An electromyographic study of uterotubal activity in the ewe

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Summary. The electrical activity of the oviduct and uterus was automatically registered on a potentiometric pen recorder in ewes fitted with an extracellular multielectrode assembly. The duration and amplitude of local non-propagating activity of the uterus increased and became progressively grouped into phases as natural oestrus progressed. Phases of contractions lasting 5–6 min were initially propagated at a rate of 2/hr. Their frequency increased within 24 hr of the decline in plasma progesterone levels. Grouped activity was then resumed for the next 24 hr. Similar changes were seen in the activity of the oviduct although this region was active earlier in oestrus.

The uterotubal activity developed in the same way 24 hr after withdrawal of a progestagen-impregnated sponge but lasted for 3 days. When another oestrus was induced by injection of a prostaglandin analogue, the activity patterns were qualitatively similar although they did not start until 36 hr after injection and lasted for only 2 days.

Introduction

The pattern of motility of the genital tract of many species undergoes marked changes during the oestrous cycle. In the ewe, endoscopic examination of the uterus revealed rhythmic coiling and uncoiling during an oestrous period of about 72 hr (Lehrer & Schindler, 1974). Observations at laparotomy indicated that the direction of uterine contractions was reversed after ovulation (Hawk, 1975). Increased pressure in the lumen of the uterus at the time of oestrus has also been described (Lightfoot, 1970). Further information has recently been provided by electromyographic exploration of the uterine smooth muscle. Using electrodes sewn onto the myometrium, Naaktgeboren *et al.* (1973) recorded a peak of activity around the time of normal oestrus. This phenomenon was also observed in ovariectomized ewes 24–36 hr after the injection of oestradiol benzoate (Rousseau & Prud'homme, 1974).

These investigations were limited, however, by the size of the insulating base of the electrodes used, which was too large to permit the placing of electrodes on the oviduct. Fine wire electrodes have been successfully implanted directly in the wall of the oviduct and uterus in cattle (Ruckebusch & Bayard, 1975), sheep (Ruckebusch & Pichot, 1975) and rabbits (Ruckebusch, 1975).

The aim of the present work was to analyse the electrical spiking activity of the oviduct and uterus of the ewe during natural and induced oestrus.

Materials and Methods

The experiments were conducted during the breeding season on 6 parous 3–4-year-old Charmoise ewes weighing about 50 kg. Oestrus was detected by the presence of a teaser ram, and after being fasted for 2 days the ewes were subjected to laparotomy under general anaesthesia (20 mg pentobarbitone sodium/kg). Electrodes were made from insulated nichrome wires 80 µm in diameter and

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100–200 cm in length (Trinamel: Johnson Matthey Ltd, London), the insulation being burnt from one end before use. They were inserted through the serosa and muscular layer of the oviduct and uterus in groups of three, 2 mm apart, using a needle as a trocar. The free end was tied off close to the wall and the electrodes were passed to the exterior through a stab wound between the shoulders. The length of the electrode wires allowed the animals to lie down and move freely in the cage without risk of damage to the recording apparatus. Six groups of electrodes were fixed bilaterally: 2 on the ampulla, 2 on the isthmus and 2 on the uterus. Insulation and fixation were achieved by proliferation of the serosa in approximately 2–4 days.

Animals were housed in individual pens and the electrical activity was monitored throughout the experiment by direct connection of two of the three electrodes of each group to a 12-channel polygraph (RC coupling, time constant 0.1 sec: Reega, Alvar, Paris). For each ewe, the output of 4 channels of the polygraph was continuously plotted over 2 months using a linear integrator (Latour, 1973). Electrical activity was summed at 30-sec intervals and values indicated via a potentiometric recorder (chart speed 2 cm/hr). Polygraph recordings were made daily from all 12 electrode sites for 8 hr.

Two ewes received no additional treatment. Four animals were maintained in dioestrus for 2 weeks by means of a Cronolone (SC 9880)-impregnated sponge (30 mg 17 α -acetoxy-9 α -fluoro-11 β -hydroxypregn-4-ene-3:20-dione: Searle, Montrouge) inserted intravaginally at surgery. Oestrus was induced 10 days later by withdrawal of the sponge and i.m. injection of 400 i.u. PMSG (Searle). Fourteen days later, another oestrus was induced by i.m. injection of 125 μ g ICI 80,996, a synthetic analogue of prostaglandin F-2 α , and 400 i.u. PMSG.

Jugular blood samples were taken daily from 14 to 20 days after surgery in the control ewes, and from 2 days before to 4 days after sponge withdrawal or prostaglandin treatment. The plasma was stored at –20°C and progesterone concentrations were measured by radioimmunoassay (Ruckebusch & Bayard, 1975).

