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Telemetric evaluation of uterine electromyographic activity and body temperature in the horse mare

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The Ohio State University, 1991
TELEMETRIC EVALUATION OF UTERINE ELECTROMYOGRAPHIC ACTIVITY AND BODY TEMPERATURE IN THE HORSE MARE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Graduate School of the Ohio State University

By

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* * * * *

The Ohio State University
1991

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INTRODUCTION

Telemetry is the application of a transmitter or telemeter to the measurement of data at a point distal to a receiver. In the biological sciences, telemetry has been used to track wildlife movements, record body temperatures, monitor heart rate and animal activity and measure skeletal electromyographic activity. These studies have encompassed a wide variety of domestic and feral animal species but not the domestic horse.

The technology has several advantages that make it an attractive alternative to traditional methodologies. Restraint of the animal is eliminated and data can be recorded more frequently than is practical or possible with other methods. Implantation of a transmitter eliminates the need for external wiring that could hinder research in a freely moving animal in a cage or stall.

The second stage of parturition in the mare is characterized by strong uterine contractions that cause the birth of the fetus within an average of twenty minutes. The successful application of radiotelemetry to the study of myometrial EMG activity at the time of parturition in the mare
could lead to utilizing the technology to study uterine contractions during early pregnancy in the mare.

Mare body temperature can also be studied with radiotelemetry. There are few studies of mare body temperature during the estrous cycle, pregnancy and at parturition. The incorporation of radiotelemetry into studies of mare body temperature may reveal variations in temperature that are dependent on the stage of the estrous cycle and pregnancy.

Therefore, the objectives of these studies were to:
1) develop a radiotelemetric system to study myometrial electromyographic activity and body temperature fluctuations in the horse mare.
2) determine the pattern of myometrial electromyographic activity in the periparturient horse mare using radiotelemetry.
3) determine the pattern of myometrial electromyographic activity in the five days preceding and following parturition in the horse mare.
4) With radiotelemetry, determine if there are fluctuations in maternal body temperature at the time of parturition.
5) determine if there is a correlation between myometrial electromyographic activity and body temperature fluctuations prior to parturition in the horse mare.
CHAPTER I

LITERATURE REVIEW

Uterine contractility

The myometrium consists of an outer layer of longitudinal and an inner layer of circular smooth muscle (Leeson et al., 1985). Contraction of the myometrium is involved in a variety of functions including parturition, uterine involution, sperm transport and intrauterine migration of embryos. The myometrium undergoes some growth during the estrous cycle (Finn and Porter, 1975; Schoenberg, 1977), with an increase in size of three to five times occurring during pregnancy due to hyperplasia and hypertrophy (Schoenberg, 1977). A complex interaction of hormones and neurotransmitters promote or inhibit uterine contractions. Estrogen and progesterone in particular influence uterine motility through regulation of other hormones and neurotransmitters, and modulation of protein synthesis in myometrial smooth muscle cells.

The basic contractile unit of the myometrium is the smooth muscle cell. Within a smooth muscle cell are thick myofilaments of myosin and thin filaments of actin and tropomyosin (Chamley-Campbell et al., 1979; Carsten and
Miller, 1987). The thick myofilaments lie parallel to the long axis of the cell, with thin filaments surrounding them. Dense bodies, which are analogous to the Z disc in skeletal muscle, are scattered throughout the myofilaments and on the cytoplasmic side of the plasma membrane (Chamley-Campbell et al., 1979; Carsten and Miller, 1987). There is also an extensive network of granular and agranular sarcoplasmic reticulum (SR; Garfield, 1984). Estrogen treatment or pregnancy causes an increase in the amount of granular SR, probably due to increased protein synthesizing capacity. The agranular or smooth SR is believed to be the primary site of intracellular calcium (Ca^{2+}) storage with the mitochondria and Golgi apparatus serving as less important intracellular Ca^{2+} sources (Carsten and Miller, 1987).

The contractile mechanism of smooth muscle is fundamentally the same as in skeletal muscle (Garfield, 1984), involving the interaction of myosin and actin to produce shortening and therefore contraction of the cell (Watterson et al., 1984; Carsten and Miller, 1987). In contrast to skeletal muscle, myosin filaments of smooth muscle lack a bare zone, which allows a greater shortening of the smooth muscle cell than can occur in skeletal muscle (Watterson et al., 1984). Increased formation of crossbridges between myosin and actin and the alignment of more crossbridges in parallel results in a stronger but slower contraction of smooth muscle (Watterson et al., 1984; Carsten and Miller, 1987).
The myosin molecule has two heavy chains forming an α helix with a globular head portion towards the NH₂ terminus and two light chains (Watterson et al., 1984; Carsten and Miller, 1987). In order for contraction to occur, phosphorylation of the myosin light chains by myosin light chain kinase (MLCK) is required (Watterson et al., 1984; Carsten and Miller, 1987). Activation of MLCK occurs after calmodulin (CaM) binds cytosolic Ca²⁺ and the CaM-Ca²⁺ complex binds to MLCK allowing phosphorylation of the myosin light chain heads and crossbridge formation with actin to occur (Vokaer, 1984; Carsten and Miller, 1987). Relaxation occurs when a cAMP dependent protein kinase phosphorylates MLCK and lowers its affinity for the CaM-Ca²⁺ complex (Vokaer, 1984; Carsten and Miller, 1987; Reimer, 1990).

For Ca²⁺ to initiate contraction of a smooth muscle cell, cytosolic concentrations need to increase from a relaxed state of 10⁻⁸ or 10⁻⁷ M to 10⁻⁶ M (Watterson et al., 1984; Carsten and Miller, 1987). Increased intracellular Ca²⁺ concentrations result from an influx of extracellular Ca²⁺ through voltage or receptor operated channels and the release of intracellular Ca²⁺ stores by stimulation of the phosphatidylinositol second messenger system (Watterson et al., 1984; Carsten and Miller, 1987; Kawarabayashi et al., 1989; Garfield et al., 1990; Sanborn and Anwar, 1990). Termination of a contraction involves the efflux of cytosolic Ca²⁺ through calcium pumps, which require a Ca²⁺-Mg²⁺ ATPase for operation and the
sequestration of Ca$_2^+$ into the intracellular stores (Carsten and Miller, 1987). Calcium channel blockers, such as verapamil, inhibit myometrial contractions by preventing the influx of Ca$_2^+$ through the channels (Corruzi et al., 1989).

The resting membrane potential (RMP) of myometrial smooth muscle cells is reported to be -50 mV (Naaktgeboren et al., 1973; Anderson, 1977; Kao, 1977; Inoue et al., 1990). Estrogen increases the RMP, making the smooth muscle cell more excitable (Marshall, 1962; Soloff, 1975; Kao, 1977). Progesterone was thought to stabilize the RMP and thus prevent uterine contractions (Marshall, 1962; Csapo and Takeda, 1965), but that view has been disputed. Studies have shown no difference in RMP between estrogen and progesterone treated myometrial smooth muscle cells (Kao, 1977).

Although electromyographic (EMG) activity can be detected throughout most of pregnancy, a lack of coordinated activity keeps the uterus in a quiescent state. This lack of coordinated EMG activity is due to few or no gap junctions being present when the myometrium is under progesterone domination (Garfield et al., 1990). Gap junctions provide one means of intracellular communication between cells and couple cells electrically and metabolically (Caveney, 1985). As progesterone levels decline near the end of pregnancy and estrogen levels increase, a low progesterone to estrogen ratio results with a concurrent increase in the formation of myometrial gap junctions (Garfield et al., 1979; Garfield et
Estrogen treatment or a high estrogen to progesterone ratio improved propagation of action potentials in rat myometria (Miller et al., 1989). Increased action potential propagation and the emergence of coordinated myometrial activity were due to the appearance of gap junctions (Garfield, 1984; Miller et al., 1989). The formation of gap junctions increased late in pregnancy or as a result of estrogen treatment (Garfield et al., 1979; Burghardt et al., 1987) and their appearance at the time of parturition improved propagation of action potentials and coordination of EMG burst activity in rat myometria (Miller et al., 1989). In late pregnant rats, the increase in gap junction formation was correlated with a decrease in maternal progesterone levels, an increase in fetal estrogen levels and increases in fetal and maternal estrogen to progesterone ratios (Garfield et al., 1979, MacKenzie and Garfield, 1986). Further evidence that estrogen stimulates gap junction formation is the prevention of gap junction formation in the myometrium of immature rats pretreated with estradiol 17β and followed by tamoxifen treatment, a nonsteroidal antiestrogenic compound (MacKenzie and Garfield, 1986). In addition to estrogen stimulation of gap formation, distension of the uterus increased the number of gap junctions in ovariectomized rats (Wathes and Porter, 1982). Uterine distension plus treatment of ovariectomized rats with estradiol 17β resulted in even greater numbers of gap junctions.
Increased formation of gap junctions is the result of estrogen stimulation of gene transcription and protein synthesis (Rexroad, 1978; Mackenzie and Garfield, 1986; Hendrix et al., 1991). This has been demonstrated in the rat uterus by treatment with cycloheximide, a protein synthesis inhibitor, and actinomycin D, an inhibitor of DNA transcription. Both compounds decreased myometrial gap junction formation in the rat uterus (MacKenzie and Garfield, 1984).

Progesterone is inhibitory to uterine contractions during the estrous cycle and pregnancy. Metabolites of progesterone in the late pregnant rat had a greater inhibitory effect on uterine contractions than progesterone itself (Putnam et al., 1991). Progesterone treatment or the high progesterone to estrogen ratio during pregnancy is inhibitory to gap junction formation possibly by suppressing expression of the gene that is responsible for gap junctions (Garfield et al., 1980a; Garfield et al., 1987; Garfield et al., 1990). Evidence for this hypothesis was demonstrated with the antiprogestosterone compound RU486, which binds the progesterone receptor and prevents the transcription of progesterone dependent proteins (Garfield et al., 1987). Treatment of pregnant Rhesus macaques with RU486 resulted in increased myometrial gap junction formation through a decrease in the inhibitory effects of progesterone (Haluska et al., 1989). Similar results have been reported in the rat (Garfield et al., 1987).
Production and assembly of connexin 43, a gap junction protein, were increased by RU486 treatment (Hendrix et al., 1991).

Prostaglandins, eicosanoids and thromboxane may also regulate gap junction formation (Garfield et al., 1980a,b). Inhibition of cyclooxygenase reduced the number of gap junctions in myometrial tissues from pregnant rats (Garfield et al., 1980a). Prostaglandins may stimulate gap junction formation by increasing membrane fluidity and aggregation of membrane bound gap junction proteins (Garfield et al., 1980b).

Nutritional deficiencies may affect gap junction formation and myometrial contractions. A deficiency of dietary zinc resulted in a decrease in the number and appearance of myometrial gap junctions in late gestation rats (Dylewski et al., 1986; Lytton and Bunce, 1986). Although oxytocin treatment induced parturition in zinc-deficient rats, the contractions were asynchronous and irregular (Lytton and Bunce, 1986). Zinc may be involved with the estrogen receptor and a deficiency would impair steroid-receptor interaction and subsequent estrogen controlled gene expression.

Estrogen stimulates the rate of protein synthesis (Anderson, 1978) and increases the number of smooth muscle cells in the uteri of ovariectomized rats (Finn and Porter, 1975). In ewes, estrogen treatment caused an increase in the uterine RNA/DNA ratio, but this effect was blocked by actinomycin D (Rexroad, 1978). Actinomycin D also blocked
the stimulatory effect of estrogen on uterine contractions (Rexroad, 1978) as did cycloheximide (Rexroad, 1981a). Estradiol 17β treatment stimulated production of myometrial estrogen and progesterone receptors in ewes, as well as an increase in cytosolic estradiol concentrations (Rexroad, 1981b). Increased interaction of estrogen with its receptor and translocation to the nucleus would result in an increased rate of gene transcription and protein synthesis (Rexroad, 1981a,b; Rexroad, 1984). In contrast, progesterone treatment decreased the amount of estrogen receptors and could affect uterine contractions by inhibiting estradiol 17β induced receptors and RNA synthesis (Rexroad, 1981a,b).

Myometrial activity varies with the stage of the estrous cycle and the predominant ovarian steroid hormone. Maximum uterine motility occurs during the estrous cycle when estrogen is dominant (Ruckebusch and Bueno, 1976; Rexroad, 1978; Taverne et al., 1979; Sigger et al., 1984; Cruz and Rudolph, 1986; Rodriguez-Martinez et al., 1987a; Claus et al., 1989; Ko et al., 1989d; Rhodes and Nathanielsz, 1990). Estradiol 17β treatment of ovariectomized ewes caused a rapid increase in EMG activity along with an increased frequency of short duration EMG events (Massmann et al., 1991). During the luteal phase, EMG activity becomes unpatterned with decreased spike amplitudes (Naaktgeboren et al., 1973; Taverne et al., 1979; Sigger et al., 1984). A lack of propagating activity is also apparent (Rodriguez-Martinez et al., 1987a).
The direction of uterine contractions or EMG activity from diestrus to estrus changes and is dependent on the ratio of estrogen to progesterone (Kern and Schill, 1982). In cattle, the direction of uterine contractions is primarily tubocervical but during the second day of estrus, the direction reverses to cervicotomy (Rodriquez-Martinez et al., 1987a). Reversal of the propagation of EMG activity to a cervicotomy direction also occurs during estrus in ewes (Naaktgeboren et al., 1973). Observation of uterine contractions in anesthetized ewes have revealed a change in direction towards the oviducts during estrus (Croker and Shelton, 1973; Hawk, 1975; Rexroad, 1978). Treatment of ovariectomized ewes with estradiol 17β caused an increase in contractions moving in a cervicotomy direction (Croker and Shelton, 1973; Hawk, 1975; Rexroad, 1978). The origin of the contractions appeared to be in the posterior uterus, particularly the body (Hawk, 1975; Rexroad, 1978). Progesterone treatment had the opposite effect of estradiol 17β by increasing the number of tubocervical uterine contractions in ewes (Croker et al., 1973). The reversal of the direction of uterine contractions during estrus would serve to facilitate sperm transport (Rexroad, 1978).

