THE UNIVERSITY OF MANITOBA

THE EFFECT OF TIME OF FEEDING ON TIME OF PARTURITION AND ON STEROID PROFILES DURING THE LAST MONTH OF PREGNANCY IN THE EWE

BY

JUDITH CHIKONKA NGALANDE LUNGU

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ABSTRACT

The Effect of Time of Feeding on Time of Parturition and on Steroid Profiles During the Last Month of Pregnancy in the Ewe

Judith Chikonka Ngalande Lungu

Two trials were conducted in two consecutive years to determine if lambing time and maternal steroid profiles could be altered by a change in time of feeding during the last month of pregnancy. At 4-6 weeks prior to expected delivery, 12 ewes were divided into two groups of 6 each. Each group was kept under constant light and fed twice daily. Group 1 was fed at a new time (1230h and 2100h), while group 2 continued to be fed at the regular times (0800h and 1630h). Blood samples collected at 2, 3 and 4 weeks after start of the trial and during the last 7 days prior to expected delivery, were assayed by radioimmunoassay techniques.

Time of lambing was not significantly altered ($P>0.05$) by time of feeding. Lambing was concentrated during the daytime (between 0700h and 2100h) in both groups with daytime lambing of 78% and 64% in groups 1 and 2, respectively. More ewes in group 1 (67%) lambed between the two feeding times than in group 2 (27%).

Mean gestation length of 146.3 days for group 1 and 147.3 days for group 2 were not different ($P>0.05$). Breed gestation length of 144.6 days (Finnish Landrace), 147.5 days (Suffolk) and 147.6 days (Crossbreds) were also not significantly ($P>0.05$) different.

Progesterone (P_4), cortisol (F) and estrogen concentrations and profiles at 2, 3 and 4 weeks after the start of the trial, which was at 4 weeks (trial 1) and 2 and 3 weeks (trial 2) prior to expected delivery and also during the last week of pregnancy were not different ($P>0.05$) between the groups. No relation between time of feeding and progesterone or estrogen concentrations could be found. However, cortisol concentrations seemed to increase in response to feeding in both groups at 2, 3 and 4 weeks after the start of the trial and during the last week of pregnancy. A diurnal pattern in cortisol concentration was found; suggestive of a daily rhythm. Cortisol concentrations were highest during the late night to early morning hours (0100h to 0800h).

Progesterone concentration significantly ($P<0.05$) decreased during the last 7 days before delivery in both trials decreasing from an overall mean of 16.5±0.8 ng/ml at 7 days before delivery to 3.2±0.7 ng/ml at lambing in trial 1.

Estrogen concentrations increased ($P<0.001$) dramatically during the last 24 to 36h before lambing in both trials increasing at a rate of 1 pg/ml/hr at about 16h before delivery. Estrogen concentration increased from a mean of 17±1.2 pg/ml at 7 days before lambing to 59.0±11.5 pg/ml at the time of lambing in trial 1. Estrogen concentration decreased after lambing. This increase in estrogen and decrease in P_4 indicates a change in the steroid synthesizing capacity of the placenta. Cortisol concentrations increased ($P<0.05$) modestly during the last 36h in both trials, increasing from 61.6±10.8 ng/ml at 7 days before lambing to 94.4±6.5 ng/ml at lambing in trial 1.

Estrogen, P_4 and F concentrations were very variable at 2 and 3 weeks prior to delivery with each steroid displaying as many as 10-12 peaks/24h period. The intra animal variability of all the steroids decreased as time of parturition approached, decreasing to 4-6 peaks/24h period during the last week of pregnancy. The peaks were also more sustained, lasting about 2-3h as compared to 1.5h at 3 weeks prior to parturition.

There was no maternal prepartum estrogen surge in a ewe that gave birth to dead lambs, indicating that a live fetus was essential for this rise in circulating estrogen concentration.

The conclusion was that time of feeding did not alter parturition time in the ewes in this study, and did not alter P_4 or estrogen concentrations. Feeding, but not necessarily time of feeding, appeared to increase cortisol concentrations at all times of pregnancy observed in this study. Also, in addition to the specific time trends for each steroid, there was less intra animal variability in all the three steroids as parturition approached .

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INTRODUCTION

Many ewe and lamb deaths during parturition could be prevented if a herdsman was present to help ewes with dystocia and lambs of poor vitality. A herdsman needs to keep a 24 hr watch at the ewes during the lambing season because lambing seems to be distributed over a 24 hr period. It would be easier and cheaper for the farmer if most of the lambing was concentrated during the daylight hours.

The modifying effect of feeding time on time of parturition has recently received considerable attention. Konefal (1980) reported that an altered feeding time would cause Hereford cows to deliver during the daylight hours. According to Konefal (1980), feeding cows twice daily during the last month of pregnancy at around 1200h and 2100h instead of the usual 0800h and 1600h feeding times, resulted in most calvings occurring between 0700h and 1900h. Since a daytime lambing resulting from a system of feeding like that of Konefal in cows would be advantageous in sheep, the Konefal system was tested with sheep. In addition, the experiment was designed to determine if this change in feeding time during the last month of gestation altered the daily pattern of the maternal steroid hormones involved in the initiation of parturition, and whether the steroid patterns bore any relationship to the lambing times. Serial measurements of hormones from the same ewe were made at close intervals during a 24hr period after the ewes were put on the new feeding time, and also during the last week of pregnancy.

II

LITERATURE REVIEW

2.1 INTRODUCTION

This review will be concerned with fetal and maternal roles in the processes leading to parturition. The mechanisms triggering parturition should begin when the fetus is sufficiently mature, so that it can survive an independent life outside the uterus. Parturition is a very complex process and it is heralded by dynamic hormone changes in the fetal and maternal circulation. The fetus is thought to play a major role in the commencement of events leading to parturition. During the last 2 to 3 weeks of pregnancy, the fetal adrenal is believed to go through a maturational process which leads to an increase in fetal cortisol production (Thorburn and Challis, 1979). The increase in fetal cortisol is believed to trigger the events leading to parturition. The rising fetal cortisol is thought to be responsible for the fall in maternal progesterone (P_4) concentration and the immediate prepartum rise in estrogen concentration (Anderson et al., 1975; Challis et al., 1977b; Thorburn et al., 1977a; Liggins et al., 1977; Anderson et al., 1981), through an effect on placental steroidogenesis (Anderson et al., 1975; Flint and Ricketts, 1979; Ricketts et al., 1980a). The fetal cortisol rise is also thought to function as a 'tuner' that synchronizes and coordinates those maturational processes required for extra-uterine life (Challis et al., 1977b). Such processes include maturation of fetal

organs such as the lung, and biochemical differentiation of fetal tissues for glycogen deposition and insulin release (Liggins et al., 1979).

Progesterone is essential for maintenance of pregnancy in all domestic species (Thorburn et al., 1977a). Parturition is preceded by regression of the corpus luteum (CL) and a decrease in the concentration of P_4 in maternal plasma (Thorburn et al., 1977a). Csapo (1956) hypothesized that P_4 blocked myometrial activity. Progesterone is thus believed to have a dampening effect on myometrial contraction, while permitting transfer of nutrients to the fetus and removal of waste products from the fetus.

Progesterone produced by the CL is responsible for maintenance of pregnancy until about day 50 in sheep (Linzell and Heap, 1968). The average gestation length in sheep is 149 days (Hulet and Shelton, 1980). After day 50, the placenta secretes most of the P_4 involved in the maintenance of pregnancy (Linzell and Heap, 1968, Heap et al., 1973; Ricketts and Flint, 1980). These authors deduced this based on the research showing that neither hypophysectomy nor ovariectomy after day 50 of pregnancy leads to abortion in sheep. Plasma P_4 concentration increases steadily from days 50 to 90 reaching maximum levels around days 125 to 130 (Thorburn et al., 1977a). Progesterone concentration then decreases in a variable manner over the last 3 to 7 days of pregnancy (Bassett et al., 1969; Fylling, 1970; Liggins et al., 1973; Elsner et al., 1980). Thus, the progesterone withdrawal preceding parturition removes the block to myometrial contraction.

Estrogens are thought to play a major role in events immediately prior to parturition (Liggins et al., 1973, Thorburn and Challis, 1979). Estrogens increase the capacity of the uterus to contract and promote the synchronization of the contractile units (Fuchs and Fuchs, 1981). This effect is through stimulation of the synthesis of numerous enzymes, and also by stimulation of prostaglandin synthesis. Estrogens are also implicated in processes associated with fetal lung maturation (Mendelson et al., 1980). Although there is evidence of estrogen production in early gestation (Carnegie and Robertson, 1978; Challis and Patrick, 1981), concentration of unconjugated and conjugated estrogens in fetal tissues and fetal and maternal plasma remain low throughout pregnancy, increasing only during the last 24 hr before parturition (Challis, 1971; Robertson and Smeaton, 1973; Rawlings and Ward, 1976; Challis and Patrick, 1981).

Ovine placental lactogen (oPL) is a hormone with a function in pregnancy which is not fully understood. Ovine placental lactogen is thought to be important in fetal growth and maintenance of pregnancy in sheep and is also believed to be important in inhibiting the synthesis of prostaglandins (PG) (Thorburn, 1977). Ovine placental lactogen is thought to be synthesized in the binucleate cells of the fetal chorionic epithelium (Martal and Djiane, 1977). It has been detected in the fetal and maternal circulation by many investigators (Chan et al., 1978; Gluckman et al., 1979; Taylor et al., 1980,1982a). Ovine placental lactogen is first detected in maternal plasma at about 45 to 50 days of pregnancy, and the concentration rises to reach peaks on days 120 to 130 followed by a significant fall before delivery. Despite the similarity

in the profiles between P_4 and oPL in the pregnant ewe, the secretion of the two hormones is not regulated by the same mechanisms (Taylor et al., 1982a). Dexamethasone or synthetic adreno-cortical hormone (ACTH-(1-24)) depresses P_4 concentration and induces premature parturition in sheep (Strott et al., 1974), but oPL does not decline under these conditions (Taylor et al., 1983), and may not be important in events leading to parturition. This hormone will not be included in the following discussion.

2.2 FETAL ROLE IN PARTURITION

Changes in the fetal pituitary-adrenal relationship trigger events leading to parturition in sheep and other domestic animals (Liggins et al., 1977; Thorburn et al., 1977a,b). Involvement of the fetal pituitary-adrenal system in the initiation of parturition was first observed in the field by sheep farmers in Idaho in the early 1960's. They noticed that sheep grazing in mountain pastures at certain times of the year had prolonged pregnancies. The cause of the prolonged pregnancy was the weed Veratrum californicum , which the ewes had consumed during early pregnancy. The alkaloid in this weed caused impairment in development and function of the fetal adrenal glands and the fetal pituitary (Binns et al., 1963,1964). The congenital malformations of the fetus were later reproduced experimentally (Liggins et al., 1967, 1973; Drost and Holm, 1968). Fetal hypophysectomy (Liggins et al., 1967), fetal pituitary section (Liggins et al., 1973) or bilateral adrenalectomy (Drost and Holm, 1968) at day 100 of pregnancy all led to prolonged pregnancy.

At about the same time, there were rapid advances in the techniques for determination of hormone concentration in fluids, and maintaining fetal catheters in utero. Bassett and Thorburn (1969) showed that the fetal plasma concentration of cortisol increased enormously during the last 7 to 10 days prepartum. This has been repeatedly confirmed (Comline et al., 1979; Magyar et al., 1980b, 1981; Thorburn and Challis, 1979; Hennessy et al., 1982). Earlier, Comline and Silver (1961), had observed a marked fetal adrenal hypertrophy in late pregnancy. Liggins and others showed that infusion of ACTH-(1-24), or a glucocorticoid but not a mineralocorticoid to the fetus caused premature parturition (Liggins, 1968, 1969; Liggins et al., 1973). Comparable infusions to the mother were ineffective in inducing parturition. Several other investigators have induced premature parturition by intrafetal infusion of high doses of ACTH-(1-24) (Strott et al., 1974; Kendall et al., 1977), or physiological levels of ACTH-(1-24) (Cabalum et al., 1982) or cortisol (Anderson et al., 1975). Moreover, intrafetal infusion of glucocorticoids into adrenalectomized fetuses (Flint et al., 1976), or ACTH into hypophysectomized fetuses (Challis et al., 1977a) induced premature parturition. Furthermore, premature parturition induced by ACTH (Jones et al., 1978a) and dexamethasone (Jones et al., 1978b) infusion into the fetus was preceded by a rise in fetal cortisol concentration. Thus the fetal pituitary-adrenal function, and particularly changes in cortisol secretion appeared intimately involved with the onset of labor.

2.2.1 Maturation of the ovine fetal pituitary-adrenal function

Fetal sheep adrenals can respond to ACTH and corticosteroids with an increase in cortisol output as early as day 50 of pregnancy (Wintour et al., 1975; Challis et al., 1979; Glickman and Challis, 1980; Manchester and Challis 1982). At this stage of gestation, the ovine fetal adrenal has the full capacity for steroidogenesis, with all the enzymes necessary for cortisol synthesis. Endogenous peripheral plasma levels of cortisol are high at this time (Wintour et al., 1975). Between days 90 and 130 of pregnancy, the sheep fetal adrenals are not responsive to ACTH stimulus and endogenous plasma cortisol levels are low. The fetal plasma concentration of cortisol in bilaterally adrenalectomized fetuses is not different from those of intact fetuses between 90 and 120 days of pregnancy (Wintour et al., 1980). The placental transfer of cortisol from the mother to the fetus is very high at this time (90-120 days), and accounts for all the cortisol measured in both adrenalectomized and intact fetuses (Hennessy et al., 1982). Large rises in ACTH-(1-39) (a naturally occurring ACTH) concentrations in fetal plasma evoke very small changes in cortisol concentration at this time (Jones et al., 1977b). After day 130, the sensitivity of the sheep fetal adrenals to ACTH is restored. The adrenals now respond to ACTH stimulus with an increase in cortisol production. Thus, the maximum production rate of cortisol by fetal adrenal cells is lower between 90 and 130 days of pregnancy than earlier or later in gestation.

It is known that the burst of adrenocortical activity prepartum is under the influence of hypophyseal factors, because removal of the fetal ovine pituitary gland abolishes the prepartum rise in fetal cortisol

(Challis et al., 1977b), while intrafetal infusion with ACTH is capable of inducing fetal adrenal production of cortisol and premature parturition. While it is known that an intact fetal pituitary and adrenal are a necessity for parturition, and that ACTH is capable of prematurely inducing the increase in fetal cortisol and thus causing parturition, there is no evidence available that suggests that a rise in fetal plasma ACTH is responsible for the naturally occurring increase in fetal plasma cortisol. The evidence available indicates that ACTH concentrations in the fetus increase only in the very last days (3-4 days) before parturition when cortisol levels are already elevated (Rees et al., 1975a; Jones et al., 1977a; Challis et al., 1977a; Durand et al., 1980; Wintour et al., 1980; Rose et al., 1982). In view of these findings, it has been stated that there are maturational changes taking place in the fetus that cannot be simply explained by increases in ACTH levels. It has been suggested that there is maturation of the fetal adrenal response pattern to ACTH in later pregnancy (Liggins et al., 1977; Nathanielsz, 1978; Thorburn and Challis, 1979). This maturation is determined by the ability of exogenously administered or endogenously released ACTH to stimulate an increase in cortisol output from the fetal adrenal in vivo or in vitro.

The question is, "what causes the fetal adrenal to be nonresponsive to ACTH stimulation between days 90 to 130, and what triggers the responsiveness to ACTH to be restored at the end of gestation?" This question has been explored by many researchers, and attempts to answer it will be presented below.

The factors responsible for the alteration in fetal adrenal cortisol output have been studied extensively (Anderson et al., 1972; Davies and Ryan, 1973; Madill and Bassett, 1973; Wintour et al., 1975; Jones et al., 1977a,b; Glickman and Challis, 1980; Magyar et al., 1980a; Durand et al., 1981a,b,c, 1982a,b, 1984, 1985; Manchester and Challis, 1982; Cathiard et al., 1985; Challis et al., 1985) but are still poorly understood. It has been suggested that the factors leading to the fetal adrenal-pituitary maturational changes might originate in the fetal adrenal, pituitary, hypothalamus or from another part of the body.

2.2.2 Fetal adrenal factors

Histologically, the fetal adrenal gland at day 60 contains predominantly one cell type aligned in 4-6 cell thick cords arranged radially within the gland (Robinson et al., 1979). The cells have some vesicles within the mitochondria and some smooth endoplasmic reticulum (SER). The fetal adrenal gland is capable of steroid synthesis at this time and responds to ACTH stimulation with a high corticosteroid secretion (Wintour et al., 1975). Between the 90 to 120 day period, a distinct outer zone (glomerulosa) forms, but the inner zone (zona fasciculata) becomes disorganized and the cells look immature. The fetal adrenal cells fail to respond to ACTH stimulation with a high cortisol output at this time but respond to ACTH stimulation with a high progesterone output (Challis et al., 1979; Glickman and Challis, 1980; Manchester and Challis 1982). It is only after day 120 that significant numbers of mature zona fasciculata cells begin to appear (Robinson et al., 1979; Durand et al., 1980; Webb, 1980). This zone can be stimulated by ACTH to secrete corticosteroids (McDougall et al., 1980).

Fetal adrenal cells possess fewer corticotrophin receptors during the nonresponsive state to ACTH than at the end of pregnancy (Durand, 1979). The number of receptors per cell increases dramatically from day 140 to term (Durand, 1979; Durand et al., 1981a), which correlates closely with the increasing sensitivity of the fetal adrenal to ACTH (Durand et al., 1981a). However, the low number of binding sites before day 140 does not seem to prevent ACTH stimulation of progesterone production (Glickman and Challis, 1980; Manchester and Challis 1982) by fetal adrenal cells from midgestation fetuses, but prevents cortisol production. Thus it appears that cell changes and receptor modulation are some of the factors involved in the fetal adrenal maturational processes associated with cortisol production.

2.2.3 Hypophyseal factors regulating fetal adrenal function

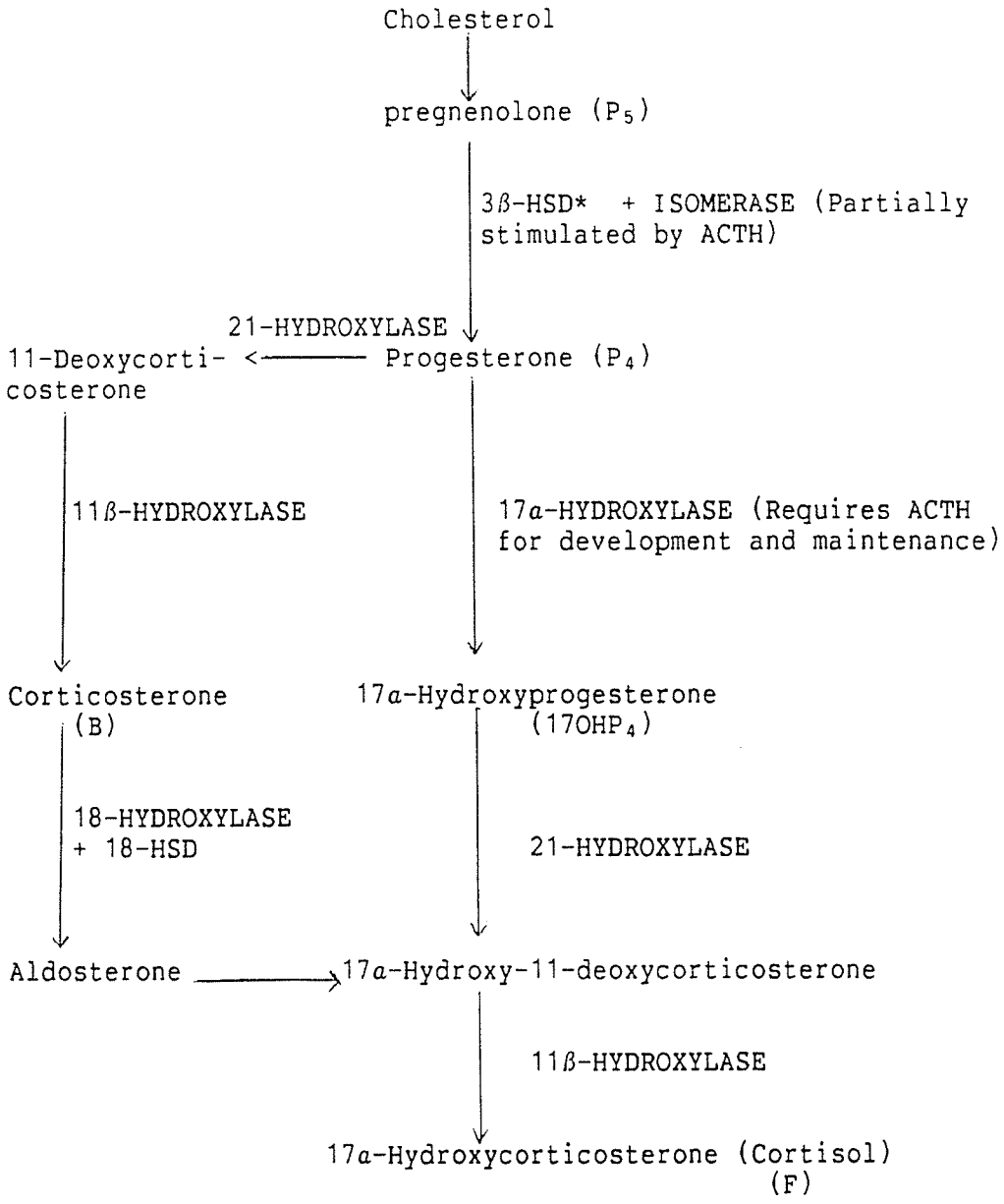
The fetal pituitary secretes, in addition to ACTH-(1-39) (MW 4,500), some high molecular weight peptides A, B, and C (MW 50,000, 30,000 and 20,000) which react with anti-ACTH-(1-24) (Silman et al., 1979). These high molecular weight forms of ACTH-like peptides are found in high concentration (150 pg/ml) in fetal plasma as compared to ACTH-(1-39) (50 pg/ml) from days 120 to 135 of pregnancy (Silman et al., 1979; Jones and Roebuck, 1980). Roebuck and others (1980) deduced from their in vitro experiments that these high molecular weight forms of ACTH-like peptides, which are in such high concentrations, were inhibiting fetal adrenal responsiveness to ACTH between days 90 to 130 of pregnancy. In contrast to results of other researchers who found a poor response to ACTH of day 90 to 130 fetal adrenal cells (Wintour et al., 1975; Challis

et al., 1979; Glickman and Challis, 1980; Manchester and Challis, 1982), Roebuck et al., (1980) showed that fetal adrenal cells from day 120 to 135 fetuses responded to a low ACTH concentration with an increase in cortisol production. They also demonstrated that the high molecular weight forms of ACTH-like peptides were steroidogenic at a low concentration (1 ng/ml) but at high concentration, prevented ACTH stimulated cortisol release from these fetal adrenal cells. Roebuck et al. (1980) hypothesized that the high molecular weight peptides, provided they contain 15-18 region of the ACTH molecule should effectively compete for ACTH receptors on the fetal adrenal and thus block accessibility of ACTH-(1-39) to steroidogenic sites. They concluded that in vivo the high molecular weight forms of ACTH-like peptides were suppressing the ability of fetal adrenals to respond to circulating ACTH between days 90 to 130. They reasoned that the fetal adrenal sensitivity is restored at the end of pregnancy when these ACTH-like peptides decrease.

The suggestion that the high molecular weight forms of ACTH secreted by the pituitary between 90 to 130 days of pregnancy might be inhibiting fetal adrenal responsiveness to ACTH have been partly disproved by other researchers (Glickman and Challis, 1980; Durand et al., 1985). Durand et al. (1985) showed that when midgestation fetal adrenal cells were repeatedly stimulated (2 hr/day for 4 days) by ACTH or fetal pituitary extracts (FPE which are considered to contain the high molecular weight peptides) from 124 day old fetuses, the response to ACTH stimulation in cAMP and corticosteroid levels during the 5 days of culture was similar to the response to FPE stimulation. In other studies, Glickman and

Challis (1980) showed that the fetal pituitary cells of different gestational ages (day 50, 100, 130, and term) stimulated cortisol and P_4 production from maternal adrenal cells of all gestational ages tested. However, similar coincubations of fetal pituitary cells with fetal adrenal cells, stimulated cortisol production of only day 50, 130 and term fetal adrenal cells but not day 100 cells. Progesterone production on the other hand was stimulated in all the fetal adrenals tested. Thus the results of Durand and others (1985) and those of Glickman and Challis (1980) do not support the physiological significance of the blockade of steroidogenic action of ACTH by some high molecular weight forms of ACTH as demonstrated in the ovine fetus by Roebuck et al., (1980). It appears that Roebuck et al., (1980) demonstrated a reduction in ACTH-induced steroidogenesis when the ratio of high molecular weight forms of ACTH/ACTH-(1-39) was about 10, but this ratio in fetal pituitary is always less than 4 (Silman et al., 1979) and even lower in fetal blood (Jones and Roebuck, 1980). The lack of fetal adrenal cortisol production at day 100 is thus not due to inhibiting substances from the fetal pituitary, since cortisol production from maternal adrenal cells can be stimulated by fetal pituitaries, nor lack of fetal adrenal ACTH receptors since P_4 was stimulated (Glickman and Challis, 1980). It was then suggested that there was a major enzyme block in the cortisol biosynthetic pathway distal to P_4 formation in the fetal adrenal at days 90 to 130 of pregnancy (Glickman and Challis, 1980). The suggestion was that 17α-hydroxylase activity was the major rate limiting step (Fig 1).

Figure 1: Pathways for fetal cortisol synthesis in the ovine fetal adrenal at parturition



*3ß-Hydroxysteroid dehydrogenase

Contents of the fetal pituitary and ACTH in particular, have been shown to be important in the regulation of fetal adrenal function. The effect is at least partially a direct one on the fetal adrenal cells (Liggins and Kennedy, 1968; Anderson et al., 1972; Madill and Bassett, 1973; Liggins et al., 1973; Wintour et al., 1975; Jones et al., 1977a; Brown et al., 1978; Durand, 1979; Magyar et al., 1980a; Durand et al., 1981a,b,c, 1982a,b, 1984, 1985; Rose et al., 1982). Hypophysectomy (Hx) of the ovine fetus leads to atrophy of the fetal adrenals and impairment of steroidogenesis (Liggins and Kennedy, 1968). Although the infusion of ACTH-(1-24) into Hx fetal sheep induced parturition, maximum corticosteroid concentration achieved was far below that of intact fetuses (Jones et al., 1978b). Moreover the time interval between infusion of ACTH-(1-24) and parturition at 124-129 days of pregnancy was longer in fetuses Hx at 115 days than in intact fetuses (Challis et al., 1977b).

Experiments in vitro have shown that adrenal cells from Hx fetuses, had lower cAMP and corticosteroid production in both the presence or absence of ACTH-(1-24) during the first 3 days of culture (Durand et al., 1985). The total enzyme activities involved in the steroidogenic pathway were lower in fetal adrenal cells from Hx fetuses than controls (Cathiard et al., 1985). When fetal adrenal cells from Hx and intact fetuses were cultured for 6 days with ACTH-(1-24), corticosterone production was the same in the two groups, but cortisol production by cells from Hx fetuses was one half that from intact fetuses (Cathiard et al., 1985), indicating that Hx prevented the development of 17α-hydroxylase. The in vivo and in vitro experimental results thus

indicate that the fetal pituitary is essential for the development of fetal adrenal function and that it stimulates adrenal trophic and steroidogenic responses.

2.2.4 Substrate limitation

To examine further the factors limiting cortisol production by the fetal adrenal cells, Manchester and Challis (1982) determined cortisol, corticosterone and progesterone output from dispersed fetal adrenal cells obtained at days 50, 100, 130 and 143 of pregnancy. They incubated the fetal adrenal cells for 4 hr with either ACTH, guanosine 5'-triphosphate (GTP); a non-hydrolyzable GTP analog, or dibutyryl adenosine 3',5'-monophosphate (dbcAMP) and potential substrates for corticosteroid production (pregnenolone (P_5), progesterone, 17α-hydroxypregnenolone ($17OHP_5$), or 17α-hydroxyprogesterone ($17OHP_4$)). They hoped to overcome the intracellular block to cortisol production by providing appropriate substrates of the cortisol biosynthetic pathway and to bypass a block at the receptor and adenylate cyclase level by provision of a GTP analog and dbcAMP, respectively. On day 50, all substrates used were converted to cortisol. But cortisol output from fetal adrenal cells in midgestation (days 100 and 130) was significantly higher in the presence of $17OHP_4$ than in the presence of progesterone or pregnenolone, indicating that 17α-hydroxylase was a limiting enzyme (Fig 1). At term, Δ_4 substrates (P_4 and $17OHP_4$) were more effectively converted to cortisol than Δ_5 substrates (P_5 and $17OHP_5$) indicating that 3β-Hydroxysteroid dehydrogenase (3β-HSD) was the rate limiting enzyme at this time. Also conversion of P_4 and $17OHP_4$ to cortisol, and cortisol

output in the presence of cAMP and GTP, were lowest at midgestation and high at days 50 and 143, suggesting that many enzymes of the cortisol biosynthetic pathway are limiting cortisol biosynthesis in midgestation. They also suggested that there was a defect in GTP and cAMP generation.

2.2.5 Extrapituitary factors

Spontaneous (Anderson et al., 1972; Liggins et al., 1973; Wintour et al., 1975; Jones et al., 1977a; Brown et al., 1978; Glickman and Challis, 1980; Magyar et al., 1980a; Durand et al., 1981a,b,c, 1982a,b, 1984, 1985; Manchester and Challis,1982; Rose et al., 1982) and ACTH-induced (Durand et al., 1981a,c) in vivo and in vitro maturation of ovine fetal adrenals is characterized by an increased steroidogenic responsiveness to ACTH. This maturation involves an increased activity of several enzymes involved in the steroidogenic pathway especially that of 3ß-HSD and 17α-hydroxylase and enhancement of the adenylate cyclase system.

In short-term culture experiments involving fetal adrenal cell incubations of up to 4 hr, fetal adrenal responsiveness to ACTH was demonstrable only at day 50 and after day 130 of pregnancy (Glickman and Challis, 1980; Magyar et al., 1980a; Manchester and Challis, 1982). But long-term midgestation fetal adrenal cell culture of up to 6 days in the absence or presence of ACTH resulted in an increase in the steroidogenic enzyme activities during the 6 day culture (Durand et al., 1982a,b, 1984, 1985; Cathiard et al., 1985). The response to ACTH of these midgestation fetal adrenal cells was higher on day 6 than day 1. In ACTH-free media the increase in steroidogenic activity was due mainly to

the development of the pathway leading to corticosterone synthesis (Fig 1) while in the presence of ACTH (2h/day for 5 days) cortisol synthesis was stimulated more than corticosterone (Durand et al., 1984). It was suggested that the burst of enzyme activities in these fetal adrenal cells in vitro was due to the absence of inhibiting factors that block enzyme activity in vivo (Durand et al., 1982a, 1984, 1985; Cathiard et al., 1985). However, no such putative inhibitory factors have been found. Durand et al. (1981b,c, 1982a) in vivo, and Durand et al., (1984) in vitro, showed that these inhibitory factors can be overcome by ACTH infusion of 115 day old fetuses, or ACTH-(1-24) treatment of cultured fetal adrenal cells. Infusion of 115 day old ovine fetuses with ACTH-(1-24) for 5 days induced a marked development of 3ß-hydroxysteroid dehydrogenase and isomerase and to a lesser extent 21- and 11ß-hydroxylase activities (Anderson et al., 1972; Durand et al., 1981b,c, 1982a) (Fig 1). Development of the 17α-hydroxylase activities was slower than the development of the other enzyme activities in the steroidogenic pathway and therefore required a longer period of cell culture or ACTH infusion into the fetus (Cathiard et al., 1985).

2.2.6 Fetal adrenal modulation by glucocorticoids

Factors responsible for changes in fetal adrenal function are complicated by the finding that glucocorticoids might enhance adrenal function (Liggins et al., 1977; Wintour, 1984; Challis et al., 1985). Liggins et al. (1977), showed that the output of cortisol by fetal sheep adrenals in response to an ACTH challenge in vivo, was greater in fetuses that had been pretreated with dexamethasone for 48hr. More

recently, Challis et al., (1985) showed that dispersed fetal adrenal cells from fetuses infused in utero for 100hr with either pulsatile ACTH (P-ACTH) only, or metopirone (to block 11ß-hydroxylase activity) and cortisol or metopirone and dexamethasone at 127 and 131 days of pregnancy had similar cortisol output. But those that had been pretreated with PACTH and metopirone showed reduced cortisol output. It appears that the presence of glucocorticoids overcame the metopirone blocking effect, and stimulated cortisol production. However in adults, several studies have documented suppressive effects of glucocorticoids on adrenal function, either through a negative feedback on pituitary ACTH release or directly on the adrenal gland (reviewed by Keller-Wood and Dallman, 1984). The studies in the fetus raise the possibility that glucocorticoids might increase adrenal function. It is not known how this glucocorticoid-enhanced adrenal fetal function would contribute to the increased fetal adrenal sensitivity seen after day 130.

2.2.7 Conclusion

The change from the nonresponsive fetal adrenal, to the fetal adrenal highly sensitive to ACTH at the end of pregnancy is termed 'Maturation of the Fetal Adrenal Function' (Liggins et al., 1977; Thorburn and Challis, 1979; Wagner et al., 1979). This maturation of adrenal responsiveness which occurs during the last three weeks of pregnancy appears to involve an increase in the number and size of cortical cells in the zona fasciculata (Robinson et al., 1979; Durand et al., 1980; Webb, 1980), an increase in the number of ACTH receptors per adrenal cell (Durand, 1979; Durand et al., 1981a), development of the ACTH

sensitive adenylate cyclase pathway (Durand et al., 1981a,c, 1982b; Manchester and Challis, 1982), and an increased activity of several enzymes of the steroidogenic pathway (Durand et al., 1981a,b,c, 1982a,b; Anderson et al., 1972; Manchester and Challis, 1982; Cathiard et al., 1985; Durand et al., 1985). ACTH is an absolute requirement for development and maintenance of 17α-hydroxylase activity in vitro (Durand et al., 1984; Cathiard et al., 1985), and is essential in vivo.

