Chapter

Animal Models in Myometrial Activity Research: Morphofunctional Features, Role of Oxytocin

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Abstract

One of the main functions of the reproductive system is providing the physiological process, which occurs by caudal spread of excitability of smooth muscle tissue and ensures delivery of the fetus. The results of this work confirm the importance of blood supply in automatism of the ovarian horn areas, which are the leading regions in propagation of electrical waves and coordination of other rhythmogenic loci. Morphofunctional methods have shown that the ovarian horn areas have strong vascularization, which is confirming the pronounced electrical activity of these loci. Oxytocin has a central role in changing of excitation patterns. Increasing hormone concentrations (10^{-2} μg/kg, 10^{-1} μg/kg, 1 μg/kg, 10 μg/kg) resulted in increase of the bursting activity duration of all studied myometrial areas. At the same time, rise in the frequency of spike rhythmogenesis was observed only at a dose of 1 μg/kg. Morpho-histochemical analysis revealed the existence of atypical cells with a high level of Ca^{2+}-dependent acid phosphatase in both distal rhythmogenic ends of the horn. However, the ovarian horn area had the greatest enzymatic activity. Thus, the obtained data give good reason to conclude that the ovarian horn area has a leading role in the myometrium.

Keywords: uterus, ovarian horn area, cervical horn area, spontaneous activity, oxytocin action, uterine artery ischemia, pacemaker activity, myometrium

1. Introduction

In all varieties of human internal organs, the uterus has a unique position not only because of differences in the structure and response to various environmental factors but also because of its special function compared to other visceral organs. The main functional importance for uterus has the coordinated contractile activity creating conditions for an orientation of waves to the uterine cervix [1, 2]. It has also been known that it is the spontaneous electrical activity is associated with uterine contractility.

Premature and also pathologically proceeding childbirth can lead to serious consequences up to the death of the fetus and mother. One of the possible methods of their prevention is the ability to control initiated uterine contractility. In this regard, the study of mechanisms providing this process will help to find out ways the solution this problem. Especially, it is important to reveal both the rhythmogenic regions and the driver pacemaker area providing the coordinated contraction of the organ. Several authors [3, 4] have noninvasively recorded the human uterine electrical activity from the abdominal surface, which allows the monitoring of parameters and their changes during rhythmogenesis, including discoordination of automatism, which leads to pathological consequences. Experimental resulting obtained in animal models, particularly, in rats, can provide a basis for similar clinical development.

The spontaneous electrical activity of nonpregnant rats is registered both from the uterine corpus and different areas of uterine horns and consists of intermittent bursts of action potentials resulting from the cyclic depolarization of the cell membranes [2, 5–7]. During pregnancy, especially in the later period, significant changes in generation of spontaneous activity in the rhythmogenic regions are observed, stimulating and coordinating the contractile activity of the uterus [3, 5, 8]. The study of the electrophysiological properties of all types of pacemaker activity presented in this organ will help to reveal the mechanisms providing their coordination during labor.

It is known that for normal functioning of the tissue optimal blood supply is necessary. Particularly, certain dependences of the electrical activity and, consequently, the process of delivery from a bloodstream are revealed for uterine smooth muscle tissue. Moreover, during pregnancy, occlusion of blood vessels [9, 10] is observed that also can matter in clinical practice. Based on this, morphofunctional research on studying the blood supply process in rhythmogenic regions of the uterus, providing generation of its contraction is necessary, which has not been presented in the literature yet.

Pregnancy is preceded by hormonal changes in the body, giving rise to certain changes in the excitability of myometrium [11–14] and, consequently, in the mechanisms of genesis of spontaneous activity. The solution of the aforementioned problem is most convenient to carry out in the conditions providing their activation. Oxytocin belongs to the strongest stimulators of uterine contractility during the birth process. It leads to the depolarization of membrane in myometrial cells and increase of frequency of spike discharges [3]. Oxytocin is produced in the hypothalamus and released into the maternal blood. It is also produced in the uterine placenta in the later period of pregnancy, and its concentration increases before delivery [15, 16]. Despite such important role of oxytocin during pregnancy and childbirth, the mechanism of its influence on the pacemaker activity has not been fully investigated.

The purpose of this work was to study the morphofunctional characteristics of rhythmogenic regions in the rat myometrium, as well as the identification of driver pacemaker areas under the influence of oxytocin.

2. Materials and methods

It is well-known that the sensitivity of the myometrium to hormones and mediators is raised in pregnancy, which is affecting autonomous spontaneous rhythmogenesis [17]. This is the reason why the experiments were done on nonpregnant rats.

Experiments were carried out on female animals (200–250 g) narcotized intraperitoneally with Nembutal (50–55 mg/kg) under the conditions *in situ*. The experiments were acute, and at the end of the recording the animals were killed. The peritoneal cavity was opened, and the uterine corpus with the uterine horns from two sides was exposed. The uterus was denervated by sectioning the nerves (plexus hypogastricus, uterinus, uterovaginalis). Spontaneous electrical activities were recorded simultaneously from the uterine corpus, uterine cervix, ovarian, and cervical ends of uterine horns. The activity of the uterine cervix was recorded by inserting a monopolar silver electrode into this area. Spontaneous electrical activity of the remaining areas was recorded from the surface of these regions with bipolar electrodes (interelectrode distance was 2 mm). The registration areas are schematically shown in **Figure 1**.

Oxytocin (5 units/ml, Biolek, Ukraine) was diluted in distilled water and injected intravenously at various doses: 10^{-2}, 10^{-1}, 1, and 10 μg/kg. Depending on the animal weight, such concentrations were possible to administrate by the introduction of different injection volumes — from 0.3 to 0.2 ml. Only one dose of oxytocin was studied on each animal in particular experiments.

Ischemia of the uterine artery was performed by an elastic cord prior to the ovary and its corresponding horn.

The results were recorded without a Faraday cage, and the level of valid signal was 10 μV. Statistical analysis of the data was performed by LabView, Origin8.5, and DIAdem programs.

Corresponding norms of parameters were registered and analyzed in each subsection of the present work, considering seasonal changes in the activity characteristics.

In order to study the morphofunctional properties of cellular structures the activity of Ca^{2+}-dependent acid phosphatase has been revealed [18]. This methodological approach is based on the detection of intracellular phosphorous-containing

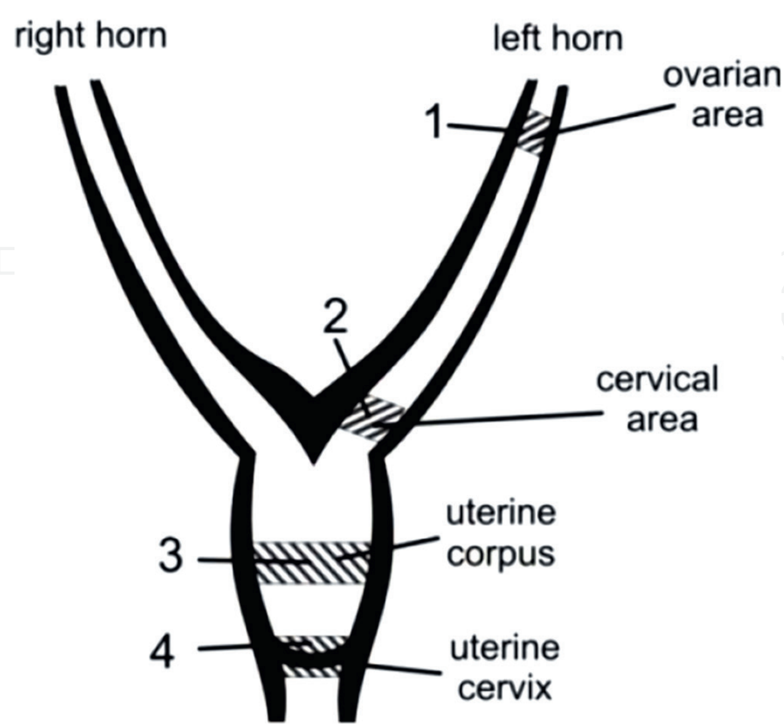

Figure 1.
Scheme of electrical activity registration in the ovarian horn area (1), cervical horn area (2), uterine corpus (3), and uterine cervix (4).

compounds taking key positions in the energy exchange processes directed on the preservation and self-reproduction of vital systems.

The vascular system of the rat uterus was detected using the calcium adenosine triphosphate histoangiological method of Chilingaryan [19]. The method is based on the selective phosphorus deposition segregated from ATP by calcium ions. Subsequently, the reaction product is converted to black lead sulfide. This method provides a clear selective detection of vascular-capillary network, and the major advantage is the possibility of simultaneous differentiation of the various links of the microcirculatory bed (arterioles, capillaries, and venules).

3. Results and discussion

3.1 Electrophysiological characteristics of different areas of the uterus and uterine horns

It is known that the electrical activity can be registered from both pregnant and nonpregnant uterus, but these activity discharges are scarce and have shorter duration in nonpregnant organisms [2, 3, 20].

According to the detailed electrophysiological analysis, rat uterine horns also have an ability to generate spontaneous electrical activity. Pacemaker areas are located in the ovarian and cervical ends of the uterine horn [21]. The uterine cervix also has autonomic spontaneous rhythmogenesis, which is completely asynchronous with activity of the uterine corpus in nonpregnant rats and is presented by slow-wave oscillations in membrane potential [2, 22, 23].

