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SOME ASPECTS OF UTERINE MOTILITY IN THE MARE AS MEASURED BY MYOMETRIAL ELECTROMYOGRAPHY

A THESIS PRESENTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
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DENISE M. JONES
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"Difficult though it may be to record uterine activity in a unicorn the data obtained is no more reliable than similar data derived from the study of one mouse".

Finn and Porter (1975)

ABSTRACT

The purpose of this study was to determine how uterine motility as measured by both electromyographic techniques (emg), and to a lesser extent by intra-uterine pressure changes (IUP), is influenced by steroid hormones, uterine stimulants and relaxants, infused intra-uterine fluids, natural breeding and the first 20 days of pregnancy.

Two intact and two ovariectomised mares had uterine emg activity measured from 3-8 hours/day over a period of 1-5 months. Simultaneous IUP recordings, using an open tipped catheter, were periodically taken. One intact mare during anoestrus and both spayed mares were given exogenous courses of oestradiol and progesterone to simulate oestrous cycle activity. Oxytocin, cloprostenol, propantheline bromide and clenbuterol were administered to each mare during anoestrus, transition, oestrus, and dioestrus, where applicable. Quantities (60-1000ml) of sterile double distilled water were infused intra-uterine into each mare at various cycle stages. One intact mare was bred on four occasions and followed through the first 20 days of her pregnancy.

Mares in oestrus recorded synchronous short bursts (3-5 min) of high amplitude emg activity following a crescendo-decrescendo pattern. In dioestrus burst duration increased (15-25 min) and amplitude decreased with increasing plasma progesterone levels. Emg results during anoestrus and transition were intermediate. During early pregnancy emg characteristics varied depending on whether the conceptus was in the oviduct, migratory or fixed. It is proposed that in oestrus emg changes manifest as contractions, while in dioestrus as increased uterine tone.

Oxytocin and cloprostenol caused uterine responses at all cycle stages with the most pronounced response during oestrus where drug administration was followed by prolonged emg activity (10-25 min) initially and then followed by short burst activity. The least response was seen during dioestrus. Propantheline bromide decreased emg activity especially in dioestrus and is an effective uterine relaxant; clenbuterol however caused minimal measurable change.

Infused intra-uterine fluids resulted in a single spike pattern of emg activity which was generally asynchronous between electrode sites during the first infusion and depressed uterine activity following a subsequent second infusion.

Natural service resulted in minimal emg changes similar to those seen after rectal palpation, ie a short term (5-10 min) burst of densely grouped action potentials. This response is so short it seems unlikely either endogenous oxytocin and/or prostaglandins would have any significant influence on sperm transport in the mare; it is suggested that the emg change seen at this time is more in the nature of a local response to vaginal stimulation by the penis of the stallion, and is similar to that seen during palpation per rectum.

Electrode site emg variation was common, especially during dioestrus and early pregnancy.

The emg activity recorded in early pregnancy is different to that found in the non-pregnant dioestrus mare and probably related to the position of the embryo; it is suggested that abnormal uterine motility could be a cause of early embryonic death in this species.

During the oestrous cycle there was little correlation either statistically or visually between emg and IUP with or without drug treatment, but IUP increased with uterokinetic drugs and decreased with relaxants.

IUP changes may not be a reliable method of measuring uterine activity in the mare. This is supported by the finding that there was no statistical difference in IUP parameters measured between cycle stages, whereas there were important emg variations. As the experimental mares experienced signs of intestinal discomfort after administration of the uterine stimulants, and propantheline bromide is a known intestinal relaxant, the author argues that IUP results recorded after drug treatment could be influenced by the effects of these substances on intestinal motility rather than solely the consequence of a direct uterine response.

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I INTRODUCTION AND LITERATURE REVIEW

I.1 INTRODUCTION

Myometrial contractions are thought to be essential for most uterine functions; sperm and ovum transport (Nalbandov,1976), movement of the conceptus necessary for embryo spacing in the sow (Scheerboom *et al.*,1987), early pregnancy recognition in the mare (Ginther,1983a), parturition, expulsion of the foetal membranes and uterine involution. Coordinated contractions of the uterus are also helpful in reducing contamination after natural mating and parturition thereby ensuring an optimal environment for conception (Peterson *et al.*,1969).

In the mare, many researchers have explored the cellular aspects of uterine defense (Liu and Cheung,1986; Asbury,1987; Watson,1988). Less attention has been given to the mechanical ability of the equine uterus to reduce contamination. Evans *et al.*(1987) using acyclic mares of varying ages (2-26 y.o.) administered exogenous oestradiol and progesterone to simulate oestrus and dioestrus. Each mare, under each hormonal milieu, was then subjected to intrauterine inoculations of either bacteria plus microspheres or microspheres alone. After 5 hrs the clearance of the microspheres was evaluated. Young mares in oestrus were consistently found to have the highest rate of clearance. This was not the case in the progesterone dominated uterus, perhaps because of cervical closure. In another study (Evans *et al.*, 1986) oestrogen treated mares and anovulatory mares, which may have had relatively high oestrogen levels (Oxender *et al.*,1977), began clearing markers within 2 hrs and all markers were cleared by day 3. This was again not true of the progesterone treated mares. More recently Le Blanc *et al.*(1989) using a similar technique, introduced intrauterine bacteria and markers during the pre-ovulation period in a group of mares and studied clearance in the post-ovulation period on days 0,3 and 5. No disruption in the clearance mechanism was found in young healthy mares post-ovulation, as compared to older mares with a history of chronic endometritis and infertility.

Attempts at assessing uterine motility in healthy cycling mares via transrectal ultrasound (Cross and Ginther ,1987, 1988), intrauterine pressure changes (Goddard *et al.*,1985;Goddard and Allen,1985) and emg recordings (Taverne *et*

al.,1979) have been published. However, no long term project following motility patterns throughout normal oestral cycles and under varying treatment conditions has been published. The study reported in this thesis was undertaken to further clarify patterns of uterine motility in the mare under the above conditions. Recording of myometrial emgs was the method chosen as this technique allows long term investigation from consistent locations in the same animal, and also allows each animal to serve as its own control. Furthermore, emg recordings have been shown to correlate with simultaneous intrauterine pressure recordings in other species (Naaktgeboren *et al.* 1973; Porter, 1970,1974; Scheerboom *et al.*,1987). Since this correlation has not been documented in the mare, intrauterine pressure recordings were also taken from time to time in an attempt to assess such a relationship.

I.2 GROSS ANATOMY OF THE UTERUS OF THE MARE

The uterus is a hollow, thick walled, muscular organ located intra-abdominally between the rectum and the bladder. It is suspended by the broad ligament from the level of the third or fourth lumbar vertebra to the fourth sacral vertebra and is supported and surrounded by intestinal coils. The uterine wall is anatomically divided into three layers: the outer perimetrium, middle myometrium and inner endometrium. The myometrium can be further divided into a thick inner layer of circular smooth muscle, a vascular layer and thinner outer layer of longitudinal smooth muscle. The perimetrium, vasculature and longitudinal smooth muscle are continuous with corresponding tissues of the broad ligament (Sisson,1975; Ginther,1979).

Uterine arteries carry hormones for regulation of uterine functions. The middle uterine artery is the main vascular supply, but uterine branches of the vaginal artery (caudal uterine artery) and ovarian artery (cranial uterine artery) also contribute. The uterine artery supplies branches to the mesometrium.Three vessels comprise the venous return; uterine branches of the vaginal and ovarian veins, and the uterine veins. There are extensive anastomoses within both arterial and venous systems (Ginther,1979), but the mare lacks the close apposition between the ovarian artery and utero-ovarian vein found in the ewe and the cow, although in all three species the uterus and ovary are drained by the common uterine branch of the ovarian vein (Ginther,1981).

There is an extensive lymphatic drainage, especially in the broad ligament, about which little is known.

The uterine innervation is autonomic. Parasympathetic fibres come from the sacral area via pelvic nerves, while sympathetic innervation is from the caudal mesenteric ganglion and plexus(Ginther,1979).

Neurohumoral control of reproductive function is the responsibility of the hypothalamus, pituitary, and possibly the pineal gland. Reviews of this area can be found in several texts (Nalbandov,l976; Ginther,1979; Roberts,1986).

I.3 FUNCTIONAL ANATOMY OF THE UTERUS

The myometrium consists of smooth muscle cells resting in a network of connective tissue composed of collagen, fibroblasts, macrophages, mast cells and elastic fibres (Finn and Porter, 1975). Knowledge of the structure of individual cells, how they communicate,and the function of the intercellular matrix is essential to the understanding of myometrial contraction.Each smootli muscle cell is less than 1mm long, considerably smaller than a striated muscle cell (Gabella, 1979). They are grouped in cord like bundles, separated by connective tissue, within the longitudinal and circular layer of the myometrium (Finn and Porter,1975). Communication between these small cells is enhanced by several anatomical adaptions:

(i) Caveolae

Caveolae are numerous flasked shaped invaginations of the cell membrane grouped in rows parallel to the long axis of the cell. The total surface of all caveolae increases the total surface area of the cell by about 70%. The caveolae communicate with the extracellular space through lumina in their necks. Their association with the sarcoplasmic reticulum and other intracellular structures is less well developed in the myometrium than in intestinal smooth muscle The exact function of caveolae is unknown. It is probable they control cell volume, serve as stretch receptors, and are involved in calcium transport and storage (Finn and Porter,1975; Gabella ,1979,1981).

(ii) Dense Bands

Dense bands, composed of electron dense material on the cytoplasmic side of the cell membrane, are located between rows of caveolae. They are penetrated by intracellular filaments and form cell to stroma and cell to cell connections. An intercellular bridge, known as an intermediate junction, is formed by two matching dense bands in adjacent cells held together by intercellular cement (Gabella 1979,1981).

(iii) Gap Junctions or Nexuses

The nexus is an area of close apposition between the membrane of two cells. The minute gap is accessible to the extracellular space and is spanned by intercellular ionic channels. These gaps are probable sites of electronic coupling between muscle cells (Gabella, 1979,1981).

(iv) Collagen

Collagen is the major extracellular component. There is three times more collagen in smooth than striated muscle. These collagen fibrils may be in contact with the sarcolemma (Gabella 1979,1981). Uterine collagen, which is synthesised by smooth muscle cells, contains a large portion of type 3 fibres, found in organs needing a high degree of compliance (Bornstein and Sage,l980).

(v) Sarcoplasmic Reticulum

Sarcoplasmic reticulum,abundant in the smooth muscle cells of the myometrium,is arranged in tubules covered with cisternae running parallel to the cell axis. In granular sarcoplasmic reticulum the cisternae are studded with ribosomes, and may be important for synthesis of the major cellular components. In areas of the cell wall rich in caveolae there are often fenestrated sacs of sarcoplasmic reticulum in close approximation (10nm) with the caveolae, or the reticulum may be associated with caveolae in the form of tubules running parallel to the cell axis between rows of caveolae. Sarcoplasmic reticulum is also found in association with mitochondria and myofilaments. It is probably involved in the storage of intracellular Ca^{++}, possibly in the cisternae (Gabella,1981).

(vi) Mitochondria

Mitochondria are scattered throughout the sarcoplasm with a concentration in the area beneath the cell membrane. Positioned parallel to the myofilaments they are in close proximity to the sacs and tubules of the sarcoplasmic reticulum and may have an association with the caveolae. Mitochondria, due to their ability to accumulate divalent cations, are probably important in Ca^{++} regulation (Gabella,1981).

(vii) Dense Bodies

Dense bodies are electron dense material scattered in the sarcoplasm. They are similar in composition to dense bands and may represent a disperse form of intracellular attachment of the thin filaments (Gabella,1981).

(viii) Intracellular Filaments

Three types of intracellular filaments have been identified:

a) Thick or myosin fibres - arranged in a longitudinal fashion
b) Thin or actin fibres - arranged in a longitudinal fashion
c) Intermediate fibres -often connected to dense bands.

Actin is present in a higher ratio than myosin and these two fibre types are linked by cross bridges during contraction. The myofilaments are believed to slide past each other, as in skeletal muscle, to cause shortening of the cell. The cell can shorten to less than a quarter of its resting length due to the arrangement and shape of the dense bodies. Tremendous force is generated by the sliding of the filaments. Cell to cell and cell to stromal junctions are important in the transmission of this force and during contraction the surface of the cells fold and interdigitate with adjoining cells. The intermediate junction, which receives actin and intermediate filaments from each cell involved, is a direct link from the forces in one cell to another. As these junctions are scattered over the entire surface of the cell,there is substantial mechanical coupling occurring along its length. The filaments also attach to dense bands that form stromal links,and thus force is also transmitted through the connective tissue.

During isotonic contraction the collagen fibrils change their arrangement and run transversely to the cell axis, shortening by winding in helices around the cells. This strengthens the argument that there is a link between the cell membrane and collagen and that the collagen is important in the transmission of force (Gabella,1979). At least 3 - 5 % of the cell surface has specialized areas of contact with neighbouring cells (Bulbring *et al.*,1981b). The special cell to cell and cell to stroma arrangements, in addition to the ground substance composition in smooth muscle, give it the quality of plasticity, which allows it to behave more as a viscous mass than a structured tissue (Finn and Porter,1975; Gabella,1979; Ganong,1983).

I.4 EXCITATION - CONTRACTION COUPLING

The sarcoplasm of each smooth muscle cell is surrounded by a semipermeable excitable membrane, the sarcolemma (Finn and Porter,l975; Ganong,1983). The ability of the cells to respond to changes in their environment depends on their ability to maintain an ionic gradient across the sarcolemma which has specific electrical and permeability characteristics. Small changes in permeability result in significant ion movement resulting in an action potential (Brading,1981). Individual smooth muscle cells have an interior negative charge due to an outward osmotic potassium (K+) gradient. This results in an electrochemical gradient attracting K+ inward. When these gradients are balanced the net K+ flux is zero and the membrane is stable (Ludin,1980) resulting in a membrane resting potential of -50 to -56mv (Brading,1981). As the membrane becomes more permeable to sodium (Na+) and calcium (Ca++) they move intracellularly following the electrochemical gradient, resulting in depolarization or a decreased resting membrane potential. When the membrane again becomes more permeable to K+ it moves extracellularly resulting in hyperpolarization or an increased resting membrane potential. This sequence of depolarization followed by repolarization creates an action potential (Finn and Porter,1975; Ludin,1980). For repeated responses (action potentials) cells must have mechanisms to restore the ionic gradients. These mechanisms are believed to involve active exchange pumps requiring energy (Brading,1981).

Excitation - contraction coupling is usually a result of these generated action potentials (Kuriyama,1981) and is dependent on

Ca++(Brading,1981;Casteels,1981;Kuriyama,1981). As depolarization occurs Ca++ enters the cell via the sarcolemma and is also released from intracellular stores in the sarcoplasmic reticulum, plasma membrane and mitochondria. This increase in available intracellular Ca++ is the activator for muscle contraction (Hamoir, 1977; Perry and Grand,1979; Ludin,1980). An accepted theory is that Ca++ associates with the enzyme calmodulin to activate myosin light chain kinase to catalyze the phosphorylation of myosin. The phosphorylated myosin then interacts with actin to cause crossbridge turnover and shortening, leading to the development of tension. When Ca++ is removed the kinase is not active and a phosphatase removes the phosphate resulting in actin myosin disassociation and relaxation (Hartshorn *et al.*,1977; Matsui *et al.*,1983). The rise in intracellular Ca++ also triggers the breakdown of ATP by ATP-ase. As Ca++ depletes, ATP reforms and activates the pump to stabilize the cell membrane. The muscle cell relaxes (Ludin ,1980).

Regulation of uterine contraction is through the interaction of two mechanisms. One is the myosin light chain kinase system leading to muscle contraction. The other is cyclic AMP and cyclic AMP dependent protein kinase which precipitates relaxation. The cyclic AMP system is believed to increase Ca++ uptake and ATP dependent Ca++ transport through the cell membrane as well as decrease the phosphorylation of myosin light chains (Matsui *et al.*,1983).

For a thorough review of smooth muscle contraction the reader is referred to "Smooth Muscle: an Assessment of Current Knowledge" (Bulbring *et al.*,1981a) and "Excitation - Contraction Coupling in Smooth Muscle" (Casteels *et al.*,1977).

In addition to its characteristic of plasticity, the uterus has inherent pacemaker activity and the ability to function as a syncytium. This auto-rhythmicity or pacemaker activity is aided by a low resting membrane potential (-50 mv) which tends to make it somewhat unstable resulting in spontaneous action potentials. This probably occurs in many cells, but when the number of cells producing this potential at a given place and time exceeds a critical value, synchronization occurs, the potentials generated spread by conduction causing uterine contraction (Bulbring *et al.*,1981b; Finn and Porter,1975). That this rhythmic discharge of action potentials persists in the presence of ganglionic and nerve blocking agents

is proof that the origin is myogenic . As all uterine cells are capable of initiating a contraction, the pacemaker is said to shift (Marshall,1962). Its unitary or syncytial ability is a function of its anatomical and electrical cell to cell and cell to stroma connections (Ganong,1983).

The uterine endometrium contains two types of adrenergic receptors (alpha and beta) located within the cell membrane. Transmitter substances can be excitatory or inhibitory, depending on which receptor they activate. Excitatory substances produce depolarization, an action potential and a release of intracellular Ca^{++} stores causing contraction. Inhibitory substances cause hyperpolarization and an increase in Ca^{++} uptake (Adams,1977; Kuriyama,1981) . Which receptors dominate vary with the species and hormonal status. In general, the oestrogen dominated uterus increases alpha receptors and action potential discharge. When there is already spontaneous activity and alpha receptors are stimulated the frequency and duration of the contractions increase (Bulbring *et al.*,1981b). Excitatory agents that cause contraction are known as ecbolics. Drugs that bind beta receptors to cause relaxation are tocolytics (Kern and Schill,1983).

I.5 THE REPRODUCTIVE CYCLE OF THE MARE

(i) Introduction

The majority of mares exhibit a seasonal reproductive pattern, which ensures birth of the young at a favourable time for their survival in terms of weather conditions and food supply. As mares have an 11 month gestation the breeding season and foaling season overlap in the spring to fulfil this purpose. The annual reproductive pattern of the mare can be divided into four parts. The first, or anovulatory period is characterised by sexual quiescence. Less than 25% of mares are known to ovulate during this time. This anovulatory period is followed by a transition phase to the ovulatory period. Transition begins in late winter and continues through to early spring. The ovarian and behavioural changes are gradual and are brought about by changes in the hormones produced in the hypothalamic - pituitary axis. The ovulatory or breeding season commences once the first ovulation has occurred, leading to the establishment of regular oestrous cycles. After the last ovulation of the

year, which occurs in the autumn, the mare enters another gradual transition period from the ovulatory to the anovulatory season (Sharp,1980).

(ii) Anovulatory Period

During the anovulatory period there is gonadal atrophy. Follicle activity is minimal and when present the average follicle diameter is 5 - 10 mm. Ovarian blood flow is decreased from 50-100ml/min close to ovulation to 5-10ml/min during the anovulatory period (Sharp,1980). Luteinizing hormone (LH) levels in anovulatory mares are negligible (less than 2ng/ml.). Follicle stimulating hormone (FSH) concentrations average between 5 - 10 ng/ml, but undergo wide fluctuations (Freedman *et al.*,1979). Progesterone levels are extremely low , less than 0.5ng/ml (Oxender *et al.*,1977), while oestradiol levels vary between 3-6pg/ml and oestrone between 7-10pg/ml (Sharp,1980). However oestradiol levels are occasionally quite high, reaching oestral levels (25-30pg/ml), possibly due to adrenal production, as spayed mares also maintain a fluctuating level of plasma oestradiol (Oxender *et al.*,1977). Although behavioural signs in the presence of a stallion are minimal, both anovulatory and spayed mares occasionally exhibit oestral behaviour and will stand to be mounted (Ginther,1979). Endometrial atrophy also occurs during this period (Kenny,1975).

Day length is the most likely cue for control of this reproductive seasonality (Ginther,1979). Manipulating the photoperiod, with other factors remaining constant, has shown convincingly that the ovulatory season can be advanced appreciably in the spring and the anovulatory period delayed in the autumn (Oxender *et al.*,1977; Freedman *et al.*,1979). Ginther(1979) has published an excellent review of the subject. Briefly, light through the retina stimulates neurological pathways to the pineal gland, which functions as a neuroendocrine transducer, cueing the hypothalamus to release GnRH through the portal system which activates the anterior pituitary to release FSH and LH. These gonadotrophins then act on the ovaries through the general circulation. In the mare, dark stimulates pineal gland enzymes, probably via melatonin, blocking hypothalamic stimulation. Increasing daylight reduces this pineal enzyme activity resulting in gonadal stimulation. Nutrition and ambient temperature appear capable of modifying this system. The response to either

natural or artificial light is slow, so artificial lighting programmes must be started 8-10 weeks before the effect is desired (Sharp,1980). In intact mares 16hr of light applied from the middle of the anovulatory season hastens the onset of the ovulatory season by 2 months (Oxender *et al.*,1977; Freedman *et al.*,1979).

(iii) Transition To The Ovulatory Phase

As ovulation approaches the size of the largest follicle increases to nearly 40mm diameter while the number of follicles decrease. This decrease in follicle numbers is preceded by a drop in FSH concentration to less than 2 ng/ml. Preceding ovulation, there is an increase in LH (5ng/ml) associated with the final growth and maturation of the ovulatory follicle. In the ovariectomised mare there is also a rise in LH, although the rate of increase is not as dramatic (Freedman *et al.*,1979). In spayed mares FSH levels do not decline as there is no negative gonadal feedback. A non-steroidal substance in follicular fluid (inhibin) has been found to inhibit FSH release in mares (Ginther,1979).

(iv) Ovulatory Phase

The oestrous cycle in the mare averages 21-22 days and is divided into oestrus (5-7 days) and dioestrus (15 days). In oestrus rising oestrogen levels, produced by ovarian follicles stimulate the anterior pituitary to reduce FSH and increase LH production. LH precipitates final maturation and subsequent ovulation of the predominant follicle 24 to 48 hrs before the end of behavioural oestrus, as well as formation of the corpus luteum (CL). Oestrogen produced by oestral follicles and progesterone manufactured by the dioestral CL are responsible for the behavioural and physical changes the mare experiences throughout her cycle. Post-ovulation, progesterone levels gradually increase from less than 1ng/ml to more than 5ng/ml by day 4 (ovulation = day 0). About day 14 prostaglandin is released from the endometrium causing luteolysis and a rapid decrease in plasma progesterone, returning the mare to oestrus within 3 days (Hughes *et al.*,1980; Rossdale and Ricketts,1983). A more complete discussion of luteolysis can be found in chapter I.10.(ii).

(v) Early Pregnancy

The first three weeks of pregnancy in the mare can be divided into three stages:

a) the first 6 days post-ovulation when the fertilised ovum is still in the oviduct,

b) days 7-15, when the embryo is believed to be mobile within the uterus,and

c) day 16 and after, when embryo location is believed to be fixed (Ginther,1979,1983a,1983b,1984; Leith and Ginther,1984).

During the mobility phase the vesicle has been found to change locations 1.7 times every 2 hrs. Maximal mobility occurs during days 11-14 and embryo movement is progressive, moving caudally when in a horn and cranially when in the body. Uterine contractions, possibly stimulated by a substance released from the vesicle, are believed responsible for vesicle movement (Leith and Ginther,1984).

I.6 THE STEROID HORMONES

(i) Introduction

The steroid hormones, oestrogen and progesterone, are simple chemical structures with specific action on the metabolic, morphologic and behavioural systems of mammals (Katzenellenbogen,1980). They bind to steroid specific receptors that then translocate to nuclear sites where they stimulate RNA synthesis necessary for cell growth and function (Leavitt *et al.*,1974; Hsueh *et al.*,1976; Katzenellenbogen,1980). The presence of a hormone at the target tissue can change the receptor population. Oestrogen priming of the myometrium increases the concentration of progesterone receptors (Leavitt *et al.*,1974; Stone *et al.*,1978). In the oestrogen primed uterus progesterone interferes with the availability of oestrogen receptors in the cytoplasm. There is a dose relationship in this receptor suppression by progesterone (Hsueh *et al.*,1976; Bhakoo and Katzenellenbogen,1977a,b; Katzenellenbogen,1980), and it takes 24 hrs. for the effect of progesterone to reverse (Bhakoo and Katzenellenbogen,1977a). The stimulatory effect of oestrogen on progesterone receptors is also partially blocked by the presence

of progesterone. Therefore the relative ratios of oestrogen to progesterone are important(Stone *et al.*,1978).

In the intact ewe Miller *et al.*(1977) found that the maximum numbers of oestradiol receptors were present during oestrus then declined until the end of the luteal phase. Peak progesterone receptor populations occurred during the first few days of dioestrus and began to decline past day 5. Maximal plasma concentrations of progesterone coincided with minimal myometrial progesterone receptor levels.

When physiological levels of steroid are present interaction is restricted to the specific receptor protein for that hormone, but at pharmacological levels spillover specificity or illicit occupancy of receptors may occur (Katzenellenbogen,1980).

(ii) Oestrogen

The translocation of the oestrogen receptor to the nucleus causes an alteration in gene expression (Gorski and Gannon,1976). Within one hour of exogenous oestrogen administration to the ewe the uterine blood flow increases and there is a decrease in the coefficient of oxygen utilization. This implies that the rate at which blood reaches the uterus is determined by the levels of ovarian hormones and is probably due to a direct action on the tone of smooth muscle in the arteriole wall (Huckabee *et al.*,1970). By 6hrs after injection there is an increase in RNA synthesis, fluid inhibition, glucose oxidation and 2 deoxyglucose metabolism (Katzenellenbogen,1980). DNA synthesis is next to increase. Myometrial hypertrophy and hyperplasia and an increase in actin and myosin result (Parkington and Lipton,1976).

Workers also report an increase in uterine motility under oestrogen influence. Exogenous oestrogen given to ovariectomised immature female rats raised the membrane potential of *in vitro* myometrial strips from -35 to -55mv allowing spontaneous activity leading to regular rhythmic contractions alternating with periods of quiescence. This coordinated uterokinetic response is a result of efficient conduction of electrical activity throughout the myometrium (Marshall,1962). Csapo and Corner (1952) demonstrated the staircase

phenomenon with *in vitro* strips of oestrogenised rabbit myometrium where there was a gradual increase in tension with electrical stimulation, until a maximum tension was reached and maintained.

Oestrogen is also responsible for the gross physical and behavioural changes seen during oestrus. In the mare these include hyperaemia of the reproductive tract, an increase in thin cervical and vaginal mucus, relaxation and oedema of the vulva, cervix and uterus, and acceptance of the stallion (Hughes *et al.*,1980; Neely,1983; Roberts,1986).

(iii) Progesterone

Csapo (1956) noted a blocking effect on uterine motility due to progesterone. He found that the working capacity of the myometrium was similar under oestrogen or progesterone influence, but that somewhere in the contraction sequence progesterone caused a block so that the process couldn't proceed. Even when action potentials were generated the response remained local and the impulse did not propagate due to poor conduction. After withdrawal of progesterone in the rabbit, a minimum of 18 hrs is required for the "block" to be withdrawn. A negative staircase effect exists *in vitro* , where there is a gradual decrease in tension with repeated electrical stimulation until a plateau tension is reached and maintained.

The membrane potential of the uterus under progesterone influence is about -63mv i.e. the membrane is hyperpolarized. No synchronised discharge of action potentials occurs (Marshall,1962). *In vitro* there is a decrease in the amplitude of contractions and a lack of rhythmicity. This is dose related in the rabbit with a 4-6 hr lag effect after the first injection (Csapo and Takeda,1965). Besides causing a hyperpolarization of the membrane, progesterone may also act to suppress mRNA synthesis, which codes proteins capable of modifying sarcolemmic function in respect to Ca^{++} binding (Currie,1979).

Experimental work suggests Ca^{++} is more firmly retained in or near the sarcolemma when progesterone is dominant (Csapo,1959; Torok and Csapo,1976; Currie and Jeremy,1979). Since the presence of free Ca^{++} is

critical for coupling the excitation-contraction process, a lack of it interferes with this process (Currie and Jeremy,1979). Excitability is not the same as contractility. In Ca++ free *in vitro* solution a strong electrical stimulation can bypass excitation of the membrane (action potential) and cause contraction by mobilization of Ca++ from its myoplasmic sites (Csapo,1959). The loss of excitability is delayed in uterine strips from pregnant rabbits, or when progesterone is added, supporting the Ca++ retention theory (Currie and Jeremy,1979). A uterus under the influence of progesterone may also show less pharmacological reactivity (Csapo,1956; Torok and Csapo,1976; Lye and Porter,1978). However, the progesterone block theory has not been supported in all species (Porter,1970; Porter and Challis,1974).

In the mare, progesterone causes increased uterine tone, closure and lengthening of the cervix, and a pale vaginal mucosa coated by scant sticky mucus. She is not receptive to, and often aggressive towards the stallion (Hughes *et al*,1980; Neely,1983; Roberts,1986).

I.7 THE CASTRATE

In the castrate uterus there is a loss of protein and high energy compounds reducing the working potential of the myometrium. The muscle may become structurally incapable of developing mechanical activity (Coutinho and DeMattos,1968). The myometrial cells of both the castrate and immature uterus have a low membrane potential (-35mv),resulting in little or no spontaneous discharge of action potentials rendering the muscle virtually inactive (Marshall,1962) both *in vitro* (Csapo and Goodall,1954) and *in vivo* (Melton and Saldivar,1965; Lye,1980). However, in the recently castrated rabbit (less than 6 days after surgery) there is an increase in spontaneous activity which is of high amplitude and well coordinated. This suggests a restraining effect of oestrogen on the myometrium (Coutinho and DeMattos,1968). This effect has also been found in cyclic women (Coutinho,1967), and confirmed in the rabbit where exogenous oestrogen caused a marked depression of activity when given to the spayed rabbit. The activity returned after oestrogen withdrawal (Coutinho and DeMattos,1968).

In the non-cycling anoestral or castrate mare the uterine tone is relaxed, the cervix passively open or closed and the vaginal vault pale and dry. The mare is tolerant of the stallion, but displays no overt interest (Hughes *et al.*,1980; Neely,1983; Roberts,1986).

I.8 THE MEASUREMENT OF UTERINE ACTIVITY

(i) Introduction

Various methods have been used to measure myometrial activity . *In vitro* uterine strips under varying hormonal influences have been studied (Csapo and Corner,1952; Csapo and Goodall,1954; Csapo,1956; Torok and Csapo,1976; Csapo,1977). *In vivo* methods include intra-luminal pressure measurements (Ruckebusch,1975; Porter *et al.*,1974; Lye,1980; Goddard *et al.*,1985), laparotomy (Croker and Shelton,1973; Lehrer and Schindler,1974; Hawk,1975;), intra-uterine endoscopy (Bourke and Lindsay,1988), transrectal ultrasound (Cross and Ginther,1987,1988), hysterography with contrast media (Fischel *et al.*,1978), recording from stretch (strain) gauge transducers placed circumferentially around the horn (Capraro *et al.*,1976,1977) and sutured to the uterus (Scheerboom *et al.*,1987), and electromyographic recordings (Naaktgeboren *et al.*,1973; Porter *et al.*,1974; Taverne *et al.*,1979;Scheerboom *et al.*,1987). A discussion of the two methods chosen for this study follows.

(ii) Electromyography

Myometrial cells exhibit a relative surface negativity when active. This can be recorded as a potential change of one region with respect to another (Kao,1967). Action potentials can be recorded extracellularly because the electrical activity is conducted by the surrounding tissues. The potential recorded is triphasic. The first downward deflection is due to a positive change in potential. When the action potential reaches the second electrode a rapid change from positive to negative occurs due to Na+ ion influx and an overshoot of the action potential occurs to reflect this rapid rise in intracellular potential. During repolarization there is an outflux of current as repolarization occurs causing a minor downward deflection (Ludin,1980).

The activity recorded is the combined activity of more than one cell (Kao,1967; Naaktgeboren *et al.*,1973). Inactive cells, depolarizing cells, and

repolarizing cells can summate and abolish each other's effects (Naaktgeboren *et al.*,1973). Only if neighbouring cells fire synchronously will summation occur and large spikes be recorded. Therefore the spike amplitude is an indication of the number of active cells. Rate of rise and duration of spike activity is determined by the frequency of action potential discharge (Kao,1967). A regular rhythmic pattern with high amplitude spikes of short duration is seen with good myometrial coordination (Kao,1967; Naaktgeboren *et al.*,1973). Asynchronous activity is marked by spikes of low amplitude and long duration with multiple peaks. Single spike patterns represent local contractions involving only a small group of cells (Naaktegboren *et al.*,1973). Since the electrical activity in the myometrium travels over various pathways at different velocities, these paths may crisscross and even travel in opposite directions. All this complicates the emg analysis, especially when the action potential discharge is repetitive (Melton and Saldivar,1965). Therefore many measurements must be averaged before reliable comparisons can be made. The change in pattern and amplitude will yield the most valid observations (Davis,1959).

(iii) Intra-luminal Uterine Pressure

Intra-luminal uterine pressure (IUP) can be measured through either an open or closed catheter system. The open catheter method allows for absolute pressure measurements but requires frequent flushing or a continuous fluid flow to prevent tip closure (Zerobin and Sporri,1972). If the catheter tip becomes embedded in the endometrial folds only local pressure changes might be transmitted (Finn and Porter,1975). In the closed system a fluid filled balloon is used. The catheter may be introduced through the cervix; however it is difficult to know its exact location and the violation of the vaginal vault and cervix may change intra-uterine resting pressure (Goddard *et al.*,1985). Balloons, sutured in place through the uterine wall, allow for more continuous recording and knowledge of location (Zerobin and Sporri,1972). If the transducer is not located at the exact height of the uterus true resting pressure will not be measured, therefore only changes in resting pressure are usually significant (Lye,1980). Respiration and animal movements will cause artifacts in the pressure tracings (Zerobin and Sporri,1972; Goddard *et al.*,1985).

Intra-uterine pressure records have been analyzed by a variety of techniques. The rate of rise in mm Hg/min helps to determine the contraction strength and coordination. A fast rate of rise signifies powerful coordinated contractions (Zerobin and Sporri,1972; Lye,1980). Amplitude, frequency and duration of pressure cycles have been evaluated and various indices have been developed to express them. The Montevideo unit is the contraction amplitude x frequency over a 10 min period (Zerobin and Sporri,1972). A uterine activity unit (UAU) measures the area under the contraction curve ie. duration x amplitude (Goddard *et al.*,1985). There are limitations to this type of motility index. For instance,the duration of the contraction may not provide meaningful information as, in hypotonic dystocias, the long low amplitude contractions are less effective than short ones (Zerobin and Sporri,1972). Whenever a single index is used it conceals changes in individual parameters that may be meaningful (Krishnamurti *et al.*,1982). Tonus, a continuous long lasting contraction with little oxygen consumption, can take two forms. Contractile tonus is an active basal tension maintained by continuous stimuli. This can be measured as an increase in basal resting pressure. Plastic tonus, however, cannot be determined by intra-uterine pressure changes as the uterine smooth muscle can undergo changes to attain a new basal length in response to stretch that is independent of continual stimulation (Zerobin and Sporri,1972).

There is concern by some researchers that the balloon itself might interfere with motility (Zerobin and Sporri,1972). Hawk (1970) concluded through his experiments that an intra-uterine device inserted into oestral ewes prevented fertilization by causing uterine contractions to move towards the cervix rather than towards the oviducts thereby inhibiting sperm transport into the horns and oviduct. Although fertilization may be prevented by an intra-uterine foreign body , contractions *per se* are not necessarily inhibited or provoked by the presence of a balloon. Lye (1980) noted that when ewes were ovariectomised and an intra-uterine balloon inserted the uterus became gradually quiescent over a two week period, suggesting that the device did not cause uterine stimulation; oestrogen treated spayed ewes, however, showed strong uterine contractions after injection of prostaglandin and oxytocin.

After completion of this work the uterine tissues were examined for chronic inflammatory changes, but none were found.

I.9 Uterine Motility In The Mare

(i) Introduction

Uterine motility has not been extensively studied in the mare. It appears that the first report was by Capraro *et al.*,(1976,1977) using a stretch (strain) gauge fastened circumferentially around one uterine horn. Since then, other researchers have recorded motility changes in the non pregnant mare using emg (Taverne *et al.*,1979), intra-uterine pressure recordings (Goddard *et al*,1985; Goddard and Allen,1985; Ley *et al.*, 1987; Sharpe *et al.*,1988; Ko *et al*,1989), and transrectal ultrasound (Leith and Ginther,1985; Cross and Ginther,1987). Pregnancy (Haluska,1985; Cross and Ginther 1988) and parturition (Haluska,1985) have also been monitored. A review of the results reported by the above research workers follows.

(ii) Ultrasound

Transrectal ultrasound scanning of the uterus is a non-invasive technique that has been used to monitor uterine activity. A 5 or 7.5 mHz linear scanner was placed longitudinally over the uterine body without directly manipulating it, and recordings taken for 1 minute periods at various defined intervals. Contractile activity was manifested as a flowing or streaming movement of the endometrium. A uterine activity score from on 0 - 4 was arbitrarily assigned, depending on the amount of motion observed (Cross and Ginther,1987). Unfortunately the authors did not combine this technique with any other method of motility measurement.

Seasonally anoestrous ponies were examined for motility by ultrasound. The previously described uterine activity score was used (Cross and Ginther,1987). Anovulatory mares had a score of less than 1. One group was then given 100mg of progesterone for a 20 day period. The motility score increased after 14 days to a maximum of 2.5. Another group of mares were given 1 mg oestradiol 17ß for 10 days then 100 mg progesterone for 10 days. The uterine activity peaked 4 days after oestradiol (tone score of 3) and remained elevated for the remainder of the trial. The authors measured activity for 1

min/day except on days 8,10,14 and 16 where for 2 hr periods scores were taken every 10 min for 1 min.

Another study was carried out on early pregnant mares and jennies (Cross and Ginther,1988). Uterine motility was measured for 1 min every other day. In the pregnant animals there was an increase in the uterine activity score 10 - 14 days post-ovulation. This corresponds with the stage of maximal vesicle mobility (Leith and Ginther,1984). In the pregnant mare uterine activity began to decline about day 16 to reach minimal levels by days 18-22 (Cross and Ginther,1988).

(iii) Intra-uterine Pressure

Goddard *et al.*(1985) used a solid state catheter tipped pressure transducer to measure absolute uterine pressure in a random group of mares. The overall mean absolute pressure in this group was 1.46mm Hg. No statistical difference was found between mares in anoestrus, dioestrus or oestrus, but in individual mares pressure values were higher in oestral (2mm Hg) than anoestral states (1.27mm Hg), and lowest in dioestral (1.09mm Hg) states. One mare in oestrus was noted to have frequent waves of spontaneous activity reaching 20mm Hg and lasting 2 minutes. Baseline pressure variation was large, -12mm Hg to + 20mm Hg, with larger mares tending to have negative IUP. They found an ever present baseline fluctuation of up to 3mm Hg possibly due to visceral activity and noted that respiration caused a rhythmic fluctuation with a mean change of 5.3mm Hg. Urination, snorting ,whinnying and postural changes caused dramatic increases in intra-uterine pressure (100mm Hg) of short duration (less than 30 seconds). Uterine activity units (UAU) were used as a measure of work done where 1 UAU was equal to an increase of 1mm Hg for 1 minute. The UAU method of measurement was chosen as the authors argued amplitude and frequency measurements were too heterogeneous and would cloud interpretation of results.

Similar results were found in IUP investigations of cyclic mares by Ko *et al.*(1989), also by measuring the total area under the contraction curve. They found that spontaneous uterine contractions were characterized by 0-3 strong contractions interspersed with smaller contractions over a 30 min period. The

mean IUP in the uterine body being 4.19 ±0.78mm Hg with a mean maximum pressure of 12.78 ±1.85mm Hg. No difference in activity was found between oestrus and dioestrus on the days IUP was measured.

(iv) Electromyography

Emg activity has been studied in three pony mares during spontaneous oestrous cycles and induced luteolysis (Taverne *et al.*, 1979). Analysis of the results concentrated on duration,frequency, and amplitude patterns during different stages of the cycle and as influenced by exogenous events. Three distinct patterns of emg activity could be recognized. During oestrus there were phases of high amplitude (150-600uv) densely grouped spikes(duration 2-8.5 minutes) alternating with periods (10-45 minutes) of complete inactivity. Activity tended to be synchronous between electrode sites. The dioestral pattern showed diffuse phases of low amplitude spikes (50-200uv) alternating with periods of relative inactivity, in which small bursts of spikes lasting 1-3 seconds, were prominent. Luteolysis showed a distinct third pattern. There were frequent phases of high amplitude (130-600) spikes of 1-4 minutes duration separated by equal periods of inactivity. These patterns could be modified by various stimuli. Entry to the mare's box, or even the sound of human voices had temporary but marked effects on emg activity. Rectal or vaginal examination sustained this increased emg activity but only for a few minutes after completion of the examination.

Emg studies on the pregnant mare's uterus (Haluska,1985) showed that during pregnancy the uterus becomes increasingly more quiescent. In mid-pregnancy the myometrium was electrically active 66% of the time but by late pregnancy this had decreased to 30% . Since plasma progesterone is at its lowest levels and plasma oestrogen at its highest levels during this quiet phase, Haluska suggests a factor other than progesterone is responsible, probably relaxin.

