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ORIGINAL ARTICLE

Effects of Beta-sitosterol on Isolated Human Non-Pregnant Uterus in Comparison to Prostaglandin E_2

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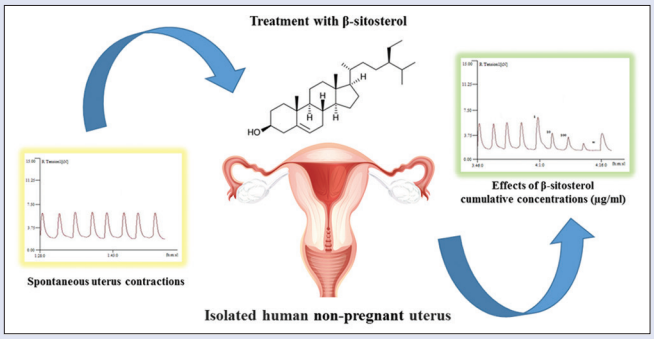
ABSTRACT

Background: Beta-sitosterol (β-sitosterol) is one of the several phytosterols widely studied for its potential to reduce benign prostatic hyperplasia and blood cholesterol levels. **Objective:** In the present study, the effects of β-sitosterol on spontaneous and agonist-induced contractions in *in vitro* nonpregnant human uterus with respect to prostaglandin E_2 (PGE_2) were investigated. **Materials and Methods:** Myometrial strips, measuring approximately 15 mm × 4 mm × 2 mm, were attained from hysterectomy samples of premenopausal women. Longitudinal muscle strips were mounted on tissue baths, under physiological conditions, to measure their isometric contraction. The effects of cumulative amounts of β-sitosterol on spontaneous motility in the absence and presence of prazosin, atropine, fulvestran, indomethacin, or ethylenediaminetetraacetic acid (EDTA), and on agonist-induced motor activity, were examined. **Results:** On strips in the follicular phase, both β-sitosterol (1–100 μg/ml) and PGE_2 (0.1–10 μg/ml) increase, in a concentration-dependent manner, muscular basic tonus and amplitude and frequency of spontaneous uterine contractions; whereas on strips obtained during periovulatory phase, β-sitosterol and PGE_2 cause inhibition of uterine motility. For contractile response, the effective concentrations (EC_{50}) were 47.8 μg/ml and 5.19 μg/ml, respectively. Unlike indomethacin, the tissue pretreatment with prazosin, fulvestran, atropine, or ethylenediaminetetraacetic acid did not affect the contractile uterine responses to β-sitosterol. Furthermore, the β-sitosterol was able to potentiate the contractile response induced by acetylcholine and vasopressin. **Conclusions:** These observations suggest that β-sitosterol may be a useful modulator of the uterine motility during menstrual cycle, facilitating female fertility.

Keywords: Beta-sitosterol, female fertility, isolated human myometrium, prostaglandin E_2, uterine contractility

SUMMARY

- On strips in the follicular phase, beta-sitosterol (β-sitosterol) and prostaglandin E_2 (PGE_2) increase muscular basic tonus and amplitude and frequency of spontaneous uterine contractions
- On strips in the periovulatory phase, β-sitosterol and PGE_2 cause inhibition of uterine motility
- Tissue pretreatment with prazosin, fulvestran, atropine, or ethylenediaminetetraacetic acid did not affect the contractile uterine responses to β-sitosterol unlike indomethacin
- β-sitosterol was able to potentiate the contractile response induced by acetylcholine and vasopressin.



Abbreviations used: PGE_2: Prostaglandin E_2; PGs: prostaglandins; EP_{1-4}: prostaglandin receptor types; EC_{50}: Half-maximal effective concentration.

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INTRODUCTION

Beta-sitosterol (β-sitosterol) [Figure 1] is the most representative compound belonging to phytosterols, a class of plant bioactive compounds widely distributed in the plant kingdom, which possesses a structure altogether similar to cholesterol. Many preclinical and clinical studies about the potential cholesterol-lowering activity and protection against benign prostatic hypertrophy of β-sitosterol were carried out.[1-4]

Despite this, few and not-in-depth studies on its potential to positively affect the reproductive system and female fertility have been performed. β-sitosterol is one of the major components (16%) of the methanolic extract of pomegranate seed and has been reported that this extract is a powerful motor activity stimulator in rat uterus.[5]

