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# The morphofunctional pattern of neuronal biogenic amines in postpartum involution period - *in vivo* study

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**Summary.** Postpartum uterine diseases are associated with significant imbalance in the levels of biogenic amines (BAs) in rat uterus. Mast cells (MCs) are the main suppliers of BAs such as serotonin, catecholamines, and histamine in uterus. There is limited evidence of the BA-positive elements involved in the physiological regulation of uterus during postpartum involution. The aim of present study is to determine the concentration and distribution of biogenic amines (BAs) such as histamine, serotonin, and catecholamines in the uterine endometrium, myometrium, and peritoneal fluid (PF) during the postpartum uterine involution. A total of 110 nulliparous outbred female nonpregnant Wistar rats of mature age were divided into eleven groups (n=10 per group) according to days of postpartum involution. Tissue specimens of uterine segments, PF were prepared. Serotonin, catecholamines, and histamine concentrations were examined by fluorescence-histochemical techniques. The fluorescence of the BA-positive elements was detected and analyzed by microspectrofluorimetry. Results were analyzed using the Kruskal-Wallis chi-squared test and pairwise Mann-Whitney-Wilcoxon tests with "Benjamini-Hochberg correction" in R 3.6.3. Mast cells in uterine segments, PF exhibited characteristic yellowish-green fluorescence. The highest MCs number was reported in corpus uteri on the 15th day of postpartum involution. Serotonin, catecholamines, and histamine levels were significantly higher in BA-positive elements in the initial days. BA content was dynamic and relies on the time elapsed after parturition. There was statistically significant difference in the levels of BAs in the cornu and cervix uteri. A single morphofunctional complex of BA supply was noticed in the reproductive system of the rats. The coupled interactions of intra- and extra-organic BA-positive elements was associated with anabolic/catabolic equilibrium in uterus through the metabolism of serotonin, catecholamines, and histamine during postpartum involution.

**Key words:** Uterine corpus, Postpartum involution, Mast cells, Histochemical analysis

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## Introduction

Physiological changes in uterus are observed in adult life typically during pregnancy. Uterus undergoes

**Abbreviations.** BAs, biogenic amines; ECBS, extra-organic complex of bioamines supply; ICBS, intra-organic complex of bioamines supply; MCs, mast cells; PF, peritoneal fluid remodeling to return to normal pre-pregnancy condition via 'uterine involution'. Damaged tissue processes across uterus can induce gynecological complications such as uterine infections, post-partum hemorrhage, reproductive and placental abnormalities (Rosen, 2008). According to WHO reports 2018, approximately 303000 women died after childbirth due to pregnancy-related uterine complications (Alkema et al., 2016). Therefore, it is essential to delineate the underlying mechanisms associated with postpartum uterine involution to produce therapeutic modalities for preventing maternal mortality, and uterine subinvolution. Several mediators such as cytokines (Galvão et al., 2011), biogenic amines (Hansson et al., 2009), β-defensins (Alan and Liman, 2012), Toll-like receptors (Alan and Liman, 2018), growth factors (Yoshii et al., 2014; Alan et al., 2015; Sağsöz et al., 2015), and matrix-degrading enzymes (Takamoto et al., 1998; Nguyen et al., 2016) are reported to be involved in uterine involution (Zheng et al., 2019). In addition, the majority of common postpartum reproductive disorders are associated with a delay in the process of uterine involution. In these disorders, a significant imbalance of biogenic amines is evident across uterine tissues. Fluorescence-histochemical analysis is reported to be a highly specific and sensitive method to unravel the formaldehyde-induced fluorescence of intra- and extra-uterine BA-positive elements as a prognostic criterion during the disruption of uterine involution (Forsythe, 2019; Dindyaev et al., 2019).

Degranulation of MCs could release neurotransmitters such as biogenic amines in response to extracellular stimuli (Forsythe, 2019). The BAs produced during this process exhibit a wide range of biological effects on organs and play a key role in the neurohumoral regulation of uterine functions (Petryankin et al., 2015). MCs also exhibit an important role as a regulator of the neuronal BAs homeostasis (Dindyaev, 2012; Menzies et al., 2012; Dindyaev et al., 2019). The changes in cellular metabolic rate of effector tissues are facilitated through BAs, and they can alter homeostatic balance in the microenvironment of tissues including uterine tissues (Upadhyaya et al., 1993; Dindyaev, 2012). Hence, anabolic and catabolic equilibrium mediating through BAs can contribute an important role in postpartum involution (Salamonsen, 2003, Berger et al., 2009). However, there is lack of evidence to demonstrate the link between intra-uterine BA-positive elements of uterine endometrium & myometrium and extra-uterine BA-positive elements such as PF and peritoneal MCs involved in regulating the physiology of postpartum uterine involution. The aim of the present study is to determine the concentration and distribution of BAs such as histamine, serotonin, and catecholamines in the rat uterus during the postpartum involution.

## Materials and methods

### *Ethical approval*

Animal ethical approval to execute the in vivo experiments were provided by Institutional Ethical Committee, Ivanovo State Medical Academy & First affiliated hospital of Zhengzhou University (Henan, China) in accordance with the International Guidelines on conducting studies involving the use of experimental animals as per the Directive 2010/63/EU of the European Parliament for animal use for scientific purpose' and 'NIH Guidelines for the Care and Use of Laboratory Animals' and the Order Number 724 of the Ministry of Higher Education (Minvuz, Soviet Union, 13.11.1984). Cervical dislocation was used to the euthanized animals.

### *Animals and sample collection*

The total number of nulliparous outbred nonpregnant female Wistar rats of 110 with mature age were segregated into eleven groups (n=10 per group). Rats were housed under standard vivarium conditions: maintained at 12-hour light and 12-hour dark cycle (light 8:00-20:00), and the ambient temperature maintained at 22±2°C with free access to water and pelleted rodent chow. The results were analyzed in periods of postpartum involution - 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 days during the Fall-Winter time frame.

### *Tissue preparation*

The uterus was isolated from the abdominal cavity. Uterine cervix and cornu were separated from the corpus as well. Subsequently, the tissue specimens were frozen in a cryostat at -25°C. The regions of uterus (corpus, cervix, and cornu uteri) were fixed in 10% formalin. After fixation, the cryostat sections of 20 μm thick were embedded in paraffin.

The isolation of MCs in PF was performed by injecting Hanks' buffered salt solution containing 1% fetal calf serum into the rat peritoneal cavity. Subsequently, PF was collected after a brief massage of the anterior abdominal wall followed by a midline incision of peritoneal cavity under deep anesthesia (Pentobarbital). Fluid with MCs was isolated with the Pasteur micropipette from the uterine parametrium, and a fluid smear was prepared for analysis.

