Spontaneous motility of the cervix in cyclic and ovariectomized ewes and changes induced by exogenous hormones

R. Garcia-Villar, P. L. Toutain, J. More and Y. Ruckebusch
Station de Pharmacologie-I.N.R.A., 180, chemin de Tournefeuille, 31300 Toulouse, France

Summary. Continuous recordings of the electrical and mechanical activities of the cervix were made. Two basic patterns of electrical signal, short spike bursts and long spike bursts, showed close mechanical relationships with the record of mechanical activity. Electrical activity was organized in myoelectrical complexes including the regular alternation of phases of irregular spiking activity and phases of regular spiking activity. The myoelectrical complexes of the cervix existed independently of the sexual status although the frequency of recurrence did vary. The highest frequency was recorded during the periovulatory period around oestrus when each complex was synchronized with a similar pattern recorded from the uterine horns. At the end of the periovulatory period, the irregular spiking activity decreased, the myoelectrical complex pattern of the cervix and uterine horns consisting mainly of regular spiking activity at a high frequency until metoestrus. During the luteal phase, only the cervix remained active, with myoelectrical complexes formed mainly by phases of regular spiking activity recurring at long intervals. This pattern was also recorded after ovariectomy in the absence of hormonal treatment.

Introduction

There have been many physiological and pharmacological studies on the motility of the uterine horns in various species during the oestrous cycle or pregnancy and parturition. In contrast, few studies have been conducted on the cervix because it was suggested, on the basis of histological studies of women, that the mechanical properties of the cervix are determined mainly by the connective tissues (collagen) (Danforth, 1954, 1980), and that the role of the cervix in parturition or in the transport of spermatozoa is a passive one. Consequently, the function of the cervix has been largely described in terms of biochemical events, e.g. in rats (Harkness & Harkness, 1959), and changes in compliance at the time of parturition in rats (Hollingsworth & Gallimore, 1981) and in sheep and goats (Fitzpatrick & Dobson, 1979). Human cervical tissue consists of an outer fourth which is mainly muscular and an inner three-fourths which are mainly collagenous with embedded smooth muscle cells (Hughesdon, 1952). It appears that the cervix contains the richest innervation of any part of the human uterus (Krantz & Phillips, 1962) and isolated strips of human (Najak, Hillier & Karim, 1970) and sheep (Edqvist, Einarsson, Gustafsson, Linde & Undell, 1975) cervix exhibit spontaneous contractility in vitro and responsiveness to different drugs. In addition, the independence of uterine and cervical motility in vivo has been demonstrated in ruminants (Chen, MacDonald & Hawes, 1966; Fitzpatrick, 1957; Stys, Clewell & Meschia, 1978). Stress conditions or general anaesthesia impair the passage of spermatozoa in the ewe (Brinsfield & Hawk, 1969).
All these data suggest that cervical smooth muscle function is of importance and must be investigated by appropriate chronic methods. Amongst the different techniques, it has been proposed that electromyography (EMG) and strain gauge transducers are suitable for long-term assessments of the electrical and mechanical activities of the cervix in cyclic as well as pregnant ewes (Garcia-Villar, Toutain & Ruckebusch, 1982).

The aim of the present work was to describe the spontaneous electrical and mechanical activities of the cervix in cyclic and in ovariectomized ewes and to evaluate the effects of oestrogen or progesterone in ovariectomized animals.

Materials and Methods

The experiments were carried out on seven 3–4-year-old Lacaune ewes, weighing 42–53 kg, during the breeding season. The ewes were fasted for 2 days and anaesthesia was induced by 20 mg thiopentone sodium/kg. A low mid-line incision was made in the ventral abdomen. Electrodes made from insulated nichrome wires, 0.12 mm diameter and 200 cm long (Trinamel, Johnson Matthey Metals Ltd, London), were implanted in groups of 3, 2 mm apart on the ventral cervix (2 groups) and on each uterine horn (1 group). After heating the tip (~1 cm) each electrode was inserted through the wall using a needle as a trocar. The free end was tied off close to the wall and the electrodes were exteriorized through a stab wound on the flank of the animal. A strain gauge transducer (Vishay type EA 06–125 BZ 350) inserted into a medical Silastic (Dow Corning) sheet (4 × 10 × 2 mm), orientated with the long axis of the cervical canal, was sutured between the 2 groups of cervical electrodes. During surgery, 3 out of the 7 ewes were ovariectomized.