Prostaglandins (PGs) possess an important role in the parturition procedure. Particularly, the endogenous PGE_2 [Figure 1] exerts a pivotal role in the onset and maintenance of human labor.[6] In fact, clinically, a PGE_2 analog is commonly employed to induce labor. PGs also appear to possess the capability to regulate myometrial contractility in a typical manner, both at the time of labor and of human reproduction.[7] Appropriate uterine motility and uterotubal transport function are necessary for the transfer of semen and gametes, as well as for effective embryo implantation; on the other hand, inappropriate

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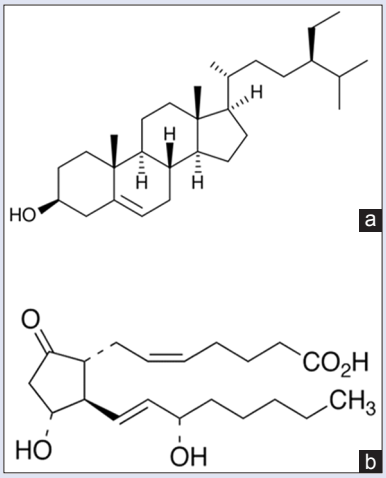

Figure 1: Structures of beta-sitosterol (a) and prostaglandin E_2 (b). Reproduction size at column width

uterine motility may cause miscarriages, ectopic pregnancies, endometriosis, and retrograde bleeding.[8-11] PGE_2 seems to have a pivotal role in the physiological uterine contractility regulation during the menstrual cycle, facilitating normal reproductive processes[12] through its receptors (EP_{1-4}) to promote angiogenesis and cell proliferation and modulate uterine activity. The excitatory effects of the PGE_2 in human uterus, in the follicular phase of the menstrual cycle, are mediated by the EP_1 and EP_3 subtypes, involving calcium release from intracellular stores, while the profound inhibitory effect of PGE_2 during the periovulatory peak is mediated by the EP_2 and EP_4 subtypes which stimulate adenylate cyclase.

The present investigation for the first time represents the evaluation of the effects of β-sitosterol on human nonpregnant isolated uterus during the follicular and ovulatory stages of the menstrual cycle, based on its potential to affect the reproductive system.

MATERIALS AND METHODS

Chemicals

β-sitosterol, PGE_2, prazosin, atropine, fulvestran, indomethacin, acetylcholine, and vasopressin were acquired from Sigma (Sigma Chemical Co., St. Louis, MO). Ethylenediaminetetraacetic acid (EDTA) and salts for the preparation of the Krebs' solution were purchased from VWR International, Radnor, PA, USA.

Tissue collection

The Local Bioethical Committee of the University of Messina approved this study. Human uterus samples were obtained from hysterectomy specimens derived from premenopausal women with no evidence of malignant uterine disease. The samples, which were obtained from the Department of Human Pathology of the Adult and of the Development Age of the University of Messina, were placed in Krebs' solution, at pH 7.4, at 4°C, and used within 3 h from collection.

Isolated uterus experiments

Uterine strips were cut up measuring about 15 mm × 4 mm × 2 mm. The longitudinal strips were prepared for isometric recording under 1 g of traction in an organ-isolated tissue bath (Schuler organ bath, type 809-Hugo Sachs Elektronik, Harvard Apparatus GmbH; March-Hugstetten, Germany) as previously reported.[13] Each strip was located in a 20-ml jacketed tissue bath containing Krebs' solution at 37°C and pH 7.4. The composition of the solution (in mmol/l) was as follows: sodium chloride: 121, potassium chloride: 4.5, sodium bicarbonate: 15.5, sodium phosphate: 1.2, calcium chloride: 2.5, magnesium chloride: 1.2, glucose: 11.5, and gassed continuously with 95% O_2–5% CO_2. A silk thread was used to connect the uterine strips to a fixed hook and an isometric force transducer (HSE F30, Type 372-Hugo Sachs Elektronik, Harvard Apparatus GmbH; March-Hugstetten, Germany). The uterine preparation was equilibrated for 90 min, during which the Kreb's solution was replaced every 15 min. Uterine motor activity (tonus, amplitude, and frequency of contractions) was measured and the data were recorded and stored using an HSE-ACAD W data acquisition software (Hugo Sachs Elektronik, Harvard Apparatus GmbH; March-Hugstetten, Germany) and displayed on a PC screen. The majority of the uterine preparation generated spontaneous motility within 40–90 min, and strips with no activity during this period were eliminated. Following generation of regular rhythmic contractions, β-sitosterol and PGE_2 were applicated in a cumulative mode at 1–100 μg/ml and 0.1–10 μg/ml concentrations, respectively. Further experiments were carried out to investigate the possible mechanisms by which β-sitosterol could modify the uterine motility. With the aim to observe whether α-adrenoceptors, PGs, muscarinic and estrogenic receptors, or extracellular calcium are implicated in the uterine response to β-sitosterol, concentration–response curves, in the presence of α-adrenoceptor blocker, cyclooxygenase inhibitor, estrogenic receptor blocker, muscarinic receptor blocker, or extracellular calcium chelator, were recorded. Muscle strips were treated with prazosin 2 μg/ml, indomethacin 3 μg/ml, atropin 5 μg/ml or fulvestran 1 mg/ml, and EDTA (1×10^{-3} M) at least 25 min before the application of β-sitosterol.

