Spectrotemporal changes in electrical activity of myometrium due to recombinant follicle-stimulating hormone preparations follitropin alfa and beta

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Objective: To investigate the effects of follitropin alfa and beta on the myoelectrical activity of rat myometrium using signal-processing techniques.
Design: Prospective, placebo-controlled study.
Setting: Animal and pharmacology laboratory at Inonu University.
Animal(s): Forty-five female Wistar albino rats.
Intervention(s): Thirty of 45 animals involved in the experiment were registered as the superovulation group. After two successive normal estrous cycles, these animals were put into three equal subgroups. Group 1 was the control; animals were given 0.9% saline. Groups 2 and 3 were treated with follitropin alfa (Gonal-f) and follitropin beta (Puregon), respectively. The other 15 animals were ovariectomized (OVX) and subjected to the same protocol. The uterine myoelectrical signals were recorded and analyzed using a Matlab environment.
Main Outcome Measure(s): Power/second, variance, and the effects of recombinant human follicle-stimulating hormone (FSH) on myoelectrical signals were assessed through temporal, spectral, and joint time-frequency analysis. The uterine endometrium and ovarian morphology were also assessed concerning primary follicles, antral follicles, and corpora lutea.
Result(s): The power and some characteristic spectral components of myoelectrical signal were reduced with the administration of follitropin alfa and beta. No statistically significant difference was detected between endometrial and ovarian histology of the rats treated with these follitropins.
Conclusion(s): Uterine myoelectrical signals change with administration of recombinant human FSH preparations. Follitropin beta and, more precisely, follitropin alfa suppress the spectral components and power of the myoelectrical signals, which provides uterine quiescence. (Fertil Steril® 2008;90:1348–56. ©2008 by American Society for Reproductive Medicine.)

Key Words: Myoelectrical signal, follitropin alfa, follitropin beta, superovulation, myometrium, endometrium, rat

The pituitary gonadotropin follicle-stimulating hormone (FSH) belongs to a family of glycoprotein hormones consisting of two dissimilar bounds forming alfa and beta subunits (1). The FSH receptor, a G-protein coupled receptor present in granulosa cell of ovary, is a unique transmembrane molecule pivotal in fertility for the purpose of folliculogenesis (2, 3). The action of FSH via these receptors is mediated by cyclic adenosine monophosphate (cAMP), a second messenger system that is induced by adenylate cyclase and G-proteins (4, 5).

The introduction of recombinant human FSH has facilitated the development of novel FSH compounds with different potency and action duration. The amino acid sequences of recombinant human FSH preparations are identical to that of human pituitary FSH (6). Two similar recombinant human FSH preparations but with different purification regimes are currently available in the forms of follitropin alfa and follitropin beta (7–9). They are similar in terms of immunopotency, in vitro biopotency, and internal carbohydrate complexity (10). However, recombinant human FSH preparations differ from pituitary FSH and each other in their degree of glycosylation and sialylation as well as the purification process, which consequently affects their safety and efficacy.

The presence of FSH receptors in nongonadal tissues has been documented in various studies (11–13). These FSH receptors in the form of mRNA transcripts and proteins have been reported in myometrium (11). Also, FSH is known to directly act on endometrium, resulting in decidualization of endometrial stromal cells in vitro (12). The FSH receptors have also been found in the cervix (13). In the cervix, FSH is speculated to cause the uterine relaxation that is essential for the normal development of the fetus and vital in certain phases of the cycle for successful reproduction (14).

Both follitropin alfa and beta are extensively used in assisted reproduction to trigger the ovaries for superovulation.

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Because FSH receptors are present in the myometrium (11), recombinant human FSH may affect the myometrial electrical activity during superovulation and thereby possibly modify myometrial action potentials. So far, the influence of these two preparations on uterine electrical signals and, accordingly, on uterine contractions has not been adequately investigated.

The rat is a widely used model for studies on the physiology of gonadotropin action (15, 16). Our experiment was designed to investigate the effects of follitropin alfa and beta on the myoelectrical properties of the rat myometrium by using a computerized recording system and dedicated signal processing methods, as had been previously used by our group (17).