Oxytocin is a powerful uterokinetic substance that can produce its effect during the estrous cycle (LeBlanc et al., 1984; Cooper and Foote, 1986; Rodriquez-Martinez et al., 1987c; Sharpe et al., 1988; Ko et al., 1989b) or to induce
labor (Jeffcott and Rossdale, 1972; Dawood, 1990). It stimulates myometrial contraction by increasing intracellular Ca\(^{2+}\) through receptor and voltage operated channels and the stimulation of the turnover of phosphatidylinositol (Zerobin and Kundig, 1980; Bade and Gardner, 1985; Edwards et al., 1986; Maigaard et al., 1986; Marc et al., 1986; Carsten and Miller, 1987; Sanborn and Anwar, 1990; Soloff, 1990). The activity of Ca\(^{2+}\)Mg\(^{2+}\)ATPase in the Ca\(^{2+}\) pumps, and therefore Ca\(^{2+}\) efflux, is inhibited by oxytocin (Tuross et al., 1987; Soloff, 1990). Phasic and tonic uterine contractions have been described in rat myometrium after oxytocin treatment (Edwards et al., 1986). Calcium influx through voltage operated channels occurred during phasic contractions and a persistent depolarization without calcium influx occurred during the tonic contractions.

Receptors for oxytocin are specific for myometrial smooth muscle and not other smooth muscle (Fuchs, 1990) and are upregulated by estrogen or by an increased estrogen to progesterone ratio (Carsten and Miller, 1987; Fuchs, 1990; Soloff, 1990). The affinity of oxytocin for its receptor is also increased by estrogen (Soloff, 1975). Oxytocin receptors reach their highest concentrations in the myometrium at term (Fuchs, 1988). Distension of the uterus also increases the number of oxytocin receptors (Fuchs, 1990). Progesterone has an inhibitory effect on the production of oxytocin receptors in several species except women (Fuchs, 1990). Regional
differences in the population of myometrial oxytocin receptors have been described in late gestation rats (Gorodeski et al., 1990). Greater numbers of oxytocin receptors were found in the uterine segment most distal to the ovary compared to the segment most proximal to the ovary. A progesterone gradient that was highest in the proximal uterine segment and lowest in the distal segment was also reported. Vasopressin crossreacts with oxytocin receptors (Rivera et al., 1990) but is 20 to 30 times less potent than oxytocin in stimulating uterine contractions (Chan et al., 1990).

Several prostaglandins also exert an influence on myometrial contractions. Uterine prostaglandin production occurs in the endometrium, decidua, amnion and chorion (Carsten and Miller, 1987; Haluska et al., 1988). Estrogen treatment of animals or the increasing estrogen to progesterone ratio in late pregnancy stimulates production of prostaglandins F$_{2\alpha}$, E$_1$ and E$_2$ (Mackenzie et al., 1983; Carsten and Miller, 1987; Massmann et al., 1991). An inhibitory effect of progesterone on prostaglandin synthetase was demonstrated by treatment of pregnant rhesus monkeys with RU 486, which caused an increase in amniotic fluid prostaglandins (Haluska et al., 1988). Prostacyclin (PGI$_2$) production is inhibited by estrogen (MacKenzie et al., 1983). Thromboxane and eicosanoids may influence uterine contractility by stimulating gap junction formation (Garfield et al., 1980b).
Prostaglandins $F_{2\alpha}$ (PGF$_{2\alpha}$), $E_1$ and $E_2$ are generally considered to stimulate uterine contractions. Increased uterine contractility following treatment of the whole animal or uterine strips in vitro with prostaglandin $F_{2\alpha}$ or an analogue has been reported in ewes (Hawk and Conley, 1985), cows (Cooper and Foote, 1986; Garcia-Villar et al., 1987; Rodriguez-Martinez et al., 1987b; Eiler et al., 1989), goats (Cooke and Homeida, 1989), mares (Capraro et al., 1977; Taverne et al., 1979; Goddard and Allen, 1985a; Cross and Ginther, 1988), rats (Tuross et al., 1987; Wainmann et al., 1988) and women (Giannopoulos et al., 1985). Evidence against a uterokinetic effect of prostaglandin $F_{2\alpha}$ has been reported in mares (Sharpe et al., 1988) and postpartum cows (Ko et al., 1989c). There is also conflicting evidence concerning the uterokinetic effects of the PGF$_{2\alpha}$ analogues cloprostenol, fenprostelene and dinoprost (Ruckebusch and Bueno, 1976; Garcia-Villar et al., 1987; Rodriguez-Martinez et al., 1987b; Eiler et al., 1989). Prostaglandin $F_{2\alpha}$ and $E_2$ affect intracellular $Ca^{2+}$ concentrations by different mechanisms. Prostaglandin $F_{2\alpha}$ causes an increase in $Ca^{2+}$ influx, release of $Ca^{2+}$ from intracellular stores and inhibition of the $Ca^{2+}$ pump (Carsten and Miller, 1987; Molnar et al., 1987; Tuross et al., 1987). Prostaglandin $E_2$ appears to inhibit $Ca^{2+}$ uptake (Carsten and Miller, 1987) and modulate cAMP concentrations (Molnar et al., 1987).
Prostacyclin produces an inhibitory effect on uterine contractions through the increased production of cAMP and possibly through closure of gap junctions (Omini et al., 1979; MacKenzie et al., 1983; Haluska et al., 1988; Cooke and Homeida, 1989; Garfield et al., 1990). Although estrogen inhibits PGI$_2$ production, PGI$_2$ treatment of estrogen treated ovariectomized animals will inhibit spontaneous uterine motility (Cooke and Homeida, 1989).

Relaxin is a protein hormone with inhibitory effects on uterine contractility (Porter and Watts, 1986; Sherwood et al., 1990; Bagna et al., 1991). It also functions to cause cervical softening and dilatation (Bagna et al., 1991). The hormone has structural but not functional similarity to insulin (Sherwood et al., 1990). The corpus luteum produces relaxin in some species such as rats and pigs (Taylor and Clark, 1987; Sherwood et al., 1990), whereas in the mare, relaxin is produced from the placenta as early as 65 days of pregnancy (Stewart and Stabenfeldt, 1981; Stewart et al., 1982). Relaxin secretion appears to be estrogen dependent (Sherwood et al., 1990) and in swine corpora lutea, PGE$_2$ increases relaxin production while human chorionic gonadotropin is inhibitory (Taylor and Clark, 1987).

Relaxin treatment in late pregnant cows has been reported to cause a decrease in progesterone concentrations and early calving (Bagna et al., 1991). Although relaxin will inhibit uterine contractions, its effect can be overridden by oxytocin
or prostaglandin $F_{2\alpha}$ (Porter and Watts, 1986; Sherwood et al., 1990). A deficiency of relaxin can result in dystocia and retained placenta (Sherwood et al., 1990).

The myometrium is innervated by the autonomic nervous system and has cholinergic, $\alpha$- and $\beta$-adrenergic receptors (Marshall, 1973; Roberts et al., 1977; Kern and Schill, 1983; Korenman and Krall, 1984; Watterson et al., 1984; Marnet et al., 1987). Cholinergic receptors on the myometrium are classified as muscarinic receptors. Carbachol is an acetylcholine agonist that causes uterine contractions (Bade and Gardner, 1985; Marc et al., 1986). It has similar effects as oxytocin and will cause an influx of $Ca^{2+}$ (Bade and Gardner, 1985) along with an increased rate of phosphatidyl turnover and inositol triphosphate (Marc et al., 1986). When used midway through parturition in sows, carbachol reduced the stillbirth rate of neonatal pigs by stimulating uterine contractions (Sprecher et al., 1975).

The populations of $\alpha$- and $\beta$-adrenergic receptors vary cyclically and with stage of pregnancy (Roberts et al., 1977; Kern and Schill, 1983; Cruz and Rudolph, 1985). Estrogen treatment caused an increase in both $\alpha_{1}$- and $\alpha_{2}$-adrenergic receptors (Kern and Schill, 1983; Marnet et al., 1987) while $\beta_{2}$-receptors were high in progesterone primed uteri (Roberts et al., 1977; Cruz and Rudolph, 1985). Prostaglandin $F_{2\alpha}$ appears to increase $\alpha_{1}$-adrenergic sensitivity in the estrogen dominated rabbit uterus (Hurd et al., 1991). Inhibition of
prostaglandin synthesis with meclofenamate decreased $\alpha_1$-adrenergic sensitivity.

Stimulation of $\alpha_1$- and $\alpha_2$-adrenergic receptors by the endogenous catecholamines, epinephrine and norepinephrine, or an agonist results in myometrial contractions (Harbert and Spisso, 1981; Marnet et al., 1987; Wu et al., 1988). Alpha$_1$ agonists cause an increase in cytosolic Ca$^{2+}$ through the phosphatidylinositol second messenger system (Exton, 1985; Reimer, 1990). The products of phosphatidylinositol turnover are diacyl glycerol and inositol triphosphate (Reimer, 1990). Diacyl glycerol activates protein kinase C and inositol triphosphate releases intracellular Ca$^{2+}$ stores. Protein kinase C may terminate a Ca$^{2+}$ signal by promoting Ca$^{2+}$ efflux (Reimer, 1990). In one study, though, several $\alpha$-adrenergic agonists failed to cause an increase in inositol triphosphate (Phillipe and Bangalore, 1989).

Alpha$_2$ agonists inhibit adenylate cyclase activity and cyclic adenosine 3',5'-monophosphate (cAMP) production (Exton, 1985; Wu et al., 1988). Several studies have demonstrated that inhibition of adenylate cyclase and cAMP production or the inhibition of cAMP phosphodiesterase have a stimulatory effect on uterine contractions (Laifer et al., 1986; Kofinas et al., 1987; Molnar et al., 1987). Xylazine, an $\alpha_2$-agonist that stimulates uterine contractions in cattle (LeBalnc et al., 1984; Rodriguez-Martinez et al., 1987c; Ko et al., 1989a,e; Ko et al., 1990a) may also function through a
receptor operated Ca\textsuperscript{2+} channel because verapamil blocked the stimulatory effect of xylazine on myometrial contraction (Ko et al., 1990b).

Beta\textsubscript{2}-adrenergic receptors predominate during pregnancy or in progesterone treated animals (Roberts et al., 1977; Kern and Schill, 1983; Abrahamsson, 1986). Progesterone treated ovariectomized rats had a three to six fold increase in the number of \( \beta_2 \)-adrenergic receptors and may also activate the gene responsible for \( \beta_2 \)-adrenergic receptor synthesis (Maltier et al., 1989). Dexamethasone may inhibit uterine contractility by increasing the number of \( \beta_2 \)-adrenergic receptors or by inhibition of norepinephrine. In rats, the population of \( \alpha_1 \)- and \( \beta_2 \)-adrenergic receptors remained constant until day 22 (day of parturition) when an increase in \( \alpha_1 \)-adrenergic receptors and a decrease in the number of \( \beta_2 \)-adrenergic receptors occurred (Legrand et al., 1987a). The change in receptor population was temporally related to the rise in estrogen and decline of progesterone concentrations. In ewes, a decrease in innervation and norepinephrine content of the uterus has been reported during pregnancy, possibly due to a greater rate of growth of the uterus compared to innervation or degeneration of axons. (Sigger et al., 1986). A decrease in uterine norepinephrine content, indicative of neuronal inactivation or degradation, occurred during pregnancy in several species (Arkinstall and Jones, 1985).
Stimulation of $\beta_2$-adrenergic receptors activates adenylate cyclase, increases production of cAMP and inactivation of myosin light chain kinase (Marshall, 1973; Zerobin and Kundig, 1980; Korenman and Krall, 1984; Harbon et al., 1984; Watterson et al., 1984; Wu et al., 1988; Garfield et al., 1990). As a result of increasing cAMP levels, there is activation of a protein kinase that phosphorylates and inactivates myosin light chain kinase with resultant relaxation of the cell (Vokaer, 1984). Beta agonists also promote inactivity of the myometrium by increasing Ca$^{2+}$ efflux and sequestration, inhibiting action potential propagation and promoting closure of gap junctions (Garfield et al., 1990). In veterinary medicine, $\beta_2$-agonists such as clenbuterol are used therapeutically to treat the premature onset of labor (Zerobin and Kundig, 1980; Kern and Schill, 1983; Menard, 1983). Beta antagonists caused an increase in frequency, duration and amplitude of uterine contractions in pregnant rhesus monkeys (Harbert and Spisso, 1981).