In a distinct group of experiments, after the equilibration period and once regular motility had generated, the spontaneous motor activity of the uterine strips was suppressed (quiescent preparation) through a reduction of the temperature of the organ bath to 30°C and by modifying the nutritional salt solution by reducing the calcium concentration to one-tenth of the amount in a normal Krebs' solution. After abolition of spontaneous motility, the effects of β-sitosterol, on acetylcholine (1 μg/ml)- and vasopressin (1 μg/ml)-induced contractions, were investigated.

Different strips from uterus sample were tested for β-sitosterol and PGE_2, and each treatment was performed in quintuplicate.

Statistical analysis

Results were expressed as the mean ± standard error of the mean of five measurements. Differences in muscular tension were tested for significance using one-way analysis of variance followed by Dunnett's test, with $P < 0.05$ as statistically significant.

RESULTS AND DISCUSSION

Effects on spontaneous uterine motility

Preliminary experiments conducted on uterine strips mounted, for isometric recording, under 1 g of traction in organ bath did not show any significant effects on uterine motility rate. The latter ones, in fact, have been maintained for several hours under control condition without any alteration until PGE_2 treatment at several increasing concentrations (positive control), which enhanced it progressively. Repeated concentration–response curves for PGE_2 were statistically stackable.

On strips in the follicular phase, both PGE_2 (concentration range: 0.1–10 μg/ml) and β-sitosterol (concentration range: 1–100 μg/ml) increase, in a concentration-dependent manner, muscular basic tonus and amplitude and frequency of spontaneous uterine motility [Table 1 and Figures 2-4, respectively]; whereas on strips obtained during periovulatory phase, β-sitosterol [Figure 5] and PGE2 cause

an inhibition of uterine motility. For contractile response, the effective concentrations (EC_{50}) were 47.8 μg/ml and 5.19 μg/ml, respectively.

Table 1: Isolated human uterus during the follicular phase of the menstrual cycle

Treatment	Concentration (μg/ml)	Spontaneous contractions		
		Basal tone increase (%)	Amplitude increase (%)	Frequency increase (%)
β-sitosterol	1	3.2±0.65	5.7±0.90	3.5±2.00
	10	9.0±1.30*	29.5±3.20*	19.5±3.40*
	100	12.5±1.00*	62.0±2.50*	27.7±2.75*
PGE_2	0.1	4.0±0.70	7.5±1.15	6.3±1.35
	1	13.0±1.10*	37.5±2.40*	31.0±3.00*
	10	18.5±1.25*	79.0±4.25*	57.8±4.10*

*P<0.05 respect to basal values. Effects of β-sitosterol and PGE_2 on spontaneous uterine motility. Results are expressed as mean±SEM (n=5). PGE_2: Prostaglandin E_2; SEM: Standard error mean

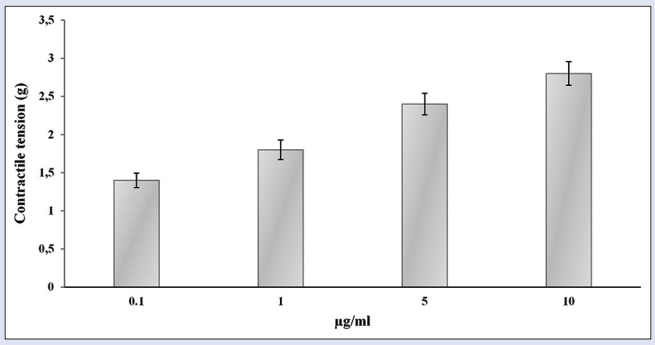

Figure 2: Concentration-dependent effects of prostaglandin E_2 on spontaneous motility of nonpregnant human uterus. Results were expressed as mean ± standard error of the mean (n = 5). Reproduction size at column width

