CHARACTERIZATION OF ELECTRICAL AND MECHANICAL ACTIVITIES OF RABBIT UTERUS ASSOCIATED WITH THE PRESENCE OF CAPACITATED AND NON-CAPACITATED SPERMATOZOA

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Abstract: To investigate the effects capacitated spermatozoa may exert upon motility of the rabbit uterus, both contractility and electrical activity (frequency and intensity) were measured in 3 distinctive uterine segments of anaesthetized does: horn (UH), uterotubal junction (UTJ) and tube (UT) after 1) natural mating, 2) infusion of either seminal plasma or PBS, 3) infusion of either capacitated or non-capacitated spermatozoa. Basal values were: 17.1, 15.7, 16.4 g (contractility, $P>0.05$); 3.5, 3.5, 3.4 Hz (frequency, $P>0.05$); 0.49, 0.50, 0.57 µV (intensity, $P>0.05$) for UH, UTJ, UT, respectively. Seminal plasma caused an increase ($P<0.05$) in the UH contractility: 26.3 *vs.* 11.7 (natural mating) and 17.0 g (PBS); it also caused a decrease ($P<0.05$) in electrical intensity at the UTJ: 0.24 *vs.* 0.67 (natural mating) and 0.58 µV (PBS). The presence of either capacitated or non-capacitated spermatozoa caused no changes in contractility and electrical frequency in any of the uterine segments. However, there was a change in electrical intensity at UTJ (0.37 *vs.* 0.57 µV for non-capacitated and capacitated spermatozoa, respectively; $P<0.05$). There were also differences between segments by treatment: UTJ (0.37) *vs.* UT (0.59 µV) for non-capacitated; UH (0.46) *vs.* UT (0.71 µV) for capacitated spermatozoa ($P<0.05$). In conclusion, use of this experimental model showed that uterine electrical activity was slightly modified by the presence of capacitated spermatozoa.

Key Words: rabbit, uterus motility and contractility, uterotubal junction, uterus tube.

INTRODUCTION

Uterine motility displays different patterns of activity throughout the reproductive life of females: pregnancy, parturition, estrus and mating. At these 2 last stages, uterine motility is involved in sperm transport and probably in sperm capacitation. After mating, spermatozoa are transported towards the uterine tube by uterine contractions; then, spermatozoa are sequestered in the isthmus by means of specific sperm-epithelial cell interactions, thus establishing the functional sperm reservoir (Hunter *et al.*, 1980). This reservoir enables the storage of a selected and privileged sperm population that eventually becomes capacitated in a time dependent fashion (Green *et al.*, 2001). In the rabbit doe, the sperm reservoir (vagina, cervix and uterus) may store subpopulations of motile spermatozoa until 16 h after mating (Scott, 2000). Hyperactivation, characteristic of capacitated spermatozoa, enables the spermatozoa to separate from tube epithelial cells and penetrate the zona pellucida; an increase in intracellular concentration of Ca^{2+} may accelerate sperm release (Suarez, 2007). Signals from pre-ovulatory follicle or hormone signals that promote ovulation may stimulate epithelial cells to secrete a sequence of factors that trigger both sperm capacitation and hyperactivation (Suarez, 2007). Most of the spermatozoa are still connected to epithelial cells until ovulation occurs (Kawakami *et al.*, 2001), but by the time of ovulation an increase in myometrial contractions could assist sperm release from epithelial cells (Katz and Yanagimachi, 1980). It is not clear whether capacitated spermatozoa are responsible for increased myometrial motility or whether it is caused by an ovarian endocrine influence via periovulatory Graafian follicles (Hunter, 2008). Sperm release seems to be related to the combined effects of

signalling between oviductal cells and the oocyte, progesterone levels, parallel capacitation events and changes in the levels of different molecules involved in the binding of sperm to oviduct that may vary throughout estrous cycle (for review see, Coy *et al.*, 2012). Previous research on uterine motility has been focused on establishing interactions between electrical and mechanical characteristics of rabbit oviductal smooth muscle and between longitudinal and circular muscle (Talo, 1974; Ruckebusch, 1975; Coons and Johns, 1982). Analysis of the electrical spiking activity of the ewe oviduct and uterus during natural and induced estrus has also been examined (Ruckebusch and Bueno, 1976), as well as the effects of hormonal manipulation on the rabbit oviduct isthmus motility with the aid of special transducers (Muller and Nelsen, 1979; Bourdage and Halbert, 1980). Other research involves the study of electromyographic activity in the mare uterus during different estrous cycle stages (Troedsson *et al.*, 1993), the study of uterine contractility (contraction frequency and the direction of propagation) by ultrasound in women (Fanchin *et al.*, 2001) and recently the development of more sophisticated *in vitro* models to elucidate the complex way sperm-oviduct interaction actually works (Miessen *et al.*, 2011). The possible association between the presence of capacitated spermatozoa and the increase in myometrial contractility has not been explained, so the objectives of this research were to investigate whether (1) *in vitro* capacitated spermatozoa exert any influence on the mechanical and electrical activities of 3 uterine segments (horn, uterus-tubal junction, tube) from mature rabbits, and (2) whether natural mating, seminal plasma or PBS, could modify rabbit uterine activities in some of the 3 segments.