The two layers of the myometrium differ in their response to estrogen, progesterone, oxytocin and prostaglandin $F_2\alpha$. Electromyographic activity of the pregnant ewe occurred more readily in the longitudinal plane (Parkington et al., 1988) and improved cell-to-cell coupling was reported in the longitudinal muscle layer of rats at term (Miller et al., 1989). A decreased contractile response to electrical
stimulation was reported in the circular muscle layer with an increased contractile response occurring in estrogen- and progesterone-treated ovariectomized guinea pigs (Adams et al., 1985). Progesterone also had a selective effect on the longitudinal muscle layer in the estrogen treated groups. The longitudinal muscle layer in rats showed a greater response to oxytocin stimulation than did the circular muscle layer (Tuross et al., 1987). Additionally, there were more $\alpha$-adrenergic receptors in circular muscle and more $\beta_2$-adrenergic receptors in longitudinal muscle until late in pregnancy (Tuross et al., 1987).

A circadian rhythm of uterine contractility has been described in pregnant monkeys with peak activity occurring during the dark phase and the nadir occurring during the light hours (Ducsay et al., 1983; Taylor et al., 1983; Haluska et al., 1987c; Ducsay and McNutt, 1989; McNutt and Ducsay, 1990; Ducsay and Yellon, 1991). A report of peak uterine activity during the daytime in pregnant monkeys (Harbert and Spisso, 1980) may have been due to a shorter length of the light phase than was used in other studies (Ducsay et al., 1983). The increase in uterine contractility at night does not appear to be due to a direct effect of melatonin (Ducsay and Yellon, 1991) or catecholamines (McNutt and Ducsay, 1990). Fetal adrenal activity may influence the circadian rhythm of uterine contractility since dexamethasone inhibition of fetal adrenal blocked the uterine activity rhythm (Ducsay and McNutt, 1989).
Pregnant rhesus monkeys kept in constant darkness developed a 24 hour variation in EMG activity in the absence of external cues (Honnebier et al., 1991). The authors concluded that, in monkeys, myometrial EMG activity has a true circadian rhythm. Myometrial EMG activity was lower in the day than at night in pony mares before parturition but the pattern was not present on the last day before parturition (Haluska et al., 1987a). No consistent daily rhythm of myometrial EMG activity was found in another study of pregnant pony mares (Dudan et al., 1987).

Maturation of the fetal hypothalmo-pituitary axis and increasing fetal adrenocorticotropin hormone concentrations are considered to be the signal that initiates parturition (Bazer and First, 1983). The resultant increase in fetal cortisol has different effects in different species. For example, increased fetal cortisol causes an increase in placental estrogen and decreased progesterone production in sheep (Bazer and First, 1983). In pigs, fetal cortisol stimulates placental production of prostaglandin F₂₅ that causes luteolysis (Bazer and First, 1983). Treatment with dexamethasone can induce parturition in the domestic livestock species, although the efficacy of treatment varies (Bazer and First, 1983; Lye and Freitag, 1991). Although dexamethasone will induce parturition in the mare, it has not been recommended for use in the mare due to dystocia (Jeffcott and Rossdale, 1972).
There are other variables that can affect uterine contractility. For example, myometrial EMG activity in the mare was reported to be influenced by environmental stimuli such as a person entering a stall or at feeding time (Taverne et al., 1979). Simultaneous recording of myometrial EMG activity and electrocardiographic (ECG) activity in mares revealed that both EMG and ECG activity increased when a person entered a stall (Haluska et al., 1987a). The ECG activity was recorded in that study to serve as a measure of sympathetic nervous system and implied that environmental stimuli have more of a general effect on the mare than a specific effect on the uterus. Human activity, such as cage cleaning, did not disturb the pattern of EMG activity of pregnant monkeys kept in constant darkness (Honnebier et al., 1991). This could represent acclimatization of the monkeys to human presence.

Surgery to implant myometrial electrodes caused an elevation in EMG activity in ewes that lasted several days before gradually recovering (Nathanielsz et al., 1982; Sigger et al., 1984). A change in posture of the dam, such as lying down, caused a transient increase in intrauterine pressure in sheep (Nathanielsz et al., 1980). In order to study the effect of fetal movement on myometrial EMG activity, curare was given to sheep fetuses (Nathanielsz et al., 1982). No difference in myometrial EMG activity was found after curare treatment.
Various methods have been developed to study uterine contractility in vivo and in vitro, including electromyography, intrauterine pressure transducers, visual observation in anesthetized animals and single cell microelectrodes. Advances in computer equipment and programming have improved data acquisition and analysis.

Electromyography measures the electrical activity of smooth and skeletal muscles. To measure myometrial EMG activity, electrodes consisting of two wires are placed close together (e.g. 5 mm apart) on the uterus. The wires may be bare and embedded into the myometrium (Sigger et al., 1984; Claus et al., 1989) or on a disk like terminus sewn to the serosa (Naaktgeboren et al., 1973; Taverne et al., 1979). Placement of multiple electrodes has revealed regional differences in myometrial EMG activity (Ruckebusch and Bueno, 1976; Sigger et al., 1984) as well as the direction of waves of contractile activity (Naaktgeboren et al., 1973; Parkington et al., 1988). For long term study, electrode wires have been routed subcutaneously to an external plug placed on the animal's back.

Analysis of myometrial EMG records usually involves the frequency, duration and amplitude of bursts of EMG activity. A hard copy of the raw signal can be analyzed for frequency and duration of events or the data can be processed by computer programming. Full-wave rectification and integration transform the raw data into a form that can be analyzed. An
arbitrary definition of what an EMG event is based on the amount of time between apparent bursts of activity. A signal that drops below a predefined threshold voltage for the length of time chosen signals the termination of an EMG event. Figueroa et al. (1985) have defined this period as the delimiter. Power spectrum or Fourier analysis of the components of the waveform is another approach to the analysis of EMG activity (Hsu et al., 1989).

Intrauterine pressure transducers have been used to study uterine contractions in several species including cattle (Rodriquez-Martinez et al., 1987), mares (Goddard and Allen, 1985a) and ewes (Goddard and Allen, 1985b). The transducers were catheters equipped with strain gauges containing an electrical circuit that converts force into an electrical signal (Rodriquez-Martinez et al., 1987). Increases in the signal are indicative of the force of contractile activity. When used in conjunction with myometrial EMG electrodes, intrauterine pressure transducers compare EMG activity with force of a contraction (Krishnamurti et al., 1982). Increases in EMG activity were accompanied by an increase in force. Data have been analyzed for the area under the contraction curve (Goddard and Allen, 1985). In a study of mare uterine contractile activity, a stretch gauge was developed using circuitry similar to that used in intrauterine pressure transducers (Capraro et al., 1977). A change in the diameter
of the uterine horn due to contractile activity was detected by the gauge and converted to an electrical signal.

Direct observation of the uterus, in anesthetized ewes of during mid ventral laparotomy, has been used to study uterine contractions (Croker and Shelton, 1973; Hawk, 1975; Rexroad, 1980). After a stabilization period and the number and direction of contractions were recorded. These studies have noted changes in the frequency and direction of uterine contractions during the estrous cycle. Observation of uterine contractile activity in the mare's uterus is possible with transrectal ultrasonography (Ginther, 1985) and has been studied in nonpregnant and early pregnant mares (Cross and Ginther, 1988; Griffin and Ginther, 1990).

The role of uterine contractions in early pregnancy has been studied in several species in which the embryo or embryos undergo a period of transuterine migration. Transuterine migration and spacing of rat embryos occurs at days 4 to 5 of pregnancy (Legrand et al., 1987a). There is also a transient increase in myometrial EMG activity, peak concentrations of estrogen at day 4 and a seven fold increase in progesterone concentrations (Legrand et al., 1989). Since disruption of embryo migration and inhibition of EMG activity can be achieved by treatment with prazosin, an $\alpha_1$-adrenergic antagonist, control of the rat uterus at this stage appears to controlled by norepinephrine (Legrand et al., 1989). Once spacing has occurred, there was a decrease in the uterine
norepinephrine content around the blastocysts compared to segments of uterus between blastocysts (Legrand et al., 1987b). The reduction in norepinephrine content would limit uterine contractions in the area of the blastocysts.

An increase in the frequency of contractions in myometrial muscle, taken from various days of pregnancy in sows, was associated with the time of embryonic migration (Pope et al., 1982a). Since swine embryos also produce estrogen (Pope et al., 1983), cholesterol and estradiol 17β impregnated silastic beads were placed in sows' uteri (Pope et al., 1982b; Pope et al., 1983). The estradiol 17β beads migrated further in the sow's uterus than did the cholesterol beads. Estrogen production by the embryos may be important in initiating embryonic migration (Pope et al., 1983).

Transuterine migration of a twin embryo from one horn to another has been reported to occur in ewes at day 14 to 15 of pregnancy (Nephew et al., 1989). The stimulus for migration was not known since it occurred before there was detectable estrogen production by the embryos. An increase in myometrial EMG activity during early pregnancy in the ewe (Rhodes and Nathanielsz, 1990) may be associated with embryo migration.

Movement of equine conceptuses has been known through studies of the location of the primary corpus luteum relative to the gravid uterine horn (Bain and Howey, 1975; Butterfield and Matthews, 1979). Later studies with ultrasonography revealed a pattern of embryonic transuterine migration or
mobility that occurs between day 9 and 15 of pregnancy (Ginther, 1983a,b). These studies also produced the observation that there appeared to be ultrasonically visible uterine contractile activity when the transducer was held in a longitudinal plane above the uterine body (Ginther, 1985). A scoring system of the uterine contractile activity was developed and applied to groups of nonpregnant and early pregnant mares (Cross and Ginther, 1988). Uterine contractile activity scores were greater in pregnant mares than nonpregnant mares between days 10 and 14 postovulation. The period of maximum equine conceptus transuterine migration and increased uterine contractile activity were temporally related to the time of maternal recognition of pregnancy in the mare, reported to occur at day 16 in one study (Hershmann and Douglas, 1979) and between days 11 and 14 in another study (Goff et al., 1987). Maternal recognition of pregnancy in domestic livestock species involves the continued production of progesterone by the corpus luteum during the time when luteolysis would normally be occurring in the nonpregnant animal. Prostaglandin \( F_{2\alpha} \) of uterine origin is considered to be the primary luteolysin in cattle, sheep, pigs and horses (Knickerbocker et al., 1988) and studies have shown that the presence of a conceptus inhibits endometrial production and(or) release of prostaglandin \( F_{2\alpha} \) in vivo and in vitro in cows (Knickerbocker et al., 1986; Gross et al., 1988), ewes (Godkin et al., 1984; Lacroix and Kann, 1986), sows (Bazer
and Thatcher, 1977; Gross et al., 1984) and mares (Sharp et al., 1984; Sharp and McDowell, 1985). In the mare, the conceptus enters the uterine horn at the early blastocyst stage at 5 or 6 days postovulation (Ginther, 1979) but, unlike the bovine conceptus, it does not remain in the ipsilateral horn to the corpus luteum. Nor does equine conceptus undergo expansion of the trophoblast but instead retains a spherical shape enclosed within an acellular capsule (Betteridge, 1987). The role of the extensive transuterine migration or mobility is believed to place the conceptus in contact with the entire endometrium and inhibit endometrial production or release of PGF₂α, and prevent luteolysis (Ginther, 1985; McDowell et al., 1988). To demonstrate the importance of conceptus mobility in the maintenance of early pregnancy, one uterine horn in a group of pony mares was ligated to restrict mobility of the embryonic vesicle. This resulted in failure of pregnancy maintenance in four of five mares (McDowell et al., 1988). In contrast, when the conceptus was allowed to contact 80% of the endometrium (control group), pregnancy was maintained in all four mares. Compared to control mares, serum progesterone levels were decreased in mares in which the conceptus was restricted to one horn.

The stimulus for uterine contractile activity in the early pregnant mare is not known. Estrogen has been investigated since it is produced by early equine embryos (Heap et al., 1982) and stimulates uterine contractile
activity in seasonally anovulatory mares (Cross and Ginther, 1987). However, estradiol 17β treatment did not increase uterine contractile activity or extend the embryonic vesicle mobility phase (Bessent et al., 1988). The equine conceptus also produces a specific array of proteins before day 14 of pregnancy (McDowell et al., 1990). Although these proteins may represent an equine trophoblastic protein, they may also function in stimulating uterine contractility during early pregnancy. A role for progesterone in the mobility of the early equine conceptus and therefore uterine contractility has been reported. Progesterone removal by ovariectomy or prostaglandin F2α treatment resulted in a decreased rate of conceptus mobility in early pregnant mares that could be restored with exogenous progesterone (Kastelic et al., 1986).

Telemetry

A telemetric transmitter contains circuitry to convert biological data input from a subject animal into electrical signals that are then transmitted to a receiver (Ko and Neuman, 1967). The device should be small with a reliable transmission range and high sensitivity (Ko and Neuman, 1967; Riley, 1970). Tissue rejection of the implanted device is a potential problem and materials such as paraffin, plastic and silastic have been used as the outermost coating (Ko and Neuman, 1967; Michael and Weller, 1968; Riley, 1970; Jungers and Stern, 1980). The circuitry is usually protected by epoxy resins or plastic. The power supply of the transmitter is a
concern because of the size of the batteries required to power the unit (Ko and Neuman, 1967; Riley, 1970). Lithium batteries are small with a life of six months of continuous use (Lefcourt et al., 1986). At the end of battery life, voltage begins to decay and affect the transmitted signal (Lefcourt et al., 1986).