ACTH infused into the fetus has been shown to increase steroidogenic enzymes involved in the cortisol biosynthetic pathway (Durand et al., 1981a,b). However preterm fetal cortisol surge precedes the ACTH rise (Rees et al., 1975b; Jones et al., 1977a; Durand et al., 1980). The results of Durand et al (1985) and Glickman and Challis (1980) indicate that the refractoriness of the fetal adrenal in midgestation is not caused by factors of pituitary origin. Extrapituitary factors have been suggested to cause inhibition of fetal adrenal maturation and therefore the refractoriness in midgestation up to the last days of pregnancy (Durand et al., 1982b, 1984; Cathiard et al., 1985), but these putative inhibitory factors have not been found. It is not known how these putative inhibitory factors are removed at the end of pregnancy in order to restore fetal adrenal sensitivity. It seems reasonable to assume that the maturation of the fetal adrenal and therefore restoration of adrenal sensitivity might involve trophic and steroidogenic factor(s) of pituitary and extrapituitary origin and possibly fetal cortisol levels transferred from the mother. Extrapituitary steroidogenic factors such as PGE and prostacyclin have been suggested to be involved in the restoration of fetal adrenal sensitivity but have been discarded (Liggins et al., 1982).

2.3 MATERNAL ROLE IN PARTURITION

2.3.1 Induction of placental steroidogenesis

The prepartum rise in fetal cortisol concentration is followed by an increase in the concentration of cortisol in the amniotic fluid (Challis et al., 1979), which is followed by a rise in maternal plasma. The reason for the maternal plasma cortisol rise which occurs during the last 2-3 days of gestation is not known (Chamley et al., 1973, Burd et al., 1976).

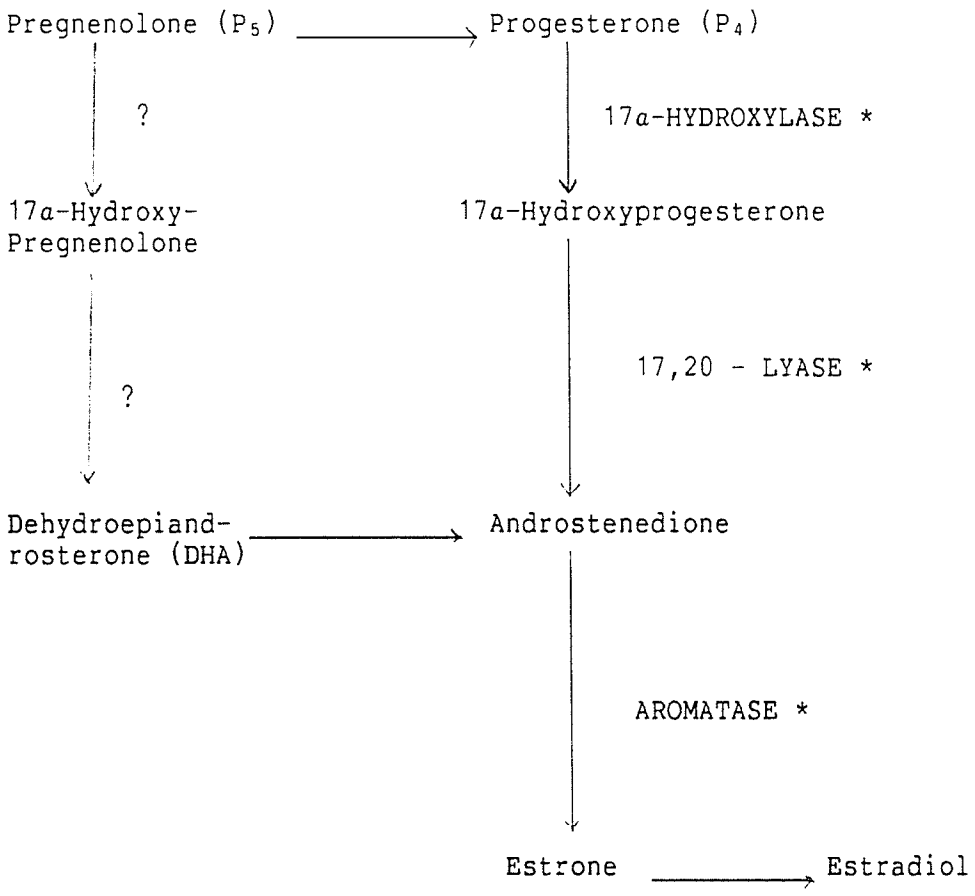
The fetal cortisol is believed to act on the placenta to cause a fall in its output of progesterone and an increase in the production of estrogens. It has been proposed that cortisol induces an increase in placental 17α-hydroxylase and 17,20-lyase activities (Anderson et al., 1975; Steele et al., 1976b; and Yu et al., 1983). According to this hypothesis, cortisol secreted by the fetal adrenal brings about parturition by altering the nature of ovine placental steroid synthesis, such that progesterone production is decreased (Bassett et al., 1969; Elsner et al., 1980), and estrogen production is increased (Challis, 1971; Nathanielsz et al., 1982).

Progesterone in both fetal and maternal plasma, but not fetal fluids (Power et al., 1982), decreases before spontaneous or induced parturition (Bassett et al., 1969; Liggins et al., 1973; Strott et al., 1974; Bedford et al., 1972a; Elsner et al., 1980; Anderson et al., 1981; Power et al., 1982). Following a decrease in plasma P_4 concentrations, maternal unconjugated and sulphoconjugated estrogens rise about 24-48hr before delivery (Challis, 1971; Currie et al., 1973; Cabalum et al.,

1982; Nathanielsz et al., 1982). The factors responsible for the reduction in P_4 concentration and a simultaneous rise in estrogen levels have been investigated by measuring enzyme activities in placental homogenates, and measuring intermediate products of enzymes in the estrogenic pathway. Decline in P_4 is partly due to the decrease in placental output (Bedford et al., 1972b), and also through the conversion of P_4 to estrogens (Anderson et al., 1975). The idea that P_4 is converted to estrogens is supported by the finding of increased activity of 17α-hydroxylase in fetal placenta tissues after intrafetal infusion of dexamethasone (Anderson et al., 1975). As mentioned earlier, the fetal placenta is the site of P_4 synthesis from pregnenolone (P_5). It was suggested that this was the first step in the placental pathway for biosynthesis of estrogens and a way of reducing P_4 concentrations (Fig 2). The increase in 17α-hydroxylase activity in vitro has been supported by the demonstration of an increase in the concentration of 17α-hydroxylated progestins and 17,20-dihydroxypregn-4-en-3-one in maternal plasma during spontaneous and induced parturition (Flint et al., 1975a,d).

Increased placental enzyme activities at term and after glucocorticoid induced parturition have been reported by various other workers. Increased 17α-hydroxylase activities (Anderson et al., 1978b; Ricketts et al., 1980a,b), c-17,20 lyase activity (Steele et al., 1976b), aromatase activity both at term (Mann et al., 1975) and after intrafetal glucocorticoid infusion (Anderson et al., 1978a) have been reported. The increase in c-17,20 lyase activities in the placenta were confirmed by the rise in androstenedione in the uterus at term (Steele

Figure 2: Pathways for estrogen synthesis at parturition in sheep placenta



* Enhancement of enzyme activity by glucocorticoids (From Anderson et al. 1981)

et al., 1976a). The rise in androstenedione parallels the rise in estrogens, implying both a role of androstenedione as an estrogen precursor, and that the pathway from P_5 through P_4 is an important route for estrogen production (Fig 2). The pathway from P_5 through dehydroepiandrosterone (DHA) also possibly plays a role in supplying precursors for estrogen synthesis since it has been shown that infusion of DHA into the fetal jugular vein results in increased concentration of estrone, estradiol and dehydroepiandrosterone sulphate (DHAS) (Rosenfeld et al., 1980 Fig 2).

High levels of sulphoconjugated and unconjugated DHA have been reported in ovine fetal plasma at term (Colas and Curet, 1978; Savoie et al., 1981; Nathanielsz et al., 1982; Liggins et al., 1985), and have been thought to originate from the maternal adrenals, ovaries and fetal testes. However ovariectomy and/or adrenalectomy of the mother or the fetus will lead to the normal prepartum estrogen rise (Bedford et al., 1972a,b; Thompson and Wagner, 1974; Flint et al., 1976; Kendall et al., 1977; Liggins et al., 1985). Both DHA and androstenedione can be aromatized to estrone by the ovine placenta exposed in vivo to cortisol (Anderson et al., 1978a,b). Pregnenolone and pregnenolone sulphate are also high in fetal plasma at the end of gestation, and the rise in fetal and maternal estrone and estrone sulphate are mirror images of the fall in pregnenolone and pregnenolone sulphate during the last 4 days of pregnancy (Nathanielsz et al., 1982). These progestins are important as estrogen precursors.

2.3.2 Activation of steroidogenic enzymes

How fetal cortisol activates steroidogenic enzymes in the fetal placenta is still unknown. It has been suggested that cortisol acts directly on the placenta (Ricketts et al., 1980a) since cortisol added to late gestation placenta explants caused activation of 17α-hydroxylase and aromatase activities. This suggestion is supported by the demonstration (Flint and Burton, 1984) of cytosolic glucocorticoid receptors in sheep placental tissues. They found high cytosolic receptor concentration in midgestation and low cytosolic receptor concentration before parturition. Based on the results of these studies, they theorized that the receptor concentrations were consistent with low fetal cortisol levels in midgestation and high fetal cortisol levels at term. At low cortisol concentrations, translocation of the receptor from cytosol to the nucleus is reduced, thereby raising cytosol receptor concentrations (Rousseau et al., 1973), while at high cortisol concentrations, binding to the cytosol receptors is high and translocation to the nucleus is high.

Fetal cortisol may not be the only factor involved in the activation of placental steroid metabolizing enzymes. It has been suggested that factors of fetal pituitary origin are required for the activation of placental enzymes, since ACTH- or dexamethasone-induced parturition in hypophysectomized fetuses causes only a slight increase in maternal unconjugated estrogens (Kendall et al., 1977).

2.3.3 Prostaglandins

Parturition in sheep is associated with increased uterine production of prostaglandins (PGs) and increased circulating concentrations of their metabolites (Thorburn and Challis, 1979). Prostaglandins increase in fetal and maternal fluids during spontaneous and induced parturition. They are elevated in maternal plasma (Thorburn et al., 1972; Currie et al., 1973; Mitchell et al., 1979; Olson et al., 1984,1985), fetal plasma (Challis et al., 1976; Olson et al., 1985), amniotic fluid (Mitchell and Flint 1977a, 1978b; Evans et al., 1982a; Olson et al., 1984, 1985), myometrium and cotyledons (Liggins and Grieves, 1971; Mitchell and Flint, 1977a; Evans et al., 1981), and in chorioallantois (Evans et al., 1982a) during labor. While both stimulatory and inhibitory PGs increase in fluids, stimulatory PGs ($PGF_2\alpha$ and PGFM) are selectively increased in maternal vena caval and aortic plasma (Lye et al., 1982; Olson et al., 1985)

Prostaglandins play a major role in the control of parturition in sheep. They are considered the final mediators of uterine contractility (Olson et al., 1985). The increase in $PGF_2\alpha$ and PGFM in utero-ovarian venous plasma, maternal vena caval and aortic plasma precedes the onset of any detectable uterine activity (Currie et al., 1973; Ledger et al., 1985; Olson et al., 1985) and thus supports the hypothesis that PGs have a causal role in uterine contraction (Flint et al., 1974). Several workers have demonstrated the effect of PGs on uterine contractility. Prostaglandin $F_2\alpha$ infused into the aorta of pregnant sheep at rates comparable to production rates (5-10 ug/min) stimulated uterine contractions similar to those seen at term without altering the

concentrations of P_4 and estrogen (Liggins et al., 1973). This treatment also increased myometrial sensitivity to oxytocin. Estrogen administration to ovariectomized nonpregnant ewes caused a cyclic pattern of uterine activity consisting of periods of high and low activities (Lye et al., 1983). The periods of high uterine activity were positively correlated with increased vena caval plasma PGF. Intrafetal infusion of ACTH induces an increase in PGs in the endometrium, myometrium, cotyledons and fetal membranes and an increase in the frequency and amplitude of uterine contractions (Evans et al., 1982b). Administration of PG synthetase inhibitors indomethacin (Evans et al., 1982b) or meclofenamic acid (Mitchell and Flint, 1978a) reduced the concentration of ACTH induced myometrial PGF and inhibited ACTH induced labor. Moreover, delivery of live lambs was prevented even though there was a decrease in P_4 and an increase in estrogen concentration (Mitchell and Flint, 1978a). Numerous studies suggest that the steroid hormone changes induced by a rise in fetal cortisol cause the release of PGs (Anderson et al., 1981). In nonpregnant ovariectomized sheep, P_4 treatment followed by estrogen administration increased $PGF_2\alpha$ release (Louis et al., 1977). Intrafetal infusion of ACTH (Evans et al., 1981) or dexamethasone (Liggins and Grieves, 1971) induced an increased concentration of both estrogen and PGF in maternal cotyledons and myometrium, and a decrease in P_4 concentration. The estrogen surge that occurs at term occurs before the PG increase (Challis et al., 1972; Nathanielsz et al., 1982). Thus it appears that the declining P_4 and increasing estrogen levels mediate PG release.

It is not known how the changes in steroid levels at term induce PG synthesis. Various studies have shown that PG release can be induced under certain experimental conditions with only a P_4 decline or an estrogen surge. Administration of a 3ß-HSD inhibitor, such as epostane (Ledger et al., 1985; Taylor et al., 1982b) or trilostane (Anderson et al., 1981), reduced maternal P_4, increased PGF and led to delivery without any significant increase in estrogen. The conclusion was that normal labor requires only a fall in P_4 long enough so as to allow release of PGs. However, infusion of estradiol into late pregnant sheep in amounts that produce physiological maternal estrogen levels, caused delivery in the presence of increased PGF release but in the absence of any significant P_4 change (Thorburn and Challis, 1979). However difficulty at parturition and increased retained placentas have been reported in the presence of high P_4 levels. In other studies, it has been shown that uterine activity and utero-ovarian venous PGF levels were suppressed with 200 mg P_4 per day during dexamethasone induced premature parturition (Liggins et al., 1973) but the rise in PGF concentrations in the myometrium and maternal cotyledons were not suppressed. They suggested that P_4 appeared to inhibit release rather than block PGF synthesis.

Prostaglandins are also important in causing cervical softening just before parturition. Administration of mefenamic acid, a PG-synthesis inhibitor, prevented cervical softening that is usually seen after a plasma PGF increase induced by intrafetal infusion of dexamethasone (Ledger et al., 1985). In addition, administration of indomethacin a PG synthesis inhibitor to animals subjected to ACTH induced labor not only

led to a reduction in PGs but also reduction in the degree of cervical softening (Evans et al., 1982b).

2.3.4 Progesterone, prostaglandins and oxytocin: maintenance of pregnancy and parturition

Parturition is caused by the interaction of hormones. Thus, the rise in fetal cortisol, which causes a fall in P_4 and a rise in estrogens and PGs, are a few of the changes preceding parturition. The ovine uterus is quiescent during pregnancy, but uterine activity begins 12-24hr before delivery (Rawlings and Ward, 1976). The role of P_4 in maintaining pregnancy appears to be through inhibition of PG release (Currie and Thorburn, 1977), or by acting as a glucocorticoid antagonist (Flint and Burton, 1984). Progesterone has been shown to be a glucocorticoid antagonist in some tissues (Samuels and Tomkins, 1970). Uptake of ^{3}H-dexamethasone by nuclei of placental minces incubated with P_4 synthesis inhibitors was increased while addition of P_4 to fetal lung minces in concentrations below those found in the placenta prevented nuclear uptake of ^{3}H-dexamethasone (Flint and Burton, 1984). Flint and Burton (1984) theorized that P_4 protects against initiation of labor, by acting as a glucocorticoid antagonist that protects the fetus against premature activation of placental enzymes by transient rises in cortisol concentration. It does this by limiting cortisol uptake by the placental glucocorticoid receptor, which is present as early as day 50.

Although P_4 withdrawal precedes normal and induced parturition, it is not clear whether this is essential since P_4 administration fails to delay parturition (Bengtsson and Schofield, 1963). Experiments of Lye

and Porter (1978) on ovariectomized nonpregnant sheep showed that estrogen treatment induced spontaneous activity in the uterine lumen. Oxytocin and $PGF_2\alpha$ stimulated the estrogen dominated uteri, but when 100 mg of progesterone was added to the estrogen dominated group, myometrial activity declined, and the uteri became unresponsive to oxytocin and $PGF_2\alpha$. Thus, the combined action of the preterm fall in P_4 and rise in estrogen on myometrial activity may be through their effects on $PGF_2\alpha$ release. The preterm estrogen rise is also thought to increase sensitivity of the uterus to oxytocin by increasing the concentration of oxytocin receptors in the myometrium as proposed for the rat (Alexandrova and Soloff, 1980; Chan, 1983). Estrogen also increases blood flow to the endometrium, myometrium, cervix and vagina (Rosenfeld and Worley, 1978), and induces uterine activity.

Plasma oxytocin concentration rises during delivery (Forsling et al., 1979). Oxytocin is secreted in response to vaginal distension (Flint et al., 1975c) and this oxytocin rise stimulates a second $PGF_2\alpha$ release (Forsling et al., 1979). Release of oxytocin is inhibited by P_4 and potentiated by estrogen (Roberts and Share, 1968, Roberts, 1975). Oxytocin stimulates stretch receptors in the cervix and is responsible for the final expulsion of the uterine contents (Forsling et al., 1979).

2.4 TIME OF PARTURITION

The initiation of parturition is thus not expected to be influenced by any one factor. Rather it depends on the interaction of many internal and external factors. The time during the day at which parturition occurs has been documented by a number of authors. In cattle, Arthur

(1961) reported a circadian rhythm in calvings with a tendency for night time calvings, while other authors (Eubank, 1963; Edwards, 1979; Yarney et al., 1979) reported a more uniform distribution of calvings over a 24h period. Lambing has also been shown to occur at all times of the day and night (Terrill, 1974). Breed differences have been reported, with more Dorset Horn ewes lambing in the day than Merino ewes (George, 1969). Still others have reported more lambings during the day than during the night (Sherafeldin et al., 1971; Younis and El-Gaboory, 1978). These differences in parturition time over a 24hr period have been attributed to external factors modifying the time of parturition. These external factors include barometric pressure (Dvorak, 1978), light rhythm and/or intensity (Younis and El-Gaboory, 1978), weather conditions such as snowstorms and rainstorms (Brackelsberg, 1985b) and farm routines (Edwards, 1979). The farm routines could include feeding, milking and other daily chores.

The modifying effect of feeding time on time of parturition has recently received wide attention. Although Sherafeldin et al. (1971) related time of feeding to distribution of lambing, it was not until recently that a system of feeding farm animals, specifically cows, was presented that could alter the parturition times (Konefal, 1980). According to the 'Konefal Method', feeding pregnant cows twice daily during the last month of pregnancy around 1200h and 2100h instead of the usual 0800h and 1600h feedings induced calvings to occur during the daylight hours.

Konefal's observations sparked both enthusiasm and skepticism. Since then a number of experiments have been designed to test the Konefal

method in both cattle and sheep. In cattle, Yarney et al. (1979) and Palmer, (1980) reported approximately 88% daytime calvings with the Konefal method, compared to about 39% of daytime calvings in cattle fed at 0800h and 1600h. By using a once a day method of feeding, Lowman et al. (1981) reported 79% daytime calvings in cattle fed at 2000h during the last week of pregnancy, as compared to 57% daytime calvings in cattle fed at 0900h. Brackelsberg (1985a) reported 91% calvings between 0500h and 2300h from 5 calving seasons (1980-1984) when the pregnant cows were routinely fed corn silage at 1615h with hay consumption ending around 2400h beginning at least two weeks before the expected start of the calving season. When the cows were fed from 0600h until 1200h, a reduction in calving frequency was found between 2300h to 0300h and between 1200h to 1600h.

However, Tucker et al. (1985) did not detect a difference in time of calving between cows fed at 0830h, 1600h and 2100h starting 30 or 60 days before expected calving time. These cows were kept in confinement pens. However, a longer interval between feeding and calving was found in cows fed during the afternoon in contrast to those fed in the morning hours.

Both successes and failures have been reported in sheep. When ewes were fed once a day at either 0800h, 1000h or 1200h Gonyou et al. (1982) and Cobb and Gonyou (1982) reported an increase in the number of ewes lambing during the day in the 1200h feeding time than when fed earlier. However, when the ewes were fed twice a day at either 0900h and 1200h or 1630h and 2100h, Jilek et al. (1985) reported no differences in lambing time between the two groups.

It seems that there have been some successes in altering time of parturition when feeding times were changed during the last few weeks of pregnancy. The reasons for the alteration in parturition time due to feeding is not known. Brackelsberg (1985b) suggested that the low nighttime lambing might be due to the reduced physical activity during the night while Sherafeldin et al. (1971) suggested that feeding delays onset of oxytocic contractions. A fall in rumen motility and rumen pressure before parturition has been reported (Dirksen and Kaufman, 1978). Palmer (1980) suggested that the secretion of some of the hormones involved in the initiation of parturition may be altered by changes in the activity patterns of animals.

That some hormone patterns might be involved in timing of parturition has been implied in some species. In monkeys, the frequency and amplitude of uterine contractions peak near 2400h in late pregnancy (Ducsay et al., 1983), and monkeys generally deliver at night or in early morning. In addition, a circadian pattern of progesterone has been reported in the monkey with highest concentrations found at 2000h (Walsh et al., 1984), a periodicity which coincides closely with the monkey's circadian uterine rhythm. Even though circulating estrogen and progesterone concentrations might be expected to be reversed at this time, estrogen levels were low and progesterone high at the time of high uterine activity. In fact these nocturnal progesterone peaks increased progressively in magnitude before parturition. In rats, parturition occurs when the plasma levels of corticosterone are low (Lincoln and Porter, 1979).

III

MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Twelve pregnant nulli and multiparous ewes of mixed breeding, and known breeding dates were used in each of two trials. At 4-6 weeks prior to expected delivery, the ewes were divided into two groups of six in each trial. Each group of six was then placed in a separate pen in isolation from the other group. The lights in the experimental rooms were then left on continuously to attempt to remove the possible influence of light and dark cycles.

Both groups in each of the two trials were fed two times daily, but the time the feed was given to each group differed. Group 1 (also referred to as the late fed group) was given feed at 1230h and 2100h. Group 2, or the early fed group, continued to be fed at the regular feeding times (0800h and 1630h). The ewes had free access to cobaltized iodized salt and water.

3.2 TRIALS

3.2.1 Trial 1

Each group was placed in a separate room and the ewes were allowed to move around freely in their pens. They were group fed 1 kg of grain (mixture of ground oats and barley) and approximately 3 kg of

grass-legume hay per ewe per feeding. Each group was comprised of four crossbreds, one Suffolk and one Finnish Landrace. Seven of the ewes were carrying single lambs, while five were carrying twins. Group 1 consisted of 4 ewes carrying singles, and 2 ewes carrying twins while group 2 had 3 ewes carrying singles and 3 ewes carrying twins.

Blood samples were collected by jugular venipuncture in 10 ml Vacutainers. The blood was kept at 4°C for about 16h and then centrifuged at 2000 X g for 20 min. Serum was decanted into vials and frozen at -20°C until assayed.

Intensive blood collection was done for 48 h beginning 4 weeks after the start of the feeding schedule (about 2 weeks prior to expected delivery). Samples taken over the first 24h were drawn every hour, while samples from 25-48h were taken every 30 min. Starting at 7 days before expected lambing time, blood samples were again collected every 2h until 2h after lambing.

3.2.2 Trial 2

In this trial the animals were placed in individual metal crates that allowed ample movement. They were individually fed 1 kg grain mixed with about 2.5 kg alfalfa pellets two times daily. Each group consisted of three Finnish Landrace and three crossbreds. Five of the ewes were carrying one lamb, three ewes were carrying twins, three ewes were carrying triplets and one was subsequently determined to be not pregnant. Group 1 consisted of 3, 1 and 2 ewes carrying singles, twins and triplets, repectively, while group 2 consisted of 2, 2 and 1 ewes carrying singles, twins and triplets, respectively.

This trial was designed to give the least possible disturbance to sheep during sampling, since venipuncture and other kinds of stress have been shown to increase corticosteroid concentration (Bassett and Hinks, 1969). To minimize disturbances to the ewes, blood samples were taken via indwelling jugular cannulae. The catheters were implanted in the ewe's jugular vein the day before the first blood sample was taken. The catheters were secured to the back of the ewe and extended through portholes to the outside of the experimental rooms (Graham, 1980). Thus blood samples were collected outside the experimental room without disturbing the ewes. Catheters were left in place from about 3 weeks prior to expected delivery until lambing and were kept patent by flushing with heparinized saline.

Intensive blood collection was done at 2 and 3 weeks after start of the feeding schedule which was about 3 and 2 weeks prior to expected delivery. Each collection was done for 30h beginning at the time the first daily feed was given to each respective group. The blood was collected every 30 min. for 4h after each feeding time, and hourly thereafter. At 3 weeks before expected delivery, blood sampling started a few hours after the first feed in group 1 because the catheters were not initially functioning well. During the last 7 days of pregnancy, blood samples were collected every hour until labor occurred.

At each sampling period 5 ml blood was collected in chilled heparinized syringes that contained glutathione/EGTA preservative solution (Peuler and Johnson, 1977). The blood was centrifuged within 30 min. of collection at 2000 X g for 20 min. at 4°C. Plasma was aspirated using chilled disposable pasteur pipettes into vials and frozen at -20°C until assayed.

3.3 HORMONE ASSAYS

Progesterone, cortisol and estrogen were measured by radioimmunoassay (RIA). All samples from each sheep for each hormone were assayed in a single batch.

3.3.1 Progesterone assay

The procedure used for progesterone assay was that described by Abraham et al. (1971) as modified by Yuthasatrakosol (1975). A highly specific antiserum (11-9/4/80) obtained from Dr. N. C. Rawlings University of Saskatchewan was used. This antiserum cross-reacted with cholesterol (0.47%), cortisol, (<0.01%), 17ß-estradiol (<0.01%) and testosterone (0.04%). The antiserum which was received in a lypholized form, was diluted with phosphate buffered saline (PBS). The PBS contained 5.38g $NaH_2PO_4.H_2O$, 16.35g $Na_2HPO_4.7H_2O$, 9.0g NaCl, 1.0g Na Azide and 1.0g Knox unflavored gelatin diluted to 1 liter with double-distilled water and the pH was adjusted to 7.0.

The non-radioactive standard progesterone used was purchased from Mann Research Laboratories, Orangeburg, New York. The working standard solutions contained 0, 25, 50, 100, 200, 400 and 800 pg of progesterone per 500 µl of solution. These were prepared by serially diluting a 640 ng/ml progesterone stock solution with PBS. These working standard solutions were freshly prepared every two weeks and kept at 4°C.

A 100 µl aliquot of ^{3}H-progesterone (containing approx. 1000 cpm) was added to four serum extraction tubes during each assay and the recovery of the added ^{3}H-progesterone was determined after the extraction was

determined. The mean percentage recovery of ^{3}H-progesterone was 76.6 ± 3.1 (n=23 assays). This value was used to correct for procedural losses during extraction.

The interassay and intraassay coefficients of variation were determined by including duplicate pool samples in every assay. The average progesterone concentration in the pool sample was 3.2 ± 0.08 ng/ml (n=23) which gave interassay and intraassay coefficients of 13.1% and 10.4% respectively.

3.3.2 Estrogen assay

The general procedure for estrogen determination was similar to that for progesterone. Estrogen concentrations were determined by an RIA procedure previously described by Yu et al. (1974), but omitting the column chromatography. A specific estradiol-17ß antiserum (76-22/7/77) purchased from Dr. N. C. Rawlings was used. This antibody crossreacted with estrone (5.6%), 17α-estradiol (0.35%), progesterone (0.001%), testosterone (0.003%) and cortisol (0.001%). The antibody was diluted with PBS to a working solution that bound about 39% of the added ^{3}H-estradiol. The standards used contained 0, 12.5, 25, 50, 100, 200 and 400 pg estradiol per 500 µl. The estradiol standard was purchased from Mann Research Laboratories.

Duplicate pooled samples were included in all assays. The average estrogen concentration in the pooled sample was 24.3 ± 0.4 pg/ml (n=22). This gave interassay and intraassay coefficients of variation of 8.8% and 7.2%, respectively.

The mean percentage recovery of added ^{3}H-estradiol was 68.8 ± 1.1. This value was used in the calculations to correct for procedural losses during extraction.

3.3.3 Cortisol assay

The RIA procedure used for cortisol assay was similar to that described by Rawlings and Ward (1978) except that an antibody instead of a binding protein was used in this assay.

A specific antiserum to cortisol (4-30/11/78), purchased from Dr. N. C. Rawlings was used. This antiserum had a 10% cross-reaction with progesterone and less than 1% with cholesterol, 17ß-estradiol, 17α-estradiol, testosterone, pregnenolone and dehydroepiandrosterone. In order to reduce the potential interference, progesterone was extracted with petroleum ether prior to cortisol extraction.

The cortisol standard was dissolved in absolute alcohol and working standards were made by evaporating the alcohol, diluting the residue with PBS to a working standard concentration of 0, 0.1, 0.2, 0.4, 0.8, 2, 4, 8, 10 and 20 ng per 200 µl. The labelled cortisol (Hydrocortisone (1,2,6,7-^{3}H(N) New England Nuclear Corporation)) in an ethanolic solution was taken to dryness and diluted with PBS to a working solution containing about 6000 cpm in 200 µl.

Duplicate samples of 200 µl each and triplicate standards of 200 µl were mixed with 1.0 ml petroleum ether and left at -20°C for 1h. The unfrozen ether extract was removed by aspiration and discarded and the few drops remaining were aspirated off. Two ml of absolute ethyl alcohol

(Canadian Industrial Alcohols and Chemicals Ltd) were added to the residue, vortexed for 30-40 sec and then centrifuged at 2000 X g for 10 min. The supernatant was transferred to correspondingly labelled tubes, and dried under a gentle stream of N_2 in a water bath at 39°C. To the dried alcohol extract was added 200 µl of antibody at a dilution that bound approximately 39% of the added ^{3}H-cortisol, and 200 µl of ^{3}H-cortisol. The tubes were vortexed and left to equilibrate overnight at 4°C (minimum time of incubation was 16h).

Separation of the bound from the free was done by adding 500 ul of charcoal solution (375 mg per 100 ml PBS), incubating for 10 min at 4°C and centrifuging at 2000 X g for 10 min. The supernatant from each tube was decanted into a scintillation vial and 10 ml of Scintiverse was added. The vials were shaken, left to equilibrate for 2h and then counted for 4 min.

The average cortisol concentration in the pooled sample included in each assay was 91.5 ± 1.2 ng/ml (n=23). The interassay and intraassay coefficients of variation were 14.3% and 9.4%, respectively.

Duplicate tubes of stripped serum with no antibody added were included in the RIA of all three steroids. This was used for calculating the nonspecific binding which was included in the calculation of steroid concentration. A value of less than 5% total binding was considered to be acceptable.

3.4 ANALYSIS OF DATA

The intra and interassay coefficients of variation for hormone determinations were calculated as described by Rodbard (1971). Differences in hormone concentration between groups, breeds and the number of fetuses a ewe was carrying were analyzed using the General Linear Model and Analysis of Variance (ANOVA) procedures outlined by the Statistical analysis system (SAS Institute Inc., 1982) for a split-plot. The difference between groups on time of lambing was tested by a Chi-square analysis (Snedecor and Cochran, 1976). The 24 hr data of samples taken every 30 min were analyzed by spectral analysis (SAS-Time Series in Econometrics, 1982), or Fourier curve to determine if the steroid levels changed in a cyclic pattern over a 24h period. Characteristics of the steroid profiles were analyzed by using the computer program Pulsar (Merriam and Wachter, 1982), an algorithmic method that identifies ultradian pulsations. This is accomplished by removing the trends from the hormone series and identifying the peaks from the residuals. One point was considered a peak if it's value was above the smoothed baseline by 7.6 times the assay error standard deviation (SD). Two to 5 points were considered peaks when each point exceeded the baseline by 5.2 times the error SD for 4 points, 3.8 times error SD for 3 points, 3.0 times the error SD for 2 points and 2.4 times the error SD for 5 points.

IV

RESULTS

4.1 LAMBING TIME

The lambing times for the two trials are shown in Table 1. In the first trial, four out of five ewes and five out of six ewes in group 1 and 2, respectively, lambed during the daytime (defined as between 0700h and 1900h) while in the second trial, three out of four and two out of five in group 1 and 2, respectively, lambed during the daytime. A Chisquare test of the combined trial 1 and trial 2 ewes showed no differences ($P>0.05$) in lambing time between the groups. In both groups, more ewes (78% in group 1 and 64% in group 2) lambed between 0700h and 1900h than during the night. More ewes (67%) in group 1 lambed between the two feeding times than in group 2 (27%), but this was not statistically ($P>0.05$) different. The majority of the ewes in both groups lambed within the period between 4h before the first feed of the day and 4h after the last feed of the day (Table 1).

The mean gestation lengths of 146.3 days for group 1 and 147.3 days for group 2 were not significantly ($P>0.05$) different. There was also no statistical difference ($P>0.05$) in mean gestation length between the Finnish Landrace (144.6 ± 0.7 days), the Suffolks (147.5 ± 0.5 days) and the crossbreds (147.6 ± 1.0 days).

TABLE 1

Time of Lambing

	No. of ewes	Between the two feeding times**	0700-1900h	1900-0700h	Between 4h before 1st feed and 4h after last feed
			TRIAL 1		
Group 1	5	5(100%)	4(80%)	1(20%)	5(100%)
Group 2	6	1(17%)	5(83%)	1(17%)	5(85%)
			TRIAL 2		
Group 1	4	1(25%)	3(75%)	1(25%)	3(75%)
Group 2	5	2(40%)	2(40%)	3(60%)	4(80%)
			COMBINED		
Group 1	9	6(67%)*a* *	7(78%)*b*	2(22%) *c*	8(89%)*d*
Group 2	11	3(27%)*a*	7(64%)*b*	4(36%) *c*	9(82%)*d*

*Values within columns with the same letter are not significantly different at p=0.05

**Group 1 1230h to 2100h
Group 2 0800h to 1630h

4.2 STEROID PROFILES AT APPROXIMATELY 2 AND 3 WEEKS PRIOR TO PARTURITION

Steroid profiles in the figures will be presented with standard errors of the mean (S.E.) wherever cluttering is not considered a problem. In addition, vertical dotted or solid lines will be included on some of the profiles to indicate the time the feed was given to the ewes.

4.2.1 Progesterone

The mean progesterone (P_4) concentrations were significantly different ($P<0.001$) between ewes in both trials. In trial 1 the concentrations ranged from 16 ng/ml in the ewe with the lowest concentration to 27 ng/ml in the ewe with the highest concentration. However the mean P_4 concentrations between treatment groups was not significantly ($P>0.05$) different. The mean P_4 profiles of the two groups (n=6) over a 48h period at about 2 weeks prior to expected delivery (4 weeks after the change in the feeding schedule in trial 1) are shown in Figs. 3A and 3B.. Both groups showed substantial short-term changes with numerous peaks. In group 1 (Fig. 3A), there was an apparent increase in P_4 concentrations starting at 0700h and peaking at about 1200h just before the first feed was given. The concentrations then fluctuated displaying about six peaks during the first 24h of the sampling period. On the second day, when the samples were taken every 30 min, P_4 peaks appeared to be less numerous than on the first day. In group. 2 (Fig. 3B), P_4 concentrations started to increase at 0900h after the first feed was given, fluctuated, and was still increasing at about

1630h when the second feed was given. On both the first and second days, the mean P_4 concentrations fluctuated greatly and showed no consistent relation to time of feeding.