The uterus is unique among smooth muscular organs in that, it has an exceptional selectivity not only to various types of mediators and ions but also to sexual hormones. As noted above, the sensitivity of this tissue to hormones considerably increases during pregnancy, and especially in its late stages [11–13], which, naturally entails changes in the generation of electrical activity and, consequently, in its parameters. In these conditions, coordination of all presented types of autonomous spontaneous electrical activities is observed, which leads to the realization of peristaltic activity in the uterus [2, 8]. To reveal the synchronization mechanisms of rhythmogenesis in different areas of this organ, a prior analysis of the electrophysiological characteristics of these activities is necessary. The study of abovementioned issues would be best to carry out on nonpregnant organisms; in this case, the activity of the uterus and uterine horns can be taken as the norm.

In the present work, with simultaneous registration of activity in different areas of the organ, we tried to analyze the characteristics of the spontaneous electrical activities of nonpregnant uterine corpus, terminal ends of uterine horns, and uterine cervix.

The study of electrical activities of both the uterine corpus and different areas of the left uterine horn allowed us to register spontaneous electrical burst discharges (**Figure 2 (1-3)**). Electrical activity in aforesaid regions is presented by typical spike activity bursts that occur against the rather unstable level of membrane potential and last 41.8 ± 3.3 sec (n = 10), 45 ± 2.1 sec (n = 7), and 34 ± 1.5 sec (n = 10) respectively, in the ovarian and cervical ends of horn, uterine corpus, and then disappear.

In contrast to the described areas, the activity of the uterine cervix was usually recorded during all times of registration and presented by slow-wave rhythmic oscillations in membrane potential (**Figure 2 (4)**). The amplitude and frequency of activity corresponded to 189.1 ± 17.8 μV and 17.4 ± 0.7 oscill./min (n = 10) in this

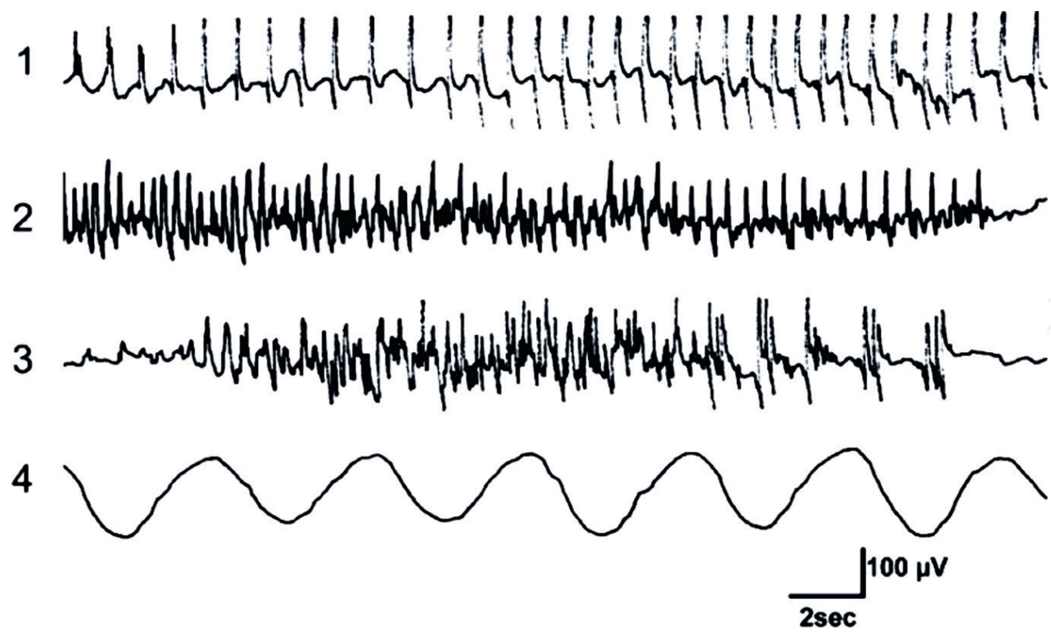

Figure 2.
Simultaneous registration of spontaneous activity in the studied uterine areas. The numbers on the left correspond to the regions of electrical activity registration presented in **Figure 1** *(n = 9).*

region. Based on our results and literature data, we can conclude about the autonomy of pacemaker activity rhythmogenesis of the uterine cervix.

Spike activity discharges in the three upper regions do not occur simultaneously in most experiments. But they work together in a certain period of time. Based on the registrations carried out continuously for 30 minutes, we have calculated the summarized periods of the active states of the uterine three upper regions (**Figure 3**). Despite the spike activity discharges lasted various time intervals, their total duration was almost the same for the cervical end of the horn and uterine corpus (10.95 ± 1.54 min and 11.8 ± 1.24 min, respectively (**Figure 3 (2,3)**). However, this parameter of activity for the ovarian locus of uterine horn was much greater than the above values (19 ± 1.12 min) (**Figure 3 (1)**).

There was a slow-wave rhythmic oscillation of the membrane potential in the uterine cervix, which has been observed during the entire period of registration.

According to the presented three upper curves in **Figure 2**, pacemaker activities of the uterine corpus and two regions of the uterine horn have the characteristics of a typical spike rhythmogenesis. Since activities of these regions alternate one another, it is difficult to establish which of them is primary in relation to the other. At the same time, comparison of these activity characteristics would help to establish the identity of mechanisms of their genesis.

The next series of experiments were carried out to examine the relationship between the frequency and amplitude of spike activities in the 1, 2 and 3 areas, presented in **Figure 1**. This correlation has an exponential characteristic in the uterine corpus (**Figure 4**). Changes in the frequency of spike activity genesis of given area are in the range of 30–200 spikes/min. Interesting is the fact, that in the range of 45–60 spikes/min frequency changes the same value of amplitude was registered (about 188.2 μV). It should be noted that these parameters are in the range of 45–80 spikes/min frequency changes, the probability of occurrence of which could be considered rather high for the uterine corpus (49% of cases), since the range of frequency changes is large.

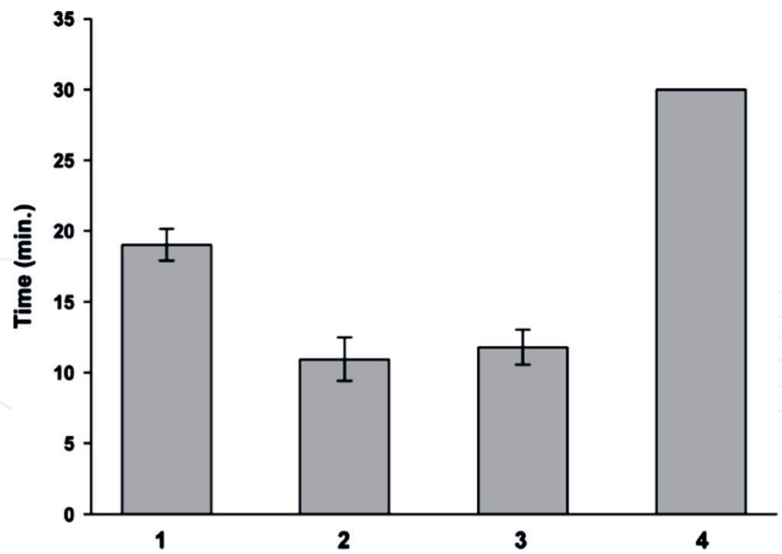

Figure 3.
Duration of the active state registration in the various areas of uterine horns and uterus. The numbers below correspond to the regions of electrical activity registration, presented in **Figure 1** *(n = 9).*

The amplitudes of spike activities are linearly dependent on the rhythm of their genesis in the cervical end of uterine horn (**Figure 5**). Changes in the frequency of automatism were almost in the same ranges (30–210 spikes/min), which corresponded to the uterine corpus activity. However, the abovementioned frequency range corresponding to the same value of the spike amplitude (≈188.2 μV) is a little shifted (55–80 spikes/min) and again in the aforementioned range of the most common frequencies (60.7%). As can be seen from comparison of the graphs presented in **Figures 4** and **5**, the spike amplitudes decreased 2.4 times in both areas in the range of all frequencies. Despite a number of identical parameters of presented graphs, the relationships in these areas have different characters.

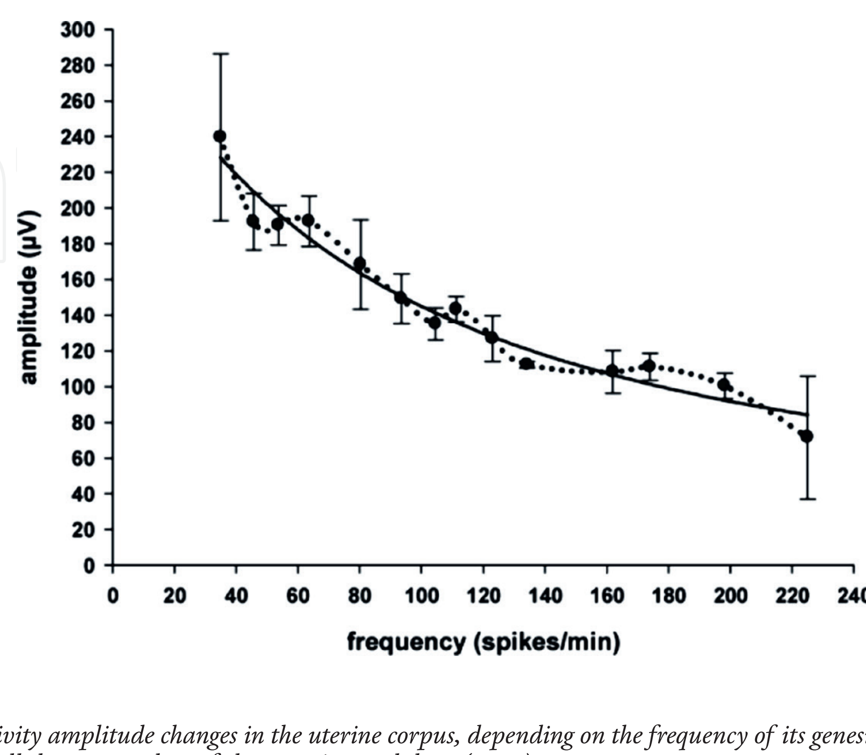

Figure 4.
The spike activity amplitude changes in the uterine corpus, depending on the frequency of its genesis. The dotted line connects all the mean values of the experimental data (n = 9).