Radiotelemetric transmitters most often use frequency-modulated (FM) or amplitude-modulated (AM) signal carrier frequencies. An FM transmitter produces a higher quality signal that will drop out suddenly when reception becomes marginal (Loeb and Gans, 1986). Many commercially available transmitters use an AM carrier frequency. The signal is highly directional and will introduce errors into the data if the transmitter orientation to the receiver changes signal reception (Ko and Neuman, 1967; Gallaher et al., 1985; Gans and Loeb, 1987). Gallaher et al. (1985) addressed this problem by utilizing a pair of closed loop antennae placed around a plexiglass rat cage to create an omnidirectional antenna system.

Radiotelemetry has been used to study intracardial electrograms (Wahlers et al., 1987), skeletal EMG activity during locomotion (George and Joseph, 1967; Ko and Neuman, 1967; Jungers and Stern, 1980), uterine contractile activity (Michael and Weller, 1968; Ducsay et al., 1983) and body temperature (Zartman and DeAlba, 1982; Gallaher et al., 1985; Lefcourt et al., 1986). Telemetered skeletal muscle EMG data
were reported to be free from the electrical noise (60 Hz) that was superimposed on data obtained from a hardwired system (Ko and Neuman, 1967). However, telemetered signals may be subjected to electrical noise interference from a variety of sources such as electric motors and fluorescent lights (Loeb and Gans, 1986).

Michael and Weller (1968) used an FM transmitter with a pair of stainless steel wire electrodes to measure myometrial EMG activity in three monkeys. The authors reported they were able to receive a strong signal with a few areas of poor signal reception that were not a problem. No follow up to this report was found in the literature. A study of circadian patterns of uterine contractile activity in monkeys included one monkey that had been fitted with a uterine pressure transducer connected to a telemetry unit placed in a backpack (Ducsay et al., 1983). The pattern of uterine activity was similar to that of restrained monkeys. No other studies that applied radiotelemetry to the study of uterine contractility were found.

**Body temperature**

The preoptic area (POA) of the hypothalamus is the site of thermoregulation with neurons in this area classified as warm sensitive, cold sensitive and temperature insensitive (Boulant, 1991). Warm sensitive neurons control heat loss and suppress heat production while cold sensitive neurons stimulate heat production. Approximately 30% of neurons in
the POA are warm sensitive, 10% are cold sensitive and the remaining 60% are temperature insensitive (Silva and Boulant, 1986). The steroid hormones and prostaglandins such as PGE₂, appear to affect thermoregulation by stimulation or inhibition of the firing rates of neurons (Boulant, 1991).

There is also a set point temperature in the POA (Burdick and Hodgson, 1990; Boulant, 1991). An increase of the POA temperature above the set point causes heat loss responses to begin to return the temperature back to the set point (Boulant, 1991). During fever, pyrogens increase the set point temperature and cause a decreased firing rate of warm sensitive neurons (Boulant, 1991).

Body heat is produced by metabolic reactions and is distributed by the circulatory system (Boulant, 1991). Shivering in animals and brown adipose tissue in neonates and cold adapted animals also produce heat. Heat loss from the body is accomplished by conduction, convection, radiation and evaporation (Burdick and Hodgson, 1990; Boulant, 1991). Skin temperature regulates all methods of heat loss except evaporation, which is the only heat loss mechanism to function when the ambient temperature becomes equal to or greater than skin temperature (Burdick and Hodgson, 1990; Boulant, 1991).

There is a diurnal variation in the body temperature of pregnant and nonpregnant animals (Dufty, 1971; Ruppenthal and Goodlin, 1982; Zartman and DeAlba, 1982; Fujimoto et al., 1988; Haluska and Wilkins, 1989). The body temperatures of
animals tend to be lower in the morning and higher in the evening. This diurnal pattern was not apparent on the day before parturition in pony mares (Haluska and Wilkins, 1989). Pregnant rhesus monkeys kept in constant darkness developed a 24 hour body temperature variation that was independent of a synchronizing cue or Zeitgeber and was in phase with myometrial EMG activity (Honnebier et al., 1991). The authors concluded that the two rhythms are interdependent or subject to the same maternal timekeeping mechanism.

In addition to a circadian or diurnal pattern of body temperature, there is also a cyclic variation. Higher body temperatures have been reported during the luteal phase when progesterone is the predominant ovarian steroid hormone (Israel and Schneller, 1950; Wrenn et al., 1958; Bane and Rajakoski, 1961; Lewis and Newman, 1984; de Mouzon et al., 1984). Progesterone treatment caused an increase in body temperature in ovariectomized women (Israel and Schneller, 1950) and cows (Wrenn et al., 1958). Also, an increase in body temperature during the luteal phase in cattle coincided with the presence of active luteal cells (Bane and Rajakoski, 1961). Progesterone caused an inhibition of the firing rate of more warm sensitive neurons in the POA than cold sensitive neurons in mouse brain slices (Tsai et al., 1988). The authors concluded that the thermogenic effect of progesterone could be due to inhibition of heat loss mechanisms and
facilitation of heat loss by inhibiting warm sensitive neurons.

An increase in body temperature in cattle has been reported during estrus (Wrenn et al., 1958; Bane and Rajakoski, 1961; Kumaran and Iya, 1966; Zartman and DeAlba, 1982; Lewis and Newman, 1984). The increase occurred on the first day of estrus and fell sharply on the second day of estrus, assumed to be the day of ovulation (Wrenn et al., 1958; Bane and Rajakoski, 1961). Lewis and Newman (1984) found that vaginal temperatures were lowest the day before estrus before increasing on the day of estrus. They concluded that the decrease could be useful as a predictor of the onset of estrus. A nadir in body temperature, related to the time of ovulation in women, has been used for family planning and artificial insemination (Israel and Schneller, 1950; Hilgers et al., 1984; de Mouzon et al., 1984). However, the wide variability of when the nadir occurs relative to ovulation has meant that it could not be used as a reliable predictor of ovulation (Hilgers et al., 1984; de Mouzon et al., 1984).

Using an intravaginal radiotelemetric device, a temperature spike found during estrus appeared to be associated with ovulation in dairy cows (Zartman and DeAlba, 1982). Subsequent studies with radiotelemetry revealed that the temperature spike was associated with the luteinizing hormone (LH) surge (Clapper et al., 1990; Mosher et al., 1990). The LH surge precedes ovulation and since the
temperature spike was associated with the surge, temperature was believed to an indicator of ovulation (Clapper et al., 1990). In the mare, no change in rectal temperature that could be used to predict ovulation was found (Ammons et al., 1989).

While progesterone tends to have thermogenic properties, estrogen appears to have the opposite effect. Estrogen treatment caused a dose dependent decrease in body temperature in ovariectomized and intact women (Israel and Schneller, 1950). When estrogen was used in combination with progesterone, progesterone overrode the estrogen effect and an increase in body temperature resulted. The effect of estrogen on body temperature is at the level of the hypothalamus. In the POA, estradiol 17β and testosterone stimulated warm sensitive neurons and inhibited cold sensitive neurons, although both rarely affected the same neuron (Silva and Boulant, 1986; Boulant and Silva, 1987).

A drop in body temperature, associated with parturition, has been reported in several species including cows (Wrenn et al., 1958; Ewbank, 1963; Dufty, 1971; Curto et al., 1987), ewes (Ewbank, 1969), bitches (Concannon et al., 1977; van der Wedyn et al., 1989) and monkeys (Ruppenthal and Goodlin, 1982). In sows, there was no predictive drop in temperature prior to parturition (King et al., 1972; Littledike et al., 1979), although King et al., (1972) reported that rectal temperatures in sows were low for the eight days prior to
parturition. Littledike et al. (1979), used telemetry to follow temperature prior to and following parturition and reported an increase in temperature about 12 hours before the delivery of the first pig.

In horse and pony mares, studies of maternal body temperature fluctuations associated with parturition have produced contradictory results. Wright (1943) reported a slight decrease in body temperature associated with parturition in horse mares. A later study of four pony mares found no changes in body temperature at the time of parturition (Cox, 1969). However, three of the four mares were not used to being handled and had to be restrained. Studies in rats have demonstrated that an increase in body temperature occurred as a result of handling (Cunningham and Peris, 1983; Gallaher et al., 1985). Jeffcott (1972) also did not find a temperature drop associated with parturition in horse mares, but the data were recorded once to four times daily. Recently, a temperature decrease associated with parturition was reported in pony (Shaw et al., 1988) and horse mares (Haluska and Wilkins, 1989). However, a similar study of maternal body temperature in horse mares did not find a change that could be useful as a predictor of parturition (Ammons et al., 1989). All of the aforementioned equine studies were based on temperature recordings made once to four times a day.
Studies in the dog have shown a decrease in body temperature at the time of parturition that was temporally associated with a decrease in progesterone values (Concannon et al., 1977; van der Wedyn et al., 1989). Since progesterone is considered to have thermogenic properties, decreased progesterone concentrations late in gestation could be responsible for a decrease in maternal body temperature (Concannon et al., 1977). Other factors may also be involved. Oxytocin caused a hyperthermic reaction in male mice even when used with β-endorphin, neurotensin and bombesin, which tend to lower body temperature (Mason et al., 1986). The authors extended the interpretation of their results to speculate that during parturition, a potential temperature drop due to increased β-endorphin levels may be offset by oxytocin. This does not explain why there is a decrease in maternal body temperature in several species at the time of parturition when oxytocin concentrations are very high. For example, there is a massive release of oxytocin associated with parturition in mares (Haluska and Currie, 1988).

The thermoregulatory ability of pregnant animals may be altered, particularly in late gestation (Kasting et al., 1978; Cooper et al., 1979; Knecht et al., 1980; Imai-Matsumara et al., 1990). In one study, three of eight late pregnant rats exposed to high environmental temperatures could not establish a rate of evaporative water loss to dissipate body heat (Knecht et al., 1980). The authors suggested that this was
evidence for a decreased thermoregulatory ability in late gestation, but did acknowledge the other rats had no thermoregulatory impairment. Cold stress caused a progressive decrease in colonic temperature in rats as pregnancy became more advanced (Imai-Matsumara et al., 1990). The greatest decrease in colonic temperature during cold stress was on the day before or on the day of parturition. Reducing the length of cold exposure from 60 minutes at 10°C to 30 minutes did not reduce or eliminate the temperature decrease near parturition. The authors concluded that decreased temperature was due to decreased maternal heat production as parturition neared but also thought that maintaining the high blood flow to the placentae could be at the expense of maternal body temperature.

Another possible factor regulating maternal body temperature in late pregnancy is arginine vasopressin (AVP), which has structural similarity to oxytocin. Production of AVP in the hypothalamus is primarily in the supraoptic nucleus and is released from the posterior pituitary, although it may be found in other areas of the brain such as in the limbic system and lateral septum (Bennet and Whitehead, 1983). The functions of AVP include increasing water permeability in the kidney and vasoconstriction of peripheral blood vessels. It also appears to be an antipyretic agent (Cooper et al., 1979; Pittman et al., 1988).
Injection of a pyrogen into nonpregnant ewes caused an increase in mean rectal temperature of 1.3°C (Kasting et al., 1978). In contrast, pyrogen injection was less effective in causing a febrile response in pregnant ewes near term pregnancy. Five hours after parturition, 11 of 17 ewes remained afebrile when challenged with the pyrogen. By two days postpartum, though, most ewes would become febrile when challenged. Based on the results of this study, a natural fever suppressing system was proposed to exist in near term pregnancy. Arginine vasopressin was subsequently found to cause an antipyretic reaction in ewes when injected into the septal region of the brain (Cooper et al., 1979). Similar fever suppressing results were found in pregnant guinea pigs treated with a pyrogen followed by AVP perfusion into the lateral septum of the brain (Merker et al., 1980). There were differences in distribution of vasopressin reactive neurons between pregnant and nonpregnant guinea pigs. Pregnant guinea pigs had a large increase in vasopressin in neurons of the median eminence and decreased vasopressin concentrations in the supraoptic and paraventricular nuclei. In contrast to these studies, Blatteis et al. (1988) found no suppression of fever in full term ewes challenged with a pyrogen and did not feel that suppression of fever in full term ewes should be considered a general phenomenon.

Further evidence of an antipyretic role for AVP was demonstrated in castrated male rats (Pittman et al., 1988).
Castrated rats reacted to pyrogen induced fever with higher temperatures that remained elevated for longer periods than intact rats. There was also a reduced AVP content in the lateral septum and ventral-septal area in the brain. Arginine vasopressin was proposed to affect fever duration in the intact animal by causing defervescence or the reduction of fever.
CHAPTER II

UTERINE ELECTROMYOGRAPHIC ACTIVITY IN HORSE MARES
AS MEASURED BY RADIOTELEMETRY

ABSTRACT

The objective of this study was to determine if radiotelemetry could be used to measure myometrial electromyographic (EMG) activity.

A radio transmitter with one pair of biopotential leads was implanted in the flank ipsilateral to the pregnant uterine horn at least five weeks prior to the expected date of parturition in two mares. The biopotential leads were implanted in the base of the pregnant uterine horn. Telemetered data were received by a pair of antennae placed at right angles in a 3.3 by 6.6m stall. Data were recorded on VHS format videocassette tapes continuously for the 24h prior to and following parturition. Simultaneous physiograph recordings were made as a hard copy reference. In addition, 10 mg of prostaglandin F2a was administered to two mares in the luteal phase of the estrous cycle.
Myometrial EMG during parturition was increased similarly to that of previously published reports that used myometrial electrodes wired directly to a physiograph. Prostaglandin $F_{2a}$ also caused an increase in myometrial EMG activity within 8 min of administration. This study demonstrated that radio-telemetry can be used for measuring myometrial EMG activity.

