GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Study of Proliferative Processes and Nuclear Estradiol and Progesterone Receptors in Myocytes in Pregnant and Postpartum Mouse Uterus

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Numerical densities of the nuclei were morhometrically evaluated in all myocytes and myocytes expressing nuclear estrogen- and progesterone-receptor complexes, which were revealed immunohistochemically with monoclonal antibodies in C57Bl/6 mice. It was shown that the above quantitative parameters of myometrial cells after the first pregnancy were similar to those in nonpregnant mice by day 10 after delivery. In the third pregnancy, especially developed after the second interrupted pregnancy, proliferation processes in the myometrium were not completed by postpartum day 10, but dramatically progressed. It was associated with a significant decrease in the fraction of myocytes carrying nuclear hormone–receptor complexes with estradiol and progesterone and their disturbed physiological relations in the myometrium during and after pregnancy probably due to dedifferentiation of a considerable part of myocytes.

Key Words: *pregnancy; postpartum involution; proliferation of myocytes; nuclear hormone-receptor complexes with estradiol and progesterone*

In physiological pregnancy, the increase in uterus size and weight is primarily attained due to intensive myocyte proliferation [10]. Postpartum involution occurs via clasmatosis and apoptosis of myocytes [1,11] with simultaneous myocyte proliferation arrest and is completed in mice by the 10th day after delivery [11]. It was also shown that proliferation in the myometrium is mainly regulated by estrogens and progesterone [8,13] via the the formation of hormone-receptor complexes consisting of cytosolic receptor proteins and the hormones followed by their binding to nuclear chromatin and expression of genes encoding various proteins including receptor proteins. However, the cells dedifferentiate during proliferation and cannot express or synthesize any proteins (including receptor). It can be hypothesized that protein synthesis and formation of estrogen- and progesterone-receptor complexes in myocytes after proliferation restores during differentiation. The dynamics of these processes at different stages of pregnancy and during postpartum involution, during the first and subsequent pregnancies, in proliferation of different intensity, and in relation to each of these sex steroid hormones remains unclear.

Here we studied the time course of proliferation processes in murine myometrium, total myocyte concentration and relative content of myocytes expressing nuclear estrogen- and progesterone-receptor complexes during the first and subsequent pregnancies and postpartum involution.

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MATERIALS AND METHODS

The work was carried out on 95 female C57Bl/6g mice weighing 20-22 g obtained from Breeding Center of Institute of Cytology and Genetics, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk. Specimens of the uteri from 5 intact mice aged 2 months served as control (group 1). Group 2 included mice with a history of one pregnancy and delivery. Group 3 comprised mice, which had undergone three pregnancies and normal delivery. Group 4 included mice that had undergone three pregnancies while the second was interrupted on day 15 of gestation by intraperitoneal administration of 0.3 ml of Enzaprost-F (5 mg/ml active agen concentration, Chinoin). The day after vaginal plug detection was considered to be the first day of gestation. The myometrium was sampled during the third pregnancy (days 10 and 20) on postpartum days 1, 3, 5 and 10 (5 animals per term). The animals were sacrificed by cervical dislocation under ether anesthesia. The samples of the myometrium for histological assay were fixed in 10% neutral formalin, dehydrated in ascending ethanol concentrations, and embedded in paraffin. The sections (5-7 μ) were sliced on an HM 355S microtome (Microm), stained with Meyer's hematoxylin and eosin, and examined under an AxioStar plus light microscope (Carl Zeiss). Nuclear estrogen- and progesterone-receptor complexes in myometrial myocytes were detected immunohistochemically using monoclonal antibodies to estradiol and progesterone receptor proteins (Novocastra).