(v) Stretch (Strain) Gauge Technique

Capraro *et al.(1977)* using a stretch gauge consisting of a length of silicone rubber tubing filled with mercury contained at both ends by sealed electrodes monitored uterine motility in mares. Four ovariectomised mares were

surgically prepared and the uterus exposed. The stretch gauge was tunnelled through the myometrium of one uterine horn of each mare near the horn-body bifurcation, placed circumferentially around the horn then sutured to the uterine serosa. An increase in the length of the mercury filled tubing caused a change in resistance that was quantitated using a modified Wheatstone bridge circuit. To verify that only uterine contractile events were being recorded a laparoscope was inserted into the abdominal cavity of one mare. Oestradiol (2mg) was administered daily to each mare for 4 days. Recordings were characterised by occasional excursions of 2-4mv lasting 3-4min .

I.10 UTERINE STIMULANTS

(i) Oxytocin

Oxytocin is a peptide hormone synthesised in the supraoptic and paraventricular nuclei of the hypothalamus, stored in and released from the posterior pituitary (McDonald,1988; Soloff,1979). Endometrial oxytocin receptor levels are regulated by the steroid hormones and vary to be maximal at oestrus and parturition and minimal at mid-dioestrus (Soloff,1979; Wathes *et al.*, 1986). Uterine sensitivity to oxytocin, in terms of its contractile ability, depends on the number of receptor sites. Near parturition contractile filaments in uterine smooth muscle also increase, and this increase coupled with the receptor increase makes the organ capable of response to small quantities of oxytocin (Soloff,1985).

The exact mechanisms by which oxytocin has its uterokinetic effect, other than its receptor interaction, are unknown. Evidence suggests that extracellular calcium is important, as calcium channel blockers inhibit both spontaneous and oxytocin induced uterine contractions . Soloff (1985) has reviewed studies carried out in this area.

Oxytocin stimulates uterine motility, turning weak irregular movements into regular forceful contractions (McDonald,1988). The *in vitro* rabbit uterus is most responsive to oxytocin when oestrogenised, and non reactive under progesterone (Torok and Csapo,1976). The guinea pig uterus, however, also responds to oxytocin in the progestational state (Porter *et al.*, 1974). In the cow, Hays and Vandermark (1953) noted a similar response to oxytocin in

IUP recordings in oestral and dioestral animals, but virtually no response in the castrate. Burton *et al.*(1988) found oxytocin in the post-partum cow was highly effective in increasing uterine motility in the first 4-5 days, but by days 7-9 doses of 20 - 40 USP units were required for a response, and after day 9 there was little increase in motility. After intravenous injection there was a 30 second lag until a response was seen. At higher doses although there was a longer duration of action, at the 40 IU dose there was an initial myometrial spasm lasting 6-10 min.

Zerobin and Sporri (1972) also noted a dose response effect in the sow. High doses given near parturition caused spasm of the myometrium. More than 10 IU caused excessive IUP and suppressed phasic uterine activity.

The uterus of the ewe responds strongly to oxytocin when in oestrus (Soloff,1979; Lye,1980). Vaginal distention also greatly increases plasma oxytocin levels in the oestral ewe. This response is inhibited under progesterone, and vaginal distention during pregnancy actually decreased plasma oxytocin (Roberts and Share, 1968,1969; Roberts,1971). Genital stimulation is believed to change the electrical activity of the hypothalamus. Oestrogen and progesterone may modify this effect (Roberts and Share,1969). A central site of action has been postulated for oxytocin release and supported by lateral ventricle infusion of progesterone, which stopped release of oxytocin (Roberts,1971). Lightfoot (1970) measured IUP changes in ewes mounted by rams. Within 1 sec uterine tonus increased. He felt this was not due to oxytocin however, because of the short latent period and the fact the pattern was dissimilar to that found after oxytocin injection. It is possible that a local nerve reflex is responsible for some changes in uterine motility (Hays and Vandermark,1953).

Goddard and Allen (1985) using IUP recording noted that all mares responded to 2 IU oxytocin intravenously. There was a rapid rise in IUP by as much as 32mm Hg . The uterine activity score was greatest for the first 10 minute period and declined over the next 50 minutes. The response did not vary with reproductive status, although oestral mares tended to show decreased contractile work in the second and fifth 10 minute period. Ko *et*

al.(1989) using the same technique but a higher dose of oxytocin (40 IU) determined that uterine pressure elevations in mares occurred within 2-3 sec of intravenous administration and gradually decreased to pre-treatment levels within 30 min in both oestrus and dioestrus. Mean maximum cycle pressures of 31.85 ±5.15mm Hg were attained.

Ley *et al.*(1987) found a similar response to oxytocin using a balloon tipped catheter system in mares. After 6 IU of oxytocin given intravenously there was an immediate response lasting 10 - 15 minutes. They noted an initial peak contraction wave of sustained duration (3-11 minutes) followed by multiple short duration contractions of 1-3 minutes. The response to oxytocin was predictable.

Uterokinetic effects of continuous infusion of oxytocin (1 IU/min for 60 min) were also found in the mare (Sharpe *et al.*,1988) when monitored by an intra-uterine balloon. An IUP response was seen within 5 min and decreased toward the end of the infusion even though infusion rate remained constant. This suggests either uterine fatigue or desensitization as noted in the ewe (Flint and Sheldrick,1985).

The results of Capraro *et al.*(1977), using stretch gauges surgically fastened circumferentially on one uterine horn, support the uterokinetic response of the mare's uterus to oxytocin. After establishment of the baseline in spayed mares given exogenous oestradiol, 80 IU of oxytocin was administered; there was an immediate increase in baseline position to 14mv which persisted for 90 min and was followed by major excursions (6-8mv) of short duration (2-3 min) for the next 4hrs.

Besides the uterokinetic effect of oxýtocin, it also appears to stimulate uterine prostaglandin release. In the ewe, oxytocin, especially in the presence of oestrogen, stimulated the synthesis of prostaglandin, although the uterotonic and PG stimulating effects of oxytocin were independent of one another (Roberts and McCracken, 1976). The ***in*** *vitro* rat myometrium and endometrium increases synthesis of PG in response to oxytocin (Campos *et al.*,1988). Oestrogen, oxytocin and prostaglandins may all be important in

luteolysis (Roberts and McCracken,1976). It has been found that oxytocin treatment in early dioestrus markedly shortens the oestrous cycle in heifers (Anderson *et al.*,1965).

King and Evans (1984) first noted the influence of oxytocin on endometrial prostaglandin production *in vitro* during the oestrous cycle of the mare. Prostaglandin production was maximal when oxytocin was added to an endometrial sample obtained on day 14, around the time of luteolysis. When oxytocin binding sites were examined (Stull and Evans,1986) more were located in the myometrium than the endometrium at all stages of the cycle, but the concentration of bonding sites fluctuated throughout the oestrous cycle, being greatest during days 14-17 post-ovulation. The affinity for oxytocin was always higher in the endometrium, and this affinity did not change in either location throughout the oestrous cycle. The increase in oxytocin receptors corresponds with a rise in endometrial PG production and luteolysis (Douglas and Ginther,1972; Neely *et al.*,1979a) and a rise in plasma oestrogen concentrations (Noden *et al.*,1975). When oxytocin was given to mares early in dioestrus (days 4-9) luteolysis failed to occur (Neely *et al.*,1979b); however this may have been due to the inhibitory effects of progesterone on oxytocin binding sites (Stull and Evans,1986). In the ewe oxytocin receptor availability has been found to be regulated by oestrogen and progesterone levels. Progesterone inhibits the oestrogen induced oxytocin binding sites but appears to lose this inhibitory ability after 10 days (Roberts *et al.,1976).*

No investigations have been reported concerning luteal secretion of oxytocin in the mare. However, Burns *et al.*(1981) measured plasma oxytocin levels during different stages of the cycle in mares. Maximal concentrations were seen during oestrus and up to day 5 post-ovulation decreasing to a low level by day 10, slightly increasing about day 15. They conclude that ovarian steroid secretion may be involved in the oxytocin secretory mechanism. Tetzke *et al.*(1987) however, found a somewhat different pattern of plasma oxytocin levels, with significantly higher levels being found during the mid to late luteal phase (day 7- 15 post-ovulation) than during ovulation or early dioestrus. The frequency of sampling used enables rapid fluctuations in plasma

oxytocin concentrations to be observed; this suggested a pulsatile secretion which they argued might be indicative of a circadian rhythm.

In the ewe, cow and woman,oxytocin is produced in the corpus luteum where synthesis begins in the granulosa cells of the pre-ovulatory follicle and increases after luteinization (Wathes *et al.*, 1986). Basal concentrations of oxytocin were lowest during oestrus and the early luteal phase, but highest during the mid-luteal phase and luteolysis in normal cycling cows. After cloprostenol injection (a synthetic prostaglandin) maximal oxytocin response occurred in the mid-luteal phase. When superovulation was stimulated and the prostaglandin injection repeated in the mid-luteal phase there was a high correlation between the numbers of CLs and the increase in oxytocin (Schams *et al.*,1985).

There appears to be clear evidence for the ovarian secretion of oxytocin, in the cow and ewe, which varies with stage of cycle, and a possible reciprocal interaction between prostaglandin and oxytocin (Walters and Schallenberger, 1984; Schams *et al.*,1985).

(ii) Prostaglandin

Prostaglandins are derived from arachidonic acid and contain an inactive intermediate ringed compound called prostanoic acid. Variations in the prostanoic ring result in the 4 major prostaglandin groups: A,F,E,and B. The subscripts 1,2, and 3 reflect the fatty acid precursor involved (Rudd,1982). Prostaglandins are produced in all tissues and have a wide range of biological actions. The reproductive tract is mainly influenced by PGE and PGF. There is evidence for their role in ovum maturation, follicular rupture, luteolysis, stimulation of gonadotropin release, and uterine motility. These characteristics of prostaglandins, especially PGF2 , have led to many clinical applications, such as oestrus synchronisation, induction of abortion, induction of parturition, and timing of ovulation. The reader is referred to a review by Karim and Hillier (1979).

Prostaglandin is not stored in the endometrium, but rather synthesised in response to various stimuli. Therefore substrate availability is important

(Ramwell *et al.*,1977). In cattle there is an increase in endometrial arachidonic acid, a principal substrate for PGf2a metabolism under the influence of progesterone (Hansel *et al.*,1975). Several workers (Vernon *et al*,1981; Watson *et al.*,1988) have also noted a greater prostaglandin production capacity in the equine endometrium under progesterone domination, which increased with lengthening progesterone exposure (Vernon *et al.*,1981).

Oestrogen is believed to be an important quick acting stimulus which triggers prostaglandin production in the mare (Vernon *et al.*,1981; Watson *et al.*,1988). This is supported by observations in the cow where tamoxifen (trans-1-(p-ß-di-methylaminoethoxy-phenyl)-1,2-diphenylbut-1-ene), which blocks oestrogen synthesis, resulted in a failure of endogenous prostaglandin release during dioestrus and therefore a prolonged luteal phase (Jacobs *et al.*,1988). In the mare, King and Evans (1988) found higher serum oestrogen concentrations occurred immediately prior to luteolysis during the normal oestrous cycle, whereas in prolonged cycles, due to a retained CL, no rise in serum oestrogen occurred near the expected time of luteolysis. This suggests that oestrogens may play a key role in the initiation of luteolysis in the mare.

Endometrial prostaglandin was proposed as the luteolysin in mares by Ginther and First (1971) when they found that hysterectomised mares maintained a functional CL for at least 30 days post-ovulation. As this was not the case after unilateral hysterectomy of either the horn ipsilateral or contralateral to the existing CL, they proposed that the effect was not through a local utero-ovarian mechanism as in the sheep (Ginther,1981), but rather through systemic release. When prostaglandin is administered exogenously in mares either intra-uterine, intramuscular, or directly into the CL , the CL regresses and there is no significant difference in the interval to subsequent oestrus or ovulation (Douglas and Ginther,1975); this is further evidence supporting prostaglandin as the luteolysin in the equine.

The CL contains prostaglandin receptors in its cell membranes (Rao,1976; Karim and Hillier,1979; Nett and Niswender,1981; Wakeling and Greene,1981), and prostaglandin binding with these receptors accounts for its luteolytic

specificity. Luteolysis may be mediated through prostaglandin disruption of LH stimulation of adenylate cyclase causing an inhibition of luteal cyclic AMP and therefore progesterone synthesis. Inhibition of cyclic AMP also precedes the loss of cellular receptor binding sites for LH. Although there is no direct competition of prostaglandin for the LH receptor sites, the close relationship between the trophic effect of LH and the lytic effect of prostaglandin on the adenylate cyclase system may account for the resistance of the new CL to prostaglandin. Near ovulation the LH receptors are probably saturated preventing the association of prostaglandin with its receptors. With time there is a slow disassociation of LH, and prostaglandin receptors are unmasked . The possibility also exists that the altered sensitivity to prostaglandin may be due to altered prostaglandin receptors in the aging CL and not due to a prostaglandin-LH receptor interaction (Karim and Hillier, 1979; Wakeling and Greene,1981).

An alternative explanation for luteolysis is that prostaglandin dramatically decreases the blood flow to the CL, while increasing the total ovarian blood flow (Nett and Niswender,1981). The finding that PGF2 concentration in the utero - ovarian vein of pregnant and cycling sheep is highest on day 15, but PGE2 is only high on day 15 in pregnant ewes, further supports the vascular hypothesis, since PGE is felt to override the vasoconstrictive activity of PGF2 , and stimulate the synthesis of cyclic AMP and therefore of progesterone (Nett and Niswender,1981). However it is not possible to elucidate whether the decrease in luteal blood flow actually causes luteolysis or is a consequence of other processes involved in luteal regression (Knickerbocker *et al.*,1988).

In the cycling mare the life span of the CL is about 14 days with functional luteolysis, denoted by a decline in progesterone secretion, occurring over a 40hr period. Endometrial PGF2 release precedes this decline by about 3-5 hrs (Stabenfeldt *et al.*,1981). A decrease in luteal weight and peripheral plasma progesterone levels is concomitant with maximal PGF2 concentrations in the uterine vein (Douglas and Ginther,1976) and uterine lumen (Zavy *et al.*,1978). The PGF2 binding capacity of luteal membrane prostaglandin

receptors is also greatest at day 14 and least before day 3 (Vernon *et al.*,1979).

During early pregnancy, although there is also a high binding capacity of PGF2 to luteal prostaglandin receptors, prevention of luteolysis is believed to be due to failure of PGF2 to reach the CL, most likely because of embryonic influences on patterns of PGF2 synthesis and/or secretion (Douglas and Ginther,1976; Sharp *et al.*,1984).

Intra-uterine infusions of saline between days 5 and 9 of the luteal phase have been used since the 1930"s to induce luteolysis in mares (Ginther,1979). A decrease in plasma progesterone occurs within 24-48 hrs with a return to oestrus occurring on average at 4.4 days post treatment (Kenny *et al.*, 1975). Recent work has demonstrated that the pH of the saline solution might be the critical factor in causing luteolysis (Pascoe *et al.*,1989).

When the uterus has been under progesterone influence for several days significant amounts of PGF2 were found to be synthesised and released from the endometrium within 5 to 10 min of saline infusion (Stabenfeldt *et al.*,1984). Likewise an increase in peripheral PGFM, the major metabolite of PGF2 , was noted within 15 min of a 500ml saline infusion during the luteal phase. PGFM remained elevated for 3 hrs and an early return to oestrus occurred (Neely *et al.*,1979a). Betteridge *et al.* (1985) flushed 10-14 day dioestral mares with 200-1200 ml of physiological saline and measured peripheral plasma PGFM. They found that cervical dilation, transrectal uterine manipulation, and vaginal distention, as well as saline infusion, caused a significant rise in plasma PGFM, but only when it was carried out 5-6 days post-ovulation. These findings are in contrast to the studies of Wilde *et al.*(1989) who in a series of experiments found no elevation of PGFM or decrease in plasma progesterone after cervical dilatation in dioestral mares.

Betteridge *et al.*(1985) also noted an 8-22 fold increase in PGFM after intravenous administration of 20 IU oxytocin in intact dioestrus mares. This rise was not seen in ovariectomised mares unless they had been treated for a minimum of 7 days with exogenous progesterone and the rise in PGFM was

greatest after 12-13 days of progesterone treatment, supporting the findings of Vernon *et al.*(1981).

The uterokinetic effects of PGF2 are most likely regulated by its mobilization of extra and intracellular Ca++ ions (Rudd,1982) and changes in the resting membrane potential (Finn and Porter,1975). Torok and Csapo (1976) found that PGF2 decreased the threshold to contraction stimulation in the post-partum rabbit uterus *in vitro*, but up to 1000 times the dose would not alter the threshold in the pregnant uterus. The results in the ewe and woman are comparable. The uterus of the ewe, under the influence of oestrogen (but not progesterone) responds to PGF2 with an increased resting intra-uterine pressure and cycle frequency (Rexroad and Barb,1975; Carrick and Cupps, 1976; Roberts and McCracken,1976). In women, PGF2 increases the contractility of the fallopian tubes and uterus under endogenous oestrogen influence except during pregnancy (Bygdeman,1981).

Results in the post-partum cow are contradictory. Burton *et al.*(1988) found that oxytocin, but not PGF2 , with or without oestrogen administration, enhanced uterine motility. Thus PGF2 does not appear to be a uterotonic agent in the post-partum cow. The findings of Eiler *et al.* (1984), using intra-uterine balloons to measure pressure changes in post-partum cows, concurred with those of Burton *et al.*(1988) in that PGF2 did not significantly increase uterine contractions at the dose rate used (25mg PGF2). However, at lower doses (4-16 mg) there was a response that decreased with increasing repetitive doses (Eiler *et al.*,1981). They concluded that this was due to refractoriness to PGF2 . Kindahl (1984), in his investigations, found that post-partum cows with the longest endogenous PGF2 release patterns experienced rapid uterine involution, and when exogenous PGF2 was given days 3-13 post-partum, the cow involuted 10 days earlier than the expected average.

In the mare post-partum administration of prostaglandin also appears to assist uterine involution. A group of mares was injected twice a day with one half a luteolytic dose of the prostaglandin analogue prostalene, beginning on the day of foaling and continuing for 10 days or until breeding. Seventy six percent of treated mares conceived to the foal heat compared with 44% of

untreated controls. It was concluded that increased uterine motility led to quicker evacuation of uterine contents and therefore earlier uterine involution in the treated mares (Ley *et al.*,1987).

When myometrial emg's were recorded from the mare, an increase in activity was found during both natural luteolysis and also luteolysis induced by exogenous prostaglandins. Following injections of luteolytic doses of prostaglandin (250ug fluprostenol) all electrodes showed continuous electrical activity for 20 - 30 minutes without a change in amplitude. The continuous activity was gradually interrupted by increasing periods of inactivity. Thus prostaglandin was found to have a marked effect on the emg pattern in mares in dioestrus (Taverne *et al.*,1979).

Using ultrasound, Cross and Ginther (1987) noted that after an injection of 3mg PGF2 their progesterone treated anovulatory mares showed an increase in their uterine activity score within 20 min that lasted up to 2 hrs. This was not true of the untreated controls. In oestrogen treated mares there was no change in the already high level of uterine activity after prostaglandin administration. In the dioestral mare maximum motility of the uterus was observed between day 14-18, a period which corresponds with prostaglandin release and luteolysis (Ginther,1979).

Goddard and Allen (1985), using IUP recordings, measured the effect of the natural prostaglandin and a synthetic analogue, cloprostenol, given intramuscularly. The doses chosen were 50% of those required to effect luteolysis in pony mares. A clear response to the prostaglandins was not seen on every occasion, but this was unrelated to stage of cycle. The IUP took 10 min to respond to the prostaglandins with cloprostenol having the longest sustained duration of action; uterine activity was still at high levels (35-40mm Hg above baseline) at the end of the hour recording session. A gradual decline from a 50mm Hg rise at 20 min post-injection to 10mm Hg rise by one hour was observed with the natural prostaglandin. Similar inconsistent responses to PG were noted by Ley *et al.*(1987) using a similar technique in the mare. Sharpe *et al.* (1988) studied the uterokinetic effect of prostaglandin using a different methodology. After insertion of intra-uterine

balloons, PGF2 was infused intravenously over one hour (200ug/hr). No uterokinetic effect was noted. Intra-uterine infusion of PGF2 also had no effect. Plasma progesterone levels indicated that most mares were not in a luteal phase during the experiments. These authors conclude that prostaglandins are not uterokinetic in the mare.

The work of Capraro *et al.* (1976), however, using the previously discussed stretch gauge technique, concluded that PGF2 is uterokinetic in the mare.

(iii) Stretch

Uterine distension results in stretch only when the elastic capacity of the wall is exceeded so that a reactive tension is developed. This tension results from the tendency of the connective tissue elements to oppose deformation (Currie,1979). Marshall (1962) found that quiescent strips of rat uterus exposed to oestrogen responded to stretch by an increase in action potential discharge. This did not occur if the uterus was under progesterone influence. Stretch also caused a significant increase in uterine PGF levels in both the pregnant and post-partum uterus of rabbits (Csapo,1977), but the IUP changes only occurred in the post-partum animals. Currie (1979) confirmed this *in vitro* where strips from a pregnant uterus, under high progesterone influence, could be stretched more than 50% with little increase in tension whereas stretched post-parturient strips exhibited extremely high contractile activity. Because of its distensibility or plasticity the progesterone treated uterus is protected from the stimulatory consequences of extensive deformation. As progesterone levels decrease, so does uterine non-reactivity to stretch (Currie,1979).

I.11 Uterine Relaxants

(i) Clenbuterol

Unlike the ecbolics which have both an endogenous physiologic and exogenous pharmacologic role, the tocolytics are pharmacological substances. Clenbuterol is a potent , quick acting tocolytic of long duration, that stimulates the adrenergic beta 2 receptor system of the myometrium. It increases cellular adenyl cyclase leading to an increase in cyclic AMP which results in muscle relaxation, apparently due to an efflux of intracellular Ca++

(Putnam *et al.*,1985). Zerobin and Kundig (1980), using intra-luminal pressure transducers and emg recordings, monitored uterine motility in several species after clenbuterol administration during both parturition and the puerperal phase, as well as in ewes under the influence of oestrogen. Pressure recording showed a cessation of contractions, lasting up to 3 hrs. Emg activity was less affected, but conduction pathways were possibly disrupted. Oxytocin, which competes for control of the Ca++ ions, can prevent the effects of clenbuterol if present at the time it is administered, and can override its effects if given when clenbuterol concentrations are beginning to wane (Kern and Schill,1983). In the cow, if part of the foetus is through the cervix, the effect of clenbuterol is not as long lasting, while in women, clenbuterol will partially or totally counteract the effect of prostaglandin given to cause labour or abortion (Kern and Schill,1983).

Al-Eknah and Noakes (1988) conducted a trial with clenbuterol in ovariectomised sheep that were exhibiting spontaneous uterine activity as measured by intra-luminal balloons. After injection there was an immediate cessation of uterine activity for at least 1 hr. Recovery was followed by a second period of depressed motility occurring about 4 hrs after the initial injection,and lasting 6-7 hrs.

Clenbuterol has clinical applications in postponing labour in cows (Greene,1981; Putnam *et al.*,1985), and relaxing the myometrium during labour (Hassett and Sloss,1984).

In the mare the effect of clenbuterol on uterine motility has been studied during early pregnancy (Leith and Ginther,1985). On day 12 or 13 post-ovulation mares were given 0.8ug/kg clenbuterol intravenously and vesicle mobility was monitored using ultrasound techniques. Examinations were made every 5 min for 1 min over a 2 hr period. Location changes occurred less frequently in treated mares and the embryonic vesicle tended to stay in one location longer than in controls. The authors suggest that the results of this trial indicate the involvement of uterine contractions in embryo mobility, as clenbuterol blocks uterine contractions (Greene,1981).

(ii) Propantheline Bromide

Propantheline bromide, a quaternary ammonium alkaloid ester, is an anticholinergic, parasympatholytic agent with actions similar to but less specific than atropine (Katzung,1982). The blockade of cholinergic receptors in the alimentary tract after intravenous propantheline administration has a dramatic and immediate effect on gut motility; it causes peristaltic inhibition due to relaxation of the musculature of the viscera and results in a decrease in tone and propulsive movements for up to 2 hrs (Merkt *et al.*,1979; Katzung,1982). The effect can be overridden to some extent by local hormones . There is no known significant effect of propantheline bromide on the uterus (Katzung,1982). The drug is used in equine reproduction to relax the rectum, allowing thorough palpation of the reproductive tract to take place.

II MATERIALS AND METHODS

II.1 ANIMALS AND HOUSING

All experimentation took place from March to November, 1988. The experimental procedures were approved by the Massey University Animal Ethics Committee. The following mares were chosen from the Massey University teaching herd as experimental subjects:

i) FLING - 3 year old intact maiden Standardbred, recorded for 5 months.

ii) SWEETIE - 7 year old intact Thoroughbred, parity status unknown,recorded for 3 months.

iii) JO - 10 year old Thoroughbred, aborted between 45- 60 days gestation, (approximately 1 1/2-2 months before being ovariectomised for the experiment), recorded for 1 month.

iv) SNOWY - 16 year old pony mare, ovariectomised at least 5 years before the experiment,parity status unknown, recorded for 3 months.

All mares had normal reproductive tracts as determined by rectal, ultrasound and vaginoscopic examination. The intact mares had normal cyclical activity during the previous breeding season and normal uterine biopsy results.

Mares were allowed to acclimatise to a straw bedded loose box in an isolated hospital wing, and were allowed to become accustomed to wearing a cover prior to surgery. An isolated environment was felt to be necessary since previous work (Taverne *et al.*,1979) had shown that external factors could influence emg activity. A mixture of corn and maize was fed at 7 a.m.and 4 p.m.. Lucerne hay was provided at each feeding, as well as during recording sessions. Water and salt blocks were freely available. Mares, wearing covers, were allowed access to small grass paddocks or concrete yards for exercise at least every other day. The two intact mares were kept under a lighting pattern of 16 hours light and 8 hours dark to promote cyclical activity. Artificial light was provided in each loose box by a 200 watt incandescent bulb controlled by an automatic timer which turned light on at 0500 hours and off at 2100 hours. Fling was started on the lighting

regime mid June. In mid July she was put on a course of synthetic gonadotrophin releasing hormone (Buserelin-Receptal, Veterinary Ethics,N.Z.) .012mg twice a day for 3 days followed by .012 mg once a day for 2 days at which time follicle development occurred and the mare came into oestrus. Fling remained under lights until the end of her experimental period. Sweetie was in seasonal transition when the surgery was performed in late August and was kept under lights until October.

II.2 ELECTRODE PREPARATION

Electrodes consisting of teflon coated multistranded stainless steel wire were used (Cooner Wire Co. #AS633 Chatsworth Ca.). The wire was cut into 2 meter lengths. One end was then threaded into a 23g 25mm needle from which the hub had been removed. The blunt needle end was then crimped to secure the wire. Approximately 150 mm from the needle hub an area of teflon coating was removed over about 2mm to expose the stainless steel surface. A knot was placed 5 mm distal to the exposed section of wire to help locate and secure the electrode when it was placed in the myometrium. For the first two mares (Snowy and Fling) a section of teflon about 2 mm long was removed before attachment to the myometrium. Due to the poor quality recordings, that could not be remedied by electronic filtering or shielding of the recording equipment, for the second two mares (Jo and Sweetie) an increased amount of electrode surface was exposed (4mm).

II.3 SURGERY

Each mare was sedated with .05mg/kg acetylpromazine maleate (ACP, Techvet Ltd. Edmunds UK). Induction was performed with 10% glycerol guiacolate (8g/100kg) (Giafen, Phoenix Pharmaceutical Distributers Inc.N.Z.) and 10% thiopentone sodium (2g) (Intraval sodium, Pitman-Moore,N.Z.). An endotracheal tube was inserted and anaesthesia was maintained on halothane (Fluothane,I.C.I., U.K.) and oxygen during the surgery.

The uterine horns and body were exposed by a mid ventral laparotomy incision. The prepared electrode wires were inserted to a depth of 5mm from the perimetrium as close as possible to the middle of each uterine horn and to the middle of the uterine body and tied in place. Three wires were inserted per site,

5mm apart in a triangular pattern to provide bipolar recording plus an earth. The ends of each set of wires were coded with knots according to site location,then exteriorized from the abdomen and tracked subcutaneously to the flank anterior to the point of the hip. Prior to inserting the electrodes the ovaries of the mare Jo were located, the pedicles ligated and the ovaries removed.

Each mare was given 1,500IU tetanus antitoxin (Glaxo Animal Health, N.Z.) and trimethoprim-sulfa (40mg trimethoprim/15kg) (Amphoprim, Virbac Laboratories,Fr.) once daily for 5 days post operatively. After recovery from anaesthesia, mares were covered and allowed to recuperate for 3-5 days. No post operative complications were encountered, other than a low grade local infection at the site of wire emergence in the flank which was treated with a 10% povidine iodine wash and spray (Biocil,Ethical Agents Ltd.,N.Z.). Electrode wires were then soldered to a 25 pin D type connector (E.C.S. Division of Air Spares N.Z. Ltd). The connector was taped to the skin of the mare. Connections were periodically checked and resoldered if loose.

II.4 RECORDING OF EMG

Mares were placed in a tie stall for recording sessions. The length of the sessions varied from 3 to 8 hrs. Each mare was recorded on a daily basis whenever possible. After removal of the mare's cover, standard 2 core screened wires, connected by banana plugs to the A.C. amplifiers (Type 122, Tetronix Inc.,USA) were attached to the D type connector on the mare. The low frequency filter response was set at 8 hz and the high frequency filter response at 50 hz. The coupling time constant was .02 sec. and the approximate voltage gain was 1000. A Gould 2400 pen recorder (Gould Inc., Ohio, USA) was used to transcribe the signals and collect the data. A chart speed of 3 - 100 mm/min. was used as circumstances dictated.

II.5 INTRA-UTERINE PRESSURE RECORDINGS

Pressure recordings were made using saline filled open tipped catheters. These were made from medical grade single lumen polyethelene tubing 1.5mm I.D. and 2.5mm O.D. (Dural Plastics and Engineering, N.S.W. Australia). Tubing was cut into 1.5 meter lengths and the intrauterine end fitted with an external polyethelene coil to aid intrauterine retention. The vulva and perineal region of

the mare was thoroughly cleaned with warm water and 2% povidine iodine scrub, then dried. The tubing was removed from cetavlon disinfectant (Savlon, ICI, N.Z.), rinsed with distilled water, inserted per vaginum through the cervix and taped externally to the buttocks to support the catheter and aid its retention in the uterus. The external end was then threaded over a blunted 14 gauge needle which was attached to a pressure transducer (Physiological Pressure Transducer 4-327-L221, Bell and Howell, Pasedena Ca.) connected to a Neotrace blood pressure D.C. amplifier (Neomedix Systems, Australia), calibrated at 50mm Hg full scale. This was used in conjunction with the amplifier and recorder as for the emg measurements. The transducer was placed at the approximate height of the uterus. The length of tubing from the mare to the transducer was allowed a degree of slack to allow for possible movement of the mare. The transducer and tubing were filled with sterile physiological saline. Prior to each recording session the amplifier was calibrated to atmospheric pressure. During recording sessions the line was periodically flushed with saline to ensure patency and the transducer recalibrated.

II.6 CYCLICAL ACTIVITY OF THE MARE

The cyclical activity of the mares was divided into four categories.

i) Anoestrus

The two ovariectomised mares, Jo and Snowy, were considered anoestrus when not under the influence of exogenous hormones. Fling was considered anoestrus during the time when plasma progesterone (P4) levels were < 1ng/ml and there was no evidence of follicular activity upon palpation and ultrasound of the ovaries. Sweetie was in seasonal transition when surgery was performed and therefore not classified as anoestrus.

ii) Transition

The two cycling mares, Fling and Sweetie, were considered transitional when plasma P4 values were < 1ng/ml, follicular activity was present on the ovaries as determined by ultrasound and palpation, and ovulation did not occur.

iii) Oestrus

In the cycling mares (Fling and Sweetie) oestrus was defined as the presence of one or more ovarian follicles greater than 35mm diameter plus overt signs of oestrus. In the ovariectomised mares (Jo and Snowy) oestrus was defined as beginning 24 hrs after an intramuscular injection of oestradiol benzoate (Intravet, Australia) at which time mares were exhibiting signs of oestrus. They were considered to be in oestrus for the duration of the oestradiol treatment, about 10 days. Three different doses of oestradiol benzoate were administered. Snowy and Fling (in anoestrus) were given 1mg/day for 4 days, followed by 2.5 mg/day for 5 days and 10 mg/day for 10 days. Josephine was injected with 2.5 mg/day for 12 days and then on a separate occasion given 10mg/day for 7 days. Each dose was given intramuscularly at 9 a.m. Oestradiol treatment always preceded progesterone treatment.

iv) Dioestrus

Fling and Sweetie were considered to be in dioestrus when P4 levels were > 2ng/ml. Jo, Snowy and Fling (when in anoestrus) were given 150 mg exogenous intramuscular progesterone in oil (Progestin, Chemavet,N.Z.) once a day for 10 days . They were considered to be in dioestrus 24 hrs post injection when P4 levels were > 2ng/ml. Dioestrus was further divided into early dioestrus (P4 1-5ng/ml) and late dioestrus (P4 > 5ng/ml).

II.7 DRUGS (OTHER THAN STEROID HORMONES) GIVEN

After normal emg and IUP patterns were established for each stage of the oestrous cycle, drugs were administered. As more than one drug was often given on the same day, time was allowed between treatments for the emg and IUP activity to return to pretreatment patterns. Each drug was given to each mare a minimum of three times per cycle stage.

Cloprostenol

One quarter the luteolytic dose of cloprostenol, 125 mcg, (Estrumate, Cooper's Animal Health N.Z.) was administered intramuscularly.

Oxytocin

Oxytocin (Oxytocin-EA, Ethical Agents Ltd. N.Z.) was administered intravenously. The "optimal" dose was determined to be 5 I U. At this dose mares exhibited no signs of discomfort and the emg response was consistent. The 5 I U dose was used exclusively on Sweetie and Jo. Snowy and Fling, the first two mares recorded, were between them given a range of doses (2 I U, 5 I U , 20 I U and 50 I.U.).

Clenbuterol

Clenbuterol (Planipart, Boehringer Ingleheim Ltd. N.Z.) is not marketed for obstetrical use in mares and the appropriate dose for mares was not given by the manufacturer. The cattle dose, .3mg, was administered intravenously.

Propantheline bromide

Propantheline bromide (Propan B, RWR Veterinary Products PTY Ltd., Australia) was given intravenously at the 100 mg dose level as recommended for rectal examination.

II.8 OTHER MANIPULATIVE PROCEDURES

Examination per rectum

To minimise disturbance of electrodes, rectal examination was performed with as little manipulation of the uterus as possible. Faeces were only back raked if necessary. The examiners hand was inserted, rested on the cervix and then moved forward over the uterine body where gentle massage of the body and horn body junctions was carried out for approximately one minute.

Uterine distention

The perineal region of the mare was washed as described under IUP catheter placement. A sterile 30 g balloon tipped catheter was introduced through the cervix and the balloon distended with 50 ml of air. One litre of sterile double distilled water (37°C) was infused through the catheter. After infusion was complete the fluid was allowed to remain in the uterus for 2 min and then allowed to drain. Rectal manipulation of the uterus was sometimes needed to ensure complete evacuation. The procedure was then immediately repeated with

a second litre of water. On 6 occasions, smaller amounts (60 and 180ml) of water were infused and not evacuated.

Breeding

Natural breeding was monitored on Sweetie on 4 occasions. During three of these services the mare and recording equipment were moved to a nearby yard and the mare remained connected to the recorder during and for 15 min after mating. She was then disconnected from the equipment, returned to the recording stall and reconnected within 3 minutes. For one breeding the mare was disconnected from the recording equipment, bred, and reconnected within 2 min of service.

II.9 ELECTROMYOGRAPHIC ANALYSIS

For the purpose of analysis emg records were divided into periods of relative inactivity, activity, and single spiking activity. The results obtained from all three electrode sites were combined to yield a mean result for uterine electrical response per hour. Variation between individual electrode sites was also examined. Due to temporary or permanent dysfunction of individual wires, not all electrode site locations were always functional.

Relative inactivity defines electrically quiet periods. These are often interrupted by small bursts (lasting 4-12 sec) of action potentials and having an amplitude range between 50 - 200μv . When they occur there are usually 2-4 such bursts per min. Periods of relative inactivity were analyzed for their average duration in min. between active phases per one hour period in each stage of the cycle. No attempt was made to quantify the bursts (Fig.1)

Active periods are defined as those characterised by close groupings of small spike bursts which are separated by less than 10 sec and last for at least one minute with an amplitude of greater than 50 μv (Fig.1). Activity periods were analysed for the mean percentage of time there was emg activity per hour (emg A/hr),the mean amplitude of the emg activity per hour, and the average duration in min of active periods within an hour. All parameters described above (emg A/hr, mean emg amplitude/hr, average duration of activity periods/hr), as well as

the average duration in min of periods of relative inactivity/hr were subjected to statistical analysis.

The numbers of action potential bursts within an active period were divided into low density (6-10 bursts/min), medium density (10-20 bursts per min) and high density (over 20 bursts/min). At slower chart recording speeds individual bursts could not always be identified, especially at the high density level, so visual assessment was substituted. Density was not examined statistically. Density changes of significance are noted in the results.

Single spiking activity consisted of single action potentials that were not organized in a burst pattern. Characteristic qualities of single spikes were their consistency in spacing and amplitude (Fig.2). No attempt at statistical analysis of this activity was attempted due to its infrequent appearance.

II.10 INTRA-UTERINE PRESSURE ANALYSIS

Before the introduction of the intrauterine catheter for measurement of the IUP, a period without uterine interference was allotted to establish the resting emg pattern for that day. No subsequent change in the emg activity was noted after catheter placement. For each recording session of IUP the baseline pressure for that session was established. Records were then divided into 10 min segments. Three IUP indices were evaluated for each segment. Cycle frequency is the number of pressure waves generated above baseline for 10 min. Mean maximum cycle amplitude is the maximum amplitude of each pressure wave divided by the number of waves per 10 min. Cycle duration is the total time in minutes that the amplitude was >5mm above baseline pressure during a 10 min period.

II.11 BLOOD SAMPLE COLLECTION AND PROGESTERONE ASSAY

Blood plasma samples in EDTA vacutainer tubes were obtained by jugular venipuncture. All samples were immediately centrifuged, the plasma separated and stored at -18°C until analysis. For all mares blood samples were drawn at the initial recording session. For the progesterone treated mares samples were taken during days 2 and 8 of progesterone therapy and 48 hrs after the last progesterone injection. In the intact mares plasma samples were randomly taken to monitor anoestrus and /or seasonal transition. Samples were also taken post-

ovulation to monitor progesterone levels, three times over 24hrs (0900, 01400, 0800hr) to monitor natural luteolysis, and after prostaglandin induced luteolysis.

Progesterone determinations were made on 500 μl of obtained plasma. Samples were extracted with 5ml toluene:hexane (1:2 v/v). The plasma was frozen overnight and solvent was then decanted into clean tubes, dried under air and redissolved in 500 μl ethanol. Duplicate 100 μl samples of ethanol extract were dispensed into plastic tubes and dried under air, as were duplicate 100 μl samples of standard ethanolic solutions of progesterone (P-1030, Sigma Chemical Co., St.Louis Missouri,U.S.A.) with concentrations corresponding to plasma progesterone levels of 0.625-40 ng/ml. A mixture containing antiserum (courtesy of Dr. J.T. France) at a final dilution of 1:18,000 (Tungsubutra and France,1978); (1,2,6,7-3H8) progesterone (TRK 413,Amersham,Bucks,U.K.) at 20,000 c.p.m./100μl; phosphate-buffered saline containing 0.02 M-EDTA and 0.1% gelatin (PBS-EG) in the ratio of 1:1.4 (by vol.) was added (600 μl) to each tube and vortexed. After overnight incubation at 4 ^{o}C, 600 μl of 2.5% (W/V) charcoal (Norit A, A.H. Thomas Co.,Philadelphia,U.S.A.) suspension in PBS-EG were added to the tubes, vortexed and then incubated at 4°C for 10 min. Tubes were then centrifuged at 3000g for 10 min at 4°C. The supernatant was decanted into scintillation vials and 6 ml toluene-triton scintillation fluid added before counting for 2 min in a Beckman LS 7500 scintillation counter. Assay sensitivity was 0.10 ng/ml. Intra-assay coefficients of variation (CV's) were 8.4 and 21.0% (n=3), and inter-assay CV's were 3.3 and 19.3% (n=2) for plasma pools containing mean progesterone concentrations of 7.40 and 0.41 ng/ml respectively.

II.12 STATISTICAL ANALYSIS

Statistical probabilities for differences between experimental groups were tested by analysis of variance. Correlation coefficients were used to measure the association between continuous variables. The computer program used for data base management and statistical analysis was PANACEA (Pan Livestock Services,1987). Unless stated otherwise, values in Tables are presented as means ± the standard deviation (SD) and the number (n) of determinations used for each mean is given in parentheses. Alphabetic superscripts are used to denote statistically significant differences ($p<0.05$) between data with the same letter

designation. Levels of significance (P values) are given in the results section of the text.