### *Fluorescence-histochemical methods*

a) Serotonin and catecholamines detection

The sections were incubated in a solution of 5% paraformaldehyde (Merck, Germany) in 0.2 mol/l phosphate buffer (pH 7.4) at room temperature for 1 hour, followed by heating of the sections at 80°C for 15 minutes. MCs with serotonin and catecholamines were detected by green-yellow fluorescence using Falck-Hillarp formaldehyde fluorescence technique in E.M. Krokhina modification (Krokhina and Alexandrov, 1969).

b) Histamine detection

The paraffin was removed from tissue samples by washing with xylene. The sections were stained with 1% O-phthalaldehyde (Merck, Germany) for 30 seconds in a desiccator. Cell suspension from PF was subjected to the centrifugation at 4°C for 10 minutes (1500 rpm) and subsequently washed with sodium borate-carbonate buffer (pH=10.6) and Tyrode's solution. PF with cells was spotted on the glass slides and covered it with cover slip coated with film of 1% O-phthalaldehyde for 30 seconds in xylene. Green-yellow fluorescence was emitted by MCs containing histamine due to metachromatic granules on microspectrofluorometry.

*Microspectrofluorimetric quantification of BAs*

The tissue sections were examined under a luminescent microscope LUMAM-I3 with a set of excitation filters matched to the modes of BAs fluorescence (JS-18, FS 1-2, SZS 24-4, BS 8-3). Fluorescence generated by the histamine, serotonin, and catecholamines was differentiated by microspectro-fluorimetry with setup FMEL-1A photometric conductor (output voltage 2100 V, FAU-73). To elucidate the concentration of catecholamines, serotonin, and histamine in the uterine segments, the 0.1 probe of the photometric attachment was used. The luminescence intensity was determined in arbitrary units of fluorescence on the scale provided by the detector. The serotonin-paraformaldehyde fluorophore was produced after formaldehyde-induced fluorescence at excitation maximum at 370-410 nm and emission maximum at 520-530 nm. Catecholamines fluorescence was observed at excitation and emission maxima 410 nm and 480-490 nm, respectively. The histamine fluorescence was recorded in the yellow region of the spectrum with a maximum of excitation at 365-410 nm and emission at 515-520 nm. BAs detected by this method have a linear relationship between the intensity of the fluorescence and their concentrations (Enerbäck and Wingren, 1980).

*Statistical analysis*

Statistical analysis was performed using R 3.6.3. The significant difference between the days of study was calculated according to the results of the Kruskal-Wallis chi-squared test. A pairwise Mann-Whitney-Wilcoxon test with "Benjamini-Hochberg correction" was used to ascertain the significant difference between each variable. Student's t-test was used to verify the significant differences exhibited between the studied groups. MCs count was expressed as the Mean ± S.E.M. The p-value was set to be <0.05.

## Results

*Distribution of MCs*

The wider distribution of MCs was observed in endometrium and myometrium of the cervix, cornu and corpus uteri in all the experimental groups. MCs in the corpus, cervix, and cornu uteri were shown by the characteristic yellowish-green fluorescence. Furthermore, MCs also distributed across the endometrium and myometrium of rat uterus, but primarily visible in the myometrial smooth muscle cells. MCs are predominantly perivascular and most of their cytoplasm is densely filled with granules. The spatial distribution of the fluorescent MCs density in the endometrium and myometrium of the corpus uteri was minimal during the 1$^{st}$ day. By the 6$^{th}$ day, the fluorescent MCs density has increased approximately 2-fold in the endometrium and 4-fold in the myometrium. The highest fluorescence intensity of MCs was observed in corpus uteri on the 10$^{th}$ day of postpartum involution (visually well differentiated by specific yellow-green intensive fluorescence of granularity in the cytoplasm and non-fluorescent nucleus. The peritoneal MCs with characteristic degranulation was effectively distinguished (Fig. 1A-D).

*Microspectrofluorimetry*

Microspectrofluorimetry results reported the presence of histamine, serotonin, and catecholamines in MCs (Fig. 2A-D). (i) The presence of BAs was observed in endometrium and myometrium of uterus, but very limited in the uterine cavity contents. (ii) The highest concentrations of histamine and serotonin were in the myometrium of the cervix uteri (Fig. 2D), while catecholamines were found in the endometrium of the cornu uteri in the initial days of the study (Fig. 2B). (iii) Subsequently, the catecholamines and histamine concentrations were peaked on 7$^{th}$ day in the endometrium and myometrium of the cornu uteri. (iv) the decline in histamine, and catecholamines were observed starting from 8$^{th}$ day, and reaching the lowest values, except for histamine in the myometrium of the cornu uteri. (v) The catecholamine levels in cervix uteri and cornu uteri varied in a similar wave-like pattern (Fig. 2B,C). (vi) Further, the serotonin concentration in uterine endometrium was increased at the end of study (Fig. 2B,D).

The serotonin concentration in the endometrium and myometrium of the cornu uteri fluctuated initially and, thereafter, followed a similar pattern (Fig. 2A,B). The concentrations of catecholamines, serotonin and histamine were identified microspectrofluorimetrically in the extra-uterine BA-positive specimens (peritoneal MCs and liquid phase of PF) during postpartum involution. During the initial days after parturition, the concentration of histamine, serotonin, and catecholamines were extensively higher in peritoneal

MCs than PF. The catecholamines and serotonin concentration followed the similar pattern of fluctuations in MCs and PF. Serotonin was increased from 4$^{th}$ to 5$^{th}$ day, and then decreased with a gradual increase after the 7$^{th}$ day. The maximum concentration of histamine was found in peritoneal MCs, but by the 15$^{th}$ day of the study, it gradually had decreased in PF. The BA content was dynamic depending on time elapsed after parturition (Fig. 3A,B).

Based on the results of the study, significant differences in the level of BAs in the cervix and cornu uteri were revealed. Kruskal-Wallis chi-squared statistical analysis delineated that there was a statistically significant difference between 'intra-uterine BA-positive elements' and 'days of postpartum involution in eleven groups of animals' ($p<0.05$, $p<0.01$, $p<0.001$, $p<0.0001$). The Mann-Whitney-Wilcoxon tests were carried out to ascertain the significant difference among the groups. The catecholamines concentration in myometrium of uterus on 4-8$^{th}$ days does not significantly differ in groups (4, p=0.46-0.91; 5, p=0.36-0.46; 6, p=0.21-0.77) (Table 1).