MATERIALS AND METHODS

Animals

This study was carried out in the Experimental Research Laboratory of the Inonu University Faculty of Medicine, complying with the approval of the ethic committee and the guidelines for care and use of experimental animals. Forty-five mature, healthy, 4-month-old female rats of Wistar strain, weighing between 200 and 250 grams and having a 4-to-5-day regular estrous cycle, were used in the study. The animals were kept under standard conditions: 12-hour light and 12-hour dark periods, 20°C constant temperature, and a humidity range between 40% and 60%. The rats had free access to standard dry pellets ad libitum and tap water until the end of the study. Daily vaginal smears were taken to determine the estrous cycle of each animal as per established protocol (18). The rats were monitored at least for two successive 5-day estrous cycles, then they were randomized and studied.

Treatment

Superovulation Rats undergoing superovulation (N = 30) were equally divided into three groups. Group 1 (control) animals were given 0.9% saline. Groups 2 and 3 were treated with follitropin alfa (Gonal-F; Serono, Aubonne, Switzerland) and follitropin beta (Puregon; Organon, Oss, the Netherlands), respectively. The treatment period was defined as dioestrus 1, 2, and 3 by unifying metestrus and dioestrus periods as described by van Cappellen et al. (15). Rats in groups 2 and 3 were respectively treated with subcutaneous injections of follitropin alfa and follitropin beta using the scheme shown in Table 1.

Ovariectomy To minimize the possible influence of estrogen and progesterone, which might have provoked myoelectrical activity in the superovulation group, a second experiment was set up with animals (N = 15) that underwent ovariectomy. Ovariectomy was performed following established protocol (18). Ten days after the surgical procedure, the ovariectomized (OVX) animals were equally divided into three groups. Group 1 (control) received 0.9% saline. Groups 2 and 3 received follitropin alfa and follitropin beta, respectively. The rats received subcutaneous injections of follitropin alfa or follitropin beta using the scheme illustrated in Table 1.

Measurement of Uterine Myoelectrical Activity

The recording of the uterine myoelectrical activity was conducted with a BIOPAC MP100 A-CE data acquisition system (model MP100; version 3.7.2; Goleta, CA) with a 1000 preamplifier gain (this preamplifier gain was compensated later through signal processing) and a sampling frequency of 500 Hz. Seventeen hours after the last injection, each rat in superovulation (on the expected day of proestrus at 10:00 hours) and OVX groups (on the 14th day of castration) underwent laparotomy once again, and bipolar electrodes were subserously implanted into their uterine horn with 1-cm interelectrode spacing. The reference electrode was placed on the left leg, and the uterine electrical activity was recorded for at least 3 minutes under anesthesia. The signals were analyzed in Matlab (version 6; The MathWorks, Natick, MA) environment.

The uterine horns and ovaries of superovulated rats were processed following standard histologic techniques (19). The uterine endometrium and ovarian morphologic features were then assessed in terms of primary follicles, antral follicles, and corpora lutea.

Signal Preprocessing

Because of being quite random and limiting the time-frequency (TF) resolution, the spikes exceeding 2σ (standard deviation) of the signal were considered as artifacts and were replaced by the value of 2σ in all off data. This did not change the content of the signal, which mostly remained in the 2σ range (20). To remove the DC part and other high frequency spikes, the signal was then filtered by a second-order Butterworth passband filter, whose cut-off frequencies were set on 0.25 Hz and 10 Hz.

Because the recordings were not based on an excite and record strategy, the temporal characteristics, such as burst potentials leading to contractions of uterus, in the individual records were not expected to be time coherent, and the time domain averaging did not provide adequate results. Therefore, a signal that holds characteristics common to all individual records was configured by the cross-convolutions implemented over the signals in each group. In the frequency domain, this driven signal is expressed as

$$c(f) = k \prod_{i=1}^{N} F\{s_i(t)\}, \quad (1)$$

where $F\{\cdot\}$is the Fourier transform and $s_i(t)$ represents the individual records from the rats' uterine horn. This is, in fact, a group-convolution process that can give a measure of spectral coherency in each group. For rationality, the amplitude of this derived signal was scaled to the value of average of involved spectrums through the constant k. The time domain

TABLE 1

Treatment protocols of the superovulated and ovariectomized rats.