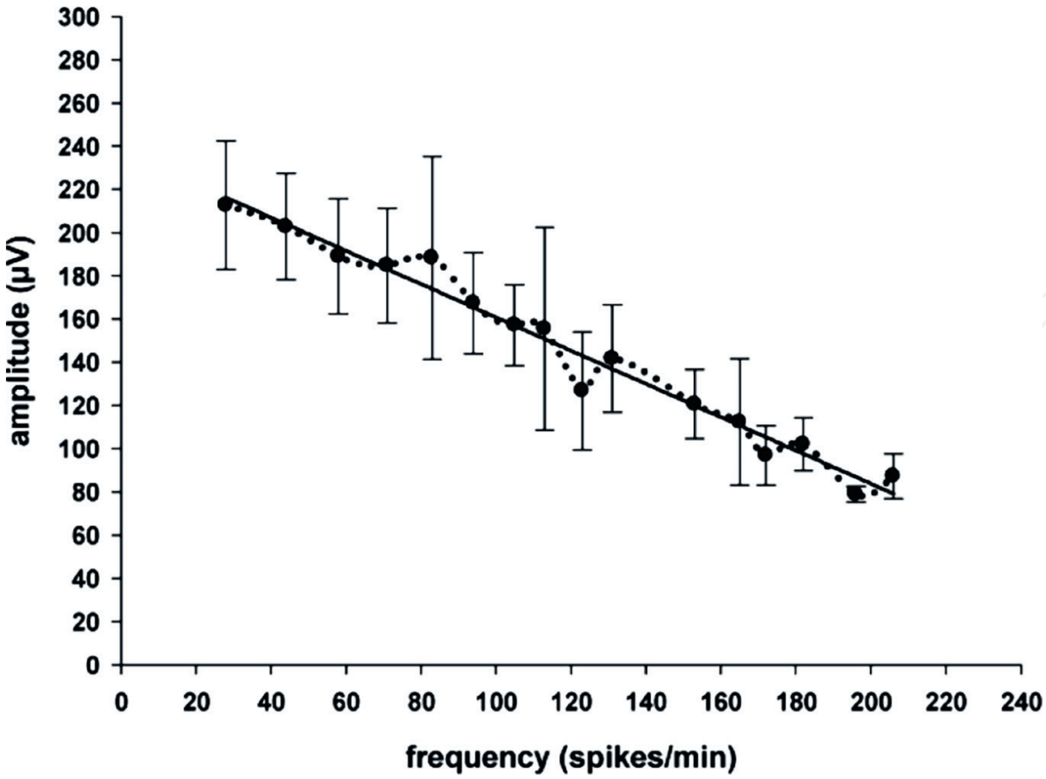

Figure 5.
The spike activity amplitude changes in the cervical horn area, depending on the frequency of its genesis. The dotted line connects all the mean values of the experimental data (n = 9).

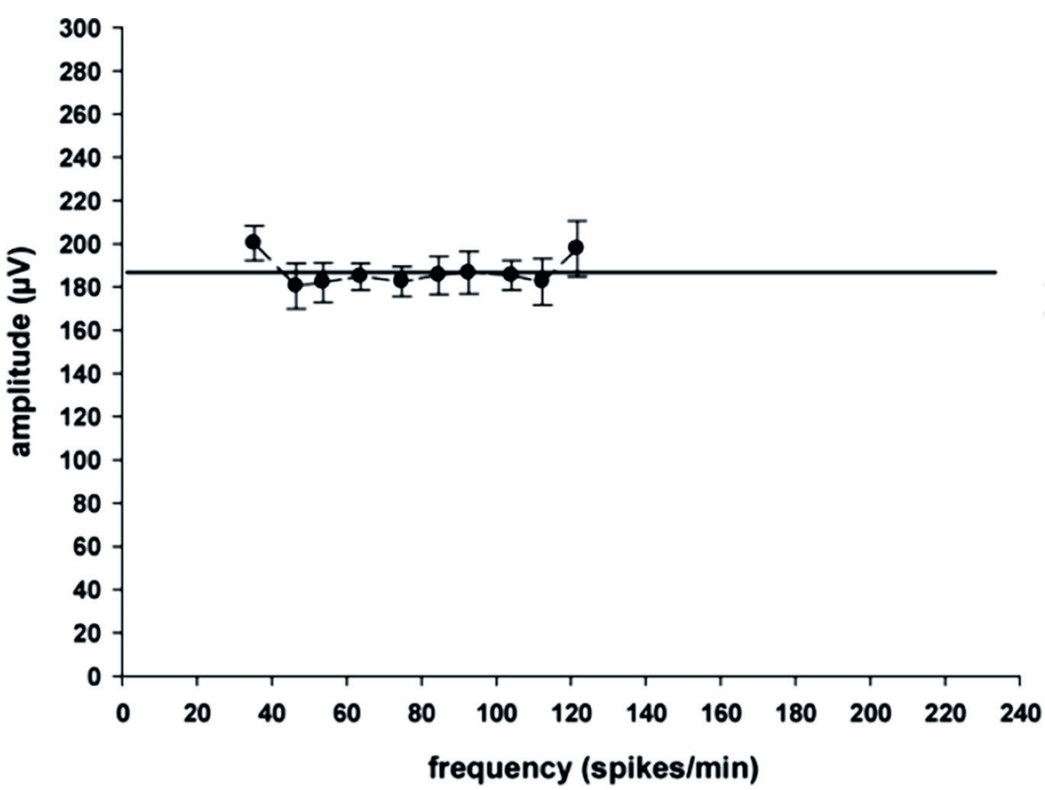

Figure 6.
The spike activity amplitude changes in the ovarian horn area, depending on the frequency of its genesis. The dotted line connects all the mean values of the experimental data (n = 10).

The correlation between amplitude and frequency is rather different in the ovarian part of horn compared to the uterine corpus and cervical part of uterine horn (**Figure 6**). Here the frequency of spikes is less scattered and mainly involves the range of 30–120 spikes/min. At the same time, the spikes having stable amplitude (186.8 ± 2.13 μV) are registered in the indicated range of frequency changes. In contrast to this area, similar amplitude value of spikes is observed within the most met frequency range (45–80 spikes/min) in the cervical end of horn and uterine corpus. Noteworthy is the fact that the frequency parameters of the same range of changes are also the most met (48.5%) in the ovarian end of horn such as the cervical end of horn and uterine corpus.

Thus, despite the obvious differences in the amplitude and frequency correlations between ovarian end of horn and downstream areas, which are also generating spike activity, the greatest probability of occurrence of frequencies corresponds to the same range of changes for all three areas.

3.2 Blood supply as a factor regulating pacemaker activity of the myometrium

It is known that the spontaneous electrical activity of smooth muscle tissue is myogenic in nature [2, 6, 21, 24]. If the myogenic rhythm plays the main role in occurrence of the peristalsis in smooth muscle, the humoral control is necessary for modulation and coordination of the contractility patterns [25]. Particularly, the blood supply due to its transport function plays an important role in this process. Thus, smooth muscle of the gastrointestinal tract is rather resistant to decrease in blood flow [26]. At the same time, anoxia rapidly decreases the rat uterine contractile activity and its complete suppression takes place within a few minutes under conditions of ischemia [27–29].

It is also known that parameters of the smooth muscle activity can vary within a rather wide range not only suggesting about the relationship of automatism and blood flow but also confirming the variability of this process [30].

Rats and mice have a duplex uterus (two separate uterine horns). The loops of the uterine artery encircle each horn separately [27, 31], while the blood flow in the right and the left horn is realized in two directions: from ovarian and cervical ends to the center [32, 33].

According to a detailed electrophysiological analysis, unlike the uterus, any part of which is able to generate spontaneous discharges, pacemaker areas are located in terminal ends of uterine horns in rats [21, 34]. The results of the previous section confirm the noticeable differences of characteristics of spontaneous activity in the ovarian end of uterine horn and downstream rhythmogenic areas. Moreover, it is shown that the total duration of generation of the electrical bursts is much higher in this area. However, there are very few special studies in the literature devoted to the experimental analysis of blood supply in the ovarian region and its importance in electrical activity generation. The following paragraph analyzes these issues. The uterine horns and the uterine artery loops that feed each horn are presented schematically in **Figure 7**. The areas of activity registration are also shown (respectively numbered). In the first series of experiments, a comparative analysis of changes of spike activity parameters in the uterine corpus and terminal parts of uterine horns was carried out under conditions of the uterine artery ischemia. **Table 1** presents the values of the parameters of rhythmogenesis of the noted areas in norm.

