Assessment of myoelectrical signal parameters in estrogen, progesterone, and human chorionic gonadotropin administered in nonpregnant rat myometrium after ovariectomy

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Objective: To investigate the correlation of myoelectrical signals with spontaneous contractile events and physiological states in the nonisolated uterine horn of rats.
Design: In vivo uterine myoelectrical activity recording study.
Setting: Animal and pharmacology laboratory at Inonu University.
Animal(s): Thirty-six female Wistar albino rats.
Intervention(s): Six animals were not castrated and served as a sham-operated control group; the other 30 were ovariectomized (OVX) and put into groups: unbiased OVX subjects, estrogen (E)-biased OVX subjects, P-biased OVX subjects, E-plus-P-biased OVX subjects, and hCG-biased OVX subjects. An MP100 A-CE was used for data acquisition, and a personal computer was used for processing.
Main Outcome Measure(s): Besides the temporal, spectral, and joint time-frequency (spectrotemporal) analysis, some quantitative measures such as standard deviation and mark to space power ratios of myoelectrical signals were measured.
Result(s): Progesterone, E, and hCG administration down-regulated the power and contraction frequency of the uterine electrical signal. The spectral concentrations that occurred around the 0.9, 0.35, and 0.7 Hz frequency ranges may be distinguishing characteristics for P, E, and hCG, respectively.
Conclusion(s): Based on the obtained results, uterine contractions change with ovariectomy and administration of hormones. Progesterone, E, and hCG particularly prolong the quiescent periods of the uterus by reducing the frequency of uterine contractions as well as the power of the myoelectrical activity. Individual or combined use of P, E, or hCG might favor quiescence of the uterine muscle and the maintenance of pregnancy. (Fertil Steril® 2008;89:188–98. ©2008 by American Society for Reproductive Medicine.)

Key Words: Myoelectrical signal, myometrium, estrogen, progesterone, hCG, wavelet

Uterine quiescence is essential for the normal development of the fetus and vital in certain phases of the cycle for successful reproduction (1, 2). Therefore, understanding, characterization, and control of the mechanisms underlying uterine contractions are necessary. The earliest recognized report regarding the contractile activity of uterine myometrium was that of Dickinson (3). In addition, regular labor-like contractions of high intensity, low frequency, and low basal pressure have been described during menstruation (4).

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These contractions originate in the subendometrial myometrium (5, 6). Adequate myometrial contractions may help in gamete or sperm transportation throughout the uterotubal cavities (7, 8), but for successful embryo implantation in spontaneous or assisted reproduction, uterine quiescence is considered to be very important for maintaining the pregnancy by avoiding the displacement of embryos from the uterine cavity (1, 7, 8).

From this standpoint, the control of uterine contractile activity is vital for successful implantation and management of IVF patients (1, 7). One of the basic mechanisms that control uterine contractions is the underlying electrical activity of spontaneously generated action potentials (9). Resting membrane potential, excitability of the cell, and the frequency and duration of action potentials in myometrium are differentially influenced by estrogen (E) and P (10).

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Subendometrial myometrium exhibits cyclic changes in E and P receptors, as has been reported by Noe et al. (11). In addition, the presence of hCG in human myometrium is acknowledged (12); however, the cyclic properties of hCG still remain ambiguous.

Studies indicate that myometrial cells are coupled together electrically by gap junctions composed of connexin proteins (9, 13). Progesterone and 17β-estradiol are thought to exert their effects on the uterus by regulating ion channels (14) and hence the density of gap junctions that influence the myometrium conductivity. While P down-regulates, E dramatically up-regulates the density of gap junctions (15, 16).

Interestingly, hCG has been reported to be one of the hormones that can directly maintain uterine quiescence by down-regulating myometrial gap junctions during pregnancy (17). It mediates its own action by increasing the myometrial biosynthesis of P (17) and consequently inhibits oxytocin-stimulated myometrial contractions (18).

Experiments using ovariectomized (OVX) rats have shown that the amplitudes of membrane potentials from myometrium after E_2 treatment were higher than those after P treatment (10), whereas simultaneous treatment with both P and E_2 produces a plateau potential of longer duration during burst discharges (10). Within certain limits, there is a dose-response relationship between the levels of these steroids in the blood and the frequency of the uterine peristaltic contractions (19).

Briefly, P seems to exert an overall control on uterine quiescence by suppressing a number of genes such as connexin 43 (Cx43) and by downregulating the density of gap junctions in the myometrium, which is essential for uterine contractility (20). A limited number of studies have been performed on in vivo recordings of uterine myoelectrical activity in nonpregnant OVX rats and its properties such as burst power, space power, and quiescence period.

The primary aim of this study was therefore to explore the nature of the electrical activity of the uterus. Furthermore, the experiment was designed to investigate the effects of conjugated equine estrogens (CEEs), P, and hCG (administered in vivo) on the myoelectrical properties of the rat myometrium by using a computerized recording system and signal processing.

MATERIALS AND METHODS

Animals

Thirty-six virgin, adult, albino Wistar rats weighing 200–250 g at 3 months of age were studied. The estrous cycles in sham-operated animals were monitored by cytological evaluation of vaginal smears. The animals were housed in plastic cages with a metal grid lid at a room temperature of 20°C; fluorescent lamps provided artificial light. The rats were kept in groups of six subjects on a 12-hour light/dark cycle, during which the lights were turned on at 6:00 A.M. Food and water were available ad libitum.

The permission for the animal tests and experiments was given by the Bioethical Board of Inonu University Medical Faculty. Thirty animals were ovariectomized bilaterally under intraperitoneal ketamine (50 mg/kg) and xylazine HCl (10 mg/kg) anesthesia. The remaining six rats underwent a surgical procedure similar to the others, but the ovaries were not removed.

Five days after the surgical procedure, the six noncastrated animals were assigned to a sham-operated control group, and the 30 castrated animals were divided into five groups with six rats in each group. The organization of groups is as follows: sham-operated control animals (group 1); OVX control animals (0.9% saline only; group 2); OVX animals treated with P in oil (2.5 μg/g IM; group 3); OVX animals treated with CEE (0.1 mg/kg/day per os gavage; group 4); OVX animals treated with P 2.5 μg/g after 7 days of pretreatment with CEE (0.1 mg/kg per day per os; group 5); and OVX animals treated with hCG (10 IU hCG IP; group 6). In groups 4 and 5, treatment with CEE was started 5 days after bilateral ovariectomy and continued for 7 days with use of a metal gavage probe.