The morphology of the sections was studied at ×400 using a closed test system consisting of 25 squares (total area 1600 μ^2). The number of myocytes in the myometrium was assessed by numerical density of their nuclei (Nai). In addition, nuclear numerical density (Nai) and relative content of myocytes expressing estrogen- and progesterone-receptor complexes were morphometrically evaluated. The total numerical density of all myocytes at each stage of the experiment was taken as 100%. Correlation relationships between the content of myocytes with nuclear estrogen- and progesterone-receptor complexes, and stages of the gestation were determined by Spearman rank correlation. The significance of differences between the means was evaluated by Student's *t* test. The differences were considered significant at $p<0.05$.

RESULTS

During the first physiological pregnancy, proliferation was activated from day 10 and peaked on day 20 of pregnancy (Fig. 1). By postpartum day 1, the concentration of myocytes in myometrium sharply decreased and by day 10 was equal to that of control mice (Fig. 1). The content of myocytes with estrogen-receptor complexes in the nucleus (ER-myocytes) 2-fold increased by day 10 of pregnancy, then decreased by ~5 times by postpartum day 1, and returned to the control level by day postpartum 10 (Fig. 1). Along with other data on structural transformations in the myometrium, this indicated completion of the post-partum involution [11]. Spearman correlation coefficient demonstrated a strong negative relationship (-0.87) between the content of ER-myocytes and pregnancy term and a strong positive relationship (+0.83) between this parameter and postpartum term, that it is quite logical. Increased total concentration of myocytes in the myometrium coincided with increased content of myocytes carrying nuclear progesterone-receptor complexes (PR-myocytes), which in turn had a strong positive correlation (+0.74), but in postpartum, weak negative correlation (-0.21) despite the "peak" on day 3 (Fig. 1). ER-myocytes predominated in the myometrium of intact mice and constituted 80%. A small fraction of myocytes had receptors to both hormones, because their sum exceeded 100% (Table 1). PR-myocytes prevailed by the end of pregnancy. However, at this stage and on day 1 postpartum, only about half of all myocytes demonstrated the presence of one or another receptor. This phenomenon was determined by myocyte dedifferentiation due to their active proliferation (Fig. 1), but later the relative number of cells expressing the nuclear receptors for investigated hormones increased (Table 1). The ER-/PR-myocyte ratio recovered on day 10 after delivery, but more than 20% myocytes carried receptors for both hormones, because their sum was more than 120% (Table 1). These findings can be explained by fusion of myocytes carrying receptors to different hormones.

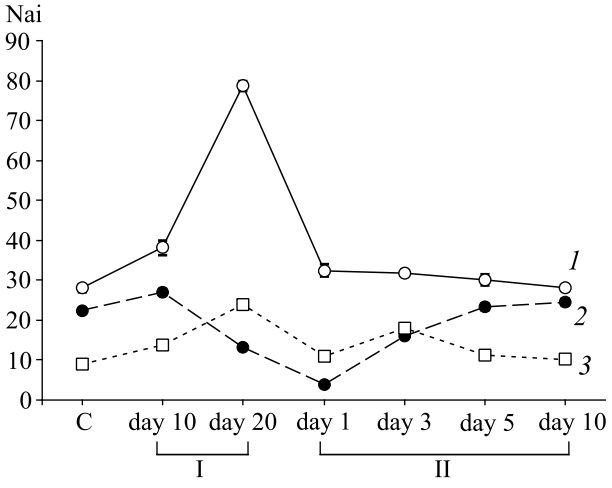

Fig. 1. Numerical densities (Nai) of myocyte nuclei (*1*) and ER- (*2*) and PR-myocyte nuclei (*3*) in the myometrium of C57Bl/6g mice during the first pregnancy (I) and after the first delivery (II). Here and in Fig. 2, 3: C: controls (intact mice).