About 20-min ischemia of the uterine artery, feeding the ovarian part of horn, led to certain changes in the activity parameters of each area, respectively (**Figure 8**). For illustrativity, all results are presented in percents related to norm.

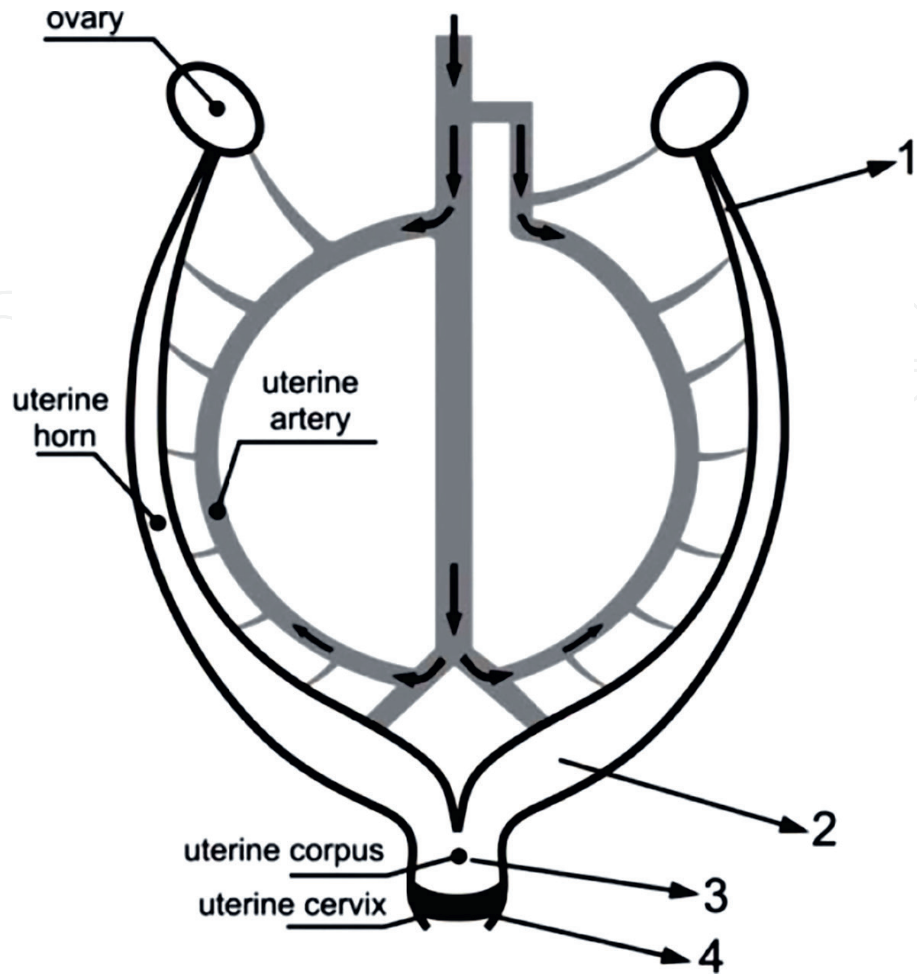

Figure 7.
The blood supply scheme of the uterus and uterine horns in rats. The numbers on the right correspond to the regions of activity registration: 1-ovarian end of horn, 2-cervical end of horn 3-uterine corpus, and 4-uterine cervix.

Areas of registration	Amplitude of spikes, μV	Frequency of spikes, spikes/min	Duration of activity discharges, sec
1	142.3 ± 4.99	84.5 ± 6.16	36.3 ± 2.66
2	126.0 ± 2.00	92.5 ± 2.29	34.9 ± 1.79
3	104.7 ± 2.40	116.9 ± 4.83	19.6 ± 4.94

Table 1.
*Parameters of spike activity in the uterine corpus and terminal parts of uterine horn in norm. 1, 2, and3 are the corresponding registration areas (***Figures 1** *and 7).*

As can be seen in **Figure 8(A)**, the amplitude of spikes in the ovarian horn area undergoes to the greatest changes. In the cervical end of horn and uterine corpus, this parameter decreases by less than a quarter of its initial value (24.4 and 23%, respectively). Interestingly, suppression levels of the amplitude in these areas are similar. At the same time, a different picture is observed for the ovarian area of uterine horn. There is a sharp, almost twice decrease in amplitude (to 54.5%).

According to **Figure 8(B)**, the frequency parameters of all three areas compared to the amplitude parameters are less subjected to changes under conditions of the ischemia. In the cervical horn area and uterine corpus, this parameter changes

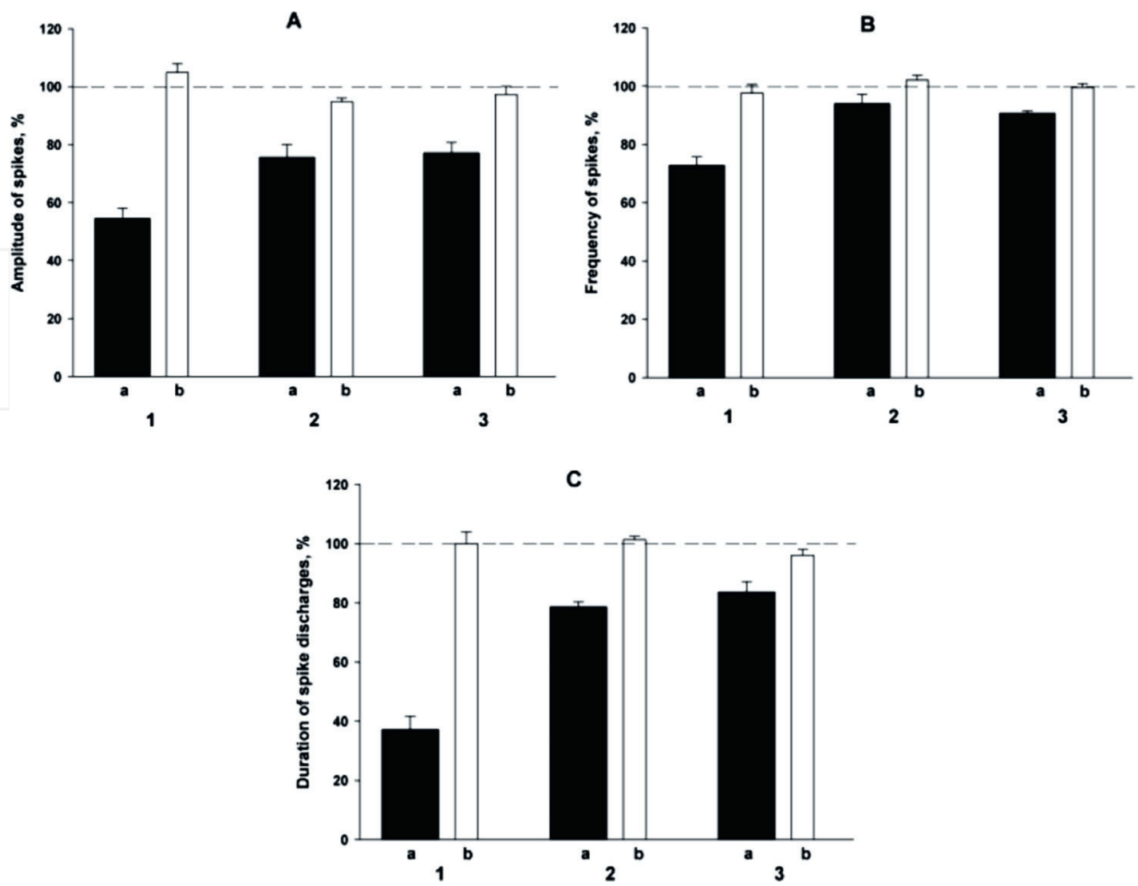

Figure 8.
The influence of ischemia on the parameters of spike activity in the uterine corpus and terminal areas of uterine horn. A - amplitude of the spikes, B - frequency of the spikes, C - duration of the spike discharges. a - uterine artery ischemia; b - restoring blood flow. The numbers below correspond to the regions of electrical activity registration presented in **Figures 1** *and* 7 *(n = 8). The dotted line demonstrates the norm.*

insignificantly (by 5.9 and 9.3%, respectively), but decrease in spike frequency reaches to 27.2% in the ovarian horn area.

A similar trend of changes in activity parameters is also shown for the duration of the spike discharges in the studied areas (**Figure 8 (C)**), in the proximal horn area, the duration of burst genesis was significantly shortened (by 62.8%), while decrease in this parameter for two subsequent areas (**Figures 1** and 7 **(2,3)**) corresponded to only 21.2 and 18.3%. Restoration of blood flow after ischemia, as a rule, increased all parameters to near-normal values.

Despite the significant suppression of activity in the ovarian area, its full inhibition was not observed. Probably, the total block of rhythmogenesis could be achieved by prolonging time of ischemia or increasing force of the cord pressure to the complete closure of the vessel lumen. But such experiments could damage the artery.

As noted above, blood enters the uterine horn from ovarian and cervical ends and flows to the middle region of it. Blood supply of the uterine corpus and uterine cervix is provided by an additional branch of the artery (**Figure 7**) [27]. Ischemia of the uterine artery, which enters the ovarian horn area, entails significant changes mainly in the activity characteristics of this region. A little inhibition of parameters in the cervical end of horn and uterine corpus under conditions of such ischemia may indicate that there is a likely certain connection between the studied rhythmogenic loci. Actually, if the cervical horn area had an autonomous blood supply, the activity parameters would remain unchanged.