Measurement of Uterine Myoelectrical Activity

The recording of the uterine myoelectrical activity was conducted with a BIOPAC MP100 A-CE data acquisition system (Goleta, CA) (model MP100; version 3.7.2) with a 1000 preamplifier gain (this preamplifier gain was compensated later through soft processing) and a sampling frequency of 500 Hz. Twelve days after the ovariectomy or sham surgery, each rat underwent laparotomy and bipolar electrodes subserously implanted into the uterine horn with 1-cm interelectrode spacing.

The reference electrode was placed on the left leg of the rat. Immediately after the replacement of electrodes, the uterine electrical activity was recorded for at least 3 minutes under anesthesia. After the recordings were successfully made in all 36 rats, the parametric measures were achieved through soft processing for each record. For each group, a segment of these biological signals that contains at least a contraction period is presented to show the temporal and spectral characteristics of the uterine myoelectrical activity.

Signal Preprocessing

The recorded biological signals from the uterus of the rats were found to be nonstationary signals and included at least three patterns of waves in general: a multicomponent nonstationary fast wave or burst wave (the contraction), the envelope of burst potentials (the contraction size), and the spontaneous or unformatted wave portion between two bursts termed as space (the silent phase between contractions).

As a general rule, most of the content of a signal remains in the 2σ (standard deviation) range. Therefore, in the recorded signals the energy spikes with amplitudes exceeding 2σ of the signal were considered to be artifacts because they were quite random and limited the signal's spectral resolution; they were replaced by those with amplitudes of 2σ to improve the signal's accuracy.

The analysis was conducted in both time and frequency domains. The transformation of the signal-to-frequency domain was carried out by the well-known fast Fourier transform. The spectral content of the signals from 0.01 to 1.2 Hz, which is the dominant area of manipulation by the hormone treatment and the most pronounced spectrum segment throughout the literature, was assessed.

Further, wavelet analysis, which has been used as an efficient method for investigating the local characteristics of the nonstationary signals, was done on the signal. Among various mother wavelets, the Morlet wavelet was chosen since it is simple and well suited to the spectral estimation of biological signals.

The Morlet wavelet is simply the product of a complex sinusoidal wave at frequency f with a Gaussian bell shape curve function centered at time τ and a standard deviation σ proportional to the inverse of the scale f. Even though wavelets are limited by dilation effects and the choice of the wavelet type for particular applications, the signal phase is retained, which may be exploited to obtain time lag–related information subsequent to biological activity.

RESULTS

The overall pattern of recorded uterine electrical activity was nonstationary, and the periodicity of rhythmic events varied considerably even during the course of the same experiment. The rhythmic spontaneous electrical bursts, whose envelope or initiator may be considered slow waves probably accompanied by contractions that appeared as intraluminal pressure and axial force, were observed to be fairly regular and of spike potentials, whereas the quiescence between two bursts was observed to be only a nonrhythmic or unformatted electrical signal.

Some parameters of the recorded electrical activity were measured and presented in Table 1, whereby a cross-comparison analysis became possible. Moreover, a segment of the myoelectrical activity delivered by a subject in each group was used as a sample signal. The 1–15 Hz filtered part (a second-order Butterworth filter was used) shows the burst potential's outline, and its 0–1.2 Hz spectrum shows the effect of each drug on the spectrum (see Figs. 1–6).

Table 1 shows the overall effects of hormonal drugs such as E, P, and hCG on the whole uterine electrical signal. The burst/contraction periods remained almost comparable (30 ms) for sham-operated subjects as well as for OVX subjects that were administrated/biased with P, E, and hCG. In unbiased OVX subjects, these periods radically decreased, but they increased for subjects with both E and P.

The quiescent phase between two bursts remained comparable (40 ms) for sham and unbiased OVX subjects, but

TABLE 1

The measured parameters of the uterine contractions in average basis.

	Sham-operated (control group)	Ovariectomized group	*P*	E	E and P	hCG
Mean record time, seconds	140	154	204	256	188	152
Mean burst period, seconds	30	18	31	30	42	34
Mean silent period, seconds	40	42	78	81	100	70
Mean signal power, W	9.313	2.45	1.423	2.38	2	2.357
Mean burst power, W	72.8	14.11	22.57	49.4	42.17	86.87
Mean space power, W	22.5	14.53	2.51	8.2	2.53	4.64
Mean SD	180.55	100.2	85.19	123.435	97.12	94.65
Mean slow-wave frequency, Hz	0.0143	0.0167	0.0092	0.0090	0.0070	0.0096
Mean mark to space time ratio	0.75	0.43	0.397	0.37	0.42	0.485
Mean mark to space power ratio	3.2	0.97	8.9671	6.02	16.64	18.75

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interestingly it almost doubled for OVX subjects biased with P as well as with E. For OVX subjects biased with both E and P, this phase extended to 100 seconds, but for subjects biased with hCG it was found to be approximately 70 seconds. Furthermore, the mark to space time ratios for OVX subjects were more or less analogous, but for sham-operated subjects they had much higher values compared with the OVX ones.

The mean burst power dramatically decreased in the unbiased OVX subjects (see Table 1). As OVX subjects were biased with hormonal drugs, the burst power increased, and, interestingly, for hCG, it drastically increased to 86.87 W. The space mean power in contrast significantly decreased as the OVX subjects were biased with drugs. Consequently, the mark to space power ratios for hormone-biased subjects increased.