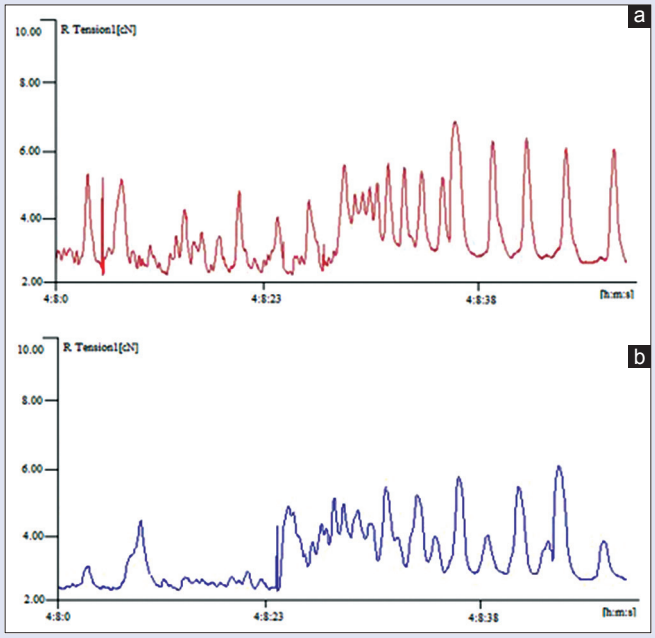

Figure 4: Representative recordings of isolated human uterus during the follicular phase of the menstrual cycle. Effects of prostaglandin E_2 (a) and beta-sitosterol (b) on basal tone, amplitude, and frequency of spontaneous motility. Reproduction size at column width

It is well known that α- and β-adrenergic and muscarinic activities, as well as PG or estrogenic activity, modulate the human uterine motility.[14] As a consequence, we investigated their potential responsibility to explain the stimulatory effects of β-sitosterol on uterine motility. EDTA, prazosin (2 μg/ml), atropine (5 μg/ml), and fulvestran (1 mg/ml), at concentrations that block the respective receptors or extracellular

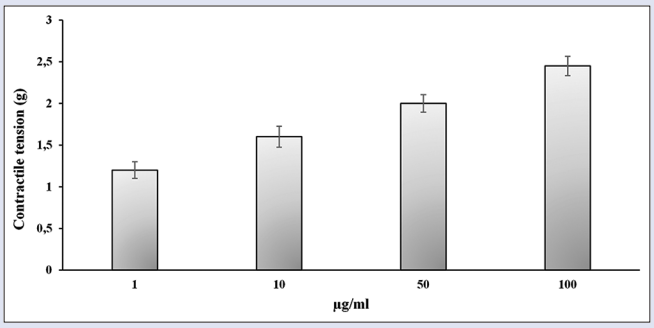

Figure 3: Concentration-dependent effects of beta-sitosterol on spontaneous contractility of nonpregnant human uterus. Results were expressed as mean ± standard error of the mean (n = 5). Reproduction size at column width

