

Development of Myometrial Electrical Activity during the First Half of Pregnancy in the Sheep

J. N. Sigger,^{A,C} R. Harding^A and A. Bailey^B

^ADepartment of Physiology, Monash University, Clayton, Vic. 3168.

^BDepartment of Physiology and Pharmacology, University of Queensland, St Lucia, Qld 4067.

^CTo whom correspondence should be addressed.

Abstract

Uterine myoelectrical activity was recorded in seven pregnant sheep covering the period between 13 and 75 days post-coitum. Activity in the myometrium was present at day 13 and took the form of intermittent spikes of low amplitude. Bursts of spikes of irregular duration became noticeable between days 25 and 40 but most were not coordinated throughout the myometrium. Coordinated bursts of myoelectrical activity, which could be recorded at several sites simultaneously, first appeared between 40 and 65 days. These bursts had similar characteristics to the myoelectrical activity associated with uterine contractions during the last third of gestation. The myoelectrical activity showed a progressive increase in amplitude during the first half of gestation. There was no relationship between plasma progesterone levels and the increase in amplitude or appearance of coordinated bursts of uterine activity.

Introduction

Low-amplitude contractions of the uterus occur throughout late pregnancy in several species, including the guinea-pig (Porter 1971), sheep (Hindson and Ward 1973), monkey (Germain *et al.* 1982) and human (Csapo and Sauvage 1968). The activity of the sheep uterus during late pregnancy consists of contractions lasting an average of 7 min which occur between one and four times per hour. During the contractions myoelectrical activity is recorded simultaneously at most recording sites on the uterus (Harding *et al.* 1982). However, there is limited information on the activity of the uterus before 90 days gestation (term is 145 days). The initial time of appearance of coordinated uterine contractions and the way in which they develop are of importance as the data may indicate the way in which uterine motility is controlled during pregnancy and parturition. Van der Weyden *et al.* (1981), in a limited study, have described some of the changes which occur in myometrial electrical activity during pregnancy in the ewe but do not mention the development of coordinated activity in the uterus. In this paper we present an account of the development of coordinated uterine activity and the relationship of this activity to plasma progesterone levels during the first half of pregnancy in chronically instrumented ewes. A preliminary account has been published elsewhere (Bailey *et al.* 1982).

Materials and Methods

Data from seven pregnant crossbred ewes of known mating dates were used, with day 1 of gestation being taken as the day following mating. Food was withdrawn 24 h before surgery to implant electromyogram (EMG) electrodes (insulated multistranded stainless steel wire; Cooner Sales Co., Chatsworth, U.S.A., Cat. No. AS632) on a range of recording sites on the horns and

body of the uterus (Table 1). The ewes were anaesthetized with 2–3% (v/v) halothane in oxygen and the uterus was then exposed via a midline abdominal incision. Pairs of EMG electrodes were sewn into the myometrium in a way which left a 1.0–1.5 mm bared portion of the wire within the muscle (Nathanielsz *et al.* 1980). The electrode leads were passed out through an incision high on the flank of the ewe. Signals were amplified and displayed using Grass polygraph equipment with high pass filtering set at 3 Hz.

Electrodes were implanted between 11 and 47 days after mating, with the exceptions of ewes 56 and 2144 (Table 1) which had the electrodes implanted 3 and 4 weeks before mating. In ewe 2144 the electrode leads were left in a subcutaneous position until after mating when they were exposed under general anaesthesia. All of the ewes (with the exception of 2132) were re-operated at 90–130 days gestation to insert implants into the fetuses for other experiments. Each ewe was carrying a single live fetus at the time of the second operation. Ewe 2132 aborted at day 54 for unknown reasons but before this time the plasma progesterone concentrations and uterine activity were similar to those in the other ewes.

In order to investigate the influence of ovarian hormones other than progesterone on uterine activity, one ewe (2213) was bilaterally ovariectomized under halothane anaesthesia at day 27 of gestation. Intramuscular injections of 10 mg medroxyprogesterone acetate (Upjohn) were given on days 27, 33, 42, 49, 56 and 64 to maintain pregnancy.