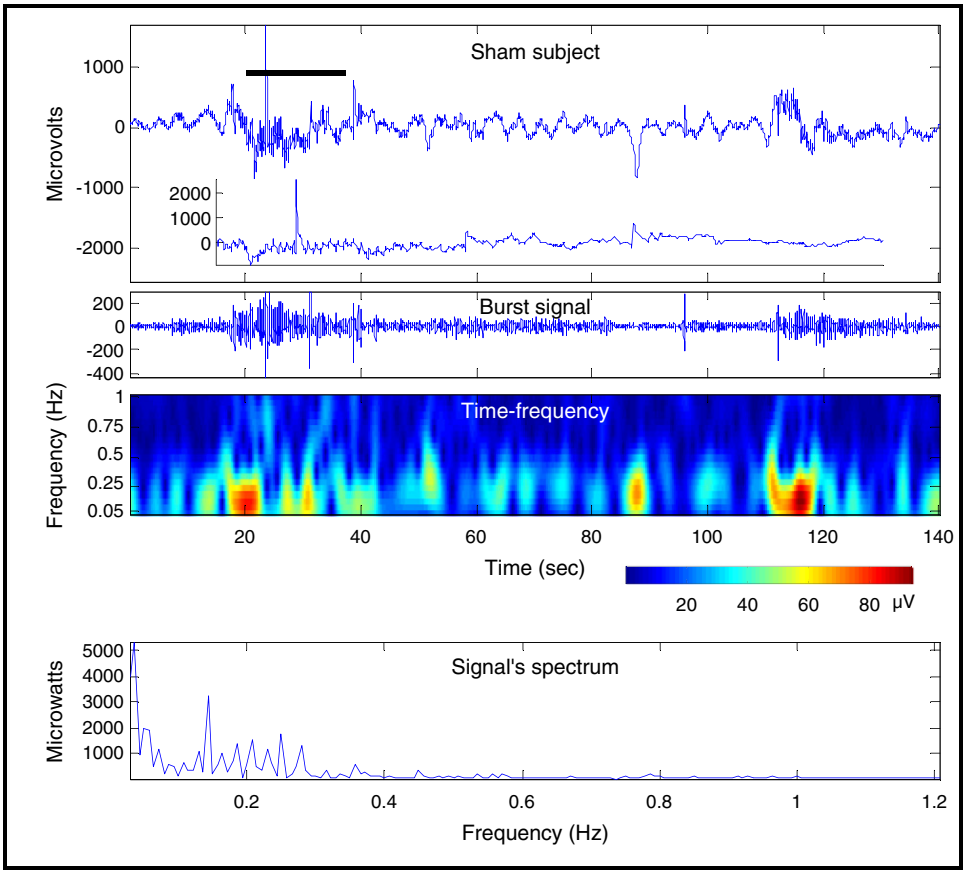
The variability of the signal that was measured by SD has reduced with ovariectomy and was even more reduced for P-administrated subjects. It was also observed that the contraction frequency was reduced in subjects biased with P, E, and hCG (typically around 0.0090 Hz) and much reduced in subjects biased with both E and P.

Figure 1 shows a segment of the electrical activity counting two bursts/contractions of the uterus from a sham-operated rat. The upper pattern in the upper panel shows the whole signal, whereas the next one in the same panel is the zoomed leading burst, which is marked by a solid line. The second panel shows the 1–15 Hz frequency band signal. Our goal in designing such figures was to show the effect of E, P, and hCG on the characteristic structure of the uterine myoelectrical signal, particularly on the pattern of contraction bursts and the lower-frequency band in which much of the effects of hormonal drugs have been shown.

As seen from upper panel, the burst signal is in the format of a train of almost regular pulses whose frequency range from about 1 to 15 Hz. From the spectral point of view, the dominant content of the whole signal remained under

FIGURE 1

The selected sample wave from our experiment delivered by sham-operated subjects as the control group. The top panel shows the recorded signal and the localized burst signal (solid line). The second panel shows the 1–15 Hz pattern of the burst signal (contractions). The third panel shows the time-frequency scheme of the signal, and the last panel shows the low-frequency spectral power density of the signal.



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1 Hz., as in the bottom panel in the figure. The high frequencies were of comparatively low amplitude, but these also need to be characterized.

Similar figures, in the same format, show the electrical activity of the uterus of the remaining unbiased and hormone-biased OVX rat groups. As seen from Figure 2, the temporal characteristic of the whole uterine electrical signal from an unbiased OVX subject particularly within the burst period became more evident. In this case, the pulse train involved in the burst energy was in a more regular form and not buried behind spontaneous pulses generated by the effect of hormones.

Unfortunately, this amount of accuracy of burst signal was not observed for all the subjects belonging to the same group. As seen from the figure, the intensive part of the spectral content of the signal was concentrated around 0.2 Hz (0.1–0.3 Hz), whereas the amplitudes of other harmonics were comparatively low. The high-frequency range of the spectrum might be interesting; this needs to be examined.

The effect of P on the uterine electrical signal is depicted in Figure 3. In this case, the contraction rate trimmed down to 0.0092 Hz on average, as shown in Table 1. The burst/contraction signal, which was in the form of a pulse train, either became irregular in amplitude or some intensive spontaneous pulses could have been generated by P and loaded on the signal, mainly in the contraction phase, as in the upper panel of the figure.

This effect was observed very explicitly in the spectral content of the signal as a significant elevation in the spectral peaks concentrated around the 0.9 Hz frequency range. This is marked by an arrow. The 0.2 Hz frequency, which was shown by unbiased OVX subjects, diminished compared with the 0.9 Hz range. However, P caused the signal's power to decrease significantly in the overall signal.

In the same manner, the effect of E on the uterine electrical signal is shown in Figure 4. The burst rate became 0.0090 Hz as in the case of OVX subjects biased with P. The burst signal, that is, the pulse train, became even more irregular in

FIGURE 2

The selected sample wave from our experiment delivered by unbiased OVX subjects as the second control group. The top panel shows the recorded signal and the localized burst signal (solid line). The second panel shows the 1–15 Hz pattern of the burst signal (contractions). The third panel shows the time-frequency scheme of the signal, and the last panel shows the low-frequency spectral power density of the signal.