**INTRODUCTION**

Uterine contractile activity in mares has been studied by a variety of methods. Bipolar electrodes implanted at various sites in the myometrium have been used to study myometrial EMG activity during estrus and diestrus (Taverne et al., 1979) and at the time of parturition (Haluska et al., 1987a). Other methods employed have included a strain gauge around the base of a uterine horn (Capraro et al., 1977), intraluminal pressure transducers (Goddard et al., 1985; Ko et al., 1989d; Ley et al., 1988) and ultrasonography (Cross and Ginther, 1988; Griffin and Ginther, 1990). The use of electromyographic electrodes or strain gauges necessitates the connection of wires from the electrodes to a physiograph (hard-wired system). Wires from the myometrial electrodes are usually tunneled subcutaneously to an exit point in the center of the back in order to put the electrical connector out of reach of the animal. A hard-wired system requires that the animal be restrained to some degree, usually in a small pen.
or in a chute. In studying a free moving subject, electrode wires were connected to a swivel in the ceiling (Taverne et al., 1979; Haluska et al., 1987a), but the animal was still physically connected by wires to a physiograph. An animal may lie down and roll, causing damage to the external wires and connector. In primate species, there is the added problem of the animal using its hands to grab the external wires.

Radiotelemetry transmitters do not require a direct hard-wired connection and have been used successfully for remote temperature sensing studies in several studies (Zartman and DeAlba, 1982; Ruppenthal and Goodlin, 1982; Gallaher et al., 1985). Some transmitters are equipped with biopotential leads designed to monitor electrocardiography (ECG) and electroencephalography as well as temperature. These transmitters have the potential to monitor EMG activity as well. Adaptation of radiotelemetry technology to the study of myometrial EMG activity would eliminate hard-wired systems and allow data collection during all reproductive states of the mare. With a radiotelemetry system, mares could be isolated from environmental disturbances and thereby minimize this effect on EMG activity (Taverne et al., 1979; Haluska et al., 1987a). Radiotelemetry systems would eliminate the potential problems of damage to external wires and allow for the study of free moving animals without external wires inhibiting natural movement. This system would eliminate the necessity of tunneling wires subcutaneously to the electrical
connector on the back and the potential infection problems at the connector site.

The objective of this study was to determine if radiotelemetry could be used to measure myometrial EMG activity. To our knowledge, this is the first report of the use of radiotelemetry to measure myometrial EMG activity in any species.

MATERIALS AND METHODS

Telemetry Equipment and Operation

The telemetry system used in this project consisted of a transmitter, antenna, receiver, a device to multiplex signals from different animals to the computer, a Hewlett-Packard (HP) Vectra ES/12 AT computer\(^1\) (IBM compatible) with IBM DOS version 3.3\(^2\) and the software system designed to filter, convert, store and analyze acquired data (DataQuest III).

The transmitters used were PhysioTel\(^3\) TA10CTA-D80-L40 transmitters, weighing 75 g and were 79 mm in diameter by 4 mm thick. The transmitters described were custom ordered from Mini-Mitter, Inc., Sunriver, OR. Each transmitter was

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\(^1\)Hewlett-Packard Corporation, Sunnyvale, CA 94086.

\(^2\)International Business Machines, Boca Raton, FL 33429-1328.

\(^3\)Data Sciences Inc., St. Paul, MN 55113-1136.
equipped with a pair of high grade stainless steel helix sensing leads, 30 cm in length, designed to measure a single channel of biopotential such as ECG or EMG activity. The biopotential signal was converted from an analog to a digital form in the transmitter. A micro-power transmitter, a device which modulated a radio frequency carrier (455 kHz) with the biopotential signal and drove the internal antenna, was located within the transmitter. The electronics portion of the transmitter was encapsulated in epoxy and hermetically sealed. A lithium battery with an estimated life of six months was used to power the electronics and transmitter. Battery life could be extended by turning off the transmitter when not in use. This was possible due to an internal magnetic switch that was activated by bringing a strong magnet close to the transmitter. Since the transmitter output frequency matched the intermediate frequency used by AM radio receivers, a small transistor radio tuned at the low end of the band was used to determine if the transmitter was on or off. The battery and electronics were surrounded by a second epoxy barrier and then coated with a medical grade silicone coating. The sensing lead wires were also coated with medical grade silicone except for approximately 2 cm at the ends, which were embedded into the myometrium. Another 30 cm of lead wire were spot welded to the existing lead wire. The extension resulted in a total lead wire length of 60 cm (24 inches) and was of sufficient length to connect the uterus to
the transmitter implant site without undue tension on the lead wires. Preliminary work revealed that an electrocardiograph waveform was superimposed upon the electrical signals from the myometrium. To eliminate this ECG signal noise, a section of coaxial cable shielding was used to cover the biopotential leads (Figure 1). The shielded leads were then enclosed in medical grade silastic tubing and sealed at both ends with silastic. A neutral wire, to ground the coaxial cable shielding to the wall of the abdominal cavity, was connected to the cable shielding at the proximal end near the transmitter and sealed with silastic. Transmitters were sterilized using ethylene oxide prior to implantation in the mares.

Two stalls (3.3 by 6.6-m) located adjacent to The Ohio State University horse barn office were equipped with a pair of closed loop antennae (Figure 2). One antenna ran the length of the stall and was 6 m long by .9 m high. A second, smaller antenna was located along the adjacent wall of the stall, with dimensions of 3 m long and .9 m high. The outer casing of each antenna was made of 1/2-inch PVC pipe purchased at a local building supplies store. Five turn loops of 26 gauge enameled magnet wire were threaded through the pipe using a 3-m piece of stiff wire. The ends of the wire exited

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4 Dow Corning Corporation, Midland, MI 48640.
5 Belden Wire and Cable, Geneva, IL 60134.
the pipe through a T-connector. One wire was soldered to the central wire of a coaxial cable; the second wire was soldered to the coaxial shielding. A 1-m piece of 1/2-inch PVC pipe was used to cover the wires up to a point in the stall above the floor where a horse could not reach. The antennae were affixed to the wall with plastic 1/2-inch hanging straps and protected by lengths of 2 by 4-inch lumber framed around each antenna. The distal end of the coaxial cable was connected to a two-conductor 2.5-mm mini-plug, with the center wire soldered to the central conductor core of the plug and the coaxial shielding soldered to the outer conductor. The effective range of the transmitter with this antenna system was 2.5 m. The size of the stall was selected after preliminary work with the antennae. To reduce radio crosstalk between the two stalls, the bare wall of the adjoining stall was covered with sheet metal and grounded to a buried water pipe.

Telemetered data were received by a CTR-86 receiver and stored on T-120 VHS videocassettes for future analysis using a VHS stereo videocassette recorder\textsuperscript{6} (VCR). The receiver also functioned to route the digital signal to the computer and reconvert the signal to an analog form for recording on a physiograph. The receiver was connected to a VR1 data

\textsuperscript{6}VR630HF VCR, RCA Victor.
recorder, a device required by the system to convert telemetered data into a form that could be recorded onto a videocassette and to play back the recorded data (Figure 2). A BCM-100 consolidation matrix was connected to the data recorder and computer. The matrix supplied power to both the data recorder and receiver and multiplexed signals from the two stalls onto cables connected to the computer. In this manner, the raw telemetered data were recorded while the waveform was simultaneously viewed on the computer screen.

A video camera, equipped with a wide angle lens, was placed between the two stalls for observation of mare behavior during recording sessions. The camera was connected to the VCR and a video monitor. Six hours of continuous data were recorded utilizing the super long play (SLP) mode on the VCR. Additionally, the VR1 data recorder was connected to a physiograph by a shielded wire cable with a 2.5-mm mini-plug at the receiver and a 5-mm phono plug at the physiograph. A high-gain coupler (Type 7171), designed to accept voltage signals from 20 mV to 1 V, was used on the physiograph. The physiograph was set to an amplifier sensitivity of 200 mV/cm, with a paper speed of 30 to 60 mm/min. Signals were filtered

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7 Data Sciences Inc., St. Paul, MN 55113-1136.
8 HVM 322 video camera, Sony Corp., Tokyo, Japan.
9 Panasonic Video monitor TR-930, Matsushita Electric Industrial Co, LTD., Osaka, Japan.
10 Physiograph Type PMP-4A, Narco Co., Houston, TX 77017.
at 30 Hz with a time constant of .03 sec. A hard copy of the raw data was obtainable in real time or from the tape.

Surgery

Two late gestation mares, weighing approximately 450 kg, were implanted with a transmitter five weeks prior to expected parturition date.

Sedation of the mare was induced with 0.6 mg/kg i.v. of xylazine\textsuperscript{11} and 0.02 mg/kg i.v. of butorphanol tartrate\textsuperscript{12}. The paralumbar region of the flank ipsilateral to the gravid uterine horn was surgically prepared including a 10 minute Betadine\textsuperscript{13} scrub. Lidocaine\textsuperscript{14} was used to anesthetize the incision site. Once an incision was made into the peritoneum, the uterine horn was exteriorized. A pair of 14-gauge hypodermic needles, joined at the hubs with silastic, were inserted through the serosa and into the myometrium parallel to the long axis of the uterine horn. The gap between the needles was set at 5 mm in accordance to previous reports that used bipolar electrodes to measure EMG (Taverne et al., 1979; Haluska et al., 1987a). Two centimeters of exposed sensing lead wire were inserted into the needles and then the needle

\textsuperscript{11}The Butler Company, Columbus, OH 43228.
\textsuperscript{12}Turbogesic, Fort Dodge Labs, Inc., Fort Dodge, IA 50501.
\textsuperscript{13}The Purdue Frederick Co., Norwalk, CT 06856.
\textsuperscript{14}The Butler Company, Columbus, OH 43228.
pair was withdrawn, embedding the leads in the myometrium. The leads were held in place by suturing the silastic tubing to the serosal surface of the uterine horn. The sensing leads were also loosely anchored to the broad ligament by a suture with a 15-cm neutral wire attached to the muscles in the flank. The transmitter was placed lateral to the transverse abdominus muscle and medial to the last rib and sutured into place (Figure 3). Activation of the transmitter was done with a magnet prior to insertion in the mare. The muscle incision was closed using a continuous suture Pattern #2 chromic catgut, and the skin incision was closed with Braunamid #2 suture material\textsuperscript{15} using a Ford interlocking suture pattern. Each mare received a single tetanus toxoid injection and 9 million units of procaine penicillin once a day for three days.

**Data Collection**

Electromyographic activity was recorded on videotape during a six-hour period between 2300 and 0500 h each day. Recording was started six days after surgery because EMG activity has been reported to be elevated until six days after surgery (Figueroa et al., 1985). The time of day for recording was selected since it was the period of the least amount of outside electrical interference and environmental

\textsuperscript{15}Braunamid, B. Braun Melsengen AG, West Germany.
influence. As a mare approached parturition, recordings were continuous for the 24 h preceding and following parturition.

To further demonstrate that radiotelemetry could be used to measure myometrial activity, 10 mg of prostaglandin F$_{2\alpha}$ were administered to each of two mares after obtaining baseline recordings for 60 min. Mares were in the luteal phase of the second postpartum estrous cycle when given PGF$_{2\alpha}$.

RESULTS

Representative myometrial EMG recordings obtained by radiotelemetry are presented in Figures 4 and 5. During parturition, there was an increase in EMG activity during periods when the mare was demonstrating expulsive effort while in lateral recumbency (Figure 4). Relaxation between observed contractions was accompanied by a decrease in the amplitude of EMG activity. Immediately after the fetus was delivered, EMG activity decreased and settled into a rhythmic pattern, with the mare laying quietly.

Intramuscular injection of 10 mg of prostaglandin F$_{2\alpha}$ resulted in a marked increase in myometrial EMG activity within 8 min of administration (Figure 5).
DISCUSSION

It has been demonstrated by this study that myometrial EMG activity can be measured by radiotelemetry. Environmental disturbances, which have been previously shown to have an effect on EMG activity (Taverne et al., 1979; Haluska et al., 1987a), were minimized. Observation of the mares without disturbing them was facilitated by the use of the video camera and monitor. Post-operative complications were minimal since there was no external connector that could serve as a source of continual irritation.

The pattern of myometrial EMG activity during parturition in the mare, as measured by radiotelemetry, was similar to that previously reported for a hard-wired system (Haluska et al., 1987a,b). Telemetered myometrial EMG recordings were also similar to those reported for ewes during parturition (Parkington et al., 1988).

Prostaglandin \( \text{F}_2\alpha \) is a known stimulator of uterine contractile activity (Anderson, 1978). In our present study, the intramuscular injection of \( \text{PGF}_2\alpha \) caused an increase in EMG activity within 8 min of administration. The increase in spiking that occurred at the time of \( \text{PGF}_2\alpha \) injection was likely due to the mare's reaction to a person entering the stall and administering the injection. Previous studies of mare myometrial activity have noted an increase in activity when
a person enters the stall (Taverne et al., 1979; Haluska et al., 1987a). In a study measuring myometrial EMG activity in pony mares with a hard-wired system, activity was increased by fluprostenol, a PGF$_{2\alpha}$ analog (Taverne et al., 1979). An increase in uterine pressure, measured by pressure transducers, occurred within 10 min of treatment with PGF$_{2\alpha}$ (Goddard and Allen, 1985). Prostaglandin F$_{2\alpha}$ increased uterine contractile activity, as determined by ultrasonography, in seasonally anovulatory mares pretreated with ovarian steroids (Cross and Ginther, 1987).