Table 1. Sites of implantation of recording electrodes in seven crossbred ewes

Ewe No.	Gestational age at surgery (days)	Locations of functional electrodes ^A
2144 ^B	12	L. tubal extremity
18	47	Base L. horn
		Mid R. horn
2232 ^B	25	Body of uterus
		Mid L. horn
		Base L. horn
		Mid R. horn
2132 ^B	15	Mid L. horn
		Base L. horn
		Mid R. horn
		Base R. horn
39 ^B	11	Mid L. horn
		Mid R. horn
56	Non-pregnant	L. tubal extremity
		Mid L. horn
		Mid R. horn
2213 ^B (ovariectomized)	27	Body of uterus
		Mid L. horn
		Body of uterus

^A L, left; R, right.

^B Blood samples taken for progesterone assay.

Plasma Progesterone Determination

Progesterone levels were measured in the plasma of five of the seven ewes by specific radioimmunoassay of blood samples obtained from jugular vein catheters implanted at the time of surgery. The days on which blood was sampled are shown in Fig. 4. The blood was centrifuged at 1500 g for 15 min at 4°C immediately after collection, and the separated plasma was stored at –15°C until assayed. Progesterone was assayed by the method of Challis *et al.* (1973) using a specific antiserum, No. 334. Cross-reactions of 11 β -hydroxy-4-pregnene-3-one, pregnenolone, medroxyprogesterone acetate or cortisol were less than 1%. The assay blank was 15 \pm 12 (s.e.m.) pg per tube. Intra- and interassay coefficients of variation were 12 and 9% respectively. The coefficient of correlation between progesterone added to ovine plasma and that recovered was $r = 0.997$ ($P < 0.001$).