The serotonin concentrations in uterine myometrium on 4-7$^{th}$ days were not statistically significant in groups such as 4, (p=0.42-0.76), 5, (p= 0.34-0.77), 6 (p=0.32-0.77) and on 10-15$^{th}$ days in groups 10, (p=0.83) and

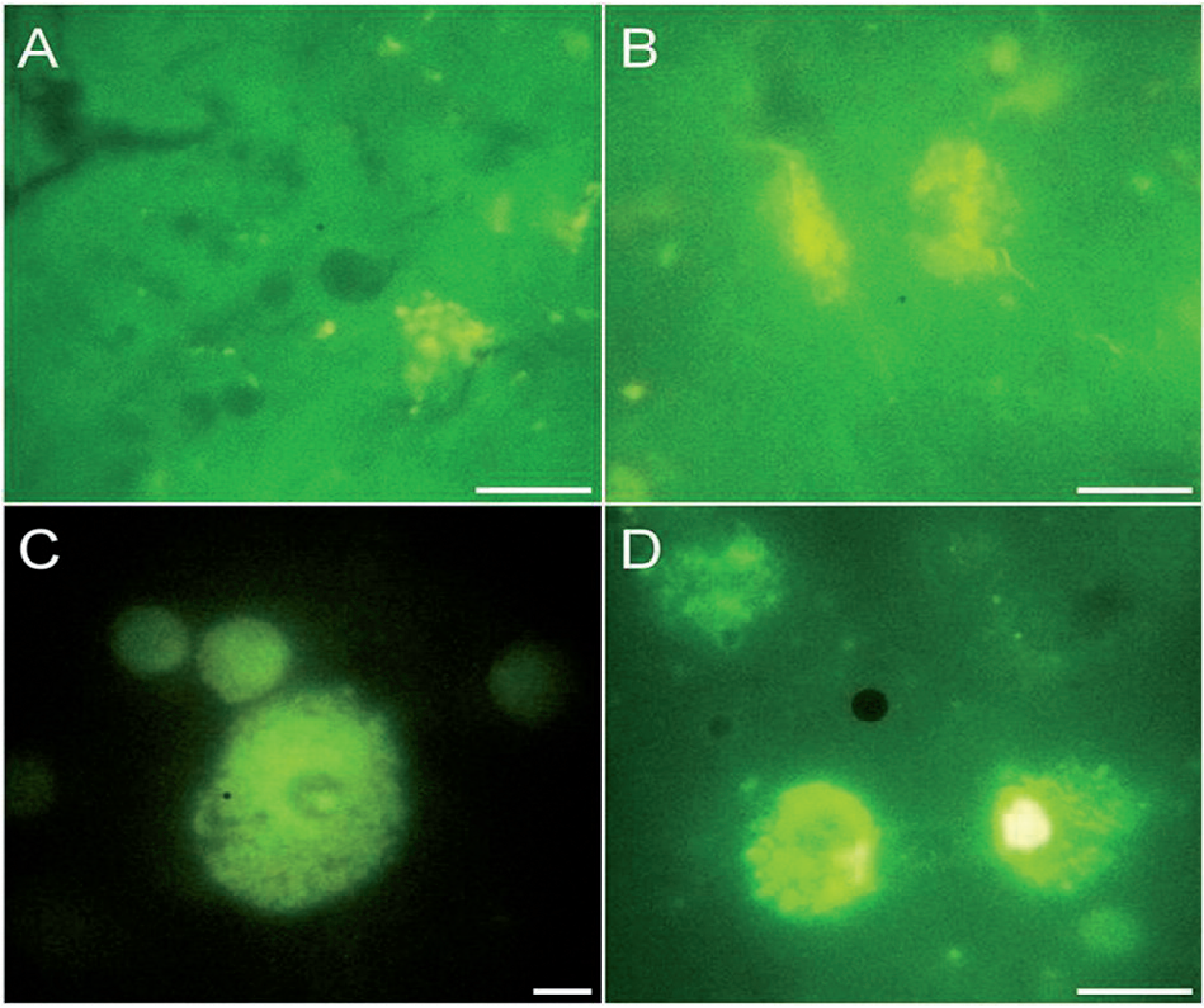


**Fig. 1.** Distribution of mast cells in the corpus uteri and the PF. Mast cells in the endometrium of the corpus uteri. The Falk-Hillarpa method in E.M. Krokhina modification, microscope LUMAM-I3. **A.** The 5th day of postpartum involution. **B.** The 10th day of postpartum involution. Peritoneal mast cells on Day 7 after parturition. Method of Cross, Evan, Rost, microscope LUMAM-I3. **C.** Lymphocytes with a characteristic greenish-yellow fluorescence around the cell in the PF (obj. 90 imm.). **D.** Degranulated mast cell (obj. 60 imm.). Scale bars: A, B, D, 100 µm; C, 20 µm.

11(p=0.96) (Table 2). In the uterine endometrium, the level of monoamine was not significantly different in group 7 (p=0.11-0.73) on days 6-7$^{th}$ and in groups 4, (p=0.41-0.58); 5, (p=0.55-0.79) on 10-15$^{th}$ days.

There was no significant difference in histamine concentrations across ‘the myometrium of the cervix uteri’ in groups 4(p=0.88), 5 (p=0.83) on the 5-6$^{th}$ days and on the 9$^{th}$ day - group 9, p=0.69 (Table 3). The histamine level in the endometrium of the cornu uteri was not statistically significant in group 5 (p=0.41) on the 6$^{th}$ day and in group 10 (p=0.62) on the 15$^{th}$ day.

Data for all the groups with ‘concentration of BAs’ and ‘days of postpartum involution’ were summarized as boxplots in Fig. 4A-H and Fig. 5A-H. Serotonin, catecholamines, and histamine levels were significantly higher in BA-positive elements on the initial days than on the 15$^{th}$ day. The serotonin level in the myometrium of the cornu uteri was not significantly different compared to the control values ($p<0.05$, $p<0.01$, $p<0.001$, $p<0.0001$). Moreover, the serotonin level was significantly different in uterine endometrium compared to the myometrium (Fig. 4B,F). The catecholamines level in myometrium slightly differed from control values and decreased in a wave-like pattern (Figs. 4E, 5E). Histamine level was reported to be fluctuated starting from 4$^{th}$ to 9$^{th}$ days in the endometrium and myometrium of cervix uteri (Fig. 5C,G).