In conclusion, we have described a technique to utilize radiotelemetry for the measurement of myometrial EMG activity. The patterns of EMG, as determined by radiotelemetry, were similar to those of published reports in the mare. The response to PGF$_{2\alpha}$, a known stimulator of myometrial contractions, was also similar to the earlier studies. Radiotelemetry eliminates the need for external wires attached to an animal and the resultant complications should an external connector be damaged. More over, this technique could be adapted to a variety of studies in different species.
Figure 1. Model TA10CTA-D80-L40 transmitter (Data Sciences, Inc., St Paul, MN) with the biopotential leads shielded and covered with silastic tubing. The neutral wire was connected to the shielding and used to ground the shielding to the abdominal musculature. Figure 1b is a schematic of the arrangement of the silastic tubing, shielding and biopotential leads.
Figure 2. Diagram of the physical layout of the radiotelemetry system. The loop antennae were connected to a CTR -86 receiver (1) located in the stall. The receiver was connected to the VR1 data recorder (2), powered by the BCM-100 (3). From here, data was simultaneously recorded on a VHS videocassette recorder (4), the physiograph (5) and the computer. The computer was used to view frequencies in real time and selectively analyze segments of data.
Figure 3. Position of the transmitter and biopotential leads within the mare. The transmitter was placed lateral to the transverse abdominus muscle and medial to the last rib. The lead wires were loosely sutured in the broad ligament and the distal ends embedded into the myometrium at the base of the pregnant uterine horn. The neutral or ground wire was placed between the peritoneum and abdominal musculature.
Figure 4. Representative EMG recording during parturition with the mare in lateral recumbency before and immediately after delivery. Myometrial EMG activity increased during observed expulsive effort by the mare (bracketed areas a and b) and decreased when she was observed to relax. After delivery of the fetus (arrow), EMG activity decreased and settled into a rhythmic pattern (bracketed area c). Paper speed was 60 mm/min.
Figure 5. Effect of prostaglandin $F_2\alpha$ on myometrial EMG activity. An increase in activity was apparent within 8 minutes of intramuscular injection of 10 mg PGF$_2\alpha$ (arrow). Paper speed was 30 mm/min.
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CHAPTER III

RADIOTELEMERIC MONITORING OF MYOMETRIAL
ELECTROMYOGRAPHIC ACTIVITY DURING PARTURITION
IN THE HORSE MARE

ABSTRACT

Myometrial electromyographic (EMG) activity was monitored in six horse mares during the prepartum and postpartum periods using radiotelemetry. Myometrial EMG events were grouped into ranges of .1 to 1 s, 1 to 10 s and 10 to 100 s duration. One hour segments of data from the 5 d prior to and following parturition were analyzed. The percentage of .1 to 1 s duration events were lower on day -5 and -4 (P < 0.05) and tended to be lower on day -2 (P < 0.1) than at the time of parturition. During the 5 d postpartum, the percentage of the shortest events decreased and were lower than at the time of parturition (P < 0.05). The percentage of EMG events that were 1 to 10 s in duration were higher on days -5 and -4 (P < 0.05) and tended to be higher on days -2 and -1 (P < 0.1) than at the time of parturition. An increase in the percentage of these events occurred by 24 h postpartum and
remained greater than at parturition for 5 d ($P < 0.05$). There was no difference in the percentage of the 10 to 100 s duration events before and after parturition.

In one mare, there was a decrease in the percentage of the shortest duration EMG events between -20 and -4 h. The percentage of the 1 to 10 s duration events and the 10 to 100 s duration events increased to a peak at 8 h prepurum before decreasing to a minimum at the time of parturition. Between -4 and -2 h, there was an increase in the percentage of the shortest duration events and a decrease in the 1 to 10 s and 10 to 100 s ranges of EMG events. During the period of -1 to 0 h, the shortest duration myometrial EMG events accounted for 75% of the myometrial activity while there was a decrease in the percentage of EMG events that were 1 to 10 s and 10 to 100s duration. In terms of total myometrial EMG activity, there was a decrease in the number of duration events from -24 to -8 h. The peak occurred at the time of parturition (-1 to 0 h).

**INTRODUCTION**

At the time of parturition in mares, there is a significant increase in the frequency and a decrease in the duration of myometrial EMG events (Haluska et al., 1987b; Dudan et al., 1987). The recording of this type of data in the mare and other animals has been done by implantation of
bipolar electrodes into the myometrium and direct connection to a physiograph (Naaktgeboren et al., 1973; Taverne et al., 1979; Dudan et al., 1987; Haluska et al., 1987b; Claus and Ellendorf, 1989).

A technique for the application of radiotelemetry to the study of mare myometrial electromyography has been described recently as a possible alternative to hard wired procedures (Cross et al., 1991a). The results of that study demonstrated that the telemetric system used was capable of recording myometrial EMG signals during parturition and after stimulation with prostaglandin F2α. In order to further demonstrate the utility of the system, the objectives of this study were to determine the pattern of myometrial EMG activity at the time of parturition and the pattern of myometrial EMG activity during the 5 days prior to and following parturition.

MATERIALS AND METHODS

The telemetric system has been previously described (Cross et al., 1991). Telemetered data were recorded onto VHS format videocassette tapes for subsequent analysis. Recording was started six days after surgery because EMG activity has been reported to be elevated until six days after surgery (Figueroa et al., 1985). Data were recorded continuously between 2300 and 0500 h each day until parturition appeared imminent and then data was recorded
continuously for the 24 h preceding and following parturition. The time of day for recording was selected since it was the period of the least amount of outside electrical interference and environmental influence (Cross et al., 1991). Recording of data was continued throughout the postpartum period.

Six horse mares, weighing approximately 450 kg, were fed according to NRC guidelines, with water and trace mineral salt available ad libitum. With the exception of when parturition was thought to be imminent, each mare was allowed a daily one to two hour exercise period.

One hour segments of EMG data were selected for analysis for the 24 h before and after parturition. Those segments were as follows: -25 to -24h, -21 to -20h, -17 to -16h, -13 to -12h, -9 to -8h, -5 to -4h, -3 to -2h, -1 to 0h, 0 to 1h, 2 to 3h, 4 to 5h, 8 to 9h, 12 to 13h, 16 to 17h, 20 to 21h and 24 to 25h. Additionally, one hour segments of EMG data from -5 to -2 days and from 2 to 5 days were analyzed. Not all segments were available for all mares at all times. Records that were judged to contain excessive noise as a result of environmental or behavioral causes were eliminated from the analysis.

Recorded EMG data were replayed and passed through a Grass P15\textsuperscript{16} preamplifier containing a band pass filter that was set at 1 Hz to .1 KHz. An inline capacitor was present.

\textsuperscript{16}Grass Instrument Co., Quincy, MA.
to eliminate noise created by DC offset voltages. The acquisition and analysis software\textsuperscript{17} sampled the data at a rate of once every 10 ms, which was considered adequate to avoid aliasing of the data. A sampling period that is too infrequent will lead to aliasing or distortion of the waveform to be analyzed (Rubin, 1985). The data were full-wave rectified to a baseline of 0 volts and then further processed through a low pass digital linear filter function of the computer software. The cutoff frequency was set at 5 Hz and the resultant record was essentially the same as an integrated record. An EMG record with known values was used to select the parameters used in the analyses. A threshold voltage that was 25\% of the average of the maximum less the minimum voltage (V) plus the average minimum voltage was used \(0.25(\Sigma V_{\text{max}} - V_{\text{min}})/n + \Sigma V_{\text{min}}/n\) where \(n\) equaled the number of segments of data within the tape used to determine the threshold. An interrupt of 50 ms was chosen to further define EMG events. If the voltage fell below the threshold voltage for longer than the interrupt setting, then an event was considered terminated. An increase in voltage occurring before the interrupt length had expired was considered part of the current event. Duration histograms were performed on one hour segments of data. Peak to peak voltages differed from mare to mare and between segments within mares. Preliminary data

\textsuperscript{17}Computerscope, RC Electronics, Inc., Santa Barbara, CA 93111
using these parameters indicated that durations events fell into a range of 20 to 100000 ms. Events that were less than 100 ms in duration were considered noise and eliminated from the analyses. The remaining events were divided into duration ranges of 100 to 1000 ms (.1 to 1s), 1000 to 10000 ms (1 to 10 s) and 10000 to 100000 ms (10 to 100 s).

Data in each range of duration events were expressed as percent of the total number of EMG reference events. All days or times were compared to the period of -1 to 0 h, with 0 defined as the time of delivery of the fetus. A one way analysis of variance was performed to determine statistical significance and Student's t test was used to detect differences between days and times. A P value of equal to or less than 0.05 was considered to be a significant difference. Values between 0.05 and 0.1 were considered to be a tendency towards a significant difference.

RESULTS

An example of a segment of raw, full-wave rectified and low pass digitally filtered EMG data is presented in Figure 6. There are six EMG events of approximately one second in duration each in the 10 second segment of data. These data were taken from the rhythmic myometrial EMG activity that occurred immediately postpartum in one mare and has been previously described (Cross et al., 1991).
The radiotelemetry system worked well for the majority of the study and in most mares. The biggest problems encountered were electrical interference at times and poor reception due to mare position in the stall. Some of the data contained distortions that were not totally eliminated by passing the data through a variety of filters. The distortions were probably due to periodically poor reception or out of range areas and those records were eliminated from analyses.

The results of the pattern of EMG events within the three duration ranges are presented in Figure 7. The percentage of EMG events in the shortest duration range were lower on days -5 and -4 (P < 0.05) and tended to be lower on day -2 (P < 0.1) than at the time of parturition. At 24 h prior to parturition, the percentage of the .1 to 1 s duration events was lowest, but there was large variation between animals and no statistical difference was detected. The percentage of shorter duration EMG events was lower on all five days postpartum than at the time of parturition (P < 0.05).

A higher percentage of myometrial EMG events that were 1 to 10 s in duration occurred on days -5 and -4 (P < 0.05) or tended to be higher on days -2 and -1 (P < 0.1) than at the time of parturition. The percentage of these events increased after parturition and remained higher than at the time of parturition for the next five days (P < 0.05). When compared
to the time of parturition, there were no differences between days in the percentage of EMG events of 10 to 100 s.

Missing data during the 24 h preceding parturition were usually due to initiation of data recording less than 24 h prepartum and behavior of mares that increased the time spent in areas of poor reception. However, one mare (#89) did have an almost complete set of data for the 24 hours preceding and following parturition. Examination of the percentage of myometrial EMG events within each duration range for mare 89 revealed that between the periods of -20 to -4 h, there was a decrease in the percentage of EMG events that were .1 to 1 s in duration (Figure 8). During the period of -20 to -4 h, the percentage of the 1 to 10 s and the 10 to 100 s duration events increased to a peak at 8 h prepartum before decreasing to a minimum at the time of parturition. During the period of -1 to 0 h, the shortest duration EMG events accounted for 75% of the myometrial activity whereas the EMG events within the 10 to 100 s in duration had decreased to 0.5%. In terms of total myometrial EMG activity, there was a decrease in the number of duration events from -24 to -8 h. The peak occurred at the time of parturition (-1 to 0 h). A similar pattern was observed in a second mare, with the nadir occurring during the period of -5 to -4 h before increasing to a maximum in the hour ending with parturition.

In the hour after parturition, the percentage of the shortest duration events decreased sharply while both the
percentage of 1 to 10 s and 10 to 100 s duration events increased. The rhythmic activity noted immediately postpartum consisted of EMG events that were approximately 1 s in duration.

DISCUSSION

The results of the present study have shown that telemetered myometrial EMG activity at parturition in the horse mare were similar to previous studies (Dudan et al., 1987; Haluska et al., 1987a,b). This pattern was evident for all mares in the days preceding parturition as well as during the 24 h prior to parturition in mare 89. In several mares, there was a large increase in total EMG activity during the last hour of pregnancy with a decrease of varying degrees occurring during the first hour following parturition.

The analysis selected for this study concentrated on EMG events with durations of less than 100 s or 1.67 m. The rhythmic activity that was clearly noted on one mare immediately postpartum and was similar to previously published data (Haluska et al., 1987b) and was the basis of the development of the analysis parameters. Previous studies have defined bursts of myometrial EMG activity differently. In one study, the duration of EMG events were divided into those that were greater than or less than 180 s (Dudan et al., 1987). Trains of action potentials were grouped into bursts of EMG
activity for analysis in another study (Haluska et al., 1987a,b). Contractions have also been defined as bursts of myometrial EMG activity lasting one minute or less and were typical of EMG activity at the time of delivery (Nathanielsz et al., 1982; Figueroa et al., 1985; Honnebier et al., 1991; Massmann et al., 1991). Contractures, which were typical of pregnancy, were defined as bursts of EMG activity lasting more than three minutes and were associated with increases in intrauterine pressure.