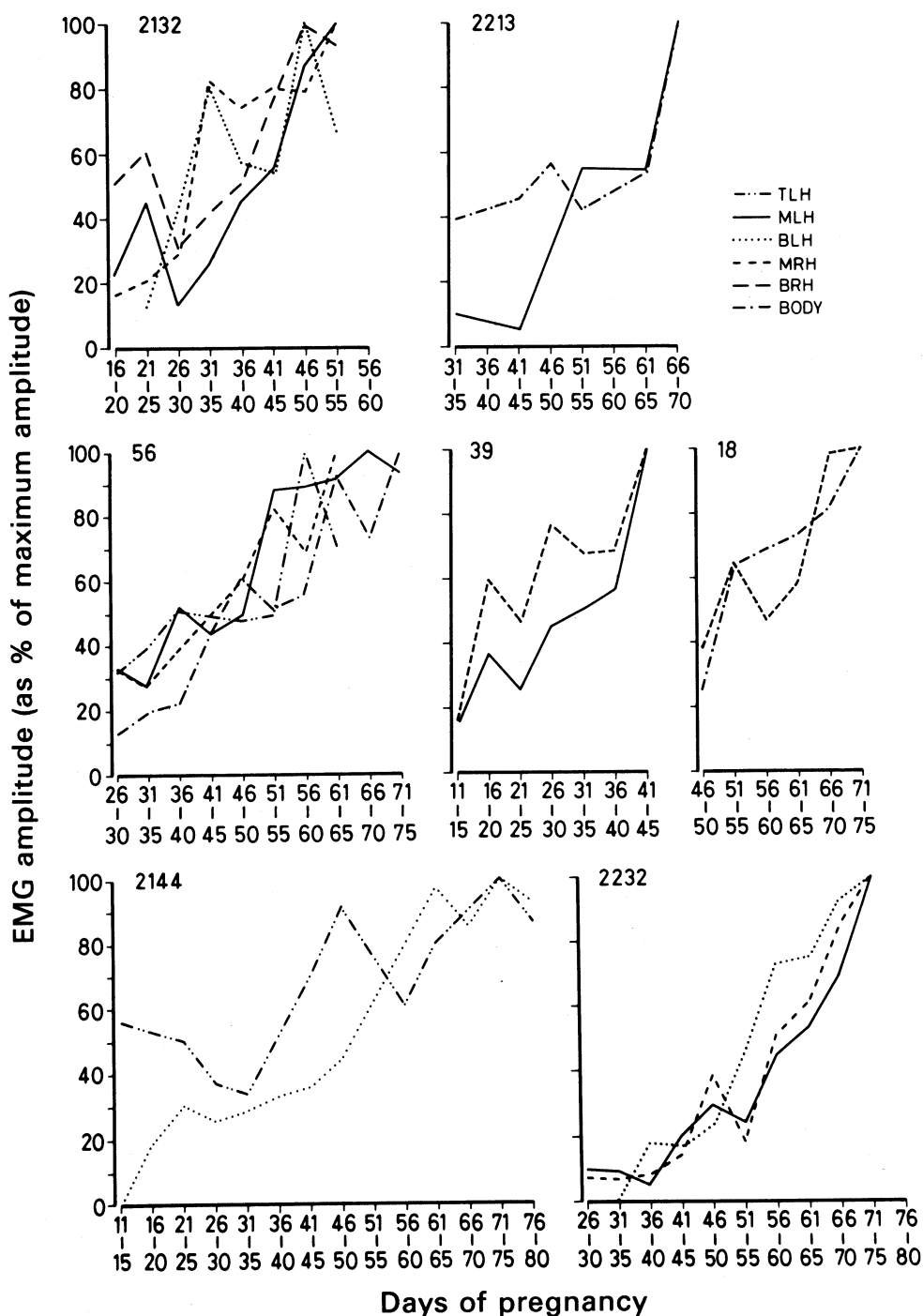


Fig. 1. Graphs showing the increase, during gestation, in EMG amplitude recorded at various sites on the uteri of the seven ewes. Amplitude is expressed as a percentage of the maximum amplitude recorded at individual sites. TLH, tip of left uterine horn; MLH, MRH, midway along left and right uterine horns, respectively; BLH, BRH, bases of left and right uterine horns, respectively; BODY, body of uterus.