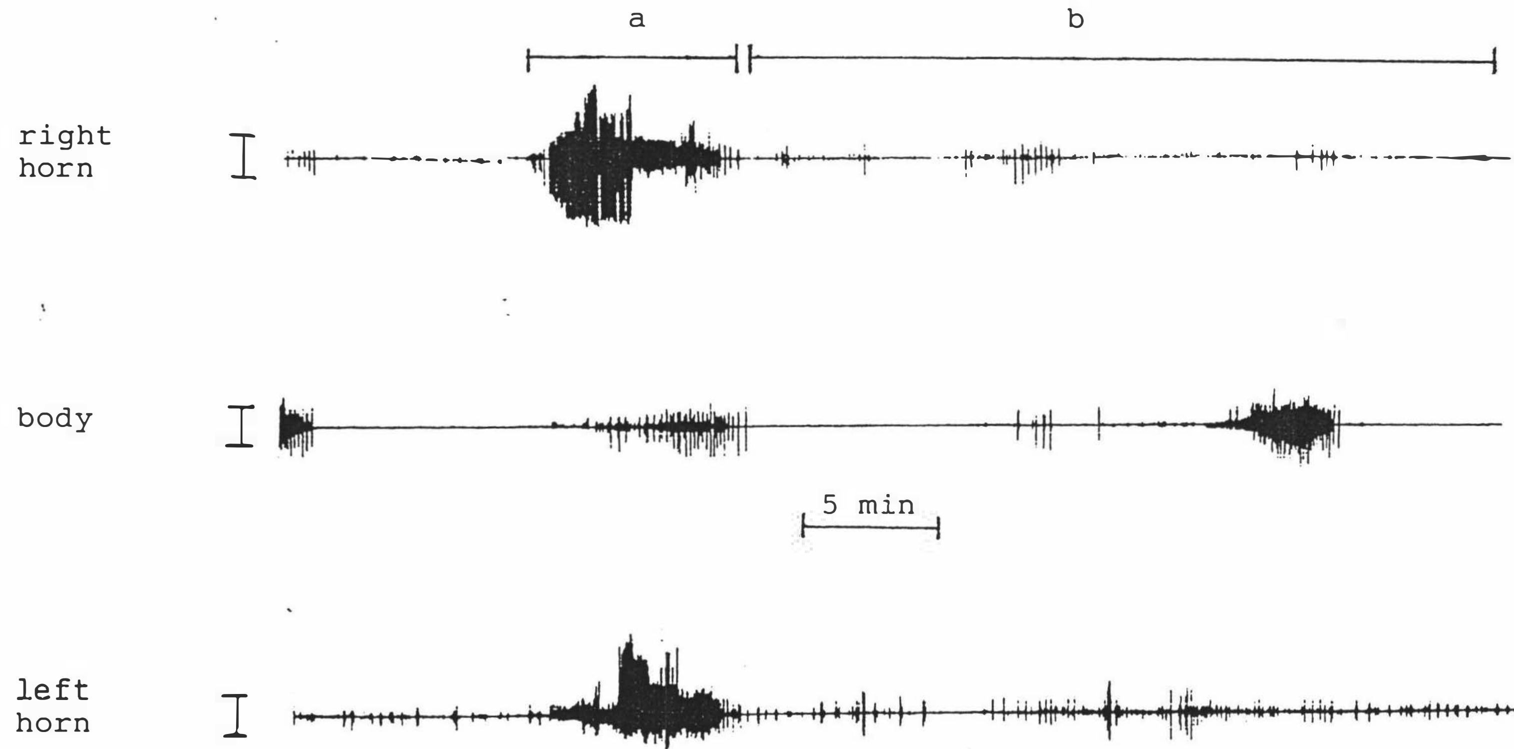


Fig. 1: Electrical activity of the myometrium depicting areas of emg activity (a) and relative inactivity (b). The vertical bars on the left represent a calibration of 200μv.

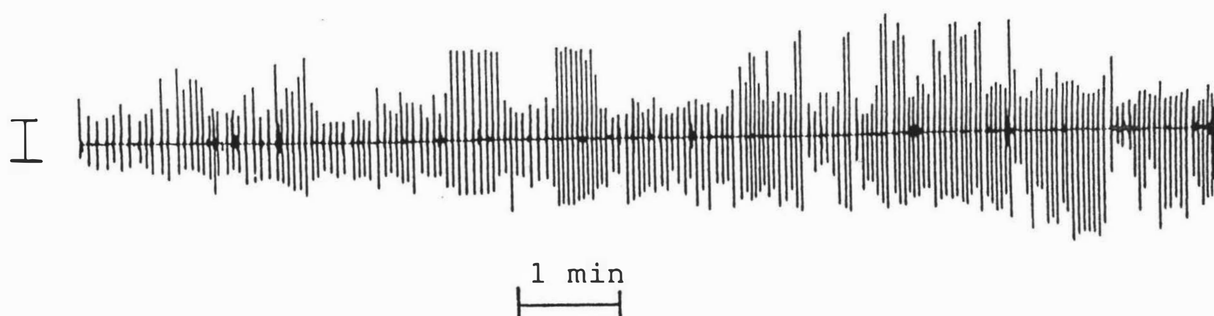


Fig. 2: Single spike activity recorded in the left uterine horn after intra-uterine infusion of a litre of sterile distilled water. Note the frequent consistency in spike spacing. The vertical bar on the left represents a calibration of 200µv.

III. RESULTS

III.1 INTACT MARES

III.1.A: Sweetie

(i) Normal Oestrous Cycle Activity (no exogenous drug influence)

Background emg activity consisting of 2-3 bursts/min of 50-200μv lasting 2-12 sec was frequently present during periods of relative inactivity with this mare.

A significant difference in emg A/hr was found between all cycle stages (transition, oestrus, dioestrus) being lowest in oestrus, increasing in transition and highest during dioestrus ($p<0.001$-Table I). With Sweetie the dioestrus emg A/hr also varied with progesterone levels, being lower when the progesterone levels were 1- 5 ng/ml and higher when progesterone levels were above 5 ng/ml ($p<0.001$-Table II).

The average length of an active period within the hour also varied in a similar manner, being least in oestrus, increased in transition and longest during dioestrus ($p<0.001$-Table I). Furthermore, in dioestrus when progesterone levels were above 5 ng/ml (late dioestrus) the average time active was significantly longer than when progesterone levels were 1- 5ng/ml ($p<0.001$-Table II).

Emg amplitude measurements followed the opposite pattern being highest at oestrus, decreasing in transition and lowest during dioestrus ($p<0.001$-Table I). Likewise when progesterone values were greater than 5ng/ml the amplitude was significantly less than the amplitude when progesterone levels were 1-5ng/ml ($p<0.01$-Table II).

During periods of activity throughout oestrus the emg record showed a crescendo-decrescendo appearance with amplitude ranging from 50 to 700μv and these active periods tended to be synchronous between electrode sites (Fig.3). Background emg was reduced or absent during the quiet periods at this stage of the cycle. During both dioestrus (Fig.4a) and transition (Fig.5)

the patterns of emg activity were similar in that the length of active periods was increased (especially in dioestrus), and there was no consistent amplitude variation . The range in amplitude was from 50 - 350μv with occasional action potentials reaching higher values (700μv). During dioestrus periods of activity, a superimposed crescendo-decrescendo pattern, similar to that found in oestrus, but without periods of relative quiet, was sometimes seen (Fig.4b).

The emg A/hr between electrode sites was analyzed and variations were found which differed with cyclic status. In dioestrus the right horn showed significantly greater emg A/hr than either the left horn or body, and the duration of a period of relative inactivity was also significantly less in the right horn ($p<0.05$-Appendix 1A). During oestrus and transition, the right horn recorded the highest emg A/hr, but not significantly higher than for the other sites; time active however, was significantly longer in the right horn during oestrus ($p<0.05$-Appendix 1A). The emg amplitude also varied between electrode sites during the different cycle stages with the emg amplitude of the right horn always being greater than for the other sites. This difference however were significant only during dioestrus and oestrus ($p<0.05$-Appendix 1A).

(ii) Pregnancy

There was a positive correlation between emg A/hr and time within the early stages of pregnancy (days 2-6, days 7-15, days 16-20) ($r=0.32, p<0.01$) and a negative correlation between amplitude and time within the same stages of pregnancy ($r= -0.54$, $p<0.001$)(Fig.6).

There were significant differences between the emg A/hr in late dioestrus and all the stages of pregnancy recorded with greatest activity between days 16-20 of pregnancy (after the location of the embryo has been fixed) and least during days 7-15 (the probable period of embryo motility) (values range between $p<0.05$ and $p<0.01$ -Table III). Although there was no significant difference in emg amplitude during the 2-6 day period compared to that observed during late dioestrus, the emg amplitude in the 7-15 day period was reduced ($p<0.01$) and in the 16-20 day period least ($p<0.001$- Table III) (Fig.6).

Emg variation between the electrode sites was then evaluated and the left horn (where the embryonic vesicle was located), was observed to have significantly less emg A/hr throughout the first 20 days of pregnancy than either the right horn or the uterine body ($p<.05$-Appendix 2A). However, when each stage of pregnancy was examined individually (days 2-6,days 7-15, days 16-20; Appendix 3A), a significant reduction in emg A/hr between the left horn and the body only occurred between the 7 - 15 day period ($p<0.001$) and the 16 to 20 day period ($p<0.05$-Fig.7 and 8). However records from all sites could not be compared at all stages since the right horn electrode did not record effectively after the first 6 days of pregnancy. During The first 6 days neither the right and left horn nor the left horn and body were significantly different in emg A/hr, although the right horn emg A/hr was greater than that of the body ($p<0.05$-Appendix 3A).

Throughout the first 6 days of pregnancy the amplitude recorded from electrodes in the right horn was higher than that of sites recorded in the left horn or body ($p<0.05$); the left horn and body recordings did not differ from each other either during this time period or from 7-15 days of pregnancy. However through the 16-20 day period of pregnancy the left horn site did show lower amplitude than the body ($p<0.001$-Appendix 3A) (Fig.8).

(iii) Drug Treatments

Uterine stimulants and relaxants were administered to Sweetie in oestrus, transition and in late dioestrus (i.e. when the progesterone levels were $>$ 5 ng/ml).

(a) **Prostaglandin**: Prostaglandin affected emg activity within 5-8 min of administration and a systemic reaction including sweating, abdominal discomfort and increased bowel movements frequently occurred.

Prostaglandin treatment, when compared to no treatment, resulted in a significant rise in emg A/hr at all the stages of the oestrous cycle

($p<0.001$-Table IV). Emg amplitude also increased, but significantly so only during oestrus ($P<0.05$-Table IV).

After prostaglandin administration there was a consistent pattern of emg response - at all stages of the cycle there was an initial prolonged burst of emg activity (10-25min), followed by synchronous bursts of shorter duration (3-8 min), separated by relative inactive periods of progressively increasing length (i.e. initially 1-4 min increasing to 2-10 min over the measured period). This pattern was most pronounced during oestrus and transition; throughout dioestrus the periods of activity and relative inactivity were less well defined and in oestrus the crescendo-decrescendo amplitude pattern was less pronounced (Figs.9a,9b,10a,and10b).

The variation in emg A/hr between electrode sites was not significant, but the left horn during transition had greater amplitude than either the right horn or the body ($p<.01$-Appendix 4A).

The effects of a single prostaglandin injection were followed for up to three hours in this mare (Table V-Fig.9b). Emg A/hr was observed to decline with time at all three stages but this was only significant during transition ($p<0.05$-Table IV). Emg A/hr at the end of the third hour was still greater than that seen before the drug was given ($p<0.05$-Tables IV and V). No significant changes in amplitude were noted at any cycle stage throughout the treatment period.

(b) Oxytocin: Treatment with 5 IU of intravenous oxytocin affected the emg pattern within 20 -50 seconds (Fig.11) and in all cycle stages there was a significant incréase in emg A/hr following the injection ($p<0.01$-Table VI). The emg A/hr parameter was then tested over each 10 min interval within the hour after treatment to determine whether there was any change in response over time. When data from all cycle stages were combined, there was a negative correlation between response (as measured by emg A/hr) and time ($r=-0.64$, $p<0.001$); analyzing the cycle stages individually the lowest negative correlation

was found during dioestrus ($r=-0.33$, $p>0.1$), and the highest during both oestrus ($r=-0.94$, $p<0.001$) and transition ($r=-0.81$, $p<0.001$). These responses can be seen in Appendix 1B and Fig.12.

A pattern of emg response similar to that seen with prostaglandin occurred during oestrus and transition. The emg activity tended to be continuous for the first 10-20 min post injection, followed by short (2-5 min), usually synchronous, activity bursts, separated by equally short periods of relative inactivity (Fig.11). During dioestrus the burst activity was more continuous and periods of activity and inactivity less well defined.

Within 40 minutes after oxytocin administration, during both oestrus and transition, the emg A/hr had returned to levels seen before treatment, whereas during dioestrus the emg A/hr remained elevated for a longer period (Fig.12). No significant variations in emg amplitude under the influence of oxytocin were recorded.

There were no significant differences in emg A/hr between electrode sites. When emg amplitude was tested for electrode site differences, the only significant variation occurred during transition, where the amplitude recorded from the left horn was higher than that of the other sites ($p<0.01$-Appendix 5A).

(c) Clenbuterol: No significant effects of clenbuterol on either emg A/hr or emg amplitude were noted at any stage of the cycle; during pregnancy (day 7-15 post ovulation) however, clenbuterol treatment resulted in a rise in emg A/hr and an increase in emg amplitude when compared to non treatment values in late dioestrus ($p<0.001$-Table VII). Significant electrode site variations in either emg A/hr or amplitude were not observed (Appendix 6A).

(d) Propantheline bromide: Within 1-2 min of intravenous propantheline administration there was a reduction in emg A/hr at all stages of the cycle but this decrease was only significantly different

from the response observed when no drug had been given during dioestrus ($p<0.001$-Table VIII). No effects on amplitude were noted.

Similarly no significant differences in the emg A/hr between the horns and body under the influence of propantheline were recorded. Amplitude variations occurred only in oestrus where the left horn recorded a greater amplitude than the right horn or body ($p<0.05$-Appendix 7A).

Background emg was usually absent under propantheline influence. Activity that did occur was seldom synchronous between electrode sites (Fig.13).

(iv) Other Observations

(a) Breeding: The emg A/hr and emg amplitude were compared in the hour after natural cover (20 ±9% and 167 ±26μv respectively) with the normal oestral values (22 ±8% and 183 ±30μv respectively). No significant differences in either parameter were observed. During breeding, and for up to 8 min after breeding, the density of the emg spikes increased from an average of 17 bursts/min pre-breeding to 40 bursts/min immediately post-breeding, then quickly declined to pre-breeding levels (Fig.14 a,b).

(b) Changes during natural luteolysis: Natural luteolysis was recorded on one occasion and confirmed by a reduction in serial progesterone values. On the day in question at 0800hr the plasma progesterone level was 13ng/ml, emg A/hr was 67 ±12% and emg amplitude 118 ±20μv. At 0900hr the emg A/hr increased to 100% at all 3 electrode sites and continued at this level for 3 hrs. At 1400hr plasma progesterone levels were 6ng/ml, the emg A/hr 76 ±18%, and emg amplitude 122 ±15μv. By 0800hr the following day (i.e. 24 hrs later) the plasma progesterone had decreased to 1ng/ml and the emg amplitude had increased to 180 ±31μv. Within a further 24 hrs the normal oestral pattern developed and plasma progesterone levels were 0.3ng/ml.

(c) **Induced Abortion**: Prostaglandin was administered to induce abortion in this mare at day 20 following breeding. The emg A/hr remained at the pre-prostaglandin administration level of 100% and the amplitude remained at the pre-prostaglandin administration level of 67 $\pm 0\mu v$ for the eight hour recording period. By the next day (24 hrs after prostaglandin administration) the % emg A/hr had decreased to dioestral levels (62 $\pm 8\%$) and the amplitude increased to 120 $\pm 17\mu v$. Within 48 hours the mare was exhibiting signs of oestrus and emg patterns typical of oestrus were established.

TABLE I SWEETIE

Mean % emg A, periods of activity, periods of relative inactivity and emg amplitudes in active periods during transition, oestrus and dioestrus.

	% emg A/hr	active periods min	periods of relative inactivity min	amplitude μV
Transition	[a]32 ± 12(72)	[b] 6 ± 3(199)	12 ± 8 (199)	[c]164 ± 38(72)
Oestrus	[a]22 ± 8 (53)	[b] 3 ± 1(135)	11 ± 8 (135)	[c]183 ± 30(53)
Dioestrus	[a]62 ± 22(49)	[b]22 ± 23(147)	10 ± 11(147)	[c]139 ± 32(49)

NOTE: The data are displayed as means ± SD with the numbers of observations in parentheses. Superscripts designate significant differences ($P < 0.05$) between values identified with the same letter for that particular parameter; P values are shown in the text. Data for all subsequent tables are displayed in the same manner.

TABLE II SWEETIE

Mean % emg A, periods of activity, periods of relative inactivity and emg amplitudes in active periods during early dioestrus (P4 1-5 ng/ml), and late dioestrus (P4 > 5ng/ml).

	% emg A/hr	active periods min	periods of relative inactivity min	amplitude μV
Early Dioestrus	[a]45 ± 12(21)	[b] 9 ± 10(63)	12 ± 11(63)	[c]164 ± 25(21)
Late Dioestrus	[a]76 ± 20(28)	[b]25 ± 22(84)	9 ± 11(84)	[c]121 ± 23(28)

TABLE III SWEETIE

Mean % emg A and mean amplitude in late dioestrus and the three phases of early pregnancy: D 2-6, D7-15, D16-20.

	% emg A/hr	amplitude µV
Dioestrus	[a]76 ± 20(28)	[b]121 ± 23(28)
Pregnancy D2-6	[a]63 ± 19(26)	[c]112 ± 34(26)
Pregnancy D7-15	[a]52 ± 24(29)	[b]102 ± 29(29)
Pregnancy D16-20	[a]92 ± 20(10)	[bc]67 ± 19(10)

TABLE IV SWEETIE

Mean % emg A and emg amplitude during transition, oestrus and late dioestrus under PG treatment and no treatment

	% emg A/hr	amplitude µV
TRANSITION		
No treatment	[a]32 ± 12(72)	164 ± 38(72)
PG	[a]80 ± 7(6)	176 ± 64(6)
OESTRUS		
No treatment	[b]22 ± 8(53)	[d]183 ± 20(53)
PG	[b]69 ± 4(3)	[d]216 ± 23(3)
DIOESTRUS		
No treatment	[c]62 ± 22(49)	139 ± 32(49)
PG	[c]97 ± 6(3)	145 ± 39(3)

TABLE V SWEETIE

Mean % emg A and amplitude changes over three hours following PG injection during transition, oestrus and dioestrus.

	% emg A/hr		amplitude µV	
Transition				
1st hr	[a]80 ± 7	(6)	176 ± 64	(6)
2nd hr	65 ± 7	(6)	193 ± 61	(6)
3rd hr	[a]60 ± 10	(3)	206 ± 34	(3)
Oestrus				
1st hr	69 ± 4	(3)	216 ± 23	(3)
2nd hr	59 ± 7	(2)	216 ± 23	(2)
3rd hr	53	(1)	200	(1)
Dioestrus				
1st hr	97 ± 3	(3)	145 ± 39	(3)
2nd hr	93 ± 12	(3)	145 ± 39	(3)
3rd hr	90	(1)	167	(1)

TABLE VI SWEETIE

Mean % emg A and emg amplitude during transition, oestrus and dioestrus under oxytocin treatment and no treatment.

	% emg A/hr	amplitude μV
TRANSITION		
No treatment	[a]32 ± 12 (72)	164 ± 38(72)
Oxytocin	[a]62 ± 28 (37)	162 ± 58(37)
OESTRUS		
No treatment	[b]22 ± 8 (53)	183 ± 30(53)
Oxytocin	[b]53 ± 30 (20)	193 ± 21(20)
DIOESTRUS		
No treatment	[c]62 ± 22 (49)	139 ± 32(49)
Oxytocin	[c]93 ± 11 (16)	127 ± 58(16)

TABLE VII SWEETIE

Mean % emg A and emg amplitude during transition, oestrus, dioestrus and pregnancy (D7-15) under clenbuterol treatment and no treatment.

	% emg A/hr	amplitude μV
TRANSITION		
No treatment	32 ± 12 (72)	164 ± 38(72)
Clenbuterol	23 ± 7 (3)	147 ± 65(3)
OESTRUS		
No treatment	22 ± 8 (53)	183 ± 30(53)
Clenbuterol	31 ± 39 (3)	189 ± 19(3)
DIOESTRUS		
No treatment	62 ± 22 (49)	139 ± 32(49)
Clenbuterol	55 ± 20 (3)	167 ± 0 (3)
PREGNANCY		
No treatment	^{a}51 ± 24 (29)	^{b}102 ± 29 (29)
Clenbuterol	^{a}74 ± 3 (3)	^{b}150 ± 10 (3)

TABLE VIII SWEETIE

Mean % emg A and emg amplitude during transition, oestrus and dioestrus under propantheline treatment and no treatment.

	% emg A/hr		amplitude µV	
TRANSITION				
No treatment	32 ± 12	(72)	164 ± 38	(72)
Propantheline	26 ± 7	(6)	200 ± 18	(6)
OESTRUS				
No treatment	22 ± 8	(53)	183 ± 30	(53)
Propantheline	16 ± 4	(4)	225 ± 17	(4)
DIOESTRUS				
No treatment	[a]62 ± 22	(49)	139 ± 32	(49)
Propantheline	[a]19 ± 16	(6)	89 ± 27	(6)

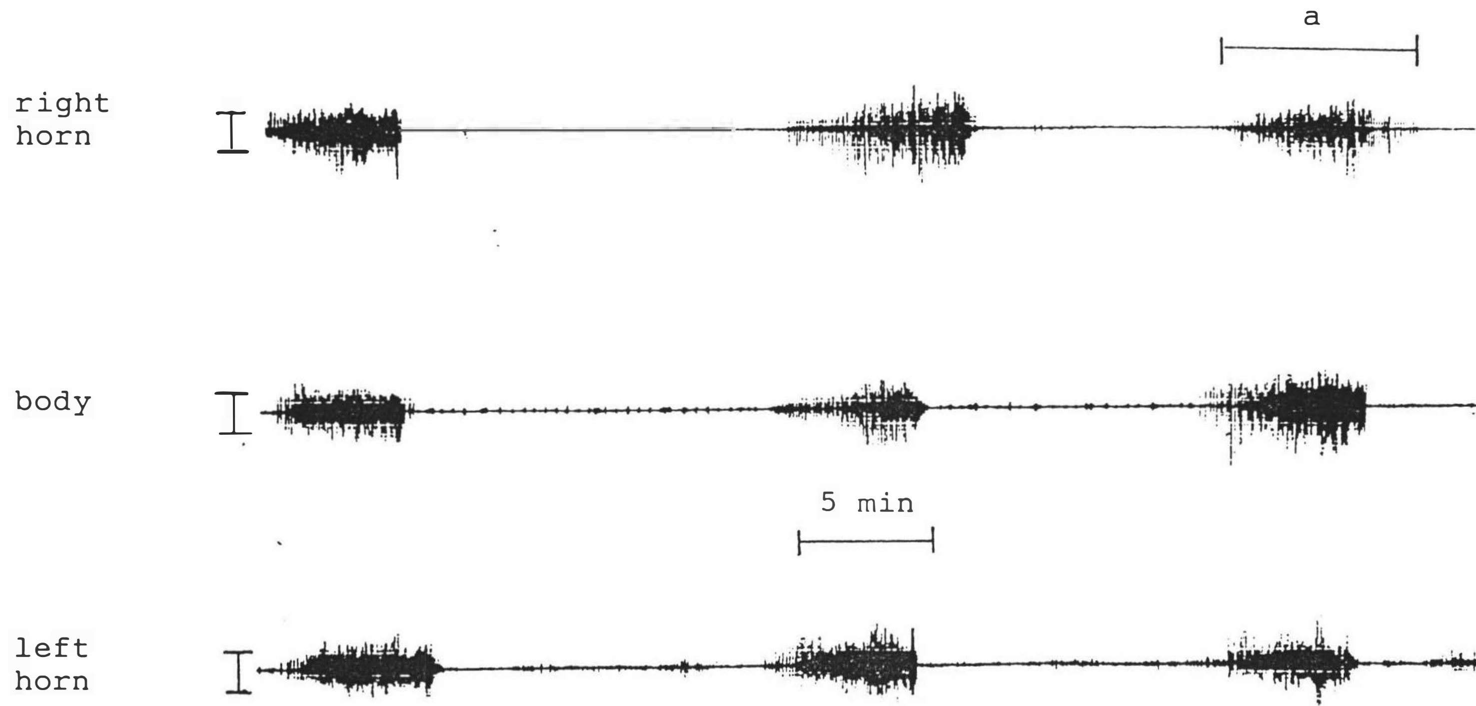


Fig. 3: Sweetie - Synchronous periods of emg activity between electrode sites during oestrus, frequently showing a crescendo-decrescendo pattern in amplitude variation (a). The vertical bars on the left represent a calibration of 200µv.

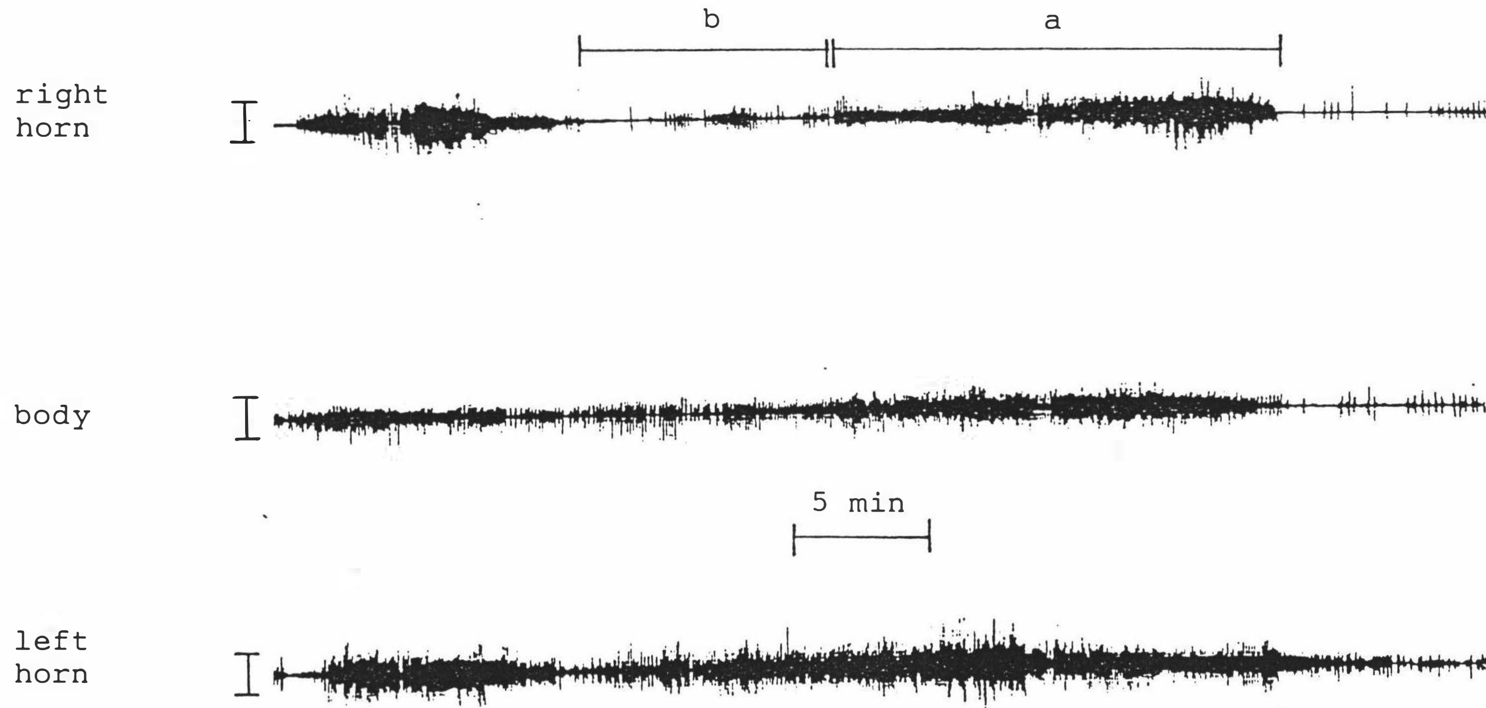


Fig. 4a: Sweetie - Periods of emg activity (a) and relative inactivity (b) during dioestrus. The vertical bars on the left represent a calibration of $200\mu v$.

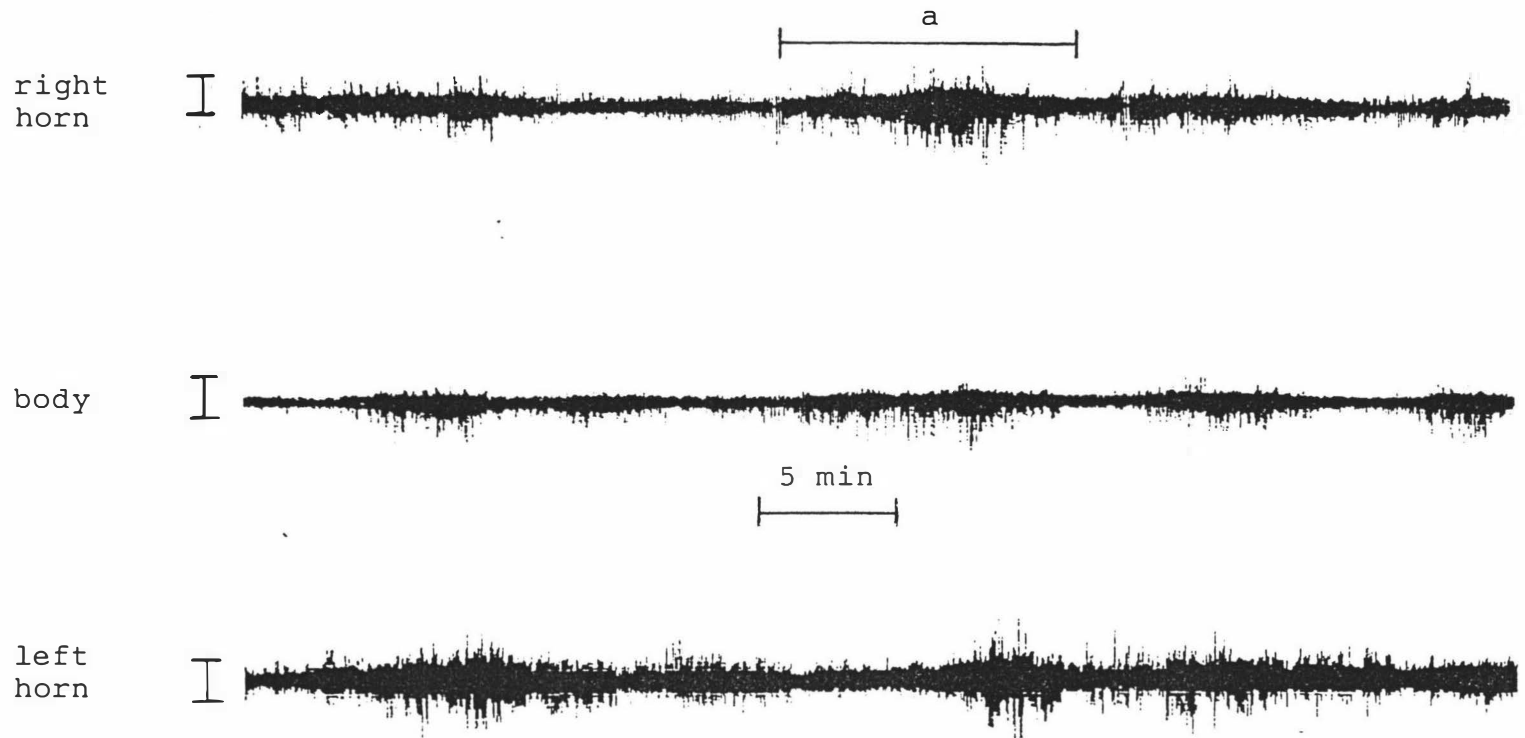


Fig. 4b: Sweetie - Periods of emg activity in dioestrus with superimposed pattern of crescendo-decrescendo amplitude changes (a), but without periods of relative inactivity. The vertical bars on the left represent a calibration of 200μv.

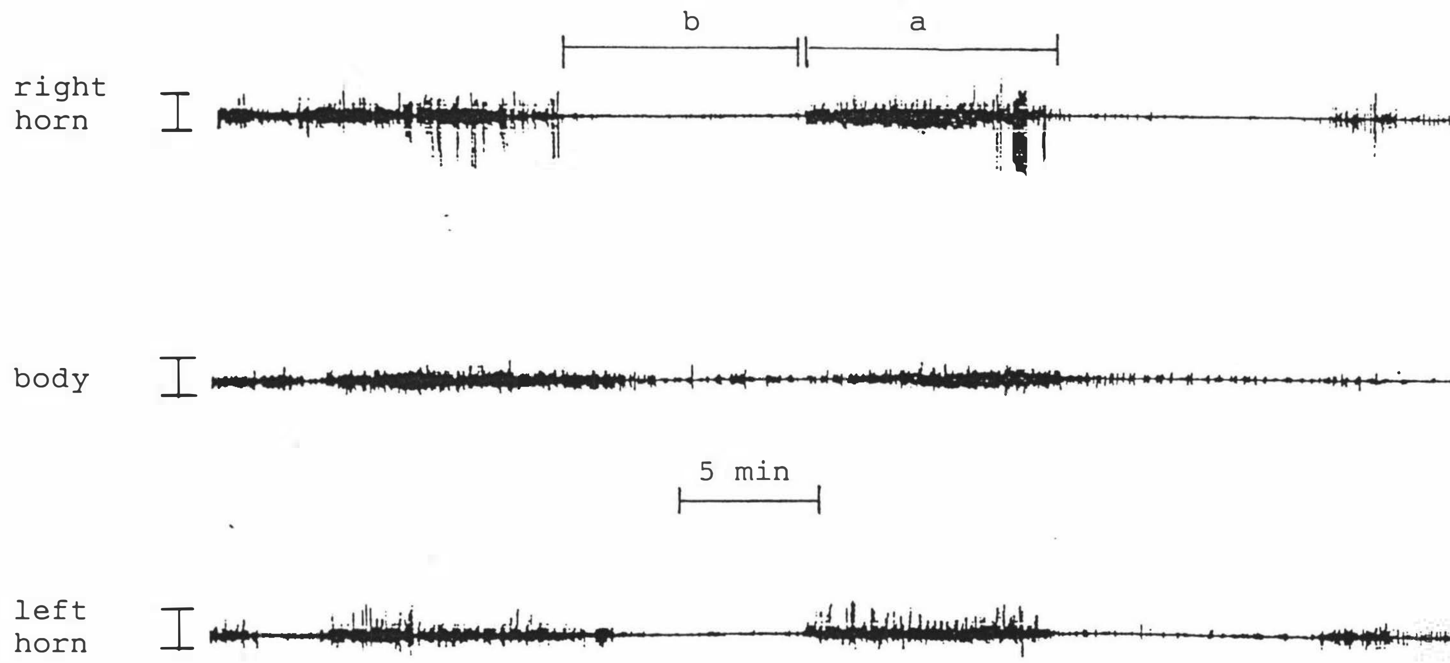


Fig. 5: Sweetie - Periods of emg activity (a) and relative inactivity (b) during transition. The vertical bars on the left represent a calibration of 200µv.

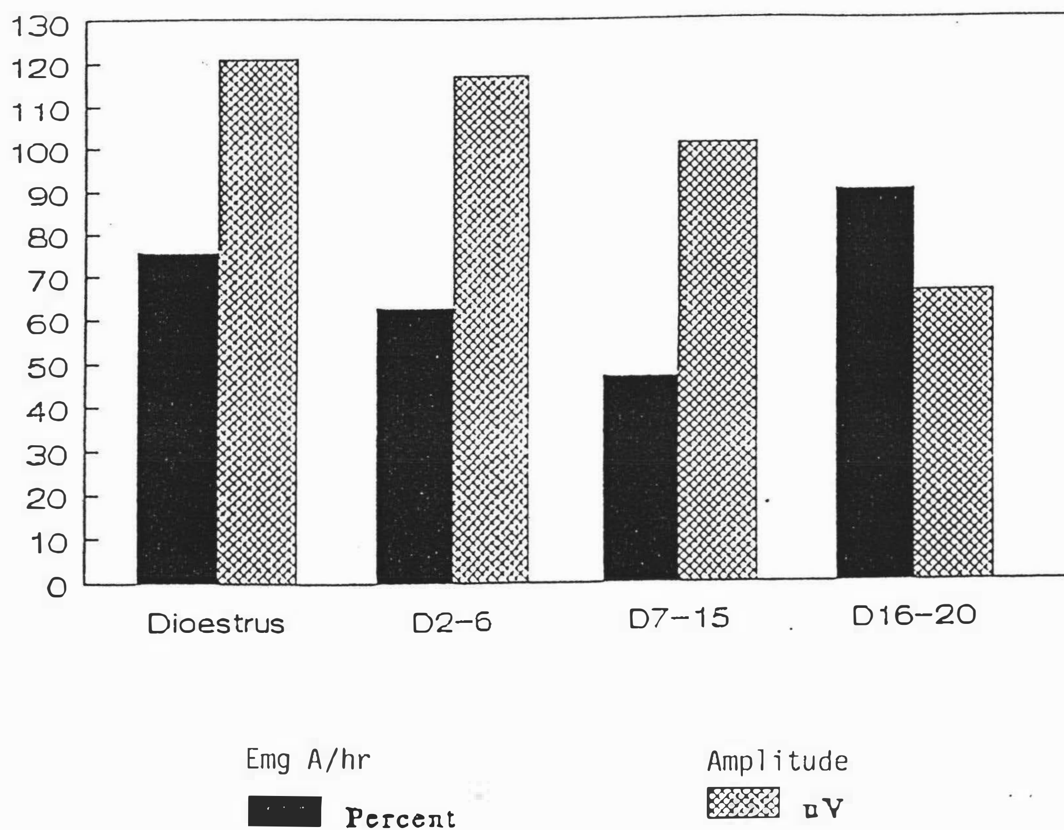


Fig. 6: Sweetie - Emg A/hr and emg amplitude in late dioestrus and three periods of early pregnancy (days 2-6, 7-15 and 16-20).

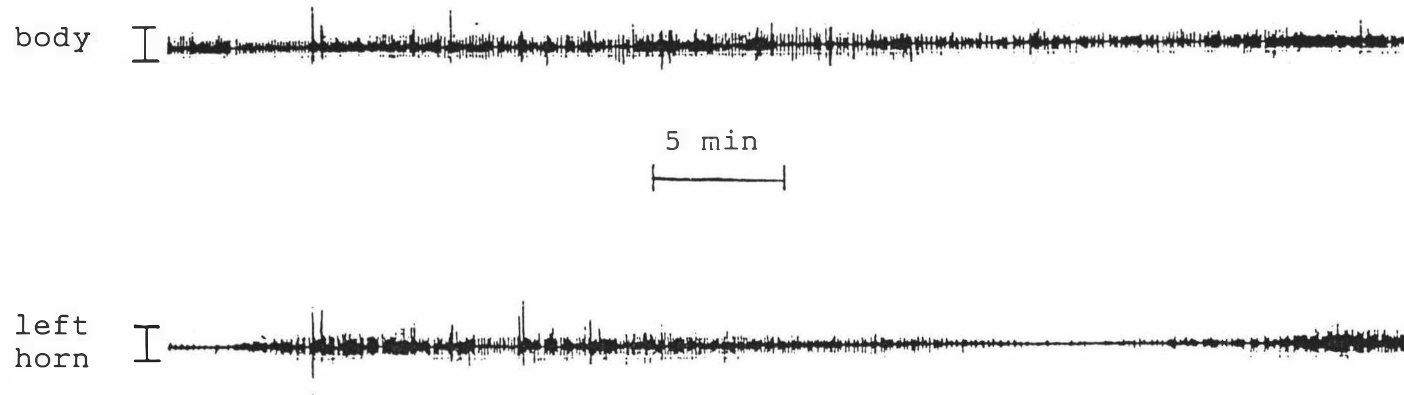


Fig.7: Sweetie - day 14 post ovulation the left horn electrode recorded a reduced emg A/hr compared to the body electrode at this stage of pregnancy. The vertical bars on the left represent a calibration of 200µv.

Fig. 8: Sweetie - Day 18 post-ovulation the left horn electrode recorded a reduced emg A/hr and a reduced amplitude compared to the body electrode. Note the difference in emg amplitude compared to dioestrus (Fig.4a,4b). The vertical bars on the left represent a calibration of 200µv.