Treatment	Dioestrus-1		Dioestrus-2		Dioestrus-3		Pro-estrus
	10.00 h	17.00 h	10.00 h	17.00 h	10.00 h	17.00 h	10.00 h
Superovulation							
Control[a]	—	—	—	—	—	—	MP100 A-CE
Follitropin alfa	2.5 IU	1 IU	1 IU	0.5 IU	0.5 IU	0.5 IU	MP100 A-CE
Follitropin beta	2.5 IU	1 IU	1 IU	0.5 IU	0.5 IU	0.5 IU	MP100 A-CE
Ovariectomy (OVX)	11th day of OVX		12th day of OVX		13th day of OVX		14th day of OVX
OVX-control[a]	—	—	—	—	—	—	MP100 A-CE
OVX + follitropin alfa	2.5 IU	1 IU	1 IU	0.5 IU	0.5 IU	0.5 IU	MP100 A-CE
OVX + follitropin beta	2.5 IU	1 IU	1 IU	0.5 IU	0.5 IU	0.5 IU	MP100 A-CE

[a] Control animals were given 0.9 % saline subcutaneously of the same volume as the groups treated with recombinant human FSH.

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version of this signal, $c(t)$, was then obtained by the inverse Fourier transform of $c(f)$. The spectrotemporal characteristics of the $c(t)$ were represented by $c(t,f)$ using continuous wavelet transform. Unfortunately, because of their low amplitude, the mimics that linked to 1.0 Hz to 2.0 Hz frequency band were imperceptible. To visualize these low amplitude components of $c(t, f)$, it was rearranged as

$$\tilde{c}(t,f) = |\widehat{c}(t,f)|\left\{\frac{c(t,f)}{|\widehat{c}(t,f)|}\right\}^{n}, \qquad (2)$$

where $\widehat{c}(t,f)$ refers to the maximum value of the modulus of $c(t, f)$, and $n \in R$ is to be in the range of [0.1,0.9]. Herein, n was chosen as 0.25.

For more convenient analysis of biological signals, Morlet wavelets were used, whose details can be found in the literature (21). Besides their ease of implementation, an exclusive advantage of wavelet-dependent tools is that the phase of the signal is retained, which can be exploited to obtain time-lag related information in consequence of the biological activity.

Statistical Analysis

Results are given as mean ± standard error of the mean (SEM). The Statistical Package for Social Sciences, version 11.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. Individual group parameters were assessed with one-sample Kolmogorov-Smirnov Z-test and were found to be abnormally distributed. Mann-Whitney U-test was used to detect statistically significant differences between the animals given saline and recombinant human FSH. $P<.05$ was considered statistically significant.

RESULTS

The analysis mainly conducted in the time, frequency, and TF domains. In the time domain, some global parameters such as the emitted power/second and variance of the recorded electrical activity were measured (Table 2), which facilitated a quantitative analysis regarding the intensity of considered gonadotropins. Momentous results that manifested uterine electrical activity induction have been obtained through the TF representation of the spectrally coherent time signal, $c(t)$, derived from the signal's spectra.

TABLE 2

Global effects of recombinant human follicle-stimulating hormone on the response of the uterine myoelectrical signal.

Treatment	Superovulation group		Ovariectomized group	
	Power/seconds	Variance	Power/seconds	Variance
Control	3.5×10^{-10}	7.6×10^{-08}	9.36×10^{-10}	1.67×10^{-07}
Follitropin alfa	1.23×10^{-10}	4.45×10^{-08}	8.85×10^{-10}	1.21×10^{-07}
Follitropin beta	5.15×10^{-10}	3.12×10^{-08}	6.51×10^{-09}	4.70×10^{-07}

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FIGURE 1

Individual time domain signals obtained from myometrium of rats.