MATERIALS AND METHODS

Animals

Mature fertile rabbit does (n=39 New Zealand and hybrids), at least 2 parturitions, 18 mo of age, 3.9 kg live weight, housing in individual cages, free access-fed with commercial food and free access-water, were used in this work.

Synchronization of ovulation

To prevent variations of uterus mechanical and electrical activities due to the moment of measurement, all the females were given equine chorionic gonadotrophin (eCG at 20 IU. Intervet, Mexico) 72 h before treatment: natural mating, seminal plasma or phosphate buffered saline (PBS); capacitated or non-capacitated spermatozoa. Previously, the effects of eCG treatment on ovarian structures were registered in 13 females. Pre-ovulatory follicles (about 6 per ovary) were the dominant structure followed by corpora lutea (about 2 per ovary) and corpora hemorrhagica (about 1 per ovary).

Semen processing and assessment

Semen was obtained from 12 fertile rabbit males by artificial vagina. Sperm motility was assessed immediately, then semen was centrifuged twice (800g 10 min, 1500g 15 min) to remove seminal plasma, which was frozen at −20°C for later use. Spermatozoa were suspended in TALP medium (Parish *et al.*, 1988), then sperm viability (Eosin-Nigrosin stain) and concentration were assessed; finally, sperm concentration was adjusted to 6.6×10^7 spermatozoa per mL.

In vitro capacitation

In a preliminary experiment, to establish the time for the majority of sperm to become capacitated, spermatozoa in TALP medium (see previous section) were incubated at 37°C in 5% CO_2 atmosphere; then, aliquots were taken at different times: 0, 3, 6, 8 and 17 h to assess 1) sperm capacitation by means of the chlortetracycline (CTC) technique and fluorescent microscopy (Hewitt and England, 1998; Green and Watson, 2001) and 2) plasma membrane-intact cells by eosin-nigrosin in parallel. On this basis, a 6 h incubation period was employed for successive experiments.

Measurement of uterus mechanical and electrical activities

A force transducer, 3 needle type electrodes (F-60 Narco, Bio-Systems, USA), a unit for physiological data acquisition (Biopac MP35, USA) and a portable computer were used for this purpose. Readings (3 repetitions per treatment, 3 min each) from both mechanical and electrical activities were simultaneously taken in 3 uterus segments from each

Table 1: Basal values of contractility and electric activity (frequency and intensity) of rabbit uterus in 3 different segments, uterine horn (UH), utero-tubal junction (UTJ) and tube (UT).

Uterine segment	Contractility (g)	Electric activity	
		Frequency (Hz)	Intensity (mV)
UH	17.1±10.7	3.5±0.5	0.49±0.32
UTJ	15.7±9.6	3.5±0.6	0.50±0.32
UT	16.4±9.4	3.4±0.5	0.57±0.45

Values are mean±standard deviation. No significant differences.

experimental unit: middle part of uterine horn (UH), uterus-tubal junction (UTJ) and tube (UT); each uterine horn from each female was considered an experimental unit. Electrical activity was measured in terms of frequency (Hz) and intensity (µV); contractility in terms of force (g).

Rabbit anaesthesia and surgical approach

Females were anaesthetized by administration of acepromazine, 0.84 mg/kg LW (Calmivet, Vetoquinol, France) as a pre-anaesthetic, followed by a mixture of tiletamine-zolazepam, 12.5 mg/kg LW (Zoletil, Virbac, France). The general anaesthesia was kept under the administration of small doses of the mixture. After laparotomy, the uterus was exposed and each segment suspended into the force transducer by means of fine surgical silk, under a restraint strain of 10 g, leaving a distance of 10 cm between them. Immediately, 3 needle electrodes were positioned within the myometrium forming a triangle, approximately 5 mm apart; then, the readings (basal values) were taken from each segment. Before taking any measurement, uterus was irrigated with isotonic saline solution at 37°C; as a result, the answer was identified in the reading outs and omitted from the analysis. At the end of this procedure, females still anaesthetized were euthanized by cervical dislocation.