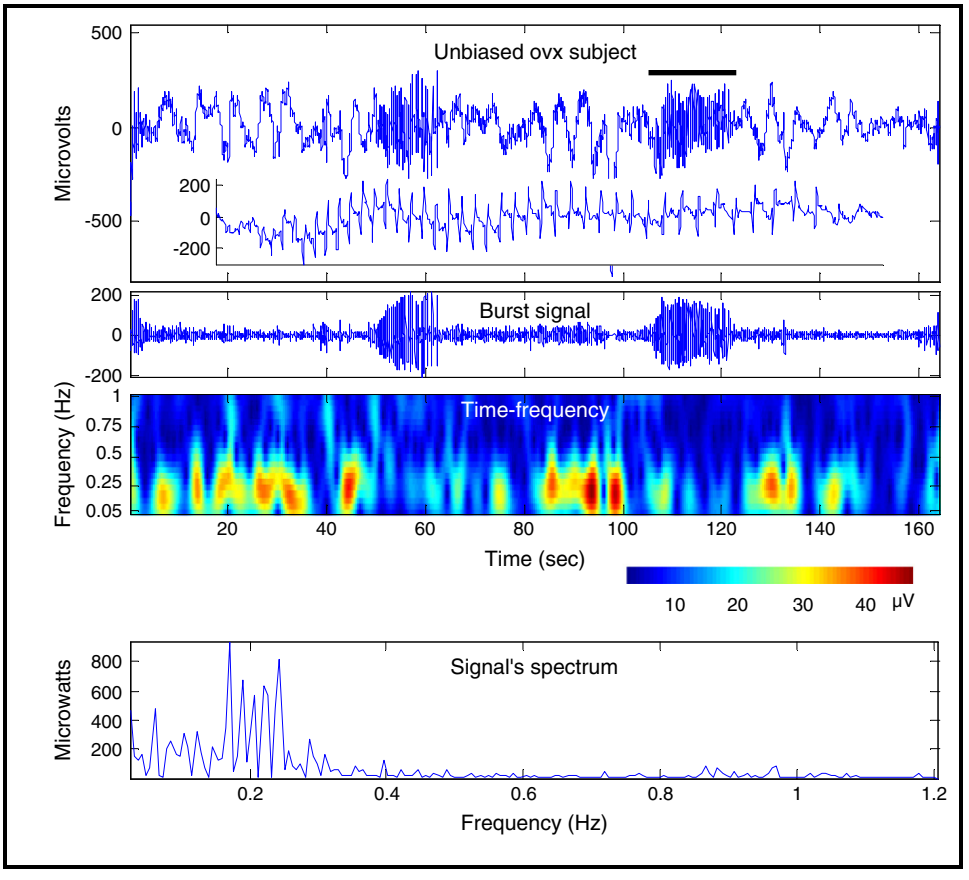

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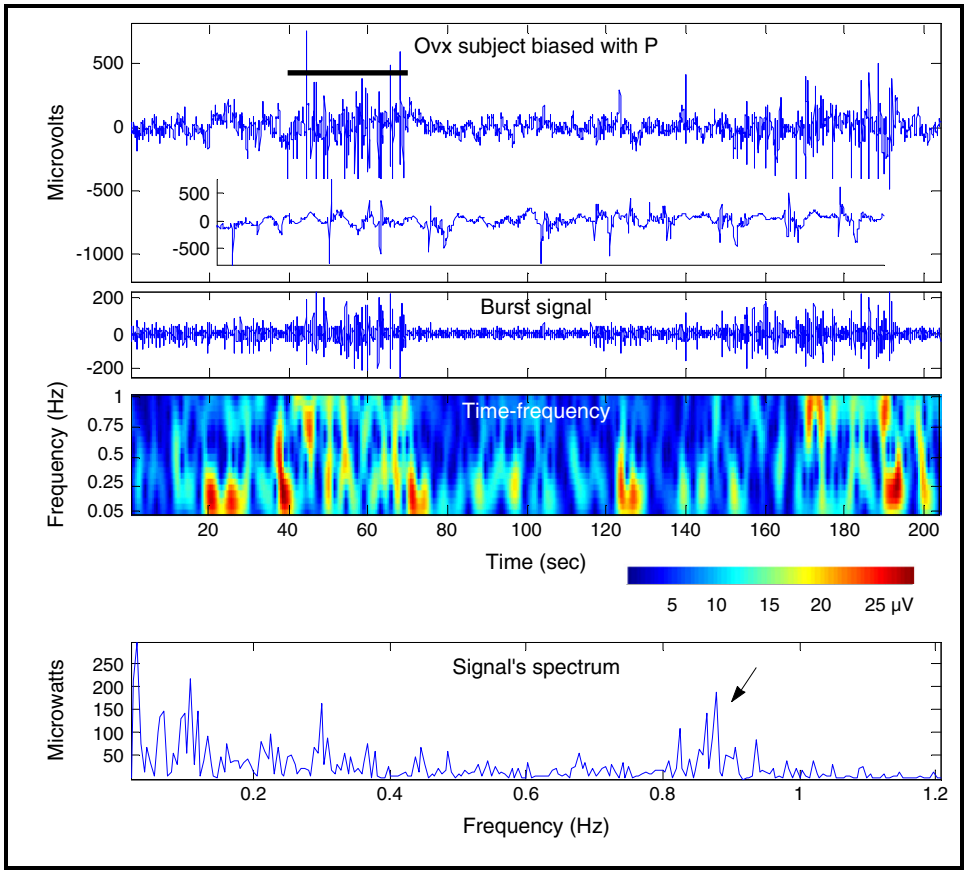
FIGURE 3

The selected sample wave from our experiment delivered by OVX subjects biased with P. The top panel shows the recorded signal and the localized leading burst signal (solid line). The second panel shows the 1–15 Hz pattern of the burst signal (contractions). The third panel shows the time-frequency scheme of the signal, and the last panel shows the low-frequency spectral power density of the signal.



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amplitude and frequency (Fig. 4). In addition to numerous low-amplitude harmonics distributed over a wide frequency band, the signal's spectrum predominantly peaked around 0.35 Hz (marked by an arrow). This seems to be a characteristic spectral parameter caused by E as it was at 0.9 Hz for P.

Similarly, the effect of E plus P was investigated (Fig. 5). In this case, the average frequency of the contractions decayed down to 0.0070 Hz. The pulses within the burst signal turned out to be fairly regular but with much higher peaks. Besides the lower frequencies, the spectral content of the signal was concentrated at about 0.3 and 0.9 Hz, which could be due to E and P, respectively, as occurred for E- and P-biased subjects. In this case the 0.2 Hz was also diminished.

In Figure 6, the same scenario is repeated for OVX subjects biased with hCG. Some irregular pulses were generated in the contraction period, and the contraction frequency also decreased to 0.0096 Hz. The average amplitude of the time domain uterine signal decayed, and the spectral content of the signal dramatically peaked at about 0.7 Hz. This particular frequency also seems to be a characteristic spectral parameter for hCG.