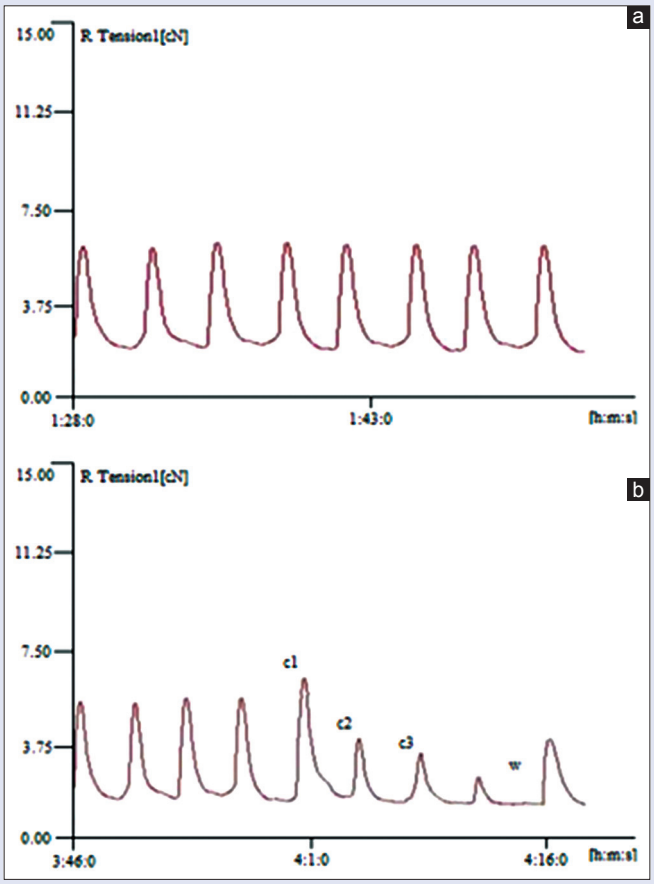

Figure 5: Effects of beta-sitosterol on myometrial motility in isolated human uterus during the periovulatory phase of the menstrual cycle. (a) Representative recordings of spontaneous contractions under control conditions. (b) Effects of cumulative concentrations (1–100 μg/ml) of beta-sitosterol. c1 = 1 μg/ml; c2 = 10 μg/ml; c3 = 100 μg/ml; w = washing. Reproduction size at column width

calcium release in the isolated human uterus,[15] did not affect the β-sitosterol effect on uterine motility (data not shown). On the contrary, pretreatment of the tissue with indomethacin, at concentrations which block the respective enzyme system (3 μg/ml), abolishes the uterine responses to β-sitosterol.

Such results suggest that the metabolic intermediates of α-adrenergic, muscarinic, or estrogenic activities do not play a pivotal role in the uterine contractile response to β-sitosterol unlike those that of the cyclooxygenase. Spontaneous uterine motility is tightly related to the cytoplasmic amount of free Ca^{2+}.[16] Drugs, which increase the intracellular availability of Ca^{2+}, stimulate the spontaneous contractility of isolated uterine muscles in humans.[15] Probably, the stimulatory action of β-sitosterol on spontaneous uterine motility is also related to the calcium ions' release from intracellular deposits. This hypothesis is supported by the findings that the observed stimulant effects of β-sitosterol on uterine smooth muscle did not appear to be mediated by α-adrenergic, muscarinic, or estrogenic receptors; furthermore, the pretreatment of the uterus preparations with EDTA did not produce significant changes in the uterine response to β-sitosterol (data not shown).

Effects on agonist-induced contraction in quiescent uterine preparation

In this experimental model, the phasic contractions of the uterus were inhibited in Ca^{2+} poor solutions, while the basal tone was preserved.

Quiescent uterine strips were contracted by the application of acetylcholine (1 μg/ml) and vasopressin (1 μg/ml) and with acetylcholine or vasopressin (1 μg/ml) + β-sitosterol (50 μg/ml). Results showed that β-sitosterol was able to increase (35%–40%) the vasopressin and acetylcholine-induced contractions significantly [Figure 6].

It is well known that, in order to induce uterine contractions, agents such as vasopressin and acetylcholine cause an increase of free intracellular Ca^{2+} (receptor mediated) by increasing Ca^{2+} influx and/or by increasing the release of Ca^{2+} from cellular depots. Consequently, it is probable that the contractile effects of β-sitosterol on precontracted uterus may be mediated through its capability to affect cellular calcium concentration.

CONCLUSIONS

In light of the results of the present research, it can be affirmed that β-sitosterol enhances the contractility of human uterine muscles in the follicular phase of the menstrual cycle, while it inhibits the uterine motility of uterine muscles in the periovulatory phase. These effects were similar and stackable to those generated by PGE_2, used as positive control, in the experimental model examined. Since the uterotonic effect of β-sitosterol was not modified by reducing the extracellular calcium concentration or by adding EDTA, it may be suggested that β-sitosterol dose dependently impairs primarily Ca release from intracellular stores as is indicated also from the similarity of the contractive effect induced by PGE_2. In addition, since the uterine response to β-sitosterol was abolished by indomethacin, it may be suggested that β-sitosterol acts likely through the stimulation of endogenous PGE_2 release. It is well known that endogenous PGE_2 plays a fundamental role in the cyclical modulation of uterine contractility during the menstrual cycle, thus facilitating pregnancy. In fact, an appropriate uterine motility and adequate uterotubal transport function are necessary for the transport of semen and gametes and for successful embryo implantation.

It can be suggested that β-sitosterol may be a useful modulator of the uterine motility during the menstrual cycle, thus facilitating female fertility.