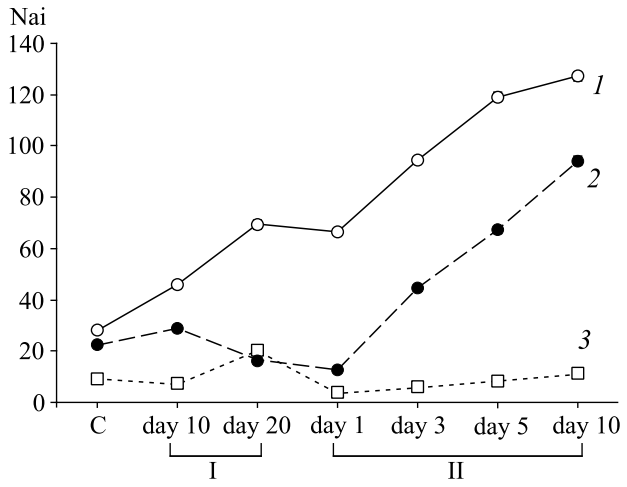

Fig. 2. Numerical densities (Nai) of myocyte nuclei (*1*) and ER- (*2*) and PR-myocyte nuclei (*3*) in the myometrium of C57Bl/6g mice during the third pregnancy (I) and after the third delivery (II).

Morphometric data published by us earlier also indicated the possibility of myocyte fusion during the postpartum period [10]. Simultaneous expression of different steroid receptors (estrogen and progesterone) in the same cell cannot also be excluded [12].

The third pregnancy and postpartum period were accompanied by active myocyte proliferation (Fig. 2). Proliferation processes competed with postpartum myocyte elimination; myocytes became smaller [10]. After delivery, changes in the content of ER-myocytes and the dynamics of changes in total myocyte concentration had a similar vector (Fig. 2). Nevertheless, rank correlation coefficients between the content of ER-myocytes and gestational stages (pregnancy and postpartum) were as high as during the first pregnancy: -0.79 and +0.97, respectively. The changes in the concentration of PR-myocytes were also similar to those reported during and after the first pregnancy

TABLE 1. Numerical Densities (Nai) of Nuclei of Myocytes Carrying Estrogen- and Progesterone-Receptor Complexes (*M*±*m*)

Condition		Studied structures				
		ER-myocytes, Nai	PR-myocytes, Nai	ER-myocytes, %	PR-myocytes, %	ER/PR-myocytes, %
Control (intact mice)		22.44±0.65	9.13±0.47	80.00±0.28	32.54±0.38	112.54±0.68
First pregnancy (group 2)	day 10	26.94±0.87*	13.85±0.47*+	70.67±0.95*+	36.33±0.69*+	107.00±1.47*
	day 20	13.20±0.55	23.30±0.82+	16.75±0.68*+	29.57±0.87*+	46.32±0.77*+
Postpartum	day 1	3.80±0.32	10.95±0.58+	11.69±0.50*+	33.71±0.73+	45.40±0.61*
	day 3	16.10±0.59	17.90±0.61	50.50±0.78*+	56.14±0.87*	106.64±1.56*+
	day 5	23.45±0.87	11.30±0.48+	78.03±0.74*+	37.60±0.76*+	115.63±1.50*+
	day 10	24.38±0.84*	10.25±0.46+	86.90±0.99*+	36.55±0.65*+	123.45±1.51*+
Third pregnancy (group 3)	day 10	28.90±0.88*#	7.21±0.39*o#	62.74±0.95*o#	15.65±0.67*o#	78.39±0.81*#
	day 20	16.54±0.42#	20.23±0.63o#	23.78±0.47*o#	29.09±0.69*o	52.87±0.58*o#
Postpartum	day 1	12.83±0.40#	3.40±0.32o#	19.29±0.54*o#	5.11±0.42*o#	24.4±0.5*o#
	day 3	44.75±0.95#	5.64±0.37o#	47.34±0.98*o#	5.96±0.43*#	53.3±0.7*o#
	day 5	67.29±1.12#	8.53±0.32o#	56.53±0.89*o#	7.16±0.39*o#	63.69±0.78*o#
	day 10	94.14±1.57*#	11.00±0.51*o#	74.02±1.42*o#	8.64±0.44*#	82.66±0.98*o#
Third pregnancy after interrupted second pregnancy (group 4)	day 10	11.42±0.48*#&	5.34±0.57*x#&	13.99±0.57*x#&	6.54±0.60*x#&	20.53±0.58*#&
	day 20	32.13±0.73#&	8.70±0.36x#&	36.45±0.81*x#&	9.87±0.43*x#&	46.37±0.62*x&
Postpartum	day 1	31.40±0.68#&	2.57±0.25x#	77.11±0.79*x#&	6.31±0.37*x#	83.42±0.67*x#&
	day 3	48.66±0.77#&	3.24±0.40x#&	53.61±0.82*x#&	3.55±0.46*x#	57.16±0.74*x#&
	day 5	52.32±0.92#&	9.14±0.26x#	42.47±0.83*x#&	7.41±0.24*x	49.88±0.53*x#&
	day 10	60.60±0.98*#&	13.08±0.65*x#&	46.38±0.74*x#&	10.01±0.52*x#&	56.39±0.66*x#&