Experimental design

In the first stage, 3 treatments were carried out: 1) natural mating (NM, n=6 females), 2) infusion of PBS into each of the 3 uterus segments (PBS, n=6 females), 3) infusion of seminal plasma into each of the 3 uterus segments (SP, n=7 females). Does from treatment 1 (NM) received natural mating from 1 male, in each case at the cage of the male; then, the doe was immediately taken to the laboratory. There, does from each of the treatments (NM, PBS, SP) were anaesthetized and laparotomy was performed as mentioned above. Before any treatment, readings of electrical and mechanical activities (basal values) from each uterine segment were taken, except for natural mating. Then, either PBS or SP at 37°C were infused into (i) uterine tube (≈50 µL), (ii) uterus-tube junction (≈100 µL) and (iii) uterus horn (≈200 µL) with the aid of a syringe and a 31-gauge needle, avoiding excessive distension. Each infused segment (2 cm long) was separated from the rest by tying it to prevent PBS or SP from spreading. After 5 min of PBS or SP infusion, readings (experimental values) from each uterine segment were also taken. In the case of NM, experimental values were taken after 60 min.

In the second stage, infusion of either 1) capacitated (n=10 females) or 2) non-capacitated spermatozoa (n=10 females), into (i) uterine tube (≈50 µL), (ii) uterus-tube junction (≈100 µL) and (iii) uterus horn (≈200 µL) was tested; previously, each uterine segment was tied up as mentioned above. Decreasing amounts of the infused liquid and spermatozoa were determined considering observations of Fateh El-Bab *et al.* (1983) in rabbits. Does from each of the treatments were anaesthetized and laparotomy was performed as mentioned above. Before treatment, readings

Table 2: Effect of natural mating, seminal plasma and phosphate buffered saline (PBS) on contractility (g) of rabbit uterus in 3 different segments, uterine horn (UH), utero-tubal junction (UTJ) and tube (UT).

Treatment	Uterine segment		
	UH	UTJ	UT
Natural mating	11.7±5.1[a]	14.2±9.7	18.5±8. 7
Seminal plasma	26.3±15.3[b]	17.0±8.7	17.4±7.0
PBS	17.0±8.6[a]	13.5±8.4	18.4±7.8

Values are mean±standard deviation.
[a,b]Different letters in columns differ significantly (*P*<0.05).

Table 3: Effect of natural mating (NM), seminal plasma (SP) and phosphate buffered saline (PBS) on electric activity (frequency and intensity) of rabbit uterus in three different segments, uterine horn (UH), uterine-tubal junction (UTJ) and tube (UT).

Treatment	Uterine segment					
	UH	UTJ	UT	UH	UTJ	UT
	Frequency (Hz)			Intensity (mV)		
NM	4.2±2.2	4.3±2.0	4.2±1.4	0.59±0.57	0.67±0.47[a]	1.4±2.1
SP	3.5±0.5	3.9±0.7	3.5±0.7	0.38±0.36	0.24±0.23[b]	0.4±0.2
PBS	3.6±0.5	3.3±0.4	3.7±0.3	0.35±0.14	0.58±0.76[a]	0.5±0.3

Values are mean±standard deviation.
[a,b]Different letters in columns differ significantly ($P<0.05$).

(basal values) from each uterine segment were taken; after 5 min of treatment, readings (experimental values) from each uterus segment were taken.

Statistical analysis

Data (basal and experimental values) were analyzed to detect possible differences due to treatment in uterine activity between segments (1, 2, 3) within horns (left *vs.* right) and segments regardless horn. Data from electrical activity were subjected to Fourier transformation before analysis. Friedman ANOVA (repeated measures) was carried out for uterine segments, one-way ANOVA was performed for treatments. Spearman correlation was carried out for mechanical and electrical activities of values grouped (segments). Statistica 5.0 (Stat Soft, UK) software was employed.

RESULTS

Preliminary experiment to determine the effectiveness of capacitation induction

Characteristics of fresh semen were (mean±standard error): 50.8±8.94% motile cells, 79.6±3.22% live cells, 16.7±2.37% abnormal cells, 211.4±50.77×10^6 spermatozoa/mL and 0.55±0.09 mL of ejaculate volume. The percentage of capacitated spermatozoa increased significantly ($P<0.05$) from 0 to 4 h (42±5.24 to 64±2.71) and from 4 to 6 h (64±2.71 to 72±1.45) of incubation, after which it remained the same at 8 and 17 h of incubation. On the other hand, percentage of live spermatozoa decreased inversely: from 0 to 4 h (66±2.86 to 60±4.40) and from 4 to 6 h (60±4.40 to 56±6.16) of incubation ($P<0.05$). Therefore, for successive experiments a 6 h incubation period was employed.