Results

Progesterone levels

In the two control ewes, plasma progesterone concentrations indicated a normal luteal function (Haresign, Foster, Haynes, Crighton & Lamming, 1975). Levels began to rise 2 days after the end of the previous oestrus and remained elevated at about 4 ng/ml for 10–12 days. Fifteen days after the previous standing oestrus, the progesterone concentration decreased to <1 ng/ml and remained depressed for 4 days (Table 1). Identical changes were observed during a second oestrous cycle.

Table 1. Peripheral plasma progesterone levels and uterotubal activity during natural and induced oestrus in ewes

<i>Natural oestrus</i>							
Days after previous standing oestrus	14	15	16	17	18	19	20
Plasma progesterone* (ng/ml)	4.0	0.7	0.7	0.6	0.6	1.2	3.7
Uterotubal activity			+	+			
<i>Induced oestrus</i>							
Time relative to treatment (days)	–2	–1	0	1	2	3	4
(1) Sponge withdrawal + PMSG							
Plasma progesterone† (ng/ml)	4.6 ± 0.8	4.2 ± 0.9	4.0 ± 1.1	0.9 ± 0.4	0.8 ± 0.7	0.6 ± 0.6	0.8 ± 0.9
Uterotubal activity					+	+	+
(2) Prostaglandin analogue injection + PMSG							
Plasma progesterone† (ng/ml)	3.6 ± 0.8	4.0 ± 0.6	4.0 ± 0.6	3.2 ± 0.7	1.6 ± 0.3	0.8 ± 0.9	3.2 ± 0.9
Uterotubal activity					+	+	

+ Indicates the onset of continuous tubal activity and subsequent phases of activity of the same magnitude.

* Mean values for 2 animals.

† Mean values ± S.E.M. for 4 animals.

The progesterone concentration also decreased in the 4 treated animals, from 5 ng/ml to <0.8 ng/ml, within 24 hr of sponge withdrawal. Levels remained low for the next 3 days. When the prostaglandin analogue was injected 14 days later, the progesterone level again fell, but more slowly and for a shorter time (Table 1).

Patterns of electrical activity

The electrical activity of the oviduct and uterus varied considerably during the course of natural oestrus. Isolated potentials predominated on the oviduct, whereas bursts of high- and low-amplitude potentials were recorded from the uterus. During dioestrus, until 16 days after the previous standing heat, only sporadic weak potentials were detected in the control ewes. More constant activity was exhibited by the isthmus near the uterotubal junction and every 2 to 3 hr, one of the oviducts displayed a pattern of regular small bursts of low amplitude. Similar patterns were observed during the 10 days of induced dioestrus (Text-fig. 1).

Text-fig. 1. The electrical activity of the oviduct and uterus of a ewe during dioestrus. Recordings were made 10 days after insertion of a progestagen-impregnated sponge. The recording sites are numbered 1 to 6 for the right oviduct and uterus in descending order, and 7 to 12 in ascending order for the opposite sites.

The onset of natural oestrus was indicated by an increase in the amplitude and frequency of the potentials within 24 hr of the decrease in progesterone levels (Table 1). Potentials, grouped into bursts lasting 5–10 sec, were recorded initially on the oviduct. They recurred at 30-min intervals, in phases lasting 5–6 min, and subsequently were also recorded on the uterus for 10–15 min periods. Withdrawal of the progestagen-impregnated sponge produced comparable changes, except that the development of bursts of activity took 36 hr, compared with 24 hr for normal oestrus (Text-fig. 2a). The uterotubal activity became progressively more continuous, especially on the isthmus. This continuous period of uterotubal activity lasted about 12 hr at natural oestrus and was again prolonged, to 24 hr, at the progestagen-induced oestrus.

Phases of activity then reappeared, lasting 5–6 min for the uterus and 2–3 min for the oviduct. Each phase consisted of 15–25 bursts of activity, approximately the same number as that recorded at the onset of oestrus, but condensed into a much shorter time (Text-fig. 2b). This pattern of activity was seen for 12 hr at natural oestrus and for 24 hr at the progestagen-induced oestrus. The end of oestrus was characterized by a progressive decrease in the amplitude of potentials and in the duration of bursts.

Text-fig. 2. Uterotubal activity during oestrus (a) 2 and (b) 3 days after withdrawal of a progestagen-impregnated sponge and injection of PMSG. The tracings are numbered as for Text-fig. 1. In (a) the series of bursts on the uterus lasts about 10 min. Continuous spiking activity is seen on the isthmus (traces 3 and 10) with a period of superimposed bursts over the whole of one oviduct. In (b) the number of bursts on the uterus is similar to that in (a) but the bursts are condensed into a 5-min period. Spiking activity on the uterotubal junction (traces 4 and 9) is increased.