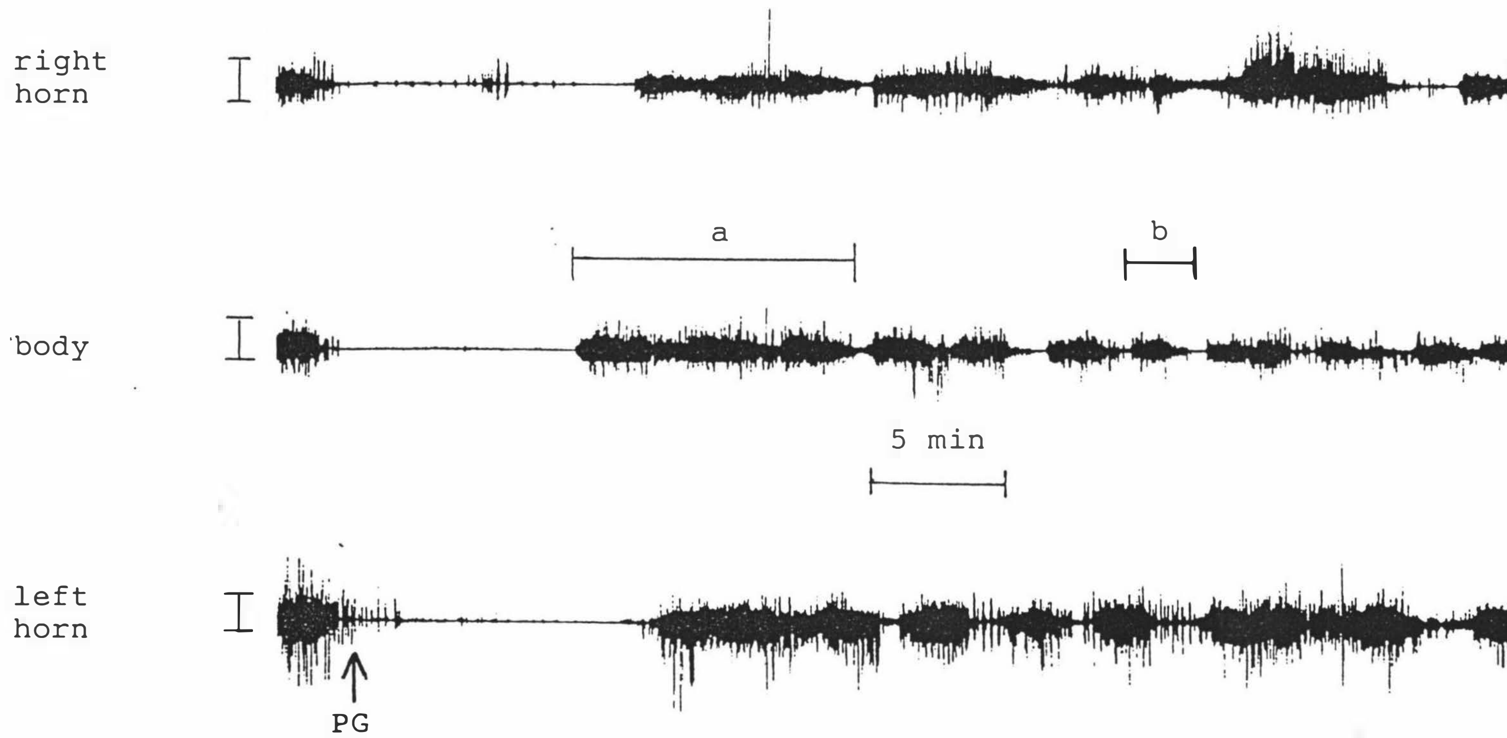


Fig. 9a: Sweetie - Pattern of emg activity after PG administration in oestrus. Note initial prolonged burst of emg activity (a) followed by a short burst pattern (b). The vertical bars on the left represent a calibration of 200µv.

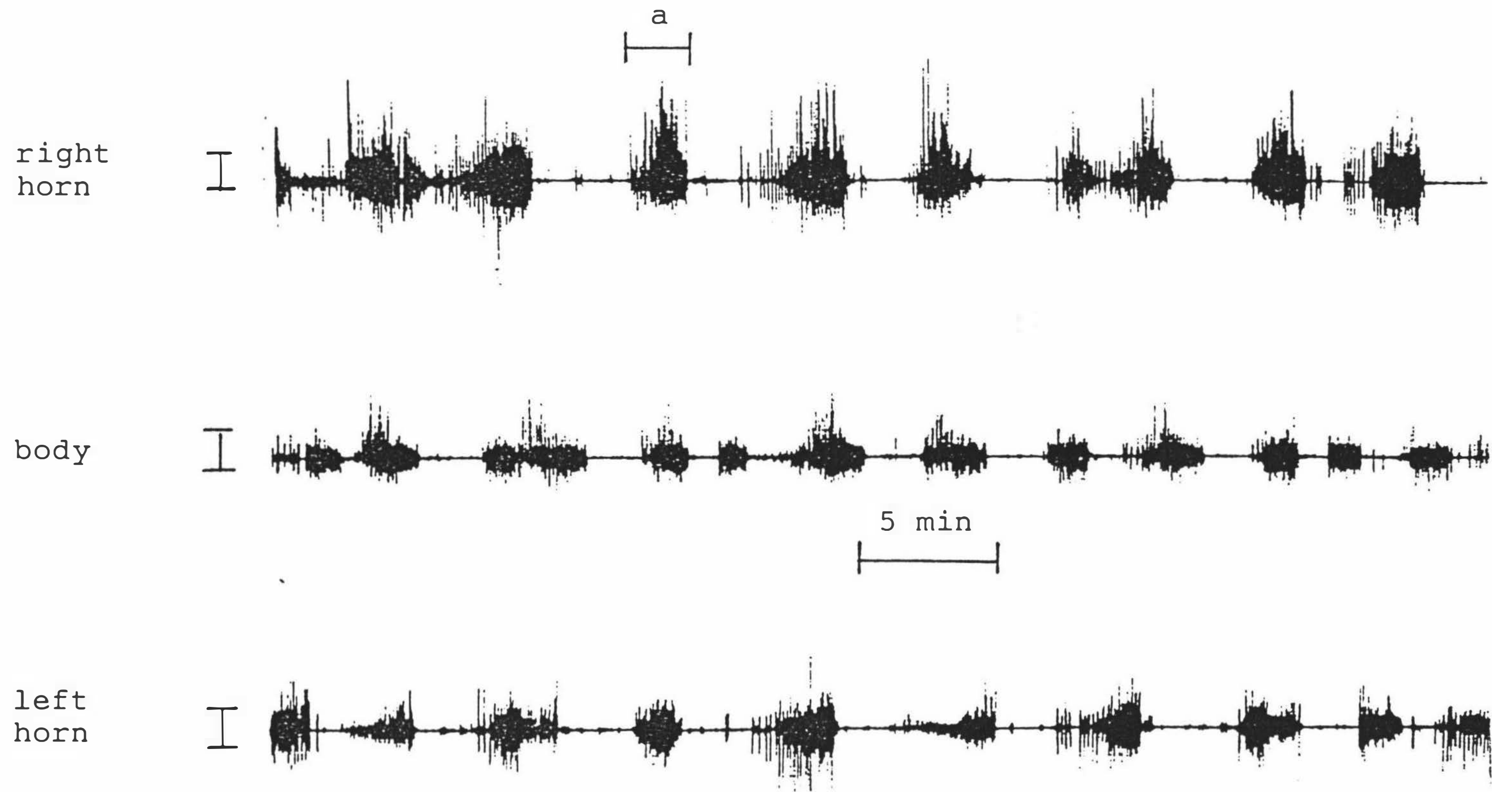


Fig. 9b: Sweetie - Emg activity the third hr after PG administration during oestrus. Note the short burst activity (a) present at all three electrode sites. The vertical bars on the left represent a calibration of 200μv.

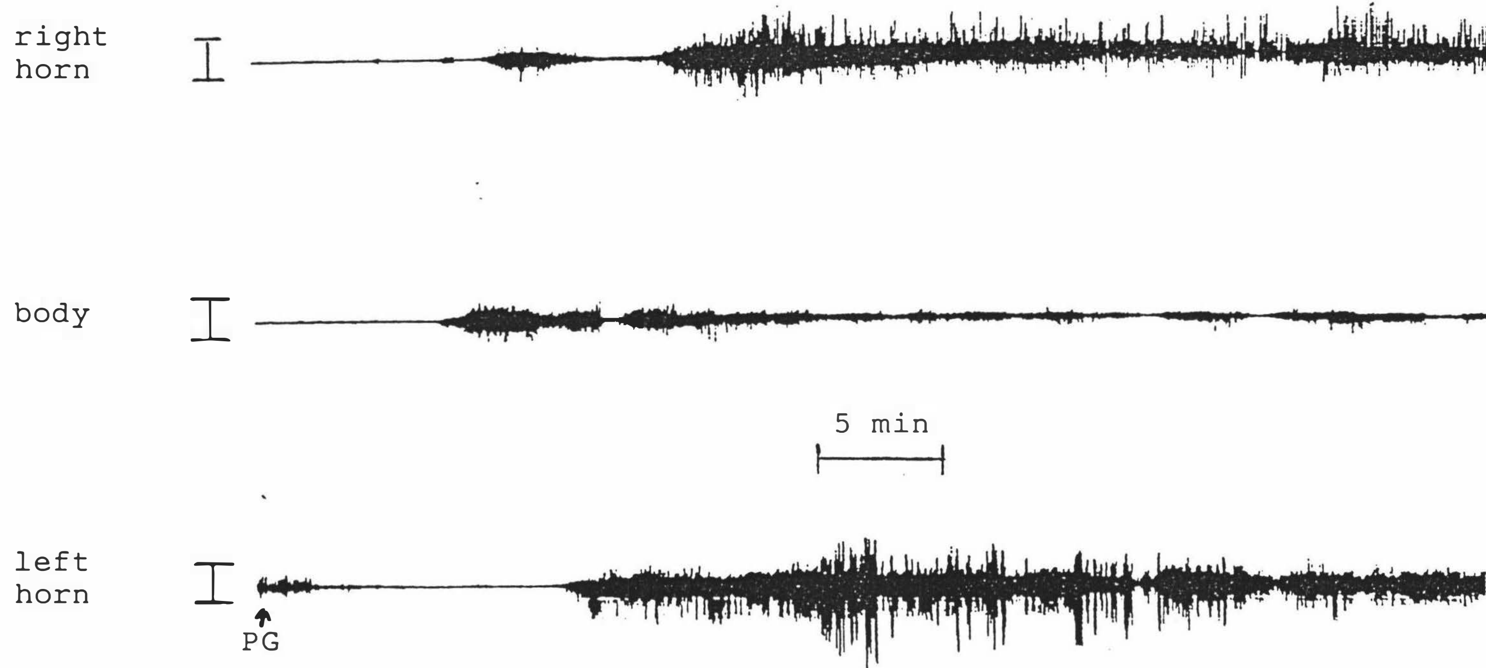


Fig. 10a: Sweetie - Pattern of emg activity after PG administration in dioestrus. Note the initial prolonged emg activity and minimal burst pattern. The vertical bars on the left represent a calibration of 200µv.

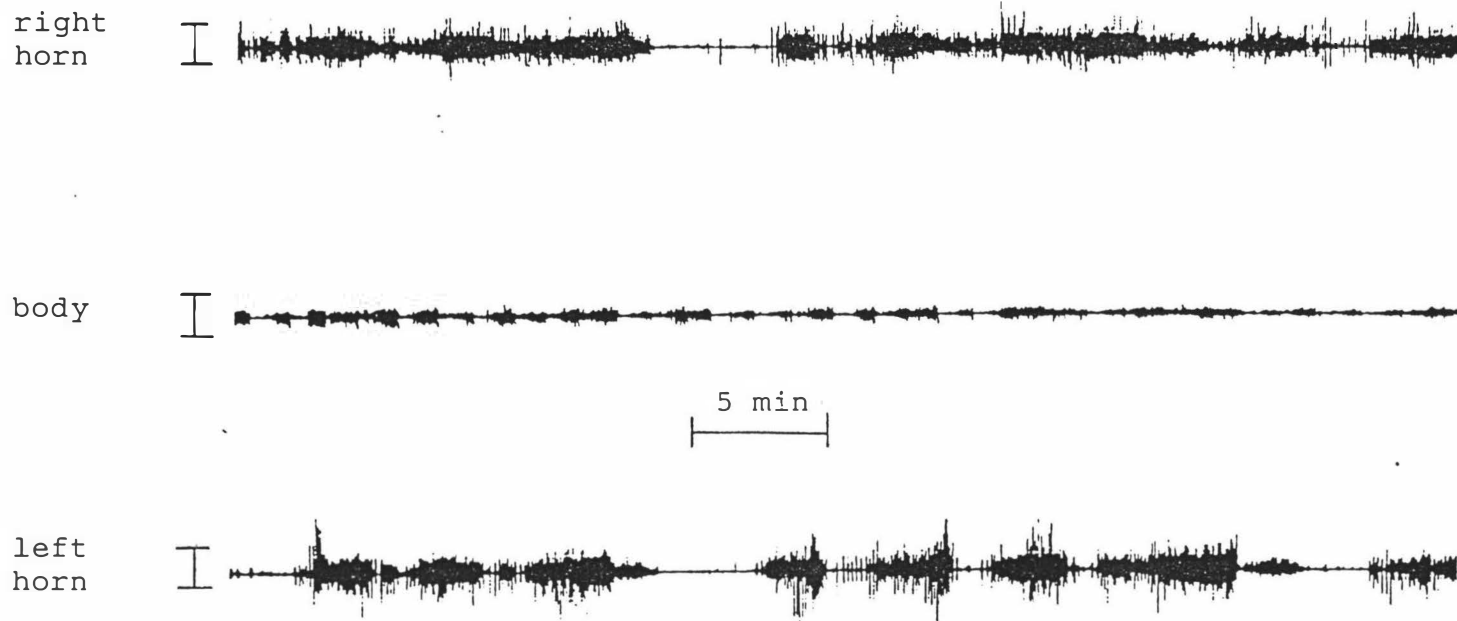


Fig. 10b: Sweetie - Pattern of emg activity in dioestrus 1 hr following PG administration. A short burst pattern, although present, is less clearly defined than in oestrus(Fig.9a,b). The vertical bars on the left represent a calibration of 200μv.

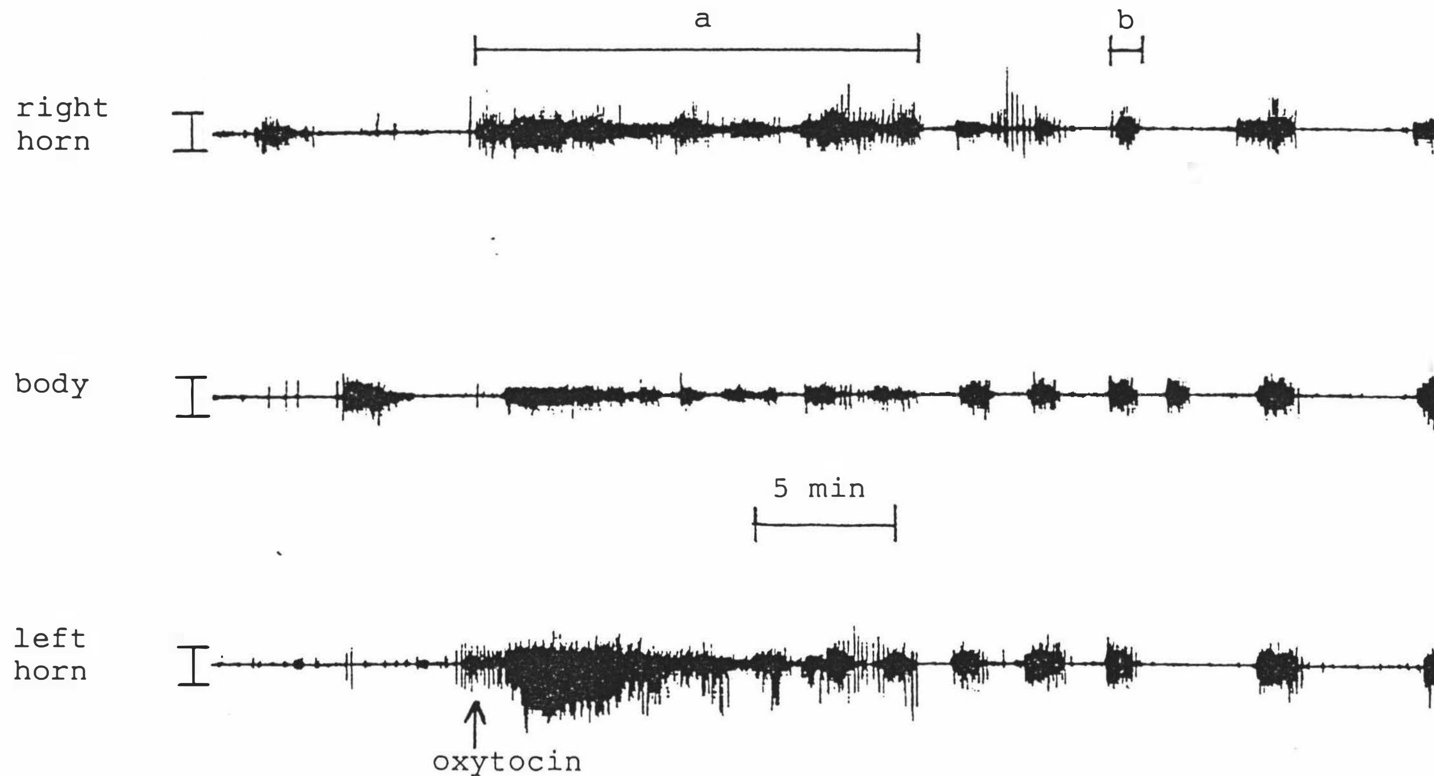


Fig. 11: Sweetie - Pattern of emg activity recorded during oestrus after intravenous administration of oxytocin (5 IU). Note the quick response and initial prolonged emg activity (a) followed by short burst pattern (b). Vertical bars on the left represent a calibration of 200µv.

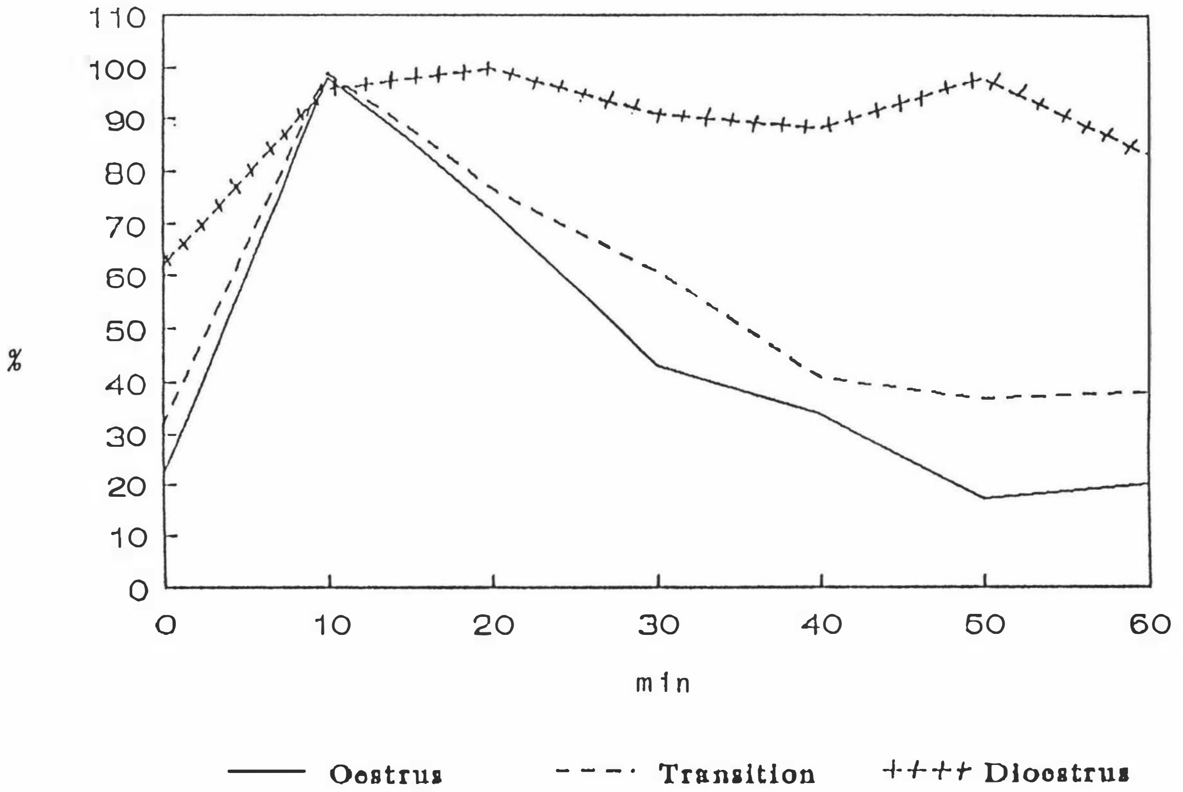


Fig. 12: Sweetie- Emg A/hr values for consecutive 10 minute intervals after administration of 5 IU oxytocin during oestrus, transition and dioestrus.

Fig.13: Sweetie - Pattern of emg activity in dioestrus following propantheline bromide administration intravenously. Note rapid reduction in emg activity recorded at all electrode sites. The vertical bars on the left represent a calibration of 200μv.

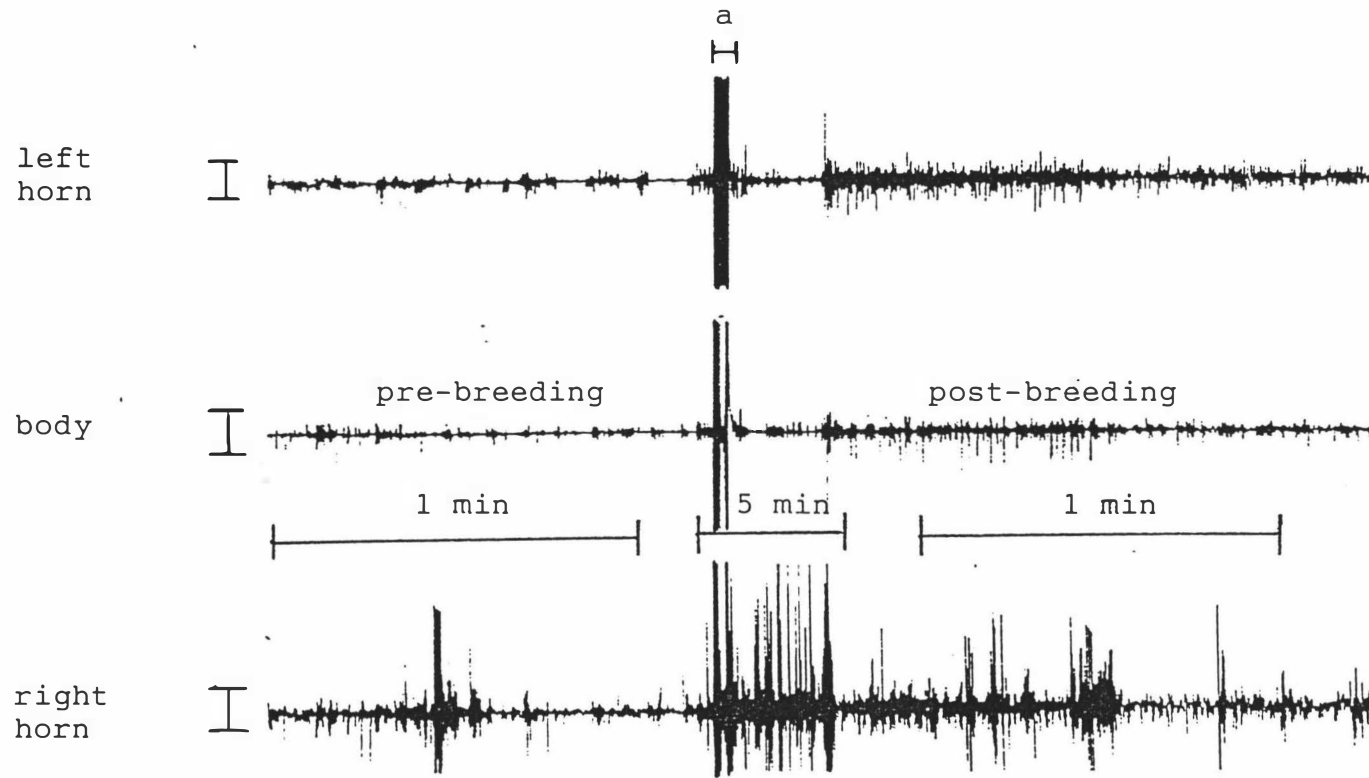


Fig. 14a: Sweetie - Emg activity pre-breeding, during natural cover (a) and immediately post-breeding. Note the increased density of emg bursts post-breeding. The activity in the pen recorder seen during natural cover (a) was probably due to a disturbance of electrode connections by the stallion. The continued disturbance in the right horn is believed due to electrode damage during breeding. The vertical bars on the left represent a calibration of 200µv.

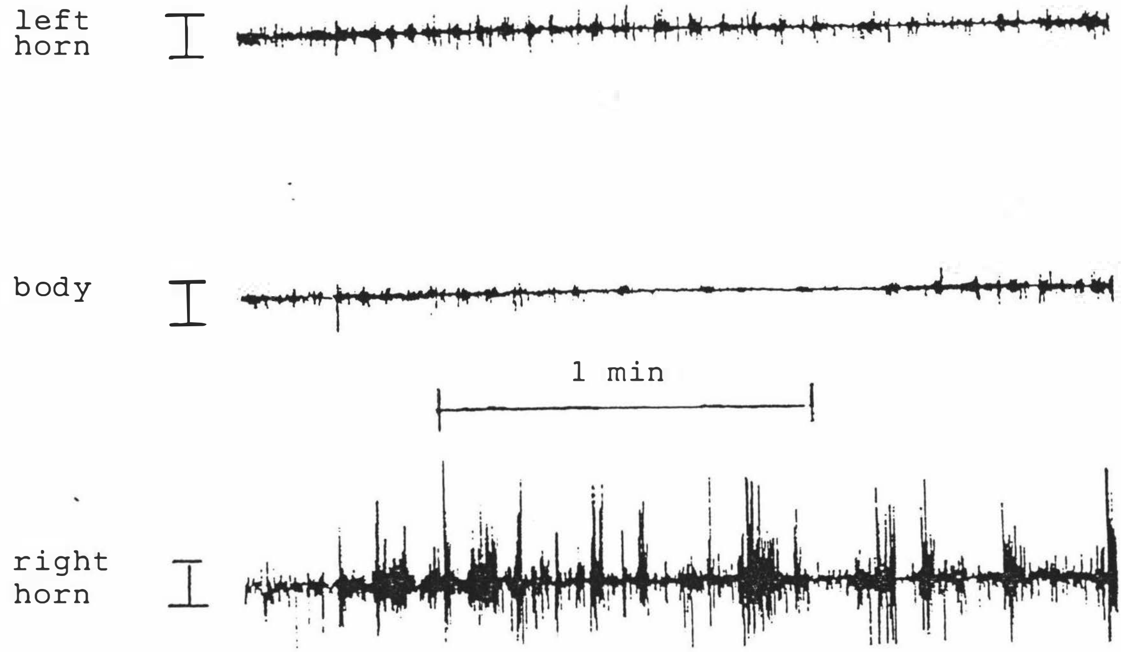


Fig. 14b Sweetie - Emg activity at approximately 6-8 min post-breeding. Note the decrease in spike density to pre-breeding levels. This was immediately followed by a resumption of the normal oestrus pattern. The right horn continues to exhibit electrode disturbance (see comment in Fig.14a). The vertical lines on the left represent a calibrationof 200µv.

III.1.B: Fling

(i) Normal Oestrous Cycle Activity (no exogenous drug influence)

The quality of emg recordings for this mare was poor compared with those obtained for Sweetie - possible reasons for this are considered in the discussion. Four cycle stages were examined, anoestrus, transition, oestrus and dioestrus.

Emg A/hr for Fling was greater during natural dioestrus than at any other cycle stage ($p<0.01$-Table IX); there were differences between the length of active periods at all stages, being longest in natural dioestrus and shortest in natural oestrus ($p<0.05$-Table IX). Periods of relative inactivity also varied being shorter in natural oestrus and dioestrus when compared to anoestrus and transition ($p<0.05$-Table IX). Emg amplitude was higher during transition than at any other stage of the cycle ($p<0.01$) and lowest in dioestrus, but not significantly so (Table IX).

When emg A/hr, periods of activity, periods of relative inactivity, and emg amplitude were analyzed according to the progesterone levels recorded during dioestrus, no differences were noted (Table X).

(ii) Artificial Oestrus and Dioestrus Induced by Steroid Hormones

(a) **Oestrogen**: Exogenous oestradiol administration during clinical anoestrus resulted in no significant change in emg A/hr either between different dose levels or compared with values recorded in natural oestrus (Table XI). When results of emg A/hr from natural and simulated oestrus were pooled no difference between the pooled value and the value for natural oestrus was observed (Table XI).

Mean amplitude of the records obtained at the 2.5mg oestradiol dose was however, greater than that seen with either other dose levels or during natural oestrus; when natural and simulated oestrus values (at all oestradiol dose levels) were pooled there was no difference between this value and that found in natural oestrus ($p>0.05$-Table XI).

During both natural and simulated oestrus a crescendo-decrescendo amplitude pattern of emg activity was recorded; this was less pronounced than that seen with Sweetie.

(b) Progesterone: When exogenous progesterone was administered during anoestrus the parameters recorded did not differ from those observed during natural dioestrus, nor did the combined values of natural and simulated dioestrus vary from natural dioestrus. However, induced dioestrus varied from anoestrus in the same manner as natural dioestrus ($p<0.01$-Table XII).

Variation between electrode sites occurred. During transition the emg A/hr of the body site was significantly less than in either of the horns ($p<0.05$) and periods of relative inactivity were longest ($P<0.05$-Appendix 8A). During oestrus and transition the emg amplitude recorded from the body electrode was less than that recorded from the uterine horns ($p<0.05$-Appendix 8A).

(iii) Drug Treatment

Since the emg A/hr and amplitude differences between natural and simulated oestrus were minimal, and the same was true for natural and simulated dioestrus, a combined value was used and no attempt was made to separate the effects of drug treatments between responses obtained in either the natural or simulated cycle.

(a) Prostaglandin: After the administration of intramuscular prostaglandin a change in emg activity was noticed within 8-10 min. Fling frequently exhibited signs of mild colic, sweating and an increase in loose bowel movements.

Emg A/hr increased during all cycle stages after prostaglandin treatment when compared to no treatment; however the difference was only significant during oestrus and anoestrus and could not be tested in transition due to insufficient data ($p<0.001$-Table XIII). After PG

administration there was an emg change similar to that found in Sweetie, except that after the initial prolonged burst of emg activity the subsequent short burst pattern was not as well defined.

When variation between electrode sites was examined no significant variation in emg A/hr occurred in either oestrus or anoestrus; in dioestrus the right horn emg A/hr was less than that of the left horn and the uterine body ($p<.05$-Appendix 9A).

(b) **Oxytocin**: An emg response was noted after oxytocin injection within 40 - 60 sec. This drug elicited sweating and signs of abdominal discomfort. Oxytocin was not given to this mare during the stage of transition.

In anoestrus 2 IU,5 IU and 50 IU of oxytocin were administered. After the 2 IU and the 5 IU dose there was an increase in emg A/hr compared to no drug treatment ($p<0.05$ and $p<0.001$ respectively). However, after the 50 IU dose there was no change (Table XIV).

When 2 IU,5 IU, or 20 IU doses of oxytocin were administered during oestrus the emg A/hr increased compared with none treatment oestral levels ($p<0.01$). This increase was greatest after the 2 IU dose and least after the 20 IU dose ($p<0.05$-Table XIV).

During dioestrus 2 IU and 5 IU of oxytocin were administered; only the 5 IU dose increased emg A/hr ($p<0.05$-Table XIV).

Emg A/hr was then evaluated for changes within treatment by examining variations in 10 minute increments post injection. Through oestrus there was a significant negative correlation between emg A/hr and time within treatment over 50 min after the 2 IU ($r=-0.62$) and 5 IU dose ($r=-0.77$) ($p<0.001$-Appendix 2B,3B) (Fig.15). After the 20 IU dose, although a negative correlation between emg A/hr and time within treatment also occurred, the decrease was not significant ($r=-0.15$).

The emg pattern after oxytocin administration was similar to that found with Sweetie -for the first 10 to 20 min emg activity was continuous followed by short bursts. In dioestrus the burst activity was less well defined and emg activity remained at a high level throughout this stage of the cycle.

When emg A/hr was compared between electrode sites in anoestrus the right horn site had the least activity at each dose level of oxytocin, but the difference was only significant at 2 IU ($p<0.001$) and 5 IU ($p<0.05$) (Appendix 10A). During oestrus only the 20 IU dose resulted in site variation, the right horn recording less activity than the other sites ($p<0.01$-Appendix 10A). In dioestrus the 2 IU dose of oxytocin resulted in the right horn recording the least activity ($p<0.001$), although the left horn activity was also less than that of the uterine body ($p<0.05$). At the 5 IU dose the right horn electrode was not operational, but the emg A/hr of the uterine body was less than that of the left horn ($p<0.05$-Appendix 10A).

Through oestrus the emg amplitude after the 2 IU dose of oxytocin was higher than the amplitude recorded in oestrus with no drug treatment ($p<0.05$); it was also higher than that recorded with other oxytocin doses ($p<0.001$-Table XIV). The 20 IU dose resulted in the lowest amplitude and the emg amplitude after both the 20 IU and 5 IU doses was less than the non treatment oestrous amplitude ($p<0.05$). In dioestrus the 5 IU dose resulted in a decrease in amplitude when compared to no treatment ($p<0.05$-Table XIV).
When amplitude variation between electrode sites was examined after 2 IU oxytocin in dioestrus the right horn recorded the lowest amplitude but that of the left horn was also lower than the body ($p<0.05$). In anoestrus, following 5 IU of oxytocin, the right horn recorded the highest amplitude ($p<0.05$-Appendix 10A).

(c) Clenbuterol: After clenbuterol treatment there was no statistically significant difference in emg A/hr or emg amplitude compared to no treatment through any oestrous cycle stage (Table

XV).

When emg A/hr between electrode sites was examined the right horn had reduced activity in every case recorded. In anoestrus the emg A/hr of the right horn was 0% and that of the left horn was also decreased. Due to the small number of suitable records, statistical evaluation for differences between electrode sites was not carried out (Appendix 11A).

(d) Propantheline Bromide: After propantheline injection changes in the emg were noted within two minutes and there was a decrease in emg A/hr in all cycle stages; this decrease was only significant during dioestrus ($p<0.01$-Table XVI).

Through anoestrus, dioestrus and oestrus the amplitude was decreased when propantheline was administered from non drug levels ($p<0.05$-Table XVI).
When variation between electrode sites was examined no significant differences were found in either emg A/hr or amplitude (Appendix 12A).

TABLE IX FLING

Mean % emg A, periods of activity, periods of relative inactivity and emg amplitudes in active periods during transition, oestrus and dioestrus.

	% emg A/hr	active periods min	periods relative inactivity min	amplitude μV
Anoestrus	[a] 32 ± 18(22)	[d] 5 ± 4(57)	[e] 13 ± 15(57)	[g] 128 ± 49(22)
Transition	[b] 35 ± 17(48)	[d] 8 ± 11(128)	[f] 15 ± 13(128)	[ghi] 171 ± 39(48)
Oestrus	[c] 35 ± 9(33)	[d] 4 ± 2(99)	[ef] 6 ± 3(99)	[h] 140 ± 52(33)
Dioestrus	[abc] 69 ± 21(9)	[d] 13 ± 16(27)	[ef] 5 ± 10(27)	[i] 99 ± 18(9)

TABLE X FLING

Mean % emg A, periods of activity, periods of relative inactivity and emg amplitudes in active periods during early dioestrus (P 1-5 ng/ml) and late dioestrus (P4 >5 ng/ml)

	% emg A/hr	active periods min	periods relative inactivity min	amplitude μV
Early Dioestrus	79 ± 6(4)	17 ± 19(19)	3 ± 2(19)	98 ± 16(4)
Late Dioestrus	60 ± 27(5)	6 ± 6(13)	9 ± 16(13)	101 ± 21(5)

TABLE XI FLING

Mean % emg A and emg amplitude after 1mg, 2.5 mg and 10 mg doses of oestradiol to stimulate oestrus during anoestrus, values in natural oestrus and in simulated and natural oestrus combined.

	% emg A/hr		amplitude µV	
1 mg oestradiol	40 ± 10	(4)	[a] 217 ± 19	(4)
2.5 mg oestradiol	38 ± 11	(5)	[abcd] 248 ± 21	(5)
10 mg oestradiol	44 ± 11	(4)	[b] 192 ± 10	(4)
Natural oestrus	35 ± 9	(33)	[c] 140 ± 52	(33)
Natural and simulated oestrus combined	37 ± 9	(46)	[d] 163 ± 59	(46)

TABLE XII FLING

Mean % emg A and emg amplitude after 125 mg P4 to simulate dioestrus during anoestrus, values in natural dioestrus and in natural and simulated dioestrus combined.

	% emg A/hr		amplitude µV	
Anoestrus	[abc] 32 ± 18	(22)	128 ± 49	(22)
125 mg P4	[a] 70 ± 20	(5)	106 ± 13	(5)
Dioestrus	[b] 69 ± 21	(9)	99 ± 18	(9)
Natural and simulated dioestrus combined	[c] 69 ± 20	(14)	101 ± 16	(14)

TABLE XIII FLING

Mean % emg A and amplitude in anoestrus, transition, oestrus and dioestrus under PG treatment and no treatment.

	% emg A/hr		amplitude µV	
ANOESTRUS				
No treatment	[a]32 ± 18	(22)	128 ± 49	(22)
PG	[a]68 ± 23	(7)	116 ± 24	(7)
TRANSITION				
No treatment	35 ± 17	(48)	171 ± 39	(48)
PG	89	(1)	108	(1)
OESTRUS				
No treatment	[b]35 ± 9	(33)	140 ± 52	(33)
PG	[b]71 ± 17	(9)	187 ± 40	(9)
DIOESTRUS				
No treatment	69 ± 21	(9)	99 ± 18	(9)
PG	77 ± 14	(11)	140 ± 95	(11)

TABLE XIV FLING

Mean % emg A, and amplitude during anoestrus, oestrus and dioestrus following varying doses of oxytocin (2 IU, 5 IU, 20 IU and 50 IU) and no treatment.

	% emg A/hr		amplitude μV	
ANOESTRUS				
No treatment	[ab] 32 ± 18	(22)	128 ± 49	(22)
2 IU oxytocin	[a] 59 ± 37	(9)	100 ± 10	(9)
5 IU oxytocin	[b] 73 ± 28	(32)	128 ± 41	(32)
50 IU oxytocin	33 ± 35	(21)	150 ± 109	(21)
OESTRUS				
No treatment	[ab] 35 ± 9	(33)	[cd] 140 ± 52	(33)
2 IU oxytocin	[ab] 88 ± 13	(18)	[cd] 203 ± 50	(18)
5 IU oxytocin	[a] 73 ± 18	(20)	[c] 118 ± 37	(20)
20 IU oxytocin	[b] 54 ± 19	(12)	[d] 90 ± 24	(12)
DIOESTRUS				
No treatment	[a] 69 ± 21	(9)	[b] 99 ± 18	(9)
2 IU oxytocin	51 ± 34	(60)	79 ± 38	(60)
5 IU oxytocin	[a] 96 ± 8	(20)	[b] 72 ± 5	(20)

TABLE XV FLING

Mean % emg A and amplitude in anoestrus, transition, oestrus and dioestrus following clenbuterol treatment and no treatment.

	% emg A/hr		amplitude μV	
ANOESTRUS				
No treatment	32 ± 18	(32)	128 ± 49	(22)
Clenbuterol	15 ± 21	(2)	75 ± 106	(2)
TRANSITION				
No treatment	35 ± 17	(48)	171 ± 39	(48)
Clenbuterol	58	(1)	100	(1)
OESTRUS				
No treatment	37 ± 9	(46)	163 ± 59	(46)
Clenbuterol	40 ± 11	0(2)	130 ± 15	(2)
DIOESTRUS				
No treatment	69 ± 21	(9)	99 ± 18	(9)
Clenbuterol	46 ± 27	(3)	104 ± 19	(3)

TABLE XVI FLING

Mean % emg A and amplitude in anoestrus, oestrus and dioestrus following propantheline treatment and no treatment.