The length of the electrode wires and the connecting cables of the gauges allowed the animal to lie down and move in its cage when connected to the input panel of the polygraph. At 24 h after surgery, the electrical activity was recorded with an 8-channel ‘direct writing’ recorder (Reega Minihuit TR, Alvar Electronic, France) at a paper speed of 0.5 to 20 cm/min, and a time constant of 0.1 sec. Concurrent summation, at 20-sec intervals, of the electrical activity from 3 electrode sites (1 cervix group and each uterine group) was obtained by a linear integrator circuit (Latour, 1973) connected to a 3-channel potentiometric recorder (J. J. Lloyd Instruments, CR 553) at a chart speed of 1 mm/min. Integration was used 24 h/day as a monitoring procedure throughout the experimental session. Direct records were also continuously obtained at the time of oestrus and during hormone treatment of ovariectomized ewes. During the luteal phase and between drug treatments of ovariectomized ewes, direct records were obtained for 6–8 h/day.

To obtain the relationship between mechanical and electrical activities, both were simultaneously recorded on a rectilinear pen polygraph (Dynograph, type R 411) equipped with voltage/pulse/pressure couplers (type 9853-A) and A.C. couplers (type 9806-A) (Beckman Inst. Inc., U.S.A.). Before implantation the strain-voltage relationships were evaluated for each gauge using weights of 5, 10 and 20 g.

Controls using endogenous hormones or behavioural detection of oestrus were not performed. The different phases of the oestrous cycle were recognized by the well-established patterns of uterine activity during the oestrous cycle, i.e. the absence of activity during the luteal phase, in contrast to the enhanced activity of the uterine horns during pro-oestrus and oestrus i.e. the preovulatory period. According to these criteria, 13 cycles were analysed. The length of the oestrous cycle varied between 14 and 19 days.

Only ovariectomized ewes were used in order to evaluate the action of hormones on cervical motility. At 30 days after ovariectomy, oestradiol benzoate (Intervet, France) was injected intramuscularly (1 mg/day for 4 consecutive days). Four weeks later, a sponge impregnated with 40 mg fluorogestone acetate (Chrono-Gest: Intervet, France) was inserted intravaginally and remained in situ for 4 days.
Results

Analysis of electromyograms

Basic EMG signals and associated mechanical activity. The elementary signals recorded on the cervix were described on the basis of amplitude, duration and morphology. According to these criteria, 2 basic signals were recorded. The first consisted of single short spikes or very short spike bursts of a mean duration of 0.1 sec and a high amplitude ranging from 200 to 500 µV (Text-fig. 1a). The second basic signal, which occurred less frequently, corresponded to bursts of spikes of a longer duration (15–40 sec) and a lower amplitude (20–60 µV), and was thus termed long spike bursts (Text-fig. 1b). The two cervical signals were recorded from cyclic and ovariectomized ewes, and differed sharply from those recorded from the uterine horns, i.e. only spike bursts of high amplitude (up to 500 µV) and a duration of 5 to 20 sec during the active periods, e.g. around oestrus.

Text-fig. 1. Electrical spiking activity and associated mechanical activity of the cervix. The electrical activity was recorded (a) as short spikes (○) and short spike bursts (○) or (c) long spike bursts; isolated short spikes are related to relatively longer elevations of small amplitude of the mechanogram; isolated long spike bursts are related to high sustained elevations of the mechanogram. When short spikes occurred in series (b), a tonic rise of the base-line was observed.

In all the records, there was a close relationship between the EMG and mechanical activity as measured by strain gauge recording (Text-fig. 1). During low-frequency short spikes, variations in the muscular activity elicited relatively long (5–8 sec) increases of small amplitude (2–4 g) in the base-line of the record (Text-fig. 1a). When short spikes occurred in series at a high frequency, the mechanical activity of each isolated short spike was summated and hence the base-line was elevated (5–10 g) for 4–7 min (Text-fig. 1b). During long spike bursts, the mechanical activity was of longer duration and high amplitude (10–20 sec and often > 10 g) (Text-fig. 1c).

Spiking occurrence. The 24-h direct recordings of cyclic and ovariectomized ewes showed a spiking frequency of 0–23 cycles/min for short spikes in a typical pattern of alternate low and high spiking frequency and a random occurrence of long spike bursts.