The level of serotonin in the cervix (myometrium) exhibited similar characteristics as in the cornu uteri ($p<0.05$, $p<0.01$, $p<0.001$, $p<0.0001$). On the 10-15$^{th}$ days, the serotonin level was significantly higher than other values in endometrium of uterus. The changes in indicator of relative BA (serotonin-catecholamines) saturation (G-score) of BA-positive structures were depicted in Figs. 4H, 5H. In all studied samples, the G-score was increased on the 15$^{th}$ day. The G-score value

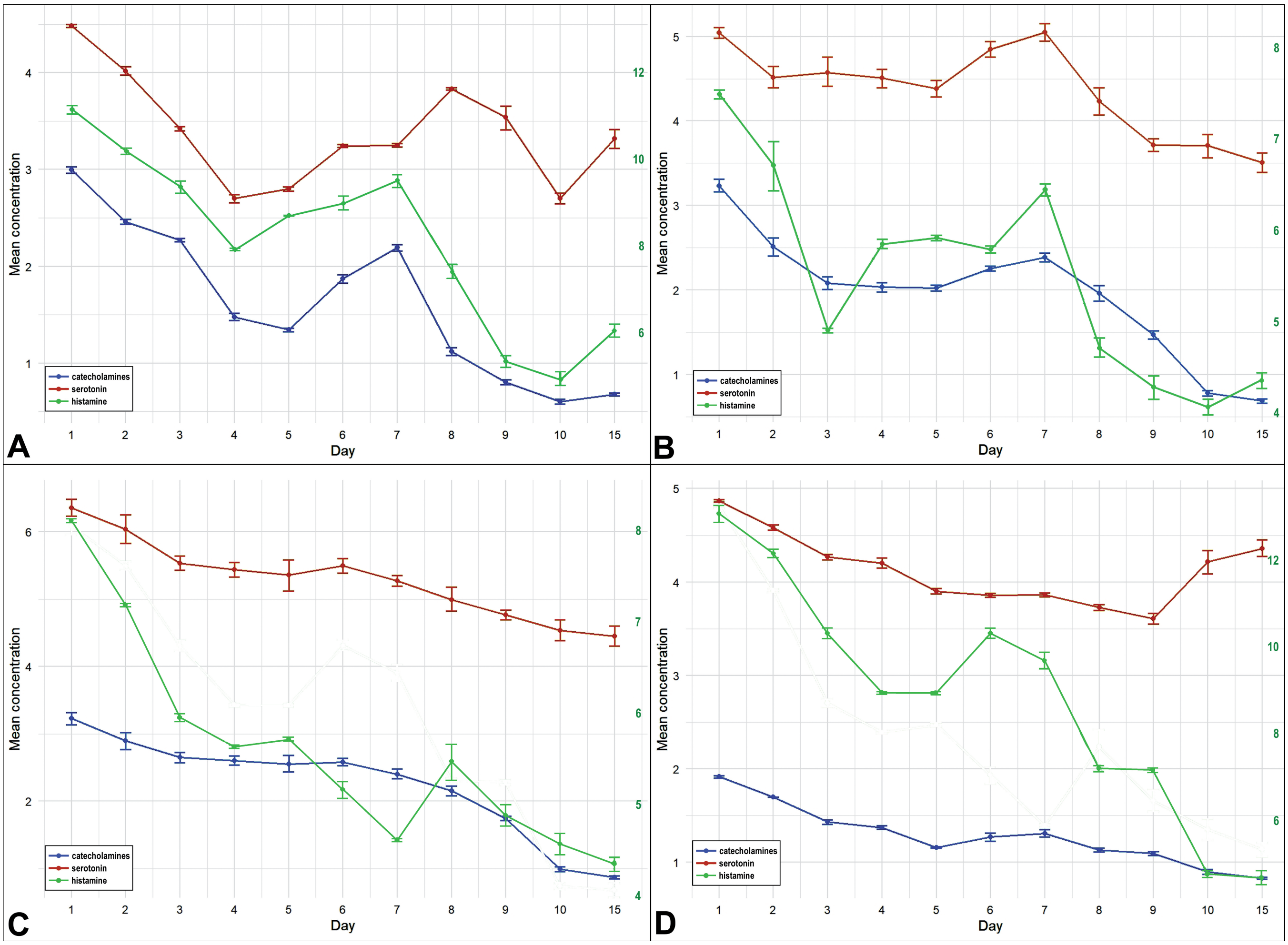


**Fig. 2.** Distribution of serotonin and catecholamines in rat uterus during the postpartum involution (p<0.05). **A.** Serotonin, catecholamines and histamine concentrations inthe myometrium of the cornu uteri. **B.** Serotonin, catecholamines and histamine concentrations in the endometrium of the cornuuteri. **C.** Serotonin, catecholamines and histamine concentrations in the myometrium of the cervix uteri. **D.** Serotonin, catecholamines and histamine concentrations in the endometrium of cervix uteri.

of the endometrium of the cervix and cornu uteri decreased significantly on the 6-7th days due to an increase in the catecholamines concentration and a decrease in serotonin that increased greatly on the 15th day. The highest values of G-score were registered in the uterine myometrium on 15th day.

## Discussion

In women, the uterus undergoes postpartum physiological involution after childbirth during a period of 6 to 8 weeks (Zheng et al., 2019). Several reports delineated the molecular mechanisms to foster the acceleration of postpartum involution to prevent post-partum complications. However, the molecular mechanisms associated with biogenic amines and their co-relation with postpartum uterine involution are not significantly unraveled. The results of the present study confirm our prior findings (Dindyaev, 2012; Dindyaev et al., 2019) on intra- and extra-uterine bioamine-containing specimens' co-location in one complex of bioamine supply (ICBS) and intra- and extra-organic complex of bioamines supply (ECBS). We hypothesized that these results could be related to morphometric characteristics of the uterus accompanied by the involvement of biogenic amine regulation during postpartum uterine involution. Spatial organization of BAs in ICBS is characterized by the non-equivalence of estimated parameters in our results section across different uterine segments during the postpartum involution. Spatial distribution of BAs at cellular level could be considered as a probe for determining a broad spectrum of cell physiological parameters; the dynamic alteration of BAs concentration was more evident in neurobiology (Unsicker, 1974; Wreford and Smith, 1982; Felten et al., 1990; van Noorden, 1991; Leger et al., 2001; Hara et al., 2016) cancer biology (Hara et al., 2016), and reproduction (Zupko et al., 2005; Chávez-Genaro et al., 2006). In our study, the fluorescence of intra- and extra-uterine BA-positive elements were identified by this histofluorescence technique and analyzed by microspectrofluorimetry and their results delineated a higher correlation between serotonin and catecholamines concentrations in BA-positive specimens. At the beginning of the postpartum period, the correlation between serotonin and catecholamines concentrations is moderate and followed by their enhancement in their levels.