Regardless of the definition used, a characteristic of myometrial EMG activity in most species is a shift from longer duration events to the shorter duration events as parturition approaches (Krishnamurti et al., 1983; Sigger et al., 1983; Taylor et al., 1983; Figueroa et al., 1985; Dudan et al., 1987; Haluska et al., 1987b; Parkington et al., 1988). In periparturient ewes, the frequency of EMG events that were one minute in duration greatly increased from 18 h to 0.5 h before parturition while at the same time there was a decrease in the frequency of the longer duration events (Parkington et al., 1988). There was also a corresponding decrease in the interval between bursts of activity. A progressive decrease in the frequency and duration of medium and long bursts of myometrial activity occurred in pony mares during the last stage of pregnancy (Haluska et al., 1987a) and Dudan et al. (1987) reported a tendency for the frequency of long events (> 180 s in duration) to decrease as parturition approached.
In the present study, the percentage of the shorter duration events was lower for most of the days prior to than at the time of parturition. The opposite was true for the percentage of the 1 to 10 s duration events. Immediately after parturition, there was a large decrease in myometrial activity in mare 89 and in other mares for which a record was available. This corresponds to the decrease in EMG activity that has been previously reported (Haluska et al., 1987b).

In a recent study (Cross et al., unpublished) the mean maternal body temperature of the same group of mares began to decrease 4 h prior to parturition, reached a nadir at delivery of the foal and began to increase by one hour postpartum. It is interesting to note that there appears to be an inverse relationship between body temperature to the percentage of the shortest duration EMG events in mare 89 and a direct relationship between temperature changes and the longer duration events, particularly the events that were 1 to 10 s in duration. Decreasing progesterone levels may be associated with body temperature changes at parturition since progesterone has thermogenic properties (Concannon et al., 1977; Tsai et al., 1988). In mares, there is a significant decrease in progesterone levels that occurs during the 24 h preceding parturition as well as increases in prostaglandin $F_{2\alpha}$ (Haluska and Currie, 1988). Progesterone is the predominant hormone during pregnancy when contractures of long duration are most frequent (Figueroa et al., 1985) and the
shift towards shorter duration myometrial events as parturition approaches is probably related to the decreasing progesterone levels or a decreasing progesterone to estrogen ratio. Other hormones, such as the prostaglandins, have effects on both body temperature and myometrial function and the increased or decreased presence of one or more hormones associated with parturition may cause changes in body temperature.

A previous study noted that during the last 24 h of pregnancy, there was a period of two to four hours of decreased myometrial EMG activity prior to rupture of the chorioallantois (Haluska et al., 1987b). In the present study, there was a decrease in myometrial EMG activity between 24 and 8 hours before parturition in the one mare with the nearly complete set of periparturient data. Myometrial EMG activity began to increase at 4 and 2 hours prepartum with a large increase in the total number of EMG events at the time of parturition. The raw foaling EMG record from this and other mares in the present study were qualitatively similar to those presented by Haluska et al. (1987b).

Several problems were encountered during the course of this study. Movement artifacts were noted on several occasions and usually consisted of large spikes. Changes in orientation of the transmitter to the antennae also caused artifactual or noise problems. The transmitters in the present studies used an amplitude modulated (AM) carrier
frequency, with one pair of biopotential leads (Cross et al., 1991). An AM frequency is highly directional and may result in occasional signal losses due to changes in orientation of the animal to the antenna or the presence of null points (Gallaher et al., 1983). Null points, where the signal strength is lower than normal, are difficult to eliminate because it is difficult to construct an omnidirectional antenna (Loeb and Gans, 1986). A pair of closed loop antennae were placed around the exterior of a cage creating what was considered to be an omnidirectional antenna and this greatly minimized the problem. However, in the present studies, this was much harder to accomplish given the size of the stalls.

Observation of one late pregnant mare before the camera was available revealed that apparent fetal activity was accompanied by increased EMG activity. Movement in the flank area of the abdomen and a shifting of the rear legs when the mare standing quietly were related to increased pen activity on the physiograph and were considered to be the result of fetal movement. The sheep fetus has periods of sleep and activity as measured by forelimb skeletal EMG activity and fetal eye movements (Nathanielsz et al., 1980; Natale et al., 1982). Irritation of the myometrium by fetal movement was considered to cause myometrial EMG activity (Nathanielsz et al., 1982) but when sheep fetuses were treated with curare to eliminate movement, the authors found no change in the level of myometrial EMG activity. Fetal activity has been
implicated in the circadian variation of short duration EMG events because fetal death prevented the increase in amplitude characteristic of predelivery (Taylor et al., 1983).

Transmitted signals are subject to a variety of electrical noise (Rubin, 1985; Loeb and Gans, 1986). The most extensive source of electrostatic noise is from 60 Hz power lines, including fluorescent lighting (Rubin, 1985). The studies were conducted at The Ohio State University Horse Center located, with the other Animal Science animal units, next to the University airport. Electrical interference from machinery and fans from within the horse barn and from the other units was noted. As much as possible fans and air conditioning units at the horse barn were kept turned off since electrical noise (60 Hz) appeared to be the primary source of electrical interference. The temperature data that was being collected at the same time did not appear to be as affected as the EMG data except when the signal became weak. The selection of 2300 to 0500h as the daily period to record EMG was based on the discovery that this time period was subjected to the least amount of electrical interference. Peak activity at the animal science barns was between 0500 and 1800 h. The startup of an electrical grinder at 0500 in the swine barn caused an immediate increase in noise on the EMG trace. At the airport, peak radio traffic occurred between 0700 and 0900 and between 1600 and 1800 h during the week but did not appear to be a factor. When electrical or radio
frequency interference was noted, another source was usually identified. A nondirectional radio signal located at an airport structure one-quarter mile from the barn was operated 24 hours a day and was constant for all animals and times.

Another source of electrical interference that had a significant effect on signal transmission were electrical storms. An increase in noise on the EMG trace could be noticed before the storm was sighted. The system was shut down and unplugged when any storm was overhead. The antennae apparently were picking up atmospheric radio frequencies from the storms as well as telemetered data.

In conclusion, the telemetered myometrial EMG activity was similar to previous studies that used hard wired methods. Due to periodic poor reception or out of range values, much of the data from the period of -24 to 24 h was distorted by electrical noise. Further refinement of the system used in horse mares is required to address the problems that were encountered.
Figure 6. An example (10 s) of radiotelemetered myometrial EMG data. The raw data (a) were subjected to full-wave rectification (b) and then processed through a low pass digital linear filtering function to produce an integrated record (c).
Figure 7. Myometrial EMG activity during one hour periods for the 5 d prior to and following parturition. Hour 0 represents the hour ending with birth of the foal. The percentage of EMG events that were .1 to 1 s in duration were highest at hour 0 whereas the percentage of events of 1 to 10 s duration were lowest at the time of parturition.
Figure 8. Myometrial EMG activity for one mare during the 24 h prior to and 4 h following parturition. The percentage of EMG events that were 0.1 to 1 s in duration peaked during the period of -1 to 0 h and the percentage of the longer duration events became minimal.
REFERENCES


CHAPTER IV

TELEMERIC MONITORING OF BODY TEMPERATURE
IN THE HORSE MARE

ABSTRACT

The objective of this study was to develop a radiotelemetric system capable of frequent monitoring of mare body temperature.

A radio transmitter was implanted in the flank of each of four mares. Telemetered data were received by a pair of antennae placed at right angles in a 3.3 x 6.6-m stall and stored on a computer hard disk. The data were recorded every 5 minutes except when mares were out of the stall for a 1- to 2-hour exercise period. No effect of environmental temperature, ranging from 5°C to 30°C, on mare body temperature was apparent.

The radiotelemetric system used in this study was effective for frequent measurement of mare body temperature.
INTRODUCTION

Recently, changes in body temperature relative to ovulation or parturition monitored by radiotelemetric systems have been reported in the cow (Zartman and DeAlba, 1982; Zartman et al., 1983), monkey (Ruppenthal and Goodlin, 1982) and sow (Littledike et al., 1979). The use of radiotelemetry has eliminated the need to restrain animals in order to obtain temperatures with a clinical or digital thermometer, and has made frequent temperature recording more practical.

Few published reports are available concerning temperature changes relative to reproductive events in horses, and all existing results were based on rectal temperatures. The results of three studies are contradictory concerning temperature changes prior to parturition. No change was found in two studies (Cox, 1969; Ammons et al., 1989), whereas a significant drop in mare body temperature prior to parturition was reported in a third study (Haluska and Wilkins, 1989). Rectal temperatures were taken three to four times a day in these studies. There was also no reported change in mare rectal temperature associated with ovulation (Ammons et al., 1989). In cattle, a temperature spike, detected by radiotelemetry, has been associated with ovulation (Zartman and DeAlba, 1982; Mosher et al., 1990).
The objective of this study was to establish a radiotelemetric system for monitoring mare body temperature.

MATERIALS AND METHODS

Telemetric Equipment and Operation

The telemetric system used in this project consisted of a transmitter, antenna, receiver, a device to multiplex signals from different animals to the computer, a Hewlett-Packard (HP) Vectra ES/12 AT computer\textsuperscript{18} (IBM compatible) with IBM DOS version 3.3\textsuperscript{19} and the software system designed to filter, convert, store and analyze the acquired data (DataQuest III)\textsuperscript{20}.

The transmitters used were PhysioTel\textsuperscript{21} TA10CTA-D80-L40, 75 g in weight and 79 mm in diameter by 4 mm thick. The transmitters described were custom ordered from Mini-Mitter, Inc., Sunriver, OR. Each transmitter contained a negative temperature coefficient thermistor for temperature sensing. A thermistor is a type of variable resistor that changes the voltage through a circuit as the temperature changes (Rubin, 1987). In response to body temperature changes, the

\textsuperscript{18} Hewlett-Packard Corporation, Sunnyvale, CA 94086.

\textsuperscript{19} International Business Machines, Boca Raton, FL 33429-1328.

\textsuperscript{20} Data Sciences Inc., St. Paul, MN 55113-1136.

\textsuperscript{21} Data Sciences Inc., St. Paul, MN 55113-1136.
thermistor in the transmitter caused a change in the average rate at which the transmitter sent radio frequency pulses. For example, an increase in temperature resulted in an increased rate of radio frequency pulses. Each transmitter was supplied with temperature calibration values and had a radio frequency carrier of 455 kHz. The electronics portion of the transmitter was encapsulated in epoxy and was hermetically sealed. A lithium battery with an estimated life of six months was used to power the electronics and transmitter. Battery life could be extended by turning off the transmitter when not in use. This was possible due to an internal magnetic switch that was activated by bringing a strong magnet close to the transmitter. Since the transmitter output frequency matched the intermediate frequency used by AM radio receivers, a small transistor radio tuned at the low end of the band was used to determine if the transmitter was on or off. The battery and electronics were surrounded by a second epoxy barrier and then coated with a medical grade silicone coating. Transmitters were sterilized using ethylene oxide prior to implantation in the mares.

Two stalls (3.3 x 6.6 m) located adjacent to The Ohio State University horse barn office were equipped with a pair of closed loop antennae. One antenna ran the length of the stall and was 6 m long by 0.9 m high. A second, smaller antenna was located along the adjacent wall of the stall with dimensions of 3 m long and 0.9 m high. The outer casing of
each antenna was made of 1/2-inch PVC pipe purchased at a local building supplies store. Five turn loops of 26-gauge enameled magnet wire\textsuperscript{22} were threaded through the pipe using a 3-m piece of stiff wire. The ends of the wire exited the pipe through a T-connector. One wire was soldered to the central wire of a coaxial cable and the second wire was soldered to the coaxial shielding. A 1-m piece of 1/2-inch PVC pipe was used to cover the wires up to a point in the stall above the floor where a horse could not reach. The antennae were affixed to the wall with plastic 1/2-inch hanging straps and protected by lengths of 2 x 4 inch lumber framed around each antenna. The distal end of the coaxial cable was connected to a two-conductor 2.5-mm mini-plug, with the center wire soldered to the central conductor core of the plug and the coaxial shielding soldered to the outer conductor. The effective range of the transmitter with this antenna system was 2.5 m. The size of the stall was selected after preliminary work with the antennae. To reduce radio cross talk between the two stalls, the bare wall of the adjoining stall was covered with sheet metal and was grounded to a buried water pipe.

Telemetered data were received by a CTR-86 receiver\textsuperscript{23} located in the stall. The receiver was connected to a BCM-

\textsuperscript{22} Belden Wire and Cable, Geneva, IL 60134.

\textsuperscript{23} Data Sciences Inc., St. Paul, MN 55113-1136.
100 consolidation matrix\textsuperscript{24}, which supplied power to the receiver and multiplexed signals from the two stalls onto cables connected to the computer. Telemetered data were recorded onto the hard disk of the computer; however, the data acquisition system could be configured to write temperature data directly to a floppy disk. Each temperature data file was automatically assigned a file name by the DataQuest III software and was coded for identification, date and time that the recording started. To analyze a data set with DataQuest III, the start and stop dates and inclusive times as well as the sampling interval were required. The system could be configured to record temperatures at a variety of sampling intervals.

**Surgery**

Four horse mares, weighing approximately 450 kg, received transmitter implants. The mares were sedated with 0.6mg/kg i.v. of xylazine\textsuperscript{25} and 0.02mg/kg i.v. of butorphanol tartrate\textsuperscript{26}. Presurgical preparation of the paralumbar region of one flank included a 10-minute Betadine\textsuperscript{27} scrub. Lidocaine was used to anesthetize the incision site. The transmitter was placed lateral to the transverse abdominus muscle and

\textsuperscript{24} Data Sciences Inc., St. Paul, MN 55113-1136.

\textsuperscript{25} The Butler Company, Columbus, OH 43228.

\textsuperscript{26} Turbogesic, Fort Dodge Labs, Inc., Fort Dodge, IA 50501.