	% emg A/hr		amplitude μV	
ANOESTRUS				
No treatment	32 ± 18	(22)	[a] 128 ± 49	(22)
Propantheline	11 ± 7	(2)	[a] 59 ± 12	(2)
OESTRUS				
No treatment	35 ± 9	(33)	[b] 140 ± 52	(33)
Propantheline	13 ± 24	(6)	[b] 83 ± 70	(6)
DIOESTRUS				
No treat ment	[c] 69 ± 20	(9)	[d] 99 ± 18	(9)
Propantheline	[c] 16 ± 13	(2)	[d] 52 ± 26	(2)

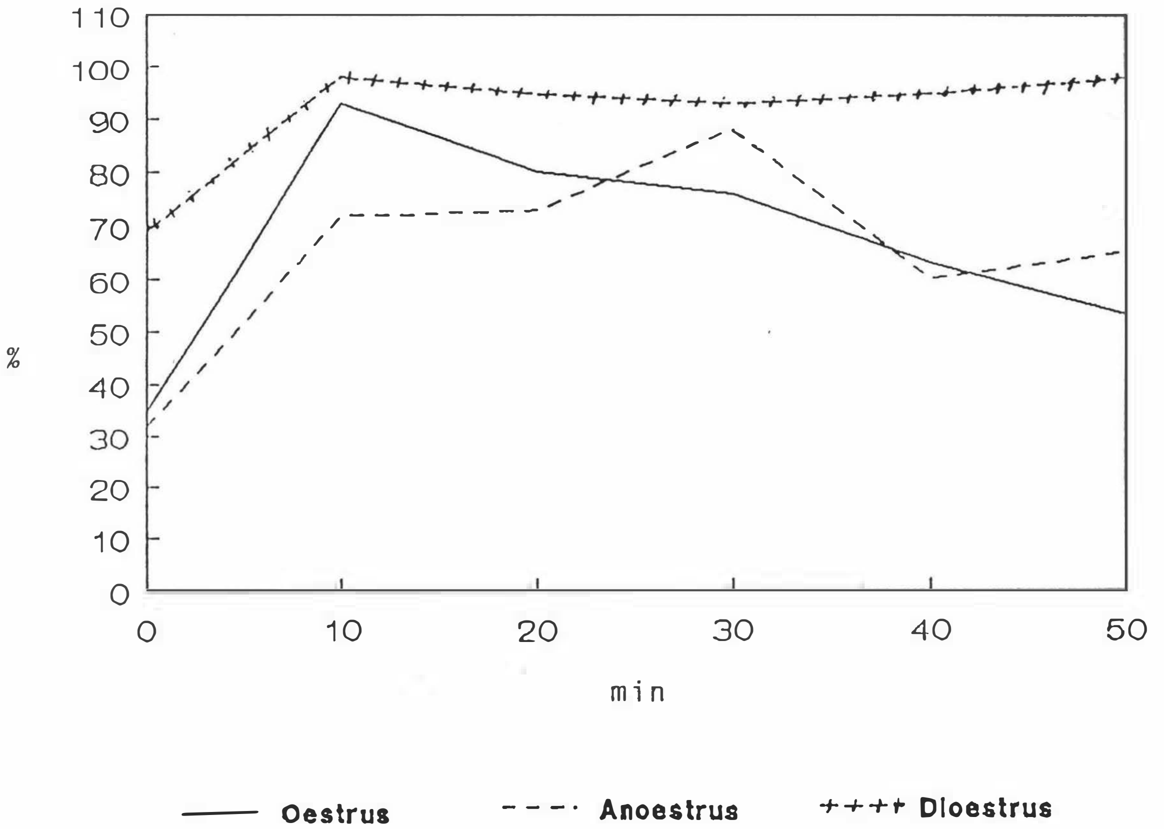


Fig. 15: Fling - Emg A/hr values for consecutive 10 minute intervals after administration of 5 IU oxytocin during oestrus, anoestrus and dioestrus.

III.2 OVARIECTOMISED MARES

III.2.A: Jo

(i) Simulated Cycle Stages

As with the intact mares background emg activity was frequently present during periods of relative uterine inactivity. Records were made during 'anoestrus' (as represented by the spayed state), simulated 'oestrus' (using exogenous oestrogens) and simulated 'dioestrus'(using exogenous progesterone).

The emg A/hr and the length of active periods were decreased under the influence of oestrogen administration ($p<0.05$-Table XVII); amplitude of the response was decreased when progesterone was given ($p<0.01$-Table XVII). No significant effects of dose of oestrogen were recorded for the parameters measured (Table XVIII); with both oestrogen and progesterone administration however, a change in the pattern of emg activity (oestrogen) and emg activity and amplitude (progesterone) could be seen on the traces within 24 hrs of the time of injection. In simulated 'dioestrus' plasma progesterone levels ranged from 5-7ng/ml.

The emg activity under the influence of oestrogen showed a pattern of crescendo - decrescendo amplitude change similar to those found with the intact mares; this was not apparent in either 'dioestrus' or 'anoestrus'. In 'oestrus' the background emg in the quiet periods was either absent, or occurred with less frequency than during 'dioestrus' or 'anoestrus'. During 'oestrus' the maximum amplitude reached was often 700-800μv during an active phase whereas in 'anoestrus' and "dioestrus", although occasional bursts contained action potentials of that amplitude, most of the activity was confined to a narrow range (50 -350μv) (Fig.16,17,18).

Variations in electrode site emg A/hr, mean lengths of active periods, relative inactive periods, and amplitude were compared for all cycle stages. In 'anoestrus' and 'dioestrus' the electrodes located in the uterine body registered a reduction in all parameters recorded (except for periods of relative inactivity) compared to the electrodes in the other sites, but this was

only significant for emg A/hr and length of active periods during 'dioestrus' ($p<0.05$-Appendix 13A). In 'oestrus' the emg amplitude recorded from the body electrode was significantly less than that recorded from the right horn ($p<0.05$-Appendix 13A).

(ii) Drug Treatment (non steroid hormones)

(a) Prostaglandin: Following intramuscular administration of prostaglandin there was an 8-10 min time lapse before the effect on emg characteristics became apparent. The mare began to sweat, and was uncomfortable, and had an increase in the frequency of bowel movements.

Emg A/hr was higher under prostaglandin influence at all stages of the cycle when compared with no treatment ($p<0.001$-Table XIX).

The amplitude changes under the influence of PG in 'oestrus' did not show the marked crescendo-decrescendo pattern as was seen prior to the drugs's influence . After PG administration, as with the intact mares, there was an initial prolonged burst of emg activity lasting 10-20 min followed by a shorter burst pattern (5-10min), which was synchronous between electrode sites. The active periods were separated by relatively quiet periods which increased from 2-4 to 5-10 min over the time the emg was recorded. This pattern was least pronounced during 'dioestrus' where periods of activity and relative inactivity were less definite (Fig.19,20,21)

In 'anoestrus' and 'dioestrus', the emg A/hr recorded from the uterine body was less than for either of the horn sites but this difference was only statistically significant for emg A/hr in ,anoestrus' ($p<0.05$-Appendix 14A). In oestrus and dioestrus the amplitude recorded from the uterine body electrode was less than for the horn electrode sites, but the difference was not significant (Appendix 14A).

(b) Oxytocin: After intravenous administration of oxytocin the effects on emg characteristics were noted within 30 to 50 sec. Occasional signs of sweating and abdominal discomfort occurred.

Oxytocin increased the emg A/hr compared to no treatment at all cycle stages ($p < 0.001$-Table XX). When the emg A/hr was examined in 10 min increments over an hour, there was a negative correlation between emg A/hr with time during all cycle stages ($r = -0.71$)($p < 0.05$-Appendix 4B)(Fig.22). Greatest effects were noted at 'oestrus'($r = -0.90, p < 0.001$) and least during 'dioestrus' ($r = -0.57, p < 0.01$) with 'anoestrus' falling in between ($r = -0.86, p < 0.001$) - see Appendix 4B. In all cases values returned to normal levels for that particular cycle stage within 30 min after drug administration. During 'dioestrus' the emg A/hr recorded from electrodes in the uterine body was significantly less than for either horn site ($p < 0.01$-Appendix 15A).

During 'oestrus', following oxytocin administration, the emg amplitude did not show the pronounced crescendo - decrescendo changes that occurred prior to drug administration.

Through 'oestrus' and to a lesser extent 'anoestrus,' after an initial 10-20min period of nearly continuous emg activity, shorter bursts (3-5min) occurred separated by short quiet periods (2-5min) just as had been found with the response of the intact mares. This pattern was not as distinct during 'dioestrus' where emg activity remained higher throughout (Fig.23,24).

(c) Clenbuterol: Records for the possible effects of clenbuterol (Table XXI) were available for only one treatment during 'dioestrus' and 3 treatments during 'oestrus'. The emg amplitude was reduced during 'oestrus' compared with control values ($p < 0.05$-Table XXI); it was also reduced in 'dioestrus' but statistical analysis was not possible.

Emg A/hr was also reduced during both 'oestrus' and 'dioestrus' following clenbuterol treatment but the reduction was only significant in 'oestrus' ($p<0.05$-Table XXI). The few records analyzed in 'dioestrus' were insufficient to verify the validity of this observation from a statistical point of view.

Emg A/hr and amplitude from the electrodes inserted in the uterine body were less than for recordings from the horns at both cycle stages for which records were available, however statistical analysis was only possible in 'oestrus' ($p<0.05$-Appendix 16A).

(d) **Propantheline Bromide**: Propantheline given intravenously was observed to cause emg changes in less than 2 min following injection (Fig.25). Records were available for the use of propantheline once during 'oestrus' and 'anoestrus' and 6 times during 'dioestrus'.

Both emg A/hr and amplitude were reduced after propantheline administration compared with no drug treatment in all stages of the cycle recorded; statistical analysis, because of the limited number of recordings, could only be carried out in 'dioestrus' where reduction in activity was significant for both emg A/hr ($p<0.01$) and amplitude ($p<0.05$) - see Table XXII.

When variations between electrode sites were examined recordings from the uterine body showed a reduction in both emg A/hr and amplitude compared with the horn sites. This difference was either not significant ('dioestrus'), or could not be statistically evaluated ('anoestrus' and 'oestrus') (Appendix 17A).

TABLE XVII JO

Mean % emg, periods of activity, periods of relative inactivity and emg amplitudes in active periods during 'anoestrus', 'oestrus' and 'dioestrus'.

	% emg A/hr	active periods min	periods relative inactivity min	amplitude μV
No treatment 'Anoestrus'	[a] 52 ± 25(11)	[c] 16 ± 5(27)	16 ± 13(27)	[e] 193 ± 51(11)
Oestradiol 'Oestrus'	[ab] 36 ± 19(29)	[cd] 6 ± 2(43)	12 ± 6(43)	[f] 218 ± 15(29)
Progesterone 'Dioestrus'	[b] 54 ± 13(10)	[d] 13 ± 11(30)	11 ± 7(30)	[ef] 135 ± 11(10)

TABLE XVIII JO

Mean % emg A, and emg amplitudes after 1mg, 2.5mg and 10mg oestradiol

Oestradiol dose	% emg A/hr		amplitude μV	
1mg	33 ± 11	(6)	208 ± 80	(6)
2.5mg	40 ± 11	(7)	271 ± 54	(7)
10mg	36 ± 23	(16)	198 ± 89	(16)

TABLE XIX JO

Mean % emg A and amplitudes in 'anoestrus', 'oestrus' and 'dioestrus' under PG influence and no treatment.

	% emg A/hr		amplitude μV	
'ANOESTRUS'				
No treatment	[a]52 ± 25	(11)	193 ± 51	(11)
PG	[a]74 ± 5	(3)	125 ± 25	(3)
'OESTRUS'				
No treatment	[b]36 ± 19	(29)	218 ± 15	(29)
PG	[b]66 ± 20	(3)	228 ± 42	(3)
'DIOESTRUS'				
No treatment	[c]54 ± 13	(10)	135 ± 11	(10)
PG	[c]79 ± 18	(6)	118 ± 33	(6)

TABLE XX JO

Mean % emg A and amplitude in 'anoestrus', 'oestrus' and 'dioestrus' under oxytocin (5 IU) influence and no treatment.

	% emg A/hr		amplitude μV	
'ANOESTRUS'				
No treatment	[a]52 ± 25	(11)	193 ± 51	(11)
Oxytocin	[a]74 ± 25	(14)	190 ± 75	(14)
'OESTRUS'				
No treatment	[b]36 ± 19	(29)	218 ± 15	(29)
Oxytocin	[b]50 ± 32	(17)	212 ± 56	(17)
'DIOESTRUS'				
No treatment	[c]54 ± 13	(10)	135 ± 11	(10)
Oxytocin	[c]74 ± 24	(22)	124 ± 51	(22)

TABLE XXI JO

Mean % emg A and amplitude in 'oestrus' and 'dioestrus' under the influence of clenbuterol treatment and no treatment.

	% emg A/hr		amplitude μV	
'OESTRUS'				
No treatment	[a] 36 ± 19	(29)	[b] 218 ± 15	(29)
Clenbuterol	[a] 20 ± 8	(3)	[b] 83 ± 17	(3)
'DIOESTRUS'				
No treatment	54 ± 13	(10)	135 ± 11	(10)
Clenbuterol	26	(1)	67	(1)

TABLE XXII JO

Mean % emg A and amplitude in 'anoestrus', 'oestrus' and 'dioestrus' under the influence of propantheline bromide treatment and no treatment.

	% emg A/hr		amplitude μV	
'ANOESTRUS'				
No treatment	51 ± 26	(11)	193 ± 51	(11)
Propantheline	23	(1)	117	(1)
'OESTRUS'				
No treatment	36 ± 19	(29)	218 ± 15	(29)
Propantheline	7	(1)	118	(1)
'DIOESTRUS'				
No treatment	[a] 54 ± 13	(10)	[b] 135 ± 11	(10)
Propantheline	[a] 15 ± 12	(6)	[b] 43 ± 41	(6)

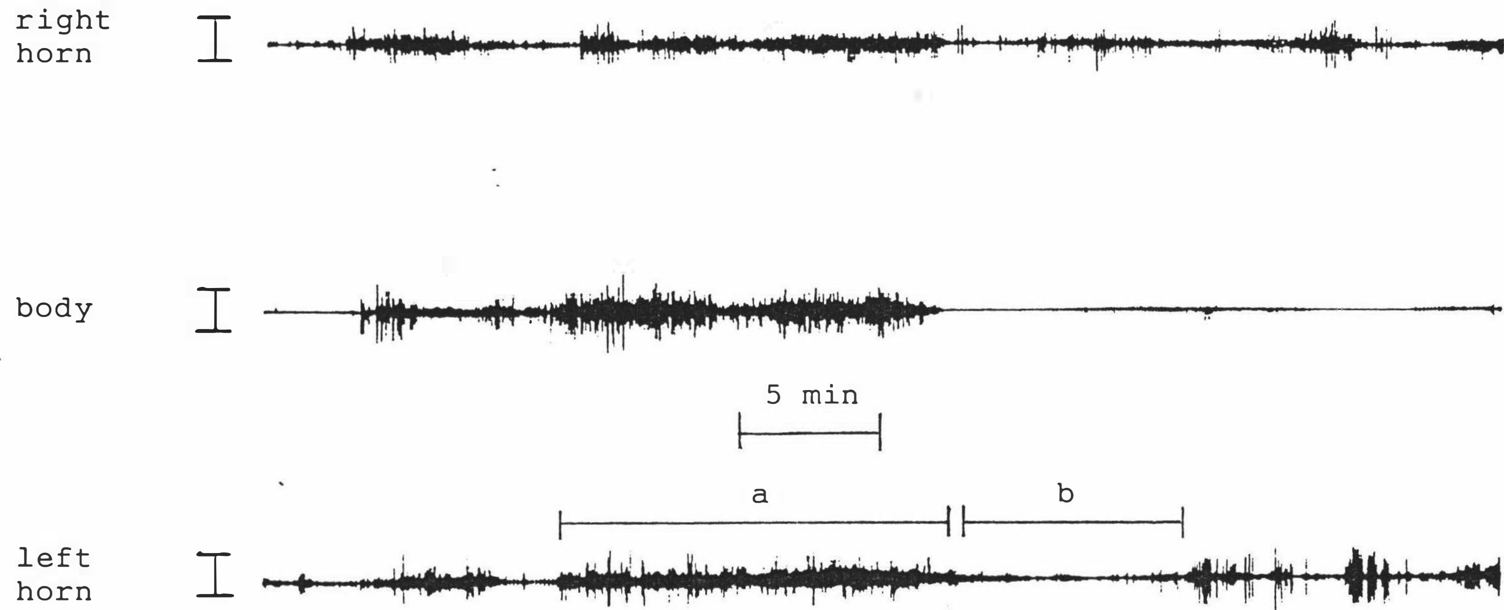


Fig. 16: Jo - Periods of emg activity (a) and relative inactivity (b) in 'anoestrus'. The vertical bars on the left represent a calibration of 200µv.

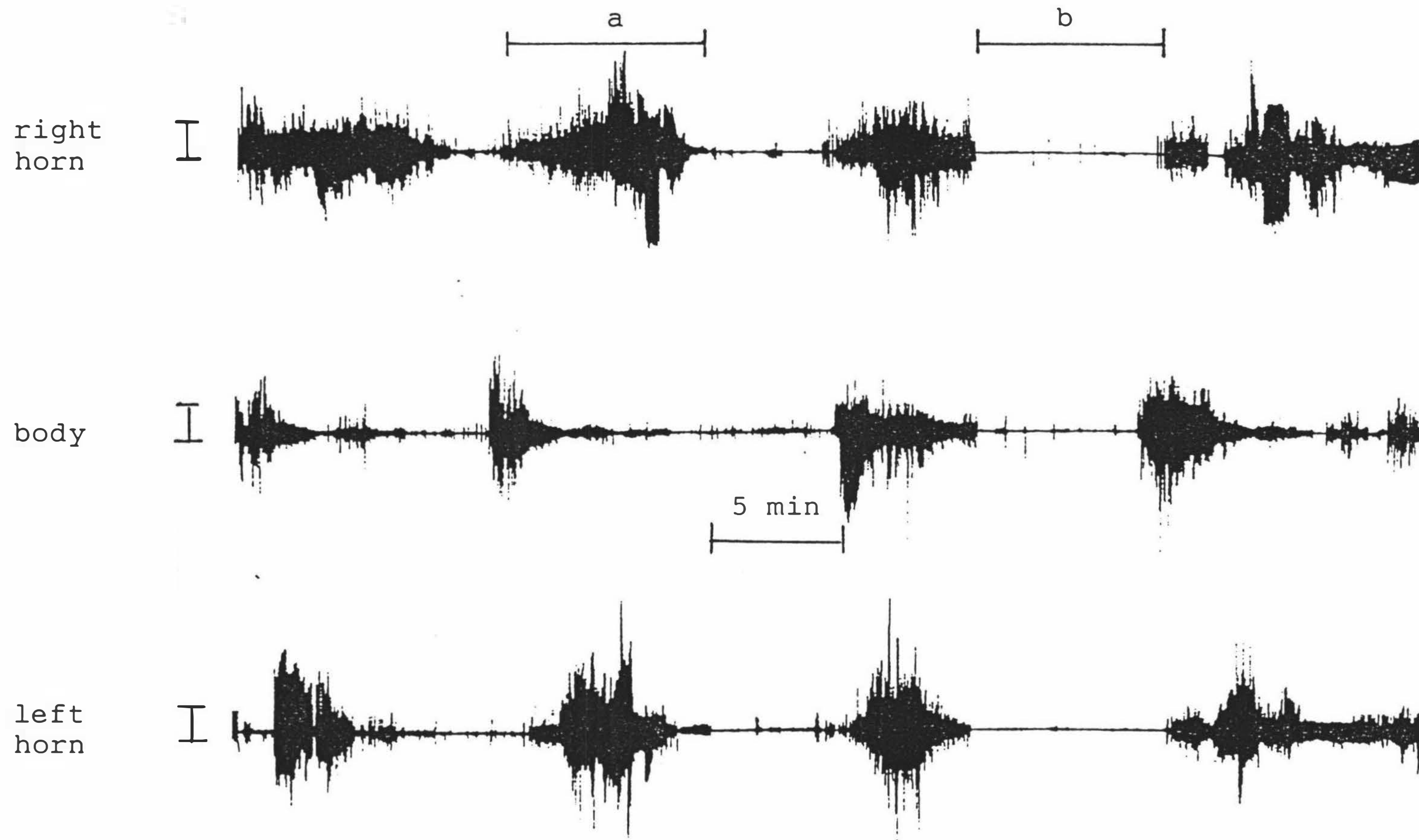


Fig. 17: Jo - Pattern of emg activity (a) and relative inactivity (b) during 'oestrus'. Note the crescendo-decrescendo changes in amplitude during active periods. The vertical bars on the left represent a calibration of 200μv.

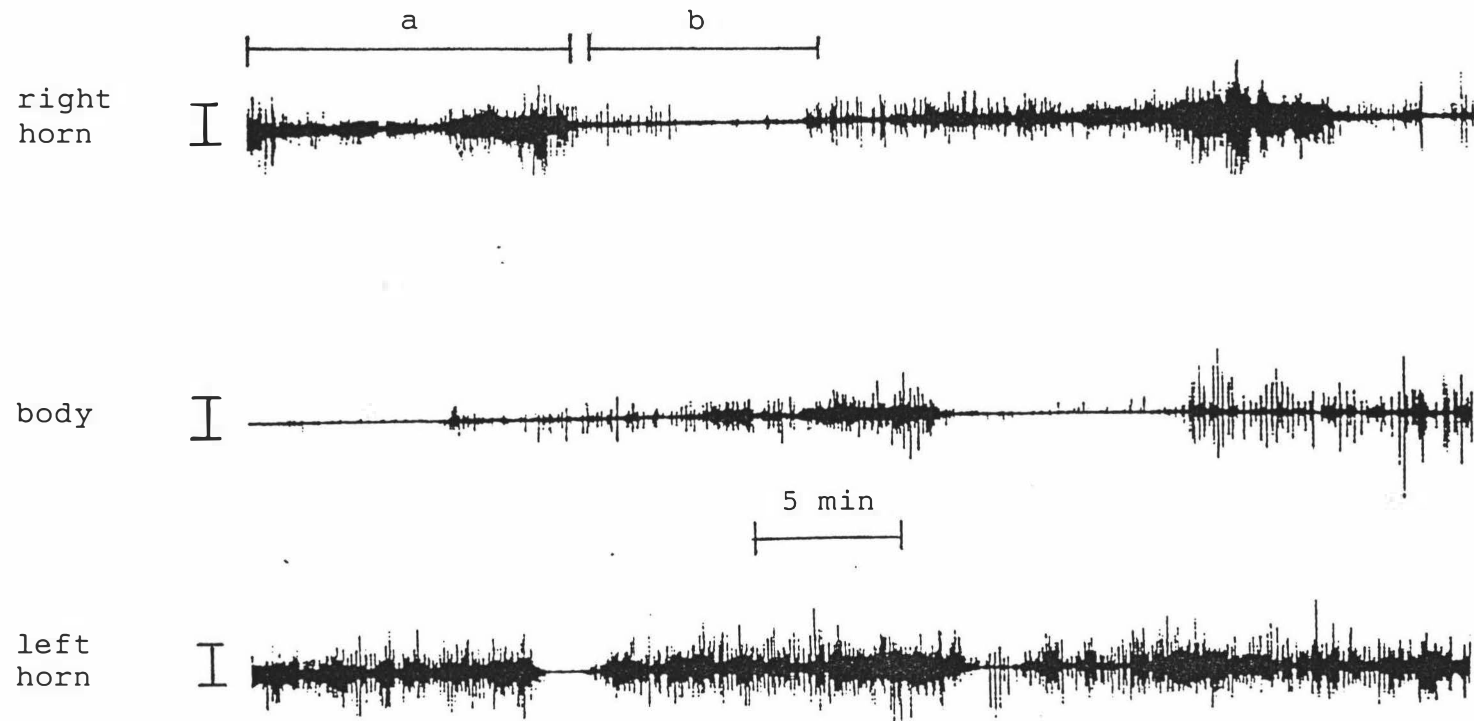


Fig. 18: Jo - Patterns of emg activity (a) and relative inactivity (b) during 'dioestrus'. The vertical lines on the left represent a calibration of 200µv.

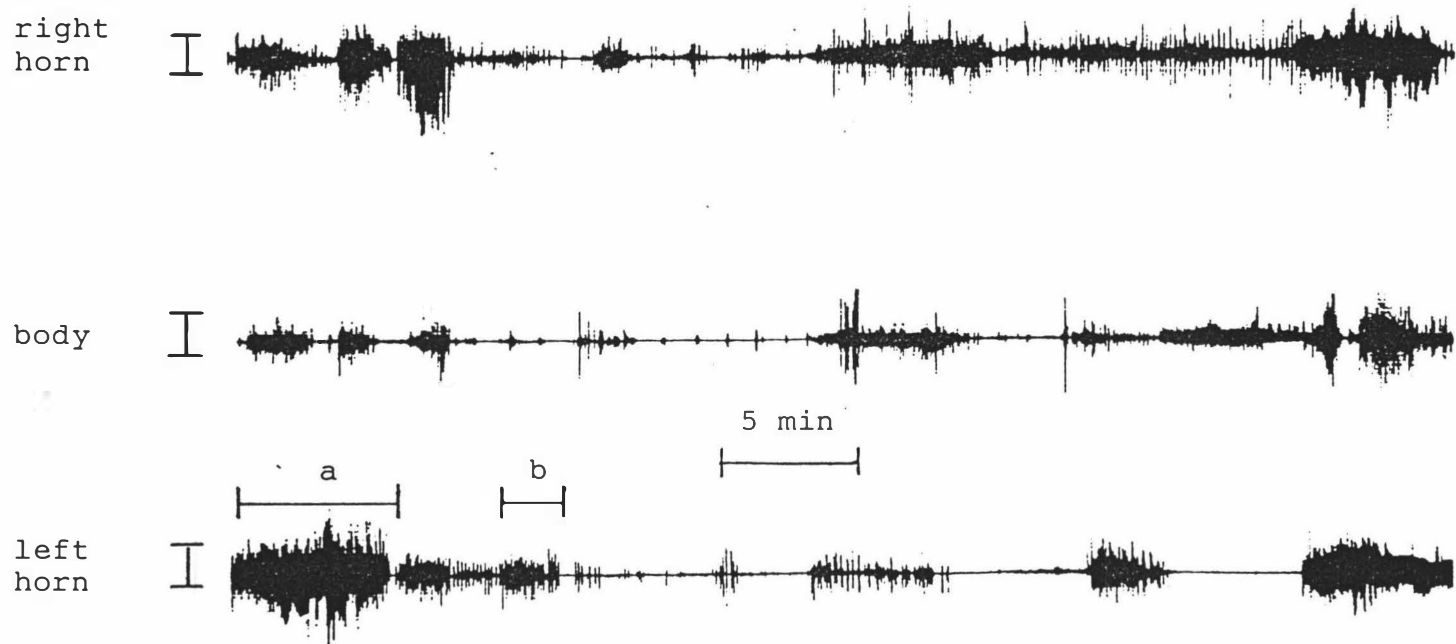


Fig. 19: Jo - Pattern of emg activity after PG administration 8 min previously in 'oestrus'. Note initial prolonged activity (a) followed by a shorter burst pattern (b). The vertical bars on the left represent a calibration of 200 µv.

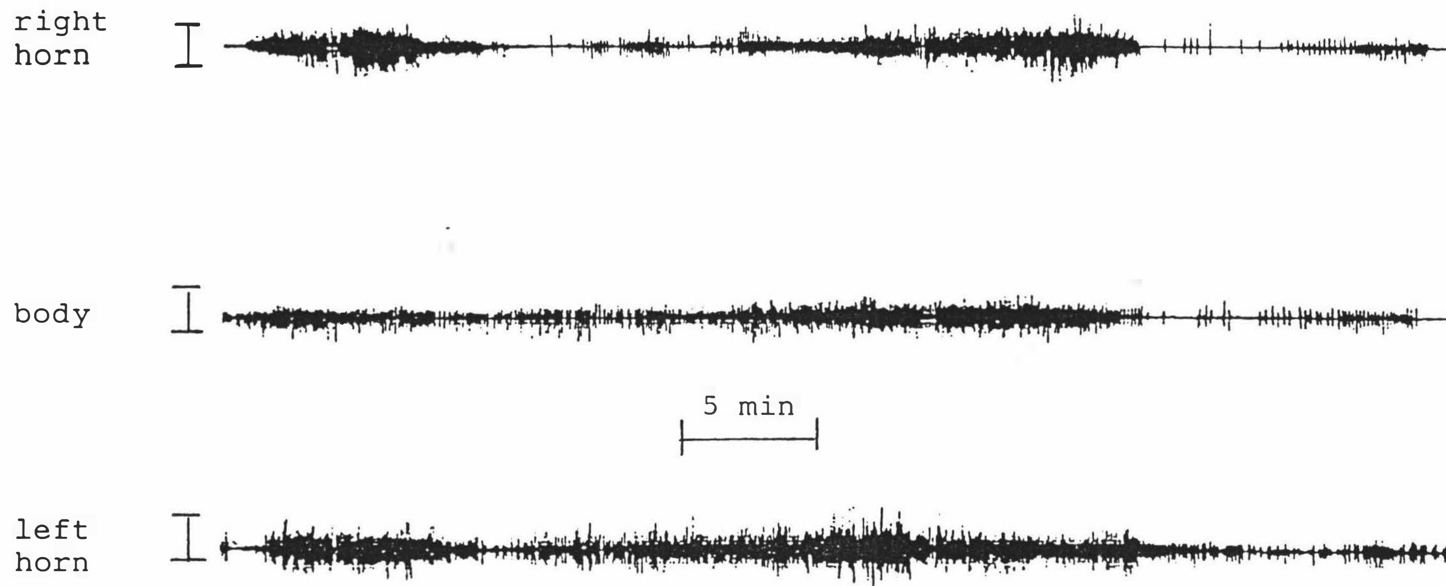


Fig. 20: Jo - Pattern of emg activity 13 min after PG administration in 'anoestrus'. Note prolonged emg activity with a minimum of burst activity. The vertical bars on the left represent a calibration of 200µv.

Fig. 21: Jo - Pattern of emg activity 15 min after PG administration in 'dioestrus'. Note prolonged emg activity with a minimum of burst activity. The vertical bars on the left represent a calibration of 200µv.

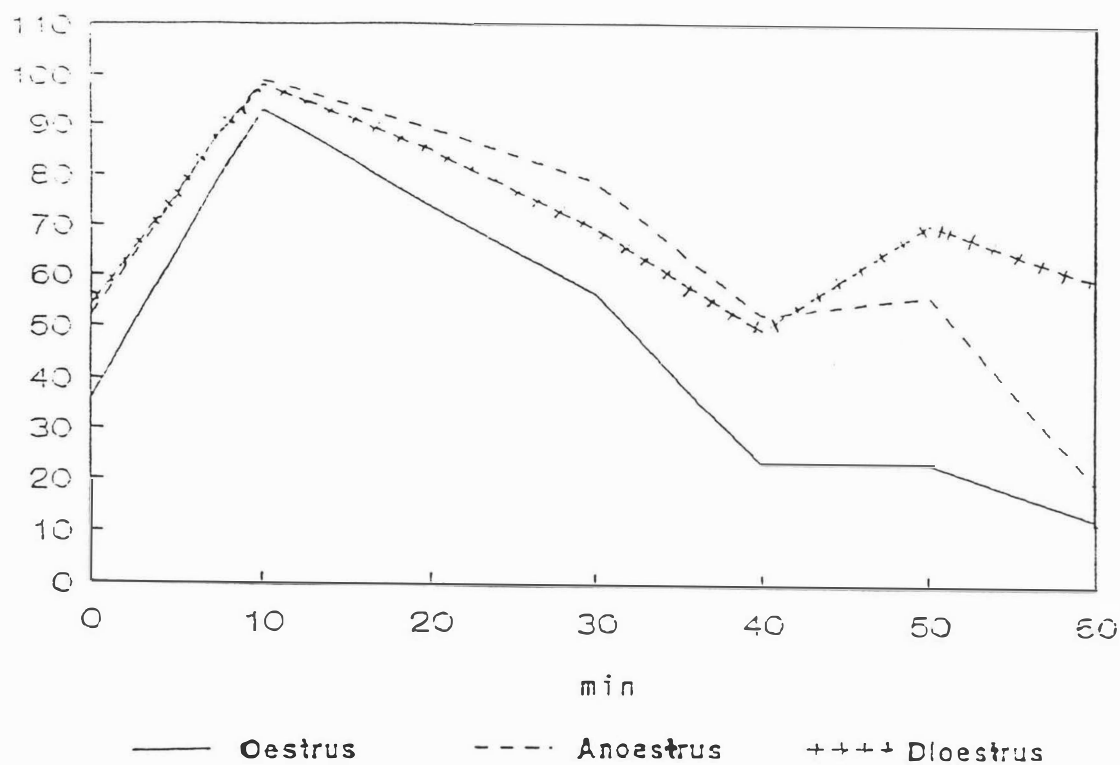


Fig. 22: Jo - Emg A/hr values for consecutive 10 minute intervals after administration of 5 IU oxytocin during 'oestrus', 'anoestrus' and 'dioestrus'.

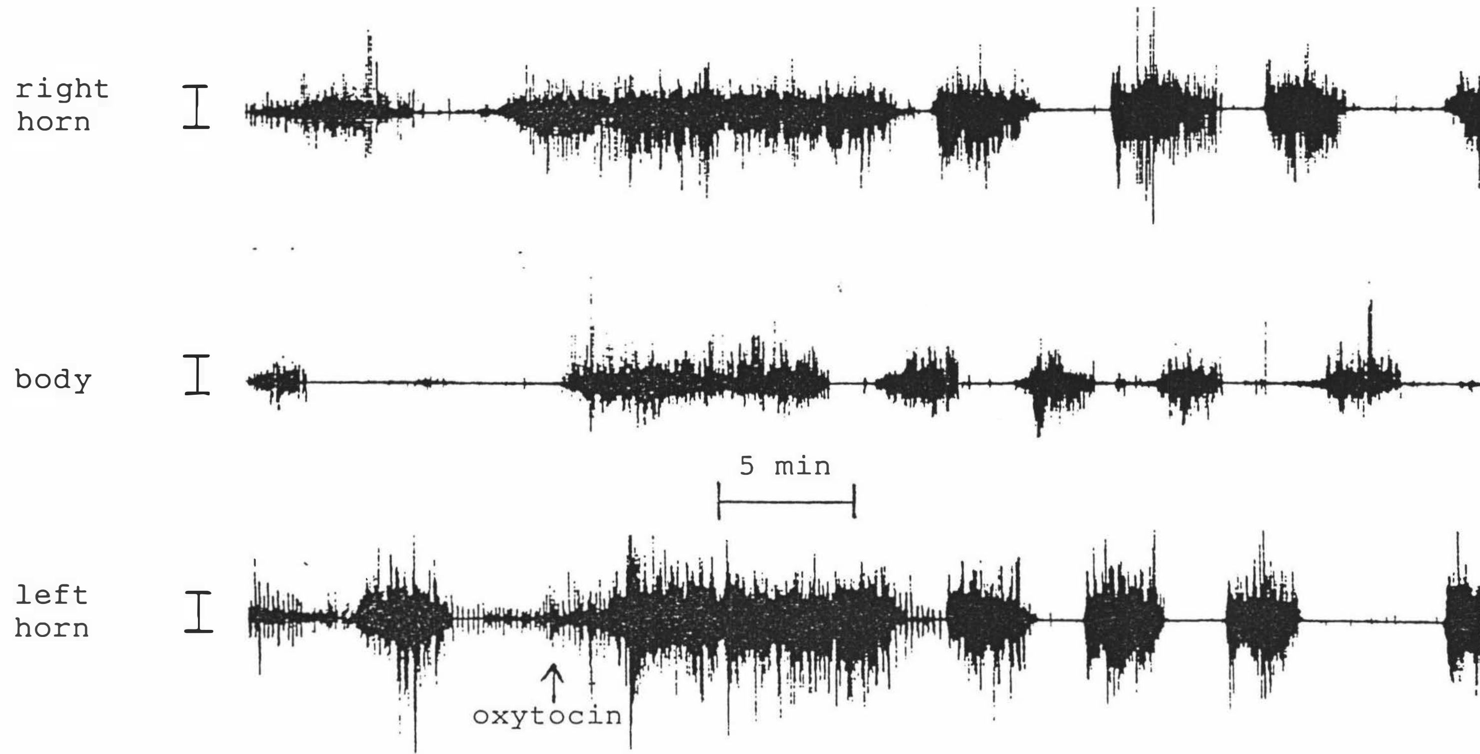


Fig. 23: Jo - Pattern of emg activity after intravenous administration of 5 IU oxytocin in 'oestrus'. Note the initial prolonged activity followed by a burst pattern. The vertical bars on the left represent a calibration of 200µv.

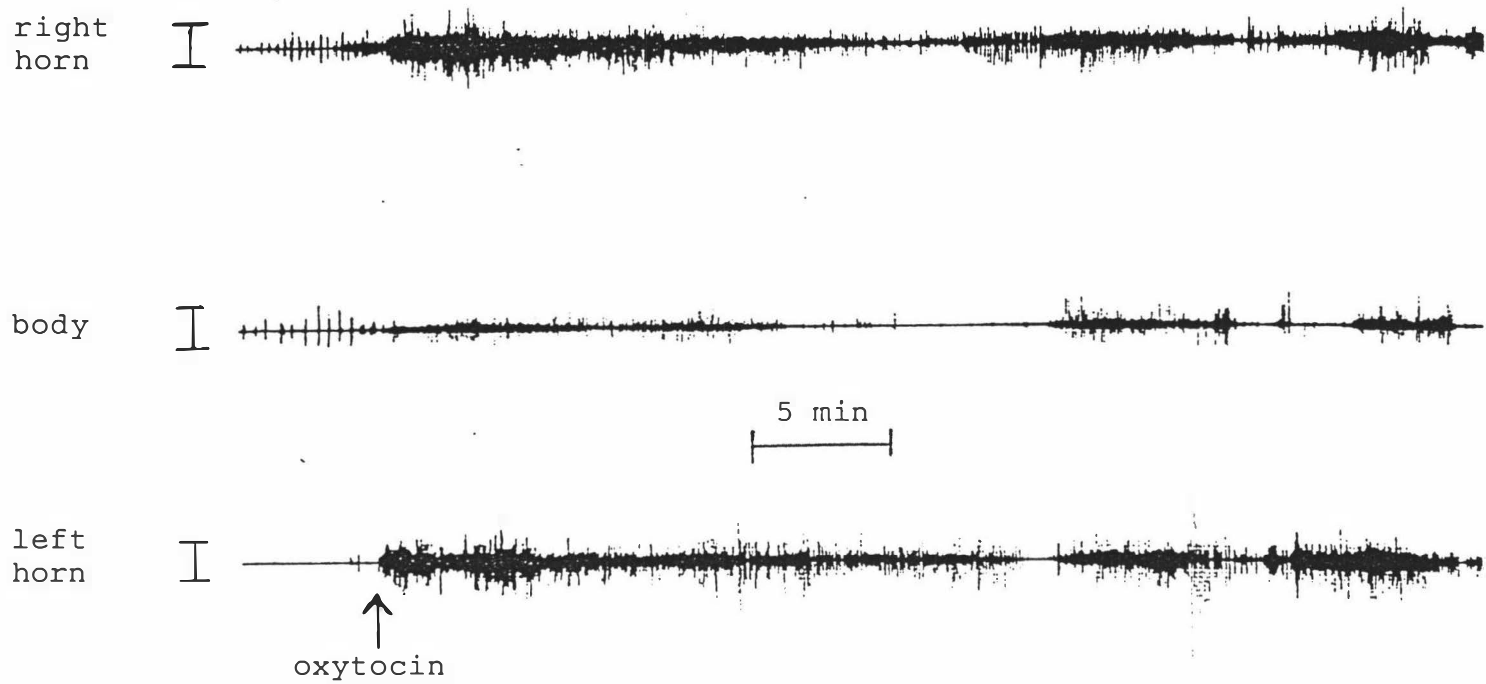


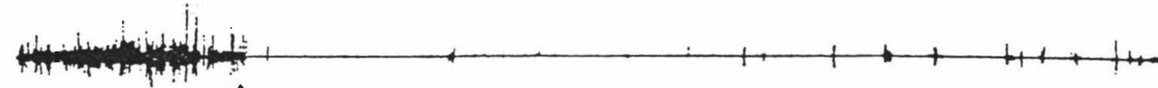
Fig. 24: Jo - Pattern of emg activity after intravenous administration of 5 IU oxytocin in 'dioestrus'. Note the prolonged emg activity with an absence of the burst pattern recorded in 'oestrus'. The vertical bars on the left represent a calibration of 200µv.

right
horn

body

5 min

left
horn



propantheline

Fig. 25: Jo - Pattern of emg activity after intravenous administration of propantheline bromide in 'dioestrus'. Note an almost total lack of emg activity shortly after propantheline administration. The vertical bars on the left represent a calibration of 200µv.

III.2.B: Snowy

(i) Simulated Cycle Stages

The stages of the cycle examined were as for Jo, 'anoestrus,' 'oestrus' and 'dioestrus'. Background emg activity was pronounced in this mare at all cyclic stages (Fig.26).

Emg A/hr varied little remaining at 31-32 ±23% at all stages of the cycle examined (Table XXIII). There was however a variation in activity pattern with periods of relative inactivity being significantly longer in 'anoestrus' than in either 'oestrus' or 'dioestrus' ($P<0.05$-Table XXIII) and amplitude being lowest during 'dioestrus' (83 ±29μv), highest in 'anoestrus' (176 ±46μv) and intermediate in 'oestrus' (143 ±16μv) ($p<0.05$-Table XXIII).

No significant variations were found in the parameters measured between electrode sites except that the right horn electrodes in 'oestrus' recorded a longer period of relative inactivity than did electrodes in the body ($p<0.05$-Appendix 18A).

No differences were noted in either emg A/hr or amplitude between the three doses of oestradiol used to simulate 'oestrus' (Table XXIV). Consistent crescendo - decrescendo amplitude patterns were not observed in 'oestrus' although the periods of relative inactivity appeared to have less background activity than during other stages. The plasma progesterone during simulated 'dioestrus' ranged from 8.8-10.5 ng/ml.