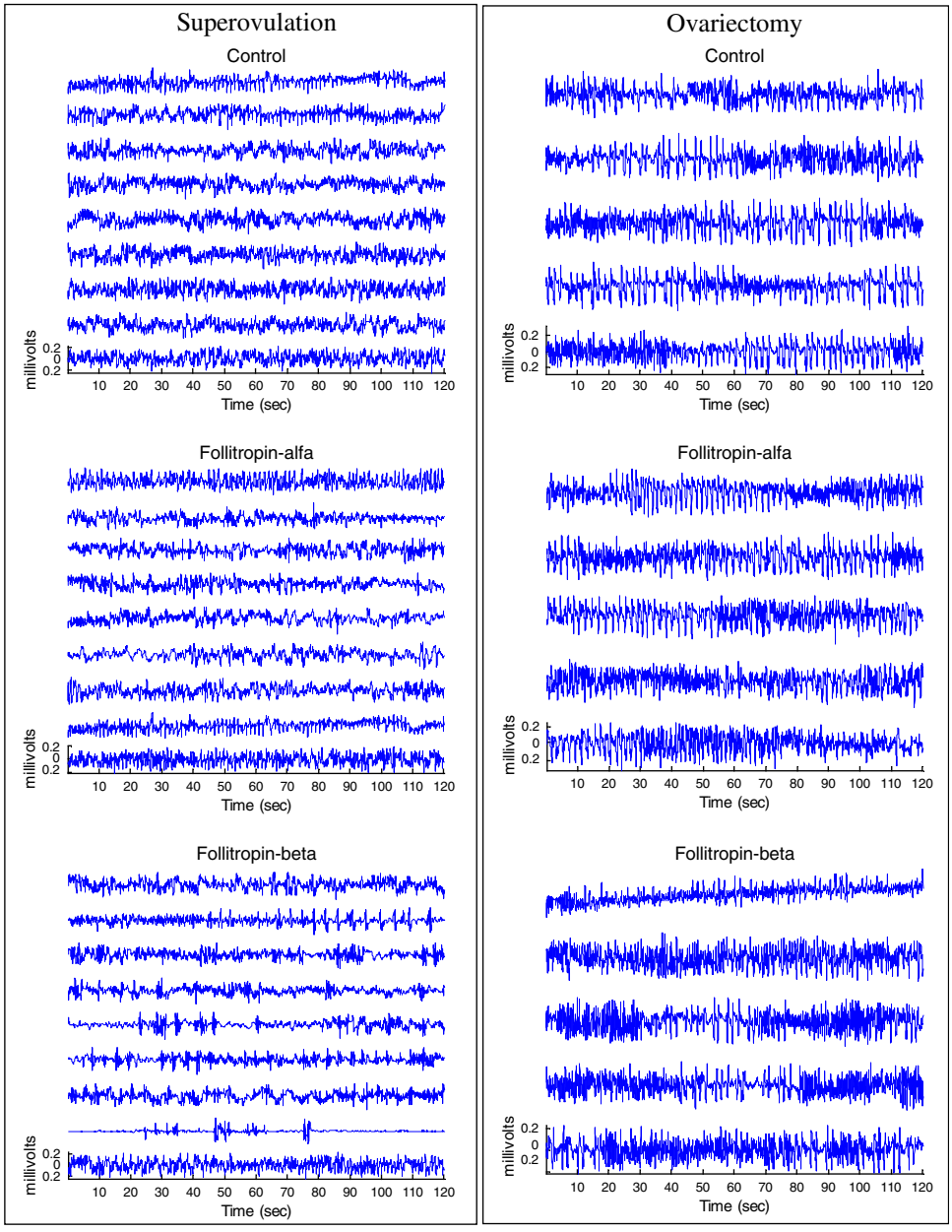

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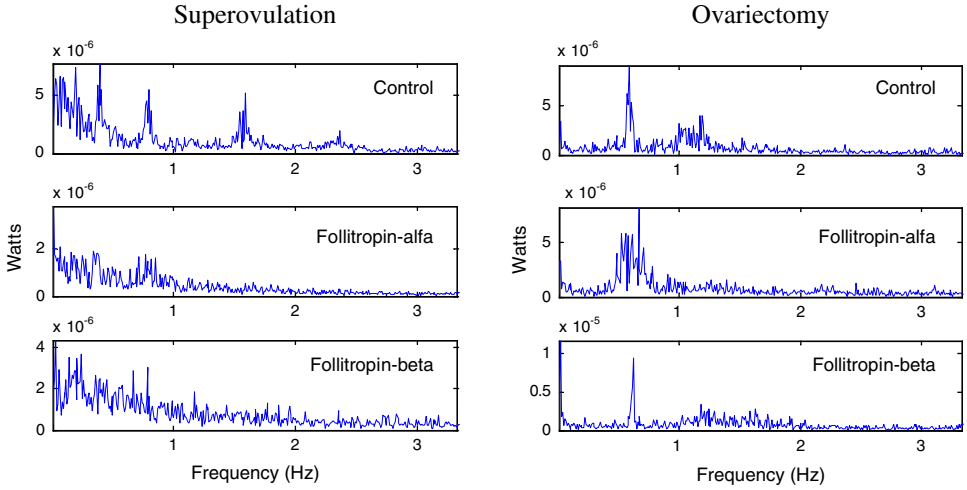
Figure 1 shows the recorded time domain signals for the control and the follitropin alfa and beta administrated subgroups of the superovulation and ovariectomy groups. Note that in each subgroup of superovulation one of the records was not satisfactory, so they were taken out of assay. Despite both recombinant human FSH preparations primarily regulating follicular maturation (as can be recognized in the figure), they exhibit hardly perceptible effects on the myoelectrical signals presented in the time domain.

The derived spectrally coherent signals, $c(f)$, are shown in Figure 2 for the superovulation and ovariectomy groups. In the figure, the upper panel shows the $c(f)$ for the superovulation control group. Besides very low frequency components, three other main spectral components were detected over 0.4, 0.8, and 1.6 Hz. The middle and lower panels show the follitropin alfa and beta injected groups, respectively. Besides a general reduction in whole power, the 1.6 Hz component was entirely reduced and other characteristic spectral components appearing over 0.4 Hz and 0.8 Hz were partly reduced by the effect of the received remedy. However, the signal's power in the follitropin beta group was not reduced as much as it was in the follitropin alfa group.

FIGURE 2

The spectrally coherent signals, *c*(*f*)s, derived from each group. The *c*(*f*) which associated to a particular group is labeled on the top of the panel.