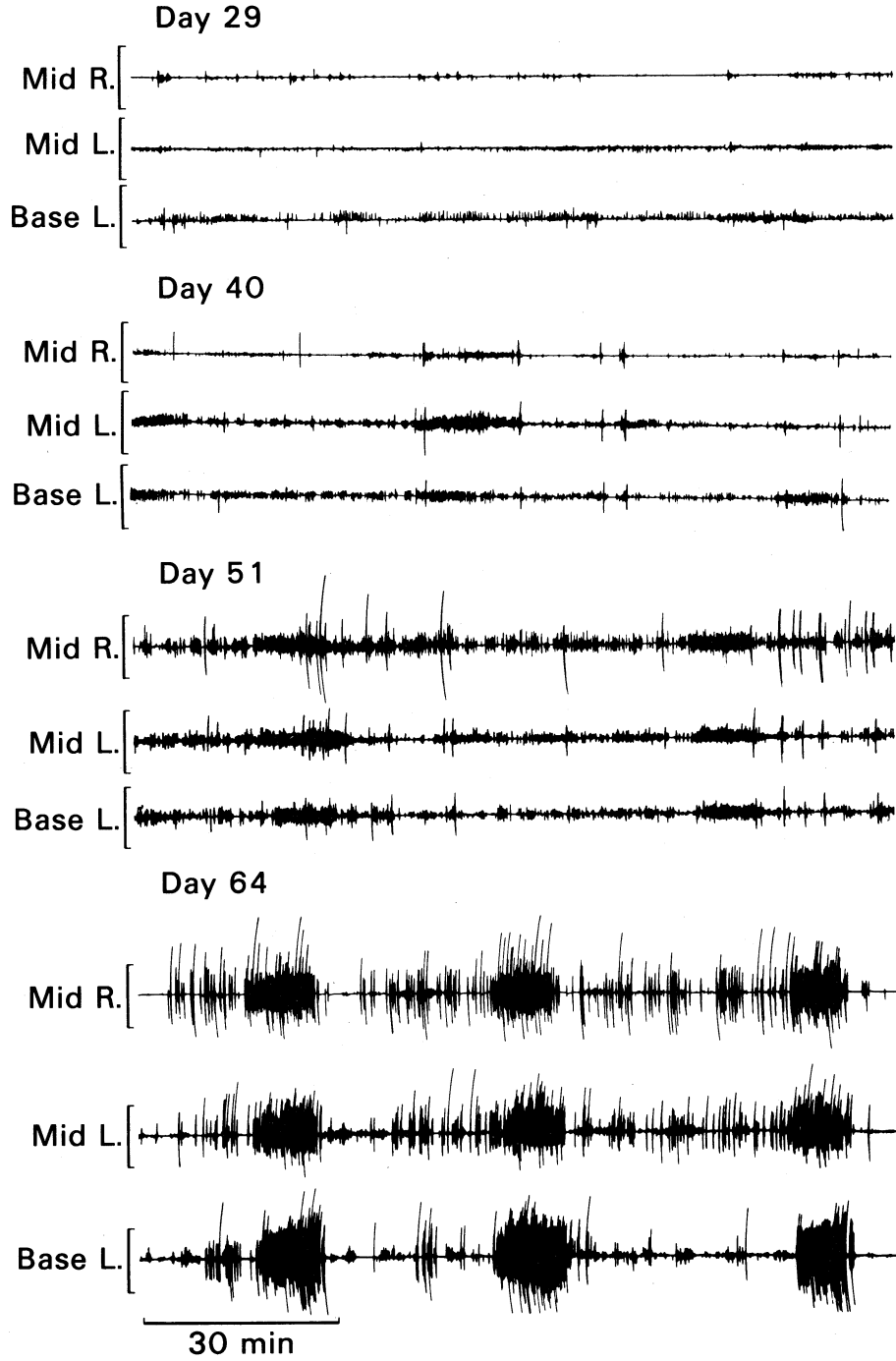


Fig. 2. A series of records from ewe 2232 showing uterine EMG activity recorded midway along the right and left uterine horns and at the base of the left horn on days 29, 40, 51 and 64 of pregnancy. Calibrations: days 29 and 40—all channels 400 μ V; days 51 and 64—mid R. horn 200 μ V, other channels 400 μ V.

Data Analysis

For the purposes of data analysis the gestation period was divided into 5-day blocks, starting at day 10 of pregnancy. Continuous recordings of at least 8 h duration were available from individual animals during each 5-day period, and at least one recording period was selected from each block for analysis. Data recorded within 3 days of surgery were not analysed. During early pregnancy, when myoelectrical activity was unpatterned, the amplitude of the activity was calculated by averaging the amplitude of 10 impulses, each approximately 2 min apart. At a later stage of pregnancy, after patterned activity had appeared in the uterus, EMG bursts were analysed for their duration, amplitude and frequency. The mean amplitude of the bursts was calculated by averaging the amplitude of single spikes occurring at the end of the first minute and at the start of the last minute of each burst. At least five EMG bursts were analysed to represent each 5-day period. Results are expressed as means \pm 1 s.d.

Results

EMG Recordings

The earliest recordings of the uterine EMG, obtained from three ewes starting at between days 13 and 16 of gestation, showed very little activity apart from intermittent low amplitude ($<100 \mu\text{V}$) spikes. There was no evidence of synchrony of activity between recording sites. In the period up to day 25 of gestation there were no clear changes in the incidence of EMG activity, although there was an increase in amplitude at most of the recording sites (Fig. 1).

Recordings were obtained from six ewes at various times between days 25 and 40 of gestation. During this period the amplitude of impulses continued to rise at most recording sites (Fig. 1) and variations in the frequency of the impulses became more pronounced, so that irregular bursts separated by relatively quiescent periods became noticeable (Fig. 2). Most bursts were not coordinated across the myometrium so that activity recorded at one site was rarely detected at other sites.

Between days 40 and 65 the amplitude of the EMG activity continued to increase at most sites. During this period coordinated bursts of activity first appeared in the uterus. These were recognized as an increase in frequency superimposed on an irregular background discharge which appeared at two or more sites on the uterus. The bursts recorded in the five ewes had a mean duration of 8.69 ± 1.68 min ($n = 64$ data sets) and occurred at a mean frequency of $1.28 \pm 0.39 \text{ h}^{-1}$. These values are similar to those recorded from ewes during late gestation (>90 days; duration 7.2 min, frequency 1.29 h^{-1}) by Harding *et al.* (1982). In three of the ewes recordings were made from three or four sites on the dorsal surface of the uterus. In all of these animals coordinated activity first appeared at just two of the recording sites and between 5 and 8 days later started at the remaining sites. Ewe 2132 initially showed regular coordinated bursts at the base of the left uterine horn, and midway along the right horn, but activity at the base of the right horn, and midway along the left horn remained unpatterned for approximately a further 8 days. During the period when coordinated contractions were becoming established there were frequent occasions when one or other of the recording sites remained quiescent or showed an attenuated EMG burst while the remaining sites showed synchronous bursts of myoelectrical activity (Fig. 3). There was no evidence from any of the ewes that bursts in one region of the uterus consistently started before activity in other regions.