When the data were pooled into 4h intervals, P_4 concentrations did not differ ($P>0.05$) between the 4h intervals in each group (Table 2), or when the groups were pooled (Table 3).

In the second trial, mean P_4 concentrations did not change between the samples taken at 2 and 3 weeks after the start of the trial, which was about 3 and 2 weeks prior to expected delivery, respectively. They were 16.7 ± 0.6 ng/ml (n=6) in group 1 and 26.1 ± 0.7 ng/ml (n=5) in group 2, 2 weeks after the start of the trial. A week later the concentrations were 15.0 ± 0.5 ng/ml (n=6) in group 1 and 27.1 ± 0.7 ng/ml (n=6) in group 2. The P_4 concentrations were significantly higher ($P<0.05$) in group 2 than in group 1. This difference is probably related to differing number of live fetuses carried in the two groups of ewes and will be discussed further in the section on effect of twinning on maternal steroid profiles.

The mean P_4 profiles of ewes at 2 and 3 weeks after the start of trial 2 are shown in Figs. 3c and 3d, respectively. They showed no consistent trends in relation to feeding time. The change in P_4 concentration over time was significant ($P<0.05$) only at 2 weeks but not at 3 weeks after the start of trial 2. Progesterone concentrations were again significantly ($P<0.01$) different between the individual ewes with concentrations ranging from 9.0 ± 0.4 ng/ml to 37.7 ± 0.4 ng/ml. In both trials (figs. 3A,B,C,D), feeding did not seem to alter P_4 concentrations.

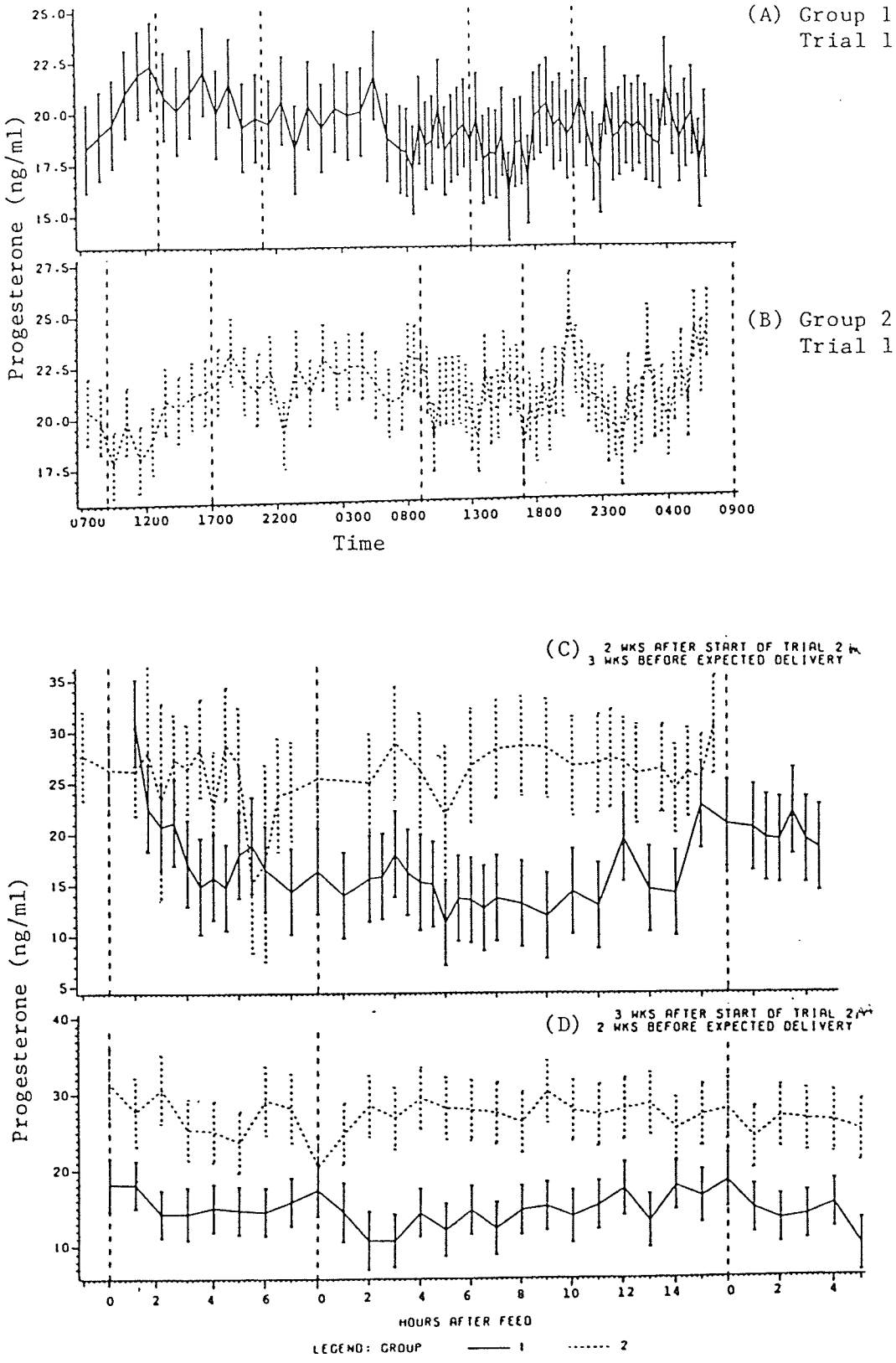


Figure 3: Progesterone (mean±S.E.) profiles at two and three weeks prior to expected delivery in trials 1 and 2

TABLE 2

Steroid concentrations at 4h intervals during a 48h period at 2 weeks prior to expected delivery (4 weeks after the start of trial 1)

	PROGESTERONE (ng/ml)		CORTISOL (ng/ml)		ESTROGEN (pg/ml)	
Hrs after feeding	GROUP 1	GROUP 2	GROUP 1	GROUP 2	GROUP1	GROUP2
1st Feeding						
0-4	20.6	19.2	58.0	65.4	16.1	13.7
4-8	20.1	20.3	64.2	61.2	13.1	16.2
2nd Feeding						
0-4	19.5	22.1	67.1	60.3	12.6	12.5
4-8	19.7	21.4	78.4	68.3	13.0	12.3
8-12	18.6	22.2	69.8	69.8	11.4	11.5
12-16	18.8	21.3	63.1	69.1	10.5	12.0

TABLE 3

Pooled steroid concentrations in relation to feeding during a 48h period 2 weeks prior to expected delivery, (4 weeks after the start of trial 1)

±SEM (n=12)

Hrs after feeding	0-4	4-8	2nd feed	0-4	4-8	8-12	12-16
P_4 ng/ml	20.1±0.5$_a$	20.2±0.7$_a$		20.8±0.6$_a$	20.5±0.6$_a$	20.2±0.6$_a$	20.1±0.1$_a$
Cortisol ng/ml	60.1±1.6$_b$	62.7±2.1$_b$		63.7±2.3$_b$	73.3±2.1$_c$	69.7±2.3$_c$	66.2±0.8$_b$
Estrogen pg/ml	15.3±0.7$_d$	14.6±0.7$_d$		12.5±0.7$_e$	12.6±0.5$_e$	11.4±0.4$_e$	11.3±0.1$_e$

* Values within the same row with the same letter are not significantly different at p=0.05.

4.2.2 Cortisol

In the first trial differences in cortisol concentration among the individual ewes were significant ($P<0.001$) with mean cortisol concentrations in individual ewes ranging from 45 ± 0.5 ng/ml to 76 ± 0.4 ng/ml. The mean cortisol concentrations between the groups were not significantly ($P>0.05$) different being 64.0 ± 1.0 ng/ml (n=6) in group 1 and 67.8 ± 0.8 ng/ml (n=6) in group 2. However, the cortisol concentrations were dependent on the time of the day the sample was collected as indicated by a significant ($P<0.05$) interaction between the groups and time.

Figures 4A and 4B shows the mean cortisol profiles over a 48h period in groups 1 and 2 4 weeks after the start of the trial (2 weeks before expected delivery). The concentrations showed large short term changes. Generally, cortisol concentrations rose or remained high at each feeding time, and were consistently high for longer periods of time during the late night to early morning hours (2400h to 0800h). The mean cortisol concentrations in group 1 (Fig. 4A) rose from 44 ± 4.1 ng/ml at 0800h to 66 ± 8 ng/ml at 1200h, at the time of the first feeding. The concentrations fluctuated showing a slight peak after the second feeding (2100h) and reached the highest concentration of 84.1 ± 4.9 ng/ml at 2400h. Concentrations declined gradually to reach nadir (45.6±6.6 ng/ml) at 0900h the next day. An hour after the first feed on the second day, the mean cortisol concentrations rose to reach a peak of 73.6 ± 10.2 ng/ml at about 1500h. The highest peak of 81.3 ± 6.0 ng/ml came around 0300h.

Group 2 (Fig. 4B) showed similar trends in relation to feeding and time of the day. From a low of 52.8 ± 5.7 ng/ml at 0700h, cortisol concentrations rose reaching a peak of 76.3 ± 4.2 ng/ml at 1100h, 3hr after the first feed. After the 1630h feed, cortisol concentrations displayed a number of peaks with a highest concentration of 80.3 ± 5.9 ng/ml at 0500h. The concentrations declined to reach lowest levels at 0800h began to rise again after the first feed, and peaked at about 1200h. On the second day, cortisol concentration peaked again at 1900hr (85.0 ± 6.8 ng/ml) and 0400h (80.3 ± 9.5 ng/ml). Thus at 0800h, mean cortisol concentrations were low in group 1 ewes (Fig. 4A), the group that got their first feed at 1230h while they were already rising in group 2 ewes (Fig. 4B), the group that received their first feed at this time (0800h). The highest concentrations in each group and on each of the two days were found in the early morning hours (0400h-0700h).

When pooled into 4h intervals after feeding, (Table 3), cortisol concentrations were significantly ($P<0.05$) higher at 4-8h after the second feed (73.3 ± 2.1 ng/ml) than at 4-8h after the first feed (62.7 ± 2.1 ng/ml). Four to 8 hours after the second feed however, is coincidental with the early morning hours.

In the second trial, the mean cortisol concentrations at 2 and 3 weeks after the start of the second trial were not different ($P>0.05$) from each other. They were 61.2 ± 1.9 ng/ml (n=6) in group 1 and 61.2 ± 1.9 (n=5) in group 2 at 2 weeks, and 55.2 ± 1.5 ng/ml (n=6) in group 1 and 59.4 ± 1.4 ng/ml (n=6) in group 2 a week later. Cortisol profiles as arranged after each feeding time (Figs. 4C and 4D) were similar between the groups and between the bleedings at 2 and 3 weeks after the start of

the trial. Generally, there were increases in cortisol concentrations after the first feed of the day and this increase was sustained for about 4-6h. After that period the mean cortisol concentrations declined and fluctuated. Cortisol concentrations also increased after the second feed, but this increase appeared at about 4-6 h after this feeding. The changes in cortisol concentrations over time interacted significantly ($P<0.05$) with groups. In both trials (Figs. 4A,B,C,D), cortisol concentration appeared to increase in response to feeding, but the highest concentrations were reached in the early morning hours. In the second trial however, the response was more dramatic in both groups at 2 than at 3 weeks after the change in the feeding schedule.

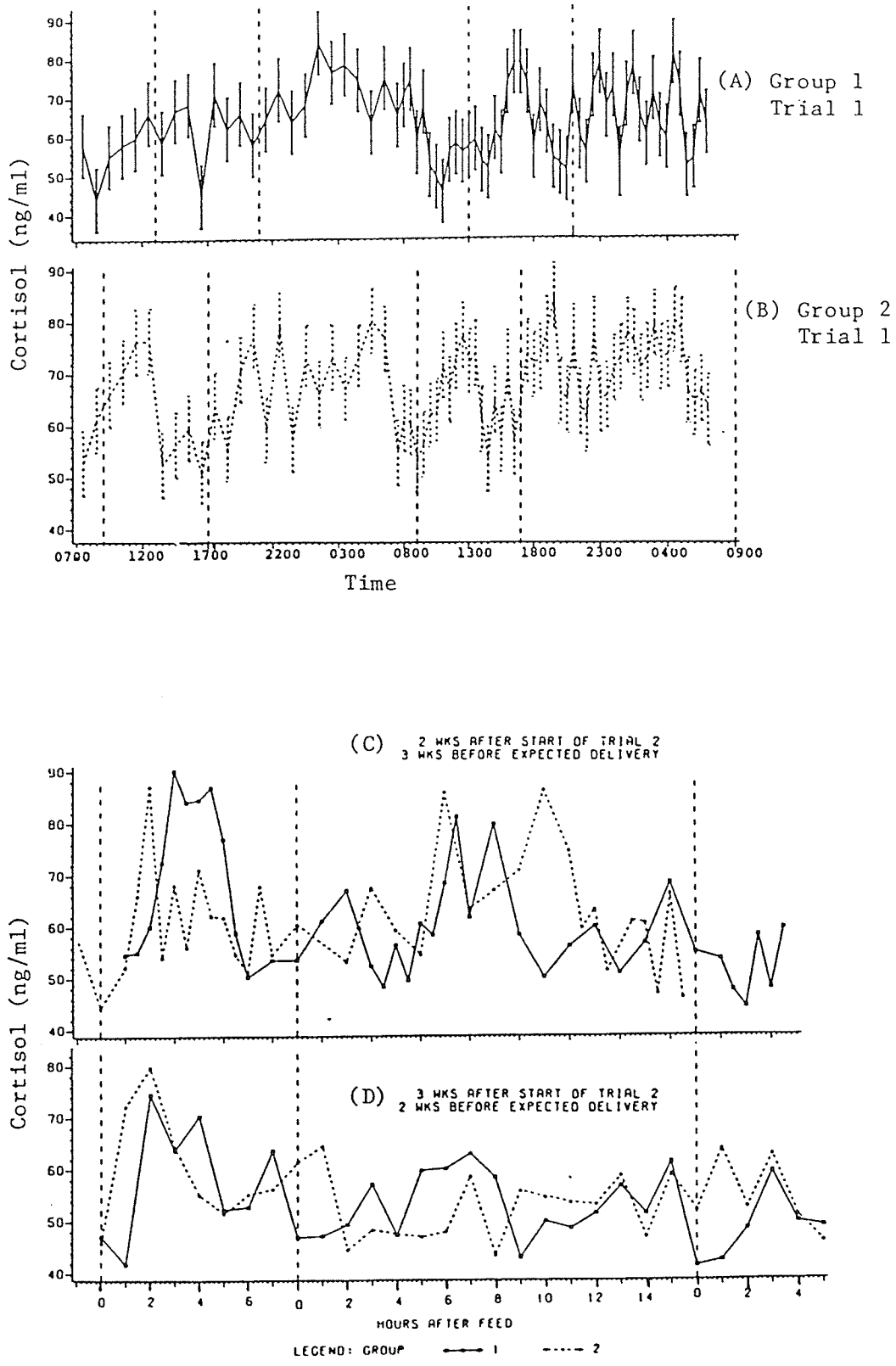


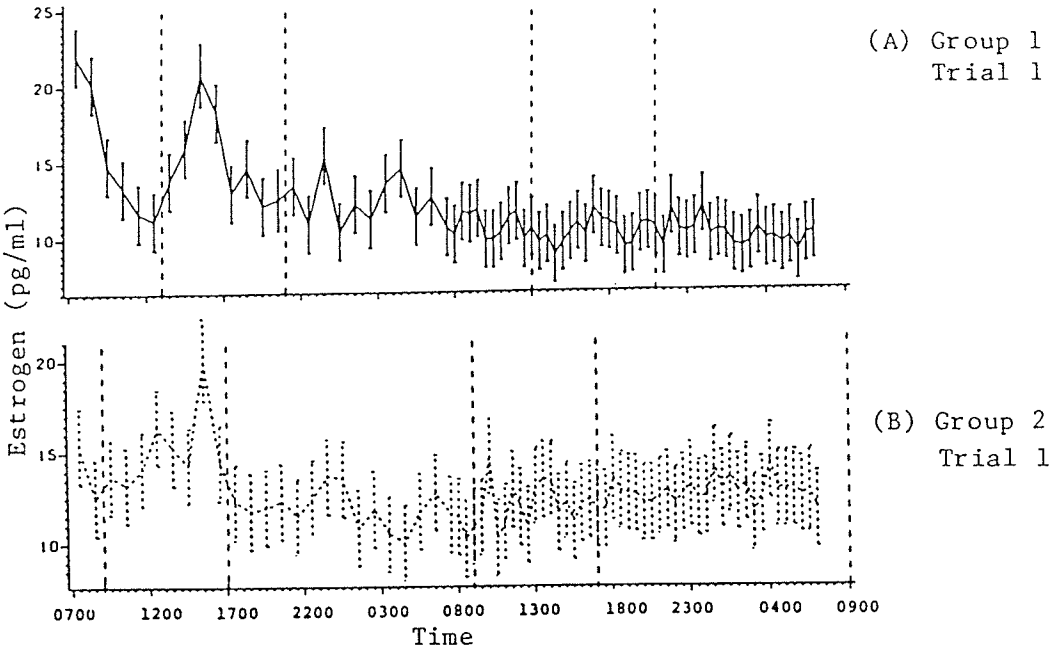
Figure 4: Cortisol (mean±S.E.) profiles at two and three weeks prior to expected delivery in trials 1 and 2

4.2.3 Estrogen

In the first trial, mean estrogen concentrations in individual ewes over a 48h period were significantly ($P<0.05$) different. They ranged from 7 pg/ml to 19 pg/ml. However, the mean estrogen concentration between the groups did not differ ($P>0.05$). The concentrations were 11.8 ± 0.2 pg/ml (n=6) in group 1 and 12.4 ± 0.2 pg/ml (n=6) in group 2. The mean estrogen concentrations changed significantly ($P<0.05$) over time during the first 24h but not during the second 24 h of the 48 h collection period.

Figures 5A and 5B show the estrogen profiles of groups 1 and 2, respectively over the 48h period in the first trial. There were fluctuations between collections, but generally the concentrations in both groups were high at the beginning of the collections, declined and maintained a steady state with numerous peaks for the rest of the sampling period. There was no consistent behavioral pattern of estrogen concentration in relation to feeding. Because of the high concentrations found during the first few hours of start of the 48h sampling period, estrogen concentrations were significantly ($P<0.05$) higher during the first 24h period than during the second 24h, and this contributed to the high estrogen concentrations seen at 0-8h after the first feed when the data was pooled into 4h intervals after feeding (Table 2).

In the second trial, estrogen concentrations between the individual ewes were significantly ($P<0.05$) different at both the second and third week after the start of the trial. They ranged from 22.9 ± 0.7 pg/ml to



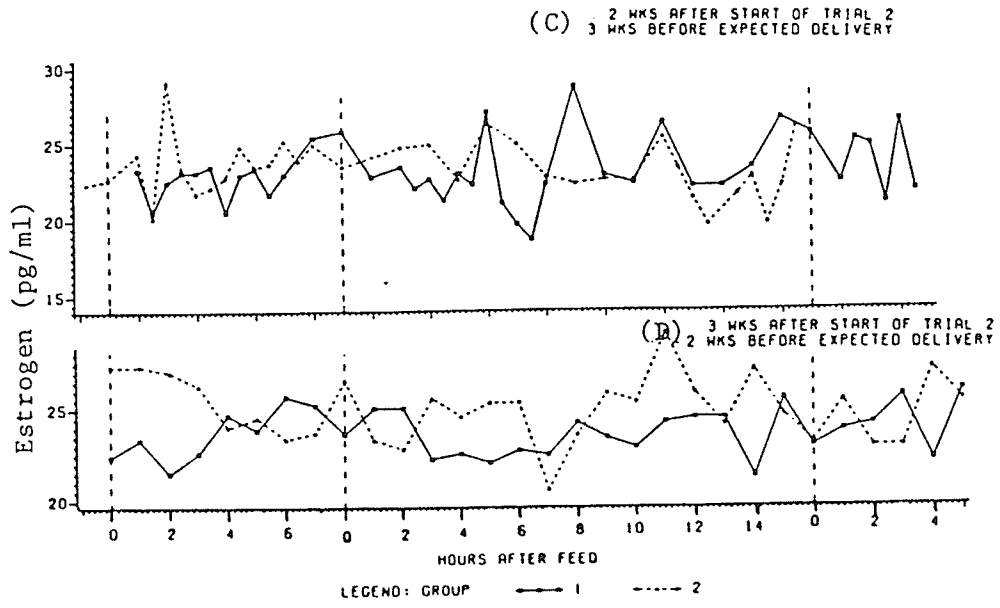


Figure 5: Estrogen (mean±S.E.) profiles at two and three Weeks prior to expected delivery in trials 1 and 2

29.1 ± 5.0 pg/ml. Mean estrogen concentrations between the two groups were not significantly (P>0.05) different. They were 22.9 ± 0.3 pg/ml and 22.6 ± 0.2 pg/ml in group 1 and 2, respectively at 2 weeks. A week later they were 23.7 ± 0.2 pg/ml and 24.9 ± 0.2 pg/ml in group 1 and 2, respectively.

The mean estrogen profiles at 2 and 3 weeks are shown in Figs. 5c and 5d, respectively. The values fluctuated greatly and no discernible change due to either feeding or time of the day could be detected. Thus in both trials (Figs. 5A,B,C,D), feeding did not alter estrogen concentrations.

4.3 COMPOSITE STEROID PROFILES AT 2 AND 3 WEEKS PRIOR TO EXPECTED DELIVERY

The composite profiles (group 1 and group 2 combined) of the three steroids during a 48h period at 4 weeks after the start of trial 1 (two weeks prior to expected delivery) are shown in Fig 6. Progesterone and estrogen profiles did not show as large variations as cortisol did. Cortisol concentrations fluctuated greatly but tended to be low around 0800h and high around 0400h.

The 24h composite steroid profiles at 2 and 3 weeks after the start of trial 2 are shown in Fig. 7. At both times, estrogen and P_4 variations bore no relationship to time of feeding while cortisol concentrations showed apparent increases immediately after the first feeding and after about 4h after the second feed. These increases after feeding were more apparent at 2 weeks than at 3 weeks after the start of the trial.

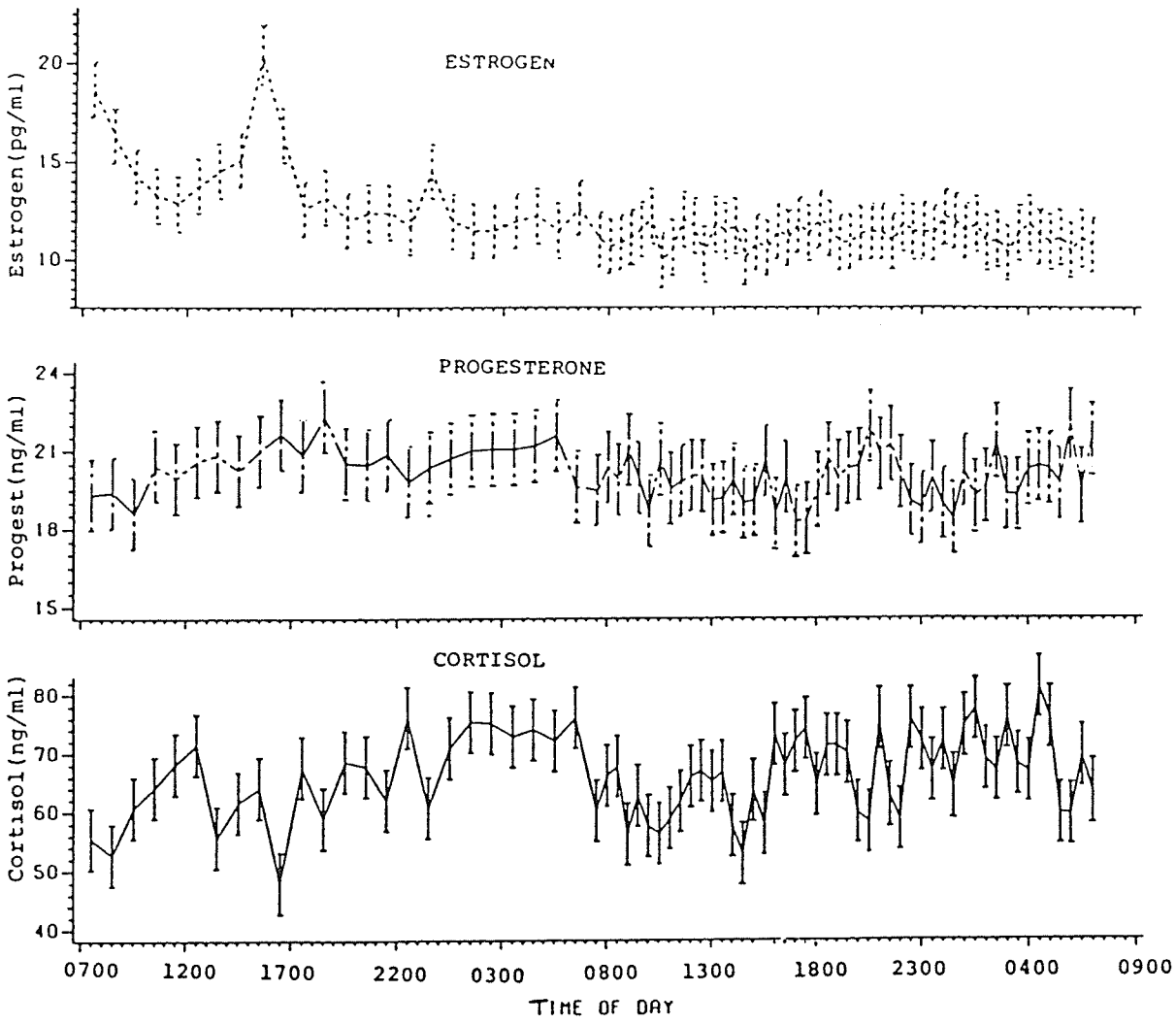


Figure 6: Composite steroid (mean±S.E.) profiles during a 48h period at 4 weeks after the start of trial 1 (2 weeks prior to expected delivery)

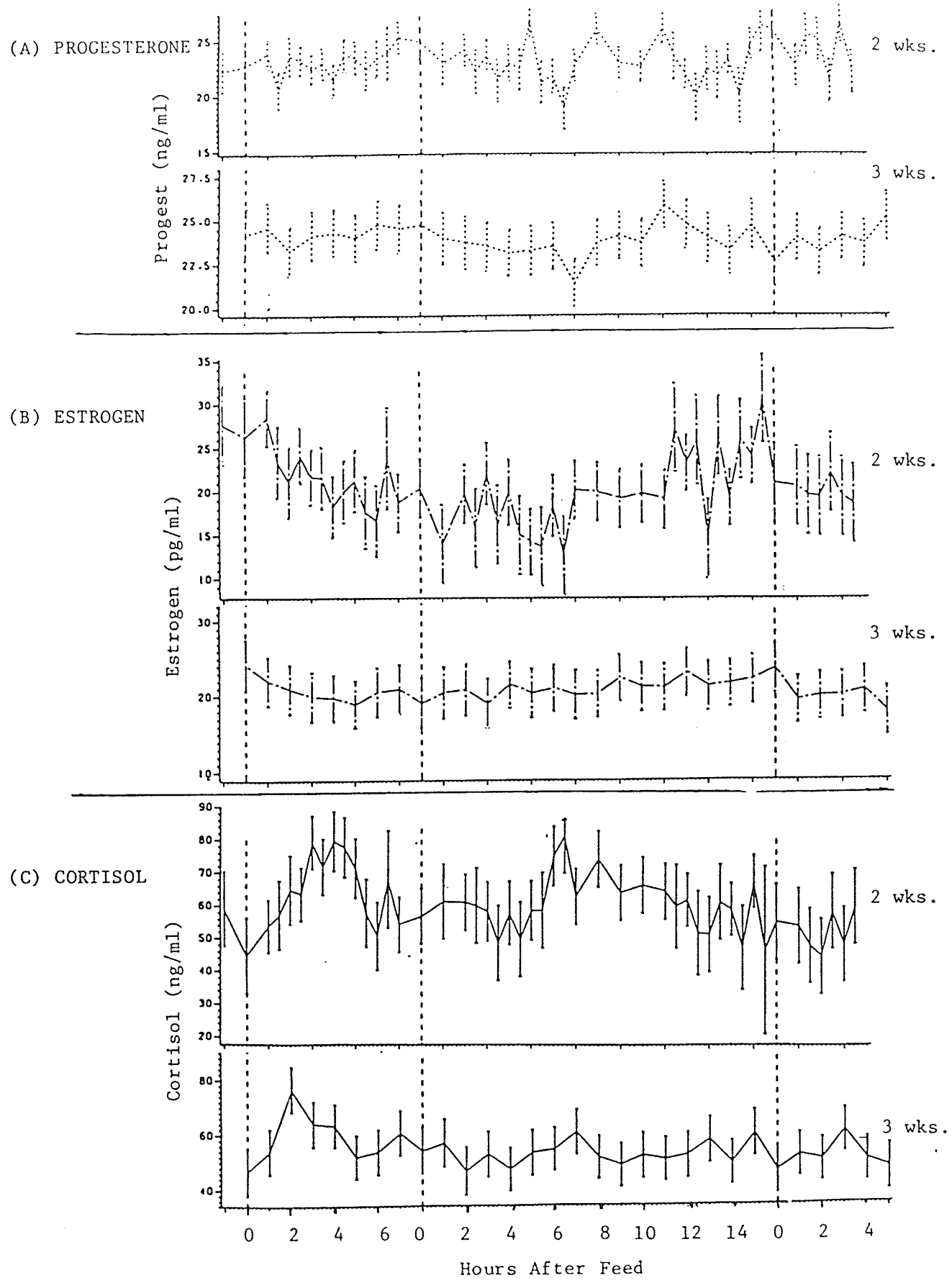


Figure 7: Composite steroid (mean±S.E.) profiles during a 24h period at 2 and 3 weeks after start of trial 2 (3 and 2 weeks prior to expected delivery)

4.4 WITHIN EWE ANALYSIS

Progesterone profiles of individual ewes in trial 1 during the 48h sampling period are shown in figure 8. The last 24h of these profiles were subjected to spectral analysis. Cyclic patterns or periodicities were found in some but not all the ewes. A 3.9h period was found in ewe 10, implying that after about every 4h the P_4 concentration pattern is repeated. Ewes 6, 7, 9 and 11 displayed a 12h cyclic period while ewes 1 and 12 had a 6h period. No specific differences could be detected in cyclic patterns or periodicities that could be attributed to feeding times.

Pulsar analysis (Table 4) showed that there were no differences in peak length, interpeak interval and the magnitude of the peaks between the groups. Moreover, there were no differences in the amplitude or number of peaks a few hours after feeding and later, in P_4 or estrogen concentrations. A mean P_4 peak amplitude of 4.9 ± 2.4 ng and a mean interpeak interval of 2 hr was found.

Progesterone profiles of some ewes from trial 2 are shown in fig. 9. Profile characteristics of the ewes at 2 and 3 weeks after start of trial 2 are shown in Table 5. Peak amplitude decreased from 5.6 ng at 3 weeks to 5.0 ng at 2 weeks prior to expected delivery. The peak length increased from 1.5±0.8h at 3 weeks to 2.2±1.0h at 2 weeks prior to expected delivery. The interpeak interval also increased from 2.6±1.5h at 3 weeks to 3.3±1.2h at 2 weeks prior to expected delivery.

The estrogen profiles of the individual ewes in trial 1 are shown in Fig. 10. Spectral analysis showed cyclic periods in some of the ewes.