(ii) Drug Treatment (non steroid hormones)

(a) Prostaglandin : While administration of PG resulted in small increases in emg A/hr at all cycle stages these increases were not statistically significant (Table XXV). Emg A/hr during 'anoestrus' was greater in recordings from the left horn electrode than from other sites ($p<0.05$-Appendix 19A). Each injection of prostaglandin caused mild signs of colic and diarrhoea.

(b) Oxytocin: When Oxytocin at doses of 2 IU, 20 IU, 30 IU,and 50 IU was given intravenously to Snowy occasional sweating and signs of abdominal discomfort resulted.

There was a significant negative correlation between the oxytocin dose and emg A/hr ($r=-0.80, p<0.001$). No differences were noted following the 2 IU dose of oxytocin compared to no treatment, but at a dose of 20 IU (administered only in 'oestrus') both emg A/hr and amplitude were decreased for one hour ($p<0.001$-Table XXVI) and at doses of 30 IU and 50 IU (given only in 'anoestrus') there was no emg activity over the following hour (Table XXVI). During 'dioestrus' the uterine body electrodes recorded increased emg activity when compared with the right horn site ($P<0.01$-Appendix 20A).

(c) Clenbuterol: Records were available following clenbuterol administration twice during 'anoestrus' and 'dioestrus' and once during 'oestrus'. Significant reductions in both emg A/hr and amplitude were noted during 'anoestrus' ($p<0.05$, $p<0.001$-Table XXVII). There were reductions in emg A/hr during the other cycle stages and an increase in amplitude, but the values were either not significant ('dioestrus') or could not be statistically tested ('oestrus'). Electrode site variation was also either not significant ('anoestrus' and 'dioestrus') or unable to be statistically examined ('oestrus'). See Appendix 21A.

(d) Propantheline Bromide: Recordings following propantheline bromide administration were available only twice in 'anoestrus' and once in 'dioestrus'. Although emg A/hr was reduced on all occasions statistical evaluation was not possible in 'dioestrus' and only the left horn was recording during 'anoestrus'; increases in amplitude during both 'anoestrus' and 'dioestrus' were also noted following propantheline but again were either not significant ('anoestrus') or could not be statistically evaluated ('dioestrus') (Table XXVIII). Under the influence of propantheline there was little or no background emg activity observed.

Insufficient data were available from this mare to test for differences between electrode sites following propantheline administration (Appendix 22A).

TABLE XXIII SNOWY

Mean % emg A, periods of activity, periods of relative inactivity and emg amplitudes in active periods during 'anoestrus', 'oestrus' and 'dioestrus'.

	% emg A/hr	active periods min	periods relative inactivity min	amplitude µV
No treatment 'Anoestrus'	31 ± 16(14)	6.5 ± 5(36)	[bc] 18 ± 16(36)	[a] 176 ± 46(14)
Oestradiol 'Oestrus'	31 ± 12(15)	5.2 ± 5(22)	[b] 9 ± 5(22)	[a] 143 ± 16(15)
Progesterone 'Dioestrus'	32 ± 23(4)	17.5 ± 26(4)	[c] 5 ± 4(4)	[a] 83 ± 29(4)

TABLE XXIV SNOWY

Mean % emg A, and emg amplitudes after 1mg, 2.5mg and 10mg oestradiol.

Oestradiol dose	% emg A/hr	amplitude µV
1mg	32 ± 9 (6)	149 ± 13 (6)
2.5mg	33 ± 17 (6)	136 ± 20 (6)
10mg	27 ± 5 (3)	150 ± 10 (3)

TABLE XXV SNOWY

Mean % emg A and emg amplitude during 'anoestrus', 'oestrus' and 'dioestrus' under PG treatment and no treatment.

	% emg A/hr		amplitude μV	
'ANOESTRUS'				
No treatment	31 ± 16	(14)	176 ± 46	(14)
PG	39 ± 10	(4)	177 ± 4	(4)
'OESTRUS'				
No treatment	31 ± 12	(15)	143 ± 16	(15)
PG	36 ± 33	(4)	125 ± 9	(4)
'DIOESTRUS'				
No treatment	32 ± 23	(4)	83 ± 29	(4)
PG	39 ± 28	(4)	72 ± 30	(4)

TABLE XXVI SNOWY

Mean % emg A and amplitude in 'anoestrus', 'oestrus' and 'dioestrus' after varying doses of oxytocin (2 IU, 20 IU, 30 IU, 50 IU) and no treatment.

	% emg A/hr		amplitude μV	
'ANOESTRUS'				
No treatment	[ab] 31 ± 16	(4)	[cd] 176 ± 46	(14)
30 IU oxytocin	[a] 0	(18)	[c] 0	(18)
50 IU oxytocin	[b] 0	(21)	[d] 0	(21)
'OESTRUS'				
No treatment	[a] 31 ± 12	(15)	[c] 143 ± 16	(15)
2 IU oxytocin	[b] 30 ± 12	(4)	[d] 169 ± 12	(4)
20 IU oxytocin	[ab] 3 ± 9	(10)	[cd] 15 ± 47	(10)
'DIOESTRUS'				
No treatment	32 ± 23	(4)	83 ± 29	(4)
2 IU oxytocin	48 ± 30	(13)	113 ± 47	(13)

TABLE XXVII SNOWY

Mean % emg A and amplitude in 'anoestrus', 'oestrus' and 'dioestrus' after clenbuterol treatment and no treatment.

	% emg A/hr		amplitude µV	
'ANOESTRUS'				
No treatment	[a] 31 ± 16	(14)	[b] 176 ± 46	(14)
Clenbuterol	[a] 3 ± 4	(2)	[b] 25 ± 35	(2)
'OESTRUS'				
No treatment	31 ± 12	(15)	143 ± 16	(15)
Clenbuterol	17	(1)	150	(1)
'DIOESTRUS'				
No treatment	32 ± 23	(3)	83 ± 29	(3)
Clenbuterol	18 ± 4	(2)	113 ± 12	(2)

TABLE XXVIII SNOWY

Mean % emg A and amplitude in 'anoestrus' and 'dioestrus' after propantheline bromide treatment and no treatment.

	% emg A/hr		amplitude μV	
'ANOESTRUS'				
No treatment	31 ± 16	(14)	176 ± 46	(14)
Propantheline	21 ± 13	(2)	200 ± 0	(2)
'DIOESTRUS'				
No treatment	31 ± 23	(3)	83 ± 29	(3)
Propantheline	11	(1)	113	(1)

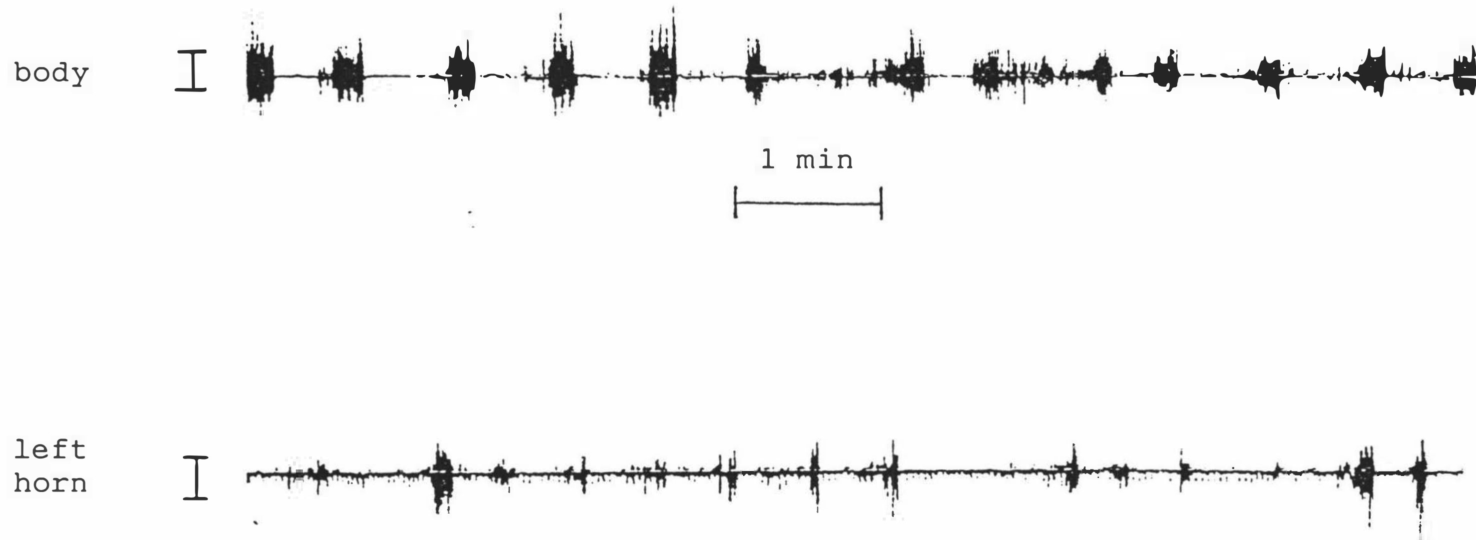


Fig. 26: Snowy - Pattern of emg background activity prominent during all cycle stages. Vertical bars on the left represent a calibration of 200µv.

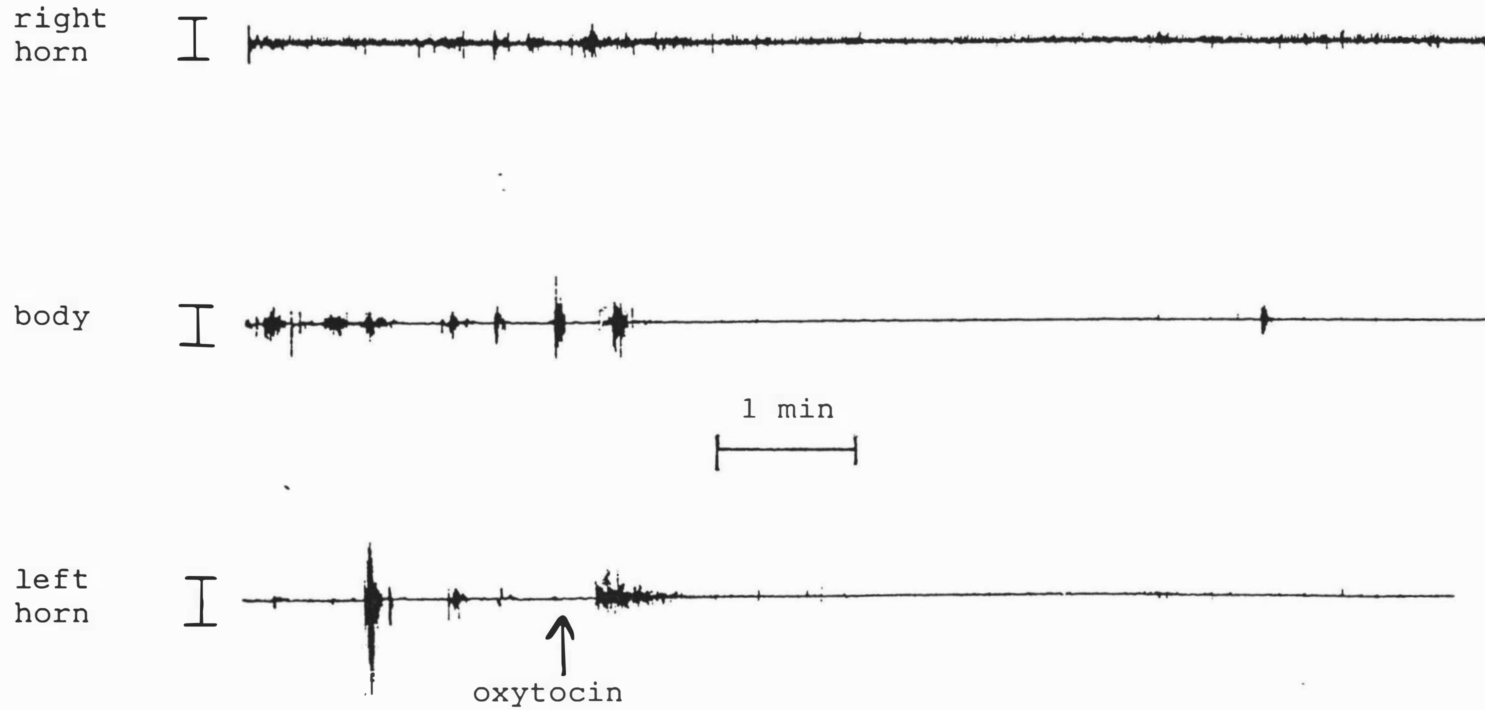


Fig. 27: Snowy - Emg pattern after administration of 50 IU intravenous oxytocin. Note reduction in emg activity. The vertical bars on the left represent a calibration of 200μv.

III.3 RESPONSES TO MISCELLANEOUS DISTURBANCES, RECTAL EXAMINATION AND UTERINE STRETCH USING DISTILLED WATER INFUSIONS:ALL MARES

(i) Extraneous Influences

After an initial period of adjustment to the recording stall and ordinary stable routine, including the presence of the author, no, or only very temporary (1-3min) variation in emg activity or amplitude was noted when a disturbance (e.g. arrival of new horse in clinic yard, entrance of examiner into stall, traffic or voices) in the mares routine occurred. More vigorous attempts to disturb the mares by the author (yelling, waving arms next to the subject) elicited only a minor emg burst (less than 1 minute) when carried out during a quiet period.

(ii) Rectal Examination

Rectal examination was performed as described in materials and methods. Rectal palpation stimulated myometrial emg activity for the period of the rectal and for up to 2 min after removal of the examiners hand. When performed during a quiet phase a brief active phase was stimulated (Fig.30b). This response could also be invoked when the uterus was under the influence of the relaxant propantheline bromide. When performed during an active phase, the amplitude and density of the emg spikes increased for the duration of the examination.

(iii) Uterine Stretch

There were two characteristic responses to the infusion of distilled sterile water into the uterus. The first was the generation of single spike activity from one or more electrode sites 76% of the time. This activity occurred with amounts of fluid ranging from 60 -1000 ml. The second was a lack of emg response to infusion of a second litre of saline immediately after evacuation of the first, and a tendency for the emg activity to remain reduced or absent for up to 15 min after evacuation of the second litre.

With Snowy the single spiking activity occurred after every initial sterile water infusion in both horns but was most pronounced in the left horn (Fig.28a), lasted 5-9 min, and reached an amplitude of 3-400μv with 20 - 40 single spikes/min. After infusion of a second litre there was a decreased emg response (Fig.28b) All infusions were done during 'anoestrus'. Vulvar washes with this mare also frequently stimulated single spike activity of a lower amplitude (50-150μv) and frequency (10 - 20 spikes/min).

Jo was infused during 'anoestrus', 'oestrus' and 'dioestrus' for a total of 5 times. Single spiking activity did not occur in 'dioestrus'. On one occasion the litre of sterile water could not be evacuated and the single spiking activity occurred for the duration of the recording session (25 min), although spike frequency and amplitude decreased towards the end of the session. A second saline infusion immediately after evacuation of the first with this mare resulted in no emg response when done during a quiet phase (Fig.29a) and no change in established emg pattern when done during an active phase. Jo showed single spike activity on some occasions when vulvar washes were performed.

Fling had fluids infused once in oestrus and 5 times in anoestrus. There was no response to one 180ml oestral infusion and one 180ml anoestral treatment. On the other occasions she responded to amounts varying between 60 - 1000 ml. The single spike activity occurred mainly in the right horn and body (average of 33 spikes/min, 3-500μv amplitude) and lasted about 6 min post-infusion. The addition of a second litre elicited little emg response and the uterine emg remained minimal (Fig.29b).

On two occasions Fling responded to a vulvar wash with single spike activity. Infusions were carried out only during oestrus in Sweetie. Single spikes were generated on 2 of 3 occasions with 1 litre amounts. Spiking activity was most pronounced in the right horn (500μv, 52 single spikes/min) lasting between 3-6 min. On the one occasion that single spiking activity was not elicited the pre-infusion emg activity continued unchanged during the procedure.

Infusion of a second litre of sterile water generated emg activity during infusion only; all electrode sites then became quiescent. There were no emg changes associated with a vulvar wash in this mare.

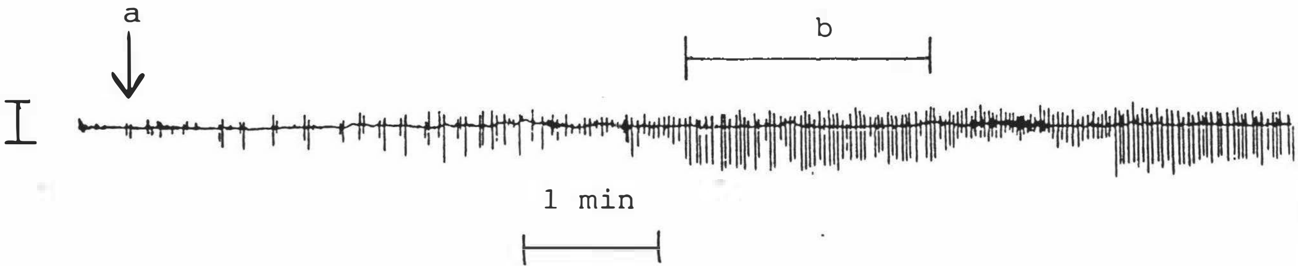


Fig. 28a: Snowy - Emg response of the left horn electrode site to vulvar wash (a) and intrauterine infusion of 1 litre of sterile distilled water (b). Note the initiation of single spike activity. The vertical bar on the left represents a calibration of 200µv.

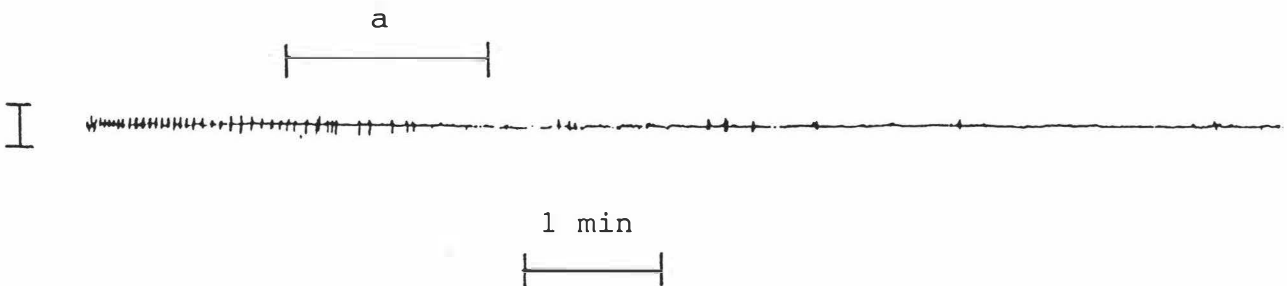


Fig. 28b: Snowy - Emg response of the left horn electrode site after intrauterine infusion of a second litre of sterile distilled water (a). Note the lack of emg activity. The vertical bar on the left represents a calibration of 200µv.

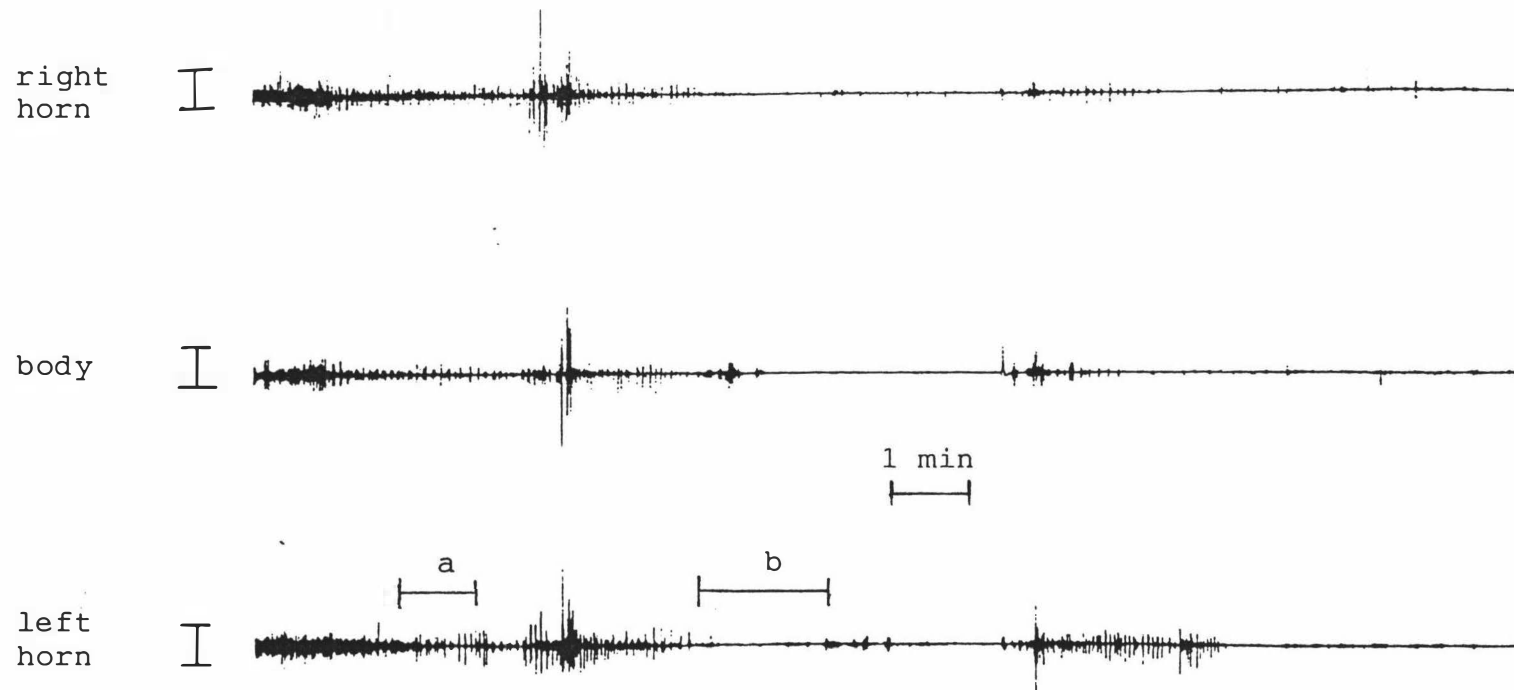


Fig. 29a: Jo - Emg response of the myometrium to infusion of sterile distilled water in 'oestrus'. Note the increase in single spike activity after infusion of the first litre (a) and minimal response to infusion of a second litre (b). The vertical bars on the left represent a calibration of 200µv.

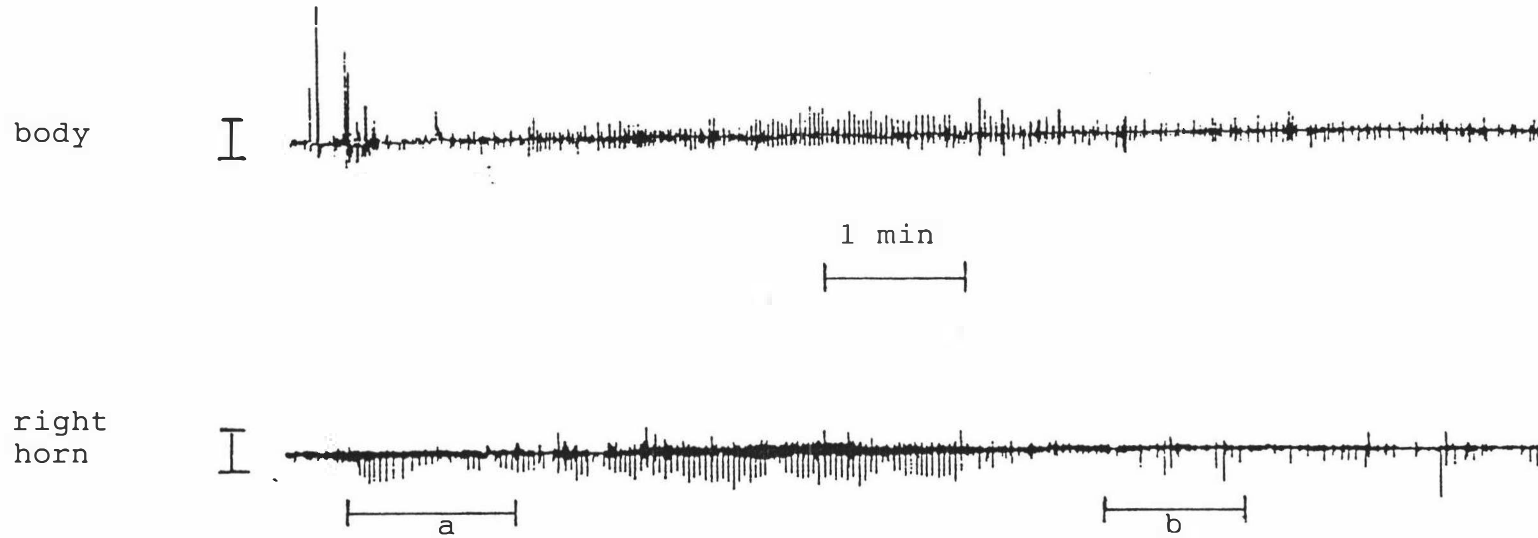


Fig. 29b: Fling - Pattern of emg activity after infusion of sterile distilled water. Note the increase in single spike activity with infusion of the first litre (a) and a decrease in activity with the second litre (b). The vertical bars on the left represent a calibration of 200µv.

III.4 SUMMARY Of EMG RESULTS

(a) All mares exhibited background emg activity during the periods of relative inactivity; this background emg decreased during oestrus or simulated 'oestrus'.

(b) In anoestrus no overall mare pattern was seen in terms of emg parameters.

(c) During transition the emg A/hr, average length of an active period and emg amplitude for the two intact mares (Sweetie and Fling) were similar.

(d) During oestrus (or simulated 'oestrus') all mares (except Snowy) had lower emg A/hr, and average length of an active period than in dioestrus whereas emg amplitude was in all cases higher in oestrus than in dioestrus.

(e) During oestrus all mares (except Snowy) had an emg record characterized by bursts of activity lasting 3-6 min and exhibiting a crescendo - decrescendo amplitude pattern; the typical pattern began at about 50μv, rose to 5-700μv, diminished again to about 50μv, variations on this were common. Furthermore the active periods during oestrus tended to occur synchronously at all electrode sites.

(f) Through dioestrus the emg pattern changed such that the length of the active periods increased while emg amplitude decreased and remained more constant; only occasional randomly occurring high amplitude spikes were noted during dioestrus.

(g) In all mares (except Snowy) there was an increase in emg A/hr that occurred within 5-10 min following PG administration; in most cases emg amplitude was unaffected.

(h) In the one mare that was examined at different cycle stages for a prolonged period following PG administration (Sweetie), emg A/hr remained elevated over non-treatment levels for at least 3 hrs.

(i) The response to oxytocin appeared to be dose dependent. A dose of 5 IU increased emg A/hr in all mares (except Snowy) within 1 min of injection while amplitude was usually unaffected. As the dose was increased emg A/hr tended to remain the same or decrease.

(j) When emg activity within treatment following oxytocin administration was examined emg A/hr values showed the quickest decline during oestrus (returning to normal pre-treatment levels within 30-40 min), and the slowest rate of decline during dioestrus often not reaching pre-treatment values by 50 - 60 min following administration.

(k) Although emg A/hr decreased after propantheline administration, falls recorded were only significant during dioestrus. Amplitude also tended to reduce, particularly during dioestrus, but there was some variation in response to this drug depending on the mare.

(l) Responses to clenbuterol administration were relatively small and only occasionally were significant differences from no treatment values observed. The trend was towards a reduced emg A/hr and a reduction in amplitude following drug administration.

(m) All mares (except Snowy) showed some significant electrode site variation in emg parameters during cyclical changes. Even when the variation was not statistically significant there was often a tendency for the same electrode site in an individual mare to vary consistently. Variation in electrode site response was sometimes affected by treatment in all mares.

III.5 INTRA-UTERINE PRESSURE

III.5.A INTRODUCTION

The question arose as to whether the method used to measure IUP in the mares examined during this investigation was actually achieving this aim. Goddard *et al.* (1985) identified a respiratory pattern in the IUP tracings from their experimental mares that consisted of rhythmic baseline fluctuations seen during periods of no wave activity. Such a respiratory pattern could be readily identified in all mares involved in this study (Fig.30a). In addition these same authors noted sharp pressure rises during urination - these were also observed in this investigation. Uterine manipulations (e.g. rectal palpation and infusion of fluids into the uterus) would be expected to vary intra-uterine pressure. In the experiments reported here uterine palpation per rectum (Fig.30b) and intra-uterine infusion of fluids (Fig.30c) raised IUP. All of these observations were considered to be evidence that the method used was achieving the objective stated.

III.5.B: CORRELATION OF IUP INDICES TO EMG A/HR AND AMPLITUDE

Because of the small number of IUP observations, data from all mares were combined and the relationship between both emg A/hr and emg amplitude was compared with the IUP indices. During dioestrus (natural and simulated) there was a positive correlation between emg A/hr and both IUP amplitude rise ($r=0.46, p<0.05$)) and cycle duration ($r=0.50, p<0.05$). No significant correlations between any other parameter were found at any cycle stage. Visually there was no consistent correlation between IUP wave activity and emg activity (Fig.31 a,b,c).

III.5.C: COMPARISON OF CYCLE STAGES

(i) Individual Mare Comparisons

Oestrous cycle stages were compared for each mare where enough data were present for statistical evaluation. The only significant difference between cycle states occurred with Sweetie where the IUP duration was longest in dioestrus ($p<0.05$-Table XXIXa) (Fig.32 a,b).

When sufficient data existed to make comparisons between mares no significant differences were noted within cycle stages. It was noted however that Snowy had the highest values and Jo the lowest in all IUP parameters in 'dioestrus' - both these mares were spayed (Table XXX).

(ii) All Mares Combined

As there was no statistical difference between mares within each cycle stage IUP data from all mares were combined. There was no significant difference in mean cycle frequency between transition, oestrus or dioestrus ($p>0.1$). However in anoestrus the mean cycle frequency was significantly higher than in transition ($p<0.05$) (Table XXIXb).

The mean amplitude of IUP cycles was significantly greater in dioestrus than in oestrus and anoestrus ($p<0.01$) and cycle duration was significantly longer in anoestrus than in either transition or oestrus ($p<0.01$) (Table XXIXb).

III.5.D: DRUG TREATMENT INFLUENCED ACTIVITY (NON STEROID HORMONE)

Data for individual mares are given in Appendix C. Due to the small numbers of satisfactory observations for each mare, data were pooled.

(i) Correlation Between IUP Indices, Emg A/hr, And Amplitude

Emg parameters were compared with IUP parameters following administration of uterine stimulants and relaxants. Under the influence of oxytocin, a number of positive correlations were observed: that between emg A/hr with IUP cycle frequency was $r= 0.32$, with cycle duration $r=0.48$, and with IUP amplitude $r=0.39$ ($p<0.001$). Emg amplitude was also positively correlated with IUP cycle duration $r=0.20$ and with IUP amplitude rise $r=0.19$ ($p<0.05$). When propantheline bromide was given there was a positive correlation between emg A/hr and duration of the IUP cycles ($r=0.63, p<0.001$). No significant correlations between emg parameters and IUP indices occurred when either prostaglandin or clenbuterol was administered.

(ii) Effect Of Individual Drugs On IUP indices (mares combined)

(a) **Prostaglandin:** When IUP parameters were examined for

response to prostaglandin treatment with all cycle stages combined (Table XXXI) there was a higher mean cycle frequency and mean cycle duration after treatment compared with no treatment ($p<0.001$). No differences in amplitude rise were noted.

Comparison of IUP with prostaglandin response between cycle stages revealed that the only significant response was between dioestrus and oestrus in IUP amplitude rise with the response in dioestrus being higher ($p<0.05$-Table XXXII).

(b) Oxytocin: When all cycle stages were combined there was an increase in IUP cycle frequency and duration under the influence of oxytocin when compared with no treatment ($p<0.001$) (Table XXXIII); amplitude rise on the other hand was less after oxytocin administration than during untreated cycles ($p<0.001$).

Comparisons of drug effect on IUP parameters within oestrous cycle stages indicated that rises in IUP cycle frequency and amplitude were lower during transition than during anoestrus ($p<0.05$ for cycle frequency) or during anoestrus, oestrus or dioestrus ($p<0.05$ for amplitude) - Table XXXIV.

(c) Propantheline Bromide: After propantheline administration, with all cycle stages combined, the IUP cycle frequency was lower and both IUP amplitude rise and cycle duration were significantly reduced ($p<0.001$ and $p<0.01$ respectively for the latter two parameters -Table XXXV).

All IUP indices remained at baseline pressure during anoestrus, i.e. no IUP waves occurred (Table XXXVI). However, IUP amplitude rise was significantly greater during transition than at other stages($p<0.01$-Table XXXVI)), as was the IUP cycle duration ($p<0.05$-Table XXXVI).

(d) Clenbuterol: After clenbuterol treatment there was a fall in IUP cycle frequency and significant falls in IUP amplitude and cycle duration ($p<0.001$-Table XXXVII). No differences in IUP parameters were noted between the various cycle stages (Table XXXVIII).

TABLE XXIXa

Frequency, duration and amplitude of IUP cycles for the intact mares (Sweetie and Fling) and the spayed mares (Jo and Snowy) during natural and simulated stages of the oestrous cycle.

	Frequency cycles/min		Mean amplitude rise mm Hg	Duration min/10min	
SWEETIE					
Transition	0.6 ± 0.47	(25)	14 ± 2.8 (25)	[a]1.2 ± 0.75	(25)
Oestrus	0.7 ± 0.02	(4)	13 ± 1.3 (4)	[b]1.1 ± 0.37	(4)
Dioestrus	0.6 ± 0.17	(6)	16 ± 2.9 (6)	[a b]2.3 ± 1.00	(6)
FLING					
Anoestrus	0.5	(1)	10 (1)	3.2	(1)
Oestrus	0.7 ± 0.35	(21)	12 ± 1.9 (21)	1.6 ± 0.70	(21)
Dioestrus	0.5 ± 0.50	(2)	15 ± 3.0 (2)	1.2 ± 0.80	(2)
JO					
'Anoestrus'	2.4 ± 0.8	(2)	13 ± 2.8 (2)	3.1 ± 2.00	(2)
'Oestrus'	0.9 ± 0.5	(2)	11 ± 2.2 (2)	2.3 ± 2.00	(2)
'Dioestrus'	0.3	(1)	10 (1)	0.7	(1)
SNOWY					
'Oestrus'	1.4 ± 1.7	(8)	7.8 ± 9 (8)	2.6 ± 3.5	(8)
'Dioestrus'	3.0	(1)	25 (1)	4.0	(1)

TABLE XXIXb

Frequency, duration and amplitude of IUP cycles in natural anoestrus, oestrus and dioestrus (Sweetie and Fling) and simulated 'anoestrus', 'oestrus' and 'dioestrus' (Jo and Snowy) for all mares combined.

	Frequency cycles/10 min		Mean amplitude rise mm. Hg		Duration min/10 min	
ANOESTRUS	[a] 1.8 ±1.3	(3)	[d] 12 ± 3	(3)	[fg] 3.0 ± 1.5	(3)
TRANSITION	[a] 0.6 ± 0.5	(25)	14 ± 3	(25)	[f] 1.2 ± 0.8	(25)
OESTRUS	0.7 ± 0.3	(27)	[e] 12 ± 2	(27)	[g] 1.5 ± 0.8	(27)
DIOESTRUS	0.8 ± 0.4	(9)	[de] 15 ± 3	(9)	2.2 ± 1.0	(9)

TABLE XXX

Frequency, duration and amplitude of IUP cycles in natural anoestrus, oestrus and dioestrus (Sweetie and Fling) and simulated 'anoestrus', 'oestrus' and 'dioestrus' (Jo and Snowy) compared.

	Frequency cycles/10 min		Mean amplitude rise mm. Hg		Duration min/10 min	
ANOESTRUS						
Fling	0.5	(1)	10	(1)	3.2	(1)
Josephine	2.4 ± 0.8	(2)	13 ± 2.8	(2)	3.1 ± 2	(2)
TRANSITION						
Sweetie	0.6 ± 0.5	(25)	14 ± 3.0	(25)	1.2 ± 0.8	(25)
OESTRUS						
Sweetie	0.7 ± 0.02	(4)	13 ± 1.3	(4)	1.1 ± 0.4	(4)
Fling	0.7 ± 0.3	(21)	12 ± 2.0	(21)	1.6 ± 0.7	(21)
Jo	0.9 ± 0.5	(2)	11 ± 2.2	(2)	2.3 ± 2.0	(2)
Snowy	1.4 ± 1.7	(8)	8 ± 9.0	(8)	2.6 ± 3.5	(8)
DIOESTRUS						
Sweetie	0.8 ± 0.4	(6)	16 ± 3.0	(6)	2.3 ± 1.0	(6)
Fling	0.5 ± 0.5	(2)	15 ± 3.0	(2)	1.2 ± 0.8	(2)
Jo	0.3	(1)	10	(1)	0.7	(1)
Snowy	3.0	(1)	25	(1)	4.0	(1)

TABLE XXXI

Frequency, duration and amplitude of IUP cycles as influenced by PG compared to no treatment, all mares (Sweetie, Fling, Jo and Snowy) and natural and simulated cycle stages (anoestrus, transition, oestrus and dioestrus) combined.

	Frequency cycles/10 min		Mean amplitude rise mm. Hg		Duration min/10min	
No treatment	[a]0.7 ± 0.5	(64)	13 ± 3	(64)	[b]1.5 ± 0.9	(64)
PG	[a]1.4 ± 1	(42)	13 ± 3	(42)	[b]2.7 ± 2.4	(42)

TABLE XXXII

Frequency, duration and amplitude of IUP cycles as influenced by PG treatment during natural and simulated anoestrus, transition, oestrus and dioestrus all mares (Sweetie, Fling, Jo, Snowy) combined.

	Frequency cycles/10 min		Mean amplitude rise mm. Hg		Duration min/10 min	
ANOESTRUS	1.4 ± 1.4	(7)	13 ± 3	(7)	2.2 ± 20	(7)
TRANSITION	1.5 ± 1.0	(13)	13 ± 3	(13)	2.5 ± 1.3	(13)
OESTRUS	1.4 ± 1.3	(13)	[a]12 ± 5	(13)	3.0 ± 3.8	(13)
DIOESTRUS	1.2 ± 0.6	(9)	[a]17 ± 5	(9)	2.7 ± 0.1	(9)

TABLE XXXIII

Frequency, duration and amplitude of IUP cycles as influenced by oxytocin compared to no treatment, all mares (Sweetie, Fling, Jo and Snowy) and natural and simulated cycle stages (anoestrus, transition, oestrus and dioestrus) combined.

	Frequency cycles/10 min		Mean amplitude rise mm. Hg		Duration min/10 min	
No treatment	[a]0.69 ± .5	(64)	[b]13 ± 3	(64)	[c]1.5 ± 0.9	(64)
Oxytocin treatment	[a] 1.3 ± 1	(124)	[b]10 ± 6	(124)	[c]3.1 ± 2.8	(124)

TABLE XXXIV

Frequency, duration and amplitude of IUP cycles as influenced by oxytocin treatment during natural and simulated anoestrus, transition, oestrus and dioestrus all mares (Sweetie, Fling, Jo, Snowy) combined.

	Frequency cycles/10 min		Mean amplitude rise mm. Hg		Duration min/10 min	
ANOESTRUS	[a]1.8 ± 1.4	(16)	[b]13 ± 8	(16)	2.6 ± 2.5	(16)
TRANSITION	[a]0.8 ± 0.9	(28)	[bcd] 7 ± 7	(28)	2.5 ± 3	(28)
OESTRUS	1.4 ± 0.9	(37)	[c]11 ± 6	(37)	2.7 ± 2.2	(37)
DIOESTRUS	1.5 ± 0.9	(24)	[d]11 ± 7	(24)	3.6 ± 3.6	(24)

TABLE XXXV

Frequency, duration and amplitude of IUP cycles as influenced by propantheline bromide compared to no treatment, all mares (Sweetie, Fling, Jo and Snowy) and natural and simulated cycle stages (anoestrus, transition, oestrus and dioestrus) combined.

	Frequency cycles/10 min		Mean amplitude rise mm. Hg	Duration min/10 min	
No treatment	0.69 ± 0.5	(64)	[a] 13 ± 2.8 (64)	[b] 1.50 ± 0.9	(64)
Propantheline	0.45 ± 0.8	(20)	[a] 6 ± 6.8 (20)	[b] 0.83 ± 1.1	(20)

TABLE XXXVI

Frequency, duration and amplitude of IUP cycles as influenced by propantheline bromide treatment during natural and simulated anoestrus, transition, oestrus and dioestrus all mares (Sweetie, Fling, Jo, Snowy) combined.

	Frequency cycles/10 min		Mean amplitude rise mm. Hg		Duration min/10 min	
ANOESTRUS	0	(2)	0	(2)	0	(2)
TRANSITION	0.75 ± 0.6	(6)	[ab] 12 ± 2	(6)	[cd] 2.00 ± 1.2	(6)
OESTRUS	0.50 ± 1.2	(6)	[a] 2.5 ± 6	(6)	[c] 0.33 ± 0.8	(6)
DIOESTRUS	0.12 ± 0.2	(6)	[b] 2.4 ± 1	(6)	[d] 0.20 ± 0.6	(6)

TABLE XXXVII

Frequency, duration and amplitude of IUP cycles as influenced by clenbuterol compared to no treatment, all mares (Sweetie, Fling, Jo and Snowy) and natural and simulated cycle stages (anoestrus, transition, oestrus and dioestrus) combined.