After day 65 there was a continuing increase in the amplitude of the bursts at most sites, but no changes were apparent in the frequency of the bursts or in their duration.

The timing and pattern of development of uterine activity in ewe 2213 which was bilaterally ovariectomized at day 27 was not noticeably different from that of the intact ewes.

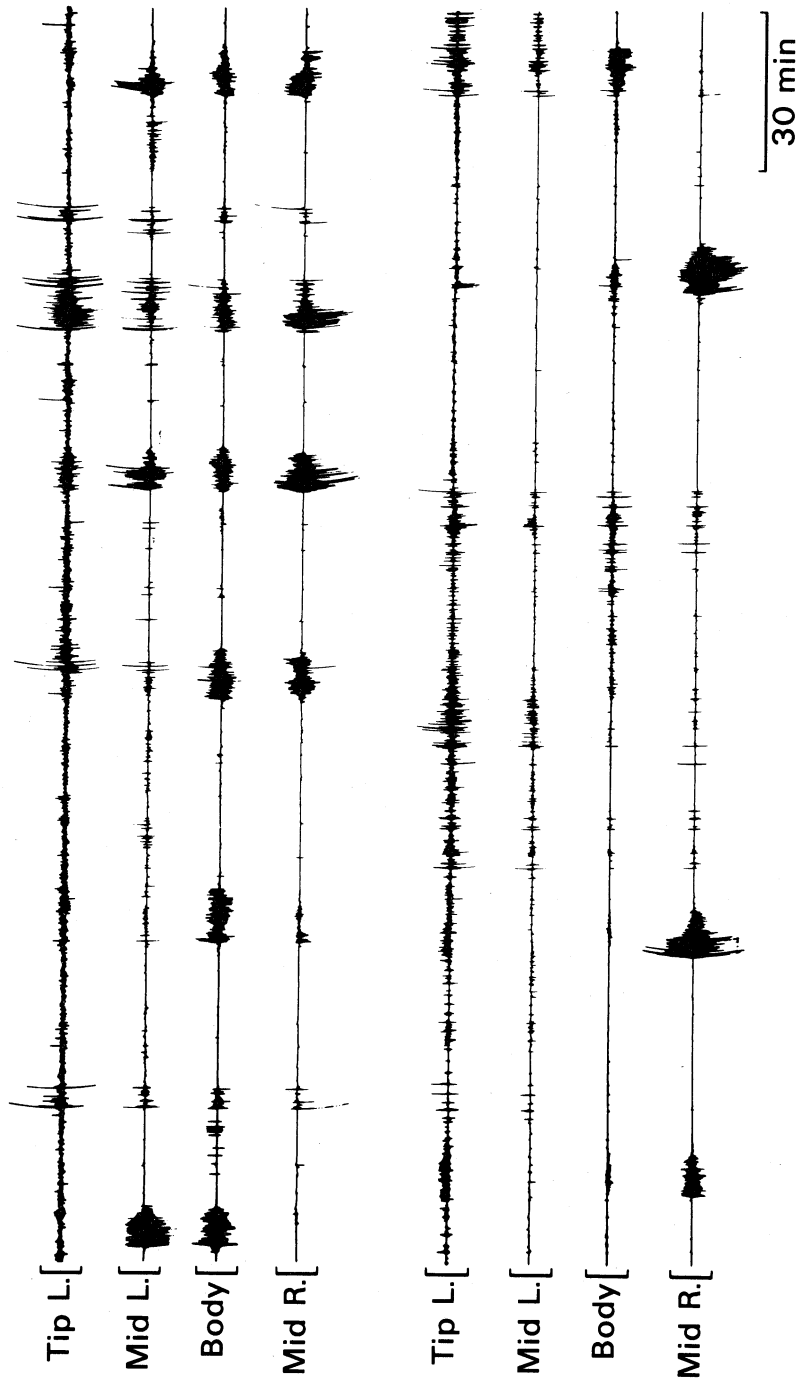


Fig. 3. Two 4-h recordings from ewe 56 at 55 days gestation. Recording electrodes were located at the tip and midway along the left horn, the body of the uterus, and midway along the right horn. Note that discrete EMG bursts are present but that individual sites may remain quiescent whilst activity is present at the remaining sites. Calibrations: 1 mV.