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In addition to the diminution of the low frequency energy, the removal of ovaries caused the components lying over 0.8 Hz and 1.6 Hz to step down in frequency (interestingly, by a factor of 0.75 in general) to 0.6 Hz and 1.2 Hz, respectively. The amplitude of these components slightly increased as their frequency reduced. In a similar fashion, both of the recombinant human FSH preparations suppressed the 1.2 Hz and reduced the 0.6 Hz components. In the OVX group, the effect of follitropin alfa on the power of the signal was also found to be higher than the effect of follitropin beta. However, these representations do not demonstrate temporal variation or TF motivation of those detected components. Therefore, as a further step, the TF scheme of the *c*(*t*) was obtained for all groups (Fig. 3). This process revealed transparent upshots caused by ovariectomy and the individual recombinant human FSH preparations on the uterus. In addition to the conclusions made for Figure 2, in these TF representations, the instant and forms of contractions (i.e., when and how) that were detected are labeled with arrows.

Through the histology of experimented rats, a prominent endometrial stromal decidual change was not observed in both control and recombinant human FSH groups (Fig. 4). Also no statistically significant difference was detected between the mean number of primary follicles in control and recombinant human FSH administrated groups. However, compared with the control group, the mean number of antral follicles and corpora lutea significantly increased in the follitropin alfa and beta treated groups. The mean number of antral follicles and corpora lutea were similar in follitropin alfa and beta groups (see Table 3 and Fig. 4).