\textsuperscript{27} The Purdue Frederick Co., Norwalk, CT 06856.
medial to the last rib and then sutured into place. Activation of the transmitter was done with a magnet prior to insertion in the mare. The muscle incision was closed using a continuous suture pattern #2 chromic catgut and the skin incision was closed with Braunamid #2 suture material\textsuperscript{28} using a Ford interlocking suture pattern. Each mare received a single tetanus toxoid injection and 9 million units of procaine penicillin once a day for 3 days.

**Data Collection**

Changes in body temperature were monitored closely for several days after surgery for evidence of post-operative complications. Body temperatures were recorded every 5 minutes except when the mares were allowed a daily 1- to 2-hour free-choice exercise period. A 5 minute sampling period was chosen because later analyses could be done in any multiple of 5 minutes; the amount of computer disk space required for data storage was minimal. For example, all the temperature data recorded for three of the mares during this study (at least 60 days of data for each mare) occupied approximately one megabyte of fixed disk space. The high density floppy disk drive on the computer used in this system was capable of storing 1.2 megabytes of data. Therefore, all the temperature data from three mares was stored on one high density floppy disk. Ambient temperature in the stalls was

\textsuperscript{28} Braunamid, B. Braun Melsungen AG, West Germany.
recorded at least three times per day. A commercially available, wall-mounted thermometer with a Celsius scale was placed in each stall where it could be easily observed. Body temperature was measured daily for approximately 60 days.

RESULTS

The radiotelemetric system used in this project successfully recorded mare body temperature changes for extended periods of time. An example of body temperature change in one mare over a 24-hour period using different sampling intervals is presented in Figure 9. The system also functioned as well in the other three mares.

The environmental temperature, which ranged between 5°C and 30°C, appeared to have no effect on body temperature.

DISCUSSION

This study has demonstrated that radiotelemetry can be used to monitor mare body temperature. This technology may be useful additionally to study temperature changes in the mare relative not only to reproductive events, but also changes resulting from the effects of pharmaceuticals, exercise, disease, and the like.

The use of radiotelemetry to transmit data that is indicative of the core or deep body temperature has been
previously reported (Riley, 1970; Littledike et al., 1979; Zartman and DeAlba, 1982; Zartman et al., 1983). In a study of sow body temperature during the periparturient period (Littledike et al., 1979), the placement of the transmitter was similar to the present study. Positioning of the transmitter between the transverse abdominus muscle and the peritoneum and posterior to the last rib in the sow was thought to be a better indication of core body temperature than rectal temperature.

One advantage to using a telemetric system for monitoring temperature is that the need to restrain the animal is eliminated. A hyperthermic reaction due to handling and insertion of a rectal probe has been reported in rats (Cunningham and Peris, 1983; Gallaher et al., 1985). In one study of changes in body temperature of pony mares prior to parturition, no change in temperature was found associated with foaling (Cox, 1969). However, the author noted that three of the four mares used were not accustomed to being handled as extensively as was required to obtain rectal temperatures, and the mares were described as distressed at being caught and placed in stocks for recording temperatures. A resultant increase in body temperature could have possibly masked a decrease in temperature associated with parturition and perhaps led to that author's conclusion.

Another advantage of the radiotelemetric system is the ability to record data without being present and recording
data more frequently than is practical with a rectal thermometer. Temperature data were recorded every 5 minutes in this study in order to allow future analysis of data in multiples of 5 minutes. A less frequent sampling period of 60 minutes would still provide 24 data points during a 24 h period for statistical analysis. This is in contrast to the three or four data points used in previous studies of mare body temperatures (Cox, 1969; Ammons et al., 1989; Haluska and Wilkins, 1989). Sampling intervals of 5 to 15 minutes may be useful in studying the effects of exercise or pharmaceuticals on horse body temperature.
Figure 9. An example of body temperature in one mare during a 24-hour period using sampling intervals of a) 60 minutes, b) 30 minutes, c) 15 minutes and d) 5 minutes.
REFERENCES


CHAPTER V

BODY TEMPERATURE FLUCTUATIONS IN THE
PERIPARTURIENT HORSE MARE

ABSTRACT

The objective of this study was to determine the pattern of mare deep body temperature fluctuations associated with parturition using biotelemetry.

A radio transmitter was implanted in one flank in each of 6 mares. Telemetered data were received by a pair of antennae placed at right angles in a 3.3 x 6.6-m stall and stored on a computer hard disk. Hourly temperature data were recorded for the period of -168 through 168 h postpartum.

A decrease of 0.76 °C in body temperature began at 4 hours prior to parturition (P < 0.1), then decreased rapidly between the 3 hours prior to and the time of parturition (Time 0). The lowest mean body temperature recorded was at the time of parturition (36.58 ± 0.16 °C; P < 0.001). A supranormal increase in mean body temperature began one hour postpartum and peaked at 38.02 ± 0.08 °C and remained elevated for 48 h.
postpartum until gradually decreasing to the level of the prepartum mean by 106 h.

INTRODUCTION

A decrease in maternal body temperature associated with parturition has been reported in cows (Wrenn et al., 1958; Fujimoto et al., 1988), ewes (Ewbank, 1969), bitches (Concannon et al., 1977), monkeys (Ruppenthal and Goodlin, 1982) and women (Goodlin and Chaplin, 1983). In mares, conflicting results have been reported. Studies of both pony and horse mares have reported a decrease in body temperature associated with parturition (Wright, 1943; Shaw et al., 1988; Haluska and Wilkins, 1989) but other studies using similar methods did not find a significant decrease in body temperature (Cox, 1969; Jeffcott, 1972; Ammons et al., 1989). The results of those studies of mares used clinical or digital rectal thermometers with sampling intervals of 2 to 4 times a day.

Telemetry has been used in a variety of species for remote body temperature study. A radio transmitter with a temperature sensitive circuit is usually implanted in an animal and an antenna and receiver collect the signal containing the deep body temperature data. The advantages of a telemetric system to study body temperature are that more frequent recordings can be made than is practical with a
rectal thermometer and the animal is not disturbed while collecting data. In reproductive studies, telemetry has been used to measure body temperature during the estrous cycle in cattle (Zartman and DeAlba, 1982; Clapper et al, 1990; Mosher et al, 1990) and at parturition in swine (Littledike et al., 1979) and macaques (Ruppenthal and Goodlin, 1982). However, to our knowledge, there are no published reports using telemetry to study mare body temperature fluctuations at the time of parturition. Therefore, the objective of this study was to determine the body temperature fluctuations associated with parturition in the horse mare using biotelemetry.

MATERIALS AND METHODS

Six horse mares, weighing approximately 450 kg, were used in this study. Mares were fed according to NRC guidelines, with water and trace mineral salt ad libitum.

The telemetric system employed in this study has been described previously (Cross et al., 1991). A pair of closed loop antennae, placed at right angles, were located in each of two 3.3 x 6.6-m stalls at The Ohio State University horse barn. A receiver was located in each stall and connected to a computer located in the barn office. Telemetered data were automatically recorded onto the computer hard disk. Each temperature data file was assigned a filename and coded for identification, date and time the recording started.
The surgical procedures for implanting the transmitters in the mares have been previously described (Cross et al., 1991). After sedation of the mare and presurgical preparation of the paralumbar region of one flank, the transmitter was placed lateral to the transverse abdominus muscle and medial to the last rib and then sutured into place. Each mare received a single tetanus toxoid injection and 9 million units of procaine penicillin once a day for 3 days.

Data Collection and Analysis

Body temperatures were recorded once every 60 minutes. Time 0 was defined as the time when the foal's hips were delivered (Shaw et al., 1988). Since there was wide variation in the time of parturition between mares, temperature data for all mares were standardized to time 0 regardless of time of parturition.

An overall mean temperature of 37.54 °C was obtained from the data for the 6 days prior to parturition (-168 to -25 h) and was defined as the prepartum mean body temperature. Hotelling's $T^2$ test for equality of means (Gill, 1978) was used to determine differences between body temperature during the time period of -24 to +168 h and the prepartum mean. The analysis was performed using the Statistical Analysis System General Linear Model (SAS Handbook, 1985). Temperature data are expressed as mean ± SEM. Differences in body temperature were considered significant if $P < 0.05$. 

RESULTS

Mean body temperatures during the prepartum period of -168 to -25 h were relatively constant. In all mares studied, a decrease in body temperature was apparent prior to foaling. The time of the onset of the decrease in mean body temperature was 4 hours prior to parturition (P < 0.1) with the greatest decrease occurring between -3 h and 0 h (P < 0.001; Figure 10). Between individual mares, the onset of the temperature decrease varied. A drop in temperature was apparent regardless of the time of day that the mare foaled (Figure 11). The lowest mean body temperature (36.58 ± 0.16 °C; P < 0.001) occurred at Time 0, when parturition occurred. Individually, the lowest body temperatures in four of 6 mares, were recorded at the time of parturition. In the other 2 mares, body temperature continued to decrease for 2 hours postpartum before increasing. Time of foaling or season appeared not to be a factor in these 2 mares because both foaled late at night (at 2217 and 0306) and one foaled in August and the other in early April.

Mean body temperature began to increase by one hour postpartum and continued to increase until peaking at 38.03 ± 0.08 °C 6 h postpartum. Since mean body temperature remained elevated above the prepartum mean body temperature (P < 0.05) through 24 h postpartum (Figure 10), temperature
data were analyzed for the period of 25 to 168 h postpartum. Mean body temperatures remained elevated from 25 to 48 h but then fluctuated between significant and not significant comparisons to the prepartum mean until 105 h postpartum. From 106 h onward, there were no differences between the prepartum mean and postpartum body temperatures.

DISCUSSION

To our knowledge, this is the first reported use of telemetry to study mare body temperature at the time of parturition. The ability to remotely record data has the advantages of not disturbing or handling an animal in order to get a recording and to record data more frequently than is practical using a rectal thermometer.

The results of the present study are in agreement with previous studies that reported a prepartum decrease in maternal body temperature in horse and pony mares (Wright, 1943; Shaw et al., 1988; Haluska and Wilkins, 1989). A sampling interval of 60 minutes using telemetry has allowed better definition of the characteristics of body temperature fluctuations around the time of parturition, but does not explain why there are differences between the previous studies. In two recent reports, using similar methods, a temperature decrease in the majority of horse mares studied was found by one group (Haluska and Wilkins, 1989), whereas
no change in body temperature that could predict parturition was found in the other study (Ammons et al., 1989). Similar conflicting results have been reported in pony mares. A precipitous drop in temperature occurring a short time prior to parturition, as reported in this study, could be missed if temperature readings are taken infrequently and in only a few mares. Another factor that could affect results is a possible hyperthermic reaction to restraint and insertion of a rectal thermometer, similar to that reported in rats (Cunningham and Peris, 1983; Gallaher et al., 1985). One pony mare temperature study reported at least 3 of the 4 mares were not used to handling and were stressed during temperature determinations (Cox, 1969).

Reasons for a decrease in body temperature prior to parturition have been discussed previously (Concannon et al., 1977; Haluska and Wilkins, 1989). The reproductive steroid hormones have effects on temperature regulating neurons in the hypothalamus (Boulant, 1991). A decrease in progesterone was correlated to decreased maternal body temperature in bitches (Concannon et al., 1977). A significant decrease in progesterone in pony mares has been reported on the last day of pregnancy (Haluska and Currie, 1988) and this could explain the steep drop in temperature prior to parturition found in this study. Other factors that have potential thermoregulatory properties should also be investigated, including prostaglandins and estradiol.
The supranormal increase in maternal body temperature following parturition in this study is similar to that reported in other studies (Concannon et al., 1977; Shaw et al., 1988; Haluska and Wilkins, 1989). An elevation of body temperature above the prepartum mean continued for at least 48 h in the present study before gradually returning to the level of the prepartum mean. A previous report in horse mares found that the supranormal increase in temperature had returned to the normal prepartum level within 24 h (Haluska and Wilkins, 1989). In bitches, body temperature remained elevated for at least 4 days before returning to normal (Concannon et al., 1977). The increase in temperature postpartum may indicate a resetting of the thermoregulating mechanisms in the hypothalamus, as suggested previously (Concannon et al., 1977; Haluska and Wilkins, 1989). Another possibility may be that an elevated postpartum temperature is a response to uterine inflammation, including the infiltration of neutrophils and macrophages that occurs shortly after parturition (Katila, 1988). In response to inflammation, endogenous substances such as some prostaglandins (e.g. PGE₂) are released that can cause an increase in temperature (Boulant, 1991). Telemetry could easily be incorporated into future studies of increases in postpartum maternal body temperature.

In conclusion, a decrease of 0.76 °C in maternal body temperature occurred in this study beginning 4 hours prior to
parturition and reaching a nadir at the time of parturition. A supranormal increase in mean body temperature began within an hour of parturition and remained elevated above the prepartum mean for at least 48 h before gradually returning to the level of the prepartum mean.

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Figure 10. Mean hourly body temperatures of six horse mares during the 24 h prior to and following parturition. All temperatures were compared to a prepartum mean of 37.54 °C obtained from the period of -168 to -25 h. Mean body temperature began to decrease relative to the prepartum mean 4 h prior to foaling (P < 0.1) and reached the lowest level at parturition (P < 0.001). A supranormal increase in temperature began at 1 h post partum and peaked at 6 h postpartum.
Figure 11. The pattern of hourly body temperature fluctuations in two mares 12 h before and after parturition. Mare 47 foaled at 00:33 h and Mare 305 foaled at 14:10 h.
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