Data comparison between uterine horns (right *vs.* left) revealed no significant differences, so for successive analysis data were pooled.

Stage 1

Basal values of contractility and electrical activity (frequency and intensity) from the 3 uterine segments are displayed in Table 1. There were no differences between segments in any of the variables. Seminal plasma caused an increase of contractility at the UH: 26.3 *vs.* 11.7 and 17.0 g for natural mating and PBS respectively ($P<0.05$). However, none of the treatments caused significant changes of contractility in the other segments (Table 2).

Seminal plasma also caused a decrease of electrical intensity at the UTJ: 0.24 *vs.* 0.67 and 0.58 µV for natural mating and PBS respectively ($P<0.05$). However, none of the treatments led to significant changes in the other segments. Regarding electrical frequency, there were no changes by treatment or between segments (Table 3).

Table 4: Effect of non-capacitated or capacitated spermatozoa on contractility (g) of rabbit uterus in 3 different segments, uterine horn (UH), uterine-tubal junction (UTJ) and tube (UT).

Treatment	Uterine segment		
	UH	UTJ	UT
Non-capacitated spermatozoa	23.0±20.3	12.7±7.3	14.1±8.9
Capacitated spermatozoa	17.5±12.5	64.3±212.7	11.5±7.8

Values are mean±standard deviation. No significant differences.

Stage 2

There were no changes of contractility or electrical frequency caused by non-capacitated or capacitated spermatozoa through the uterine segments and treatments (Tables 4 and 5). However, there was a change of electrical intensity at the UTJ: 0.37 *vs.* 0.57 µV, non-capacitated *vs.* capacitated spermatozoa (*P*<0.05). Likewise, there were differences between segments within treatments: UTJ (0.37 µV) *vs.* UT (0.59 µV) for non-capacitated (*P*<0.05); UH (0.46 µV) *vs.* UT (0.71 µV) for capacitated spermatozoa (*P*<0.05). Two representative profiles of electric intensity and force are displayed in Figures 1 and 2. There was a significant correlation between contractility and electrical activity (frequency) at the UTJ under the effect of capacitated spermatozoa (r=−0.75, *P*<0.01). In contrast, there was no significant correlation at any segment for non-capacitated spermatozoa.

DISCUSSION

This research was conducted with the aim of obtaining knowledge on the possible role capacitated spermatozoa could play in modifying both mechanical and electrical activity of rabbit uterus. In this research, there were no differences in mechanical and electrical activity between different segments of the rabbit uterus. Basal values of electrical activity (intensity) of the tube (0.119 mV, non transformed data) are similar to those reported previously (0.100-0.200 mV); in contrast, values obtained from the uterine horn are lower: 0.158 mV (non transformed data) *vs.* 0.200-0.250 mV (Ruckebusch, 1975). It should be noted that the data were recorded during the pre-ovulatory phase in both cases; it has been reported that the uterine motility of many species undergoes pattern changes throughout the estrous cycle (Ruckebush and Bueno, 1976; Coy *et al.*, 2012). During mating, an increase in mechanical and electrical activity of the uterus is expected due to the arrival of spermatozoa. An increase in this type is responsible for rapid sperm transport towards the uterine tube after mating; the increased contractility of the myometrium is associated with intraluminal pressure to allow sperm migration and establishment of reservoirs (Scott, 2000). Since this process may take from 1.5 to 6 h, the moment when uterine activity was measured (1 h after mating) was probably inappropriate. In this sense, Talo (1974) measured *in vitro* rabbit oviduct activity and observed that at 5 h after mating the frequency of electric bursts was highest at the ampullary-isthmic junction and at
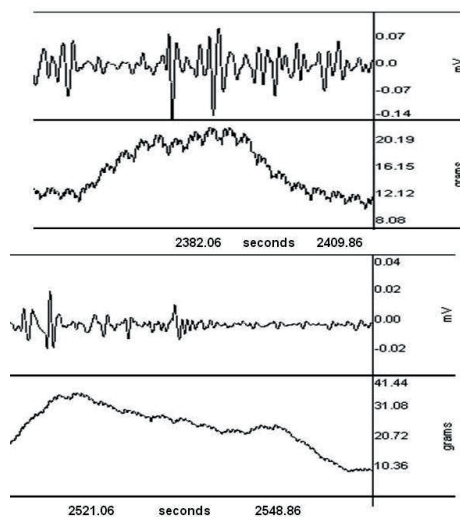