DISCUSSION

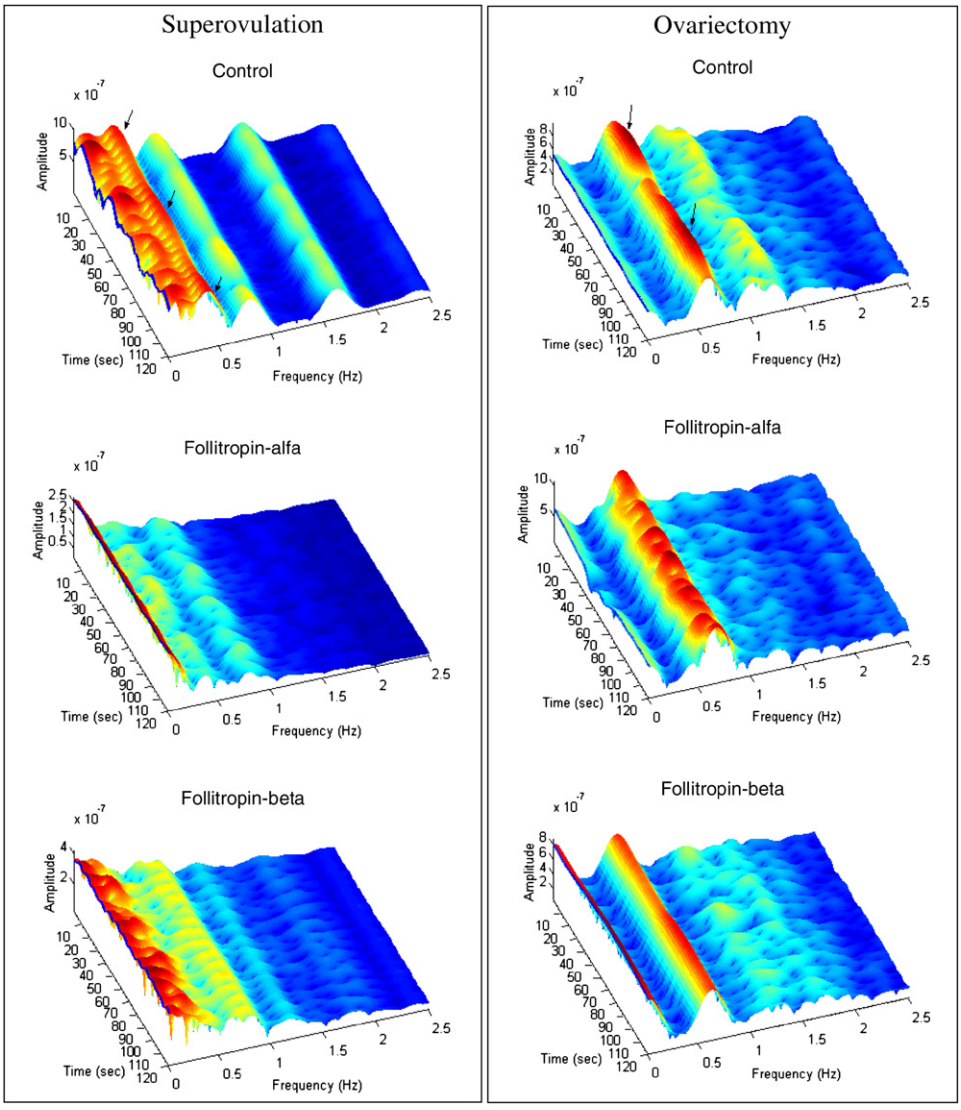
The overall myoelectrical waveform was in the form of nonstationary and multicomponent signals incorporating both slow waves (very low frequency components) and higher spectral components. The OVX rats manifested throbbing signals owing to the higher variance values, as seen in Table 2. Due to OVX, in addition to the suppression of low frequency components, the frequencies of 0.8 Hz and 1.6 Hz components (detected in non-OVX animals) were slightly demoted. This spectral change might be due to the decreased serum levels of estrogen and progesterone or, conversely, to the increased level of endogenous FSH in the myometrium after OVX. The effects of OVX, estrogen, and progesterone on myoelectrical activity were reported previously elsewhere (17). In our study, the focus was on the effects of FSH on uterine myoelectrical signals.