Progesterone Levels

Plasma progesterone levels, which were measured in five of the seven ewes studied (Table 1), fluctuated in individual animals, but there was an overall increase in each animal during the period of EMG recording (Fig. 4). Progesterone levels dropped precipitously in ewe 2213 in the 24 h following bilateral ovariectomy at day 27 and remained below 0.1 ng ml^{-1} until day 50. By day 72 the progesterone concentration in this animal had risen to 0.87 ng ml^{-1} and continued to rise rapidly thereafter, reaching 3.28 ng ml^{-1} on day 95, the last day of sampling (Fig. 4).

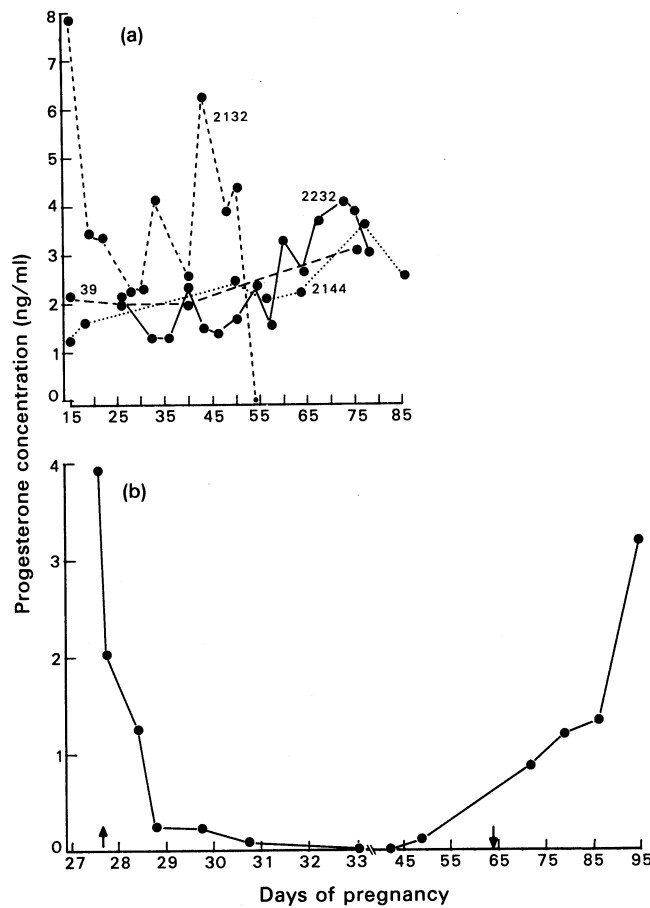


Fig. 4. (a) Plasma progesterone concentrations during early pregnancy in ewes 2144, 39, 2132 and 2232. (b) Plasma progesterone concentrations in ewe 2213, ovariectomized at day 27 of pregnancy. Note the expansion of the abscissa between days 27 and 33. The arrows indicate the time of ovariectomy (\dagger) and the time at which medroxyprogesterone acetate treatment was discontinued (\downarrow).

Discussion

The results demonstrate that the uterus is not completely quiescent during the first half of pregnancy; myoelectrical activity steadily increases in amplitude during this period despite the presence of plasma progesterone levels comparable with those occurring during the luteal phase of the oestrous cycle (Bassett *et al.* 1969; Thorburn *et al.* 1969).

Between approximately 15 and 25 days gestation myoelectrical activity recorded at one site on the uterus is not associated with activity at other sites, indicating that impulse propagation through the myometrium is limited. The contractile force generated in the myometrium by the intermittent impulses is probably minimal and is unlikely to raise intra-uterine pressure or have any mechanical effects on the embryo. Records of intra-uterine pressure obtained from experiments on anaesthetized sheep at day 15 of pregnancy support this conclusion (Fleet and Heap 1982).

The most significant change in the pattern of uterine activity takes place between 40 and 65 days post-coitum when regular coordinated bursts of impulses start to appear at multiple sites on the myometrium. The coordination of bursts of impulses across the myometrium takes place gradually over a period of days and there is no evidence that bursts are consistently initiated at one location and then spread throughout the myometrium. This pattern of activity is similar to that recorded throughout late gestation, from day 90 until the start of labour (Hindson and Ward 1973; Harding *et al.* 1982). In late gestation these coordinated contractions raise intra-uterine pressure by 0.67–1.07 kPa (5–8 mmHg) and it is probable that the contractions recorded during mid-gestation in this study also caused an increase in intra-uterine pressure.