A statistical analysis of the occurrence of short spikes in oestrous ewes was performed to characterize objectively this pattern and to suggest an appropriate terminology. The direct
records were scored as 1-min epochs and the number of spikes within each epoch was counted. Text-figure 2 shows the distribution of epochs classified according to the frequency of spikes within each; 2 modes were observed (0–1 and 15 cycles per min). The analysis of the first half of the curve revealed that the mean frequency of occurrence of spikes during one epoch was 0.966 ± 1.07 (mean ± s.d. calculated for 22 records of 24 h in 4 oestrous ewes). The ratio between mean and standard deviation was not significantly different from 1 (χ² test, p > 0.01). This suggests that the firing rate of the low frequency short spikes shows a Poisson distribution.

![Text-figure 2](Image)

Text-fig. 2. Distribution of 1-min epochs according to the frequency of short spikes. (a) The occurrence of short spikes with a low frequency (0–5/min) had a Poisson distribution. (b) The occurrence of short spikes with a high frequency (10–20/min) was distributed normally.

In contrast, the second half of the curve showed a normal distribution (χ² test, p < 0.05) with a mean frequency of occurrence of spikes during one epoch of 15.7 ± 4.49 (mean ± s.d. for 22 records of 24 h in 4 oestrous ewes). According to these two distributions, we suggest the patterns of spiking activity are termed irregular spiking activity and regular spiking activity. Both patterns were present in the direct records (see Text-fig. 3a).

The inspection of the integrated EMG record of the cervix during oestrus, the luteal phase and after ovariectomy showed that irregular activity and regular activity did not occur randomly but alternately, the period of which depended on the sexual status. We therefore suggest that the regular succession of irregular activity and regular activity be termed the myoelectrical complex of the cervix (Text-fig. 3b).

Attempts to perform a similar analysis for long-spikes bursts were fruitless as they were recorded more irregularly and less frequently than short spikes. Nevertheless, they were randomly distributed and therefore would generally be included in the irregular activity.

**Activity profile of the cervix in cyclic ewes**

During the periovulatory period (72–96 h) both uterine horns and cervix showed a typical increase in motility which can be divided into 3 main phases (Text-fig. 4).

(1) From 0 to 24–36 h, the activity of the uterus increased progressively in the form of an uninterrupted succession of spike bursts, each lasting 2–6 sec at a frequency of 2.5/min; at this time, the activity of the cervix was organized in myoelectrical complexes, i.e. recurring phases of irregular and regular activities. However, the time between successive complexes was markedly variable (21–70 min).
Text-fig. 3. Electromyogram (EMG) of the cervix in an oestrous ewe. (a) Direct EMG shows a phase of irregular spiking activity (ISA), including short spikes occurring at low frequencies and randomly occurring long spike bursts, followed by a phase of regular spiking activity (RSA) consisting mainly of short spikes occurring at high frequencies. (b) Integrated EMG displays the cyclic recurrence of long periods of ISA followed by 4–7 min of RSA and very short quiescent periods. This regular alternation of ISA and RSA is termed the myoelectrical complex of the cervix.

Text-fig. 4. Integrated EMG of the uterus (U) and cervix (C) during the oestrous cycle. From 0 to 24–36 h, uterine activity increased progressively while the cervix showed a recurring pattern formed by myoelectrical complexes with a relatively long period. From 24–36 to 48–60 h, the uterus and cervix displayed a synchronized and short period of myoelectrical complex pattern. From 48–60 to 72–96 h, uterine and cervical myoelectrical complexes presented a dramatic drop of irregular spiking activity. During the luteal phase, the uterus was almost quiescent while the cervix still displayed a pattern mainly formed by phases of regular spiking activity recurring at long intervals. The amplitude of the uterine horn record was reduced during the periovulatory period. Each vertical bar = 2 mV.
From 24–36 to 48–60 h, both uterus and cervix displayed a similar pattern of myoelectrical complexes; at this time, the irregular and regular activities of the cervix were maximal and synchronized to those of the horns. The frequency of occurrence of the complexes was higher and less variable than from 0 to 24–36 h, and the mean ± s.d. cycle duration was $39.3 \pm 10.1$ min (840 complexes distributed throughout the 13 oestrous periods under study in the 4 cyclic ewes).

From 48–60 to 72–96 h, there was a marked decrease in irregular activity of both horns and the cervix; the activity sometimes consisted only of regular activity. This diminution of irregular activity was more marked in the horns than the cervix. The cycle duration was $27.6 \pm 9.9$ min.