	Frequency cycles/10 min	Mean amplitude rise mm. Hg	Duration min/10 min
No treatment	0.69 ± 0.50 (64)	[a]13 ± 3 (69)	[b]1.50 ± .90 (64)
Clenbuterol	0.54 ± 0.90 (11)	[a] 7 ± 6 (11)	[b]0.69 ± 0.90 (11)

TABLE XXXVIII

Frequency, duration and amplitude of IUP cycles as influenced by clenbuterol treatment during natural and simulated anoestrus, transition, oestrus and dioestrus all mares (Sweetie, Fling, Jo, Snowy) combined.

	Frequency cycles/10 min	Mean amplitude rise mm. Hg	Duration min/10 min
TRANSITION	0.33 ± 0.15 (3)	12 ± 1 (3)	0.79 ± 0.40 (3)
OESTRUS	0.72 ± 1.30 (5)	4 ± 6 (4)	0.73 ± 1.30 (5)
DIOESTRUS	0.44 ± 0.50 (3)	7 ± 6 (3)	0.53 ± 0.50 (3)

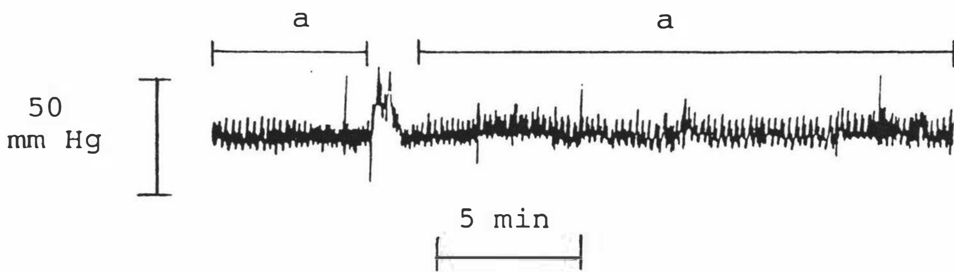


Fig. 30a: Jo - IUP pattern as influenced by the respirations of the mare. Note the rhythmic baseline fluctuations (a).

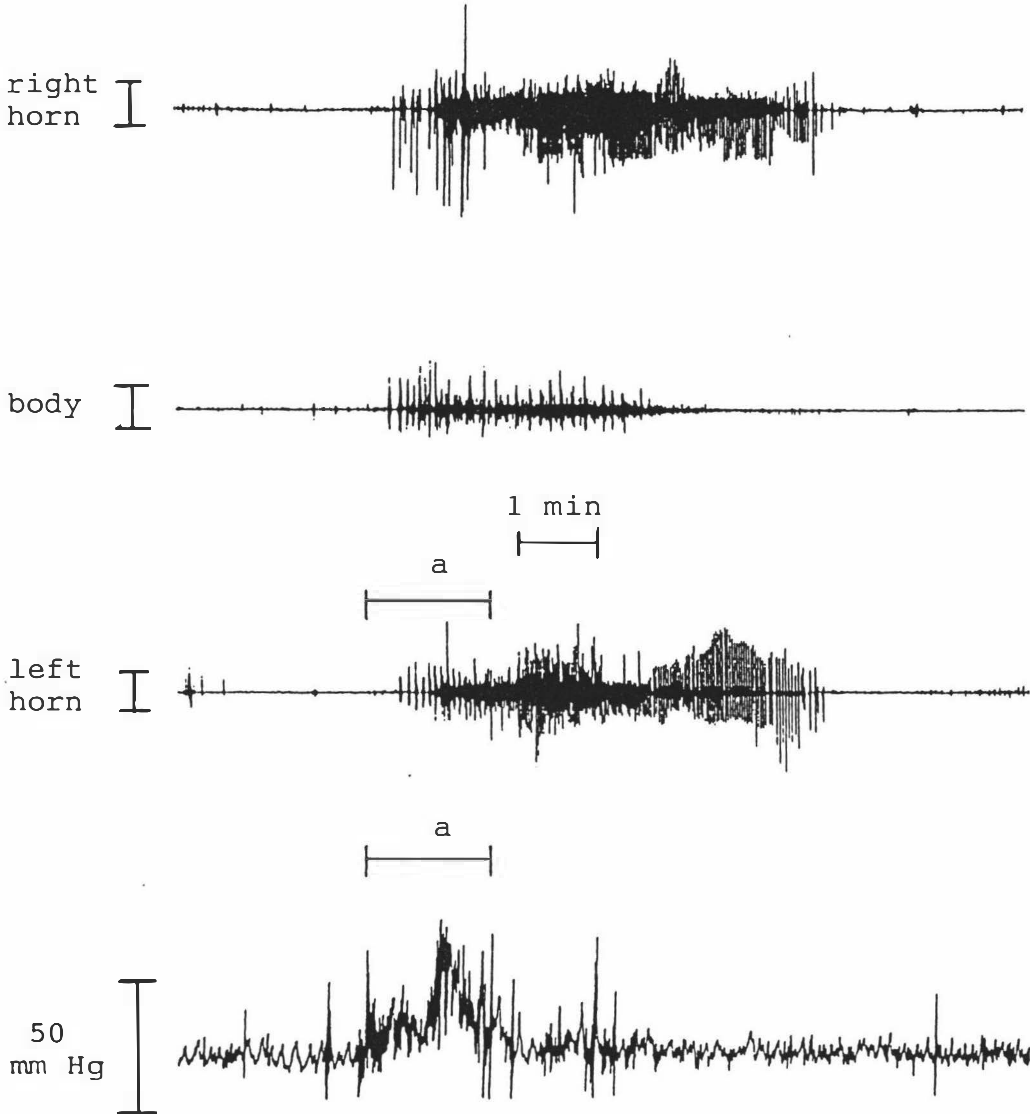


Fig. 30b: Sweetie - IUP (bottom tracing) and emg responses (upper tracings) to uterine examination per rectum (a). The vertical bars to the left represent a calibration of 200µv.

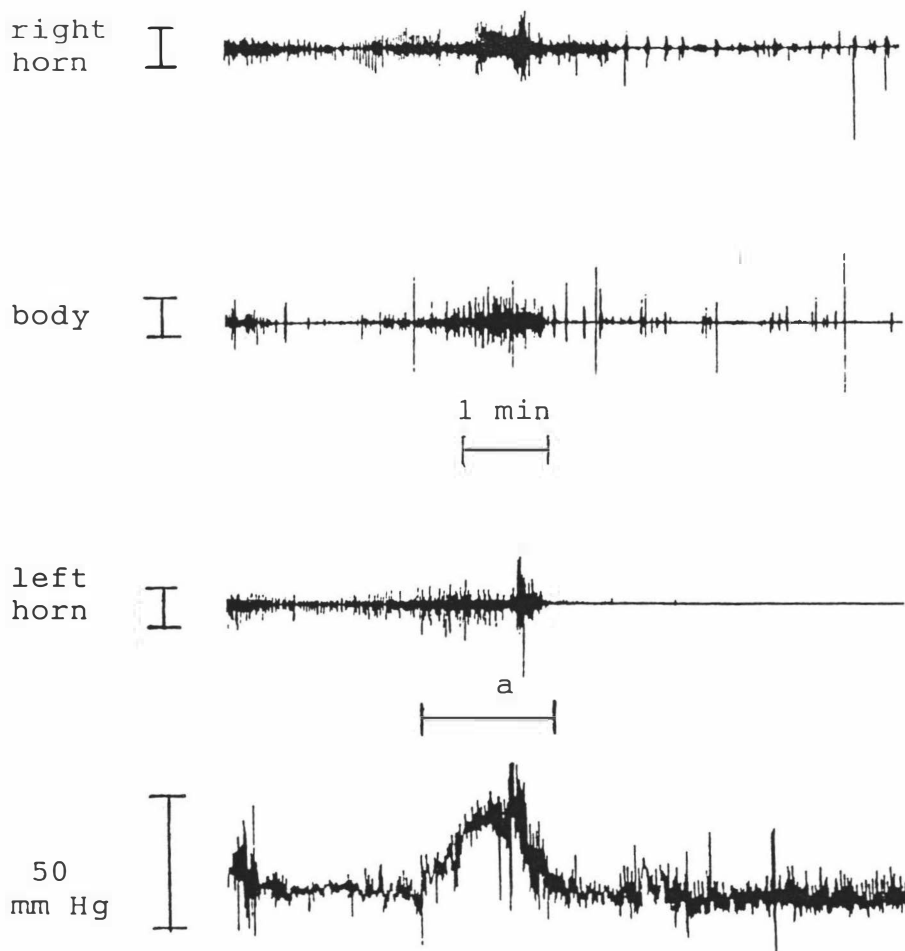


Fig. 30c: Jo - IUP response (bottom tracing) to infusion of a litre of sterile distilled water (a). This is the second litre infused - note the reduced emg response following infusion in the top three tracings as compared to the response seen after the infusion of the first litre (Fig.28a). The vertical bars to the left represent a calibration of 200µv.

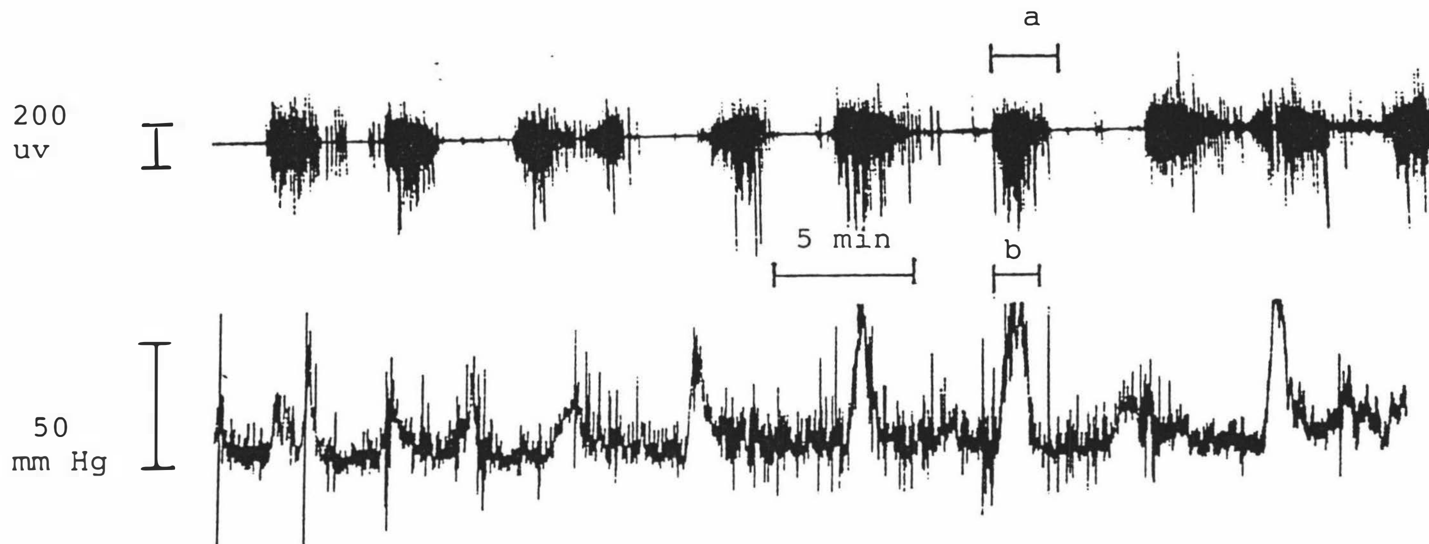


Fig. 31a: Sweetie - The bursts of emg activity (upper tracing) (a) are only occasionally associated with an IUP wave (lower tracing) (b).

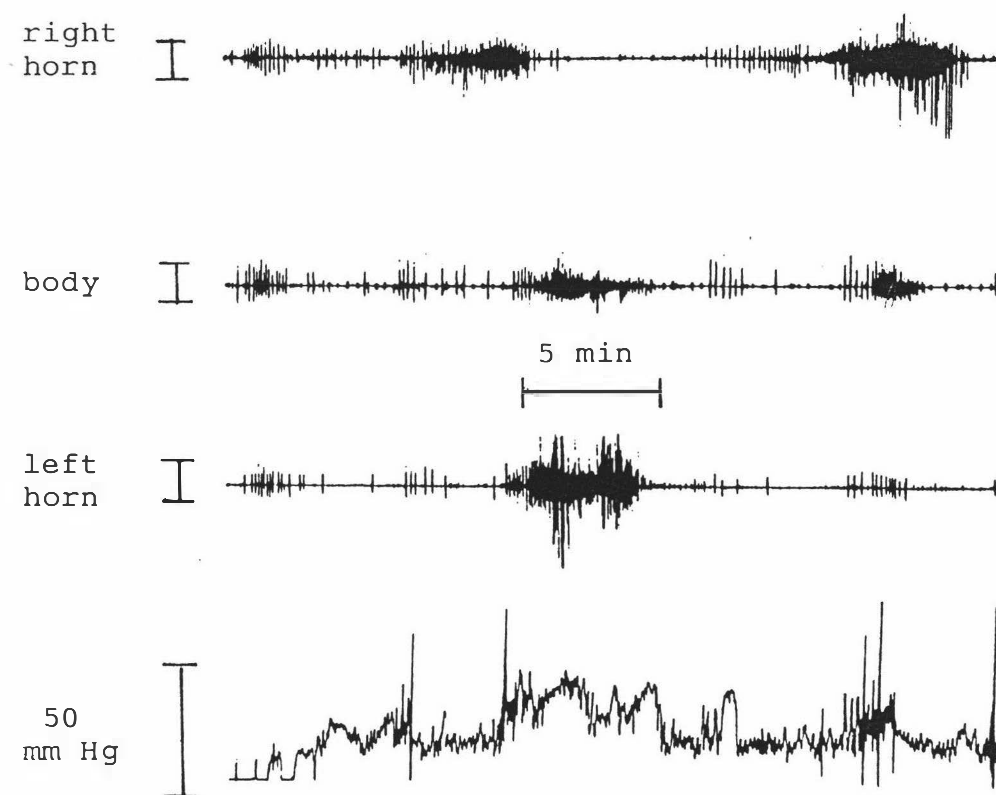


Fig 31b: Jo - These tracings show the inconsistent relationship between emg activity (top three tracings) and IUP activity (lower tracing). The vertical bars to the left of the emg tracings represent a calibration of 200µv.

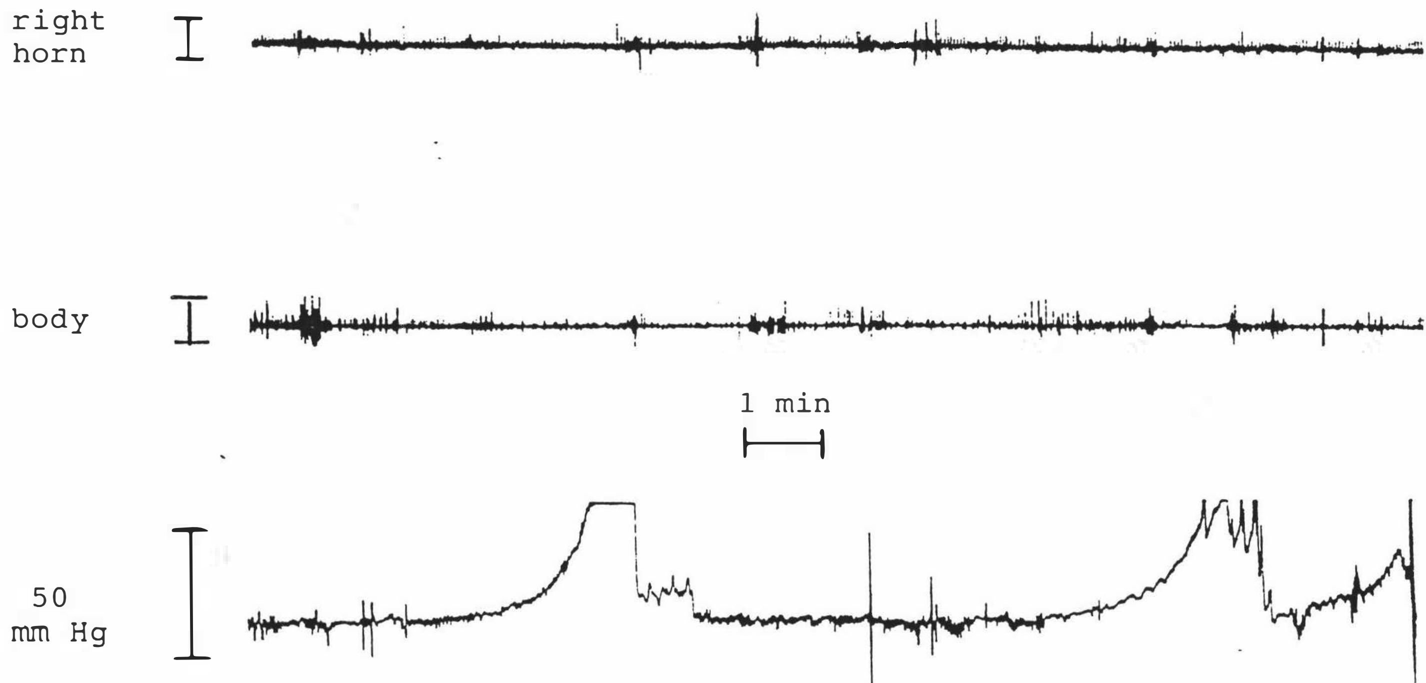


Fig.31c: Snowy - These tracings demonstrate the inconsistent relationship between IUP changes (lower tracing) and emg activity (upper tracings). The vertical bars to the left of the emg tracings represent a calibration of 200µv.

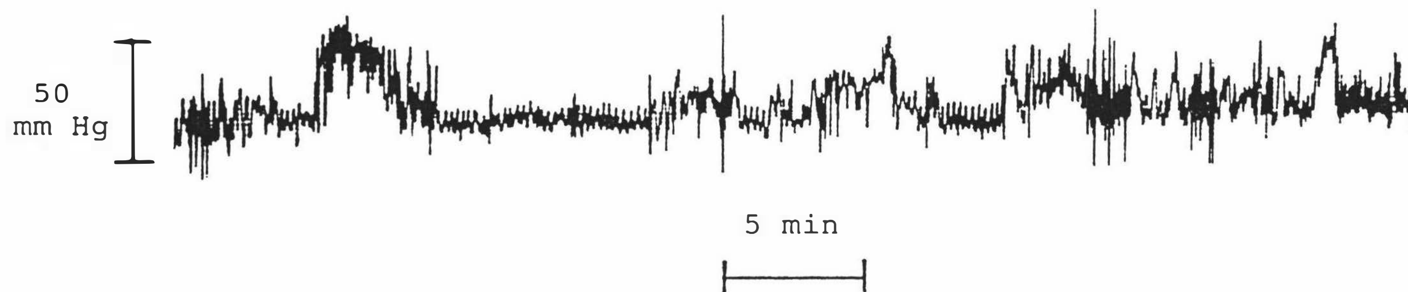


Fig. 32a: Sweetie - IUP wave pattern noted during oestrus.

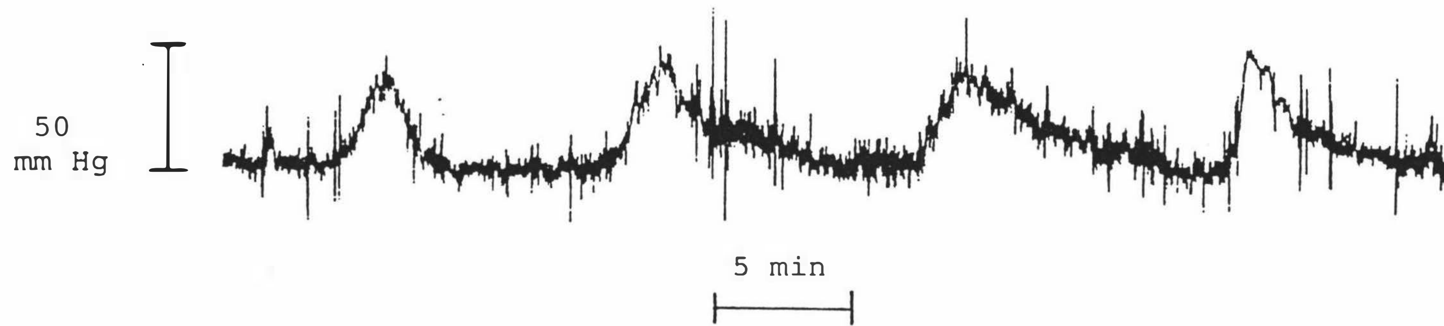


Fig. 32b: Sweetie - IUP wave pattern noted during dioestrus.

IV DISCUSSION AND CONCLUSIONS

IV.1 GENERAL DISCUSSION

Laboratory and domestic animals exhibit variations in uterine emg patterns that are strongly affected by sexual status, each oestrous cycle stage usually being identified with a specific repeatable emg pattern. When in oestrus,the uterus of the rabbit (Ruckebush ,1975) and the ewe (Naaktegeborn *et al.*,1973) exhibits high amplitude bursts of emg activity, while in dioestrus, there is minimal emg activity and that which does occur is sporadic and of low amplitude. The myometrium of the sow in oestrus also produces high amplitude (1500μv) emg activity of low frequency spiking separated by quiet periods, with one active and quiet period lasting less than 10 min. In dioestrus the activity becomes asynchronous, the previous pattern being present only at the cervix and horn bifurcation, while horn electrode sites exhibit low voltage (<150μv) high frequency activity with a complete active-quiet cycle lasting more than 10 min (Scheerboom *et al.*,1987).

The guinea pig uterus however, is distinctly different. When myometrial contractions were investigated using simultaneous emg and IUP recordings (Porter,1970; Porter *et al.*,1974) the conclusions were :

i) The electrical activity between intact and ovariectomised animals treated with exogenous oestrogen and progesterone were identical.

ii) There was excellent correlation between emg and IUP measurements.

iii) Both the oestrogen and progesterone dominated uterus exhibited identical emg and IUP patterns of regular burst activity separated by quiet periods. This pattern was considered indicative of highly coordinated contractions.

The cyclical rat's myometrial emgs also differed from other species but were similar to those observed in the mare (Taverne *et al.*,1979). *In vitro* experiments by Melton and Salvidar (1965) concluded that in the rat:

i) Mechanical activity conformed to emg activity.

ii) Regular contractions with complete relaxation are the consequence of abruptly beginning and ending bursts of spikes.

iii) Oestral myometrial strips exhibited bursts of high amplitude spikes that began and ended abruptly and the periods between the bursts were silent.

iv) In dioestrus there was a fairly continuous low amplitude electrical discharge without silent intervals. The mechanical measurements showed incomplete relaxation and uncoordinated contractile activity resulting in a variable resting tension.

In the mare uterine tone, assessed by rectal palpation, is flaccid during the anovulatory period and oestrus. During dioestrus tone increases to reach maximal levels in early pregnancy. These tonus changes are associated with changes in the steroid environment. Upon daily administration of exogenous progesterone to seasonally anovulatory mares the tone of the uterus gradually increases to that found during dioestrus. After progesterone withdrawal the flaccid tone recurs within 3 days (Hayes and Ginther,1986). During normal cyclical activity uterine tone was found to stabilise at maximal dioestrous levels 4-5 days post-ovulation and remain there until CL luteolysis causes a reduction in circulating progesterone. This early dioestrus increase in tone is paralleled by a rise in progesterone levels (Holtan *et al.*,1975). The opposite occurs in the cow. During oestrus uterine tone is maximal, minimal during dioestrus and intermediate in metoestrus (Roberts,1986).

Emg findings in the mare and cow can be correlated with these changes in uterine tone. During the study reported in this thesis the uterus of the mare in dioestrus exhibited prolonged bursts of low amplitude emg activity 50-70% of the time with periodic short bursts common during periods of relative inactivity. The increase in emg A/hr and uterine tone in the mare Sweetie paralleled the rise in plasma progesterone while the mean emg amplitude decreased. It is probable that this increase in emg activity is responsible for the increased uterine tone. Although the other intact mare, Fling, also had decreased emg amplitude and increased emg activity and uterine tone in dioestrus, the emg A/hr did not increase with increasing progesterone levels in this mare, nor did the emg amplitude decrease. Since only one natural cycle was recorded in Fling the relatively few observations could account for this result. In the dioestrous cow on the other hand there is only brief irregular emg activity (Ruckebusch and

Bayard,1975) and infrequent low amplitude pressure cycles were found to decrease with increasing progesterone levels (Al-Eknah and Noakes,1989).

During oestrus, the emg A/hr of the mare was approximately half that of dioestrus and the uterus flaccid. By contrast there is a relative increase in emg activity in the cow which is possibly responsible for the increase in uterine tone (Ruckebusch and Bayard,1975; Al-Eknah and Noakes,1989).

In the study reported in this thesis, the mare during oestrus exhibited well defined periods of emg activity, separated by periods of relative inactivity containing few emg potentials. This pattern is indicative of contractions rather than an increase in generalized tonus according to Melton and Salvidar (1965), a conclusion that is supported by the work of Ruckebusch and Bayard (1975) who placed either a strain gauge or an intra-uterine balloon at the level of one set of myometrial electrodes. They noted that during long bursts of action potentials there was an increase in IUP baseline and muscular tone. Coincident with short burst emg activity, however, pressure changes were brief but strong, quickly returning to baseline after the burst, suggesting a sequence of contraction followed by relaxation. The crescendo - decrescendo emg pattern in the oestral mare provides further evidence that contractions are occurring as only during periods of good electrical conductance will neighbouring cells fire synchronously, causing summation and the recording of high amplitude potentials (Naakteboren *et al.*,1973). Under such conditions one would expect a contraction to be a smooth increase to a peak exertion and then a gradual decrease until total relaxation is attained.

During the anovulatory period the uterus is flaccid on palpation. In Fling this was reflected by a reduced emg A/hr when compared to the emg A/hr in dioestrus. The same was not true for Jo, whose emg A/hr was equal to that found during dioestrus post surgery; emg amplitude in this mare was not reduced as in dioestrus. Jo had numerous accessory CLs when spayed (105-120 days post-service). As she was known to have been pregnant (aborted between the 45-60 days post-service examinations), it is possible that the presence of endometrial cups, producing equine chorionic gonadotrophin, could have been responsible for the high emg activity. Other factors however, may be involved, since when IUP

parameters were measured in recently ovariectomised cows, 2 of 3 maintained a relatively high level of uterine contractions (although less than in oestrus) for up to 100 days at which point recording ceased (Al-Eknah and Noakes,1989). Unfortunately in this study mares were infrequently palpated during anoestrus and the uterine tonus was not noted.

Through the transition period the emg A/hr was more similar to oestrus than dioestrus,but the active periods were of longer duration and without the crescendo- decrescendo pattern seen during oestrus. The periods of relative inactivity also contained more emg short burst activity than in oestrus, making the difference between active and quiet period less distinct and possibly reducing the ability of the myometrium to contract synchronously. Because mares in transition have variable plasma oestrogen values, but a plasma progesterone of less than 1ng/ml (Hughes *et al.*,1980), it is not surprising that the emg pattern should resemble that of oestrus rather than dioestrus.

In the spayed mare Snowy, there was little response to either exogenous hormone administration or to treatment with other drugs. Since the mare is subject to a yearly seasonal anovulatory period,short term castration could be expected to mimic this natural occurrence. In the long term castrate however, as represented by Snowy, there could have been a loss of protein and high energy compounds thus reducing the working potential of the myometrium making it incapable of developing mechanical activity (Coutinho and DeMattos,1968). With Snowy the emg A/hr did not vary with exogenous hormone administration. Nevertheless, under the influence of progesterone the mean length of the active periods tripled and the emg amplitude was reduced by almost half when compared to that seen when oestrogen was given. Thus it seems likely that the uterine musculature of Snowy was not totally incapable of response and it is possible that if this mare had been subjected to longer term alternating treatments with oestrogen and progesterone there may have also been a change in emg A/hr activity as seen in the other mares.

Snowy's results were in sharp contrast to the spayed mare Jo who, except for anoestrus, had emg results similar to the intact mares. Jo had only been spayed

for one week when the experiments began whereas Snowy had been a castrate for many years.

IV.2 VARIATION BETWEEN ELECTRODE SITES

Variation between electrode sites in one or more emg parameters in untreated mares occurred during most cyclical stages. In oestrus, major variations were found in mean amplitude, whereas in dioestrus the greatest variation in emg parameters occurred, especially in emg A/hr, an observation that supports the conclusion that myometrial activity is uncoordinated under the influence of progesterone (Melton and Salvidar,1965). With Snowy on the other hand there was little variation between electrode sites, probably reflecting the uniformly poor response of the myometrium to the hormonal treatments. The small number of observations made may also contribute to the result, with this mare.

The variation in activity noted between electrode sites may be of clinical significance since in a study where varying amounts of sterile saline (60-1000ml) were infused into the uterus of 15 mares, the fluid distribution was unequal when monitored by ultrasound in the majority of the experimental subjects (Jones,D. and Rawlinson,R., unpublished).

IV.3 EARLY PREGNANCY

During the first 6 days post-ovulation Sweetie's uterus had an emg A/hr that was consistent with overall dioestral values but the emg amplitude was reduced. Although no emg activity has been measured from the oviducts in mares, in the cow oviduct electrical activity parallels uterine activity (Ruckebusch and Bayard,1975). Since the fertilised ovum is still in the oviduct of the mare during the first 4-5 days following ovulation (Ginther,1979), this reduced emg amplitude may permit only very localized contractions, sufficient to move the fertilized ovum, but not interfere with intra-uterine entry.

During days 7-15 post-ovulation, the emg A/hr decreased and the amplitude further declined. This decrease in uterine emg activity in early pregnancy, at a time when it is increasing in the non-pregnant dioestrous mare, possibly allows for a relative relaxation of uterine tone enhancing embryo movement. The

decrease in amplitude, resulting from poor conductance of electrical activity (Marshall,1962), perhaps encourages only local contractions that ensure that the embryo moves slowly from area to area. After day 15 post-ovulation, the dramatic increase in emg activity which was observed, and consequent increase in uterine tone coupled with the decrease in emg amplitude, could provide a mechanism that assists the fixation of the embryo. The comparatively small variation in emg amplitude during this time is presumably associated with minimal local emg conductance and thus minimal contractile ability. Furthermore, the paucity of quiescent periods,, especially by day 19 when emg activity is continuous, also makes contractile activity unlikely.

In the pregnant mare endometrial PGF2 production has been reported to increase after day 16 post-ovulation at a time it is decreasing in the non-pregnant mare (Vernon *et al.*,1981). This increase in PG may be responsible in some way for the continuous emg activity that is seen at this time. Certainly PG in the investigations described in this thesis, and in the report by Taverne *et al.* (1979), increase emg activity in the uterus of the mare (see section later in this discussion).

The site variation observed in emg activity during pregnancy lends support to the above suppositions. Unfortunately the right horn electrode became non functional during days 2-6. The left horn, however, which was the site of vesicle fixation had the lowest emg A/hr and amplitude after day 6. It seems reasonable to conclude that the embryo becomes trapped in the horn exhibiting the least tone and poorest ability to contract.

IV.4 EXTRANEOUS INFLUENCES

Other researchers (Taverne *et al.*,1979) have noted a dramatic effect of environmental stimuli, such as entering'the stall, on myometrial emg activity. For this reason the mares in this project were kept isolated from the daily routine of the veterinary hospital. However, once the mares in the present study became accustomed to their environment, little or no change in emg activity occurred in response to human entry. Familiarity with the author in this investigation, who frequently spent prolonged periods in the recording stall, may account for the difference observed in the two studies.

Rectal examination caused only a minor short lived increase in electrical activity probably insufficient to affect normal physiological events, a finding which agrees with that of Taverne *et al.* (1979).

IV.5 UTERINE DISTENTION

Uterine distention in the rat, rabbit, and guinea pig causes an increase in PGF2 and action potential discharge, the effect being most marked at oestrus (Marshall,1962; Poyser *et al.*,1970; Csapo,1977). Furthermore intra-uterine infusions of saline in the dioestral mare usually cause uterine PG synthesis and release within 5-15 min (Stabenfeldt *et al.*,1984). In the study reported in this thesis uterine distention with sterile double distilled water generally elicited a short myometrial emg response after both small (60ml) and large volumes (1000 ml) were infused. Since the response to fluid infusion was immediate it is unlikely to have been due to PG release by the endometrium; moreover the single spike pattern seen after fluid infusion (Fig.28a) was not the same as that seen after PG administration (Fig.9a). Single spike responses were also noted at stages of the cycle other than dioestrus when prostaglandin precursors could not have been expected to be present. The possibility remains however, that any endogenous hormone release may have been in such minute amounts that the emg activity elicited would vary from that elicited by exogenous doses given in the experiment reported in this study.

Recent evidence suggests that it is not expansion of the uterus by the volume of fluid infused, but rather the pH of the infusion, that causes PG release (Pascoe *et al.*,1989). Acidic solutions are most effective. As the pH of physiologic saline solutions can vary from 4.5-7 this may explain the inconsistent results found when attempting to return dioestral mares to oestrus using intra-uterine saline infusions. The pH of the sterile distilled water used in the experiments reported in this thesis was 6.7, and therefore unlikely to stimulate PG synthesis.

Emg response also varied between electrode sites when uterine infusions were carried out; it is not known whether this variation was due to unequal fluid distribution or some other factor as an ultrasound examination, which is capable of showing where the infused fluid is deposited, was not carried out. Recent investigations by the author clearly demonstrate that an even distribution of fluid

is generally not achieved when fluid is infused into the uterus of the mare (Jones,D. and Rawlinson,R., unpublished data).

There was a consistent lack of response to a second fluid infusion within minutes of the first; in addition there was a tendency for the uterine emg to remain quiescent for a period of time after the second infusion had been removed from the uterus.

These results support the hypothesis that the emg response to temporary uterine distention as observed in the mares in this study, is caused by a mechanical reaction to stretched muscles rather than by hormone mediated action. The lack of response to the second infusion could be due to the fact that it caused no reactive tension in a myometrium that was still stretched from the first infusion. The positive responses to fluid infusion seen in Snowy, whose myometrium responded minimally to hormone treatment, adds further support to this proposition.

Stimulation of the clitoris (Petroff and Serteff,1956) and vagina in the oestral cow (Ruckebusch and Bayard,1975) were noted to cause an increase in uterine motility. Ruckebusch and Bayard (1975) reported that this response lasted from 5 to 30 min. A similar effect was found during oestrus in the ewe (Roberts and Share, 1969; Lightfoot 1970). In the present experiments washing of the vulva and clitoris was inconsistent in invoking myometrial electrical changes; when a response did occur it was usually the single spike activity found during uterine distention and lasted only 3-6 minutes. Furthermore it is curious that the least responsive mare, Snowy, showed the most obvious response to vulvar stimulation. Her emg reaction to vulvar stimulation is more likely to have been due to a local nerve reflex proposed by Hays and Vandermark (1953), a reflex which may from time to time be readily overridden by varying hormonal and environmental factors such as stress induced by the authors manipulations.

IV.6 BREEDING

Fertilization of the ovum by the sperm occurs in the ovarian third of the oviduct in mares, as it does in other species. Sperm transport is believed to be due to

uterine contractions, currents of luminal fluids and sperm motility (Ginther,1979), and takes only one to two minutes for spermatozoa to reach the oviducts following mating (Nalbandov,1976). Hays and Vandemark (1953) have shown that oxytocin is released at the time of mating in the cow and that the IUP response pattern at mating is similar to that provoked by exogenously administered oxytocin in the oestrous animal. Prostaglandins have been found to be produced in the seminal vesicles of rams (Rexroad and Barb,1975), and man (Bygdeman,1981). Stallion semen is also suspected of containing prostaglandins as infusion into dioestrus mares has a potent luteolytic effect (Taverne *et al.*,1979) However, in man, experimental evidence to show that seminal prostaglandins aid sperm transport is lacking (Bygdeman,1981), and in the ewe it is questionable (Horton,1979). In the rabbit PGE but not PGF2a was found to increase the rate of sperm transport when added to seminal plasma (Chang *et al.*,1973). Hays and Vandemark (1953) also raise the possibility that a purely nervous reflex might be responsible for the increase in uterine motility, while Taverne *et al.* (1979) suggest that the simple stretching effect caused by the volume of the inseminate, or an irritant influence of the semen, could also increase uterine motility.

The emg response seen during and after breeding in Sweetie on each occasion was similar to that observed during rectal examination and no post breeding change from normal cyclical activity was observed. When Taverne *et al.* (1979) artificially inseminated a pony mare 4 times with varying volumes of fresh or diluted semen they noted a similar response to Sweetie's in one trial, and an increased frequency of high amplitude active phases that persisted 2-7 hrs in the remaining trials. Unfortunately they do not state in their report in which trial(s) a diluent was used.

It seems unlikely that uterine stretch alone was responsible for the results observed with Sweetie, as when a 60ml volume of saline was placed into the uterus, the response was quite different to the response seen at breeding. Furthermore the responses to endogenous and exogenous PG (Fig.10a) with this mare, as well as the response to exogenous oxytocin (Fig.11), were also different from that seen during natural breeding (Fig.14a,b). While the possibility exists that endogenous hormone release during breeding would evoke a response that

differs from that observed during the other experiments carried out in this study, the extremely short nature of the uterine emg response seen during natural cover seems unlikely to have been associated with hormonal influences. On the other hand the similarity of the emg reaction during breeding to that of a rectal examination suggests that a local reflex could be the mechanism that is involved. As sperm are transported to the oviduct within such a short time this may be all that is necessary to facilitate syngamy and subsequent fertilisation.

IV.7 UTERINE STIMULANTS

Both oxytocin and prostaglandin have been found to be uterokinetic in all species studied (see review of literature), although results at different stages of the oestrous cycle were variable between species and in the various research reports. Drug influenced emg activity in the mare has only been examined for prostaglandin (Taverne *et al.*,1979).

All mares, except Snowy,responded to both exogenous prostaglandin and oxytocin with an increase in emg A/hr. The lack of response in Snowy was perhaps due to uterine changes during the very long period that had elapsed after she had been spayed.

After administration of both oxytocin (5 IU) and prostaglandin (125mg cloprostenol) the records showed an initial prolonged period of sustained emg activity (up to 20 min) indicating tonus rather than contraction. This prolonged emg response was followed by a shorter bursting pattern similar to, but more frequent than, that seen in oestrus. Although observed in all stages of the cycle the short burst pattern was least pronounced in dioestrus after administration of both drugs. (In two separate experiments, rectal examination of mares was carried out immediately after administration of 5 IU intravenous oxytocin and 10 minutes after 125mg intramuscular PG (cloprostenol) respectively. In both cases a palpable increase in uterine tone was detected - Jones,D., unpublished data). These PG results are similar in many respects to those found by Taverne *et al.*(1979), although they also noted an increase in emg amplitude after PG administration and found that a similar pattern (i.e. prolonged emg activity followed by a short burst pattern) occurred during natural luteolysis. An important difference observed by the writer of this thesis was that, in the one

mare followed for a substantial time period during natural luteolysis, emg A/hr remained at 100% for three hours before any reduction occurred and the normal oestrous pattern was resumed. This latter observation is similar to other findings in the present study when after a bolus administration of PG in dioestrus the short burst pattern was either absent or ill defined. According to Stabenfeldt *et al.*(1981) luteolysis occurs over 40hrs in the mare and PG release precedes the plasma progesterone decline by 3-5 hrs. A combination of these two factors (prolonged PG release and high progesterone levels) could contribute to the high level of emg activity noted during luteolysis.

The failure of either PG or oxytocin to affect emg amplitude suggests that in states other than oestrus, where amplitude remains high, synchronous firing of adjacent cells to cause summation seldom occurs. The probable consequence of this is that only weak uterine contractions result. This makes sense in that the need for the uterus to contract during dioestrus, when the cervix is closed, is minimal.

Following oxytocin and PG administration the lack of a crescendo-decrescendo pattern of emg activity, as seen in oestrus, suggests that the contractions caused by these drugs are less well controlled and more violent in nature. This may not be the case following endogenous (physiological) release.

Although Snowy failed to show a positive emg response to oxytocin, high doses of this hormone caused a negative response, and even a total cessation of emg activity. A similar result occurred in Fling where higher oxytocin doses had little or no effect on emg activity. Such a response is unlikely to be due to uterine fatigue, as there was no initial response to the drug; a more likely explanation is that the non pregnant uterus under certain conditions could be refractory to high doses (more than 20 IU of oxytocin) due to changes in receptor affinity. This negative response in uterine motility to high doses of oxytocin was not found by Ko *et al.*(1989). They monitored IUP in mares for 30 min after an intravenous injection of 40 IU oxytocin and noted significant pressure elevations after drug administration during both oestrus and dioestrus. In the series of experiments described in this thesis the higher oxytocin doses were not given frequently enough, nor in all cyclic states,nor to all mares, to demonstrate an

unequivocal response. . Further investigations are needed to verify the reasons for the varied responses seen.

IV.8 UTERINE RELAXANTS

Clenbuterol is an effective uterine relaxant in the cow and sheep (Zerobin and Kundig,1980; Greene,1981). Little is known about its effectiveness in the mare. Likewise there are no reports on the use of propantheline bromide as a uterine relaxant. Unfortunately insufficient records were available for proper statistical analysis of the effects of these drugs at all stages of the cycle to be carried out.