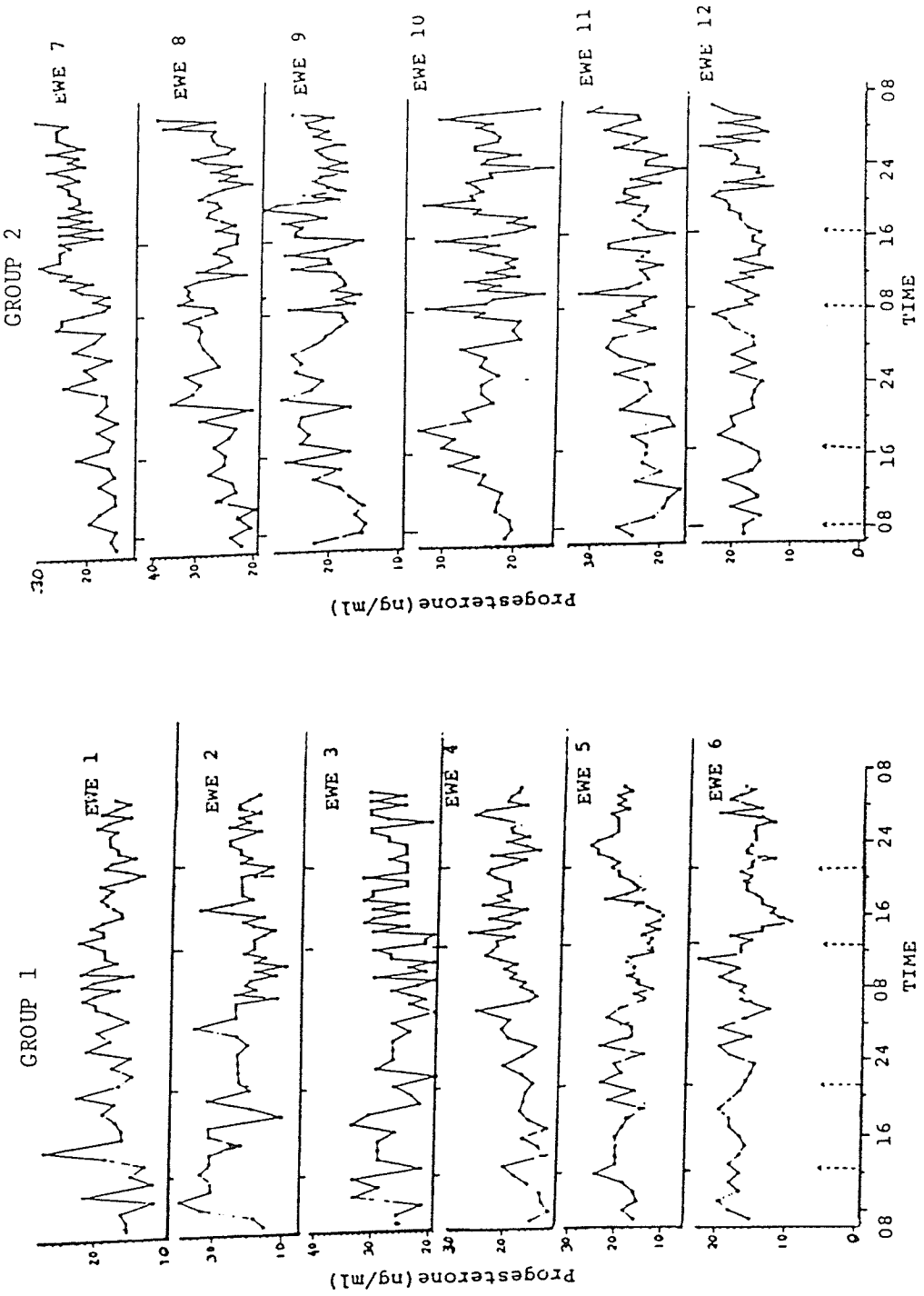


Figure 8: Progesterone profiles of ewes at 4 weeks after the start of trial 1 (2 weeks prior to expected delivery)

TABLE 4

Progesterone and estrogen characteristics (mean±S.E.) at 2 weeks prior to expected delivery (4 weeks after the start of trial 1)

Sheep No.	PROGESTERONE Amplitude ng/ml	Peak Length hr	Interpeak interval hr	ESTROGEN Amplitude pg/ml	Peak Length hr	Interpeak interval hr
			Group 1			
1	4.1±2.3	1.5±0.8	2.1±0.7	2.7±1.8	1.2±0.7	1.9±0.9
2	4.8±2.3	1.3±0.7	2.0±1.1	4.9±4.6	1.3±0.7	2.2±1.5
3	6.9±2.4	1.1±0.8	1.8±0.9	4.6±3.5	1.5±1.2	2.3±1.5
4	4.6±2.2	1.5±0.9	2.0±1.1	6.4±6.7	1.9±1.0	2.0±0.9
5	4.5±2.7	1.3±0.9	2.1±1.1	4.3±1.8	1.4±0.9	2.2±1.3
6	3.2±1.7	1.4±1.2	2.1±1.6	5.0±4.4	1.3±0.9	2.1±0.9
			Group 2			
7	3.7±1.7	1.1±0.7	2.1±0.7	2.5±2.0	1.6±1.3	2.3±0.9
8	5.7±2.4	1.3±0.7	2.1±0.8	3.8±2.8	1.7±0.9	2.3±1.4
9	5.6±2.8	1.3±0.7	2.1±1.2	8.3±4.3	1.8±1.2	2.5±1.2
10	6.1±3.5	1.0±0.5	1.6±0.6	3.7±2.3	1.3±0.7	2.1±1.2
11	5.2±2.0	1.4±0.7	2.2±1.1	3.0±1.9	1.3±0.6	2.1±1.1
12	5.1±2.7	1.3±0.7	2.0±1.0	4.1±2.1	1.4±0.9	2.1±1.5
MEAN	4.9±2.4	1.3±0.8	2.0±1.0	4.4±3.1	1.5±0.9	2.2±1.2

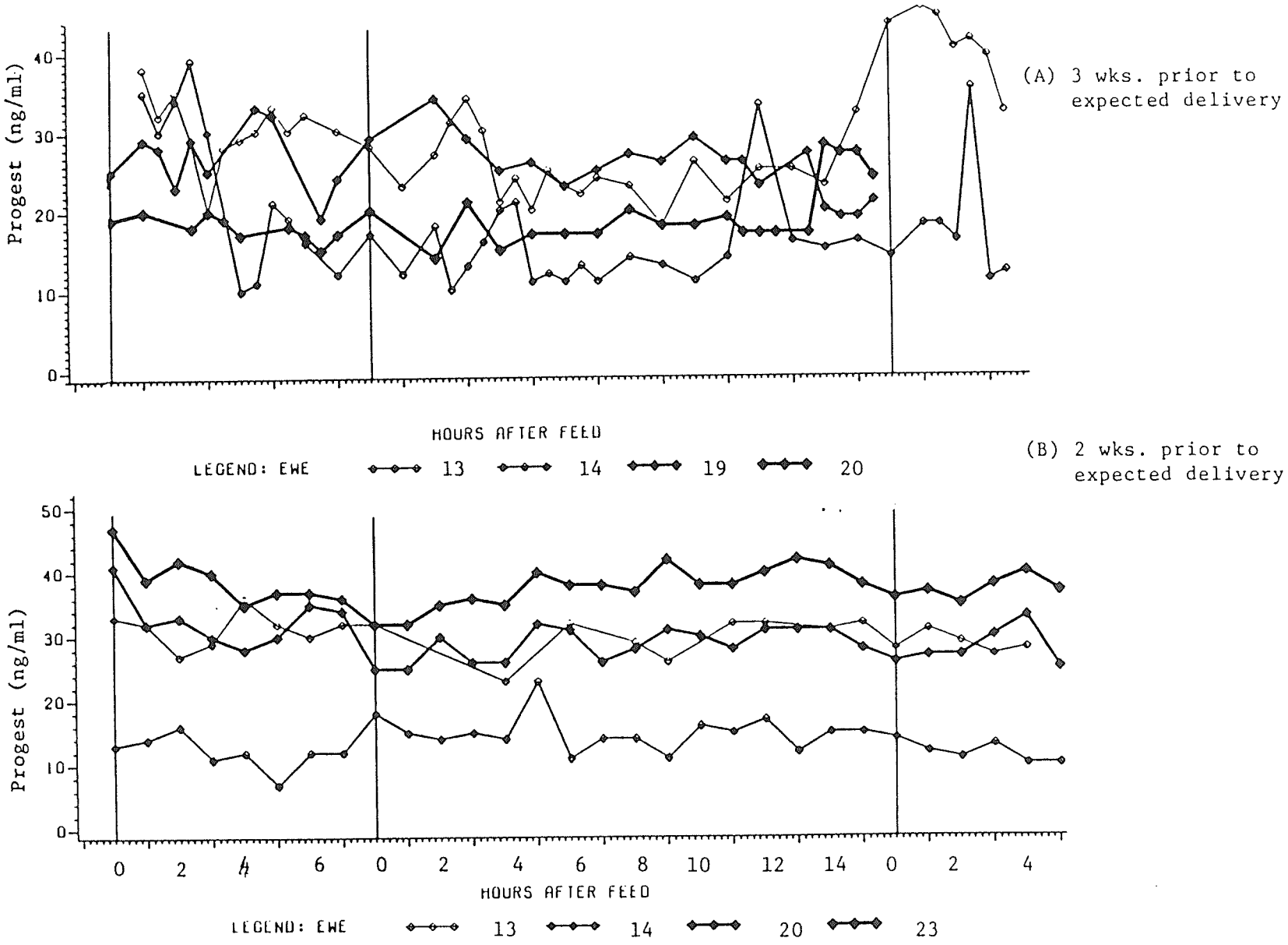


Figure 9: Progesterone profiles of ewes at 2 and 3 weeks after the start of trial 2 (3 and 2 weeks prior to expected delivery)

TABLE 5

Progesterone profile characteristics (mean±S.E.) at 3 and 2 weeks prior to expected delivery in trial 2

Sheep No.	3 weeks before expected delivery			2 weeks before expected delivery		
	Amplitude ng/ml	Peak Length hr	Interpeak interval hr	Amplitude ng/ml	Peak Length hr	Interpeak interval hr
			Group 1			
13	5.9±3.8	1.5±0.8	2.5±1.7	5.2±2.1	2.5±1.5	4.0±1.8
14	9.1±4.6	1.4±0.9	2.2±1.1	4.6±2.6	1.8±1.2	2.8±1.1
15	5.0±4.2	1.2±0.5	2.1±1.1	4.5±2.5	2.0±0.8	3.1±1.1
16	4.4±3.8	1.9±0.8	3.1±1.4	2.3±1.3	2.5±1.5	3.4±1.4
17	5.5±4.1	1.5±1.0	2.3±1.0	4.4±4.2	1.8±0.6	3.0±0.7
*18	-	-	-	6.2±2.9	1.9±1.1	2.9±1.4
			Group 2			
19	5.5±2.7	1.4±0.4	2.8±1.6	5.5±2.6	2.4±1.2	3.9±1.4
20	4.7±3.2	1.3±0.6	2.8±1.7	6.6±4.1	2.4±0.9	3.1±1.5
*21	-	-	-	7.4±4.5	1.8±0.8	3.0±1.1
*22	-	-	-	4.9±2.1	2.5±0.9	3.7±1.2
23	5.0±3.3	1.7±1.1	2.6±2.1	3.7±1.5	2.6±0.9	3.7±1.0
	5.6±3.7	1.5±0.8	2.6±1.5	5.0±2.8	2.2±1.0	3.3±1.2

*Ewes were excluded because of some missing data

TABLE 6

Estrogen profile characteristics (mean±S.E.) at 3 and 2 weeks prior to expected delivery in trial 2

Sheep No.	3 weeks prior to expected delivery			2 weeks prior to expected delivery		
	Amplitude pg/ml	Peak Length hr	Interpeak interval hr	Amplitude pg/ml	Peak Length hr	Interpeak interval hr
			Group 1			
13	4.6±1.4	1.9±0.8	2.8±0.6	3.8±2.2	1.7±1.2	2.6±1.3
14	7.8±2.7	1.4±0.7	3.4±1.6	4.4±2.2	1.7±0.8	2.8±0.8
15	7.6±3.3	1.2±0.5	2.8±1.2	5.8±3.2	1.9±1.3	3.1±1.0
16	5.2±2.1	1.5±0.9	3.3±1.1	6.4±3.1	1.6±1.0	2.8±0.8
17	6.8±2.1	1.3±0.6	2.2±0.8	4.6±1.8	1.7±0.8	2.9±0.8
*18	-	-	-	5.1±1.9	2.0±1.2	3.3±1.0
			Group 2			
19	5.7±2.3	1.4±0.8	2.9±1.4	9.9±4.2	1.8±0.9	2.7±0.7
20	3.2±1.6	1.2±0.5	2.2±1.1	7.1±2.8	2.6±1.1	3.7±1.4
*21	-	-	-	8.1±2.4	2.2±1.2	3.5±1.1
*22	-	-	-	5.3±2.6	2.2±1.2	3.4±1.4
23	4.1±3.3	1.4±0.5	2.9±1.4	-	-	-
	5.6±2.5	1.4±0.7	2.8±1.2	6.1±2.7	1.9±1.1	3.1±1.0

*Ewes were excluded because of some missing data

Ewes 3 and 12 displayed 12h periodicities while ewes 2 and 9 displayed 8h and 4h periodicities, respectively.

Peak amplitudes and interpeak intervals were not different between the groups (Table 4). A mean peak amplitude of 4.4±3.1 pg with a mean interpeak interval of 2.2±1.3h was found in trial 1 ewes at 2 weeks prior to expected delivery (Table 4).

Estrogen profiles of some ewes from trial 2, at 2 and 3 weeks after the change in the feeding time (3 and 2 weeks prior to expected delivery) are shown in Fig. 11, and the profile characteristics are shown in Table 6. The peak amplitudes were 5.6±2.5 pg and 6.1±2.7 pg at 2 and 3 weeks, respectively. The interpeak intervals were 2.8±1.2h at 2 weeks and 3.1±1h at 3 weeks while the peak length was 1.4±0.7h at 2 weeks and 1.9±1.1h at 3 weeks after the start of the trial.

The cortisol profiles of group 1 ewes in the first trial are given in Fig. 12, while those of group 2 ewes are displayed in Fig. 13. Ewes 3 and 12 showed a 1.8h cyclic period. Ewe 1 had a 2.4h period, ewe 10 a 6h period and ewes 2, 5, 9 and 11 had an 8h cyclic period. It appeared that any cyclicity revealed by the spectral analysis could not necessarily be associated with feeding time.

A mean cortisol peak amplitude (Table 7) of 24.6±10.3 ng and a 2h interpeak interval was found in trial 1 at 2 weeks prior to expected delivery.

Cortisol profiles of some trial 2 ewes at 2 and 3 weeks after the change in the feeding schedule (3 and 2 weeks prior to expected

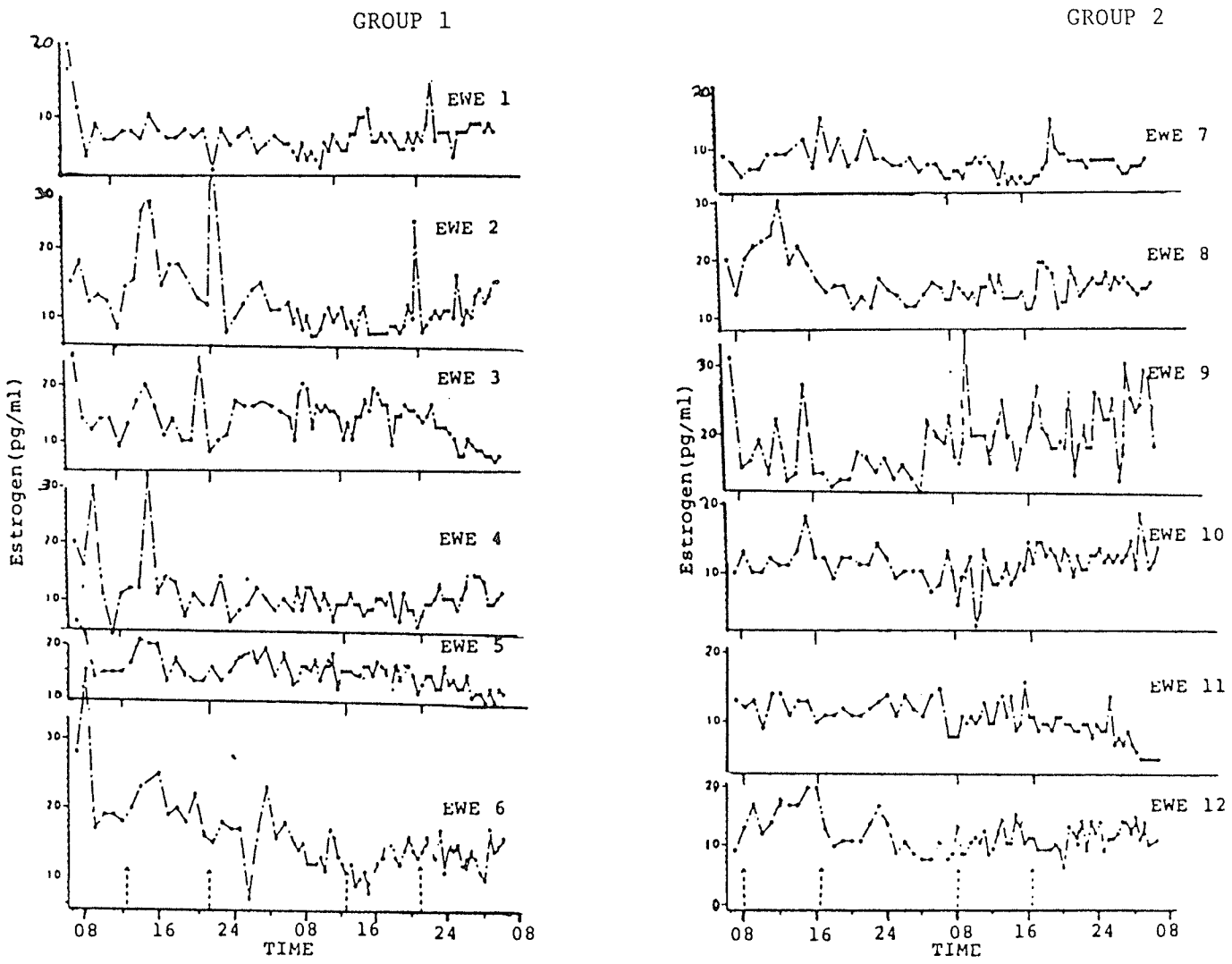


Figure 10: Estrogen profiles of ewes at 4 weeks after the start of trial 1 (2 weeks prior to expected delivery)

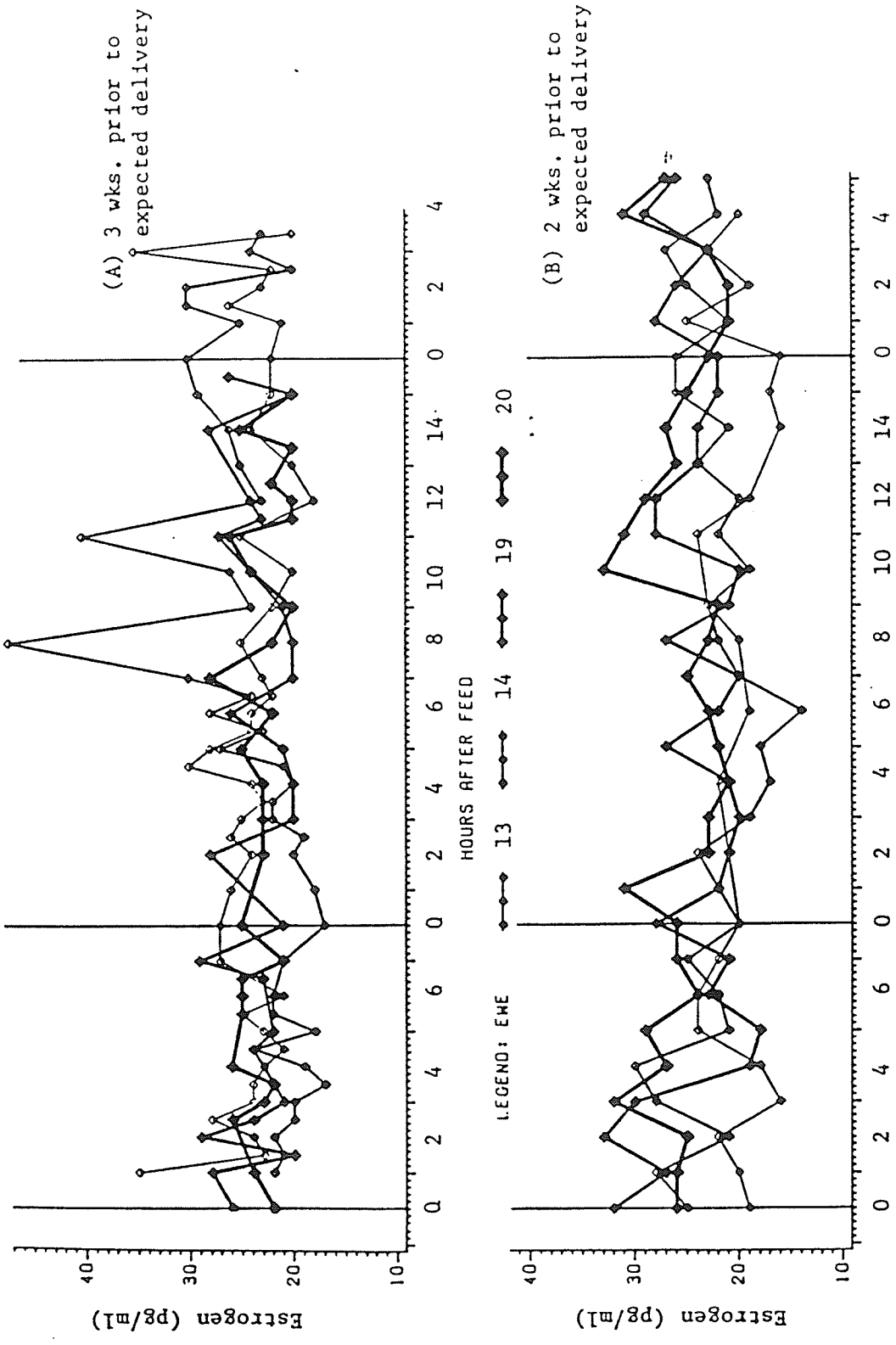


Figure 11: Estrogen profiles of ewes at 2 and 3 weeks after the start of trial 2 (3 and 2 weeks prior to expected delivery)

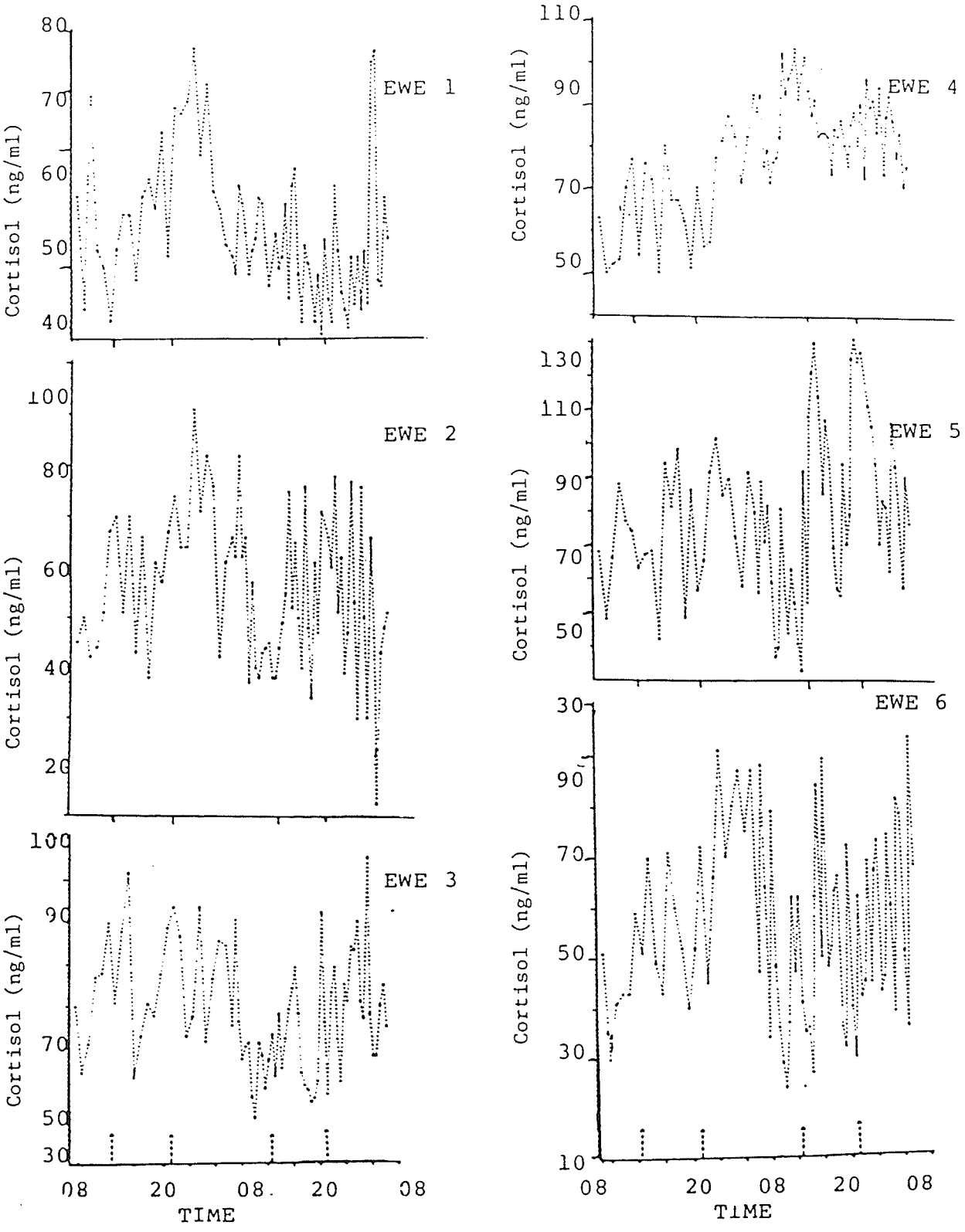


Figure 12: Cortisol profiles of group 1 ewes at 4 weeks after the start of trial 1 (2 weeks prior to expected delivery)

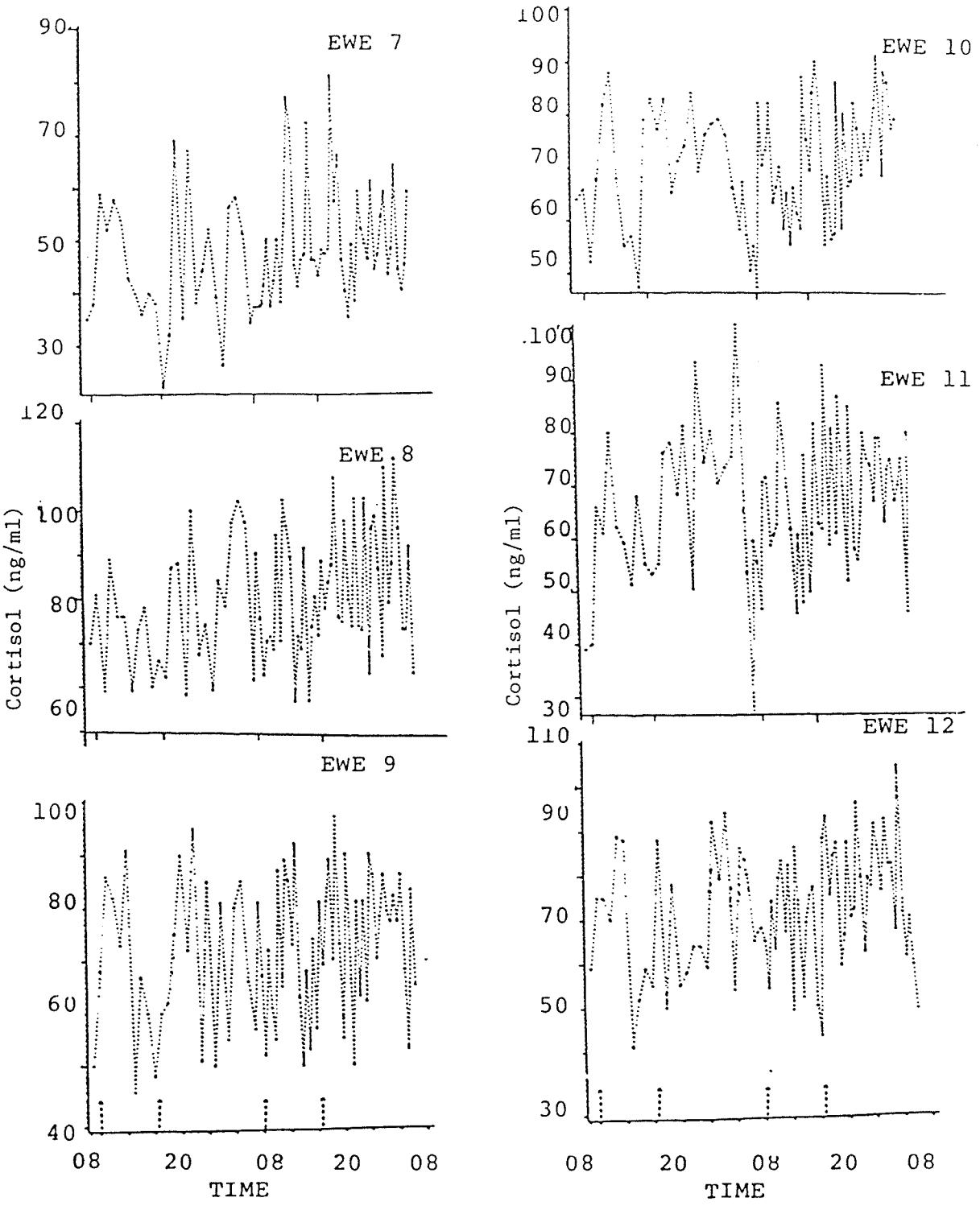


Figure 13: Cortisol profiles of group 2 ewes at 4 weeks after the start of trial 1 (2 weeks prior to expected delivery)

TABLE 7

Cortisol profile characteristics (mean±S.E.) at 2 weeks prior to expected delivery (4 weeks after the start of trial 1)

Sheep No.	Amplitude ng/ml	Peak length hr	Interpeak interval hr
		Group 1	
1	24.9±12.1	1.7±0.7	2.4±1.1
2	26.4±10.9	1.3±0.7	2.4±1.1
3	16.7±9.8	1.4±0.8	2.1±1.0
4	15.6±6.5	1.3±0.7	2.1±1.3
5	36.5±13.8	1.8±1.2	2.6±1.4
6	33.66±10.9	1.4±0.5	2.0±1.0
		Group 2	
7	22.1±11.1	1.7±0.8	2.4±1.2
8	25.7±10.5	1.1±0.5	1.9±0.9
9	26.6±8.1	1.2±0.5	1.8±1.0
10	16.5±9.7	1.4±0.8	2.1±1.3
11	25.7±10.3	1.4±1.0	2.1±1.3
12	24.4±10.5	1.2±0.5	1.9±0.8
Mean	24.6±10.3	1.4±0.6	2.2±1.3

TABLE 8

Cortisol profile characteristics (mean±S.E.) at 2 and 3 weeks prior to expected delivery in trial 2

	3 weeks prior to expected delivery			2 weeks prior to expected delivery		
Sheep No.	Amplitude ng/ml	Peak Length hr	Interpeak interval hr	Amplitude ng/ml	Peak Length hr	Interpeak interval hr
			Group 1			
13	13.7±7.6	1.2±0.7	2.2±1.3	13.6±6.1	2.7±1.7	4.1±1.8
14	43.6±13	1.1±0.5	2.0±0.8	16.7±7.3	1.6±0.7	2.7±1.1
15	30.0±14	1.4±0.7	2.6±1.1	22.4±6.8	1.9±0.7	2.8±0.8
16	27.6±14	1.4±1.1	2.3±1.6	27.7±15.0	2.6±1.7	3.6±1.5
17	57.1±21	1.4±1.1	2.1±1.1	48.9±18.7	3.0±1.4	4.0±1.7
*18	-	-	-	21.4±9.5	2.6±1.1	3.7±1.0
			Group 2			
19	33.3±15	1.4±0.5	2.1±0.9	40.9±13.7	2.2±1.2	3.4±0.9
20	24.3±16	1.6±0.7	2.1±0.4	12.7±5.7	1.8±1.5	3.0±2.1
*21	-	-	-	15.8±6.8	2.4±1.2	3.4±1.0
*22	-	-	-	28.9±10.4	2.1±1.3	3.5±1.5
23	34.1±13	1.4±0.5	2.1±1.4	23.2±6.4	2.2±1.5	3.3±1.3
MEAN	33.0±16	1.4±0.7	2.2±1.1	24.7±9.6	2.3±1.3	3.4±1.3

*Ewes were excluded because of some missing values

delivery) are shown in Fig. 14 and the ewe profile characteristics are shown in Table 8. The magnitude of the peaks declined from 33.0±16 ng at 2 weeks to 24.7±9.6 ng at 3 weeks. The peak lengths on the other hand increased from 1.4±0.7h at 2 weeks to 2.3±1.3h at 3 weeks, and the interpeak interval increased from 2.2±1.1h to 3.4±1.3h at 2 and 3 weeks, respectively. In both trials, the peak lengths in individual ewes were longer, but not statistically different ($P>0.05$) a few hours after feeding than later.

Smoothed profiles of individual ewes after the peaks have been removed by pulsar analysis are shown in Fig. 15. The trends of the profiles indicate that all the steroids undergo some daily fluctuations. However, the time trends for individual ewes and different steroids showed considerable variation and no consistent pattern could be discerned.