Figure 1: Profiles of electric intensity (mV) and contraction force (g) *vs.* time (s) from rabbit uterus horn before (top panel) and after (bottom panel) treatment with capacitated spermatozoa (≈1.3×10^7 cells in ≈200 µL). Sperm in TALP were infused into a 2 cm long segment tied up to prevent them from spreading. After 5 min of sperm infusion, readings were taken.
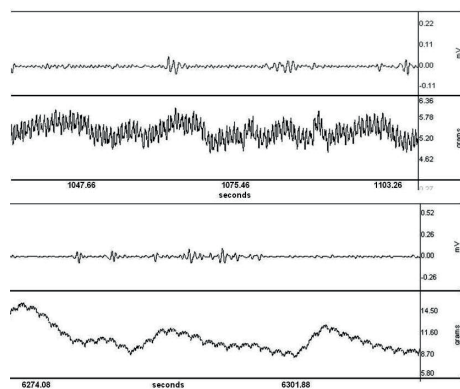

Figure 2: Profiles of electric intensity (mV) and contraction force (g) *vs.* time (s) from rabbit uterus-tubal junction before (top panel) and after (bottom panel) treatment with capacitated spermatozoa (≈7.5×10^6 cells in ≈100 µL). Sperm in TALP were infused into a 2 cm long segment tied up to prevent them from spreading. After 5 min of sperm infusion, readings were taken.

Table 5: Effect of non-capacitated or capacitated spermatozoa on electric activity (frequency and intensity) of rabbit uterus in three different segments, uterine horn (UH), uterine-tubal junction (UTJ) and tube (UT).

| Treatment | Uterine segment | | | | | |
| | UH | UTJ | UT | UH | UTJ | UT |
	Frequency (Hz)			Intensity (mV)		
Non-capacitated spermatozoa	3.4±0.5	3.2±0.6	3.4±0.5	0.48±0.32[xy]	0.37±0.29[a,x]	0.59±0.32[y]
Capacitated spermatozoa	3.3±0.5	3.3±0.4	3.4±0.4	0.46±0.43[x]	0.57±0.43[b,xy]	0.71±0.50[y]

Values are mean±standard deviation.
[a,b; x,y]Different letters in columns (ab) and rows (xy) differ significantly ($P<0.05$).

the ampullary end of the isthmus. Moreover, Hunter (2008) reported that surgical instillation of freshly ejaculated spermatozoa directly into the caudal isthmus of sows shortly before ovulation results in little or no sperm binding. Nevertheless, there were differences regarding electrical intensity at the UTJ level caused by the infusion of seminal plasma. In natural mating, some uterine segments (horns) may be exposed to bigger amounts of seminal plasma than others, and thus to the effect of prostaglandins; however, in other uterine segments (uterus tubal junction, tubes) spermatozoa more than seminal plasma could affect contractility when interacting with oviduct epithelial cells (Katz and Yanagimachi, 1980). Indeed, an increase of isthmus contraction frequency after 2 h of mating in rabbits has been reported (Bourdage and Halbert, 1980). The mere presence of spermatozoa is enough to stimulate a number of responses in oviductal epithelial cells (Murray and Smith, 1997; Yao *et al.*, 2000). One contradictory observation from this work was that the force of contraction increased in the uterine horns following addition of seminal plasma, with no changes in the electrical activity. The most feasible explanation may be instrument sensitivity; that is, the employed electrodes were not able to detect such a small electric variation, which was enough to produce the contractile response. Moreover, electrodes made of stainless steel may not avoid any possible electromagnetic interference when they make contact with uterine tissue. In this research, a significant correlation between contractility and electric activity was detected at the UTJ under the influence of capacitated spermatozoa only; regardless of the short time that spermatozoa were allowed to establish an interaction with oviductal epithelial cells, it seems that capacitated spermatozoa were able to modify the uterus activity. The UTJ is certainly critical for blocking the entry of already-capacitated spermatozoa, so it could be that capacitated spermatozoa are making some kind of stimulation that would preferentially block the UTJ (Holt and Fazeli, 2010). When considering these results, one should bear in mind the effects of acepromazine on uterine muscle, which may modify its mechanical and electrical activity; although this drug has adrenergic antagonist effects that can reduce uterine contractility (Katila, 2007), we were able to detect some small changes on uterus activity provoked by seminal plasma and capacitated spermatozoa.

In conclusion, under this experimental model uterine electrical activity was slightly modified by the presence of capacitated spermatozoa; also, seminal plasma modified uterine contractility. These data on mechanical and electrical activities of rabbit uterus under different experimental conditions may serve as reference values for future research.

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