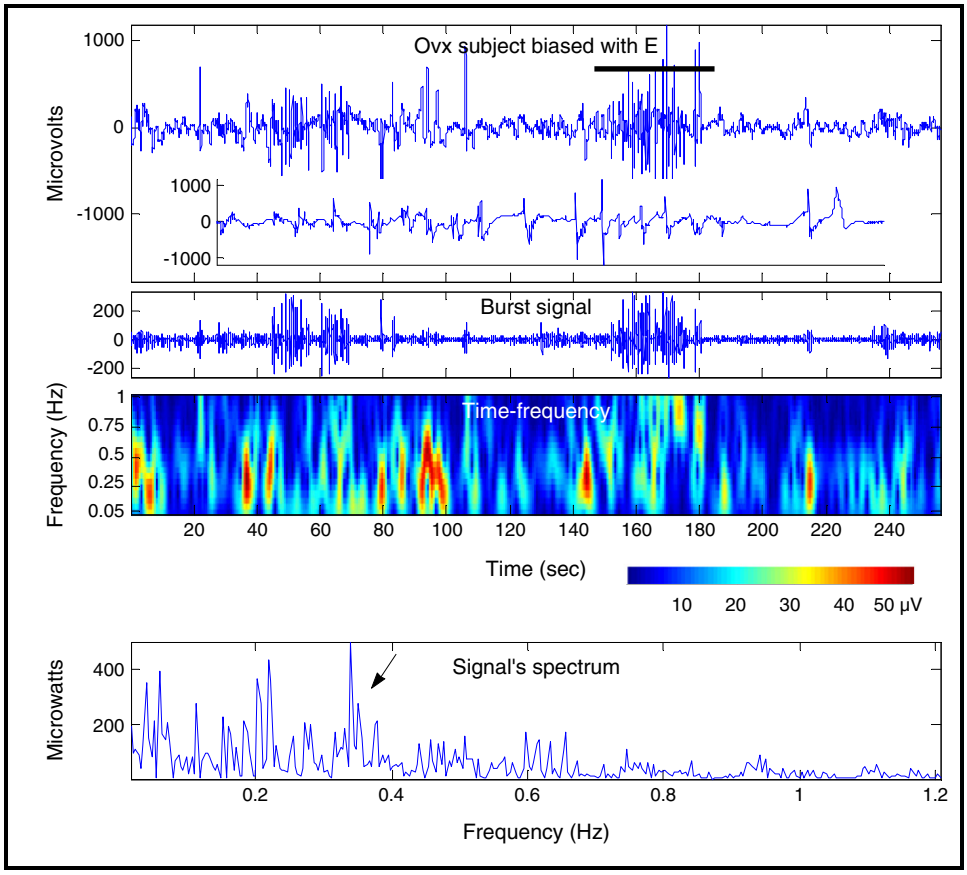
These results show that P, E, and hCG down-regulate the power and contraction frequency of the uterine electrical signal. In addition, the spectral concentrations occurred around the 0.9, 0.35, and 0.7 Hz frequency ranges, which were considered to be distinguishing characteristics for P, E, and hCG, respectively. The wavelet-based time-frequency schemes showed that the energy within the uterine signal is in the format of the nonstationary time signals and particularly that the burst periods contain higher frequencies, which needs to be further analyzed for better understanding.

DISCUSSION

Despite progress in understanding the mechanism of uterine smooth-muscle contraction, the potential to identify and treat possible dyskinetic changes in uterine contractility, particularly in women suffering from infertility, is still limited (8). We studied the myoelectrical activity of the OVX uterine

FIGURE 4

The selected sample wave from our experiment delivered by OVX subjects biased with E. The top panel shows the recorded signal and the localized lagging burst signal (solid line). The second panel shows the 1–15 Hz pattern of the burst signal (contractions). The third panel shows the time-frequency scheme of the signal, and the last panel shows the low-frequency spectral power density of the signal.



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horn of nonpregnant rats. The gross effects of hormonal drugs such as E, P, and hCG on the whole uterine electrical signal were quantified (Table 1). The characteristic patterns of burst potentials generated during the spontaneous contractions as well as the low-frequency spectral content of uterine electrical signal were presented and analyzed for both unbiased and drug-biased subjects.

Our experimental results showed that the mean contraction time in the uterine signal obtained for OVX subjects independently biased with P and E remains almost the same as the contraction time found for sham subjects. While this term decreased for unbiased OVX subjects, it increased for subjects biased with both E and P and with hCG. The quiescent or space periods for OVX subjects biased with drugs were very prolonged compared with those unbiased OVX subjects.

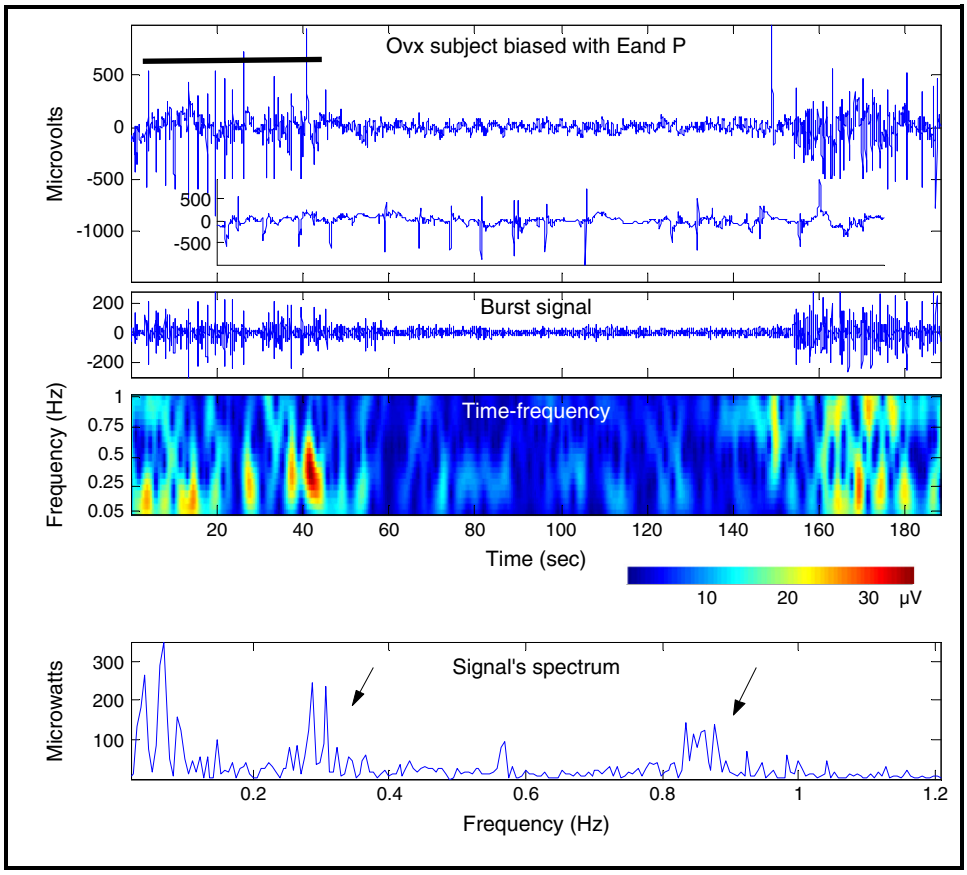
From the power perspective, the mean burst/contraction power dramatically decreased in unbiased OVX subjects (e.g., 14.11 W on average) compared with sham-operated subjects. Interestingly, the burst power more or less doubled for OVX subjects biased with P and tripled for OVX subjects biased with E and with both P and E compared with the unbiased OVX control group. In hCG-biased subjects, the burst power increased to 86.9 W. On the other hand, the mean power in quiescent periods of drug-biased OVX groups was measured to be below that in the unbiased OVX control group.

