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Morphological Study of the Main Mechanisms of Myometrium Involution after Repeated Pregnancies in Mice

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Elimination of "excessive" myocytes and their structures during involution of the myometrium after the first and third pregnancies was realized by clasmocytosis (eliminating the greatest volume of myocyte cytoplasm fragments), apoptosis, and necrosis (equal percentage by volume). In contrast to the first pregnancy, involution after the third one was not over by day 10 because of inhibited elimination of functionally lost myocytes by necrosis and apoptosis mechanisms. Presumably, this was caused by slower hydrolysis of apoptotic bodies by macrophages. The concentration of macrophages in the myometrium on day 10 of the involution period in females after the third delivery was 4-fold higher than in intact mice and in females after the first delivery during the same period.

Key Words: *consecutive pregnancy; myometrium; necrosis; apoptosis; clasmocytosis*

Postpartum involution of the uterus involves all its structures: the endometrium [4] and myometrium [5,6] with involvement of the myometrial interstitium components: collagen and vessels [3]. It was shown that during 5 days of the involution period after the first pregnancy rats develop myocyte necrosis and apoptosis, autophagocytosis and clasmocytosis of their cytoplasm; each of these processes can be regarded as a mechanism of physical and functional elimination of myometrial structures "excessive" for that period [5,6]. Autophagocytosis cannot be an effective mechanism of the myometrial structures elimination because of its initial intensity [5]. Clasmocytosis is preferable as the mechanism of elimination, because it is not related to reduction of myocyte counts, labilization of lysosome membranes, and inflammation, because myocytes only shrink in size. They can be restored during regeneration hypertrophy during consecutive pregnancy and, presumably, without damage to the force of myocardial contractions. However, it is known that the most incident complications of repeated pregnancies are uterine atonia and inertia; possible causes of these disorders are still not quite clear.

We studied structural manifestations of possible mechanisms of the postpartum involution of the myometrium in mice after the third pregnancy.

MATERIALS AND METHODS

Specimens of the myometrium from 5 intact 2-month-old C57Bl/6 females (20-22 g) served as control 1 (group 1). Specimens of the myometrium from females of the same strain, age, and body weight (n=60;

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5 animals per period of observation) were examined in the middle and at the end (days 10 and 20) of the first (control 2, group 2) and third (group 3) pregnancies and during the early and late periods of involution of the uterus (days 1, 3, 5, and 10) after normal delivery in females of groups 2 and 3. All animals were obtained from Breeding Center of Institute of Cytology and Genetics (Novosibirsk). The animals received standard laboratory ration with free access to water and food. Pregnancy term was evaluated from the day of the vaginal plug detection. The mice were sacrificed by decapitation under ether narcosis during the above listed periods. Specimens of the interplacental zone (between the placentas of adjacent fetuses) were collected for histological studies. Specimens of the myometrium for histological studies were fixed in 10% neutral formalin, dehydrated in ascending alcohols, and embedded in paraffin [2]. The sections (5 μ) were sliced on an HM 355S type microtome (Microm), stained by Meyer's hematoxylin and eosin, and examined under an AxioStar plus light microscope (Carl Zeiss). The morphology of the sections was studied at ×400 using a closed test system of 25 squares (total area 1600 μ^2). Volume densities (Vv) of necrotic myocytes, conglomerations of vacuolated structures (myocyte cytoplasm clasmocytosis products) located in the interstitium, apoptotic myocytes identified by visual cytological signs (karyopyknosis, karyorrhexis, karyolysis, apoptotic body) were examined. From these data, the summary volume densities of physically and functionally eliminated myocytes or their structures (ES), that is, of myocytes dead by necrosis, apoptosis, and clasmocytosis ("interstitial conglomerations") were estimated (by summation) at every stage of observation. The myometrium vascularization coefficient was calculated as the proportion of volume density of vessels in the myometrium to volume density of myocytes in it (expressed in fractions). Volume density of apoptosis-modified myocytes was evaluated using immunohistochemical label for detection of caspase-3 expression [2]. The results were compared with the data of the same parameter evaluation by the cytological methods as described above. The numerical density (Nai) of macrophages in the myometrial interstitium was evaluated. Analysis of correlations in the data (morphometric values) was carried out. The significance of differences in the means was evaluated by Student's test. The differences were considered significant at $p<0.05$.