Note. $p<0.05$: *in comparison with the control; between the indices of group +2, o3, x4; #with group2, &with group 3.

(Fig. 2), but Spearman correlation coefficient for their concentration and appropriate gestational stages were different: +0.87 in pregnancy and +0.72 postpartum. However, high levels of proliferation and apparently associated with them large proportion of dedifferentiated myocytes in postpartum population determined reduced relative concentration of ER- and PR-myocytes as in general and in their ratios, especially on day 1 after delivery (Table 1). By postpartum day 10, their concentrations did not return to the control level (Table 1), although the processes of elimination of "excessive" structures via mechanisms of clasmatosis and apoptosis were already finished, *i.e.* their parameters corresponded to those in intact mice [11].

High level of myocyte proliferation and sharply reduced myocyte number on day 1 after delivery were detected during the third pregnancy occurring after second pregnancy interruption like in two above experiments (Fig. 3). Similarly to the third pregnancy, myocyte proliferation in the myometrium was re-activated. Numerical density of ER-myocytes decreased by 50% by day 10 of pregnancy and then gradually increased and 2.5-fold exceeded the control value by the 10th day postpartum (Fig. 3). Rank correlation coefficients between the content of ER-myocytes and gestational stage were high and positive: +0.87 in pregnancy and +0.81 after delivery.

In this experiment, the absolute concentration of PR-myocytes (Nai) during pregnancy and postpartum involution was within the normal range (intact animals) or significantly lower (Fig. 3). Rank correlation coefficients between the content of PR-myocytes and gestation stage had similar sign, but differ in magnitude from those of the previous experiment (third pregnancy): +0.37 in pregnancy and +0.80 postpartum. The relative concentrations of both types of myocytes differed considerably. On the 10th day of pregnancy, their summary value far exceeded 20% and on the 10th day after delivery it slightly surpassed 50% (Table 1). Involution was not completed by day 10 after delivery [6].

In contrast to the first physiological pregnancy, repetitive pregnancy is accompanied by obvious changes in hormonal regulation and proliferative and involutive processes in the myometrium. However, interruption of pregnancy is apparently associated with gross violations of the regulation of myometrial proliferation and involution creating non-optimal conditions for the beginning and development of subsequent pregnancy.

The above processes (pregnancy and postpartum involution) are physiologically similar under different experimental conditions, but at the cellular level they significantly differ by their magnitude and the dynamics of the investigated parameters during pregnancy and especially during the postpartum period. This implies the existence of a nonspecific factor initiating proliferation and involution in addition to estrogen, progesterone, prolactin, adrenal steroid hormones, and extraembryonic organs [5,15,13]. This factor may be active oxygen metabolites formed due to hypoxia and oxidative stress occurring in the myometrium during pregnancy, delivery, and immediate postpartum period. They can initiate proliferation [9,2,7], apoptosis [3], blebbing [4] and clasmatosis. It should be noted that the vascularity of the myometrium during the third pregnancy and especially after birth was significantly lower than in intact mice and in the first pregnancy, and lowest at the third pregnancy occurring 2 weeks after the second interrupted pregnancy [6,11].