After 96 h, when the uterine horns became quiescent (luteal phase), the cervix remained active and still displayed cyclic patterns of myoelectrical complexes. The length of the cycles was longer and more variable than during oestrus (60–90 min), and the phases of regular activity were separated by periods of quiescence or weak irregular activity. In addition, the number of short spikes of each regular activity was generally lower than during oestrus (8–13 versus 15–23

![Text-fig. 5](image-url)

**Text-fig. 5.** Integrated EMG of the uterus (U) and cervix (C) in an ovariectomized ewe and the effect of exogenous hormones. (a) In the absence of hormonal treatment the cervix is the only active part and presents myoelectrical complexes mainly in the form of phases of regular spiking activity, recurring at long intervals. (b) The insertion of an intravaginal sponge of fluorogestone acetate (40 mg) inhibits the activity of the cervix which reappears rapidly after its withdrawal. (c) The intramuscular administration of oestradiol benzoate (1 mg) elicited a triphasic motor response, i.e. an 8-h inhibitory phase followed by a progressive rise in activity which is present in the form of an almost continuous spiking activity 12 h after the injection, and in the form of myoelectrical complexes synchronized for the cervix and uterine horns 24 h after the injection. The amplitude of the uterine horn record was reduced. Each vertical bar = 2 mV.
per min), which explained the lower amplitude of the integrated activity recorded from the cervix during the luteal phase (Text-fig. 4).

Activity profile of the cervix in ovariectomized ewes and the effects of exogenous hormones

By 6–8 days after ovariectomy, the activity of the uterine horns was always weak or totally absent. In contrast, the cervix continued to display a cyclic activity similar to that described during the luteal phase (Text-fig. 5a), i.e. a cycle length of 60–90 min. Each regular activity consisted of 40–110 short spikes during 4–7 min; only a low level of irregular activity was recorded. The same motor profile was recorded for up to 5 months after ovariectomy although time between successive complexes gradually increased (up to 140 min) and generally only regular activity was recorded.

Insertion of a fluoroestrogene acetate sponge (40 mg intravaginally) completely inhibited the motility of the cervix, from 1–2 h after the insertion. After the withdrawal of the sponge, the motility of the cervix reappeared progressively and, in less than 24 h, it was similar to that described during the control period (Text-fig. 5b).

The first injection of oestradiol benzoate (1 mg/day for 4 days) elicited, after a short delay (30–70 min), a complete inhibition of cervical motility for 7–10 h (Text-fig. 5c). Thereafter, an increasing activity appeared in the cervix and uterine horns. During the first 6 h of activity only irregular activity formed by short spikes was recorded, synchronized throughout the whole tract; the motor pattern was organized in myoelectrical complexes similar to those observed during natural oestrus, i.e. alternate phases of irregular and regular activity. When the effect of oestrogenic stimulation was maximal, i.e. after the 3rd or 4th injections as suggested by the highest level of electrical activity recorded, the cervix and horns displayed a continuous activity from which it became impossible to recognize irregular and regular phases. In addition, short spikes were replaced by bursts of spikes of 10–20 sec from the horns, while long spike bursts lasting 15–40 sec, randomly intermingled with short spikes, appeared from the cervix. When oestrogen treatment was stopped this high level of continuous activity decreased progressively in cervical and uterine tissues and again became organized in myoelectrical complexes similar to those observed 12–24 h after the 1st injection. By 8 days after the cessation of the treatment, the uterine horns were quiescent while the cervix displayed regular myoelectrical complexes similar to those described during the control period.

Discussion

As far as we know, the spontaneous motility of the cervix during the oestrous cycle or after ovariectomy, and its changes after hormone treatment, have not been reported in sheep or in other domestic species. In a preliminary report (Garcia-Villar et al., 1982), electromyography (EMG) was shown to be a valuable technique to assess cervical motility, with clear evidence of the close relationship which existed between electrical and mechanical events, as measured by strain gauges; it allowed long-term continuous recording (24 h/day) in the same animal without major disturbances as shown by the return of uterine activity to the pattern typical of oestrus at 14–18-day intervals.

The most important finding of the present experiments was that the cervix displayed its own spontaneous motility, which cannot be considered as the transmission of the upper uterine activity to a passive cervix. This was demonstrated by recording the EMG of the whole genital tract during the luteal phase and after ovariectomy: the horns were quiescent while the cervix remained active and organized in the so-called myoelectrical complexes. The EMG signals of the cervix mainly consisted of short spikes or short spike bursts of high amplitude, and more rarely, long spike bursts of low amplitude. This pattern differed markedly from that of the uterine horns
which displayed spike bursts of high amplitude and long duration only during active periods (e.g. oestrus). In addition, after hysterectomy, the cervix continued to display the same activity (preliminary observation).