In addition to ovarian stimulation, we found that the emitted signal power/second decreased in the rats administered follitropin alfa and beta (see Table 2), which means that both follitropins have physiologic roles in regulating relaxation or contraction of uterus (22, 13). Gonadotropin receptors in the uterus are associated with the same transduction pathways as in the gonads (23, 24), and FSH stimulates the production of cAMP and inositol phosphate signaling pathways (18); accordingly, it encourages cyclooxygenase-2 (COX-2) to increase the emission of prostaglandin E_2 (PGE2), which has a role in relaxing myometrium (22, 25–27).

If the results obtained for superovulation group are solely analyzed, it can be observed that in addition to a marginal dispersion in the whole energy of the signal the spectral

FIGURE 3

The time-frequency representations of *c*(*t*)s derived from each group. The *c*(*t*) which associated to a particular group is labeled on the top of the panel.



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components (0.8 Hz and 1.6 Hz) diminished with the administration of follitropin alfa or beta (see Figs. 2 and 3). From these results, two hypotheses may be put forward to explain these spectral changes caused by exogenous recombinant human FSH: [1] it is due to the increase of estrogens and progesterone in the myometrium as a consequence of the increase in their secretion in ovaries; or [2] it is due to the increase of FSH level in the smooth muscle cells of the myometrium.

From cross-analysis of TF images obtained for superovulation and OVX animals, the genuine cause of these changes can be derived. As the occurrence of this spectrotemporal effect is common to both groups, the suppression of these spectral components and of the overall signal power is prominently due to the increase in the FSH level in myometrium rather than the change in the estrogen or progesterone levels. The increased FSH level in the circulatory system and thereby in the myometrium that follows recombinant human FSH administration probably suppresses the 0.8 and 1.6 Hz components in superovulation and correspondingly the 0.6 and 1.2 Hz components in the OVX groups. This verifies that the presence of FSH receptors in the myometrium makes it possible for recombinant human FSH preparations to modify myometrial action potentials independent of estrogen and progesterone (11). Therefore, these findings serve as a direct correlation to the capacity of the drug not only in superovulation but also in suppressing uterine contractility.

FIGURE 4

A-B, Multi follicular development in follitropin alfa and beta treated animals (H&E. × 100 respectively). **C**, Smooth endometrial surface with cuboidal epithelium (arrowhead) and stromal deciduoid change (arrow) in follitropin alfa administrated rats (H&E, × 200). **D**, Tortuous endometrial surface and columnar epithelium (arrow), compact stroma in follitropin beta group (H&E, × 200).