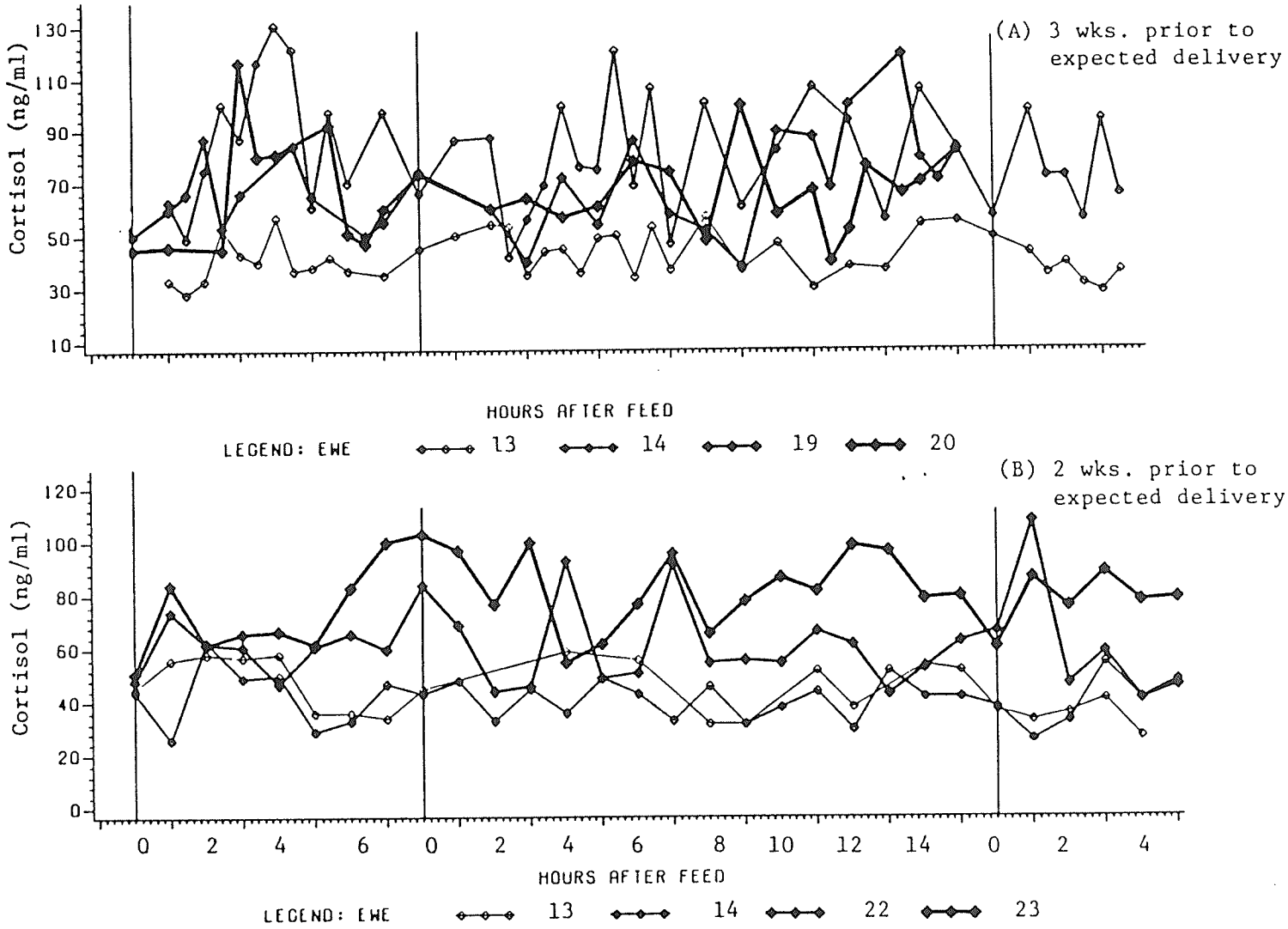


Figure 14: Cortisol profiles of ewes at 2 and 3 weeks after the start of trial 2 (3 and 2 weeks prior to expected delivery)

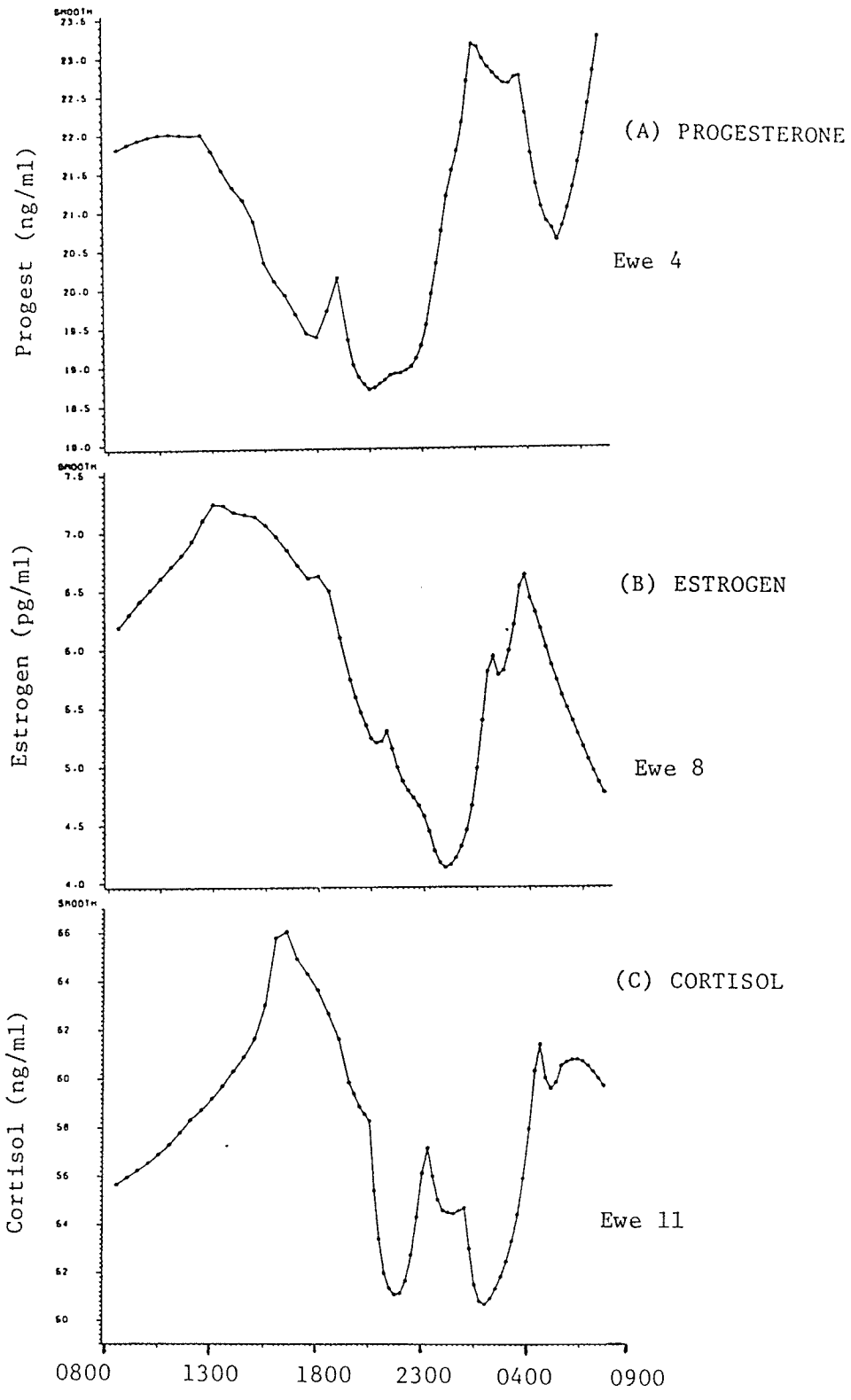


Figure 15: Steroid profile trends for certain ewes after the peaks have been removed

4.5 EFFECT OF TWINNING ON MATERNAL STEROID CONCENTRATIONS AND PROFILES

4.5.1 Progesterone

In the first trial, group 1 had 4 ewes carrying single fetuses and 2 ewes carrying twins while group 2 had 3 ewes carrying singletons and 3 ewes carrying twins. In trial 2, group 1 had 3 ewes with single fetuses, 1 ewe with twins and 2 ewes with triplets. Group 2 had 2, 2 and 1 ewe(s) with singles, twins, and triplets, respectively. Table 9 shows the mean steroid concentrations in ewes carrying either single, twin or triplet fetuses at 2 weeks prior to expected delivery. The mean P_4 concentrations in mothers carrying twins and triplets were significantly higher ($P<.01$) than in mothers carrying single fetuses in both trials. However the difference in P_4 concentrations between those carrying twins and those carrying triplets was not significantly ($P>0.05$) different although it was lower in ewes carrying triplet fetuses than those carrying twins.

The P_4 profiles of ewes carrying singletons or twins in trial 1 and those of singletons or twins and triplets in trial 2 are shown in Fig. 16. In the first trial (Fig 16A), both groups showed minor peaks at 2000h and 0400h on both days. In the second trial (Fig 16B) in which the profiles are plotted with respect to feeding time, the mean P_4 profiles showed no relation to time of feeding. The means used for the profiles in Fig. 16B excludes one ewe with twins and one ewe with triplets. Ewe 16 which was carrying twins and had a P_4 concentration of 9.0 ng/ml aborted dead lambs a few days later (Table 10), and ewe 15, which was carrying triplets and had a mean P_4 level of 13.8 ng/ml, gave birth to one live lamb one week later and is assumed to have been carrying only

TABLE 9

Effect of number of fetuses on steroid concentrations (mean±S.E.) in trials 1 and 2 at 2 weeks prior to expected delivery

Number of lambs	N	Progesterone ng/ml	Cortisol ng/ml	Estrogen pg/ml
		Trial 1		
Single	7	17.6 ± 0.2a	64.4 ± 0.9c	12.1 ± 0.2d
twins	5	23.9 ± 0.2b	68.1 ± 0.8c	12.0 ± 0.2d
		Trial 2		
single	5	16.5 ± 0.4a	47.0 ± 2.5e	24.3 ± 0.3f
twins	3	25.3 ± 1.4b	66.6 ± 1.9c	24.5 ± 0.4f
triplets	3	23.0 ± 1.0b	52.8 ± 2.4c	23.4 ± 0.4f
Steroid concentrations after the ewes with dead lambs were removed				
twins	2	33.8 ± 0.7	70.1 ± 2.3	25.7 ± 0.6
triplets	2	29.1 ± 0.7	41.8 ± 2.6	22.7 ± 0.6

*Values within columns with the same letter are not significantly different at p=0.05

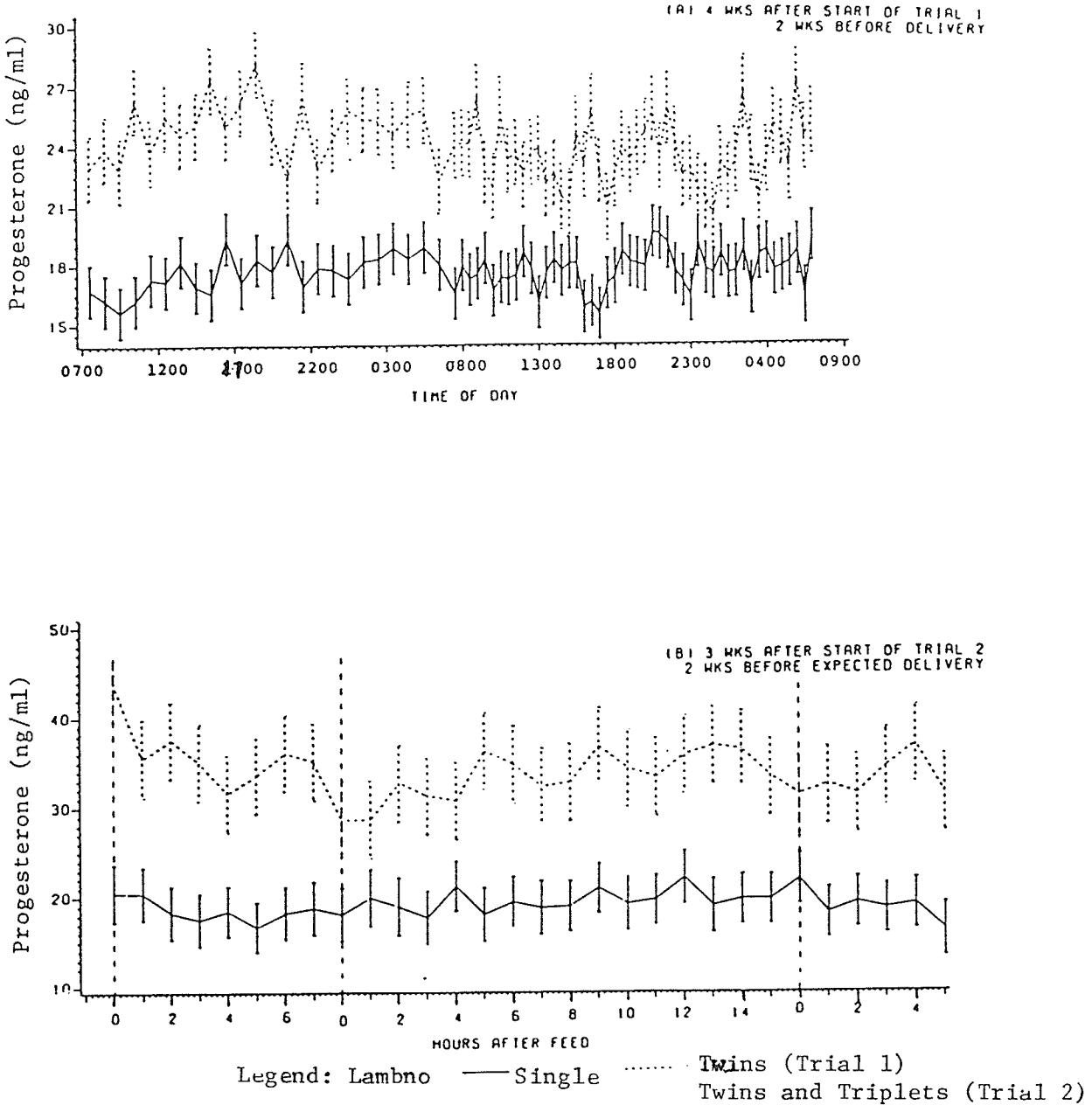


Figure 16: Effect of type of pregnancy on maternal progesterone (mean±S.E.) profiles in trials 1 and 2 at 2 weeks prior to expected delivery

TABLE 10

Effect of number of live fetuses on progesterone concentrations in trial 2 at 2 weeks prior to expected delivery

PROGESTERONE (ng/ml)	STATE OF LAMBS AT BIRTH
Singletons	
17.8 ± 1.3	Live
13.5 ± 0.7	Live
18.7 ± 0.6	Live
15.9 ± 0.8	Live
16.6 ± 0.7	Live
Twins	
9.0 ± 0.4	Both dead
30.3 ± 0.8	Both live
37.7 ± 0.4	Both live
Triplets	
13.8 ± 1.1	Two dead
29.3 ± 1.1	All live
26.1 ± 0.6	All live

one live fetus at the time of sampling. Thus with these two ewes excluded from the mean, the mean concentrations were found to be 16.5 ± 0.4 ng/ml (n=5) in singletons and 31.4 ± 0.6 ng/ml (n=4) for ewes carrying twins and triplets combined.

4.5.2 Cortisol and estrogen

Cortisol concentrations in both the first and the second trials were not different between the ewes carrying singletons, twins or triplets at 2 weeks prior to expected delivery (Table 9). The mean cortisol concentrations in the first trial were 64.4 ± 0.9 ng/ml in ewes carrying one lamb and 68.1 ± 0.8 ng/ml in ewes carrying twins. In the second trial, the concentrations were 47.0 ± 2.5 ng/ml in singletons, 66.6 ±1.9 ng/ml in twins and 52.8 ± 2.4 ng/ml in mothers of triplets. Figures 17A and 17B show cortisol profiles in the first and second trials, respectively, in ewes carrying single fetuses or more than one fetus. No significant differences ($P>0.05$) in cortisol profiles due to type of pregnancy were found, however, cortisol concentrations tended to be higher in ewes carrying twins in trial 2 (Fig. 17B).

Estrogen concentrations were also not different between the single, twin or triplet carrying ewes. In the first trial estrogen concentrations were 12.1 ± 0.3 pg/ml in singletons and 12.1 ± 0.2 pg/ml in twins (Table 9) and in the second trial they were 24.3, 24.5 and 23.4 pg/ml in single, twin and triplet pregnancies, respectively. Figs. 17c and 17d show the effect of type of pregnancy on estrogen profiles for both the first trial and the second trial at 4 and 3 weeks after the change in the feeding schedule (2 weeks prior to expected delivery). No

differences in profiles were found between the different types of pregnancies. No apparent association could be found between the type of pregnancy, feeding and estrogen concentrations.

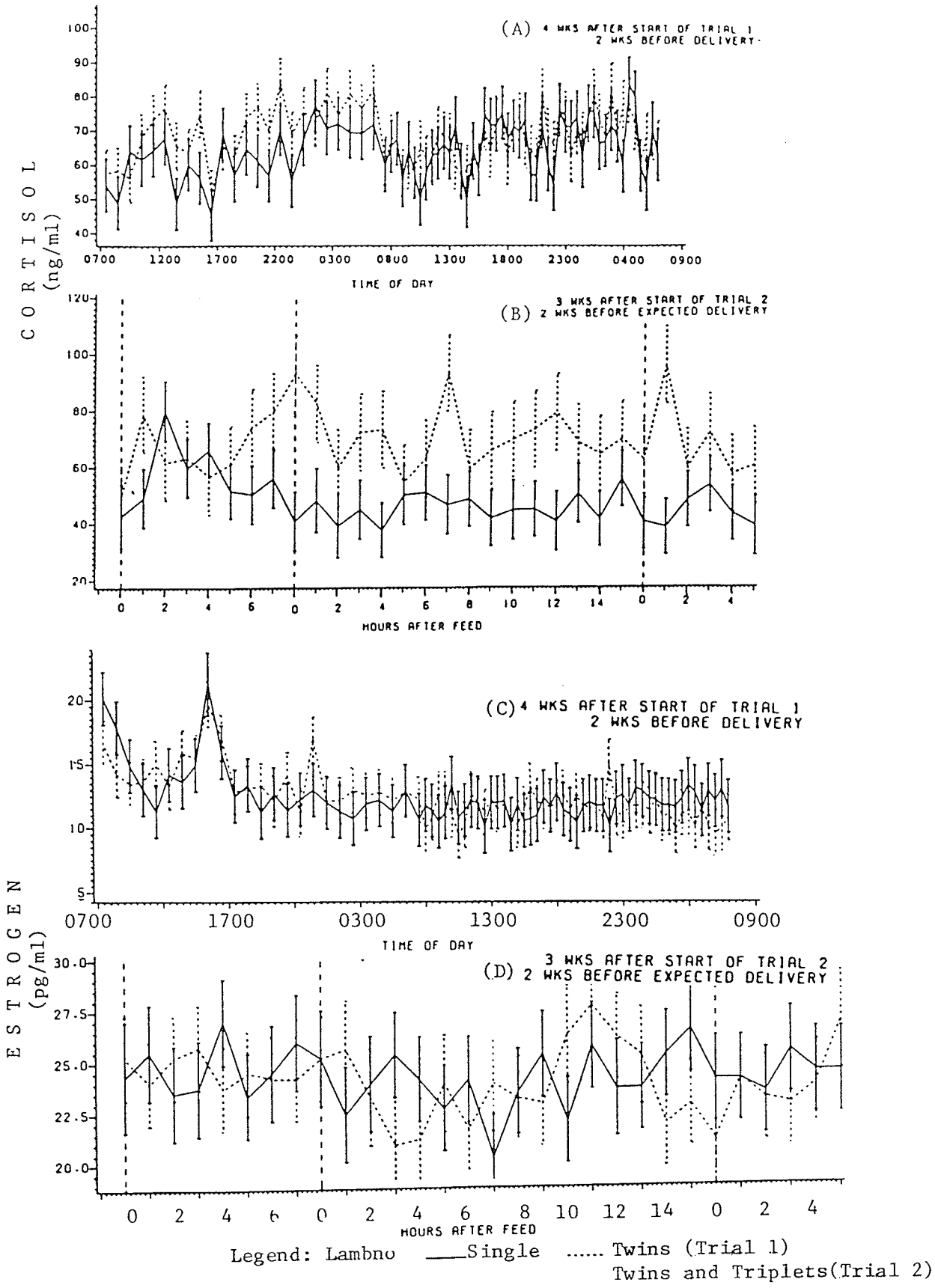


Figure 17: Effect of type of pregnancy on maternal cortisol and estrogen (mean±S.E.) profiles in trials 1 and 2 at 2 weeks prior to expected delivery

4.6 EFFECT OF BREED ON STEROID CONCENTRATIONS

Eight of the ewes in the first trial were crossbreds of mixed breeding, while two were Suffolks and two were Finnish Landrace. Table 11 shows the mean steroid concentrations of each breed in the two trials. Progesterone concentrations were not significantly ($P>0.05$) different between the breeds, although the concentrations in the Suffolks tended to be higher. In the second trial, six of the ewes were Finnish Landrace and five were crossbreds. Progesterone concentrations tended to be higher in the Finnish Landrace in this trial but these differences were not significantl ($P>0.05$).

Cortisol concentrations tended to be higher in crossbreds in both the first and second trials but were not significantly ($P>0.05$) different. Cortisol concentrations were 71.4 ± 7.3 ng/ml and 64.4 ± 2.2 ng/ml in the crossbreds during the first and second trials, respectively, while in the Finnish Landrace, the concentrations were 58.2 ± 1.4 ng/ml and 45.9 ± 1.7 ng/ml in the first and second trials, respectively. The mean values in the Suffolks were 51.4 ± 1.1 ng/ml.

Estrogen concentrations tended to be higher in the crossbreds in the first trial (Table 11), but no such tendencies were noticed in the second trial when the crossbreds were compared with the Finnish Landrace.

TABLE 11

Effect of breed on steroid concentrations (mean±S.E.) at 4 and 3 weeks after the start of trials 1 and 2

TRIAL 1 (at 4 weeks)

Breed	N	Progesterone ng/ml	Cortisol ng/ml	Estrogen pg/ml
Crossbred	8	19.9 ± 1.9a*	71.4 ± 7.3b	13.6 ± 0.2c
Finn	2	19.5 ± 3.9a	58.2 ± 1.4b	8.9 ± 0.3c
Suffolk	2	22.0 ± 0.3a	51.4 ± 1.1b	8.8 ± 0.4c
		TRIAL 2 (at 3 weeks)		
Crossbred	5	18.7 ± 0.9d	64.4 ± 2.2e	24.2 ± 0.4f
Finn	6	22.3 ± 0.6d	45.9 ± 1.7e	24.0 ± 0.4f

*Values within columns with the same letter are not significantly different at p=0.05.

4.7 STEROID PROFILES AT THE END OF PREGNANCY

4.7.1 Composite steroid profiles during the last 7 days of pregnancy in trials 1 and 2

Zero hour is considered as the time of lambing in all the results presented below. Figures 18a and 18b show the overall time trend changes of maternal plasma steroids during the last 7 days of pregnancy in the first (n=11) and second (n=10) trials, respectively. The major changes in steroid concentrations began about 60h before lambing, when P_4 concentrations began to decline and cortisol concentrations began to increase. Estrogen concentrations started to increase gradually at first and then dramatically at about 20-10h before lambing. Concentrations of both plasma estrogen and cortisol declined precipitously within 2 h after lambing (Fig 18A trial 1).

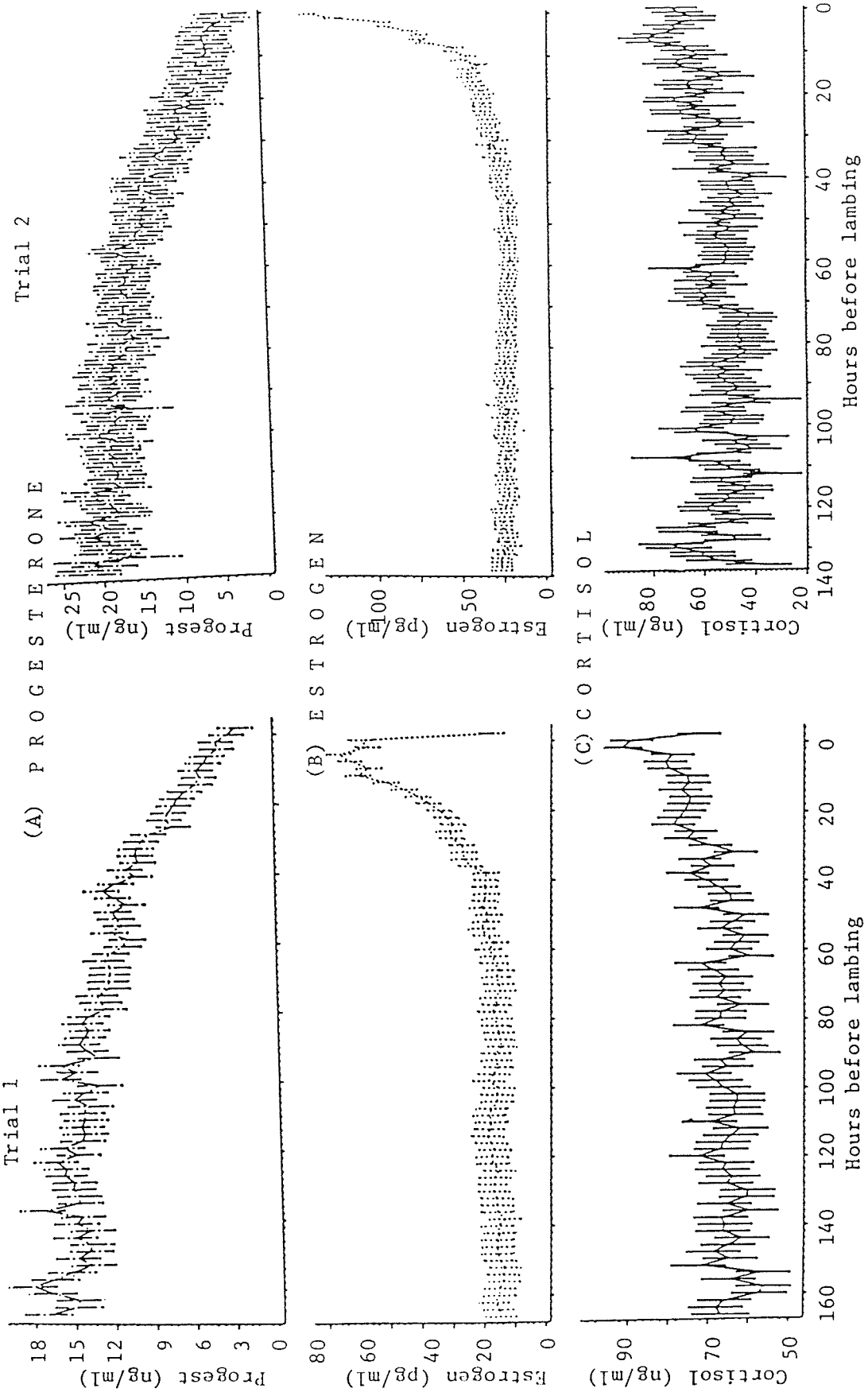


Figure 18: Composite steroid (mean±S.E.) profiles during the last 7 days of pregnancy in trials 1 and 2

4.7.2 Effect of treatment on steroid profiles

4.7.2.1 Progesterone profiles

In the first trial (Table 12), P_4 concentrations tended to be higher in group 1 than in group 2 but the differences were not significant ($P>0.05$). However in the second trial (Table 13), the concentrations were significantly higher in group 2 ($P<0.05$). The P_4 concentrations were significantly ($P<0.01$) different over time (Table 12 and 13) in both trials. Although there were differences in P_4 concentrations 132h (Table 13) before lambing, there was no significant ($P>0.05$) difference in P_4 concentration between the groups at lambing. In the first trial (Table 12) P_4 concentrations were 19.9 ± 1.4 ng/ml (n=5) in group 1 and 15.4 ± 0.3 ng/ml (n=6) in group 2 at 7 days before lambing. This reduced to 3.6 ± 0.9 ng/ml in group 1 and 2.8 ± 0.5 ng/ml in group 2 at lambing. In the second trial (Table 13), P_4 values decreased from 14.3 ± 4.3 ng/ml at 132h before lambing to 2.0 ± 0.7 ng/ml at lambing, and also from 28.5 ± 9.5 ng/ml at 132h before lambing to 6.2 ± 1.0 ng/ml at lambing in groups 1 and 2 , respectively. The P_4 profiles of the groups are shown in Fig 19A and the last 48h are magnified in Fig. 20A. Progesterone decreased in a somewhat variable manner during the last 3-4 days of pregnancy and decreased further after lambing. There were no differences in the profiles between the groups.

4.7.2.2 Estrogen profiles

Estrogen concentrations were not significantly ($P>0.05$) different between the groups in either trial (Tables 12 and 13). However, the concentrations were significantly ($P<0.001$) different over time. The

TABLE 12

Steroid concentrations (mean±S.E.) during the last 7 days of pregnancy (Trial 1)

Days before lambing	Progesterone Grp[2] 1	Progesterone Grp 2	Cortisol Grp 1	Cortisol Grp 2	Estrogen Grp 1	Estrogen Grp 2
7	19.9±1.4	15.4±0.3	64.6±15.2	58.6±6.4	14.0±2.0	14.6±4.0
6	17.2±1.9	12.6±0.6	71.3±10.6	51.6±3.3	14.6±1.2	16.6±1.8
5	16.9±2.0	12.1±1.7	70.6±6.9	75.0±8.5	19.6±0.8	15.6±3.5
4	17.1±2.7	12.3±1.1	67.6±11.5	74.6±0.3	18.6±1.7	15.3±2.4
3	13.6±3.7	10.5±1.1	63.3±01.1	63.3±5.3	18.0±0.5	16.0±2.0
2	11.7±3.2	9.8±1.4	86.3±2.0	66.5±7.1	23.6±5.3	19.0±2.5
1	9.1±2.1	6.5±0.5	87.2±4.3	73.5±3.6	36.0±8.8	26.0±3.3
0	3.6±0.9	2.8±0.5	97.2±7.2	87.6±6.0	60.0±11.7	57.6±12.4
2h after lambing	2.6±0.3	2.2±0.5	71.8±8.2	76.6±5.7	21.0±5.7	13.6±3.9

ANOVA

Source	df	F value		
Grp	1	1.8 ns	4.1 ns	0.4 ns
Lamb number	1	0.0 ns	0.03 ns	0.28 ns
Breed	2	0.03 ns	0.10 ns	0.25 ns
Time	13	33.9 **	2.12 **	8.9**
Grp*Time	13	0.02 ns	0.06 ns	0.08 ns

Each value is a mean for 24h samples taken every 2h

** Significant at P=0.01
ns not significant
[2]Grp=Group

TABLE 13

Steroid concentrations (mean±S.E.) during the last 7 days of pregnancy (Trial 2)

Hrs before Lambing	Progesterone Grp$_2$ 1	Progesterone Grp 2	Cortisol Grp 1	Cortisol Grp 2	Estrogen Grp 1	Estrogen Grp 2
132	14.3±4.3	28.5±9.5	68.3±11.0	83.5±10.5	26.0±1.1	31.0±2.1
120	11.6±2.3	24.3±3.9	70.0±18.5	75.0±15.5	24.6±2.3	29.0±4.0
108	11.7±2.0	24.0±6.0	92.0±27.0	74.5±11.8	21.7±2.6	22.6±4.4
96	8.0±0.0	22.2±3.0	60.5±10.5	73.2±17.5	21.0±3.0	21.6±3.1
84	7.5±1.5	24.6±9.5	77.5±33.5	48.0±17.7	20.0±4.0	26.0±3.0
72	9.5±2.5	22.3±3.3	69.7±21.1	58.6±18.4	19.5±2.2	23.5±2.5
60	10.2±3.4	21.0±4.2	77.7±20.9	49.2±7.5	18.7±3.1	25.0±2.5
48	7.6±1.7	23.0±4.5	76.4±1.7	56.7±12.9	20.4±1.3	21.3±2.4
36	4.0±0.5	18.0±3.3	61.3±13.3	58.2±12.2	20.0±3.4	23.7±5.0
24	3.6±0.8	13.2±2.2	76.6±13.3	77.0±17.7	22.3±5.7	30.2±8.7
12	4.0±0.7	9.4±1.6	74.7±11.1	100.2±5.8	28.0±7.8	34.0±9.3
0	2.0±0.7	6.2±1.0	95.2±17.5	100.5±6.9	105.7±8.1	126.3±24

ANOVA Source	df	F value		
Grp	1	3.3 ns	0.3 ns	6.3 ns
Lamb no.	2	0.1 ns	1.1 ns	3.9 ns
Breed	1	4.5 ns	0.3 ns	4.6 ns
Time	24	41.3 **	6.2 *	125.5 **
Grp*Time	24	0.4 ns	0.04 ns	0.04 ns

Each value is a mean for 24h samples taken every hour.

* Significant at P=0.05
** Significant at P=0.01
ns Not significant at P=0.05
$_2$Grp=Group

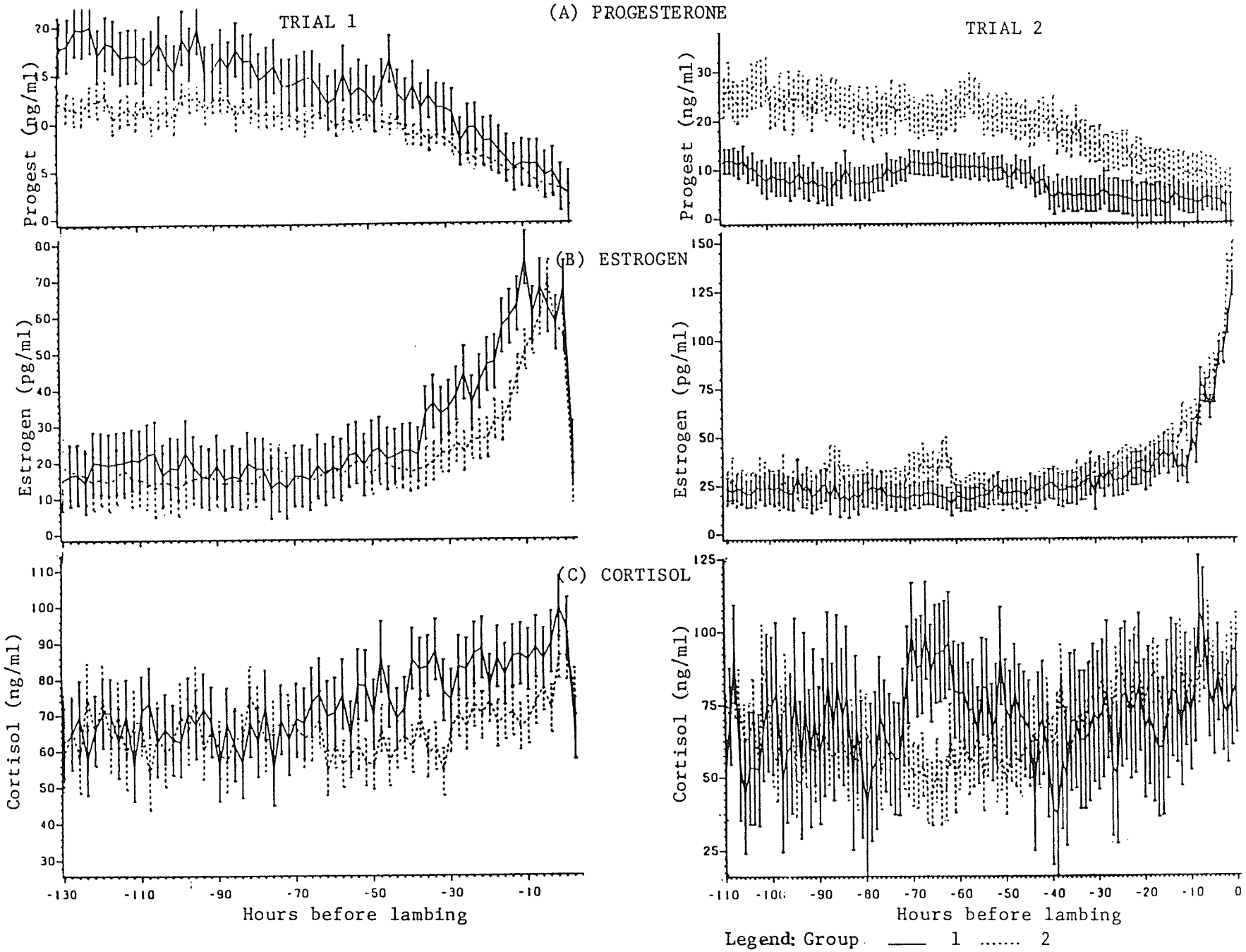


Figure 19: Steroid (mean±S.E.) profiles during the last 7 days of pregnancy in trials 1 and 2

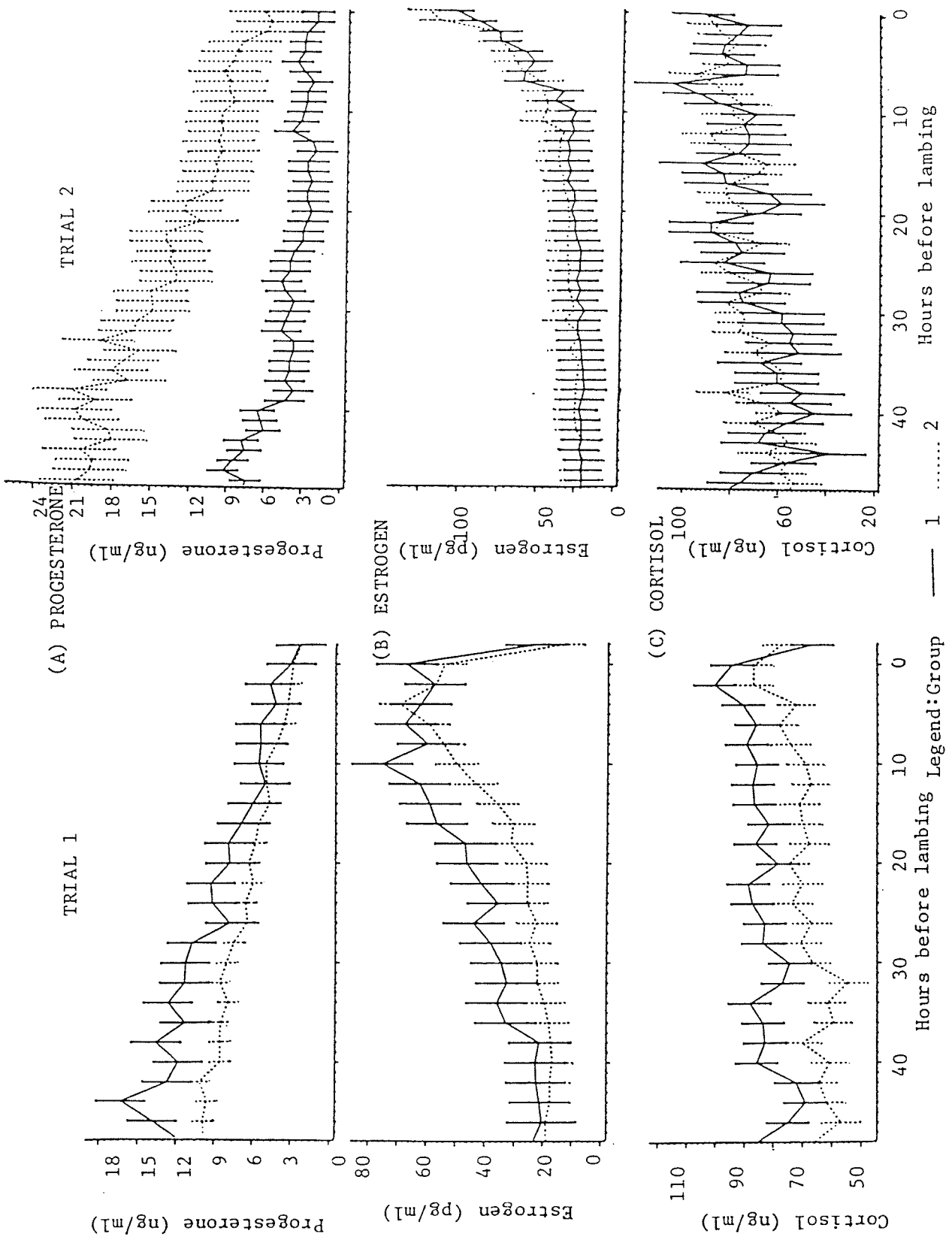


Figure 20: Steroid (mean±S.E.) profiles during the last 48h of pregnancy in trials 1 and 2

values changed from 14.0 ± 2.0 pg/ml in group 1 and 14.6 ± 4.0 pg/ml in group 2 at 7 days before lambing in the first trial to 23.6 ± 5.3 pg/ml and 19.0 ± 2.5 pg/ml in group 1 and 2, respectively, 2 days before lambing. They increased further to 60.0 ± 11.7 pg/ml and 57.6 ± 12.4 pg/ml in groups 1 and 2, respectively, at lambing (Table 12). In the second trial, the estrogen concentrations were higher than those found in the first trial. The concentrations increased from 26.1 ± 1.1 pg/ml in group 1 and 31.0 ± 2.1 pg/ml in group 2 at 132 h before lambing to 105.2 ± 8.1 pg/ml in group 1 and 136.3 ± 24 pg/ml in group 2 at lambing (Table 13). The estrogen profiles in the two groups were similar in both trials. There was a general upward trend with the steepest slope occurring during the last 20h before lambing (Figs. 19B and 20B), and a rapid decline after lambing (Trial 1). Estrogen concentrations were increasing at a rate of 1 pg/ml/hr at about 20h before lambing in trial 1 and at about 13h before lambing in trial 2.