Thus, under the above experimental conditions, the main rhythmogenic region of uterine horn undergoes the greatest activity inhibition compared with the below located pacemaker areas.

In the next series of experiments, we analyzed the activity parameter changes in the uterine cervix under similar conditions of ischemia (**Figure 9**). As mentioned above, activity of the uterine cervix is completely different from that of all other areas of this organ. In norm, slow-wave sinusoidal-type activity is registered in this area with a frequency of 15.1 ± 0.34 oscill./min and amplitude of 282.1 ± 14.5 μV, which is fully autonomous. Even some authors have described the cervix as an entirely separate from uterus organ [2, 35, 36].

As can be seen by comparing **Figures 8(A)** and **9(A)**, the opposite changes in the amplitudes of the ovarian horn area and uterine cervix were noted under conditions of ischemia. Twice decrease in the spike amplitudes of the horn is accompanied by one and half time increase in the slow-wave amplitudes of the uterine cervix (up to 152.3%). The frequency characteristics of activities in the abovementioned areas in both cases decreased and cervical rhythmogenesis slowed by only 14.4% compared to the norm (**Figure 9 (B)**). All changes in characteristics of the slow-wave activity occur against a background of its continuing genesis.

It is known that slow-wave oscillations of the membrane potential recorded from the uterine cervix in norm provide its closure by the involvement of circular muscle layers [2]. Significant increase in amplitude of the cervical waves in ischemia indicates the activation of this process that stimulates the cervical closure. During the labor, the longitudinal muscle layers of the cervix, which are able to generate spike activity, are involved in the longitudinal polar contractions provided by the main rhythmogenic region (ovarian area of the horn).

Thus, there is a certain connection between rhythmogenesis in the ovarian horn area and uterine cervix. Probably, the results obtained in this work related to the opposite changes in the activity parameters of the terminal uterine areas under

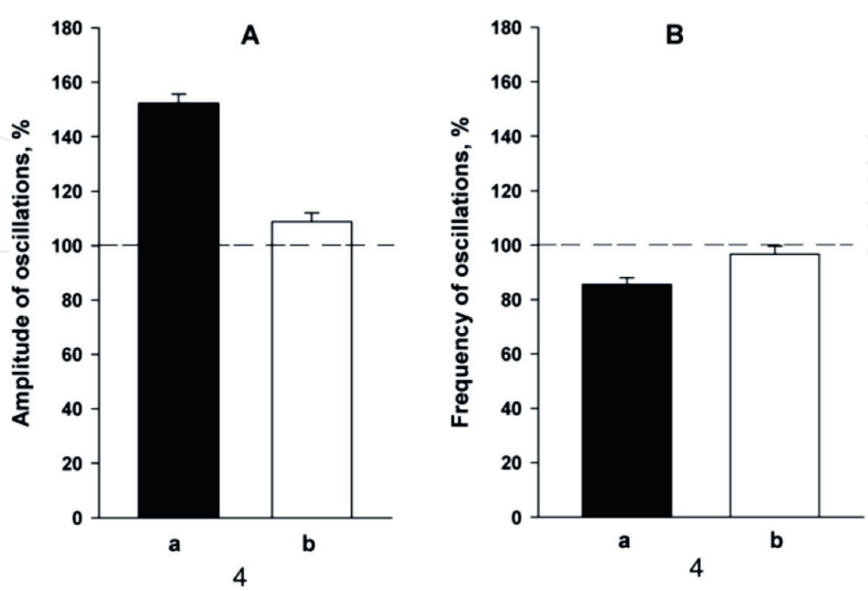

Figure 9.
The influence of ischemia on the parameters of slow-wave activity in the uterine cervix. A - amplitude of the waves, B - frequency of the waves. a - uterine artery ischemia; b-restoring blood flow. The numbers below correspond to the regions of electrical activity registration presented in **Figures 1** *and* 7 *(n = 8). The dotted line demonstrates the norm.*

conditions of the ischemia can be explained by the presence of such interaction between them [37].

In the next series of experiments, the microcirculatory bed of the ovarian region was investigated. In parallel with the vascularization of aforesaid area, the vascular bed of the ovarian nearby area and of distally located middle horn area were also studied. A strong vascularization with highly branched arterial system was detected on the longitudinal sections of the ovarian locus (**Figure 10(A)**). Therefore, there is a huge amount of the microvessel cross sections in the slices. The transition zone between the ovaries and uterine horn is characterized by less twisted blood vessels (**Figure 10(B, C)**). In comparison with the mucous and serous membranes, the muscular layer is highly vascularized in the horn area closest to the ovary. Blood vessels from the surface enter the uterine horn directly, almost without twisting, are assembled in a bunch (shown the blacked-out surface), and by this, way creates a strong vascularization of this region (ovarian end of the horn). These vessels take again a strongly twisting direction in the middle region of uterine horn. That is why there is a large number of their cross sections along with the short microvessels in the slices (**Figure 10(D, E)**).

The strong vascularization of the ovarian end of uterine horn is one of the possible factors, providing strongly pronounced automatism in this region. Vessels take a twisting direction in the middle area of uterine horn, and therefore their close contact with the tissue is less expressed. Such blood vessel location

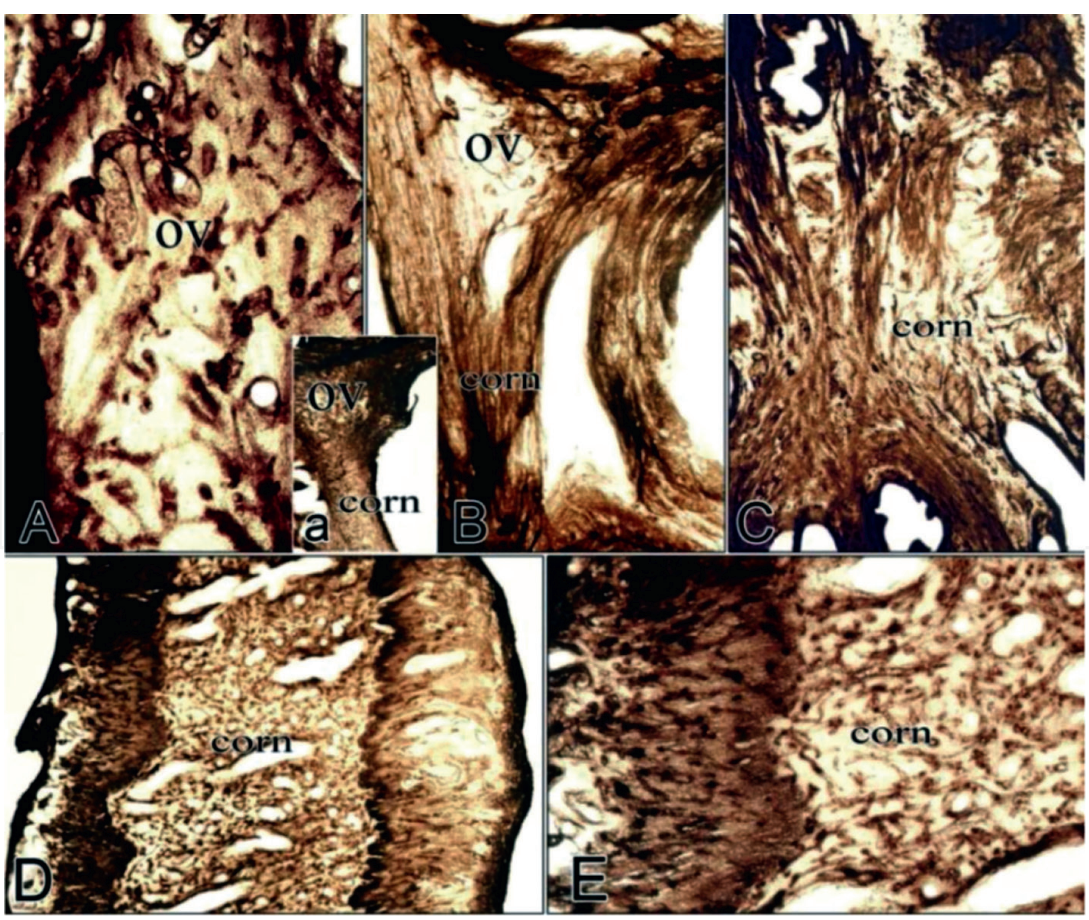

Figure 10.
Blood vessels of the microvasculature in the longitudinal sections of ovary (A, a), the initial region of the uterine horn (B, C) and the middle region of the uterine horn (D, E). ov-ovary; corn-uterine horn. Zoom: ocular 10, objective 2.5 (a), 6.3 (B), 10 (C, D, E), 16 (A).

throughout the uterine horn is specific to immature and nonpregnant females. Throughout pregnancy further development of the bloodstream and, respectively, the vascularization is observed. Branching in the vascular system occurs, providing blood flow to individual uterine segments associated with fetuses. Each uterine segment has an abundance of closely located blood vessels (unpublished data). It can be assumed that during reproductive organ formation, there are appropriate stages of the circulatory system development. Perhaps such vascular system was developed in the process of evolution to provide individual feeding of each segment by the local automatism, though the latter is coordinated with the main rhythmogenic region.

3.3 Role of oxytocin in activation of spontaneous electrical activities of the uterine corpus and uterine horns

Oxytocin is considered as one of the main regulatory substances of contractile activity in the process of childbirth [38]. Early studies have shown that under the effect of oxytocin, membrane depolarization is observed in myometrial tissue, leading to membrane excitability and conductivity, which results in increased activity and the occurrence of contractile events [3, 39, 40]. For this reason, the given substance is easy to use for the identification and study of changes in the characteristics of spontaneous electrical activity, leading to its coordinated activity for the generation of the subsequent contraction.