A high positive correlation was observed between the serotonin and catecholamines concentrations in BAs-positive structures of uterus throughout the postpartum involution. These BAs level was restored during the involution, reflecting a general pattern of anabolic/catabolic equilibrium within the homeostasis of cells, tissues, and organs. These BAs were reported to act as antagonists in many aspects of their biological action (Berger, 2009), and their concentration should always be well-balanced in a normally functioning uterus (Wilcox et al., 2000). The optimal BAs ratio created a balance among metabolic processes, regenerative and functional activity in the "working cells" microenvironment consequently facilitates the adaptation. The localization of BA metabolism reflected the unequal distribution of structures as tissue cytoarchitecture components in different uterine segments during postpartum uterine involution. A high saturation of BAs was noticed in myometrial MCs, starting from 5th day of uterine involution. This may be related to their extensive activity during the parturition as MCs release serotonin, histamine, catecholamines, and other bioactive substances (Berger, 2009). An increase in serotonin and histamine levels in the blood in turn has declined their metabolic inactivation in the uterus, which further foster the 'bioavailability of

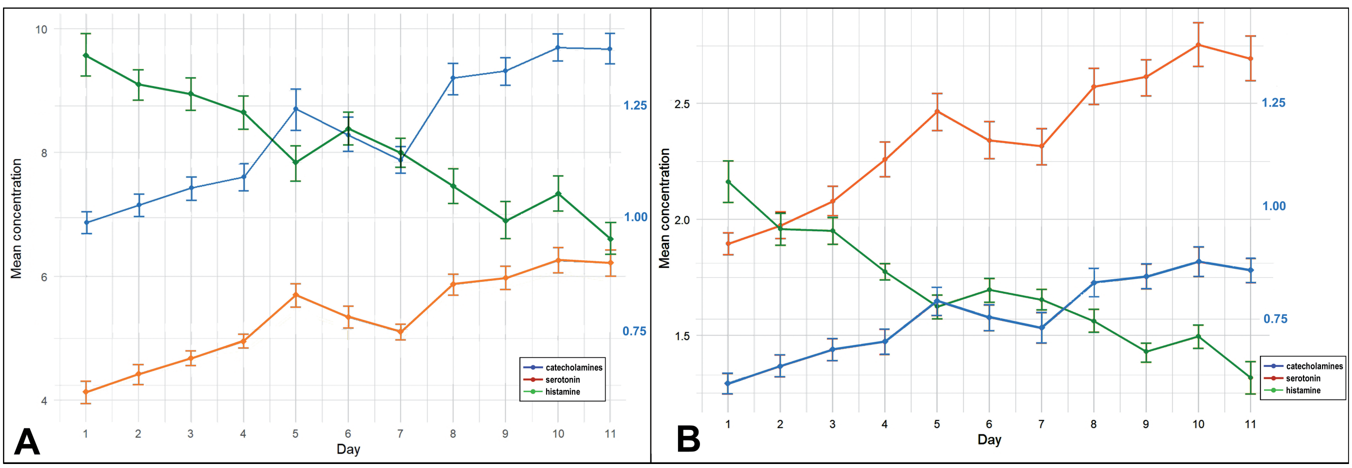


**Fig. 3.** Dynamics of BAs concentration in peritoneal mast cells and liquid phase of peritoneal fluid during the postpartum involution ($p<0.05$). **A.** The catecholamines, serotonin, and histamine concentrations in peritoneal MCs. **B.** The catecholamines, serotonin, and histamine concentrations in the liquid phase of peritoneal fluid.

serotonin' in uterine tissues.

MCs play a crucial role in the regulation of histophysiological processes in the uterus under normal and pathological conditions (Wilcox et al., 2000; Rudolph et al., 2004). Furthermore, MCs are the main source of histamine, serotonin, and catecholamines for

**Table 1.** Comparative statistical analysis of catecholamines concentration in the cornu and cervixuteri during the postpartum uterine involution (Results presented with the statistical analyses using Kruskal-Wallis chi-squared test and pairwise Mann-Whitney-Wilcoxon).

Catecholamines in the endometrium of the cornu uteri. Kruskal-Wallis chi-squared=508.8, df=10, p-value <0.0001

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | <0.0001 | | | | | | | | | | |
| 2 | <0.0001 | <0.0001 | | | | | | | | | |
| 3 | <0.0001 | <0.0001 | <0.0001 | | | | | | | | |
| 4 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | | | |
| 5 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | | |
| 6 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | |
| 7 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | |
| 8 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | |
| 9 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | |
| 10 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| 15 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

Catecholamines in the myometrium of the cornu uteri. Kruskal-Wallis chi-squared=903.41, df=10, p-value <0.0001

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | <0.0001 | | | | | | | | | | |
| 2 | <0.0001 | <0.0001 | | | | | | | | | |
| 3 | <0.0001 | <0.0001 | <0.0001 | | | | | | | | |
| 4 | <0.0001 | <0.0001 | <0.0001 | <0.05 | | | | | | | |
| 5 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.001 | | | | | | |
| 6 | <0.0001 | <0.0001 | <0.0001 | <0.001 | <0.0001 | <0.0001 | | | | | |
| 7 | <0.0001 | <0.0001 | <0.05 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | |
| 8 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.05 | <0.05 | <0.0001 | <0.0001 | | | |
| 9 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | |
| 10 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| 15 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

Catecholamines in the endometrium of the cervix uteri. Kruskal-Wallis chi-squared=489.99, df=10, p-value <0.0001

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | <0.0001 | | | | | | | | | | |
| 2 | <0.0001 | <0.0001 | | | | | | | | | |
| 3 | <0.0001 | <0.0001 | <0.0001 | | | | | | | | |
| 4 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | | | |
| 5 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | | |
| 6 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | |
| 7 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.01 | <0.0001 | <0.05 | | | | |
| 8 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.0001 | <0.0001 | | | |
| 9 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.01 | | |
| 10 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| 15 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

Catecholamines in the myometrium of the cervix uteri. Kruskal-Wallis chi-squared=849.72, df=10, p-value <0.0001

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | <0.0001 | | | | | | | | | | |
| 2 | <0.0001 | <0.0001 | | | | | | | | | |
| 3 | <0.0001 | <0.0001 | <0.001 | | | | | | | | |
| 4 | <0.0001 | <0.0001 | <0.001 | <0.05 | | | | | | | |
| 5 | <0.0001 | <0.0001 | <0.001 | <0.05 | <0.05 | | | | | | |
| 6 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.05 | <0.05 | | | | | |
| 7 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.01 | <0.0001 | | | | |
| 8 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | |
| 9 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | |
| 10 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| 15 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

uterine myometrium, endometrium and peritoneum. This plausibly explains the specific functional co-location of mast cells in ICBS and ECBS. Mori et al. (1997) reported that the 'stem cell factor' released by myometrial smooth muscle cells regulates the proliferation and differentiation of MCs. Horiuchi et al.