In the cyclic ewe, the motility of the cervix showed a marked evolution in regard to the stage of the oestrous cycle. For convenience, the latter has been divided into a 13–15-day luteal phase and a 3–4-day periovulatory period (pro-oestrus and oestrus), according to the well-known changes in uterine activity (Naaktgeboren et al., 1973; Ruckebusch & Bayard, 1975; Prud’Homme, 1976; Ruckebusch & Buéno, 1976), which can be time-related to hormonal and behavioural changes in sheep (see Bjersing et al., 1972; Prud’Homme, 1976; Quirke, Hanrahan & Gosling, 1981).

The variation of cervical motility and its relationships with uterine motility during the periovulatory period deserve attention in regard to the transport of spermatozoa in the genital tract. In ewes, spermatozoa are deposited into the vagina and have to be carried through the cervix before reaching the uterotubal junction. The mode of transport remains obscure, and large discrepancies exist in the values given for the rate of passage, values varying from a few minutes (Mattner & Braden, 1963) to several hours (Thibault & Wintenberger-Torres, 1967). It has been clearly established that sperm motility is essential for the passage through the cervix; however, it may be assisted by muscular contractions. Although no evidence is available, it can be suggested that the efficacy of the uterine and cervical motility in assisting sperm passage depends on the direction of contractions. According to Hawk (1975), at 5 h after the start of oestrus, 67% of the uterine contractions originated at the cervix near the body and moved anteriorly; in contrast, by 48 h after the end of oestrus, 75% of the contractions originated at the uterotubal junction and moved toward the cervix. However, these results were obtained in anaesthetized ewes by visual inspection during short periods (5–10 min) did not take into account the alteration in the direction of propagation during the myoelectrical cycle suggested by Prud’Homme (1976) in the oestrous ewe. Prud’Homme (1976) used an electromyographic technique and observed a relationship between the direction of propagation and the frequency of bursts: during low frequency spiking (our irregular spiking activity), the propagation was directed mainly from the uterotubal junction to the cervix, while during high frequency spiking (our regular spiking activity), the propagation was directed mainly from cervix toward the uterotubal junction. If this concept is true, it can be suggested, but not proved, from our results that during oestrus, i.e. when the uterus and the cervix displayed a synchronized pattern of myoelectrical complexes, every 40 min a series of ascending contractions picked up spermatozoa from the cervix reservoir to the uterus during 5–7 min of regular activity. This could explain the slow release of spermatozoa into the horns over a period of hours after insemination.

The persistence of myoelectrical complexes in the cervix during the luteal phase, when the activity of uterine horns was weak or totally absent, suggested that the hormonal status does not have the same influence on the motility of the different parts of the genital tract. Similar conclusions could be drawn from ovariecotomized ewes. Indeed, after ovariecotomy, the activity of the horns disappeared progressively but the cervix remained active for more than 5 months after surgery. The activity was in the form of myoelectrical complexes similar to those seen during the luteal phase, suggesting that not only was cervical motility different from that of the horns, for which an oestrogenic stimulus was necessary, but also that endogenous progesterone levels were not sufficient to inhibit the cervix. In contrast, both hormones, administered at pharmacological levels, were able to modify the activity of the cervix. Exogenous oestradiol benzoate markedly increased the motility of both horns and cervix, although an early inhibition of 7–10 h was systematically observed before the rise of the activity level. This initial inhibition was not totally unexpected. Indeed, the administration of oestrogen to ovariecotomized post-partum rats inhibited myometrial activity in vivo (Fuchs, 1974) by reducing the frequency of intrauterine pressure cycles (Downing, Lye, Bradshaw & Porter, 1978). Moreover, Porter (1979) reported the inhibition of spontaneous uterine activity in the rat and the ewe, and suggested that this effect
may be mediated through the secretion of relaxin. On the other hand, it has been observed that treatment of ovariectomized ewes with 0.9 µg oestradiol benzoate led to a high proportion of uterine contractions of small amplitude and rapid dissipation (Crocker & Shelton, 1973). Similarly, the large amount of fluorogestone acetate given by intravaginal sponge completely inhibited the motility of the whole tract, but it remains unclear whether the complete inhibition of normal cervical activity is implicated in the lower fertility obtained after synchronization by progesterone.

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References


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