These findings, particularly the reduction in the power of the quiescent periods, are in line with much of the literature that we have scanned on this topic. For instance, it has been reported that spontaneous contractile activity was less pronounced in uterine strips from postmenopausal women than in those from premenopausal women (21). From this standpoint, the reduction in power density of the uterine signal in unbiased OVX subjects could be due to ovariectomy.

Functional evidence in animals and humans supports the idea that P treatment inhibits uterine myoelectrical activity (22, 23) and prevents the normal increase in Ca^{2+} channel expressions that are observed during pregnancy (24). The gap junction protein Cx43 expression, at least in rats, is clearly

FIGURE 5

The selected sample wave from our experiment delivered by OVX subjects biased with E and P. The top panel shows the recorded signal and the localized leading burst signal (solid line). The second panel shows the 1–15 Hz pattern of the burst signal (contractions). The third panel shows the time-frequency scheme of the signal, and the last panel shows the low-frequency spectral power density of the signal.



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inhibited by P (15, 16). Uterine contractions decrease throughout the luteal phase, which suggests that P has a negative effect on uterine contractility (8).

In contrast to P, E's effect on uterine myoelectrical activity with regard to whether it inhibits or stimulates contractility is obscure. Verhoeff et al. (13) have reported that a single dose of 17β-estradiol induces the formation of myometrial gap junctions in oophorectomized ewes. According to Lye and coauthors (25), 17β-estradiol affects uterus contractility diphasically: first it is inhibiting, and then it is stimulating. Estrogens have been shown to hyperpolarize smooth muscles by activating the outward K^+ current (26) and thereby suppress spontaneously generated muscle contractions (and so the burst power).

Estrogen also inhibits Ca^{2+} entry (27, 28) and K^+ channels in smooth-muscle cells from pregnant rat myometrium (29). These findings validate the results of the present study. Ayoubi et al. (30) showed similar uterine contraction frequencies at the end of the follicular phase of menstrual and IVF cycles. Their data have indicated a marked increase in E_2 level in IVF, but this increase did not further stimulate contractions (30).

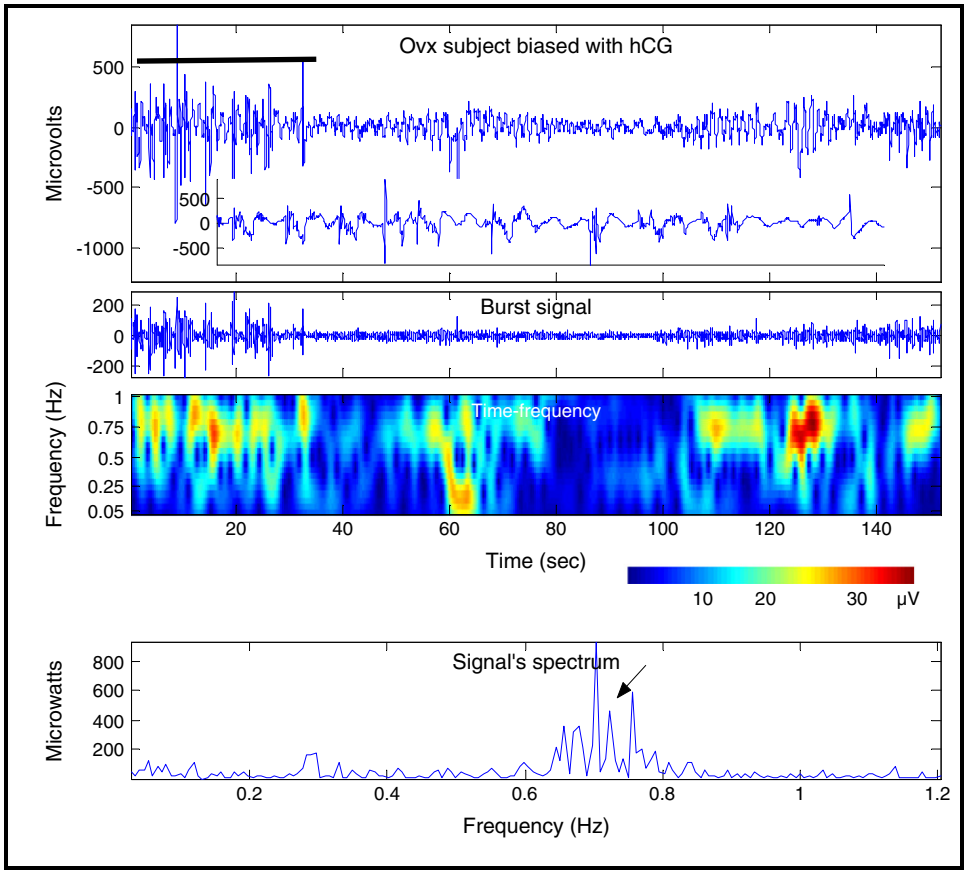
Our results showed that subjects biased with E and P had longer burst and quiescent periods and a low quiescence power than with P alone. This confirms Kuriyama and Suzuki's findings that the state of electrical activity is determined by the most recently given hormone in rats that were treated with E and P sequentially (10).