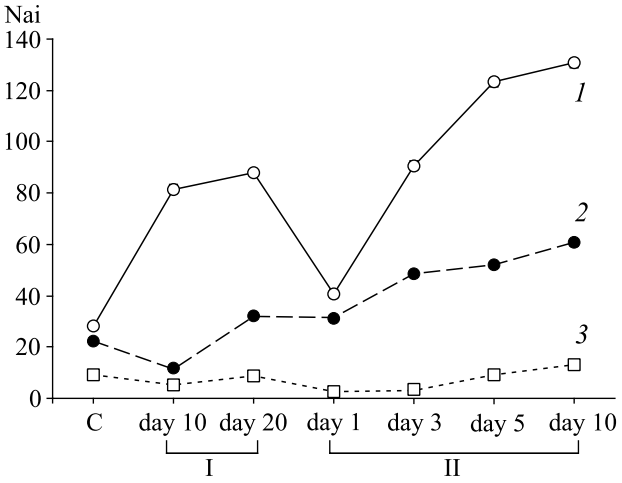

Fig. 3. Numerical densities (Nai) of myocyte nuclei (*1*) and ER- (*2*) and PR-myocyte nuclei (*3*) in myometrium of C57Bl/6g mice during the third pregnancy (I) occurring after second pregnancy interruption and after delivery (II).

REFERENCES

1. L. V. Adamyan, T. G. Borovaya, Z. V. Makiyan, and M. V. Bobkova, *Problemy Reproduktsii*, No. 6, 71-77 (2007).
2. L. V. Vanko, V. G. Safronova, and N. K. Matveeva, *Akusherstvo i Ginekologiya*, No. 2, 7-11 (2010).
3. N. K. Zenkov, V. Z. Lankin, and E. B. Menshchikova, *Oxidative Stress* [in Russian], Moscow (2001).
4. A. I. Inzhutova, A. B. Salmina, M. M. Petrova, *et al*., *Byull. Sib. Otd. Ross. Akad. Med. Nauk*, No. 1, 6-10 (2007).
5. N. E. Lenis and N. A. Koren, *Problemy Reproduktsii*, No. 3, 24-29 (2003).
6. K. S. Obedinskaya, V. A. Skurupiy, and A. P. Nadeev, *Vestn. Novosib. Gos. Univ.*, **8**, No. 3, 5-11 (2010).
7. E. V. Ozhegov, E. Yu. Zhivotova, O. A. Lebedko, *et al*., *Byull. Eksp. Biol. Med.,* **152**, No. 10, 400-403 (2011).
8. I. Yu. Torshin, O. A. Gromova, G. T. Sukhikh, *et al*., *Ginekologiya*, **11**, No. 5, 99-107 (2009).
9. K. T. Turpaev, *Biokhimiya*, **67**, No. 3, 339-352 (2002).
10. V. A. Skurupiy, K. S. Obedinskaya, and A. P. Nadeev, *Byull. Eksp. Biol. Med.,* **149**, No. 5, 487-491 (2010).
11. V. A. Skurupiy, K. S. Obedinskaya, and A. P. Nadeev, *Byull.*

Eksp. Biol. Med., **150**, No. 9, 347-351 (2010).
12. C. Ballare, M. Uhrig, T. Bechtold, *et al*., *Mol. Cell. Biol*., **23**, No. 6, 1994-2008 (2003).
13. K. D. Carpenter and K. S. Korach, *Ann. New York Acad. Sci*, **1092**, 361-373 (2006).
14. D. J. Leahy, *Adv. Protein Chem*., **68**, 1-27 (2004).
15. V. Syed, G. Ulinski, S. C. Mok, *et al*., *Cancer Res.*, **61**, No. 18, 6768-6776 (2001).