It is not clear what factors are responsible for the change in uterine activity from irregular bursts to a coherent pattern. During the oestrous cycle rhythmical uterine contractions do not start until there is a change in the oestrogen/progesterone ratio at the end of the luteal phase (Naaktegeboren *et al.* 1973; Hawk 1975). The oestrogen/progesterone ratio increases during early pregnancy and this change in ratio may be responsible for the eventual coordination of myoelectrical activity in the uterus. Porter and Lye (1983) have shown that, in ovariectomized ewes, intra-uterine administration of oestradiol-17 β overcomes the inhibition of uterine activity produced by intramuscular injections of progesterone. Before day 50 of pregnancy plasma progesterone levels approximate those in the luteal phase, but after this time the progesterone concentration rises as increasing amounts of the hormone are secreted by the placenta (Bassett *et al.* 1969; Ricketts and Flint 1980). The production of oestrogens follows a different time course. Before day 30 oestrogens are undetectable in the fetal fluid compartments, myometrium and uterine venous plasma, but after 30 days there is a rapid rise in the concentration of oestrogens, reaching a peak around day 50 (Rawlings and Ward 1976; Carnegie and Robertson 1978; Challis and Patrick 1981). After the peak has subsided, at about day 60, there is a second rise in the levels of oestrogens at these sites which is approximately parallel in its time course to the rise in plasma progesterone. A rise in the oestrogen/progesterone ratio will cause an increase in the size of smooth muscle cells (Brody and Westman 1960) and in the number of active cells (Marshall 1962), both of which would result in an increase in the amplitude of impulses recorded by the extracellular electrodes used in this study.

The development of activity in the bilaterally ovariectomized ewe followed a similar pattern to the intact ewes which indicates that hormones originating from the ovaries are not necessary for coordinated contractions to occur. Recent experiments with transplanted, isolated segments of myometrium in pregnant and non-pregnant ewes have demonstrated that coordination of uterine contractions is not achieved by the release of uterotonic substances into the maternal circulation (Sigger *et al.* 1984).

Two other factors—sympathetic innervation and fetal movements—also have the potential to influence uterine activity. The density of myometrial adrenergic innervation in the ewe is substantially reduced between 50 and 100 days post-coitum (Sigger and Parkington 1983), a period which broadly coincides with the development of distinct coordinated bursts of activity in the uterus. Fetal movements, which are present at 35 days gestation (Barcroft and Barron 1937), may provide occasional mechanical stimulation of the myometrium, but have no influence on the coordinated uterine contractions which remain unchanged when the fetus is paralysed (Nathanielsz *et al.* 1982).

Acknowledgments

We would like to express our thanks to Dr G. Jenkin and Miss R. Poxon for performing the progesterone assays and to Professor G. D. Thorburn for his advice and encouragement. Miss Jenny Buttress provided invaluable assistance with the care of the experimental animals. The work was funded by the Australian National Health and Medical Research Council.