Injection of the prostaglandin analogue and PMSG induced within 30 min an immediate stimulatory effect at all recording sites which lasted for 2–3 hr. A comparable sequence of events to those

previously described then developed within 36 hr, and continued for 36 hr. Despite this faster progression of changes, the successive involvement of the oviduct and uterus was still clear. However, the intensity of the activity was less than at natural oestrus; the potentials of the oviduct were grouped into bursts lasting only 2–5 sec and the phases of activity of the uterus consisted of only 7–10 bursts.

Temporal organization of activity

Several patterns of propagation of activity were observed on the uterus. At the onset of oestrus, series of ascending contractions predominated but these often appeared to be triggered by a descending contraction of the opposite horn. The ascending contractions frequently spread out through the uterotubal junction and involved the whole oviduct. Towards the end of oestrus synchronous descending contractions of both horns were numerous, some of them appearing to originate on the oviduct near the uterotubal junction.

Summation of the electrical activity of the uterus at 30-sec intervals showed the progressive increase and subsequent decrease of activity, which took 48, 72 and 36 hr at natural oestrus, progestagen-induced oestrus (Text-fig. 3) and after prostaglandin treatment, respectively. At the onset of natural or progestagen-induced oestrus, the contractions of the uterine horns occurred independently and the daily number of contractions for one horn was as much as one-third higher than that of the other. The more active horn was associated with the more active oviduct. The contractions then synchronized before again becoming independent at the end of oestrus (Text-fig. 3). Tubal activity of natural and progestagen-induced oestrus also showed independent patterns of activity between the two sides in early and late oestrus, corresponding with the activities of the respective uterine horns. No such asymmetry in the uterotubal activity was observed after the injection of prostaglandin analogue and PMSG.

Discussion

The results of the present experiments show that maximal rhythmic uterine activity occurs at the peak of oestrus, whether natural or induced. The direction of propagation of the uterine contractile waves was frequently reversed, as reported by Naaktgeboren *et al.* (1973). A descending contraction starting on one of the oviducts followed by several ascending contractions on the opposite horn agrees with the report of Croker & Shelton (1973) and the observations of Hawk (1975) who found that about 67% of the uterine contractions move anteriorly 5 hr after the onset of oestrus while approximately equal numbers of ascending and descending contractions occurred on both horns during late oestrus. This change may be due to a decrease in the initiatory role of the oviduct coupled with a stronger uterine activity so that the direction of contractions is controlled by isthmic activity at the beginning of oestrus and by uterine body activity towards the end. Unilateral section of the uterotubal junction in the rabbit has provided evidence for such a control mechanism (Ruckebusch, 1975).

The observation that the isthmi differed in their responsiveness to adrenergic drugs, both *in vitro* (unpublished data) and *in vivo* (Ruckebusch & Pichot, 1975), suggests a differential sensitivity of the oviducts. This asymmetry of tubal activity may be related to the presence of a corpus luteum on one ovary. A role for the corpus luteum is further supported by the disappearance of asymmetry of tubal activity after the rapid luteolysis produced by the prostaglandin treatment, this phenomenon being less marked in the case of the more gradual luteolysis at the end of a normal oestrous cycle.

Behavioural oestrus is reported to last for 51 hr after progestagen synchronization (Tomkins & Bryant, 1974). The phase of high intensity behaviour thus corresponds to the period of continuous electrical activity of the uterus. The number of bursts in each phase of activity which increased from approximately 1–3/min at the onset of oestrus to 3–4/min later correlates with the 25% increase in the rate of uterine uncoilings seen over the same period by Lehrer & Schindler (1974). Variation in the strength of the bursts may explain the partial and incomplete uncoilings described by these authors. It is likely that similar changes occurred in natural oestrus in view of the comparable

Text-fig. 3. Summation at 30-sec intervals of the uterine activity in the ewe. Tracings of the right (a) and left (b) horns over 5-hr periods from 25 to 90 hr after sponge withdrawal and PMSG injection. Full scale deflections on the ordinate correspond to $16 \mu\text{C}$. Contractions in the two horns are not synchronous until the 55th hr. After this time, quiescent periods became more marked resulting in definite periods of contractions as shown in Text-fig. 2(b).

patterns of electrical activity. With prostaglandin treatment, the relatively smaller magnitude and shorter duration of the activity may be associated with fewer uncoiling movements and possibly a reduction in behavioural receptivity, as seen by Hawk (1973).

The prolonged depression of progesterone level after sponge withdrawal was associated with an equally prolonged pattern of electrical activity. In contrast, prostaglandin injection was unable to reproduce either the intensity of electrical activity or the rapid decrease in progesterone levels ob-

served in natural oestrus. Differences in the amount and timing of the prostaglandin injection may increase the effectiveness of this treatment. The use of PMSG or its replacement by oestrogen (Crocker & Shelton, 1973) should also be considered since a transient rise in oestrogen levels successively initiates the changes in uterine motility described (Hawk, 1975). These points may be of importance in order to obtain maximum fertility.

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