4.7.2.3 Cortisol profiles

There were no significant ($P>0.05$) group differences in cortisol concentrations in either trial. Although they tended to be higher in group 1 (Table 12) in the first trial and group 2 in the second trial (Table 13) these differences were not significant ($P>0.05$). The cortisol concentrations were significantly ($P<0.01$) different over time. The concentrations were 64.6 ± 15.2 ng/ml in group 1 and 58.6 ± 6.4 ng/ml in group 2 at 7 days before lambing in the first trial (Table 12) and these increased to 97.2 ± 7.2 ng/ml in group 1 and 87.6 ± 6.0 ng/ml in group 2 at lambing. In trial 2, these values were 68.3 ± 11 in group

1 and 83.5 ± 10.5 ng/ml in group 2 at about 6 days before lambing. The values rose to 95.2 ± 17.5 and 100.5 ± 6.9 ng/ml in group 1 and 2, respectively at lambing (Table 13). Figure 19C shows the cortisol profiles of the groups during the last 6 days while Fig. 20C shows the last 48hr before lambing. Cortisol concentrations increased gradually during the last 12-36 hr before lambing in both trials. The mean profiles of the two groups did not appear to be different from each other. In both trials, the groups seemed to go through cyclic periods of highs and lows. Lambing occurred during periods of rising cortisol concentrations (Figs. 19C and 20C). After lambing (Trial 1) cortisol concentrations declined.

4.7.3 Steroid profiles in relation to feeding at a time close to parturition in trials 1 and 2

Individual profiles of some ewes at the end of pregnancy in relation to feeding are shown in Fig. 21. Mean P_4, estrogen and cortisol profiles in relation to feeding during a period close to lambing time are also shown in Fig. 22. Zero hour in these profiles represents the first feed of the day closest to parturition time for each ewe. Progesterone and estrogen concentrations did not show any changes in response to feeding, while cortisol concentrations sometimes appeared to increase after feeding (Figs. 21 and 22).

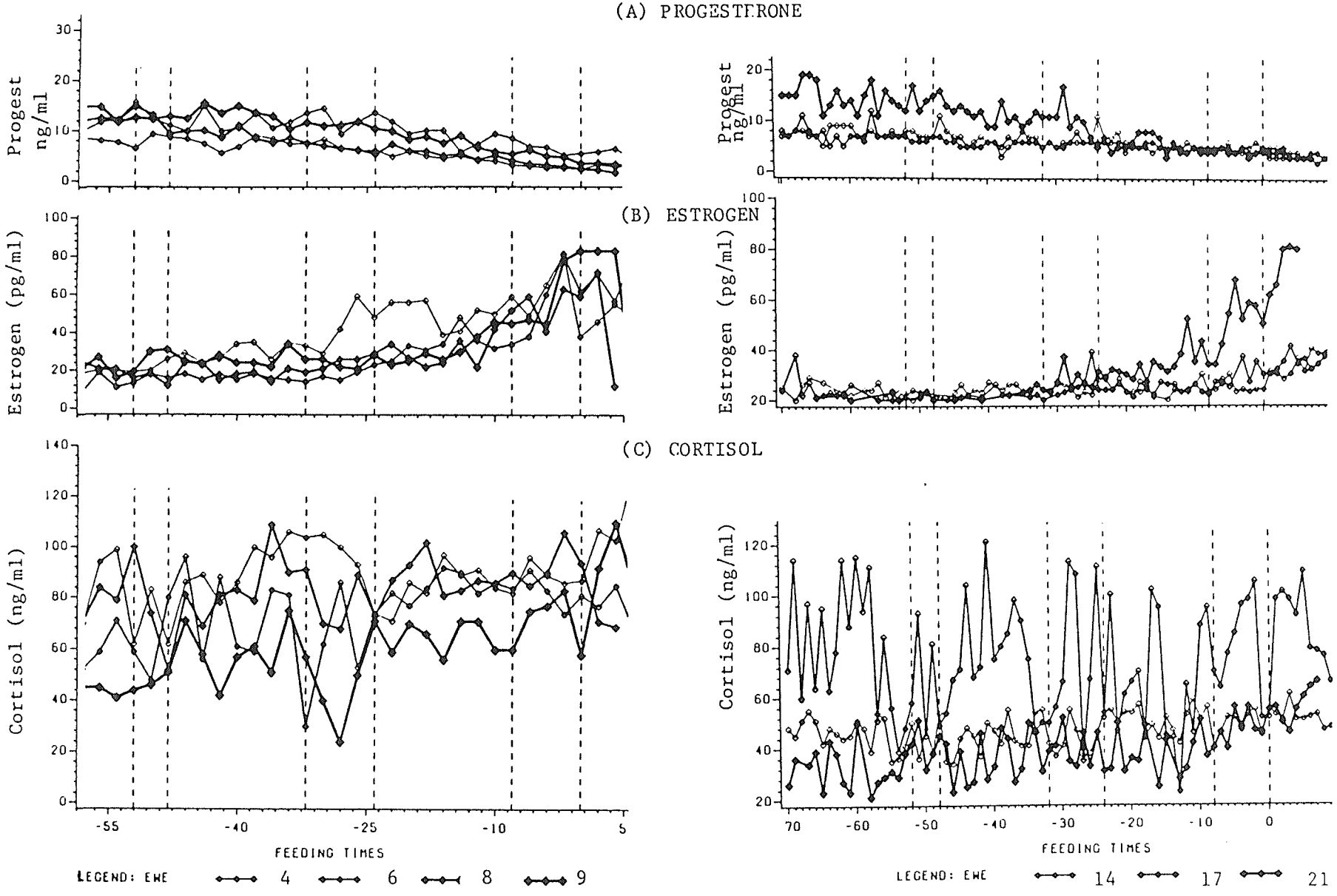


Figure 21: Steroid profiles of some ewes in relation to time of feeding at the end of pregnancy

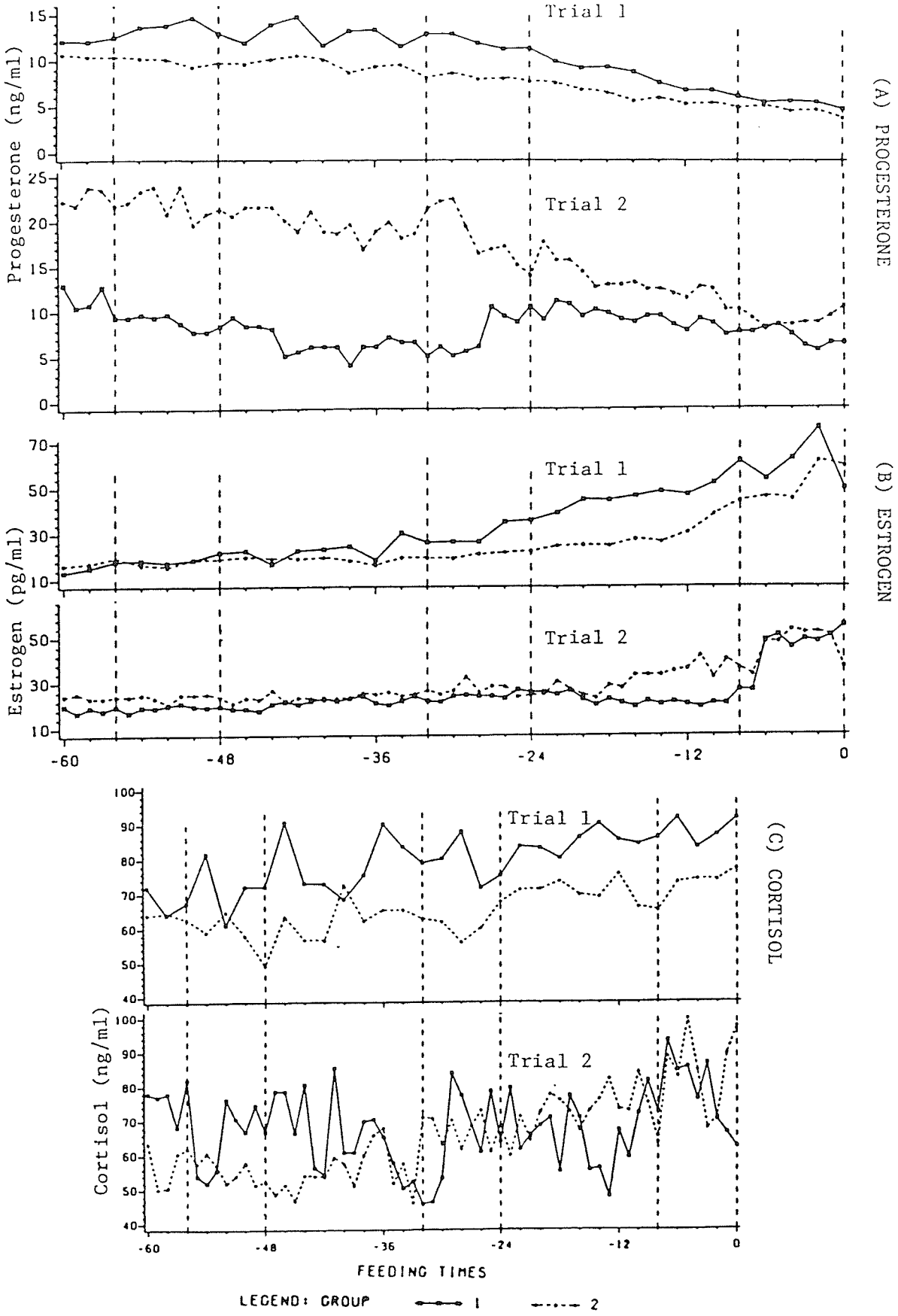


Figure 22: Effect of feeding on steroid profiles at the end of pregnancy

4.7.4 Individual ewes

The steroid profiles of some individual ewes just prior to lambing are shown in Fig. 23. The individual ewes showed the same general time trends in P_4, estrogen and cortisol profiles that were described earlier for the composite profiles. Differences in P_4 concentrations between individual ewes were nonexistent by 25h before lambing. In some ewes cortisol concentrations showed increases of a cyclic rhythm and parturitions seemed to occur at the time of increasing concentrations in all ewes that displayed a prepartum cortisol increase. A marked increase in cortisol concentrations at the end of pregnancy was not always evident. Two ewes out of the 21 ewes studied showed no such cortisol rise. On the other hand a decrease in P_4 and an increase in estrogen were always observed at the end of pregnancy except in one case in which both lambs the ewe was carrying were born dead (Fig. 24). In this ewe, there was no increase in estrogen concentrations even though a normal cortisol rise and decline in P_4 concentration was evident.

Pulsar analysis (Table 14 and 15) showed that P_4, estrogen and cortisol peaks were fewer (4 peaks/24h) at the end of pregnancy than at 2 and 3 weeks prior to lambing when there were 10-12 peaks per 24h. The P_4 peak amplitude was significantly lower ($P<0.05$) at 1.8ng in trial 1 (Table 14) and 2.8ng in trial 2 (Table 15) during the last week of pregnancy than at 2 and 3 weeks prior to parturition (Table 16). In addition the P_4 peak amplitudes progressively decreased as parturition time approached.

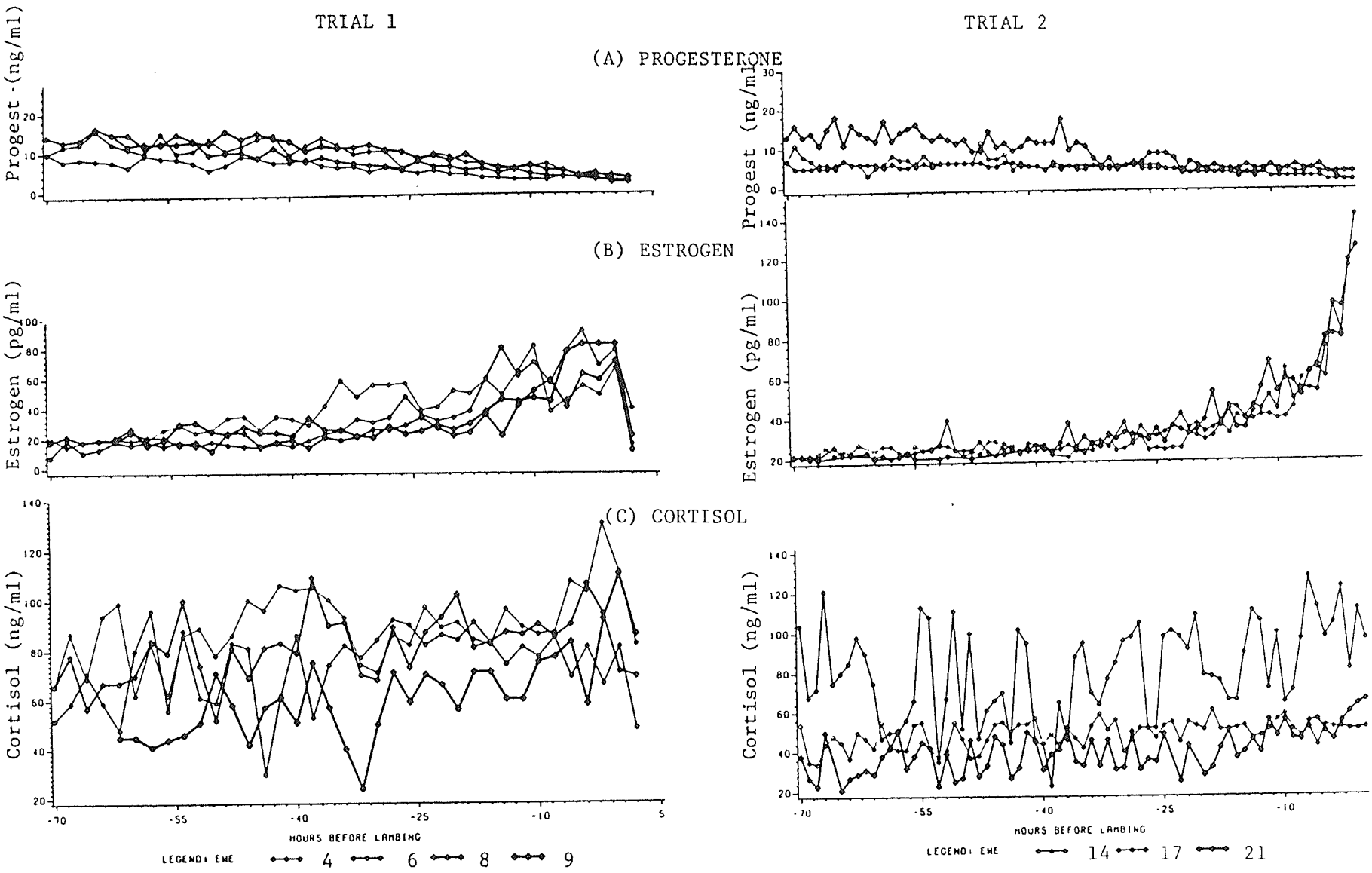


Figure 23: Steroid profiles of some ewes at the end of pregnancy

TABLE 14

Steroid profile characteristics (mean±S.E.) of each ewe during the last week of pregnancy in trial 1

Ewe	PROGESTERONE			ESTROGEN			CORTISOL		
	Amplitude ng/ml	Peak Length hr	Inter Peak Length hr	Amplitude pg/ml	Peak Length hr	Inter Peak Length hr	Amplitude ng/ml	Peak Length hr	Inter Peak Length hr
				Group 1					
2	1.8±1	3.3±1	5.1±2	9.8±3	3.5±2	6.0±2	10.6±4	2.8±0	5.4±2
3	3.1±2	2.1±1	5.3±2	6.0±4	2.1±.3	6.3±2	16.5±8	2.1±.5	5.2±2
4	1.6±1	2.0±0	5.5±2	5.6±4	2.4±1	5.3±2	11.8±7	2.0±0	5.9±2
6	2.8±1	2.2±.6	7.7±4	8.3±4	2.0±0	5.8±2	17.1±9	2.1±0	5.4±1
				Group 2					
7	2.5±2	3.1±1	5.5±2	4.6±2	3.4±1	6.7±2	14.8±5	3.8±1	6.5±2
8	1.9±1	3.3±1	6.0±2	5.3±3	3.3±2	5.8±2	17.9±9	3.3±1	6.8±3
9	1.1±1	2.8±1	5.5±2	9.2±4	3.8±1	6.2±2	14.4±6	5.3±1	7.7±3
10	1.1±1	2.0±0	5.3±2	5.5±3	2.1±0	6.1±3	13.2±8	2.3±.7	5.2±1
11	1.1±1	2.0±0	5.1±2	4.1±3	2.0±0	5.6±2	12.8±8	2.2±.5	5.3±2
12	1.9±1	2.3±1	5.6±2	3.6±3	2.3±1	6.2±2.	15.0±9	2.3±.7	7.4±3
x	1.8±1	2.5±.7	5.6±2	7.1±3	2.6±.8	5.9±2	14.4±7	2.8±.5	6.1±2

TABLE 15

Steroid profile characteristics (mean±S.E.) of each ewe during the last week of pregnancy in trial 2

Ewe	PROGESTERONE			ESTROGEN			CORTISOL		
	Amplitude ng/ml	Peak Length hr	Inter Peak Length hr	Amplitude pg/ml	Peak Length hr	Inter Peak Length hr	Amplitude ng/ml	Peak Length hr	Inter Peak Length hr
				Group 1					
14	1.7±1	2.1±1	3.5±2	5.6±3	1.9±1	3.1±1	12.1±4	2.3±1	3.3±1
15	2.1±2	2.5±1	3.9±1	5.1±3	2.6±1	5.5±2	33.3±6	5.0±3	8.2±4
16	1.5±1	2.2±1	4.1±2	4.4±3	1.6±1	2.8±1	51.2±9	2.5±2	3.8±2
17	1.7±1	2.2±1	3.5±1	8.4±2	2.2±1	3.4±1	45.6±8	2.4±1	3.7±1
18	3.1±2	3.1±1	3.6±2	5.7±3	3.3±1	3.6±1	25.9±6	4.3±3	6.4±3
				Group 2					
19	3.9±1	2.3±1	3.5±1	7.1±2	1.7±1	2.9±1	37.5±9	2.3±1	3.5±1
20	4.3±2	2.2±1	3.6±2	7.5±3	2.4±1	3.6±2	36.7±9	1.7±1	2.8±1
21	4.6±3	2.2±1	3.5±1	8.5±3	2.4±1	3.5±1	16.9±6	2.3±1	3.4±1
22	7.8±3	2.2±1	3.6±3	7.5±2	2.3±1	3.8±2	29.7±9	2.5±1	3.9±1
x	2.8±1	2.3±1	3.6±1	6.6±3	2.3±1	3.8±1	32.1±7	2.9±2	4.5±2

The peak amplitudes of cortisol at the end of pregnancy were similar to those at 2 and 3 weeks prior to expected delivery but that of estrogen increased slightly at the end of pregnancy (Table 16). Estrogen interpeak intervals increased from 2.8h and 2.6h at 3 and 2 weeks prior to expected delivery to 4.9h during the last week of pregnancy (Table 16). Cortisol interpeak intervals also increased from 2.6h and 2.8h in at 3 and 2 weeks prior to expected lambing to 5.3h during the last week of pregnancy. The peak lengths of each steroid also increased from a range of 1.4-1.5h at 3 weeks prior to expected delivery to a range of 1.7-1.8h at 2 weeks prior to expected delivery to a range of 2.4-2.8h during the last week of pregnancy.

TABLE 16

Mean steroid profile characteristics at 2 and 3 weeks prior to expected delivery and during the last week of pregnancy

	Weeks before lambing		
	3	2	Last week of pregnancy
PROGESTERONE			
Amplitude (ng)	5.6±3.7	4.9±2.6	2.2±1.5
Peak length (hr)	1.5±0.8	1.7±0.9	2.4±0.8
Interpeak interval (hr)	2.6±1.5	2.6±1.1	5.2±1.0
ESTROGEN			
Amplitude (pg)	5.6±2.5	5.2±2.9	6.6±2.5
Peak length (hr)	1.4±0.7	1.7±1.0	2.4±1.9
Interpeak interval (hr)	2.8±1.2	2.6±1.1	4.9±2.0
CORTISOL			
Amplitude (ng)	33.0±16	24.6±11.4	21.6±10.0
Peak length (hr)	1.4±0.7	1.8±0.9	2.8±1.2
Interpeak interval (hr)	2.6±1.2	2.8±1.3	5.3±2.9

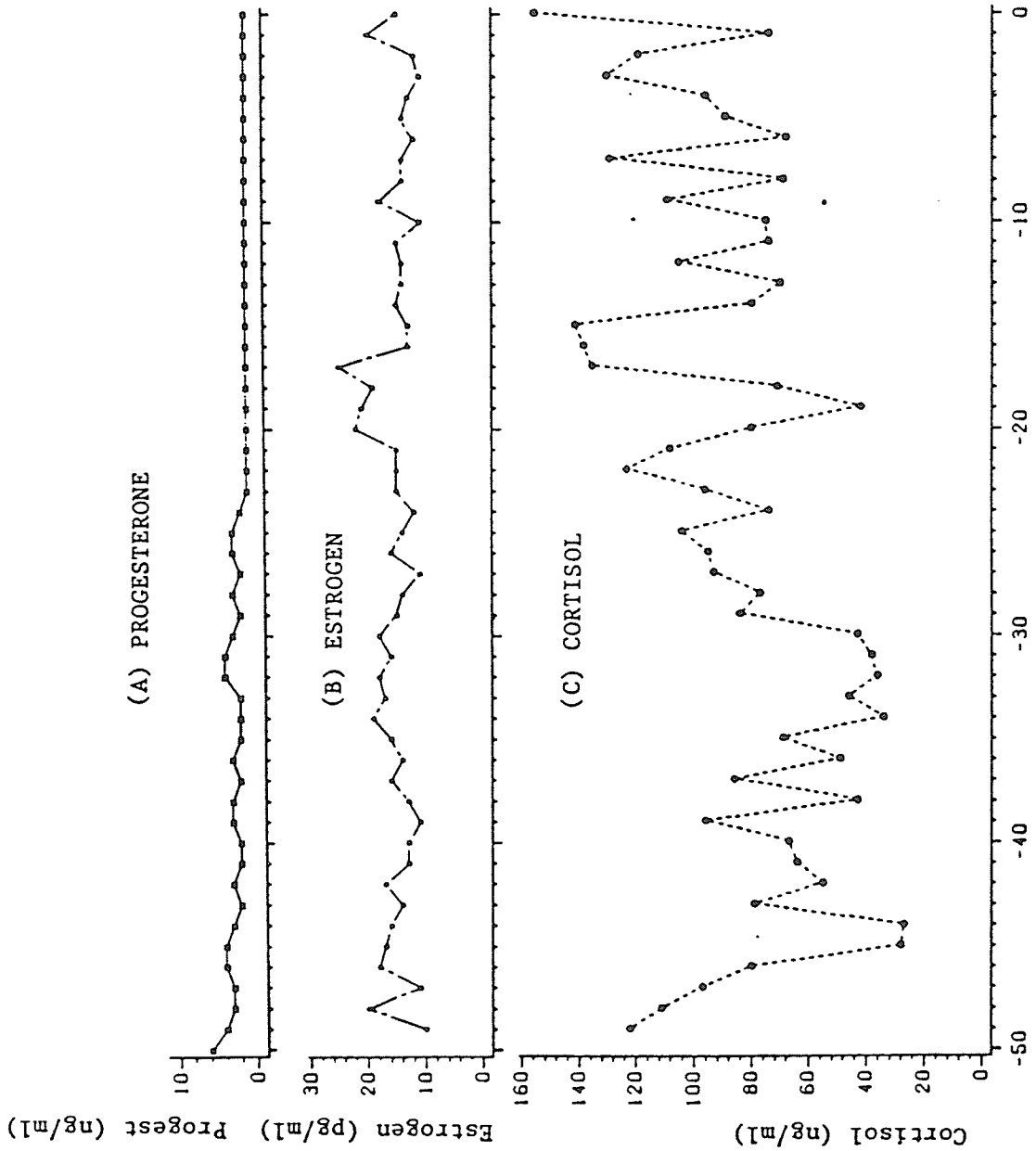


Figure 24: Maternal steroid concentrations in a ewe carrying dead fetuses before parturition

4.8 EFFECT OF NUMBER OF FETUSES CARRIED BY A EWE AND THE BREED OF THE EWE ON STEROID LEVELS AND PROFILES JUST PRIOR TO LAMBING

Progesterone concentrations in ewes bearing singles and in those bearing twins and triplets were not different at the immediate time of lambing (Fig. 25) The P_4 concentrations tended to be higher at 50h or earlier prior to lambing in ewes carrying more than one fetus. Thus P_4 decline in ewes bearing multiple fetuses was greater than in ewes bearing single fetuses. There were no differences in cortisol and estrogen concentrations between the different types of pregnancies during the last week of pregnancy which was similar to that observed at 2 and 3 weeks prior to expected delivery.

Breeds did not contribute to a significant ($P>0.05$) difference in the concentrations of P_4 (Fig 26A), cortisol (Fig 26B) or estrogen (Fig 26C) at the end of pregnancy. But in both trials, the Finnish Landrace achieved 30% higher circulating concentrations of estrogen (Fig. 26c) than the other breeds. This approached significance at 5% level.

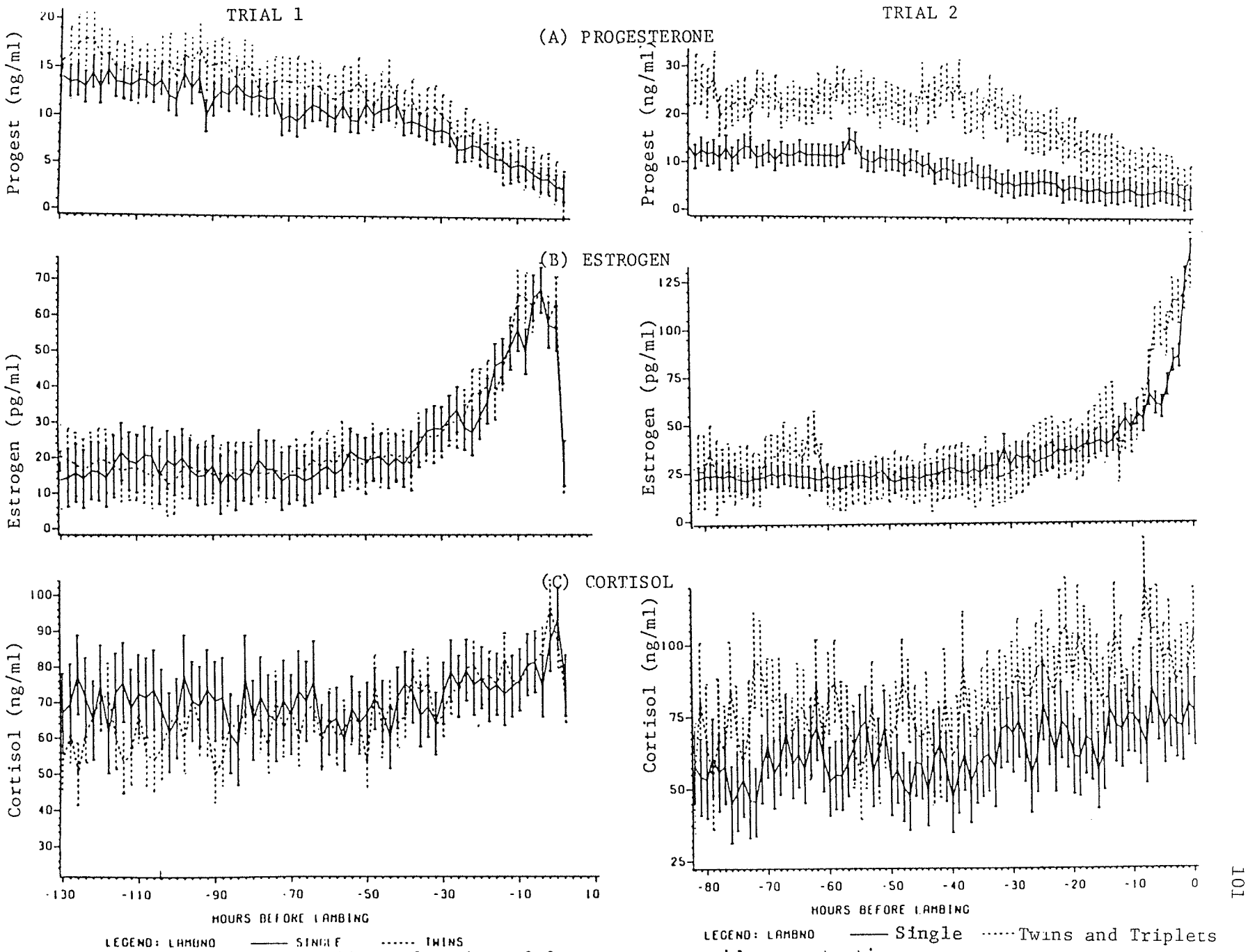


Figure 25: Effect of number of fetuses on steroid concentrations (mean±S.E.) at the end of pregnancy in trials 1 and 2

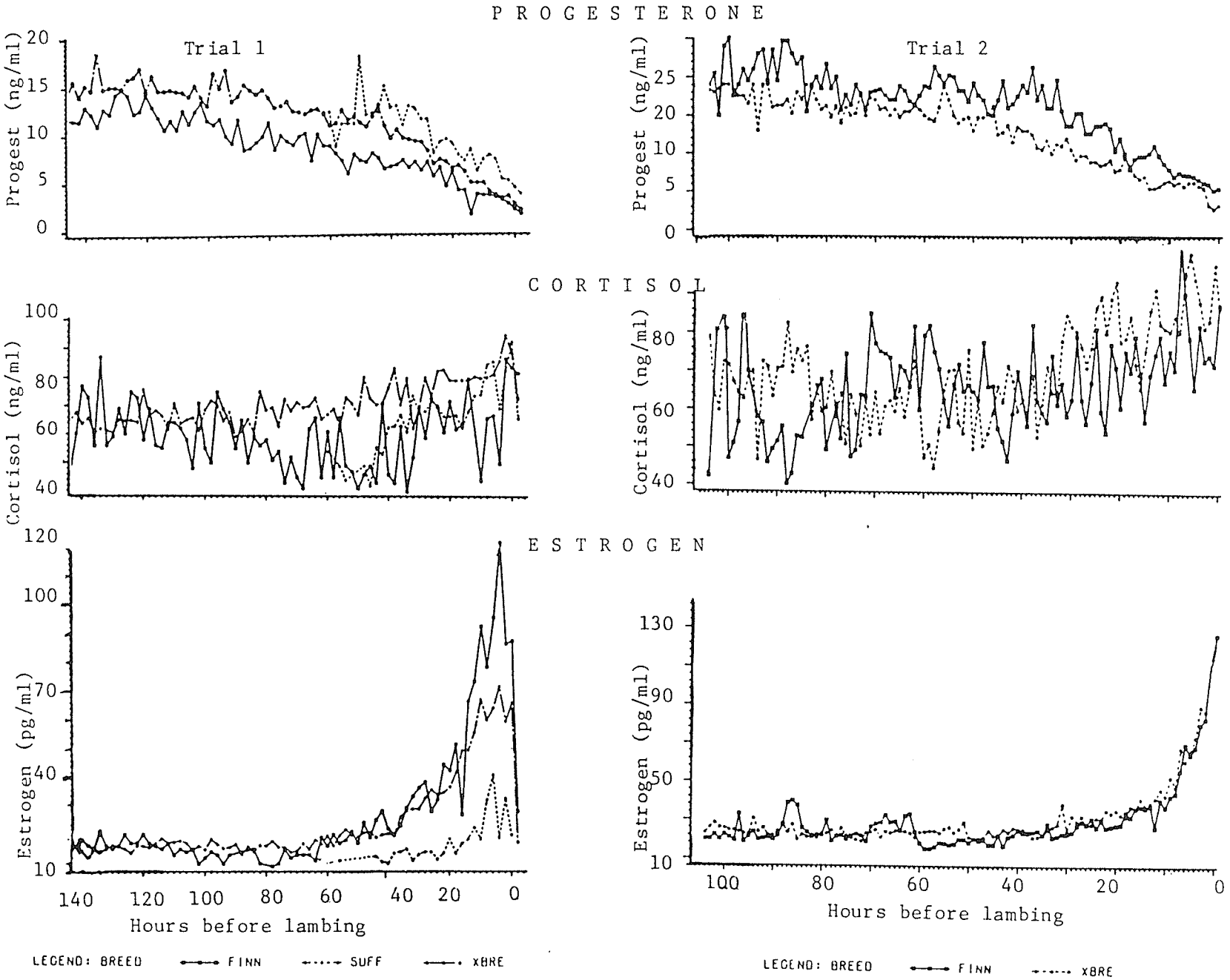


Figure 26: Breed effect on steroid concentrations at the end of pregnancy in trials 1 and 2

V

DISCUSSION

5.1 LAMBING TIME

Lambing was concentrated during the daylight hours in both groups. There was no effect of time of feeding on time of parturition although more ewes (67%) in group 1 lambed between the two feeding times than group 2 (27%). A Chi square test however failed to show significant differences because of the small number of ewes involved. Sherafeldin et al., (1971) reported lowest lambing times during periods of concentrate feeding and proposed that imminent parturitions were postponed as a result of increased adrenaline levels at this time. Although adrenaline was not measured in this study, lambing occurred even at the time of feeding and significantly increased adrenaline release has been shown to occur mostly after onset of labor (Eliot et al., 1981). Adrenaline levels may thus not be the determining factor for onset of labor.

