.8±0.1+	1.4±0.1	1.1±0.1	1.4±0.1	1.2±0.1	1.5±0.1	1.2±0.1	1.5±0.3	1.5±0.2

2). In multigravida mice, the proliferative processes became less intensive, because the number of myocytes in the test area surpassed that in intact mice by only 60%, whereas in primigravida mice these values differed by 2.5 times (Table 2). However, by day 10 postpartum the number of myocytes in multigravida mice was 2.5-fold higher than on day 20 of pregnancy and 4.2-fold higher than in intact mice. Similar dynamics was observed for the number of myocyte nuclei in the myometrium of multigravida mice in the postrartum period. The size of both myocytes and their nuclei decreased compared to the corresponding values in intact mice (Table 2). At the same time, myocytes referred by us to types 3 and 4 [4] (in the state of vacuolar and balloon degeneration) and sometimes necrotic myocytes were found during the involution period in multigravida mice. The relative counts of type 3 and 4 myocytes in the myometrium were maximum before delivery and were similar in primigravida and multigravida mice. However, this parameter sharply increased during the postpartum period in multigravida compared to primigravida mice. For instance, the relative count of type 3 myocytes in multigravida mice was higher by 70% (day 1) and then by 3.9 (day 3), 4.9 (day 5), and 6 times (day 10 postpartum) compared to primigravida mice during the corresponding periods (Table 1). In multigravida mice, the relative count of type 4 myocytes was similar to that in primigravida mice on postpartum day 1, but more than 2-fold surpassed that on day 10 of the involution period (Table 1). These findings suggest that degenerative changes in the myometrium before and after delivery were preceded by proliferative processes.

Thus, these findings attest to activation of proliferative processes in the myometrium before delivery. In primigravida mice, the number of myocytes by postpartum day 10 returned to the level observed in intact animals. The mechanism of elimination of excessive myocytes deserved special investigation. In multigravida mice, the involution process was prolonged, because no morphological signs of normalization of myometrium structure was observed by postpartum day 10. On the contrary, we observed quantitative signs of active myocyte proliferation preceding the development of degeneration processes. It can be

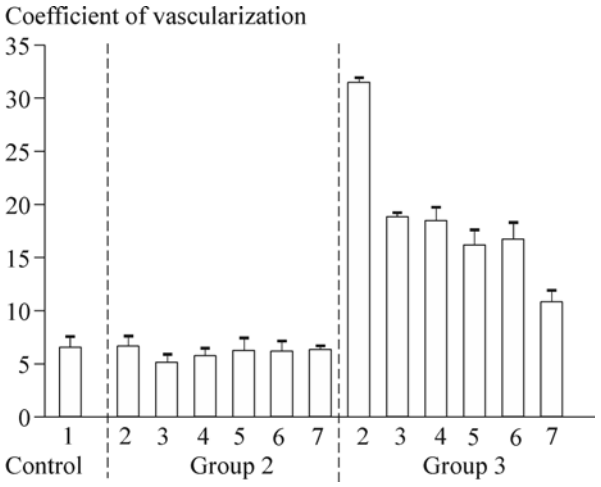

Fig. 1. Dynamics of myometrium vascularization during pregnancy and postpartum period in primiparous and muliparous mice. *1*) control; *2*) day 10 of pregnancy; *3*) day 20 of pregnancy; *4*) postpartum day 1; *5*) postpartum day 3; *6*) postpartum day 5; *7*) postpartum day 10.

hypothesized that proliferative processes in the myometrium have adaptive (reserve) character and their initiation, similarly to destructive processes (degeneration, necrosis, apoptosis), is associated with hypoxia and oxidative stress leading to intensive production of reactive oxygen metabolites. The effects of the latter (proliferation, necrosis, and apoptosis) depend on their concentration [1]. These processes are probably related to peculiarities of blood supply to the myometrium during delivery and to changes in hormone regulation of this process. Obviously, mechanical strength of the uterus in multiparous mice did not return to normal by postpartum day 10 due to reduced collagen content in the interstitium and reproductive capacity of multiparous mice decreased (5.9±0.3 fetuses *vs.* 7.4±0.3 fetuses in primiparous mice).

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