RESULTS

Possible mechanisms of elimination of myocytes or fragments of their cytoplasm, mentioned above, have been detected in the intact female myometrium (Table

TABLE 1. Results of Morphometric Studies of Presumable Mechanisms of Myometrial Involution in Mice after the First and Third Pregnancies (*M*±*m*)

Stage of observation	Total volume of eliminated myocytes, Vv		Necrotic myocytes, Vv		Interstitial conglomerations, Vv		Apoptotic myocytes, Vv		Myocytes labeled by caspase-3, Vv		Macrophages, Nai	
	I	II	I	II	I	II	I	II	I	II	I	II
Control	2.9±0.2		0.4±0.1		0.8±0.2		1.6±0.3		1.8±0.6		0.7±0.1	
Pregnancy												
10 days	5.4±0.3[a]	5.0±0.5[a]	0.4±0.1	0.8±0.1	3.1±0.5[a]	2.2±0.9[a]	1.8±0.3	2.0±0.3	2.0±0.8	2.1±0.9	0.9±0.1	2.0±0.2[ab]
20 days	10.3±0.6[c]	13.5±0.7[bd]	2.4±0.5[c]	2.7±0.3[d]	5.0±1.0[c]	8.0±1.3[bd]	2.8±0.4	2.7±0.4	2.2±0.9	3.0±0.8	1.2±0.1	3.0±0.2[b]
Postpartum period												
day 1	22.4±0.6[c]	25.7±0.7[d]	2.9±0.4	5.2±0.5[bd]	16.0±1.0[c]	15.8±1.3[d]	3.4±0.4	4.6±0.4[d]	4.2±1.2[c]	4.4±1.0	1.8±0.2	4.6±0.1[b]
day 3	14.4±0.7[c]	23.4±1.3[b]	1.44±0.20	4.8±0.7[b]	8.0±1.0[c]	11.6±1.0[bd]	5.0±0.5[c]	7.0±0.5[bd]	6.4±1.4[c]	6.4±1.1[d]	1.1±0.1	4.7±0.3[b]
day 5	7.0±0.6[c]	8.9±0.4[d]	0.9±0.7	3.8±0.5[b]	3.7±0.8[c]	2.0±0.4[bd]	2.3±0.4[c]	3.0±0.4[d]	4.7±1.3[c]	5.4±1.2	0.8±0.1	4.2±0.2[b]
day 10	2.6±0.3[c]	5.2±0.4[ab]	0.44±0.10	2.7±0.3[ab]	0[c]	0[d]	2.1±0.3	2.5±0.4	2.7±0.6c	5.1±0.6[ab]	0.7±0.1	2.8±0.2[abd]

Note. I: first delivery; II: third delivery. [a]Compared to control, [b]between the means in groups 2 and 3, [c]compared to respective values in group 2, [d]in group 3.

1). With development of the first and third pregnancies, the summary volume (Vv) of structures eliminated by all studied mechanisms increases 24 h before delivery more than 3-fold in primiparous females and more than 4-fold in females with the third pregnancy in comparison with this parameter in intact females (Table 1). Clasmocytosis products ("interstitial conglomerations") predominated by volume among all ES in both groups (first and third delivery), the percentage of necrotic and apoptotic myocytes was about the same (Table 1). Hence, mechanisms of myocyte elimination worked in rats long before labor, as was shown previously [4], and, paradoxically, in mice during the first and consecutive pregnancy. The mechanism not involving cell death, clasmocytosis, predominated. The percentage of myometrial ES sharply increased directly after delivery (on days 1-3 postpartum): by 7.6 and 4.9 times in primiparous and by 8.7 and 7.9 times in multiparous mice, respectively (Table 1). Later, the percentage of myometrial ES decreased: in primiparous females this value normalized, while in multiparous ones this value was 87% higher than in intact females. These data indicate incompleteness of myometrial involution in the females after the third delivery by day 10 of the postpartum period. Physiologically preferable mechanism of the postpartum involution of the myometrium, clasmocytosis, predominates before and after labor, judging from the volume percent of clasmocytosis products of the total volume of all ES in both situations studied. Apoptosis ranked second among the mechanisms involved in elimination of myocytes from the myometrium. This mechanism is obviously more physiological than necrosis. On the other hand, this process was maximally active on day 3 after labor in primiparous and multiparous females. However, in the latter group the process remained active on day 10 of the involution period (Table 1; Fig. 1). The maximum volume of myocytes eliminated by this mechanism was recorded before labor and particularly during the early postpartum period (Table 1). It seems, there is a universal mechanism inducing these processes. Previous studies have shown a significant deterioration of the conditions of myometrial vascularization in rats during the postpartum period [5]. Similarly, the myocardial vascularization coefficients decreased during the postpartum period in multiparous female mice in comparison with the coefficients in intact mice and primiparous females (Fig. 2). It seems that this reduction was caused by the development of hypoxia and oxidative stress and hence, increase of blood concentrations of active oxygen metabolites [1]. Depending on their concentrations, they induce cell necrosis and apoptosis [1]. The involvement of hypoxia in initiation of necrosis and apoptosis is confirmed by coefficients of correlation between their content in the myometrium and vascularization coefficients before labor. Utilization of ES is the macrophage responsibility, and the efficiency of clearing function of these cells in the organ during the postpartum period necessitates adequate increase of their counts and activity of the phagocytic and hydrolytic functions. The summary volume percentage of all structures to be eliminated by macrophages by day 10 postpartum in primiparous mice was equal to that in intact females, similarly as the values of all components of this sum (Table 1). No "interstitial conglomerations" (clasmocytosis products) were detected. The counts of macrophages in the myometrium of these females increased and decreased in complete agreement with changes in the volume densities of each of ES, which was shown by their correlation coefficients (Table 2). By day 10 of the postpartum period, the concentration