It is known that smooth muscle tissue of myometrium, as well as the gastrointestinal tract and urinary tract, is characterized by ability to generate autonomous spontaneous activity [2]. However, there is very little data in the literature about the mechanisms underlying the generation of pacemaker activity in the uterus in contrast to above mentioned other types of smooth muscle formations in which the specialized interstitial cells of Cajal (ICC) have been found, providing generation and coordination of muscular activity [41, 42]. Some cells that are completely different from myocytes by their morphology and ultrastructure were also found in studies on uterine strips of pregnant rats [43]. The morphological studies concerning the identification of atypical cells in myometrial tissue are easier to carry out under conditions providing the activation of spontaneous rhythmogenesis.

Research on the influence of oxytocin on the automatism of different areas of the uterus and uterine horns by electrophysiological and morpho-histochemical methods can help to tackle the abovementioned problems.

As already mentioned, unlike the uterine corpus, only the terminal parts (ovarian and cervical) of uterine horns can generate spontaneous electrical activity [44].

In the first series of experiments, we analyzed the fast spike processes observed in three upper regions, which are presented in **Figure 1**. We analyzed the duration of genesis of electrical discharges and spike frequencies within the bursts for the terminal parts of horns and uterine corpus (**Figure 1(1-3)**). These parameters can be ascribed to the number of the main characteristics determining the contractile activity of the organ [45].

It is known that non-regular bursts of spontaneous electrical activity can also be observed in nonpregnant rats [11, 22, 46, 47], and automatically arising activity discharges recorded in different areas of the organ have different duration and spike frequencies. **Table 2** presents our obtained data from the upper areas of the rat uterus in norm (**Figure 1(1-3)**). Interestingly, the duration of the activity recording and frequency of the spike genesis in the cervical ends of both horns a little exceed the same

Areas of registration	Duration of activity discharges, sec	Frequency of spikes, spikes/min
Ovarian end of the left horn	50.8 ± 2.74	73.7 ± 3.78
Cervical end of the left horn	55.2 ± 4.88	84 ± 4.11
Ovarian end of the right horn	28.3 ± 5.08	59.2 ± 3.41
Cervical end of the right horn	31.2 ± 3.61	75.7 ± 3.85
Uterine corpus	32.8 ± 2.00	92.1 ± 4.27

Table 2.
Parameters of spike activity in various areas of uterine horns and uterus in norm.

parameters in the ovarian ends. In addition, the right uterine horn is less active in norm as it is characterized by significantly lower values of these parameters compared with the left horn.

In the next series of experiments, we studied the durations of the discharge genesis and spike frequencies in the uterus and terminal parts of horns, depending on the concentration of oxytocin. For illustrativity, all results are presented in percents related to norm. The presence of oxytocin in any concentration causes a significant increase in the duration of genesis of electrical discharges in the both ends of uterine horns, as well as in the uterine corpus itself (**Figure 11**). In the ovarian ends of both the right and the left horns (**Figure 1(1)**), the maximal increase (almost threefold) of this parameter was noted at the oxytocin dose of 10^{-1} μg/kg. In the cervical ends of horns, closest to the uterus (**Figure 1(2)**), as well as in the uterine corpus itself (**Figure 1(3)**), the longest duration of electrical bursts is observed at the oxytocin concentration of 1 μg/kg. It is important to note that the subsequent increase in the oxytocin concentration to 10 μg/kg led to a shortening of duration of the active state registration. Nevertheless, this parameter significantly exceeds the values in norm for all the studied areas. Interestingly, the spontaneous bursts of activity, as a rule, were not recorded in the ovarian end of the right horn in these conditions.

Thus, by increasing the oxytocin concentration in the considered limits, we have shown a significant increase of the active state duration in all studied areas.

A somewhat different picture is observed in the study of frequency parameters of the spike rhythmogenesis. According to the data of **Figure 12**, for the uterine horns and uterine corpus, the greatest increase of the spike frequency in discharges is revealed at the oxytocin concentration of 1 μg/kg. In contrast to the duration of the activity discharges (**Figure 11**), the presence of oxytocin in a concentration of 10^{-2} μg/kg caused some decrease in the frequency of the spike rhythmogenesis in terminal parts of uterine horns. The increase in the oxytocin concentration to 10^{-1} μg/kg led to an acceleration of the spike rhythmogenesis. A significant increase in the spike frequency was registered in the ovarian end of the right uterine horn at the oxytocin dose of 1 μg/kg; at the same time, in the cervical areas of both horns the frequency parameters did not undergo significant changes with increase of the administrated oxytocin concentration (**Figure 12(a, b)**).

In the uterine corpus, a small increase in the spike frequency was observed within the limits of the oxytocin concentration changes from 10^{-2} to 1 μg/kg, while at 10 μg/kg, this parameter decreased to the level slightly lower than the norm (**Figure 12(c)**).

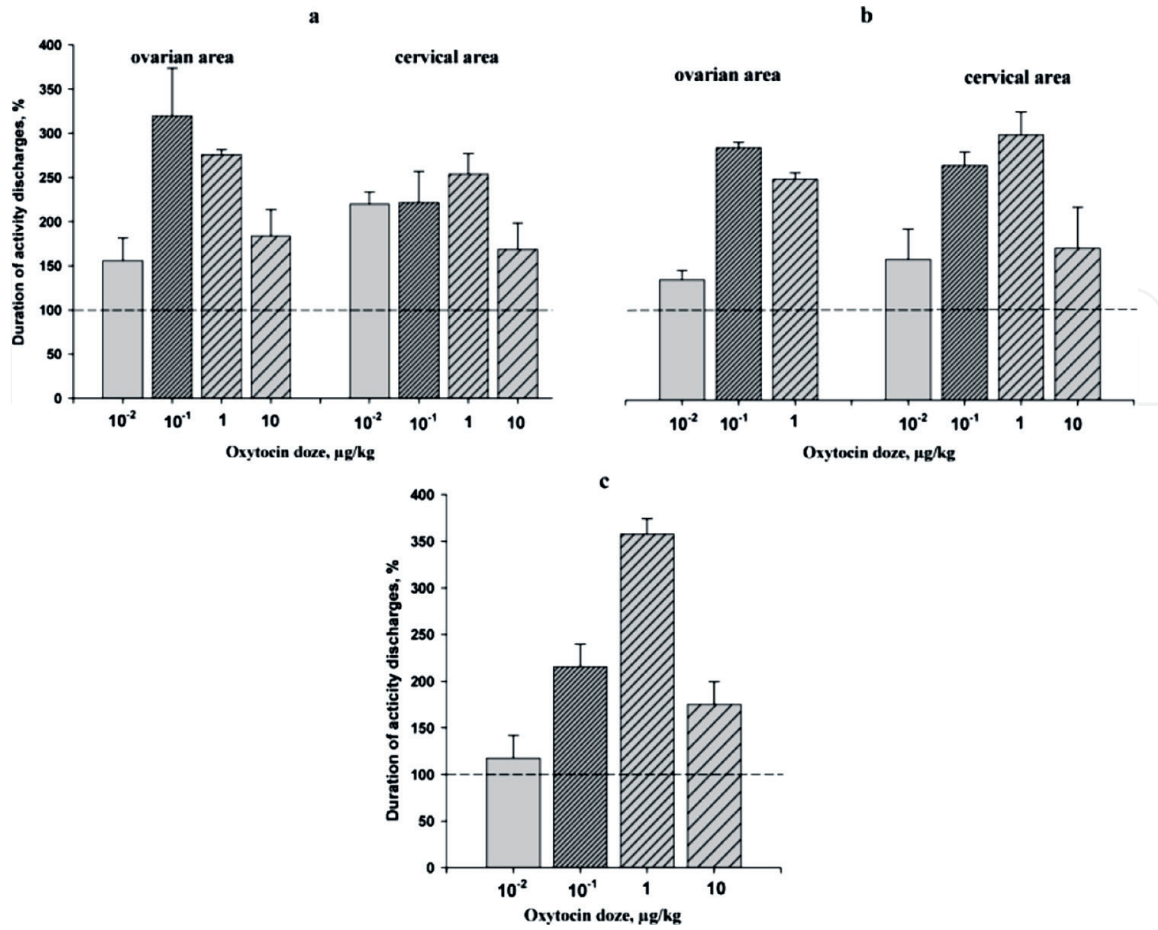

Figure 11.
The influence of oxytocin on duration of genesis of the spike activity discharges in the uterine horns and uterine corpus. The norm is shown by the dotted line (n = 19). a - the left horn, b - the right horn, and c - the uterine corpus. Oxytocin concentrations are marked by different shadings.

Small differences between the values of the spike frequency in the ovarian ends of the right and the left horns seem to be due to the high values of this parameter in the norm (**Table 2**). Unlike the duration of the genesis of the activity discharges, the frequency of the spike automatism in the studied areas is less affected by the action of oxytocin.

The uterine cervix under the influence of this agent behaved quite differently (**Table 3**). At the oxytocin concentration of 10^{-2} μg/kg, no changes were observed in the activity characteristics of this zone, only slow waves were recorded with the frequency corresponding to that in norm (16.6 ± 1.52 oscill./min). The increase in concentration of oxytocin to 10^{-1} μg/kg resulted in the disappearance of the waves, and spike activity was recorded during a certain period of time. The dependence of the duration of the spike generations and their frequency on the oxytocin concentration is presented in **Table 3**. The higher oxytocin concentration-10 μg/kg somewhat decreased these studied parameters of the uterine cervix such as the presented data for the remaining organ areas.