Change of feeding time in late pregnant cows has been shown to alter time of calving (Konefal, 1980). Konefal (1980) reported that when pregnant cows were fed at 1200h and 2100h during the last month of pregnancy instead of the usual 0800h and 1600h, more cows calved during the daytime. The cause for this alteration in time of parturition is not known. It has been suggested that the secretion of some of the

hormones associated with delivery might be altered by a change in time of feeding (Palmer, 1980). In the present study pregnant sheep in group 1 had their feeding times altered to the Konefal method during the last month of pregnancy. The results indicate that although the maternal steroid hormone milieu undergoes a substantial change during the last week of pregnancy, the time of feeding did not alter P_4 or estrogen concentrations although feeding appeared to increase cortisol concentrations.

Gonyou et al. (1982) reported an increase in the number of ewes lambing during the day when the ewes' feeding time was changed to 1200h from earlier times, while Jilek et al. (1985) reported no difference in lambing time when the ewes were fed twice a day at either 0900h and 1200h or 1630h and 2100h. Our results also showed no differences in lambing time. It is not clear whether twice feeding as opposed to once feeding was the reason for the failure to alter lambing time or whether the continual lighting affected the ewes. Konefal (1980) however used similar conditions of lighting and feeding times and found increased day time calvings. It is possible that ewes do not respond to feeding regimes in the same way as cattle. Brackelsberg (1985a) found a longer interval between feeding and calving in the cows fed during the afternoon than in the morning. Our results are opposite to those of Brackelsberg in that more ewes (67%) in the late fed group (group 1) lambed between the two feeding times than in the early fed group (27%).

5.2 HORMONE PROFILES AT APPROXIMATELY 2 AND 3 WEEKS PRIOR TO EXPECTED PARTURITION

Cortisol, progesterone (P_4) and estrogen profiles were similar in the two groups in both trials. The time of feeding did not affect P_4 and estrogen profiles in any appreciable manner although it appeared to cause an increase in cortisol concentrations. Although P_4 concentration was significantly ($P<0.05$) higher in group 2 than group 1 in trial 2, this significant increase was due to the high P_4 concentration found in the twin bearing ewes in group 2 which outnumbered those in group 1. In addition, the two ewes with P_4 concentrations that were below the average at 2 weeks prior to lambing (Table 10), belonged to group 1. One ewe was carrying twins but gave birth to 2 dead lambs while the other ewe was carrying triplets but gave birth to one live lamb. These two ewes had P_4 concentration of 9.0 ng/ml and 13.8 ng/ml, respectively. Thus the low P_4 concentrations of these two ewes significantly decreased the group 1 mean. When these ewes were excluded from the group mean, there were no differences in P_4 concentrations between the groups.

The circulating steroid concentrations varied substantially within short-time intervals of 30 min and between animals as indicated by the small interpeak intervals (10-12 peaks/24h). The variability in cortisol concentration was the largest. It varied as much as 40 ng/ml in a 30 min interval. Cortisol variations in adult sheep plasma (McNatty and Thurley, 1973) and late pregnant maternal sheep plasma (Challis et al., 1981) have previously been reported and were thought to reflect a pulsatile release. This could be the reason for the wide variability in cortisol concentration seen in this study. Stress due to venipuncture

on untrained animals increases corticosteroid levels in ovine plasma (Bassett and Hinks, 1969). The ewes used in this study were accustomed to being handled and a number of blood samples had been collected by venipuncture prior to the intensive collection. In the second trial, the effects of stress were hopefully minimized even further by chronic catheterization and collection of samples without the animals' awareness. The cortisol concentrations in trial 1 were similar to the concentrations in the catheterized trial 2 ewes showing that venipuncture may not have affected cortisol levels in this study. It could be assumed then that the effects of stress in these animals due to handling and venipuncture was minimal.

Like cortisol, estrogen levels were similar in the two trials and showed substantial intra-animal variation between the 30 min and 1h collections and there was a large inter-animal variability. However, estrogen concentrations appeared higher in the second trial than in the first trial. The reason for the higher values in the second trial is not known and the reason for the high estrogen concentration at the beginning of sampling in trial 1 is also not known either. Variability in P_4 (Bassett et al.,1969; Challis et al., 1981) and estrogen (Challis and Patrick, 1981) concentration in late pregnant sheep have been reported previously. Intra-animal variability could possibly reflect hormone release while P_4 inter-animal variability could be due to the number of fetuses being carried by a ewe. The large placental mass in a multiple pregnancy possibly secretes more P_4 than the smaller mass in single bearing ewes.

The study of time-trends in individual animals made possible by frequent sampling of the same animal showed that a circadian periodicity may be present in the secretion of cortisol by maternal adrenals in late pregnancy. Cortisol levels were highest in the early morning hours (0100h to 0500h) and fluctuated greatly during the rest of the day. In addition, cortisol concentrations appeared to increase a few hours after feeding.

Conflicting results on the circadian periodicity of corticosteroids and the effect of feeding on corticosteroid concentrations in ruminants have been reported. Bassett (1974) found no influence of feeding on plasma corticosteroid concentrations and no evidence of a circadian rhythm in wether sheep. McNatty et al. (1972) on the other hand working with anestrous New Zealand Romney ewes reported a circadian rhythm in plasma cortisol levels with evidence of episodic release. They reported highest peaks at 0900h with lowest peaks occurring during the hours of darkness. When the animals were exposed to artificial lighting at night the cortisol peaks were then randomly distributed over a 24 h period. In our study under constant light, highest peaks were noticed in the early morning hours regardless of the time the ewes were fed.

In cattle, Mills and Jenny (1979) reported elevated glucocorticoids just prior to feeding and at all times sampled throughout the 12 h post feeding period in a group fed high concentrate. An early increase in cortisol concentration after the first feed was noticed at 2 weeks after the start of the trial in this study. This was probably in response to feeding since the ewes would be expected to be hungrier at this time than at the second feeding. The delay in the appearance of cortisol

increase after the second feeding (Figs. 4C,D). might be due to the fact that the ewes did not immediately consume their feed after it was given to them. In trial 2 the ewes were in complete confinement and were fed individually. The second feed of the day was still found in the troughs 2-3h after it was given to the ewes indicating that they did not consume this second feed immediately after it was offered. However, in trial 1, where the ewes ate communally, the feed was consumed as soon as it was given. This pattern of feed consumption could account for the differences seen in cortisol concentrations in the two trials. In the first trial, in both groups, cortisol concentrations increased (Figs. 4a and 4b) soon after the feed was given to the groups.

Trenkle (1978) reported an increase in corticosteroids in ruminants when feed intake was reduced. A daily corticosteroid rhythmn has been reported in pregnant and non pregnant lactating dairy cattle (Macadam and Eberhart, 1972). They found highest corticosteroid concentrations between 0230h and 0630h with low concentrations at 1630h and attributed the rhythmn to the rigid daily management routines. They did not find any effect of pregnancy on the daily rhythm. This is similar to the high early morning cortisol concentration found in the present report. Daily routines cannot be ruled out as the cause for the cortisol rhythm found in this study. Trial 2 ewes were in complete confinement but were still disturbed at feeding time and during cleaning of the experimental rooms. Hudson et al. (1974) on the other hand found no diurnal rhythmn of cortisol concentrations in four dairy cows and explained the lack of rhythmicity to the fact that ruminants do not enter deep states of sleep and that corticosteroid activities can be related to a sleep-wake

schedule. In the present study, the early morning peaks of cortisol levels might reflect the anticipation for food, or a period of expected activity during the awakening period. Morag (1967) states that sheep fed only finely chopped grass entered states of deep sleep but when their diets were changed to unlimited amounts of hay they did not enter deep states of sleep because rumination requires both time and consciousness. The ewes in the present study were fed grain and some hay, and in the second trial they were fed alfalfa pellets and grain. It might be stated by inference that these ewes might have gone into deep states of sleep and the high morning cortisol concentrations might reflect the peak activity at awakening.

The increase in cortisol concentration after feeding, though not of equal magnitude to the early morning rise might be caused by the feeding activity. Light and food have been suggested to be the major synchronizers of the pituitary-adrenal system in the rat. Continous light abolishes the circadian periodicity of corticosterone (Takahashi et al., 1977) while time of feeding has been shown to be a more potent synchronizer of corticosterone levels with peaks appearing just before feeding time or just after feeding (Takahashi et al., 1977; Krieger and Hauser, 1978; Kato et al., 1980). The present study made no comparisons between continous light and light-dark periods. Continous lighting however did not seem to eliminate the cortisol rhythms in the present study.

In primates, in pregnant and nonpregnant states, a cortisol circadian rhythm with inverse relationship to P_4 and estriol have been reported (Reck et al., 1978; Challis et al., 1980; Junkerman et al., 1982).

Maternal cortisol levels in monkeys were reported to be highest in the early morning hours coinciding with lowest P_4 levels, while lowest cortisol levels were found around midnight coinciding with the highest P_4 levels. It was suggested that elevated maternal cortisol levels suppressed release of P_4 precursors (Challis et al., 1980), and also that elevated cortisol levels were responsible for decreasing estriol levels (Reck et al., 1978). Both P_4 and estrogen concentrations did not show any such inverse relationship to cortisol concentration in the late pregnant sheep in this study and did not show a relationship to feeding. However, smoothed estrogen and P_4 profiles showed a rhythm with lows and highs appearing at different times in different ewes suggesting the presence of a rhythm in the steroid concentration in every ewe. It is doubtful whether feeding plays a major role in determining changes in P_4 and estrogen concentrations. There have been no previous reports on effects of feeding on P_4 and estrogen levels in pregnant or nonpregnant sheep.

The breeds used in this study did not make a significant contribution to the concentration of the maternal steroids. However, the number of fetuses a ewe was carrying significantly ($P<0.05$) increased maternal P_4 concentration. Twinning increased P_4 levels by at least 30%. This is in agreement with previous reports (Bassett et al., 1969). At this stage of pregnancy, most of the P_4 in circulation is derived from the placenta (Linzell and Heap, 1968) and this increase in P_4 reflects the placental size. It is interesting to note that the ewe carrying triplets but giving birth to one live lamb (Table 10) had P_4 concentrations similar to ewes carrying single fetuses, suggesting that the placental area

carrying the dead fetus may not function fully as an endocrine organ. This is further supported by the lack of increase in estrogen concentration and complete P_4 withdrawal prepartum in ewes delivering dead lambs (Fig. 24). The ewe that gave birth to two dead fetuses had a lower P_4 concentration than ewes carrying one live fetus. This would also indicate that the placenta carrying dead fetuses was not functioning fully as an endocrine organ.

Estrogen and cortisol concentrations were not different between ewes with single fetuses and those with multiple fetuses. Previous reports have indicated conflicting results on the effect of twinning on estrogen concentrations. Adelakoun et al. (1977) reported increased estrogen concentrations in cows bearing twins while Thimonier et al. (1977) reported increase in estrogen concentrations with increase in total birth weight but not necessarily twinning.

5.3 HORMONE PROFILES AT THE END OF PREGNANCY

The frequent sampling of the same animal for 6 to 7 days before lambing done in this study, showed a steady clearcut decline in P_4 concentration, an abrupt steep rise of estrogen concentration and also a modest rise of cortisol concentration. Maternal plasma P_4 concentrations decreased during the last 6 days of pregnancy with a precipitous decline begining at 3 to 4 days before lambing. Estrogen concentration rose during the last 12-36 hr with the steepest rise occurring at about 10 hr prepartum, cortisol concentration increased consistently during the last 12-36 h. The steroid concentrations in maternal plasma found in this study and others would indicate that there

is a change in placental steroid synthesis just prior to parturition. It is believed that during the last 2 to 3 weeks of pregnancy in the sheep, the fetal adrenal goes through a maturational process which leads to an increased secretion of cortisol (Magyar et al., 1980b, 1981). The fetal cortisol is thought to act directly on the placenta (Flint and Burton, 1984) to induce enzyme activities necessary for synthesis of estrogen (Anderson et al., 1975, 1978a,b, 1981; Flint et al.,1975a; Flint and Ricketts, 1979). Thus the placenta is believed to change from an organ synthesizing primarily P_4 (Linzell and Heap, 1968), to an organ synthesizing estrogens. The change in circulating steroid levels is associated with onset of labor and delivery. Thus, the mean concentration of estrogen and P_4 in maternal plasma can provide a reasonable index of alterations in fetal adrenal secretion rate. The data indirectly supports the hypothesis that fetal plasma cortisol which begins to increase during the last 2 to 3 weeks of pregnancy (Magyar et al., 1980b, 1981), stimulates conversion of P_4 to estrogens by inducing placental 17α-hydroxylase and 17,20 lyase activities (Anderson et al., 1975, 1981; Flint et al., 1975a).

The decline of P_4 concentration reported in this study for catheterized (trial 2) and non-catheterized (trial 1) ewes is similar to previous reports (Bassett et al., 1969; Fylling, 1970; Bedford et al., 1972a; Strott et al., 1974; Burd et al., 1976; Thorburn and Challis, 1979; Carson and Challis, 1981; Cabalum et al., 1982). There was a marked inter-animal variability 6 days before lambing but this variability was reduced at the time of lambing. Thus the terminal P_4 concentrations reached at parturition were less variable between animals

and within animals compared to 6 days before lambing. The ewes with higher P_4 levels had steeper declines than the ewes with lower P_4 concentrations. The inter- and intra- animal variability has been reported by others (Bassett et al., 1969; Strott et al., 1974). The inter-animal variability in this study was mostly attributed to twinning and the number of live fetuses being carried by a ewe, while the intra animal variability could reflect P_4 secretion.

The reduction in P_4 concentration prepartum has been suggested to be by placental conversion of P_4 to pregnenolone (Anderson et al., 1975) through induction of placental 17α-hydroxylase activities. The P_4 peak amplitudes progressively decreased as parturition time approached indicating a progressive reduction in P_4 synthesis or an increased excretion rate. Withdrawal of inhibitory effects of P_4 on uterine contractility plays a part in physiological mechanisms regulating labor (Csapo, 1956). This may not be a major function at parturition since in dexamethasone-induced parturition, large doses of P_4 (150 mg/day) failed to delay onset of labor although it prevented cervical dilatation (Liggins, 1973). Progesterone withdrawal is said to play a facilitory role in parturition by allowing release of $PGF_2\alpha$ (Liggins and Grieves, 1971; Liggins et al., 1973).

Plasma estrogen concentrations increased sharply during the last 24 hr before lambing. The sharp increase in estrogen concentrations has been reported previously (Challis, 1971; Bedford et al., 1972a; Carson and Challis, 1981; Challis et al., 1981; Challis and Patrick, 1981; Cabalum et al., 1982; Yu et al., 1983) and are similar in pattern and magnitude to results reported here. The increase in estrogen

concentration at term could increase uterine blood flow (Rosenfeld et al., 1980) and/or induce $PGF_2\alpha$ production (Thorburn and Challis, 1979). It has been reported that $PGF_2\alpha$ and PGFM increase in maternal vena caval and aortic plasma at spontaneous and ACTH-induced labor (Olson et al., 1985). The sharp increase in estrogen prepartum might be mediating $PGF_2\alpha$ release which has its effects on myometrial contraction. In support of this hypothesis is the finding that when $PGF_2\alpha$ production is inhibited by meclofenamic acid (a PG synthesis inhibitor), delivery of live lambs is prevented in the presence of decreased P_4 and increased estrogen concentration (Mitchell and Flint, 1978a). In nonpregnant ovariectomized ewes, P_4 treatment followed by estrogen administration produced large and increased $PGF_2\alpha$ concentrations in caruncles and jugular venous plasma (Louis et al., 1977).

The source and nature of estrogen precursor steroids have been suggested to be placental P_4 (Anderson et al.,1975; Flint et al., 1975b, 1976), fetal and maternal plasma pregnenolone and pregnenolone sulphate (Nathanielsz et al., 1982) and androstenedione (Steele et al., 1976a). The placenta is considered the major source of the aromatizable androgens. The decrease in P_4 which precedes the increase in estrogen suggests and supports the contention that circulating P_4 may act as a precursor to estrogen synthesis (Bedford et al., 1972a) and that P_4 withdrawal at term may involve metabolism of P_4 to estrogens by activation of placental enzymes (Anderson et al., 1975, 1981).

Progesterone decline in this study preceded estrogen rise in all animals. However, in this study the mean P_4 concentrations were 20 ng/ml at about 2 weeks before lambing and decreased to 16 ng/ml at 6

days before lambing. At this time there was no concurrent increase in estrogen concentration that may have been attributed to this decline in P_4 production. The major decline in P_4 production began about a week before the onset of labour and P_4 concentration decreased to about 3 ng/ml a day before lambing. At this time the P_4 decline was followed by an estrogen rise and progesterone was probably being converted to estrogen at this time. Comparison of the P_4 peak amplitudes at 2 and 3 weeks prior to delivery (Table 16) indicates that P_4 secretion had already started to decline at 2 weeks prior to delivery. The early decline in P_4 concentration is probably due to reduction in secretion rate.

Ewes carrying twins and triplets would be expected to have more circulating estrogen precursors since they have significantly higher P_4 levels. However the concentrations of estrogen prepartum in twin and triplet carrying ewes in both trials were not significantly($P>0.05$) higher than those bearing singletons. This would possibly indicate that circulating P_4 concentrations are not the major estrogen synthesis precursors or that aromatization is the rate limiting step.

Unlike the normal live births which were associated with a prepartum estrogen surge, there was no preparturient maternal estrogen rise in the one ewe that was carrying two dead lambs (Fig. 24). In this ewe there was a complete lack of P_4 and a rise in cortisol. Labor was initiated but the ewe had to be assisted. A similar lack of preparturient estrogen surge in ewes with dead fetuses has been reported by Carter *et al.* (1976) and Carson and Challis (1981). Commencement of labor may be initiated by the abrupt drop in P_4 leading to release of the block to

myometrial contraction and also release of $PGF_2\alpha$ but normal delivery requires an estrogen surge. Progesterone withdrawal is thought to be a sufficient stimulus to induce PG release (Ledger et al., 1985) as shown by treatment of pregnant sheep with epostane, a P_4 synthesis inhibitor. In the monkey, delivery of a dead fetus was associated with an increase in PGF production (Mitchell et al.,1976).

Maternal cortisol showed a significant increase during the last 2 days of pregnancy although two of the ewes did not show this terminal rise. Increased maternal cortisol prepartum has been reported previously (Chamley et al., 1973; Thompson and Wagner, 1974; Burd et al., 1976; Magyar et al., 1981; Cabalum et al., 1982) although Drost et al. (1973) did not detect it. Cabalum et al. (1982) found a terminal cortisol rise but with no overall time-trend pattern. The increase in cortisol concentrations in this study began 36 h to 48 h before lambing. The interpeak intervals were about 6h compared to 2h at 2 and 3 weeks prior to delivery. Parturition was always associated with an increasing cortisol concentration. The cyclic periods probably reflect episodic release. It is not clear what the relation is between the increasing cortisol levels in the cycle and parturition but could be due to labor. The increase in cortisol production prepartum is due to an increase in the responsiveness of the maternal adrenals to ACTH prepartum (Glickman et al., 1980). The cause for the increased responsiveness of maternal adrenals to ACTH is not known. It has been suggested that the concentrations of different steroids in maternal plasma at the end of pregnancy might influence adrenal responsiveness to ACTH or there might be a change in the properties or number of ACTH receptors on the

maternal adrenal (Glickman et al., 1980). In support of these two suggestions is the demonstration in rats by Parkes and Deansley (1966) that the adrenal response to stress is lower in the male than the female and the finding that the stimulatory effect of ACTH-(1-24) on maternal adrenal cells was partially antagonized by α -melanocyte stimulating hormone (α-MSH) at term but not at other times during pregnancy. Thurley and McNatty (1973) reported that stress induced a greater rise in plasma corticosteroid concentrations in pregnant sheep than in non-pregnant sheep. The increase in maternal cortisol prepartum may thus be due to increased cortisol release from the highly sensitive maternal adrenals to ACTH. Another possibility is that a placental corticotrophin hormone such as that reported in man (Rees et al., 1975a) influences responsiveness of maternal adrenals and withdrawal of this factor after delivery leads to a decrease in cortisol production (Fig. 18 trial 1).

Progesterone and estrogen were not altered by feeding during the last few days before lambing but cortisol appeared to increase after feeding. The magnitude of the P_4 peaks progressively decreased as parturition time approached and the variability within the animal and between animals reduced considerably. This reflects the declining production rate of P_4. Estrogen peaks on the other hand increased somewhat in magnitude (Table 16) as parturition approached probably reflecting their active secretion. The peak amplitude of cortisol remained about the same but there were large standard errors in peak amplitudes because of "split" peaks. The interpeak intervals in all the three hormones increased as parturition approached. Due to the large interval between

sample collection (1hr), frequent variations in the steroid profiles were probably missed, but the data clearly shows that the steroid concentrations were more stable (as shown by the large interpeak intervals) as parturition time drew nearer than at 2 and 3 weeks before parturition.

Gestation lengths were similar in the three breeds used in the study. The highly fertile Finnish Landrace have been reported to have shorter gestation length of about 144-145 days while crossbreds have a longer gestation length of about 148-149 days (Terrill, 1974). The gestation lengths found in this study (144.6 d for Finnish Landrace, 147.5 d for Suffolks and 147.6 d for crossbreds) agree with the literature, but the number of animals used in the study were too few to give any statistical significance.

VI

CONCLUSION

Lambing was concentrated in the daytime in both groups with 78% and 64% daytime (between 0700-1900h) lambings in trials 1 and 2, respectively. Time of feeding did not affect time of lambing in these ewes. The data does not support the contention that time of lambing is altered by time of feeding, but lambing between the two feeding times was higher in group 1 (67%) than in group 2 (27%). The number of animals involved in the study were too few to make a definitive conclusion on the effect of feeding time on lambing time.

The data clearly indicates that the steroid-hormonal milieu in maternal plasma undergoes substantial changes as parturition approaches. It would support the hypothesis that there is a change in placental enzyme activities just prior to lambing with an increase in enzymes and factors leading to synthesis of estrogen. The overall steroid concentration changes in maternal plasma in late pregnancy were not different between the ewes in the two different feeding groups. Time of feeding did not affect progesterone and estrogen concentrations but appeared to increase cortisol concentration both at 2 and 3 weeks prior to expected lambing and during the last week of pregnancy. Differences in P_4 concentration existed between the two groups in trial 2, but this difference was a result of between group differences in number of lambs per ewe.

Feeding did not significantly ($P>0.05$) alter the steroid peak amplitudes, peak length or interpeak interval although the peak length for cortisol was a little longer for a few hours after feeding. Peak progesterone amplitudes declined from 4.9ng at 2 weeks prior to delivery to 1.8ng a few days before delivery in trial 1. The decrease in P_4 amplitude reflects declining P_4 production. Cortisol peak amplitudes did not change over time, estrogen peak amplitudes slightly increased ($P>0.05$) as parturition approached. The peak length and the interpeak interval of each steroid progressively increased as parturition approached thereby resulting in less variability in circulating concentrations of these steroids within each ewe.

A pattern of variation in plasma cortisol concentration was found, suggestive of a daily rhythmn with higher concentrations being seen in the early morning hours (0100h to 0500h) with lower concentrations observed between 0700h-0900h. This increase in cortisol concentration is suggested to be an endogenous rhythm due to expected awakening. It is suggested that a circadian periodicity may be present in the secretion of cortisol by maternal adrenals in late pregnancy and that this periodicity (night time high cortisol concentration) is not affected by time of feeding. The effect of feeding on cortisol concentration is separate from the early morning rise. The progesterone and estrogen profiles between the groups were similar at 2 and 3 weeks after the start of the trial and later. The appearance of the profiles after removal of the peaks suggest that both P_4 and estrogen have circadian rhythms but with peaks and troughs appearing at different times in different ewes.

Ewes carrying twins and triplets had higher progesterone levels than ewes carrying singletons. The progesterone concentration depended on the number of live fetuses being carried by a ewe. Breeds on the other hand did not have a significant effect on the hormone concentrations until about four hours before lambing when estrogen levels were 30% higher in the Finnish Landrace than in the other breeds.

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Appendix A

STEROID CONCENTRATIONS AT 4 WEEKS AFTER START OF TRIAL 1
(2 WEEKS PRIOR TO EXPECTED DELIVERY)

(Each value is a mean of two determinations)

TIME	EWE 1 LANDRACE FINN LAMB NUMBER - SINGLE PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML	EWE 2 SUFFOLK LAMB NUMBER - TWINS PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0700	15.8	52	19	22.5	45	15
0800	15.8	33	11	24.0	50	18
0900	16.5	69	5	31.5	42	12
1000	12.3	43	9	34.5	44	13
1100	21.0	40	7	30.0	51	12
1200	12.3	31	7	30.0	67	8
1300	15.0	43	8	31.5	70	14
1400	13.2	49	8	30.0	51	15
1500	18.0	49	7	30.0	70	25
1600	25.5	38	10	25.5	43	28
1700	15.8	52	8	30.0	66	14
1800	15.8	55	7	30.0	38	17
1900	16.5	50	7	19.5	61	17
2000	18.0	63	8	24.0	57	
2100	16.5	42	7	30.0	67	12
2200	21.0	67	8	24.0	74	11
2300	15.8	66	3	25.5	64	36
2400	14.1	68	8	25.5	64	7
0100	16.5	77	6	25.5	91	9
0200	14.1	59	7	25.5	71	11
0300	19.5	71	8	24.0	82	13
0400	16.5	53	5	25.5	76	14
0500	18.0	50	6	31.5	42	10
0600	14.1	44	7	25.5	61	10
0700	16.5	42	6	25.5	66	11
0730	18.0	39	6	25.5	62	8
0800	18.0	54	5	19.5	82	10
0830	19.5	51	4	25.5	62	7
0900	18.0	46	6	22.5	66	9
0930	15.0	39	4	24.0	37	6
1000	19.5	43	5	23.3	57	6
1030	18.0	45	4	19.5	40	7
1100	13.2	52	3	22.5	38	9
1130	19.5	51	6	18.0	43	10
1200	18.0	44	5	22.5	44	8
1230	15.0	37	7	21.0	45	9
1300	16.5	41	6	24.0	38	10
1330	16.5	46	5	22.5	38	7
1400	16.5	40	5	22.5	44	8
1430	18.0	42	7	22.5	49	6
1500	19.5	51	7	21.0	55	9
1530	16.5	38	9	19.5	75	10
1600	16.5	54	9	22.5	52	6
1630	18.0	57	10	24.0	65	6
1700	16.5	39	6	21.0	50	6
1730	14.1	31	6	25.5	40	6
1800	14.1	44	7	30.0	76	6
1830	15.0	41	6	25.5	48	7
1900	15.8	37	7	22.5	34	7
1930	16.5	31	6	24.0	61	6
2000	15.8	39	5	24.0	47	7
2030	15.0	29	5	24.0	71	10
2100	16.5	45	7			8
2130	13.2	35	5	24.0	66	22
2200	11.1	31	7	21.0	60	6
2230	13.2	54	6	19.5	78	7
2300	15.8	43	8	24.0	51	8
2330	15.0	36	14	22.5	62	9
2400	12.0	33	6	24.0	39	8
2430	14.1	30	7	24.0	47	9
0100	14.1	42	7	25.5	77	9
0130	15.0	34	7	25.5	53	8
0200	15.0	42	4		30	14
0230	15.0	33	7	21.0	76	7
0300	14.1	43	7	25.5	50	9
0330	16.5	34	7	22.5	30	8
0400	15.0	75	8	24.0	66	11
0430	12.3	77	8	21.0	46	12
0500	15.8	38	8	24.0	13	10
0530	14.1	37	7	24.0	43	11
0600	12.3	52	8	22.5	48	13
0630	14.1	45	7	21.0	51	13

TIME	EWE 3 CROSSBRED LAMB NUMBER - TWINS PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML	EWE 4 CROSSBRED LAMB NUMBER - SINGLE PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0700	25.5	70	25	15.3	63	20
0800	25.5	54	14	12.1	50	16
0900	21.0	61	12	13.3	52	30
1000	33.0	77	14	13.6	53	11
1100	28.5	78	14	15.7	70	4
1200	33.0	90	9	18.0	77	11
1300	21.0	71	13	19.8	54	12
1400	28.5	88	17	12.8	76	12
1500	28.5	102	20	13.8	72	32
1600	28.5	53	16	16.5	50	11
1700	25.5	63	11	12.2	80	14
1800	33.0	71	14	15.4	67	13
1900	30.0	68	10	16.9	67	7
2000	21.0	78	10	16.3	62	11
2100	22.5	89	25	15.9	51	9
2200	25.5	94	8	14.6	70	9
2300	18.0	87	10	16.6	56	14
2400	28.5	63	11	19.7	57	6
0100	25.5	68	17	16.3	77	8
0200	25.5	94	16	13.7	81	9
0300	25.5	62	16	18.8	87	12
0400	22.5	77	17	19.9	82	10
0500	25.5	86	16	18.5	71	8
0600	18.0	85	15	24.1	82	10
0700	22.5	66	14	15.2	92	8
0730	19.5	91	10	13.8	88	12
0800	22.5	67	18	14.7	92	8
0830	25.5	58	20	16.6	75	12
0900	21.0	61	19	15.8	79	12
0930	18.0	62	12	18.2	71	10
1000	28.5	49	16	17.4	76	8
1030	19.5	44	15	19.3	77	11
1100	22.5	62	16	17.2	82	9
1130	18.0	59	15	19.9	102	6
1200	25.5	51	15	22.4	92	9
1230	25.5	58	14	21.3	96	9
1300	28.5	64	10	20.4	98	9
1330	19.5	54	13	20.6	103	11
1400	19.5	69	10	17.7	91	9
1430	18.0	56	14	25.1	97	9
1500	28.5	63	14	18.5	101	7
1530	22.5	70	17	16.7	93	8
1600	30.0	75	15	21.7	87	8
1630	28.5	80	19	18.2	91	10
1700	22.5	69	18	15.4	82	10
1730	28.5	55	16	22.8	83	9
1800	22.5	52	16	18.5	83	11
1830	30.0	51	9	20.3	82	7
1900	28.5	48	14	18.1	73	6
1930	28.5	49	14	18.3	84	11
2000	22.5	53	16	18.3	82	8
2030	22.5	93	15	20.7	86	8
2100	30.0	67	15	22.0	80	5
2130	22.5	50	14	21.4	75	7
2200	22.5	74	13	17.9	85	9
2230	22.5	80	14	15.5	88	9
2300	25.5	63	16	21.3	80	10
2330	22.5	53	12	13.0	90	12
2400	22.5	76	12	15.4	72	10
2430	22.5	72	12	18.7	96	10
0100	25.5	85	11	14.9	89	10
0130	25.5	84	10	17.7	91	8
0200	28.5	91	7	17.9	83	10
0230	28.5	72	7	16.4	94	12
0300	18.0	68	10	19.8	73	17
0330	25.5	106	9	23.9	87	14
0400	28.5	69	8	22.1	92	14
0430	28.5	59	8	15.2	86	13
0500	22.5	59	7	18.4	77	9
0530	28.5	71	7	17.9	83	9
0600	22.5	76	6	16.6	70	10
0630	28.5	66	7	16.2	75	11

EWE 5 CROSSBRED
LAMB NUMBER - SINGLE

TIME	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0700	15.7	68	25
0800	18.0	48	22
0900	15.0	66	13
1000	15.7	88	14
1100	18.0	77	14
1200	24.0	74	14
1300	19.5	63	16
1400	19.5	67	21
1500	19.5	68	20
1600	19.5	42	20
1700	18.0	94	12
1800	16.5	81	17
1900	13.2	98	14
2000	21.0	48	12
2100	15.0	86	12
2200	22.5	56	15
2300	18.0	65	12
2400	19.5	91	14
0100	13.2	101	17
0200	22.5	84	18
0300	15.7	89	16
0400	15.7	72	19
0500	21.0	57	13
0600	18.0	91	17
0700	12.3	79	11
0730	15.0	55	12
0800	11.1	88	15
0830	14.1	70	15
0900	14.1	81	14
0930	16.5	58	16
1000	15.0	36	12
1030	15.0	39	15
1100	16.5	80	15
1130	15.7	57	18
1200	11.1	43	10
1230	11.1	62	14
1300	12.0	52	14
1330	11.1	40	14
1400	13.2	32	13
1430	9.6	91	13
1500	12.3	52	15
1530	9.6	107	15
1600	8.7	120	13
1630	9.6	129	16
1700	12.0	113	15
1730	13.2	84	14
1800	21.0	106	10
1830	16.5	95	15
1900	14.1	68	12
1930	14.1	56	15
2000	15.7	54	15
2030	18.0	93	13
2100	18.0	69	9
2130	19.5	78	11
2200	18.0	124	13
2230	19.5	130	13
2300	22.5	123	10
2330	22.5	126	10
2400	24.0		15
2430	22.5	110	11
0100	19.5	104	12
0130	18.0	93	10
0200	18.0	69	10
0230	18.0	82	13
0300	18.0	80	8
0330	19.5	61	9
0400	15.7	105	9
0430	18.0	92	7
0500	16.5	75	10
0530	18.0	56	7
0600	15.0	89	10
0630	16.5	75	9