**Table 2.** Comparative statistical analysis of serotonin concentrationin the cornu and cervixuteri during the postpartum uterine involution (Results presented with the statistical analyses using Kruskal-Wallis chi-squared test and pairwise Mann-Whitney-Wilcoxon).

Serotonin in the endometrium of the cornu uteri. Kruskal-Wallis chi-squared=437.83, df=10, p-value <0.0001

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | <0.0001 | | | | | | | | | | |
| 2 | <0.0001 | <0.0001 | | | | | | | | | |
| 3 | <0.0001 | <0.0001 | <0.0001 | | | | | | | | |
| 4 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | | | |
| 5 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | | |
| 6 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | |
| 7 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 | | | | |
| 8 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | |
| 9 | <0.0001 | <0.0001 | <0.0001 | <0.001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | |
| 10 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.01 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| 15 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.0001 | <0.0001 | <0.05 | <0.05 | <0.0001 | <0.001 | <0.0001 |

Serotonin in the myometrium of the cornu uteri. Kruskal-Wallis chi-squared = 521.04, df = 10, p-value <0.0001

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | <0.0001 | | | | | | | | | | |
| 2 | <0.0001 | <0.0001 | | | | | | | | | |
| 3 | <0.0001 | <0.0001 | <0.01 | | | | | | | | |
| 4 | <0.0001 | <0.0001 | <0.05 | <0.05 | | | | | | | |
| 5 | <0.0001 | <0.0001 | <0.05 | <0.05 | <0.05 | | | | | | |
| 6 | <0.0001 | <0.0001 | <0.0001 | <0.001 | <0.0001 | <0.0001 | | | | | |
| 7 | <0.001 | <0.05 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.001 | | | | |
| 8 | <0.0001 | <0.0001 | <0.05 | <0.05 | <0.01 | <0.05 | <0.0001 | <0.0001 | | | |
| 9 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | |
| 10 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 | |
| 15 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.01 | <0.05 |

Serotonin in the endometrium of the cervix uteri. Kruskal-Wallis chi-squared = 471.32, df = 10, p-value <0.0001

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | <0.0001 | | | | | | | | | | |
| 2 | <0.0001 | <0.0001 | | | | | | | | | |
| 3 | <0.0001 | <0.0001 | <0.0001 | | | | | | | | |
| 4 | <0.0001 | <0.0001 | <0.0001 | <0.001 | | | | | | | |
| 5 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | | |
| 6 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.001 | | | | | |
| 7 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.01 | <0.05 | | | | |
| 8 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.0001 | <0.0001 | | | |
| 9 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.001 | | |
| 10 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.05 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| 15 | <0.0001 | <0.0001 | <0.0001 | <0.01 | <0.001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 |

Serotonin in the myometrium of the cervix uteri. Kruskal-Wallis chi-squared = 465.44, df = 10, p-value <0.0001

| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | <0.0001 | | | | | | | | | | |
| 2 | <0.0001 | <0.01 | | | | | | | | | |
| 3 | <0.0001 | <0.0001 | <0.0001 | | | | | | | | |
| 4 | <0.0001 | <0.0001 | <0.0001 | <0.05 | | | | | | | |
| 5 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.05 | | | | | | |
| 6 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.05 | <0.05 | | | | | |
| 7 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.05 | <0.01 | | | | |
| 8 | <0.01 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.01 | <0.0001 | <0.0001 | | | |
| 9 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 | | |
| 10 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| 15 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 |

(1999) and Mason et al. (2003) delineated the interaction between the MCs and smooth muscle cells of the uterus as well. These statements are consistent with our data as indicated by the significant positive correlation between changes in the content of 'histamine' and 'catecholamines' during postpartum uterine involution.

**Table 3.** Comparative statistical analysis of histamine concentration in the cornu and cervixuteri during the postpartum uterine involution (Results presented with the statistical analyses using Kruskal-Wallis chi-squared test and pairwise Mann-Whitney-Wilcoxon).

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Histamine in the endometrium of the cornu uteri. Kruskal-Wallis chi-squared = 642.18, df = 10, p-value <0.0001 | | | | | | | | | |
| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| 1 | <0.0001 | | | | | | | | | | |
| 2 | <0.0001 | <0.0001 | | | | | | | | | |
| 3 | <0.0001 | <0.0001 | <0.0001 | | | | | | | | |
| 4 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | | | |
| 5 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | | |
| 6 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.0001 | | | | | |
| 7 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | |
| 8 | <0.0001 | <0.0001 | <0.0001 | <0.001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | |
| 9 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | |
| 10 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.001 | |
| 15 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.0001 |
| | | Histamine in the myometrium of the cornu uteri. Kruskal-Wallis chi-squared = 839.8, df = 10, p-value <0.0001 | | | | | | | | | |
| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| 1 | <0.0001 | | | | | | | | | | |
| 2 | <0.0001 | <0.0001 | | | | | | | | | |
| 3 | <0.0001 | <0.0001 | <0.0001 | | | | | | | | |
| 4 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | | | |
| 5 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.001 | | | | | | |
| 6 | <0.0001 | <0.0001 | <0.0001 | <0.001 | <0.0001 | <0.0001 | | | | | |
| 7 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.0001 | <0.0001 | <0.0001 | | | | |
| 8 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.001 | <0.0001 | <0.0001 | <0.0001 | | | |
| 9 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | |
| 10 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| 15 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | | Histamine in the endometrium of the cervix uteri. Kruskal-Wallis chi-squared = 611.9, df = 10, p-value <0.0001 | | | | | | | | | |
| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| 1 | <0.0001 | | | | | | | | | | |
| 2 | <0.0001 | <0.0001 | | | | | | | | | |
| 3 | <0.0001 | <0.0001 | <0.0001 | | | | | | | | |
| 4 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | | | |
| 5 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | | |
| 6 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | |
| 7 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | |
| 8 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.01 | <0.01 | <0.0001 | | | |
| 9 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.001 | <0.0001 | <0.0001 | | |
| 10 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.01 | <0.0001 | <0.0001 | |
| 15 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 |
| | | Histamine inthe myometrium of the cervix uteri. Kruskal-Wallis chi-squared = 686.5, df = 10, p-value <0.0001 | | | | | | | | | |
| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| 1 | <0.0001 | | | | | | | | | | |
| 2 | <0.0001 | <0.0001 | | | | | | | | | |
| 3 | <0.0001 | <0.0001 | <0.0001 | | | | | | | | |
| 4 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | | | | |
| 5 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 | | | | | | |
| 6 | <0.0001 | <0.0001 | <0.0001 | <0.05 | <0.0001 | <0.0001 | | | | | |
| 7 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | | |
| 8 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | | | |
| 9 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 | | |
| 10 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| 15 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.05 |