At the dose level chosen clenbuterol only rarely showed a significant reduction in any emg parameter, however there was a trend towards emg amplitude reduction that was not consistent between cycle stages. This reduction in amplitude, whether or not there was a concomitant reduction in emg activity, suggests poor conductance of action potentials and therefore minimal coordinated contractile ability. Emg activity in the cow is also little affected by clenbuterol administration, although it is known to be clinically effective in this species (Zerobin and Kundig,1980).

In this present study, when clenbuterol was administered during the embryo mobility phase of pregnancy (day 7-15) there was an increase in emg A/hr and emg amplitude. Cross and Ginther(1988) reported that clenbuterol administration during this stage of pregnancy resulted in increased embryonic death (as determined by ultrasound). It is possible that the increased uterine tone (due to a rise in emg A/hr) could interfere with embryo motility and the increased emg amplitude result in local contractions that could damage the embryo causing greater risk of pregnancy loss. Further studies are clearly needed to confirm the effect of clenbuterol on uterine emg activity during pregnancy.

Propantheline bromide caused a consistent reduction in emg A/hr, although the results were only statistically significant in dioestrus where emg activity was in general reduced by two thirds. The mean amplitude was also frequently significantly reduced. This reduction in emg activity of the uterus to the low levels observed most likely results in poor uterine tonus. As one would expect, this change was most dramatic when the uterus had high pre-treatment tone as

in dioestrus. Relaxation in uterine tone can also be noted clinically on rectal examination of the uterus following propantheline bromide administration (Jones,D., unpublished data). The lack of synchronous activity between electrode sites, coupled with decreased emg amplitude, suggests very poor contractile ability. If propantheline has the same effect in the pregnant mare it may prove a useful treatment in dystocia.

IV.9 INTRA-UTERINE PRESSURE

The purpose of measuring IUP in this study was to support the contention that emg activity is a true measure of uterine motility in the mare, as has been shown in other species (see literature review). When normal cyclical IUP results were examined there were few significant differences in most IUP parameters between oestrus and dioestrus and significantly higher IUP parameters were often found in anoestrus. The method of analysis used, i.e. examining individual IUP indices (frequency, duration and maximum cycle amplitude), instead of measuring the total area under the pressure curve may have minimized differences between cycle stages. Ko *et al.*(1989), however, using the latter method of analysis also found no significant differences in IUP between oestrus and dioestrus. The results in the present study do not correlate with the emg findings where, especially between oestrus and dioestrus, there were dramatic differences in emg parameters and patterns. Furthermore emg burst activity and IUP cycles did not consistently correspond.

One possible explanation for the poor correlation between IUP and emg parameters is the lack of constant sensitivity of the method chosen for the IUP measurements. Although the uterus is considered a hollow tubular organ, in reality the endometrium of the opposing walls in the undistended uterus is in contact. Thus a small diameter tube that does not distend the uterus (like the balloon method) may not be sensitive to small changes in pressure. The author believes this unlikely however, since Goddard ***et al.*** (1985), successfully measured intra-uterine pressure changes using a more sophisticated but similar system (balloonless). Although every effort was made to keep the tip of the catheter just proximal to the internal os of the cervix during the experiments reported in this thesis, it is possible, due to mare movement, that the tip could have shifted and become embedded in an endometrial fold, thus blocking the transmission of

pressure changes. Periodic infusion of a small amount of sterile physiological saline was made through the system to ensure patency. This infusion procedure was also performed prior to drug treatment or if the pressure recordings appeared not to be varying. Although catheter tip blockage is a possibility occasional activities such as urination, whinnying, shifting a leg position and respiration were all identifiable in this study by pressure changes just as had been described by Goddard *et al.*(1985).

A further possible explanation for the poor correlation is that contractions that occur during normal cyclical stages are not transferred as pressure changes throughout the uterus; this would seem unlikely especially in oestrus.

After the administration of the drugs oxytocin and propantheline bromide there appeared to be a correlation between emg A/hr and one or more of the IUP parameters; visually however, chart recordings of emg activity and IUP cycles did not always occur synchronously. The same could not be said following PG and clenbuterol administration where there was no statistical correlation between the two methods used.

When IUP changes were examined independently of emg activity a drug response was seen that agreed with the emg findings in that both oxytocin and PG increased two IUP indices over non treatment states, whereas propantheline and clenbuterol decreased two of the IUP indices. The results for the uterokinetic agents agree with the reports of Goddard and Allen(1985b), Ley *et al.*(1987), Ko *et al.*(1989),but differ from those of Sharpe *et al.*(1988) for PG. Perhaps the prolonged infusion used by Sharpe's group resulted in an increase in uterine tonus, but not contractions; actual IUP recordings were not presented in their paper.

Yet a further explanation for variation in IUP response during the normal cycle and following drug treatment exists. As noted in the results, all mares responded to treatment with the uterokinetic agents by showing mild abdominal discomfort, and with PG by an increase in bowel movements. The intestinal tract of the mare as in other species is composed of smooth muscle. Oxytocin binding sites have been found in the equine duodenum (Stull and Evans,1986) and the PGs have a

well known kinetic effect on the intestinal musculature (Goldyne,1982). Likewise, propantheline bromide causes inhibition peristalsis in gut and is commonly used for this purpose. The action of clenbuterol on gut motility is likely also to be one of relaxation as the intestinal tract contains beta 2 as well as beta 1 receptors (Weiner and Taylor,1987). Since the mare's uterus is nestled within intestinal coils, factors that affect intestinal motility may also be transmitted to the adjacent uterus. This can be visualised on ultrasound examination where a uterine horn can be seen to be buffeted and compressed by activity of the underlying intestine. It is possible that some of the pressure changes recorded by the IUP technique reflect this influence and explain why IUP cycles and emg activity patterns do not consistently correspond. The writer argues that the lack of variation in IUP cycles between oestrus and dioestrus supports this supposition, just as changes in hind leg position, neighing and urinating, all of which probably affect intra-abdominal pressure do. Further investigations combining sensitive techniques for measuring IUP coupled with emg monitoring, and perhaps strain gauge measurements, as well as the placement of intra-abdominal pressure transducers, need to be carried out.

Although ultrasound evaluation of uterine motility offers a relatively easy non invasive technique it has several possible drawbacks. Firstly, rectal manipulation, no matter how slight, increases emg activity and therefore may temporarily affect motility; furthermore ultrasound waves themselves could influence the electrical conductance and motility of the uterus. Secondly, ultrasonography has the same potential problem as IUP recordings in that intestinal motility can interfere with what is being observed. Thirdly, the ultrasound technique used to date (Cross and Ginther,1987,1988) only measures motility for very short periods (1 min); this is unlikely to be representative of an organ that goes through alternating active and passive episodes that may be of prolonged duration.

IV.10 CRITIQUE OF EXPERIMENTAL DESIGN

Two different electrode sizes were used (2mm and 4mm) in the experiments reported in this thesis. The larger surface electrode used in Sweetie and Jo gave clearer recordings. The less satisfactory results in Fling and Snowy, using the smaller exposed electrode surfaces,could have been due to mare factors,electrode defects associated with their preparation, surgical placement or aftercare.

However,it seems unlikely that electrode defects would have been responsible for poor results in all three uterine sites. In retrospect the difference in the records from Snowy, compared to all other mares, is more likely to be attributable to her long term status as a castrate. As she was the first mare recorded her lack of response to hormones and therapeutic drug treatment was frustrating. Fling, the second mare recorded, had poor records in that there appeared to be erratic electrical activity throughout,although distinct patterns could be recognised. It was at this point that a decision was made to enlarge the electrode surface to gain access to the electrical discharges of a greater number of cells in the hope of recording more coordinated activity and reducing contributions by small groups of cells in an isolated area. This approach needs to be repeated to determine if there is an optimal electrode size that will yield better information.

Greater numbers of intact and spayed experimental animals need to be studied to confirm the results, including investigations on recently spayed mares as well as long term castrates. Experimentation on intact mares was begun in the winter in the hope of following them through anoestrus, transition and oestrus. This required long term maintenance of wires and a great deal of patience on the part of the mares. Much information on cycling mares could be gained in a shorter time if surgery was carried out when normal oestrous patterns were already established. In addition it would be ideal if electrodes could be implanted and signals recorded via a radio transmitter negating the problem of exteriorized wires.

The method of measuring IUP was not optimal and it was difficult to keep mares confined closely enough to prevent catheter displacement. Because an open system was used it required constant monitoring to keep catheters from becoming blocked. This often did happen resulting in the loss of many records. Not enough IUP measurements were taken during normal activity, especially in dioestrus and anoestrus, in this study.

IV.11 FUTURE STUDIES

In the literature in general, there is a lack of information concerning IUP parameter variations in mares not under the influence of therapeutic drugs. Although this has recently been addressed by Ko *et al.*(1989) recording periods

were brief (30 min) and therefore may not have accurately reflected total IUP patterns.

Electrodes need to be implanted in the oviducts in series throughout both the horns and body. This would allow assessment of direction of contractile activity, variations in tone and synchrony of response between the different horns and body both naturally as well as under the influence of various treatment regimes. This could be particularly useful in the study of early pregnancy.

Myometrial emg activity in the first and second trimester of pregnancy needs investigation. The work of Haluska (1985) has shown that myometrial emg activity is quite different in late pregnancy compared to findings in the non pregnant mare and the first 20 days of pregnancy. The specific effect on emg activity of hormones such as equine chorionic gonadotrophin, oestrone sulphate and relaxin need to be clarified.

The role of therapeutic drugs on uterine motility needs to be re-examined. There is controversy as to the effect of the uterokinetic drugs, especially PG, in both the cow and horse. As these agents have potential in aiding uterine involution in the postpartum animal, and perhaps in treatment of uterine infections due to their uterine stimulant effects, they need to be more closely investigated. Results reported in this thesis suggest that low doses of oxytocin (5 IU) could prove to be more effective than higher doses in the non-pregnant mare. Additional trials using varying doses of both PG and oxytocin are needed.

Therapeutic agents for use as uterine relaxants are invaluable for relief of dystocia. In the mare few investigations have been carried out in this area. Propantheline bromide at low doses seems capable of reducing myometrial activity. Clinical trials in foaling mares are needed to determine its efficacy in these situations. Clenbuterol is known to be effective in delaying parturition in several farm animal species. Although at the dose used it appears to have little effect on uterine emg activity further experimental and clinical trials should be implemented at various dosages and under varying conditions.

In vitro experiments using equine myometrium are sadly lacking and could offer a relatively quick method of screening drugs and doses that could then be applied to the live animal.

IV.12 CONCLUSIONS

The electrical activity of the equine uterus appears to vary under the influence or absence, of the steroid hormones progesterone and oestrogen. When no steroid hormone environment exists the myometrium discharges shorts bursts of action potentials even in the long term castrate. This burst activity is also evident in the cycling mare interspersed between the regular pattern indicative of that particular cycle stage. Perhaps these bursts can be attributed to myometrial pacemaker activity.

Under the influence of oestrogen the electrical activity follows a cyclical pattern of long burst activity (3-5min) that increases to a maximal amplitude and then decreases (crescendo-decrescendo), followed by a relatively quiescent period of approximately 10 min. Activity is usually synchronous within each horn and the uterine body. This pattern is consistent with uterine contractions. In dioestrus the emg activity is more continuous, and the periods of relative inactivity and activity more difficult to define. Emg responses of this type are indicative of uterine tonus. Emg findings paralleled palpable changes in uterine tone in dioestrus, oestrus and early pregnancy.

In pregnancy the emg amplitude and emg A/hr followed a pattern representative of events occurring to the fertilised ovum. The emg activity increased greatly after day 16 of pregnancy. Abnormal uterine motility in the first 20 days of pregnancy may be an important factor in early embryonic death.

Uterine stimulants, prostaglandin and oxytocin, initially caused an increase in emg activity followed by a cyclical emg pattern similar to but more frequently repeated than that in oestrus. The latter response is most marked in oestrus and least marked in dioestrus. Prostaglandin has the most long lasting influence, up to three hours, whereas oxytocin influence is relatively short lived, about 30min. The induced cyclical activity pattern is indicative of contractions and is most pronounced in oestrus.

Uterine relaxants give a more variable result. Propantheline bromide is most consistent in reducing uterine emg especially in dioestrus. Clenbuterol, at the dose used, has minimal effect on emg activity.

The progesterone block theory of Csapo (1956), appears valid in the mare. Even though progesterone does not abolish or reduce emg activity in the mare, as it does in most species studied, the activity that is generated is uncoordinated and appears only capable of producing weak uterine contractions, even under the influence of uterine stimulants.

IUP measurements do not appear to vary between normal cyclical stages but do increase under the influence of uterine stimulants and decrease with propantheline bromide and clenbuterol. As IUP wave and emg activity show little relationship, the validity of the intra-uterine pressure recording technique used in this experiment, and those used in general in the mare, are questionable. Emg analysis however gives consistent repeatable results throughout all cycle stages and therapeutic treatments. Implantation of electrodes does not interfere with normal physiological events of the uterus. This is verified by the fact that the intact mares cycled normally, one (Sweetie) became pregnant during the experiment and the other (Fling), from which the electrodes were not removed, has recently foaled from a breeding which took place after completion of this research.

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APPENDIX A: ELECTRODE SITE VARIATION

1A Sweetie

Electrode site variation between the right horn, body and left horn in emg A/hr, periods of activity, periods of relative inactivity and amplitudes during transition, oestrus and dioestrus.

	% emg A/hr	active perlods mln	periods relative Inactivity min	amplitude μV
TRANSITION				
Right horn	34 ± 14 (55)	6 ± 3(55)	11 ± 8(55)	[a]177 ± 39(55)
Body	31 ± 12(72)	5 ± 2(72)	12 ± 7(72)	[ab]148 ± 38(72)
Left horn	31 ± 14(72)	6 ± 4(72)	13 ± 10(72)	[b]175 ± 72(72)
OESTRUS				
Right horn	24 ± 10(37)	[ab]4 ± 2(37)	13 ± 12(37)	[cd]208 ± 50(37)
Body	23 ± 9(52)	[a]3 ± 1(52)	10 ± 5(52)	[c]179 ± 24(52)
Left horn	20 ± 8(46)	[b]3 ± 1(46)	12 ± 5(46)	[d]185 ± 37(46)
DIOESTRUS				
Right horn	[ab]71 ± 24(49)	23 ± 22(49)	[ab] 6 ± 6(49)	[a]153 ± 44(49)
Body	[a]60 ± 28(49)	19 ± 20(49)	[a] 9 ± 10(49)	[a]141 ± 35(49)
Left horn	[b]57 ± 26(49)	14 ± 17(49)	[b]10 ± 11(49)	[a]124 ± 37(49)

2A Sweetie

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude throughout the first 20 days of pregnancy.

	% emg A/hr		amplitude μV	
Right horn	[a] 72 ± 23	(14)	[c] 140 ± 43	(14)
Body	[b] 66 ± 24	(65)	[c] 105 ± 28	(65)
Left horn	[ab] 57 ± 25	(65)	[c] 92 ± 30	(65)

3A Sweetie

Electrode site variation between the right horn, body and left horn in emg A/hr, and amplitude throughout days 2-6, 7-15, 16-20 of pregnancy.

	% emg A/hr		amplitude μV	
Day 2-6				
Right horn	[a] 72 ± 23	(14)	[bc] 140 ± 43	(14)
Body	[a] 56 ± 17	(26)	[b] 107 ± 33	(26)
Left horn	64 ± 16	(26)	[c] 101 ± 16	(26)
Day 7-15				
Body	[d] 62 ± 23	(29)	109 ± 23	(29)
Left horn	[d] 41 ± 21	(29)	97 ± 33	(29)
Day 16-20				
Body	[e] 100 ± 0	(10)	[f] 83 ± 12	(10)
Left horn	[e] 83 ± 26	(10)	[f] 50 ± 0	(10)

4A Sweetie

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude after PG treatment in transition, oestrus and dioestrus.

	% emg A/hr		amplitude μV	
TRANSITION				
Right horn	73 ± 14	(15)	[a]160 ± 43	(15)
Body	71 ± 10	(15)	[b]163 ± 51	(15)
Left horn	67 ± 13	(15)	[ab]243 ± 96	(15)
OESTRUS				
Right horn	60 ± 9	(5)	200 ± 0	(5)
Body	64 ± 9	(5)	200 ± 0	(5)
Left horn	61 ± 8	(5)	240 ± 55	(5)
DIOESTRUS				
Right horn	95 ± 6	(7)	131 ± 49	(7)
Body	92 ± 12	(7)	136 ± 24	(7)
Left horn	95 ± 8	(7)	136 ± 24	(7)

5A Sweetie

Electrode site variation between the right horn, body and left horn in emg A/hr, and amplitude after oxytocin administration (5 IU) in transition, oestrus and dioestrus.

	% emg A/hr		amplitude μV	
TRANSITION				
Right horn	63 ± 30	(37)	[a]162 ± 56	(37)
Body	62 ± 29	(37)	[b]134 ± 55	(37)
Left horn	58 ± 29	(37)	[ab]191 ± 82	(37)
OESTRUS				
Right horn	54 ± 32	(20)	180 ± 62	(20)
Body	53 ± 30	(20)	200 ± 0	(20)
Left horn	54 ± 29	(20)	200 ± 0	(20)
DIOESTRUS				
Right horn	94 ± 14	(16)	138 ± 50	(16)
Body	94 ± 8	(16)	138 ± 50	(16)
Left horn	91 ± 16	(16)	106 ± 75	(16)

6A Sweetie

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude after clenbuterol administration in transition, oestrus and dioestrus.

	% emg A/hr		amplitude μV	
TRANSITION				
Right horn	34 ± 14	(3)	125 ± 66	(3)
Body	22 ± 7	(3)	158 ± 72	(3)
Left horn	13 ± 2	(3)	158 ± 72	(3)
OESTRUS				
Right horn	33 ± 43	(3)	200 ± 20	(3)
Body	25 ± 31	(3)	167 ± 58	(3)
Left horn	36 ± 42	(3)	200 ± 28	(3)
DIOESTRUS				
Right horn	45 ± 20	(3)	200 ± 60	(3)
Body	59 ± 5	(3)	175 ± 52	(3)
Left horn	46 ± 36	(3)	158 ± 38	(3)

7A Sweetie

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude after propantheline bromide treatment in transition, oestrus and dioestrus.

	% emg A/hr		amplitude µV	
TRANSITION				
Right horn	29 ± 10	(6)	200 ± 22	(6)
Body	25 ± 9	(6)	200 ± 35	(6)
Left horn	24 ± 5	(6)	200 ± 20	(6)
OESTRUS				
Right horn	16 ± 4	(4)	[a] 200 ± 18	(4)
Body	19 ± 3	(4)	[b] 200 ± 26	(4)
Left horn	14 ± 4	(4)	[ab] 275 ± 50	(4)
DIOESTRUS				
Right horn	29 ± 29	(6)	100 ± 30	(6)
Body	11 ± 11	(6)	83 ± 40	(6)
Left horn	16 ± 13	(6)	83 ± 40	(6)

8A Fling

Electrode site variation between the right horn, body and left horn in emg A/hr, periods of activity, periods of relative inactivity, and amplitude during anoestrus, transition, oestrus and dioestrus.

	% emg A/hr	active periods min	periods relative inactivity min	amplitude µV
ANOESTRUS				
Right horn	33 ± 29(19)	5 ± 4(19)	19 ± 21(19)	117 ± 72(19)
Body	35 ± 17(19)	4 ± 4(19)	9 ± 7(19)	142 ± 52(19)
Left horn	30 ± 17(22)	5 ± 6(22)	13 ± 14(22)	135 ± 55(22)
TRANSITION				
Right horn	[a] 38 ± 19(42)	9 ± 15(42)	[c] 10 ± 6(42)	[e] 207 ± 46(42)
Body	[ab] 25 ± 20(45)	7 ± 12(45)	[cd] 22 ± 17(45)	[ef] 116 ± 47(45)
Left horn	[b] 35 ± 16(41)	7 ± 4(41)	[d] 12 ± 7(41)	[f] 198 ± 68(41)
OESTRUS				
Body	36 ± 10(30)	3 ± 1(30)	6 ± 2(30)	[a] 115 ± 21(30)
Left horn	34 ± 11(32)	3 ± 1(32)	6 ± 3(32)	[a] 157 ± 81(32)
DIOESTRUS				
Right horn	48 ± 40(7)	12 ± 21(7)	14 ± 21(7)	77 ± 47(7)
Body	77 ± 19(11)	19 ± 21(11)	2 ± 2(11)	114 ± 28(11)
Left horn	67 ± 19(14)	8 ± 5(14)	3 ± 2(14)	103 ± 22(14)

9A Fling

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude after PG treatment in anoestrus, transition, oestrus and dioestrus.

	% emg A/hr		amplitude μV	
ANOESTRUS				
Right horn	58 ± 4	(3)	100 ± 0	(3)
Body	67 ± 28	(7)	121 ± 27	(7)
Left horn	65 ± 29	(7)	110 ± 29	(7)
TRANSITION				
Body	86	(1)	90	(1)
Left horn	92	(1)	125	(1)
OESTRUS				
Right horn	75 ± 19	(4)	200 ± 41	(4)
Body	69 ± 22	(9)	178 ± 26	(9)
Left horn	75 ± 18	(9)	206 ± 73	(9)
DIOESTRUS				
Right horn	[ab] 51 ± 29	(6)	167 ± 143	(6)
Body	[a] 87 ± 11	(11)	155 ± 150	(11)
Left horn	[b] 78 ± 13	(11)	133 ± 106	(11)

10A Fling

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude after varying oxytocin doses (2 IU, 5 IU, 20 IU and 50 IU) in anoestrus, oestrus and dioestrus.

	% emg A/hr		amplitude µV	
ANOESTRUS (2 IU)				
Right horn	[ab]10 ± 0	(3)	100 ± 0	(3)
Body	[a]80 ± 10	(3)	100 ± 0	(3)
Left horn	[b]87 ± 6	(3)	100 ± 0	(3)
OESTRUS (2 IU)				
Right horn	90 ± 9	(6)	175 ± 61	(6)
Body	88 ± 15	(6)	217 ± 41	(6)
Left horn	87 ± 16	(6)	217 ± 41	(6)
DIOESTRUS (2 IU)				
Right horn	[a]18 ± 23	(20)	[b] 43 ± 37	(20)
Body	[a]79 ± 26	(20)	[b] 105 ± 21	(20)
Left horn	[a]59 ± 21	(20)	[b] 90 ± 21	(20)
ANOESTRUS (5 IU)				
Right horn	[ab]35 ± 21	(2)	[cd] 200 ± 0	(2)
Body	[a]72 ± 23	(16)	[c] 120 ± 43	(16)
Left horn	[b]81 ± 28	(16)	[d] 123 ± 28	(16)
OESTRUS (5 IU)				
Body	76 ± 19	(10)	125 ± 26	(10)
Left horn	70 ± 18	(10)	108 ± 46	(10)
DIOESTRUS (5 IU)				
Body	[a]92 ± 9	(10)	70 ± 5	(10)
Left horn	[a]99 ± 10	(10)	74 ± 3	(10)
OESTRUS (20 IU)				
Right horn	[ab] 8 ± 15	(4)	63 ± 75	(4)
Body	[a]65 ± 39	(4)	121 ± 37	(4)
Left horn	[b]85 ± 19	(4)	75 ± 0	(4)
ANOESTRUS (50 IU)				
Right horn	17 ± 33	(7)	121 ± 118	(7)
Body	47 ± 34	(7)	157 ± 105	(7)
Left horn	35 ± 38	(7)	171 ± 115	(7)

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude after clenbuterol treatment in anoestrus, oestrus and dioestrus.

	% emg A/hr		amplitude µV	
ANOESTRUS				
Right horn	0	(1)	0	(1)
Body	30 ± 20	(2)	150	(1)
Left horn	14	(1)	75 ± 106	(2)
TRANSITION				
Left Horn	58	(1)	100	(1)
OESTRUS				
Right horn	29	(1)	100	(1)
Body	45 ± 13	(2)	113 ± 18	(2)
Left horn	43 ± 21	(2)	150 ± 0	(2)
DIOESTRUS				
Right horn	37 ± 5	(2)	108 ± 60	(2)
Body	86 ± 27	(20	113 ± 18	(2)
Left horn	46 ± 6	(3)	100 ± 10	(3)

12A Fling

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude after propantheline bromide treatment in anoestrus, oestrus and dioestrus.

	% emg A/hr		amplitude μV	
ANOESTRUS				
Right horn	3 ± 4	(3)	38 ± 53	(2)
Body	8 ± 4	(2)	50 ± 71	(2)
Left horn	22 ± 9	(2)	88 ± 18	(2)
OESTRUS				
Right horn	40 ± 41	(2)	150 ± 0	(2)
Body	10 ± 22	(5)	135 ± 48	(5)
Left horn	14 ± 27	(6)	108 ± 86	(6)
DIOESTRUS				
Body	20 ± 10	(2)	65 ± 0	(2)
Left horn	11 ± 16	(2)	38 ± 53	(2)

13A Jo

Electrode site variation between the right horn, body and left horn in emg A/hr, periods of activity, periods of relative inactivity and amplitude during 'anoestrus', 'oestrus' and 'dioestrus'.

	% emg A/hr	active periods min	periods relative inactivity min	amplitude µV
'ANOESTRUS'				
Right horn	57 ± 33(11)	21 ± 20(11)	14 ± 8(28)	220 ± 62(11)
Body	40 ± 19(11)	10 ± 5(11)	14 ± 12(57)	168 ± 51(11)
Left horn	52 ± 30(19)	15 ± 8(9)	22 ± 16(28)	191 ± 57(11)
'OESTRUS'				
Right horn	35 ± 14(7)	7 ± 2(7)	13 ± 4(7)	[a] 314 ± 63(7)
Body	36 ± 19(29)	6 ± 4(29)	13 ± 7(29)	[a] 213 ± 85(29)
Left horn	46 ± 11(7)	7 ± 3(7)	9 ± 3(7)	264 ± 48(7)
'DIOESTRUS'				
Right horn	[a] 61 ± 9(10)	[c] 20 ± 16(10)	8 ± 3(10)	148 ± 30(10)
Body	[ab] 41 ± 6(10)	[cd] 8 ± 03(10)	11 ± 8(10)	118 ± 31(10)
Left horn	[b] 59 ± 8(10)	[d] 12 ± 06(10)	14 ± 7(10)	140 ± 39(10)

14A Jo

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude after PG treatment in 'anoestrus', 'oestrus' and 'dioestrus'.

	% emg A/hr		amplitude μV	
'ANOESTRUS'				
Right horn	[a] 90 ± 9	(3)	167 ± 14	(3)
Body	[ab] 53 ± 6	(3)	134 ± 29	(3)
Left horn	[b] 80 ± 7	(3)	134 ± 29	(3)
'OESTRUS'				
Right horn	58 ± 18	(3)	267 ± 58	(3)
Body	66 ± 23	(3)	167 ± 29	(3)
Left horn	73 ± 20	(3)	250 ± 50	(3)
'DIOESTRUS'				
Right horn	86 ± 23	(6)	117 ± 41	(6)
Body	51 ± 27	(6)	112 ± 20	(6)
Left horn	71 ± 20	(6)	125 ± 42	(6)

15A Jo

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude after oxytocin treatment (5 IU) in 'anoestrus', 'oestrus' and 'dioestrus'.

	% emg A/hr		amplitude μV	
'ANOESTRUS'				
Right horn	74 ± 26	(14)	207 ± 75	(14)
Body	72 ± 27	(14)	173 ± 86	(14)
Left horn	77 ± 25	(14)	189 ± 71	(14)
'OESTRUS'				
Right horn	56 ± 34	(17)	229 ± 47	(17)
Body	42 ± 36	(17)	141 ± 87	(17)
Left horn	51 ± 31	(17)	264 ± 63	(17)
'DIOESTRUS'				
Right horn	[a]82 ± 29	(21)	143 ± 62	(21)
Body	[ab]55 ± 35	(21)	83 ± 36	(21)
Left horn	[b]85 ± 25	(21)	150 ± 76	(21)

16A Jo

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude after clenbuterol treatment in 'oestrus' and 'dioestrus'.

	% emg A/hr		amplitude μV	
'OESTRUS'				
Right horn	[a] 27 ± 14	(3)	[c] 100 ± 0	(3)
Body	[ab] 11 ± 6	(3)	[cd] 50 ± 0	(3)
Left horn	[b] 22 ± 17	(3)	[d] 100 ± 50	(3)
'DIOESTRUS'				
Right horn	37	(1)	100	(1)
Body	0	(1)	0	(1)
Left horn	42	(1)	100	(1)

17A Jo

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude after propantheline bromide treatment in 'anoestrus', 'oestrus' and 'dioestrus'.

	% emg A/hr		amplitude μV	
'ANOESTRUS'				
Right horn	33	(1)	200	(1)
Body	0	(1)	0	(1)
Left horn	36	(1)	150	(1)
'OESTRUS'				
Right horn	15	(1)	200	(1)
Body	0	(1)	0	(1)
Left horn	5	(1)	150	(1)
'DIOESTRUS'				
Right horn	56 ± 46	(6)	55 ± 45	(6)
Body	27 ± 43	(6)	26 ± 43	(6)
Left horn	46 ± 51	(6)	46 ± 51	(6)

18A Snowy

Electrode site variation between the right horn, body and left horn in emg A/hr, periods of activity, periods of relative inactivity and amplitude during 'anoestrus', 'oestrus' and 'dioestrus'.

	% emg A/hr	active periods min	periods relative inactivity min	amplitude µV
'ANOESTRUS'				
Right horn	31 ± 16(9)	7 ± 2(9)	15 ± 9(9)	188 ± 33(9)
Body	23 ± 21(13)	5 ± 5(13)	25 ± 20(13)	154 ±78(13)
Left horn	35 ± 23(14)	7 ± 6(14)	13 ± 14(14)	171 ±66(14)
'OESTRUS'				
Right horn	26 ± 16(6)	5 ± 4(6)	[a]13 ± 6(6)	133 ± 38(6)
Body	34 ± 12(15)	5 ± 3(15)	[a] 8 ± 3(15)	143 ±24(15)
Left horn	38 (1)	4 (1)	4 (1)	250 (1)
'DIOESTRUS'				
Right horn	51 ± 23(2)	4 ± 0(2)	5 ± 3(2)	100 ± 0(2)
Body	29 ± 21(3)	31 ± 35(2)	6 ± 3(2)	83 ± 29(3)

19A Snowy

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude in 'anoestrus', 'oestrus' and 'dioestrus' after PG treatment.

	% emg A/hr		amplitude μV	
'ANOESTRUS'				
Right horn	[a] 20 ± 3	(12)	200 ± 0	(2)
Body	[b] 21 ± 18	(4)	138 ± 92	(4)
Left horn	[ab] 63 ± 23	(4)	200 ± 0	(4)
'OESTRUS'				
Body	36 ± 36	(3)	167 ± 144	(3)
'DIOESTRUS'				
Right horn	51 ± 10	(3)	100 ± 0	(3)
Body	39 ± 29	(4)	69 ± 47	(4)

20A Snowy

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude in 'oestrus' and 'dioestrus' after oxytocin treatment (2 IU and 20 IU).

	% emg A/hr		amplitude μV	
'OESTRUS'(2 IU)				
Body	30 ± 12	(4)	169 ± 12	(4)
'OESTRUS'(20 IU)				
Right horn	0	(3)	0	(3)
Body	0	(3)	0	(3)
Left horn	7 ± 14	(4)	38 ± 75	(4)
'DIOESTRUS'(2 IU)				
Right horn	[a]25 ± 19	(4)	65 ± 50	(4)
Body	[a]80 ± 15	(5)	100 ± 0	(5)

21A Snowy

Electrode site variation between the right horn, body and left horn in emg A/hr and amplitude in 'anoestrus', 'oestrus' and 'dioestrus' after clenbuterol treatment.

	% emg A/hr		amplitude μV	
'ANOESTRUS'				
Right horn	0	(2)	0	(2)
Body	0	(2)	0	(2)
Left horn	8 ± 11	(2)	75 ± 106	(2)
'OESTRUS'				
Body	17	(1)	150	(1)
'DIOESTRUS'				
Right horn	12 ± 3	(2)	150 ± 0	(2)
Body	23 ± 7	(2)	75 ± 0	(2)

22A Snowy

Electrode site variation between the right horn and body in emg A/hr and amplitude in 'anoestrus'and'dioestrus' after propantheline bromide treatment.

	% emg A/hr		amplitude μV	
'ANOESTRUS'				
Left horn	*21 ± 13*	*(2)*	*200 ± 0*	*(2)*
'DIOESTRUS'				
Right horn	8	(1)	75	(1)
Body	13	(1)	150	(1)

APPENDIX B: RELATIONSHIP OF OXYTOCIN RESPONSE WITH TIME

1B Sweetie

Emg A/hr values for consecutive 10 minute intervals after 5 IU oxytocin injection in transition, oestrus and dioestrus.

	% emg A					
10 min intervals	Transition		Oestrus		Dioestrus	
1st	99 ± 4	(7)	98 ± 5	(4)	96 ± 8	(3)
2nd	77 ± 10	(7)	73 ± 8	(4)	100 ± 0	(3)
3rd	61 ± 7	(7)	43 ± 14	(4)	91 ± 15	(3)
4th	40 ± 12	(7)	34 ± 3	(4)	88 ± 12	(3)
5th	37 ± 27	(6)	17 ± 3	(5)	98 ± 2	(2)
6th	38 ± 33	(3)	20	(1)	83 ± 24	(2)

2B Fling

Emg A/hr for consecutive 10 minute intervals following 2 IU oxytocin in anoestrus, oestrus and dioestrus.

	% emg A					
10 min intervals	Anoestrus		Oestrus		Dioestrus	
1st	57 ± 42	(3)	93 ± 8	(6)	52 ± 41	(12)
2nd	60 ± 44	(3)	97 ± 6	(6)	51 ± 39	(12)
3rd	60 ± 44	(3)	100 ± 6	(6)	53 ± 34	(12)
4th			70 ± 10	(3)	50 ± 29	(12)
5th			77 ± 6	(3)	56 ± 34	(9)
6th					47 ± 38	(3)

3B Fling

Emg A/hr for consecutive 10 minute intervals following 5 IU oxytocin in anoestrus, oestrus and dioestrus.

	% emg A					
10 min intervals	Anoestrus		Oestrus		Dioestrus	
1st	72 ± 27	(9)	93 ± 5	(4)	98 ± 5	(4)
2nd	73 ± 38	(9)	80 ± 8	(4)	95 ± 10	(4)
3rd	88 ± 12	(9)	78 ± 17	(4)	93 ± 9	(4)
4th	60 ± 18	(6)	63 ± 17	(4)	95 ± 6	(4)
5th	65 ± 25	(6)	53 ± 13	(4)	98 ± 5	(4)

4B Jo

Emg A/hr for consecutive 10 minute intervals following 5 IU oxytocin in 'anoestrus', 'oestrus' and 'dioestrus'.

	% emg A					
10 min intervals	Anoestrus		Oestrus		Dioestrus	
1st	99 ± 2	(3)	93 ± 8	(3)	98 ± 3	(4)
2nd	89 ± 19	(3)	74 ± 11	(3)	85 ± 10	(4)
3rd	78 ± 19	(3)	57 ± 3	(3)	70 ± 16	(4)
4th	53 ± 9	(2)	24 ± 22	(3)	50 ± 32	(3)
5th	57 ± 9	(2)	24 ± 18	(3)	71 ± 30	(3)
6th	20 ± 0	(1)	13 ± 10	(2)	60 ± 21	(3)

APPENDIX C: EFFECT OF DRUGS ADMINISTERED ON IUP PARAMETERS AT DIFFERENT STAGES OF THE OESTROUS CYCLE

1C Sweetie

Frequency, duration and amplitude of IUP cycles as influenced by PG, oxytocin, propantheline bromide and clenbuterol during transition, oestrus and dioestrus.

Drug	Stage of cycle	Frequency cycles/min	Mean amplitude rise mm Hg	Duration min/10 min
PG	Oestrus	2.1 ± 1.20(5)	14 ± 3(5)	2.4 ± 1.3(5)
PG	Transition	1.5 ± 1.00(13)	13 ± 3(13)	2.5 ± 1.3(13)
PG	Dioestrus	1.2 ± 0.40(4)	20 ± 4(4)	2.8 ± 1.1(4)
Oxytocin	Oestrus	1.4 ± 6.00(15)	14 ± 6(15)	2.7 ± 1.8(15)
Oxytocin	Transition	0.8 ± 0.90(8)	7 ± 7(28)	2.4 ± 3.0(28)
Oxytocin	Dioestrus	1.3 ± 0.68(10)	14 ± 6(10)	4.9 ± 3.6(10)
Propantheline	Oestrus	0.8 ± 1.50(4)	4 ± 7 (4)	0.5 ± 1.0 (4)
Propantheline	Transition	0.8 ± 0.60(6)	12 ± 3(6)	2.0 ± 1.2(6)
Propantheline	Dioestrus	0.7 ± 2.90(2)	15 ± 2(2)	1.2 ± 1.4(2)
Clenbuterol	Oestrus	0.3 ± 0.42(2)	6 ± 8(2)	0.3 ± 0.5(2)
Clenbuterol	Transition	0.3 ± 1.50(3)	12 ± 2(3)	0.8 ± 0.4(3)
Clenbuterol	Dioestrus	1.0 (1)	10 (1)	0.7 (1)

2C Fling

Frequency,duration and amplitude of IUP cycles as influenced by PG, oxytocin, propantheline bromide and clenbuterol during anoestrus, oestrus and dioestrus.

Drug	Stage of cycle	Frequency cycles/min	Mean amplitude rise mm Hg	Duration min/10 min
PG	Oestrus	0.58 ± 0.76(4)	11 ± 1.4(4)	3.50 ± 6.00(4)
PG	Anoestrus	0.57 ± 0.42(5)	12 ± 2.0(5)	1.50 ± 2.00(5)
PG	Dioestrus	0.90 ± 1.40(2)	12 ± 1.4(2)	2.60 ± 0.40(2)
5 IU oxytocin	Oestrus	1.90 ± 1.20(9)	10 ± 4.0(9)	2.90 ± 2.20(9)
5 IU oxytocin	Anoestrus	1.30 ± 1.20(8)	8 ± 5.0(8)	2.50 ± 3.30(8)
5 IU oxytocin	Dioestrus	1.80 ± 0.50(8)	11 ± 2.0(8)	3.60 ± 1.10(8)
2 IU oxytocin	Anoestrus	1.00 ± 0.00(3)	18 ± 6.0(3)	1.00 ± 0 (3)
2 IU oxytocin	Dioestrus	0.67 ± 0.50(6)	8 ± 6.0(6)	1.40 ± 1.20(6)
Propantheline	Oestrus	0 (2)	0 (2)	0.00 (2)
Propantheline	Dioestrus	0 (1)	0 (1)	0.00 (1)
Propantheline	Anoestrus	0 (1)	0 (1)	0.00 (1)
Clenbuterol	Oestrus	0 (1)	0 (1)	0.00 (1)
Clenbuterol	Dioestrus	0.17 ± 2.20(2)	05 ± 7.0(2)	0.45 ± 0.67(2)

3C Jo

Frequency, duration and amplitude of IUP cycles as influenced by PG, oxytocin, propantheline bromide and clenbuterol during 'anoestrus', 'oestrus' and 'dioestrus'.

Drug	Stage of cycle	Frequency cycles/min	Mean amplitude rise mm Hg	Duration min/10 min
PG	'Oestrus'	1.1 ± 1.3(2)	10 ± 0 (2)	2.6 ± 2.6(2)
PG	'Anoestrus'	3.4 ± 1.4(2)	17 ± 2 (3)	4.0 ± 2.0(2)
PG	'Dioestrus'	3.2 ± 1.3(5)	19 ± 8 (5)	4.0 ± 1.1(5)
Oxytocin	'Oestrus'	1.3 ± 0.9(8)	08 ± 5 (8)	3.3 ± 3.2(8)
Oxytocin	'Anoestrus'	3.2 ± 1.3(5)	19 ± 8 (5)	4.0 ± 1.1(5)
Oxytocin	'Dioestrus'	1.5 ± 0.9(15)	11 ± 6 (15)	5.2 ± 3.7(15)
Propantheline	'Anoestrus'	0 (1)	0 (1)	0 (1)
Propantheline	'Dioestrus'	0 (2)	0 (2)	0 (2)
Clenbuterol	'Oestrus'	0 (1)	0 (1)	0 (1)

4C Snowy

Frequency, duration, and amplitude of IUP cycles as influenced by PG, oxytocin, propantheline bromide and clenbuterol during 'anoestrus', 'oestrus' and 'dioestrus.'

Drug	Stage of cycle	Frequency cycles/min	Mean amplitude rise mm Hg	Duration min/10 min
PG	'Oestrus'	2.00 ± 2.8(2)	11 ± 15 (2)	5.00 ± 7.1(2)
PG	'Dioestrus'	3.00 (1)	25 (1)	4.00 (1)
2 IU oxytocin	'Oestrus'	1.00 (1)	10 (1)	4.00 (1)
20 IU oxytocin	'Dioestrus'	0.75 ± 1.5(4)	20 ± 10 (4)	0.89 ± 1.8(4)
Clenbuterol	'Oestrus'	3.00 (1)	10 (1)	3.00 (1)