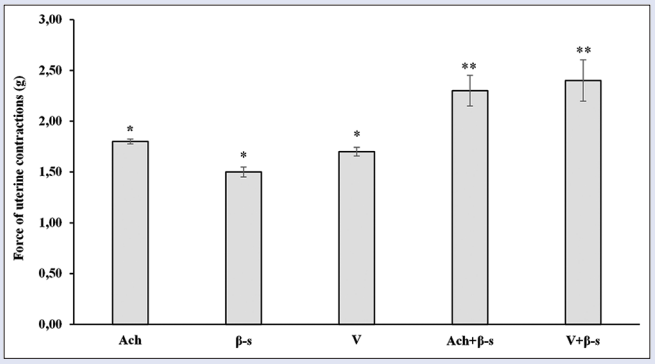

Figure 6: Quiescent uterine preparation. Effects of beta-sitosterol (β-s, 50 μg/ml) on the contractile response induced by acetylcholine (Ach, 1 μg/ml) and vasopressin (V, 1 μg/ml). Data are expressed as mean ± standard error of the mean (n = 5). *P < 0.01 with respect to basal values. **P < 0.01 with respect to agonist-treated preparations. Reproduction size at column width

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Pagano E, Laudato M, Griffo M, Capasso R. Phytotherapy of benign prostatic hyperplasia. A minireview. Phytother Res 2014;28:949-55.
2. Preuss HG, Marcusen C, Regan J, Klimberg IW, Welebir TA, Jones WA, *et al.* Randomized trial of a combination of natural products (cernitin, saw palmetto, B-sitosterol, Vitamin E) on symptoms of benign prostatic hyperplasia (BPH). Int Urol Nephrol 2001;33:217-25.
3. Calpe-Berdiel L, Escolà-Gil JC, Blanco-Vaca F. New insights into the molecular actions of plant sterols and stanols in cholesterol metabolism. Atherosclerosis 2009;203:18-31.
4. Cabeza M, Bratoeff E, Heuze I, Ramírez E, Sánchez M, Flores E, *et al.* Effect of beta-sitosterol as inhibitor of 5 alpha-reductase in hamster prostate. Proc West Pharmacol Soc 2003;46:153-5.
5. Promprom W, Kupittayanant P, Indrapichate K, Wray S, Kupittayanant S. The effects of pomegranate seed extract and beta-sitosterol on rat uterine contractions. Reprod Sci 2010;17:288-96.
6. Astle S, Thornton S, Slater DM. Identification and localization of prostaglandin E2 receptors in upper and lower segment human myometrium during pregnancy. Mol Hum Reprod 2005;11:279-87.
7. Sykes L, MacIntyre DA, Teoh TG, Bennett PR. Anti-inflammatory prostaglandins for the prevention of preterm labour. Reproduction 2014;148:R29-40.
8. Bulletti C, DeZiegler D, Stefanetti M, Cicinelli E, Pelosi E, Flamigni C, *et al.* Endometriosis: Absence of recurrence in patients after endometrial ablation. Hum Reprod 2001;16:2676-9.
9. Pinto V, Matteo M, Tinelli R, Mitola PC, De Ziegler D, Cicinelli E, *et al.* Altered uterine contractility in women with chronic endometritis. Fertil Steril 2015;103:1049-52.
10. Fanchin R, Ayoubi JM. Uterine dynamics: Impact on the human reproduction process. Reprod Biomed Online 2009;18 Suppl 2:57-62.
11. Kissler S, Siebzehnruebl E, Kohl J, Mueller A, Hamscho N, Gaetje R, *et al.* Uterine contractility and directed sperm transport assessed by hysterosalpingoscintigraphy (HSSG) and intrauterine pressure (IUP) measurement. Acta Obstet Gynecol Scand 2004;83:369-74.
12. Chiossi G, Costantine MM, Bytautiene E, Kechichian T, Hankins GD, Sbrana E, *et al.* The effects of prostaglandin E1 and prostaglandin E2 on *in vitro* myometrial contractility and uterine structure. Am J Perinatol 2012;29:615-22.
13. Occhiuto F, Pino A, Palumbo DR, Samperi S, De Pasquale R, Sturlese E, *et al.* Relaxing effects of *Valeriana officinalis* extracts on isolated human non-pregnant uterine muscle. J Pharm

Pharmacol 2009;61:251-6.

14. Wray S. Insights from physiology into myometrial function and dysfunction. Exp Physiol 2015;100:1468-76.
15. Wray S, Burdyga T, Noble D, Noble K, Borysova L, Arrowsmith S, *et al.* Progress in understanding electro-mechanical signalling in the myometrium. Acta Physiol (Oxf) 2015;213:417-31.
16. Rabotti C, Mischi M. Propagation of electrical activity in uterine muscle during pregnancy: A review. Acta Physiol (Oxf) 2015;213:406-16.