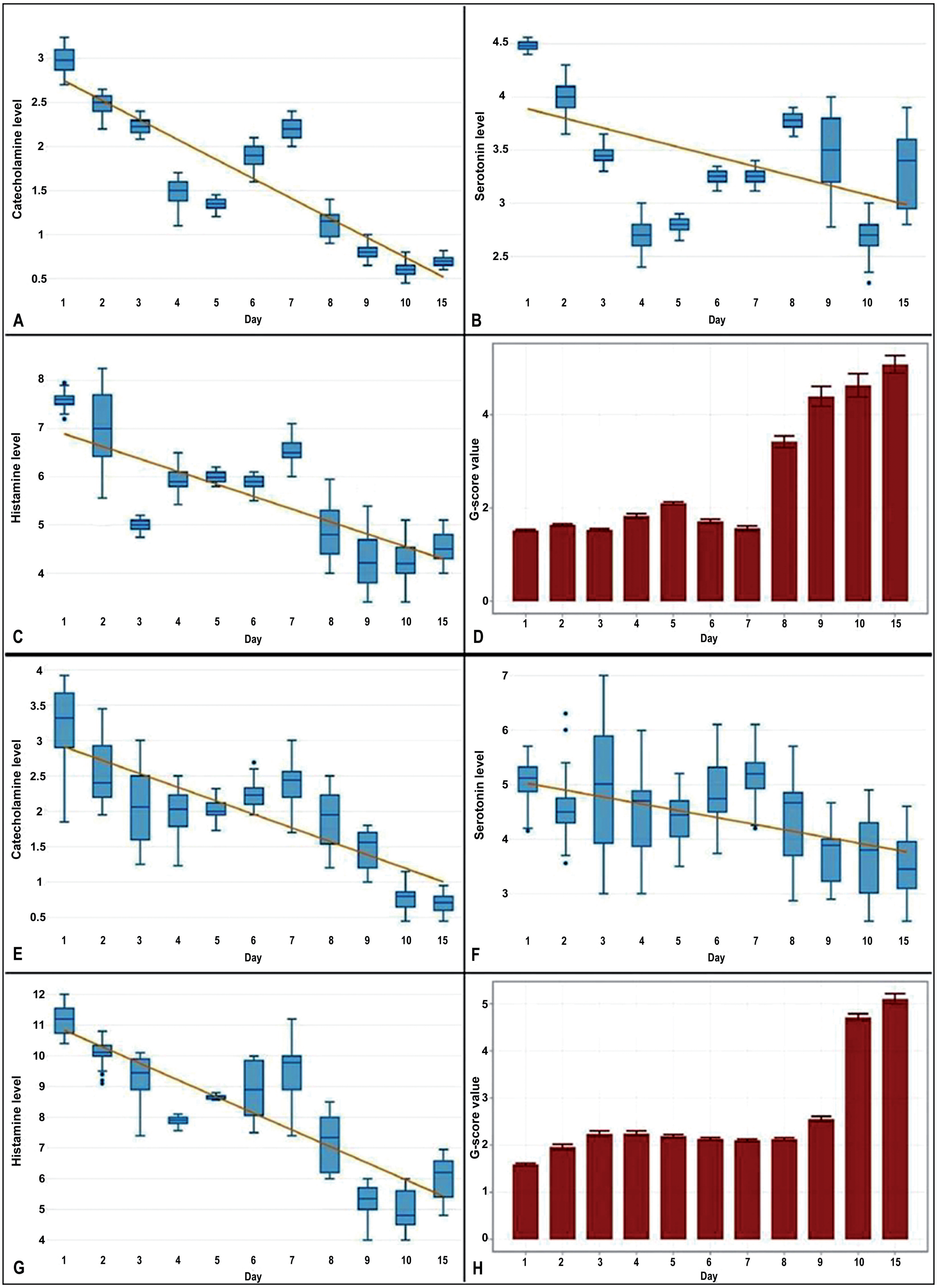


**Fig. 4.** Boxplots showing the relative BAs concentration of cornu uteri according to groups and days of postpartum involution. **A.** Catecholamines level in the endometrium of the cornu uteri. **B.** Serotonin level in the endometrium of the cornu uteri. **C.** Histamine level in the endometrium of the cornu uteri. **D.** G-score is forthe endometrium of the cornu uteri. **E.** Catecholamines level in the myometrium of the cornu uteri. **F.** Serotonin level in the myometrium of the cornu uteri. **G.** Histamine levels in the myometrium of the cornu uteri. **H.** G-score for myometrium of the cornu uteri.