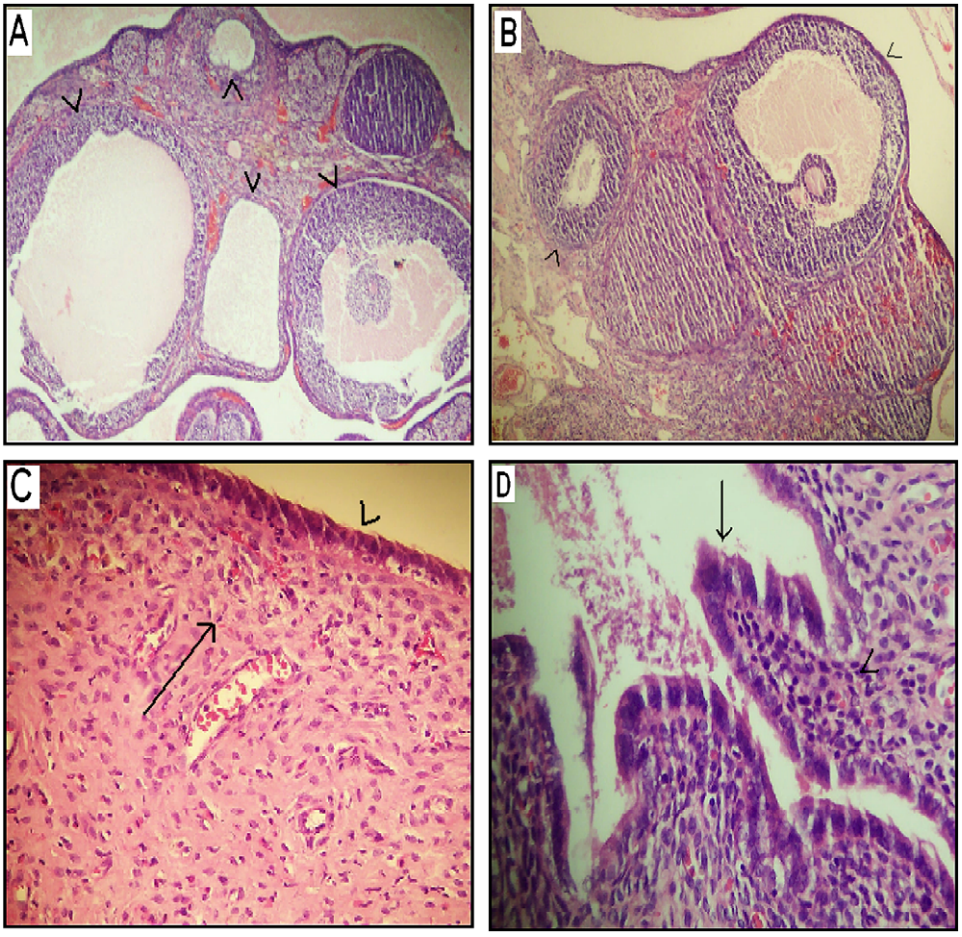

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TABLE 3

Number of primary follicles, antral follicles, and corpora lutea in rats treated with decreasing doses of recombinant human follicle-stimulating hormone and in saline-treated control rats.

Groups	Primary follicle	Antral follicle	Corpora lutea
I. Control	3.28 ± 0.18	3.42 ± 0.20	2.57 ± 0.20
II. Follitropin alfa	3.71 ± 0.28	5.71 ± 0.86	4.14 ± 0.26
III. Follitropin beta	3.37 ± 0.37	5.00 ± 0.42	4.37 ± 0.26
I vs. II	0.244	0.017	0.003
I vs. III	0.803	0.010	0.001
II vs. III	0.542	0.631	0.678

Note: Values are mean ± SEM.

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If the two follitropins are compared in terms of their aptitude in suppressing the spectral components, follitropin alfa was more effective in both superovulation and OVX animals. It has also been shown that follitropin alfa has more consistent action than follitropin beta, even from batch to batch (28). This difference may be due to the ultrafiltration of follitropin alfa followed by five chromatographic stages (6).

A comparable number of primary follicles were found in all groups involved in this experiment. In contrast to the primary follicles, the number of antral follicles and corpora lutea increased in the groups given recombinant human FSH. This result reveals that, although the beginning of follicle development is independent of gonadotropins, the advanced follicle development depends on FSH stimulation, which is in accord with previous reports (19, 29).

From another perspective, decidualization of the endometrial stroma is critical for embryo implantation and trophoblast invasion. So the decrease in stromal decidualization is a negative factor for fertility (30), and FSH could inhibit the proliferation of endometrium (31) via its receptors found in stromal cells (32). Therefore, recombinant human FSH administration may disturb the synchronization between embryo and endometrium, resulting in implantation failure (19, 30, 33). No significant decidualization was detected in individual animals, which may lead to speculation (see Table 3). Nonetheless, the 1.6-Hz and, correspondingly, the 1.2-Hz spectral components detected in myoelectrical activity may be specialized components playing a role in decidualization.

To our knowledge, ours is the first study to demonstrate the direct influence of recombinant human FSH on the electrical activity of rat myometrial smooth muscle cells in vivo. Our results give rise to questions about the possible role of recombinant human FSH in human extragonadal tissues, which could also affect in vitro fertilization success rates. The extragonadal effects of recombinant human FSH preparations, particularly on uterine quiescence and implantation, as well as the function of integrative mechanisms mediating between myometrium and endometrium must be elucidated in detail by further relevant studies.

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