The deviations from the mean value measured by SD demonstrated that on average sham subjects (SD = 180.55) are more erratic than OVX subjects (SD = 100). While E increased the SD, P, and hCG, E together with P decreased the SD of the uterine signal. Thus, the SD or variance of the electrical activity of the uterus seems to have a direct correlation to the uterine contraction dynamics.

We also determined the repetition rate of bursts. It was observed that the frequency of bursts, that is, contractions,

FIGURE 6

The selected sample wave from our experiment delivered by OVX subjects biased with hCG. The top panel shows the recorded signal and the localized leading burst signal (solid line). The second panel shows the 1–15 Hz pattern of the burst signal (contractions). The third panel shows the time-frequency scheme of the signal, and the last panel shows the low-frequency spectral power density of the signal.



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dramatically decayed down to 0.0070 Hz with an increase in irregularity as subjects were administered drugs. Throughout the literature, it has been found that in the cardiac muscle of OVX rats an increase in the sensitivity of contractile elements to Ca^{2+} could be stopped by exogenous E_2 (31). Bulletti et al. (8) showed that in the menstrual cycle the E-treated uterine contraction frequency is around 5/minute, but it is reduced to below 2.5/minute for the uterus exposed to P.

Studies have shown that hCG can directly regulate the functions of several nongonadal reproductive tissues (32, 33). Myometrium is one of the nongonadal reproductive tissues that contain hCG receptors (12). A recent study suggests that hCG could be a uterotropic hormone that maintains myometrial quiescence until labor (17). During labor, the number of myometrial hCG receptors is low compared with that during no labor in term or preterm pregnancies (34).

Exogenous hCG can cause myometrial cells to increase in both size and population (35) and can inhibit oxytocin-stimulated myometrial contractions (18). Our study demonstrated that hCG is one of the hormones that can maintain uterine quiescence by increasing the silent period and decreasing the signal power in this period.

From the physiological state, Cajal-like interstitial cells have been described outside the gastrointestinal tract. Evidence of the presence of a Cajal-like cell population in the myometrium (36, 37) has also been reported. One possible source of the uterine slow waves therefore may be the Cajal-like cells of myometrium. Interstitial cells of Cajal generate spontaneous myoelectrical slow wave and thereby initiate the electrical activity of smooth-muscle cells (38).

However, Duquette's electrophysiological study (37) did not support a role for the Cajal-like cells as generators of slow waves within the myometrium. Thus, the slow-wave activity exhibited by the myometrium remains an inherent property of the smooth-muscle cells and needs to be further studied.

From the spectral point of view, it is interesting that P, E, and hCG were exhibited at high spectral concentrations

around, respectively, 0.9, 0.35, and 0.7 Hz. Based on these results, this seems to be a distinguishing characteristic of P, E, and hCG. But these original results still need further investigation. If this is the case, by observing the spectral content of the uterine electrical signal a professional may estimate the particular drug that was given to the subject.

The wavelet time-frequency schemes, on the other hand, revealed that these particular spectral concentrations are not continuous in time and predominantly emerge in the burst (contraction) phases. The spectral concentration around 0.2 Hz, which is detected for unbiased OVX subjects, is also not continuous and emerged in silent (quiescent) phases.

In conclusion, our results are consistent with those of previous studies that show a relationship between exposure to E, P, or hCG and the inhibitory effects of these hormones on in vivo uterine myoelectrical parameters (10, 14, 17). Moreover, it is demonstrated that the uterine contraction rate could be decreased with exogenous administration of E, P, and hCG. This confirms the utero-relaxing effects of E, P, and hCG in the myometrium of nonpregnant OVX rats that were previously mentioned.

Consequently, the uterine contraction parameters change by hormone administration. This change may be attributed to genomic and nongenomic effects of E_2, P, and hCG and the interactions of these hormones.

Other parameters such as burst power, space power, and the mark to space power ratio together with an understanding of uterine wave generators could give better insight into the mechanism of uterine contraction. The characterization of different uterine contraction parameters after distinct hormonal application may help the physician in better management of IVF patients.

Thus, management of silent period and space power with sex steroids may increase IVF success rates. What is more, individual or combined use of E, P, or hCG favors quiescence of the uterine muscles in the luteal phase and so supports maintenance of pregnancy. Nevertheless, further human studies are warranted to put this important observation into practice.