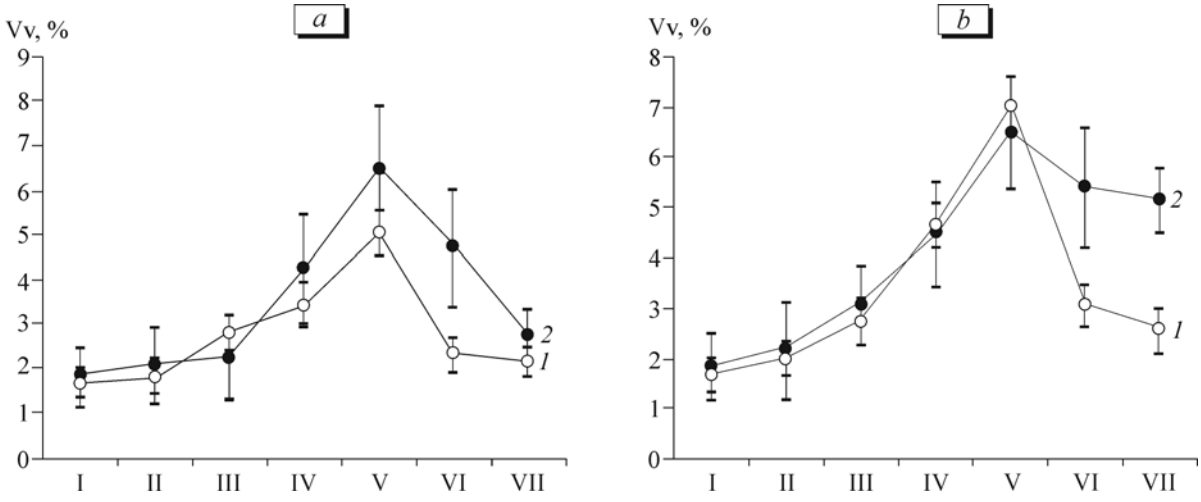

Fig. 1. Volume density (Vv) of apoptotic myocytes (*1*), evaluated by optic microscopy, and of myocytes expressing caspase-3 (apoptosis marker) (*2*) in the myometrium of female mice with the first (*a*) and consecutive pregnancy (*b*). I: control; II: pregnancy, 10 days; III: pregnancy, 20 days; IV: postpartum period (PP), day 1; V: PP, day 3; VI: PP, day 5; VII: PP, day 10.