References

- Bailey, A., Harding, R., and Sigger, J. N. (1982). Uterine motility during pregnancy in the ewe. *Proc. Aust. Physiol. Pharmacol. Soc.* **13**, 25P.
- Barcroft, J., and Barron, D. H. (1937). Movements in mid-foetal life in the sheep embryo. *J. Physiol.* **91**, 329–51.
- Bassett, J. M., Oxborrow, T. J., Smith, I. D., and Thorburn, G. D. (1969). The concentrations of progesterone in the peripheral plasma of the pregnant ewe. *J. Endocrinol.* **45**, 449–57.
- Brody, S., and Westman, A. (1960). Ovarian hormones and uterine growth. *Acta Obstet. Gynec. Scand.* **39**, 557–65.
- Carnegie, J. A., and Robertson, H. A. (1978). Conjugated and unconjugated estrogens in fetal and maternal fluids of the pregnant ewe: a possible role for estrone sulphate in early pregnancy. *Biol. Reprod.* **19**, 202–11.
- Challis, J. R. G., Davies, I. J., and Ryan, K. J. (1973). Concentration of progesterone, estrone and estradiol-17 β in plasma of pregnant rabbits. *Endocrinology* **93**, 971–5.
- Challis, J. R. G., and Patrick, J. E. (1981). Fetal and maternal estrogen concentrations throughout pregnancy in the sheep. *Can. J. Physiol. Pharmacol.* **59**, 970–8.
- Csapo, A., and Sauvage, J. (1968). The evolution of uterine activity during human pregnancy. *Acta Obstet. Gynec. Scand.* **47**, 181–212.
- Fleet, I. R., and Heap, R. B. (1982). Uterine blood flow, myometrial activity and their response to adenosine during the peri-implantation period in sheep. *J. Reprod. Fertil.* **65**, 195–205.
- Germain, G., Cabrol, D., Visser, A., and Sultan, C. (1982). Electrical activity of the pregnant uterus of the cynomolgus monkey. *Am. J. Obstet. Gynecol.* **142**, 513–19.
- Harding, R., Poore, E. R., Bailey, A., Thorburn, G. D., Jansen, C. A. M., and Nathanielsz, P. W. (1982). Electromyographic activity of the non-pregnant and pregnant sheep uterus. *Am. J. Obstet. Gynecol.* **142**, 448–57.
- Hawk, H. W. (1975). Hormonal control of changes in the direction of uterine contractions in the estrous ewe. *Biol. Reprod.* **12**, 423–30.
- Hindson, J. C., and Ward, W. R. (1973). Myometrial studies in the pregnant sheep. In 'The Endocrinology of Pregnancy and Parturition'. (Ed. C. G. Pierrepont.) pp. 153–62. (Alpha Omega Alpha: Cardiff, Wales.)
- Marshall, J. M. (1962). Regulation of activity in uterine smooth muscle. *Physiol. Rev.* **42**, 213–27.
- Naaktegeboren, C., Van der Weyden, G. C., Klopper, P. J., Kroon, C. H., Schoof, A. G., and Taverne, M. A. M. (1973). Electrophysiological observations of uterine motility during the oestrous cycle in sheep. *J. Reprod. Fertil.* **35**, 511–18.
- Nathanielsz, P. W., Bailey, A., Poore, E. R., Thorburn, G. D., and Harding, R. (1980). The relationship between myometrial activity and sleep state and breathing in fetal sheep throughout the last third of gestation. *Am. J. Obstet. Gynecol.* **138**, 653–9.
- Nathanielsz, P. W., Yu, H. D., and Cabalum, T. C. (1982). Effect of abolition of fetal movement on fetal intravascular PO₂ and incidence of tonic myometrial contractures in the pregnant ewe at 114 to 134 days gestation. *Am. J. Obstet. Gynecol.* **144**, 614–18.
- Porter, D. G. (1971). Quantitative changes in myometrial activity in the guinea-pig during pregnancy. *J. Reprod. Fertil.* **27**, 219–26.
- Porter, D. G., and Lye, S. J. (1983). Partial reversal of the myometrial progesterone block in the non-pregnant ewe *in vivo* by oestradiol-17 β . *J. Reprod. Fertil.* **67**, 227–34.
- Rawlings, N. C., and Ward, W. R. (1976). Changes in steroid hormones in plasma and myometrium and uterine activity in ewes during late pregnancy and parturition. *J. Reprod. Fertil.* **48**, 355–60.
- Ricketts, A. P., and Flint, A. P. F. (1980). Onset of synthesis of progesterone by ovine placenta. *J. Endocrinol.* **86**, 337–47.

- Sigger, J. N., Harding, R., and Jenkin, G. (1984). The relationship between electrical activity of the uterus and isolated myometrium in the pregnant and non-pregnant ewe. *J. Reprod. Fertil.* **70**, 103-14.
- Sigger, J. N., and Parkington, H. (1983). Changes in uterine innervation during pregnancy. *Excerpta Medica Asia Pacific Congress Series No. 14*. pp. 23-4.
- Thorburn, G. D., Bassett, J. M., and Smith, I. D. (1969). Progesterone concentration in the peripheral plasma of sheep during the oestrous cycle. *J. Endocrinol.* **45**, 459-69.
- Van der Weyden, G. C., Taverne, M. A. M., Dieleman, S. J., and Fontijne, P. (1981). Myometrial electrical activity throughout the entire course of pregnancy in the ewe. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **11**, 347-54.

Manuscript received 22 November 1983, accepted 11 April 1984