Comparing the effect of oxytocin on duration of the discharge genesis and frequency of the spike activity, it can be concluded that the cervical ends of uterine horns and the uterine corpus can be grouped together according to similar changes in these parameters with increase of the oxytocin concentration. The ovarian ends

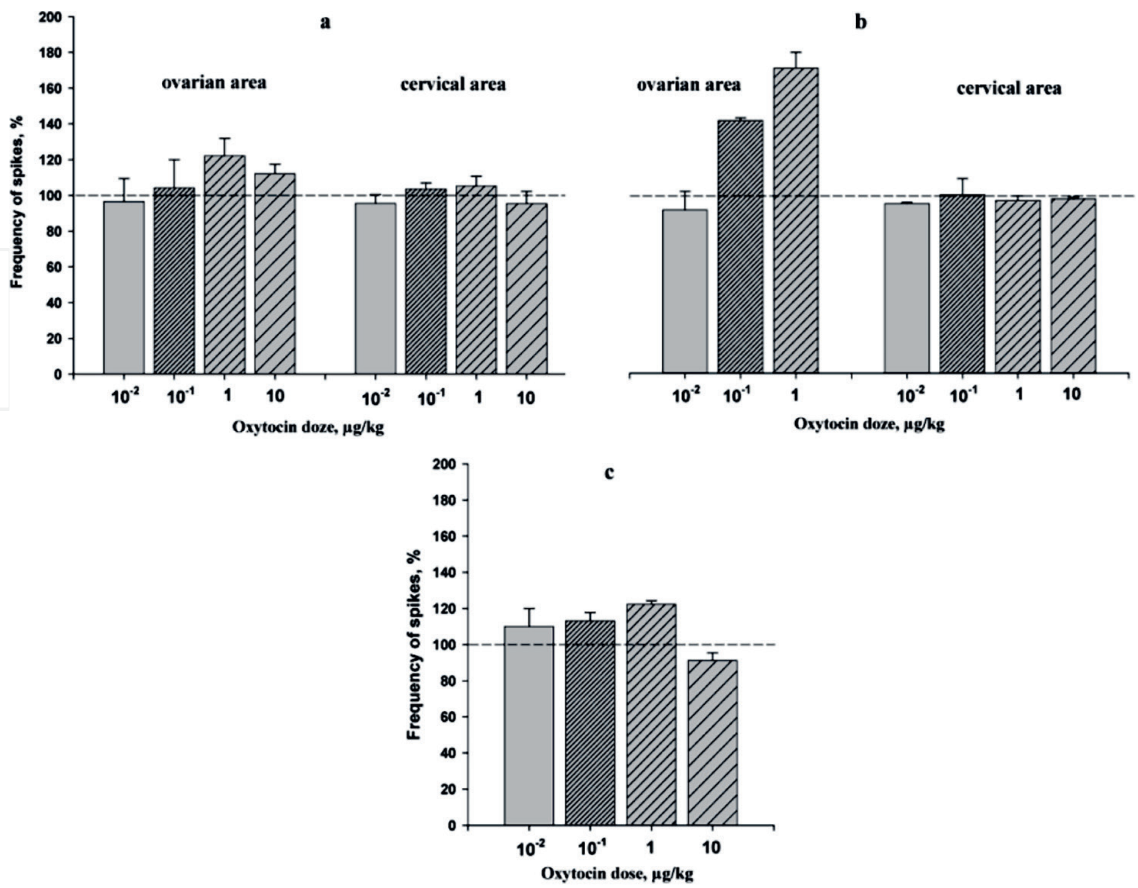

Figure 12.
The influence of oxytocin on frequency of the spike activity in the uterine horns and uterine corpus. The norm is shown by the dotted line (n = 19). a - the left horn, b – the right horn, and c – the uterine corpus. Oxytocin concentrations are marked by different shadings.

Concentration of oxytocin, µg/kg	Duration of activity discharges, sec	Frequency of spikes, spikes/min
10^{-2}	—	—
10^{-1}	50.0 ± 9.0	105.3 ± 18.7
1	66.5 ± 17.5	120 ± 12.0
10	25 ± 5.8	112.5 ± 16.8

Table 3.
Parameters of spike activity in uterine cervix under the effect of oxytocin.

of both uterine horns had somewhat different characteristics of changes in these parameters (**Figures 11** and **12**) within the same limits of the oxytocin concentration changes. Therefore, a certain interest is the comparative analysis of morphological pictures of all three upper regions presented in **Figure 1**.

Morpho-histochemical studies revealed that there are round or oval atypical cells with the high level of the Ca^{2+}-dependent acid phosphatase activity in longitudinal sections of the ovarian ends of uterine horns in intact rats (**Figure 13(Aa)**). The most clearly detected structures are cell nuclei. Gomori-positive round ovoid, coccobacillar, comma-like granulations are found on the light cytoplasmic background. Quite often these cells fuse with each other, therefore, many nuclei are revealed in the large mass of cytoplasm in the preparations. It is important to note that the greatest accumulation of these cells was detected in the ovarian ends of horns. The same cells were found in the cervical parts of horns (**Figure 13(Cc)**) and in the uterine corpus (**Figure 13(Ee)**). Unlike the atypical cells, the enzymatic activity of the acid phosphatase is significantly decreased in the typical myogenic elements of the uterine wall.

Under the oxytocin action, the enzymatic activity was sharply increased in the cytoplasm of atypical cells. In some foci, these cells were concentrated in clumps and their shape became variable, which amplified the staining intensity and giving to the location the blacked-out look. In the ovarian part of uterine horn, these cells were stained darkly, and it was impossible to determine the boundaries of the nucleus and the cytoplasm (**Figure 13(Bb)**). Atypical cells of the uterine corpus (**Figure 13(F)**) and of the cervical horn area (**Figure 13(D)**) were also intensively stained.

According to the literature data, the cells resembling the interstitial cells of Cajal were revealed in the upper urinary tract, particularly in the pyeloureteral anastomosis [43]. They were of irregular shape, with oval nucleus, and numerous contacting processes and were described as the fibroblast-like cells providing pacemaker activity.

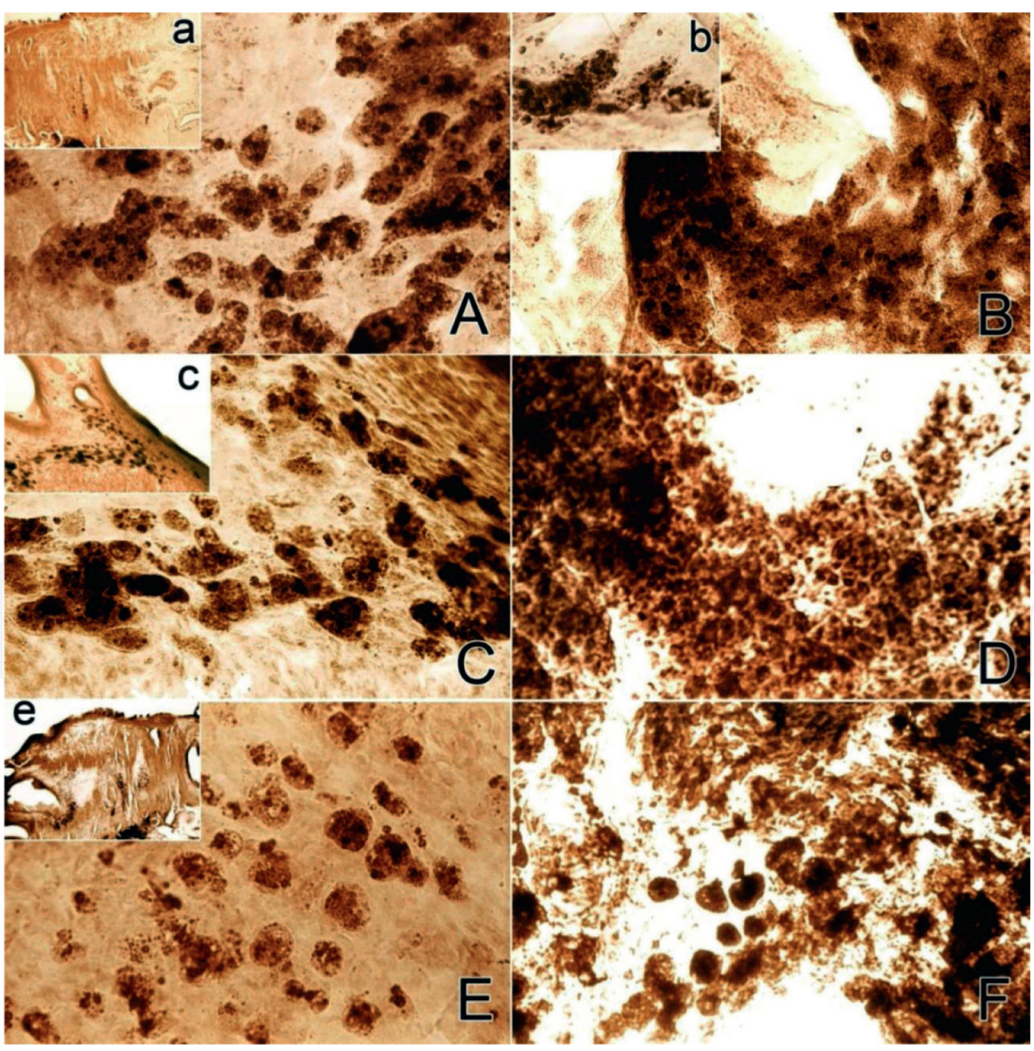

Figure 13.
Longitudinal sections of the uterine corpus and the various horn areas. Intact rats: A-ovarian area of the left horn; C-cervical area of the left horn; E-uterine corpus. At the oxytocin administration: B- ovarian area of the left horn; D-cervical area of the left horn; F-uterine corpus. Magnification: 25 × (a,c,e); 400 × (b); 1000× (A, B, C, D, E, F).