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REFERENCES

1. Bulletti C, De Ziegler D, Rossi S, Polli V, Massoneau M, Rossi E, et al. Abnormal uterine contractility in nonpregnant women. Ann NY Acad Sci 1997;828:223–9.
2. IJland MM, Evers JL, Dunselman GA, Volovics L, Hoogland HJ. Relation between endometrial wavelike activity and fecundability in spontaneous cycles. Fertil Steril 1997;67:492–6.
3. Dickinson RL. The technique of timing human ovulation by palpable changes in ovary, tube and uterus. Am J Obstet Gynecol 1937;33: 1027–33.
4. Hendricks CH. Inherent motility patterns and response characteristics of the nonpregnant human uterus. Am J Obstet Gynecol 1966;96:824–43.
5. de Ziegler D, Bulletti C, Fanchin R, Epiney M, Brioschi PA. Contractility of the nonpregnant uterus: the follicular phase. Ann NY Acad Sci 2001;943:172–84.
6. Verkauf BS. Myomectomy for fertility enhancement and preservation. Fertil Steril 1992;58:1–15.
7. Fanchin R, Righini C, Olivennes F, Taylor S, de Ziegler D, Frydman R. Uterine contractions at the time of embryo transfer alter pregnancy rates after in-vitro fertilization. Hum Reprod 1998;13:1968–74.
8. Bulletti C, de Ziegler D, Polli V, Diotallevi L, Del Ferro E, Flamigni C. Uterine contractility during the menstrual cycle. Hum Reprod 2000;15: 81–9.
9. Garfield RE, Saade G, Buhimschi C, Buhimschi I, Shi L, Shi SQ, et al. Control and assessment of the uterus and cervix during pregnancy and labour. Hum Reprod Update 1998;4:673–95.
10. Kuriyama H, Suzuki H. Changes in electrical properties of rat myometrium during gestation and following hormonal treatments. J Physiol 1976;260:315–33.
11. Noe M, Kunz G, Herbertz M, Mall G, Leyendecker G. The cyclic pattern of the immunocytochemical expression of oestrogen and progesterone receptors in human myometrial and endometrial layers: characterization of the endometrial-subendometrial unit. Hum Reprod 1999;14:190–7.
12. Reshef E, Lei ZM, Rao CV, Pridham DD, Chegini N, Luborsky JL. The presence of gonadotropin receptors in nonpregnant human uterus, human placenta, fetal membranes, and decidua. J Clin Endocrinol Metab 1990;70:421–30.
13. Verhoeff A, Garfield RE, Ramondt J, Wallenburg HC. Electrical and mechanical uterine activity and gap junctions in estrogen-treated oophorectomized sheep. Am J Obstet Gynecol 1986;155:1192–6.
14. Knock GA, Tribe RM, Hassoni AA, Aaronson PI. Modulation of potassium current characteristics in human myometrial smooth muscle by 17beta-estradiol and progesterone. Biol Reprod 2001;64:1526–34.
15. Zhao K, Kuperman L, Geimonen E, Andersen J. Progestin represses human connexin43 gene expression similarly in primary cultures of myometrial and uterine leiomyoma cells. Biol Reprod 1996;54:607–15.
16. Petrocelli T, Lye SJ. Regulation of transcripts encoding the myometrial gap junction protein, connexin-43, by estrogen and progesterone. Endocrinology 1993;133:284–90.
17. Ambrus G, Rao CV. Novel regulation of pregnant human myometrial smooth muscle cell gap junctions by human chorionic gonadotropin. Endocrinology 1994;135:2772–9.
18. Eta E, Ambrus G, Rao CV. Direct regulation of human myometrial contractions by human chorionic gonadotropin. J Clin Endocrinol Metab 1994;79:1582–6.
19. Kunz G, Herbertz M, Noe M, Leyendecker G. Sonographic evidence for the involvement of the utero-ovarian counter-current system in the ovarian control of directed uterine sperm transport. Hum Reprod Update 1998;4:667–72.
20. Chwalisz K. Role of progesterone in the control of labour. In: Chwalisz K, Garfield RE, eds. Basic mechanisms of term and preterm labour. Schering Foundation Workshop 5. Berlin: Springer-Verlag, 1993:97–163.
21. Vedernikov YP, Hartke JR, de Long MA, Saade GR, Garfield RE. Sex hormone effects in non-pregnant rat and human myometrium. Eur J Obstet Gynecol Reprod Biol 2003;108:59–66.
22. Verhoeff A, Ramondt J, Garfield RE, Wallenburg HC. Modulation of spontaneous myometrial activity in chronically instrumented ovariectomized sheep. Eur J Obstet Gynecol Reprod Biol 1985;19:113–24.
23. Buhimschi C, Boyle MB, Garfield RE. Electrical activity of the human uterus during pregnancy as recorded from the abdominal surface. Obstet Gynecol 1997;90:102–11.
24. Tezuka N, Ali M, Chwalisz K, Garfield RE. Changes in transcripts encoding calcium channel subunits of rat myometrium during pregnancy. Am J Physiol 1995;269:1008–17.
25. Lye SJ, Wathes DC, Porter DG. Oestradiol-17 beta both inhibits and stimulates myometrial activity in ewes in vivo. J Reprod Fertil 1983;67:335–41.
26. Harder DR, Coulson PB. Estrogen receptors and effects of estrogen on membrane electrical properties of coronary vascular smooth muscle. J Cell Physiol 1979;100:375–82.

27. Osa T, Ogasawara T. Effects in vitro of progesterone and estradiol-17 beta on the contractile and electrical responses in rat myometrium. Jpn J Physiol 1984;34:427–41.
28. Han SZ, Karaki H, Ouchi Y, Akishita M, Orimo H. 17 beta-estradiol inhibits Ca2+ influx and Ca2+ release induced by thromboxane A2 in porcine coronary artery. Circulation 1995;91:2619–26.
29. Okabe K, Inoue Y, Kawarabayashi T, Kajiya H, Okamoto F, Soeda H. Physiological significance of hyperpolarization-activated inward currents (Ih)in smooth muscle cells from the circular layers of pregnant rat myometrium. Pflugers Arch 1999;439:76–85.
30. Ayoubi JM, Epiney M, Brioschi PA, Fanchin R, Chardonnens D, de Ziegler D. Comparison of changes in uterine contraction frequency after ovulation in the menstrual cycle and in in vitro fertilization cycles. Fertil Steril 2003;79:1101–5.
31. Wattanapermpool J, Riabroy T, Preawnim S. Estrogen supplement prevents the calcium hypersensitivity of cardiac myofilaments in ovariectomized rats. Life Sci 2000;66:533–43.
32. Lei ZM, Toth P, Rao CV, Pridham D. Novel coexpression of human chorionic gonadotropin (hCG)/human luteinizing hormone receptors and their ligand hCG in human fallopian tubes. J Clin Endocrinol Metab 1993;77:863–72.
33. Shi QJ, Lei ZM, Rao CV, Lin J. Novel role of human chorionic gonadotropin in differentiation of human cytotrophoblasts. Endocrinology 1993;132:1387–95.
34. Zuo J, Lei ZM, Rao CV. Human myometrial chorionic gonadotropin/luteinizing hormone receptors in preterm and term deliveries. J Clin Endocrinol Metab 1994;79:907–11.
35. Kornyei JL, Lei ZM, Rao CV. Human myometrial smooth muscle cells are novel targets of direct regulation by human chorionic gonadotropin. Biol Reprod 1993;49:1149–57.
36. Ciontea SM, Radu E, Regalia T, Ceafalan L, Cretoiu D, Gherghiceanu M, et al. C-kit immunopositive interstitial cells (Cajal-type) in human myometrium. J Cell Mol Med 2005;9:407–20.
37. Duquette RA, Shmygol A, Vaillant C, Mobasheri A, Pope M, Burdyga T, et al. Vimentin-positive, c-kit-negative interstitial cells in human and rat uterus: a role in pacemaking? Biol Reprod 2005;72:276–83.
38. Ordog T, Ward SM, Sanders KM. Interstitial cells of Cajal generate electrical slow waves in the murine stomach. J Physiol 1999;518:257–69.