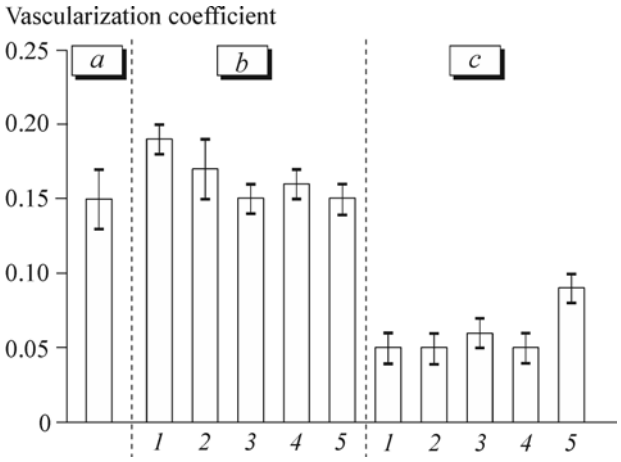

Fig. 2. Time course of myometrial vascularization in mice during the postpartum period (PP) under conditions of the first and third pregnancy. *a*) control; *b*) first pregnancy; *c*) third pregnancy. *1*) pregnancy, 20 days; *2*) PP, day 1; *3*) PP, day 3; *4*) PP, day 5; *5*) PP, day 10.

of macrophages in the myometrium was the same as in intact females (Table 1). All these facts indicate completion of myometrial involution process after the first delivery. By contrast, in multiparous females the summary volume density of ES on day 10 postpartum was 2-fold higher than in the control. This was caused by a 5-fold greater (in comparison with intact females) concentration of necrotic myocytes in the myometrium and a 52% greater concentration of apoptotic myocytes (caspase label has shown an even greater percentage of these cells; Fig. 1). Only the clasmocytosis products in the interstitium were eliminated completely, presumably because they were easier available for macrophages due to their smaller size than myocytes (Table 1). Accordingly, the concentrations of macrophages in the myometrium changed in proportion to changes in the content of all ES at all stages of observation after the third delivery (Table 1). However, it is obvious that phagocytic activity of macrophages (but not their concentration in the myometrium) had to increase significantly by day 10 of the involution period after the third delivery for effective completion of ES clearance. The total ES volume during this period was only 2-fold higher than in intact females, while macrophage count was 4-fold higher (Table 1). The causes of this phenomenon are not clear. How-

TABLE 2. Results of Analysis of Correlations between Parameters of Myometrial Structures in Mice under Conditions of the First and Repeated (Third) Pregnancy

Experiment conditions		Correlation objects				
		myometrial vessels and necrotic myocytes (Vv)	myometrial vessels and apoptotic myocytes (Vv)	macrophages and apoptotic myocytes (Nai)	macrophages (Nai) and interstitial conglomerations (Vv)	macrophages (Nai) and necrotic myocytes (Vv)
Control		0.33	0.38	0.55	0.59	0.54
First pregnancy						
P, days	10	0.33	0.61	0.65	0.60	0.57
	20	0.42	0.05	0.49	0.76	0.64
PP, day	1	0.14	0.60	0.50	0.67	0.58
	3	0.22	0.59	0.19	0.83	0.57
	5	0.31	0.48	0.37	0.62	0.76
	10	0.06	0.79	0.31	0.00	0.57
Third pregnancy						
P, days	10	0.53	0.68	0.31	0.68	0.56
	20	0.74	0.65	0.69	0.79	0.61
PP, day	1	0.52	0.39	-0.13	0.71	0.53
	3	0.48	0.31	0.36	0.76	0.57
	5	0.53	0.45	-0.08	0.65	0.47
	10	0.59	0.71	0.46	0.00	0.7

Note. P: pregnancy; PP: postpartum period.

ever, the percentage of apoptotic myocytes in ES was rather high on day 10 of the involution period and earlier (Table 1; Fig. 1). The absorption capacity of macrophages towards apoptotic bodies is presumably much lower than towards necrotic cells, which can be due to the phenomenon of peculiar blocking of the vacuolar system of tissue (myometrial) macrophages and to compensation for this condition by an increase of the count of macrophages, recruited from the bone marrow or other compartments of the mononuclear phagocyte system. The results of evaluations of the concentration of apoptotic myocytes in the myometrium (Fig. 1) by visual cytological criteria and by immunocytological visualization of caspase-3 expression are rather close.

Hence, the main mechanisms of elimination of "excessive" myocytes and their structures from the myometrium during the postpartum involution in mice after the first and third pregnancies are clasmocytosis (elimination of fragments of myocyte cytoplasm), its volume being the highest, and apoptosis and necrosis (elimination of whole cells); dead cell clearance in the interstitium is realized by macrophages. This clearance was the most effective for clasmocytosis products in both experimental situations. It seems that these mechanisms were insufficient in consecutive pregnancy, as myocytes dead by necrosis and apoptosis were not eliminated from the myometrium on day 10 of the postpartum period, presumably, because of inhibited hydrolysis of apoptotic bodies absorbed by macrophages.

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