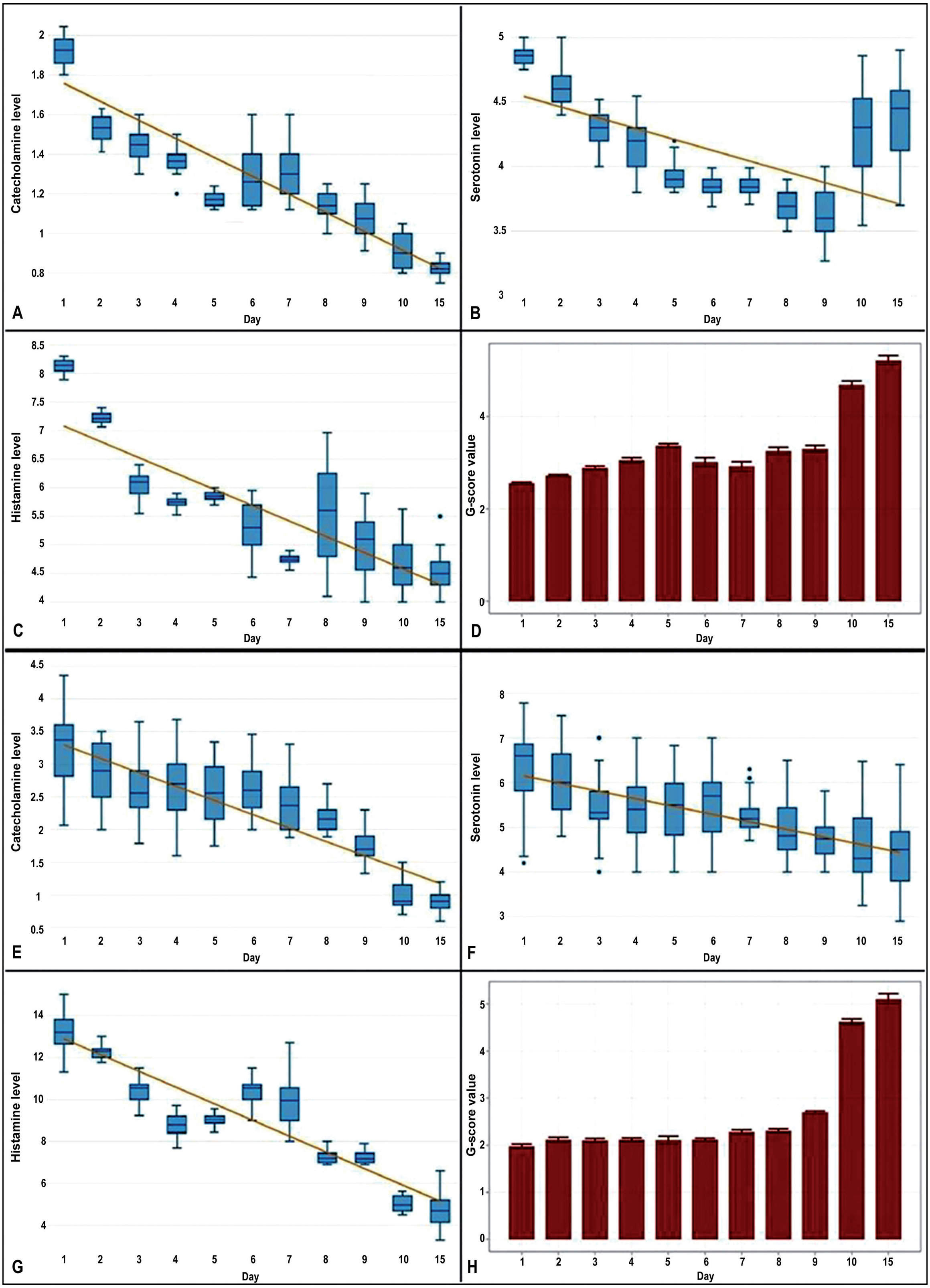


**Fig. 5.** Boxplots showing the relative BAs concentration of cervix uteri according to groups and days of postpartum involution. **A.** Catecholamines level in the endometrium of the cervix uteri. **B.** Serotonin level in the endometrium of the cervix uteri. **C.** Histamine level in the endometrium of the cervix uteri. **D.** G-score for endometrium of the cervix uteri. **E.** Catecholamines level in the myometrium of the cervix uteri. **F.** Serotonin level in the myometrium of the cervix uteri. **G.** Histamine level in the myometrium of the cervix uteri. **H.** G-score for myometrium of the cervix uteri.

Myometrial smooth muscle cells generate interstitial collagenase with serotonin stimulation to foster the postpartum involution of rat uterus (Jeffrey et al., 1991; Rydelek-Fitzgerald et al., 1993; Dumin et al., 1998). This is understood to be the reason for a significant increase in serotonin level in the myometrium of the cornu uteri from 5-9$^{th}$ days of parturition. Taking into account the aforementioned, it is determined that the induction of interstitial collagenase by serotonin influences an increase in the density of MCs in the uterus after parturition (Rydelek-Fitzgerald et al., 1993; Szelag et al., 2002; Bosquiazzo et al., 2005). Furthermore, a significant decrease in the serotonin level in endometrial and myometrial MCs immediately after parturition can be associated with its direct participation in the 'uterine tissue reconstruction' during postpartum involution.

On the 7$^{th}$ day, the population structure of myometrium of the cornu uteri is close to the parameters of prenatal uterus (Hsu et al., 2014), indicating the completion of involutional changes. Hence, the proliferation processes are adaptive in nature and are accompanied by an increase in smooth muscle cells in the population structure. Furthermore, we noticed a significant elevation of MCs degranulation in the myometrium and, subsequently, the histamine level in them on the 7$^{th}$ day after parturition. The highest levels of these monoamines are found in the myometrial MCs of cervix uteri. MCs are capable of absorbing, storing, and inactivating monoamine excesses if they have not been disposed of by "working cells" (Leon et al., 1994). Considering that MCs are major producer of histamine, serotonin, and catecholamines, the possibility of MCs functioning as a BA transporter between different uterine segments cannot be ruled out.

The total intracellular serotonin content can be estimated by a serotonin secretion index, which characterizes secretory activity of diffuse endocrine cells during postpartum involution (Hansson et al., 2009). Serotonin secretion decreases in gestation period, but it increases immediately after the parturition. Serotonin levels reach their highest value during the first few postpartum days and then serotonin secretion decreases.

Based on this, the BA status of ICBS and ECBS on the 10$^{th}$ and 15$^{th}$ days resembles the results in early and late stages of diestrus (Dindyaev et al., 2019). The uterine BA status is restored to a level corresponding to the histophysiological conditions of the sexual cycle (Dindyaev and Vinogradov, 2009). This indicates that BA-antagonists should be well-balanced exchange in the studied structures. Therefore, any imbalance can cause pathology of the female reproductive system. In ECBS, higher correlation coefficients characterize the changes in the dynamics of histamine concentration ($p<0.0001$). All correlated pairs exhibited a positive reliable relationship between changes in the BA status of mast cells in PF. PF is critical for supplying the biogenic amines across the membranes of uterine structures (Dindyaev et al., 2019). It has been observed that the significant correlations were observed in several estimated parameters of uterine BA status with fluctuations in parameters of the PF.

The present study demonstrates that there is a single morphofunctional complex of BA supply (ICBS/ECBS) in the reproductive system of rats. MCs have been reported to play a leading role in the neurohumoral regulation of histophysiological processes in the uterus during postpartum involution. The revealed patterns of dynamic changes in parameters reflect a significant coupled interaction of two links: intra- and extra-organic structures ensuring optimal BA metabolism. The ICBS is characterized by the histochemical organization's non-equivalence of estimated parameters in different uterine segments. Despite the differences in content and genesis, PF, the metabolism of uterine BAs has been leading to the synthesis, accumulation, release, functional realization, reuptake, inactivation, and transport of serotonin, histamine, and catecholamines in the uterine specimens. Therefore, this contributes to maintain the anabolic/catabolic equilibrium of BAs in the uterus to foster postpartum uterine involution.

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*Conflicts of interest/Competing interests (include appropriate disclosures).* Authors declare no conflict of interest

*Ethics approval.* Animal ethical approval to execute the in vivo experiments were provided by Institutional Ethical Committee, Ivanovo State Medical Academy and First affiliated hospital of Zhengzhou university with mutual collaboration in accordance with the International Guidelines on conducting studies involving the use of experimental animals as per the Directive 2010/63/EU of the European Parliament for animal use for scientific purpose' & 'NIH Guidelines for the Care and Use of Laboratory Animals' and the Order Number 724 of the Ministry of Higher Education (Minvuz, Soviet Union, 13.11.1984). Majority of in vivo work was executed in Russian Federation.

*Availability of data and material.* All the data included in this study will be available upon the request of journal office.

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