According to the obtained data, smooth muscle cells differed from the revealed atypical cells by the level of enzymatic activity of acid phosphatase. Thus, atypical cells had high Ca^{2+}-dependent acid phosphatase activity, although additional immunohistochemical markers are needed to determine the nature of these cells. Nevertheless, the ovarian part of horn is established to have the highest enzymatic activity of the acid phosphatase among all considered areas.

Our histochemical data confirm completely the presence of different "physiological" states in the studied organ areas. Thus, the complete correspondence of the electrophysiological and morphological results is observed.

It was shown that the duration of spike discharges is a determining factor in the generation of coordinated contractions in the uterus [48]. Analysis of the oxytocin action showed that the main effect was expressed as a significant increase of duration of the spike discharges in the studied areas of the myometrium. The rather low content of the substance in the blood (10^{-1} μg/kg) caused a significant increase in this parameter (up to threefold) compared with the norm. According to the literature data [45], oxytocin in a concentration of 2×10^{-2} μg/kg (≈0.2 nM) is sufficient to stimulate the spike activity, which is expressed by the increase of spike frequency, prolongation of discharge generation, and consequently, contraction periods. The further increase in the concentration of oxytocin causes gradual inhibition of these parameters [3, 45], which also completely agrees with our results obtained at the oxytocin concentration of 10 μg/kg not only for the uterine corpus but also for the ovarian and cervical ends of uterine horns.

Naturally, to provide the uterine contractile activity during the labor, coordination of all pacemaker areas, determining the polarity of the uterine direction, is necessary. The leading role in providing longitudinal contraction of the uterus is ascribed to the pacemakers located in the ovarian ends of uterine horns [34]. It is also shown that the duration of the electrical activity discharges is a determining parameter for the occurrence of coordinated contractions in the uterus [3]. According to the results presented in this work, it is the ovarian end of uterine horn, activation of which by oxytocin is found to be accompanied by the longest duration of genesis of the electrical discharges. Morpho-histochemical studies also confirm this fact — the greatest number of atypical cells has been found in this area, which is characterized by the highest acid phosphatase activity.

According to our preliminary results (data not shown), spontaneous electrical activity of the organ was not observed in 15–20-day-old female rats. Morphological studies also did not reveal above described atypical cells. We assume that certain rhythmogenic (atypical) cells develop during puberty, and they provide electrical activity of the uterus.

4. Conclusions

By definition Norwitz et al. [49], labor is the physiological process by which a fetus is expelled from the uterus to the outside world in the result of regular uterine contractions accompanied by cervical effacement and dilatation. It is known that uterine contractility is dependent on action potentials and their propagation along the tissue [50]. The frequency and duration of contractions are determined respectively by the frequency of the action potentials within a burst and the duration of a burst. The amplitude of contractions depends on the number of propagating action potentials [51].

The membrane potential value of myometrial smooth muscle cells is much lower than those of skeletal muscle and nerve fibers and is approaching to the threshold for spontaneous discharges. From this aspect, every cell in smooth muscle tissue can act as a pacemaker in certain conditions [52, 53]. Unlike the uterus, any part of which is able to generate spontaneous discharges [20], two rhythmogenic regions have been revealed in the uterine horns: the ovarian and cervical ends of the horns [21, 34]. The studies on nonpregnant uterus have shown that bursts of electrical discharges from the upper parts of this organ are propagated within a few mm [36]. Based on this, the spread of the excitation waves is observed in a very limited area from each rhythmogenic region and is absent along the horns. The study of the propagation rate of the electrical activity patterns along uterine horns in guinea pig showed a direct dependence on the electrical coupling between smooth muscle cells. At birth, the lowest resistance between them is observed [54].

According to recent studies, the uterine peristalsis could be provided by the association of the separate pacemaker areas in large rhythmogenic loci during labor [8]. Received data concerning such coordinated among themselves amplitude parameters of rhythmogenesis of the proximal part of horn do not exclude its leading role in providing the synchronization of spontaneous activity of the all organ. Based on the identity of detected ranges of the most met frequency parameters of action potentials with the same amplitude value (188 μB) in uterine corpus and both rhythmogenic regions of uterine horn, it is impossible to exclude the existence of coordination of their activities by the abovementioned manner.

The uterine cervix also has autonomic spontaneous rhythmogenesis. In the contrast to the uterine corpus and uterine horns, it is presented by slow-wave oscillations in membrane potential in nonpregnant individuals. The latter provides the closure of the cervical lumen due to circular muscle activity. In the late stages of pregnancy and during labor a totally synchronized spike activity of all pacemaker areas of the uterus is observed. In these conditions, the uterine cervix is able to pass spike electrical signals of proximal regions of the organ [2, 55].

Interestingly, according to the results presented in this work, ischemia of the uterine artery supplying blood to the ovarian end of uterine horn [56] leads to the opposite changes of amplitude parameters in the abovementioned region and uterine cervix. With twice decrease of amplitude value of spikes in uterine horn, this parameter of slow-wave activity in the uterine cervix increased one and a half times (to 152.3%). Thus, there is a certain correlation between rhythmogenesis in the ovarian end of horn and uterine cervix. As it was noted above, during labor occlusion of the uterine vessels is observed. Reduced blood supply (from 30 to 100%) leads to relaxation with intermittent increasing contractions during labor [21, 30]. Probably, significant increase of amplitude of slow waves in the uterine cervix under the influence of ischemia confirms the activation of that process, which stimulates compression of the cervical lumen during suppression of spike automatism in the proximal locus of horn, leading to the muscle relaxation. Possibly, this mechanism provides feedback for the normal process of delivery. The results presented here may be important for the development of a model, allowing to regulate the uterine contractility in pathological situations in clinics.

In analysis of the oxytocin action on the ovarian areas of uterine horns, it can be noted that the main effect under the influence of this hormone is expressed as a significant increase of duration of spike discharges. At the same time, rather low content of the substance in blood (10^{-1} μg/kg) caused significant (up to threefold) increase of this parameter compared to the norm. To provide the uterine contractile activity during the labor, coordination of all pacemaker areas, determining polarity of its direction, is necessary. According to literature data [34], as well as the results presented in this work, the coordinating role can be assigned to the ovarian end of horn. It is also shown that the duration of electrical activity discharges is a determining factor of the occurrence of the coordinated uterine contractions [3, 57]. The longest duration of the genesis of electrical discharges in the ovarian end of horn under the stimulation by oxytocin also confirms the leading role of pacemakers of this region. In this case, a simple registration of duration of the activity genesis from the abdominal surface of patients not requiring additional measurement of the amplitude and frequency values can help in clinics for an objective assessment of coming labor.

As it was mentioned above, nowadays the abilities of clinicians to regulate the uterine contraction, particularly, in preterm labor, remain limited. Possible solution of this problem may be the inhibition of contractions by regulation of signals, providing its genesis. Based on this, it is necessary to identify and study the mechanisms, supporting this process.

To date, the nature of the genesis of pacemaker electrical rhythms has not been fully investigated. In some types of spontaneously active smooth muscle, interstitial cells of Cajal (ICC) or ICC-like cells have been found. ICC-like cells (pacemaker cells of the gastrointestinal tract) are also found in the urethra, portal vein, urinary bladder, and ureter [57–60]. Moreover, the studies on ICC-like cells isolated from the urethra and urinary bladder showed their ability to generate spontaneous activity.

It has been experimentally shown that these cells are the main in generation and coordination of the muscular electrical activity, which creates peristalsis in the gastrointestinal tract. The existence of cells having complex geometry and terminal processes, such as the interstitial cells of Cajal, was also described in the myometrium [43]. Despite the nature of these cells has not been studied yet, we can not exclude their function similar to the interstitial cells of the gastrointestinal tract, providing generation and coordination of the contractile activity. In the recently presented data on the study of coordination of miometrial contraction, there is a conclusion about abovementioned process regulation by detected interstitial cells of Cajal in the uterus, action of which is similar to the hormonal “sensors” [61].

By morpho-histochemical studies, we have identified a great amount of round or oval atypical cells with high levels of Ca^{2+}-dependent acid phosphatase activity in longitudinal sections of the ovarian part of uterine horn in intact rats. We have also found such cells in the cervical end of uterine horn and uterine corpus, but the greatest accumulation of them was detected in the ovarian end of horn. Under the oxytocin action, a sharp increase in cytoplasmic enzyme activity of intensive-colored atypical cells was observed. These cells are colored so dark in the ovarian part of uterine horn that gives the locus of their location a completely blacked-out form, in contrast to the cervical part of the horn and uterine corpus. Thus, detection the greatest number of atypical cells in the ovarian end of horn can confirm the fact of the longest duration of electrical discharges in this area and the leading role of this particular locus in genesis and coordination of activities of the subsequent downstream active regions.

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