EWE 6 CROSSBRED
LAMB NUMBER - SINGLE

PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
15.0	51	28
18.0	30	40
19.5	41	17
16.5	43	19
18.0	43	19
16.5	59	18
18.0	51	20
16.5	70	23
15.7	49	
16.5	43	25
18.0	71	19
18.0	60	20
19.5	52	18
18.0	40	22
16.5	52	16
15.7	72	15
15.0	45	18
14.4	66	17
18.0	91	17
19.5	70	7
15.0	80	17
19.5	87	23
15.7	75	16
12.3	87	18
16.5	47	15
15.7	88	14
16.5	64	15
15.7	34	12
18.0	79	12
19.5	48	12
18.0	36	13
16.5	29	11
19.5	24	17
22.5	37	16
16.5	62	13
16.5	47	12
16.5	62	10
15.0	41	12
18.0	35	9
13.5	36	10
13.5	27	11
9.3	62	8
10.5	84	11
12.3	50	12
11.7	89	13
13.5	48	16
13.5	52	15
14.4	63	13
15.7	66	12
15.7	36	14
15.7	32	16
15.0	72	14
16.5	40	13
15.0	30	14
15.0	62	16
11.7	42	14
15.7	45	13
15.7	69	17
15.0	45	11
14.4	67	15
14.4	73	14
14.4	43	15
14.4	46	12
11.7	74	13
13.5	60	14
19.5	39	13
13.5	81	11
15.7	78	10
18.0	51	17
16.5	36	14
14.4	93	15
15.7	68	16

TIME	EWE 7 SUFFOLK LAMB NUMBER - SINGLE			EWE 8 CROSSBRED LAMB NUMBER - TWINS		
	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0700	15.7	35	9	21.9	70	20
0800	16.5	38	8	23.7	81	14
0900	15.7	59	6	20.3	59	20
1000	19.5	52	7	22.4	89	22
1100	18.0	58	7	19.3	76	23
1200	15.7	54	9	25.9	76	24
1300	15.7	43	9	22.4	59	30
1400	18.0	40	9	23.1	73	19
1500	15.7	36		27.0	78	22
1600	16.5	40	11	24.3	60	19
1700	21.0	38	7	24.0	66	16
1800	16.5	22	14	25.9	62	14
1900	15.7	32	8	23.3	87	15
2000	18.0	69	11	22.1	88	15
2100	15.0	35	7	28.2	58	11
2200	18.0	67	8	19.2	100	13
2300	16.5	38	12	33.0	67	11
2400	16.5	44	8	29.2	74	16
0100	22.5	52	8	27.8	59	14
0200	18.0	39	7	30.4	84	13
0300	19.5	26	7	24.4	78	11
0400	15.7	56	8	25.6	97	11
0500	21.0	58	6	27.0	102	13
0600	18.0	51	7	27.8	97	15
0700	16.5	34	7	27.2	61	14
0730	23.3	37	6	27.6	90	12
0800	22.5	37	5	30.2	75	12
0830	22.5	37	5	27.4	62	15
0900	19.5	41	6	24.5	70	14
0930	16.5	50	6	25.1	71	13
1000	15.7	37	5	31.0	68	12
1030	18.0	42	7	28.6	94	13
1100	15.7	50	7	29.4	70	11
1130	19.5	38	8	29.0	102	14
1200	21.0	53	7	29.8	96	14
1230	18.0	77	8	27.3	89	16
1300	22.5	71	7	19.2	56	13
1330	21.0	45	6	27.6	71	16
1400	24.0	41	4	23.8	68	12
1430	25.5	46	7	21.6	91	12
1500	22.5	47	4	22.0	56	12
1530	22.5	72	5	24.5	73	12
1600	22.5	46	4	23.6	80	13
1630	21.0	46	5	20.5	71	10
1700	22.5	43	4	20.5	88	10
1730	16.5	48	4	20.3	77	12
1800	22.5	47	5	24.0	83	18
1830	16.5	48	5	20.7	87	18
1900	22.5	81	6	22.9	107	17
1930	18.0	57	7	25.5	75	16
2000	22.5	66	13	25.1	74	10
2030	18.0	46	9	23.1	97	11
2100	21.0	40	8	23.7	79	11
2130	19.5	35	8	26.8	73	17
2200	19.5	49	7	25.1	102	15
2230	21.0	38		22.6	73	12
2300	21.0	59	7	17.7	72	13
2330	22.5	52	7	23.2	102	14
2400	19.5	49	6	20.2	62	15
2430	21.0	46	7	24.6	95	14
0100	24.0	61	7	19.4	98	14
0130	18.7	44	7	25.5	86	16
0200	21.0	47	7	27.7	66	13
0230	19.5	54	7	21.7	109	15
0300	24.0	59	7	20.4	78	14
0330	18.7	43	6	23.3	87	15
0400	21.0	48	5	24.0	111	14
0430	22.5	64	5	24.3	95	13
0500	22.5	44	6	23.6	72	12
0530	22.5	40	6	32.5	72	13
0600	21.0	45	6	23.6	91	13
0630	25.5	59	7	33.4	62	14

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EWE 9 CROSSBRED
LAMB NUMBER - SINGLE

TIME	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0700	22.0	50	31
0800	15.4	68	15
0900	14.5	86	16
1000	16.0	82	19
1100	14.8	73	14
1200	16.7	91	22
1300	18.1	45	13
1400	21.9	67	14
1500	18.0	60	27
1600	25.7	48	14
1700	16.6	60	14
1800	23.6	62	12
1900	22.4	75	13
2000	23.8	90	13
2100	23.4	72	17
2200	16.5	95	16
2300	26.1	51	14
2400	21.9	85	16
0100	20.3	50	13
0200	24.1	81	15
0300	23.2	55	13
0400	24.5	80	11
0500	21.4	85	21
0600	19.7	66	19
0700	17.5	57	18
0730	16.6	81	22
0800	17.0	67	16
0830	17.4	52	15
0900	24.8	72	18
0930	15.4	61	34
1000	18.1	55	19
1030	14.5	87	19
1100	18.8	65	19
1130	16.7	89	19
1200	16.8	85	15
1230	17.4	73	19
1300	18.1	92	21
1330	24.2	63	24
1400	18.7	50	19
1430	19.1	68	19
1500	25.2	53	14
1530	19.6	74	17
1600	16.4	57	18
1630	14.1	81	20
1700	23.7	69	22
1730	23.6	81	26
1800	22.6	89	20
1830	25.4	70	19
1900	19.3	97	17
1930	22.0	73	17
2000	27.9	55	18
2030	26.1	90	17
2100	16.8	72	25
2130	22.4	50	13
2200	16.8	81	17
2230	18.8	63	22
2300	21.2	81	17
2330	19.9	62	17
2400	16.1	90	25
2430	20.2	86	24
0100	16.2	70	21
0130	18.8	78	21
0200	19.9	86	24
0230	20.6	79	12
0300	16.9	77	16
0330	18.4	82	29
0400	20.8	77	24
0430	18.7	86	22
0500	21.5	68	23
0530	21.9	53	28
0600	18.0	83	27
0630	23.7	65	17

EWE 10 LANDRACE FINN
LAMB NUMBER - TWINS

PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
21.1	64	10
20.1	66	13
20.4	52	10
22.4	68	10
22.0	82	12
21.5	88	11
24.6	68	11
23.8	55	13
28.8	57	18
24.7	47	12
29.8	79	12
28.0	83	9
33.0	77	12
25.8	83	12
26.7	65	11
22.5	71	11
24.1	74	14
24.1	84	12
21.7	69	9
24.2	76	10
23.4	78	10
26.9	79	10
18.5	76	7
19.6	66	8
18.6	58	13
24.9	67	10
23.6	57	5
31.8	50	9
23.4	55	10
22.3	47	12
15.1	82	2
24.4	70	4
21.3	73	13
26.2	82	10
18.7	63	8
23.1	66	8
19.3	70	9
20.5	58	11
19.0	65	8
22.2	55	9
25.4	66	11
21.5	61	10
30.2	58	14
23.2	87	11
19.0	75	14
16.3	69	14
20.2	84	12
17.6	90	13
24.9	80	12
24.0	55	10
31.9	68	13
24.8	56	12
25.3	57	9
23.6	86	12
24.5	58	10
25.9	80	10
22.3	66	12
22.6	67	12
13.7	82	13
23.7	77	11
22.9	68	12
18.3	76	11
24.6	71	12
24.6	76	11
21.4	81	12
21.0	91	14
21.9	68	10
24.3	88	18
22.1	86	14
29.5	77	10
23.6	79	11
15.4		13

EWE 11 CROSSBRED
LAMB NUMBER - TWINS

TIME	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0700	23.8	39	13
0800	26.0	40	12
0900	20.7	66	13
1000	19.3	61	9
1100	18.6	80	14
1200	16.9	62	14
1300	23.2	59	11
1400	19.6	51	13
1500	22.2	68	13
1600	21.6	55	10
1700	21.5	53	11
1800	23.5	55	11
1900	17.5	76	12
2000	18.3	78	11
2100	25.1	68	11
2200	22.5	81	12
2300	20.7	50	13
2400	21.3	93	14
0100	25.8	74	11
0200	20.2	80	14
0300	25.0	70	12
0400	26.9	73	11
0500	25.9	75	14
0600	19.9	100	15
0700	25.8	53	8
0730	22.7	26	8
0800	23.9	59	8
0830	21.4	55	11
0900	21.6	46	10
0930	19.7	70	11
1000	30.6	71	10
1030	23.7	58	11
1100	22.0	60	13
1130	20.6	62	10
1200	21.1	85	10
1230	21.8	79	13
1300	18.6	69	14
1330	22.2	61	11
1400	21.5	45	14
1430	20.5	60	9
1500	26.3	47	10
1530	26.2	75	16
1600	22.6	49	11
1630	16.8	60	11
1700	20.6	81	9
1730	21.5	62	10
1800	22.6	61	10
1830	21.7	92	9
1900	20.9	58	11
1930	20.5	80	11
2000	24.9	60	10
2030	21.8	86	10
2100	23.9	69	9
2130	23.7	51	9
2200	18.6	84	10
2230	22.8	57	10
2300	18.9	55	8
2330	15.3	64	10
2400	20.7	79	9
2430	20.5	74	9
0100	17.7	73	14
0130	20.5	66	7
0200	25.0	78	8
0230	22.2	78	7
0300	20.5	62	9
0330	23.2	72	7
0400	26.4	74	6
0430	24.1	66	5
0500	21.3	69	5
0530	21.6	74	5
0600	28.7	45	5
0630	26.7	79	5

EWE 12 CROSSBRED
LAMB NUMBER - SINGLE

PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
17.8	59	9
17.8	75	13
15.0	75	17
20.0	70	12
15.5	89	14
16.9	88	18
21.3	41	17
16.4	52	17
15.1	59	20
15.3	55	20
18.2	88	13
22.2	50	10
19.4	78	11
20.0	55	11
16.1	58	11
16.4	64	14
15.9	64	17
14.7	59	14
19.9	92	9
15.8	79	11
19.7	94	9
15.9	54	8
16.1	86	8
19.4	80	11
20.8	65	8
23.0	67	9
20.3	68	14
16.3	65	9
17.4	54	9
15.1	74	11
17.1	63	12
18.8	79	11
20.7	83	13
16.1	67	9
18.1	82	10
12.6	49	13
16.2	86	15
19.1	69	11
14.9	52	11
14.9	69	16
14.1	75	14
16.0	77	11
16.0	50	12
14.6	43	12
16.7	88	10
	93	
18.1	75	10
18.1	84	10
20.1	87	11
19.9	59	10
20.6	66	7
22.9	87	14
21.7	70	13
12.2	72	11
20.8	96	15
14.1	79	10
15.4	62	15
14.9	79	13
19.6	77	15
18.5	91	10
18.8	86	12
19.8	76	12
24.9	92	13
15.1	82	15
22.0	82	15
13.7	67	13
14.7	104	16
21.7	71	12
13.0	61	15
19.0	70	11
	59	
22.9	49	12

Appendix B

STEROID CONCENTRATIONS DURING THE LAST 7 DAYS OF PREGNANCY IN TRIAL 1

(Each value is a mean of two determinations)

EWE 3

HRS BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
-2	3.1	78	28
0	5.4	78	89
2	6.0	103	74
4	5.4	83	78
6		64	83
8	5.4	88	103
10	7.2	88	125
12	6.7	89	97
14	9.0	99	79
16	11.1	92	88
18	12.3	81	85
20	10.5	65	73
22	11.1	91	77
24	14.4	92	56
26	11.7	71	53
28	13.5	76	45
30	16.5	79	35
32	14.4	71	37
34	18.0	96	38
36	12.3	76	48
38	19.5	103	16
40	15.7	75	28
42	14.4	54	22
44	28.5	79	24
46	19.5	74	12
48	17.8	90	21
50	19.5	74	24
52	22.5	85	18
54	19.5	62	20
56	21.0	75	24
58	18.0	49	15
60	18.0	71	20
62	19.5	63	11
64	19.5	76	24
66	22.5	81	16
68	22.5	58	14
70	21.0	90	19
72	21.0	53	13
74	21.0	81	12
76	19.5	49	14
78	16.5	58	17
80	22.5	46	19
82	22.5	55	23
84	22.5	46	18
86	21.0	74	13
88	25.5	43	18
90	21.0	48	17
92	22.5	61	14
94	28.5	72	17
96	22.5	67	18
98	25.5	65	22
100	19.5	69	13
102	22.5	53	12
104	22.5	68	17
106	19.5	41	18
108	18.0	54	17
110	18.0	71	19
112	21.0	45	19
114	21.0	57	20
116	19.5	56	21
118	22.5	73	24
120	21.0	72	20
122	28.5	56	23
124	28.5	37	13
126	28.5	36	18
128	22.5	41	17
130	21.0	50	14
132	22.5	47	15
134	19.5	62	17
136	28.5	66	16
138	22.5	71	10
140	22.5	54	13
142	19.5	68	17
144	21.0	71	14
146	15.7	74	11
148	16.5	45	15
150	18.0	49	14
152	15.0	67	13
154	19.5	32	11
156	15.0	60	16
158	22.5	27	14
160	21.0	47	16
162	19.5	40	18
164	21.0	48	16
166	20.0	70	16

EWE 2

HRS BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
-2	2.8	58	10
0	3.4	106	35
2	5.7	101	39
4	5.4	94	25
6	9.0	103	64
8	7.8	96	43
10	6.9	91	25
12	5.7	89	28
14	7.2	77	29
16	6.9	69	21
18	8.7	89	16
20	8.4	74	27
22	12.0	89	17
24	10.8	78	13
26	9.0	89	19
28	14.1	81	19
30	14.1	64	17
32	15.7	86	14
34	15.0	82	23
36	13.2	85	20
38	15.0	71	23
40	15.2	78	16
42	14.9	67	17
44	15.0	72	22
46	15.4	46	

EWE 4

HRS BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
-2	1.6	82	16
0	1.3	111	67
2	3.0	130	49
4	2.7	103	55
6	3.1	107	46
8	2.3	87	38
10	2.5	86	82
12	2.5	89	65
14	2.9	96	49
16	3.0	82	59
18	3.8	84	50
20	3.9	91	52
22	4.8	89	41
24	4.3	97	39
26	4.8	82	57
28	5.7	86	56
30	4.7	71	56
32	6.1	74	48
34	5.9	93	59
36	6.4	100	42
38	8.2	105	29
40	7.3	104	33
42	7.3	106	35
44	8.4	96	26
46	9.0	100	35
48	6.8	86	34
50	5.5	78	26
52	7.4	89	24
54	8.4	86	29
56	8.7	62	26
58	9.4	83	20
60	6.4	62	19
62	7.7	99	20
64	8.0	94	20
66	8.4	69	18
68	7.9	87	15
70	10.0	66	21
72	11.0	71	11
74	12.2	68	19
76	8.7	77	8
78	11.3	87	21
80	12.1	84	18
82	11.2	83	20
84	13.5	76	16
86	9.9	67	18
88	9.2	92	15
90	12.7	84	29
92	9.2	82	21
94	13.1	104	17
96	13.2	88	22
98	12.9	86	28
100	11.2	74	23
102	13.1	84	27
104	13.0	86	16
106	14.4	78	32
108	13.6	90	25
110	13.4	95	23
112	14.1	76	24
114	14.9	76	24
116	16.3	88	22
118	15.9	86	19
120	14.2	82	21
122	13.7	67	18
124	13.9	98	17
126	15.0	95	14
128	13.8	84	16
130	15.6	75	14
132	11.7	79	18
134	13.7	62	12
136	16.9	76	16
138	13.4	87	13
140	15.5	94	14
142	13.1	99	14
144	14.2	90	17
146	15.6	78	16
148	13.5	81	16
150	14.3	97	12
152	12.3	92	9
154	13.6	61	12
156	19.9	88	11
158	16.5	93	13
160	16.9	64	17
162	14.4	95	18
164	10.9	66	11
166	17.4	88	10

EWE 5

HRS BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
-2	3.7	93	11
0	6.0	110	30
2	6.9	74	52
4	8.4	93	39
6	8.7	81	23
8	7.5	92	22
10	5.4	93	27
12	10.2	85	26
14	10.2	45	15
16	12.0		25

EWE 6

HRS BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
-2	2.0	48	40
0	2.1	81	79
2	4.2	66	69
4	3.6	81	92
6	4.5	69	79
8	6.3	85	57
10	5.7	77	71
12	5.4	81	62
14	5.1	74	81
16	6.6	83	60
18	6.8	91	38
20	8.4	84	34
22	9.3	86	32
24	7.2	82	36
26	5.7	90	48
28	9.9	92	34
30	9.9	84	31
32	9.3	77	33
34	11.7	82	25
36	13.5	74	23
38	11.7	53	19
40	9.3	86	15
42	14.4	62	17
44	13.5	30	14
46	11.7	81	15
48	10.5	83	16
50	13.5	59	18
52	10.5	61	15
54	9.9	88	18
56	15.0	56	15
58	9.9	96	18
60	11.1	80	16
62	12.3	48	18
64	15.7	59	13
66	12.3	71	11
68	11.7	59	19
70	9.9	52	8
72	9.0	66	13
76	16.5	39	16
78	15.0	74	16
80	14.4	58	17
82	15.0	67	16
84	16.5	49	12
86	15.7	44	17
88	15.7	69	12
90	12.3	36	11
92	13.5	62	11
94	18.0	40	14
96	15.7	48	16
98	18.0	62	19
100	15.0	45	18
102	13.5	53	16
104	19.5	45	16
106	16.5	69	18
108	16.5	77	24
110	19.5	48	19
112	15.7	47	19
114	14.4	78	16
116	18.0	43	16
118	16.5	53	15
120	15.7	58	18
122	18.0	75	19
124	16.5	38	13
126	15.7	79	18
128	18.0	69	16
130	16.5	62	17
132	16.5	77	16
134	16.5	80	20
136	22.5	36	14
138	18.0	63	11
140	16.5	40	20
142	16.5	55	14
144	16.5	53	13
146	16.5	59	13
148	18.0	80	20
150	18.0	70	20
152	18.0	70	17
154	18.0	54	18
156	21.0	51	15
158	21.0	41	20
160	19.5	51	17
162	19.5	39	15
164	18.0	51	17
166	22.5	36	16

EWE 7

HRS BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
-2	4.6	74	8
0	5.4	80	7
2	4.6	65	28
4	5.2	45	15
6	5.7	69	19
8	7.8	74	23
10	7.8	58	14
12	6.6		19
14	9.6	60	10
16	7.5	56	15
18	6.9	45	13
20	9.6	59	12
22	6.9	44	12
24	7.5	63	12
26	6.9	55	11
28	9.0	56	.
30	9.0	72	11
32	9.6	62	10
34	10.8	40	9
36	8.7	49	10
38	10.8	55	6
40	10.2	47	6
42	15.0	39	6
44	9.6	39	5
46	9.0	38	6
48	12.0	49	9
50	18.0	39	7
52	11.1	33	8
54	11.1	44	9
56	11.1	49	12
58	8.7	38	7
60	12.3	54	6
62	12.3	34	7
64	11.9	36	8
66	13.3	50	8
68	12.5	45	8

EWE 8

HRS BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
-2	1.6	69	12
0	2.1	71	72
2	2.3	94	59
4	2.9	106	63
6	3.1	90	41
8	3.3	85	59
10	3.9	90	52
12	4.9	86	42
14	4.1	87	22
16	5.3	83	36
18	4.9	81	24
20	5.8	102	22
22	5.9	93	27
24	7.2	87	34
26	5.3	73	29
28	6.2	89	26
30	6.4	68	26
32	7.0	70	21
34	7.5	91	19
36	8.6	90	21
38	7.6	109	14
40	8.4	79	19
42	11.1	83	18
44	8.7	81	15
46	10.1	69	24
48	9.9	81	24
50	9.5	52	12
52	13.2	74	18
54	14.8	100	16
56	12.1	79	21
58	12.5	84	21
60	11.9	70	24
62	14.7	67	19
64	16.4	67	19
66	13.4	57	18
68	12.9	78	22
70	14.2	66	18
72	13.7	88	22
74	15.5	68	22
76	13.9	58	19
78	13.9	71	15
80	14.9	103	20
82	19.6	101	15
84	11.9	75	19
86	18.3	66	26
88	16.0	43	15
90	18.2	40	18
92	17.7	62	22
94	18.5	51	22

EWE 9

HRS BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
-2	2.8	86	22
0	3.3	110	83
2	3.5	92	83
4	3.4	58	83
6	4.6	83	78
8	4.8	77	45
10	6.1	75	47
12	5.3	60	45
14	5.7	60	46
16	6.5	71	38
18	8.9	71	30
20	7.4	56	26
22	8.7	66	29
24	8.1	70	25
26	9.8	59	23
28	10.4	71	28
30	11.7	50	21
32	11.1	24	22
34	10.9	40	26
36	11.6	57	26
38	10.3	75	34
40	12.9	51	22
42	13.6	61	24
44	14.9	57	24
46	13.5	42	28
48	15.5	58	23
50	12.4	71	25
52	12.9	51	31
54	12.3	46	30
56	12.6	44	19
58	11.7	41	16
60	14.7	45	27
62		45	21

EWE 11

HRS BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML	HRS BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
-2	1.7	67	6	94	11.2	58	14
0	2.9	91	33	96	13.2	74	20
2	3.3	100	31	98	9.5	62	15
4	2.6	88	48	100	10.9	77	13
6	2.5	81	25	102	10.9	68	15
8	3.2	64	38	104	11.7	56	16
10	5.6	62	31	106	11.7	63	11
12	5.4	79	20	108	13.3	50	15
14	3.5	69	25	110	13.4	67	18
16	5.5	64	13	112	13.3	58	14
18	6.4	74	23	114	11.8	48	13
20	4.4	66	23	116	13.0	68	15
22	4.5	68	22	118	13.8	75	15
24	4.3	69	23	120	13.3	82	17
26	5.5	57	17	122	12.5	69	13
28	6.0	61	21	124	13.0	73	18
30	7.2	54	19	126	9.6	54	15
32	10.8	36	20	128	11.4	75	24
34	7.1	70	19	130	12.0	51	31
36	9.1	47	14	132	13.3	52	16
38	9.3	64	14	134	12.9	54	15
40	6.5	53	18	136	13.1	37	21
42	8.5	45	13	138	11.9	42	18
44	9.2	73	18	140	12.1	61	19
46	8.9	69	21	142	11.9	38	17
48	9.2	86	17	144	13.9	54	19
50	10.6	46	16	146	11.5	65	19
52	10.6	51	17	148	12.2	83	12
54	9.8	52	23	150	11.3	52	11
56	10.0	32	26	152	13.0	59	10
58	8.0	62	15	154	14.2	62	8
60	8.3	63	18	156	12.7	65	5
62	8.6	58	20	158	15.7	46	7
64	6.6	68	23	160	12.5	57	11
66	8.2	72	16	162	9.0	73	11
68	9.1	62	19	164	13.0	89	13
70	8.6	73	10	166	12.1		9
72	11.1	57	14				
74	8.5	93	16				
76	9.6	70					
78	10.1	74	21				
80	10.2	68	18				
82	9.1	63	17				
84	10.9	67	13				
86	12.3	68	14				
88	12.2	66	15				
90	9.8	56	17				
92	10.8	63	18				

EWE 12

HRS BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML	HRS BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
-2	1.4	82	5	96	9.6	75	12
0	1.4	82	43	98	12.8	85	13
2	2.1			100	8.6	78	12
4	2.5	84	87	102	9.6	48	15
6	2.3	77	100	104	8.8	74	13
8	2.4	78	84	106	7.9	74	11
10	3.2	84	65	108	10.8	47	13
12	3.9	82	62	110	8.3	74	13
14	3.9	70	48	112	9.5	82	14
16	4.4	82	55	114	10.9	73	25
18	3.6	74	47	116	6.3	87	16
20	4.6	93	32	118	12.1	45	9
22	5.3	89	20	120	8.7	85	9
24	5.8	82	29	122	11.8	53	12
26	5.3	85	27	124	8.4	78	12
28	5.9	88	25	126	10.2	59	15
30	7.5	85	20	128	8.7	56	11
32	5.8	83	27	130	10.1	64	10
34	4.6	89	17	132	11.2	51	14
36	5.5	51	16	134	11.5	51	10
38	6.4	76	17	136	11.7	86	11
40	6.6	89	14	138	7.7	65	10
42	6.5	88	17	140	9.3	69	10
44	7.6	79	19	142	9.2	78	12
46	9.9	76	17	144	12.4	56	13
48	5.6	79	16	146	11.2	59	11
50	7.3	88	20	148	10.1	60	12
52	5.3	87	22	150	14.2	64	13
54	5.9	79	23	152	11.3	75	11
56	8.1	86	13	154	11.0	79	14
58	8.2	70	15	156	14.0	73	15
60	5.9	79	20	158	15.1	67	16
62	6.9	78	15	160	13.7	71	11
64	9.5	75	16	162	11.2	83	14
66	10.2	71	17	164	11.2	88	13
68	8.5	74	18	166	13.4	80	14
70	10.1	82	16				
72	8.5	75	15				
74	9.2	68	18				
76	9.6	84	26				
78	9.9	54	22				
80	8.7	53	10				
82	10.4	83	12				
84	9.7	49	12				
86	10.5	73	13				
88	12.4	53	10				
90	9.7	91	14				
92	7.0	77	13				
94	10.9	63	13				

Appendix C

TWENTY-FOUR HOUR STEROID CONCENTRATIONS AT 2 WEEKS AFTER THE START OF TRIAL 2 (3 WEEKS PRIOR TO EXPECTED DELIVERY)

(Each value is a mean of two determinations)

EWE 13 LANDRACE FINN
LAMB NUMBER - TRIPLETS

PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
FEED		
38	33	35
32	28	23
35	33	24
29	53	28
20	43	24
28	40	24
29	57	23
30	37	21
33	38	23
30	42	25
32	37	21
30	35	27
28	45	27
23	50	26
27	54	24
FEED		
31	53	26
34	35	25
30	44	23
21	45	24
24	36	30
20	49	28
25	50	24
23	34	24
22	53	22
24	37	23
23	57	25
18	37	22
26	47	20
21	30	25
25	38	18
25	37	20
23	54	24
32	55	22
43	49	22
45	43	21
FEED		
44	35	26
40	39	23
41	31	22
39	28	35
32	36	20

EWE 14 CROSSBREED
LAMB NUMBER - SINGLE

PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
FEED		
35	63	22
30	49	21
34	75	22
39	100	20
30	87	20
	116	17
10	130	19
11	121	24
21	61	18
19	97	22
16	70	22
12	97	21
17	66	17
12	86	18
18	87	20
FEED		
10	42	19
13	56	22
16	69	22
20	99	20
21	76	21
11	75	27
12	120	23
11	69	28
13	106	24
11	47	30
14	100	47
13	61	24
11	82	26
14	106	40
33	93	23
16	56	25
15	105	26
16	83	29
14	57	30
18	97	25
FEED		
18	72	30
16	72	30
35	56	20
11	93	24
12	65	23

EWE 15 LANDRACE FINN
LAMB NUMBER - TRIPLETS

PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
FEED		
50	75	20
32	69	20
13	78	30
14	68	27
11	88	27
12	105	29
14	106	24
9	110	19
13	77	33
18	43	21
13	48	28
14	49	19
14	75	33
13	57	19
11	52	23
FEED		
13	29	18
13	50	20
15	47	18
11	40	22
12	71	20
9	57	21
11	40	20
14	50	19
11	78	18
13	46	17
9	64	28
8	40	25
12	30	21
9	36	28
13	76	24
10	39	22
6	36	16
19	83	29
12	42	20
14	37	20
FEED		
12	36	19
14	39	22
13	54	17
13	51	26
15	65	23

EWE 16 CROSSBRED
LAMB NUMBER - TWINS

PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
FEED		
11	54	18
8	26	16
9	65	15
10	43	18
10	95	19
8	46	16
8	55	16
8	60	16
8	90	18
8	53	18
8	51	15
5	49	32
8	44	27
12	43	25
FEED		
11	39	27
12	105	25
9	30	23
8	39	19
8	41	25
6	33	25
7	60	28
8	43	16
9	76	10
8	41	12
8	38	14
8	37	20
6	34	20
8	33	20
7	66	20
7	48	23
9	39	25
9	43	25
15	74	25
13	39	25
8	39	25
FEED		
7	41	26
6	36	24
9	50	22
17	33	24
19	31	21

EWE 17 CROSSBRED
LAMB NUMBER - SINGLE

PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
FEED		
20	48	21
10	103	22
12	49	21
13	98	22
14	137	25
11	113	31
17	74	20
15	106	34
14	118	24
13	45	28
10	37	27
14	37	24
9	69	25
10	102	22
FEED		
12	69	21
20	90	22
11	42	23
11	31	14
8	61	30
11	38	21
9	112	16
8	127	16
11	140	26
10	140	22
13	119	22
13	58	23
12	42	17
18	44	21
11	83	17
16	45	24
30	46	26
21	87	29
16	51	19
FEED		
15	53	23
19	35	23
11	99	22
15	34	21
13	100	20

EWE 19 CROSSBRED
LAMB NUMBER - SINGLE

PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
25	55	20
28	105	24
25	50	26
29	60	28
FEED		
28	66	20
23	87	29
29	53	24
25	66	21
33	84	24
32	65	22
19	50	23
24	55	29
29	74	21
34	60	28
29	40	20
25	72	20
26	54	21
23	86	26
25	58	20
27	52	20
26	38	21
29	89	24
26	87	27
FEED		
26	68	23
23	99	24
27	118	.
20	79	28
19	71	.
19	82	20
21		26

EWE 20 LANDRACE FINN
LAMB NUMBER - TRIPLETS

PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
24	53	27
21	48	23
19	45	22
20	46	24
FEED		
18	45	26
20	116	23
19	80	22
17	81	26
18	92	25
17	51	25
15	47	25
17	60	21
20	73	25
14	60	23
21	64	23
15	57	23
17	61	25
17	78	22
17	74	28
20	48	22
18	99	20
18	58	24
19	67	26
FEED		
17	40	20
17	52	20
17	76	22
17	66	20
28	70	25
27		
27	82	20
24		

EWE 21 LANDRACE FINN
LAMB NUMBER - SINGLE

PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
9	45	24
24	18	26
21	20	25
22	20	28
FEED		
20	20	27
13	40	27
19	25	21
14	42	22
15	31	28
12	16	24
12	17	22
16	34	24
13	17	26
13	35	20
17	25	16
FEED		
17	29	21
19	25	19
12	16	19
11	20	20
16	28	19

EWE 22 LANDRACE
LAMB NUMBER - TWINS

PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
35	28	17
30	64	19
28	26	19
24	39	20
FEED		
32	79	16
33	65	16
36	55	22
35	59	19
29	88	18
29	46	25
23	86	17
27	73	21
FEED		
27	76	20
36	57	22
28	43	17
26	48	24
26	54	20
31	43	15
29	65	24
29	46	22

EWE 23 CROSSBRED
LAMB NUMBER - TWINS

PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
42	58	19
35	59	19
38	81	21
35	96	21
FEED		
37	73	23
40	53	21
39	64	23
36	90	20
38	71	22
36	104	24
37	106	23
39	69	25
39	77	21
38	57	27
36	98	31
38	48	24
.	48	32
39	93	26
35	62	23
37	79	28
39	99	23
35	111	24
34	70	26
FEED		
38	54	29
40	82	23
40	57	17
41	47	22
34	83	20
40	53	23
34	73	26
47		29

Appendix D

TWENTY-FOUR HOUR STEROID CONCENTRATIONS AT 3 WEEKS AFTER START OF TRIAL 2 (2 WEEKS PRIOR TO EXPECTED DELIVERY)

(Each value is a mean of two determinations)

EWE 12 FINNISH LANDRACE
LAMB NUMBER - TRIPLETS

	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
Feed	33	45	19
	32	56	20
	27	58	22
	29	57	16
	36	58	18
	32	36	24
	30	36	24
	32	34	22
Feed	32	45	20
	23	59	22
	32	56	19
	29	32	20
	26	32	23
	32	52	24
	32	38	19
	31	54	16
	32	52	17
	28	37	16
	31	33	25
	29	36	19
	27	41	23
	28	27	20

EWE 14 CROSSBRED
LAMB NUMBER - SINGLE

	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
Feed	13	44	25
	14	26	28
	16	63	21
	11	49	28
	12	50	30
	7	29	21
	12	33	22
Feed	12	47	25
	18	43	20
	15	48	22
	14	33	24
	15	45	19
	14	36	17
	23	49	18
	11	43	14
	14	33	20
	14	46	22
	11	32	23
	16	38	19
	15	44	22
	17	30	20
	12	52	24
	15	42	21
	15	42	26
Feed	14	38	26
	12	26	21
	11	33	25
	13	55	27
	10	41	22
	10	48	23

EWE 15 FINNISH LANDRACE
LAMB NUMBER - TRIPLETS

	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
Feed	12	55	24
	10	47	23
	10	78	21
	10	78	25
	11	83	31
	14	56	27
	10	68	26
	16	76	28
Feed	14	59	27
	14	75	24
	13	75	26
	10	46	20
	14	62	21
	7	81	23
	14	59	23
	12	77	27
	16	76	23
	17	95	26
	13	73	26
	9	70	28
	13	82	29
	16	96	26
	19	59	21
Feed	11	80	28
	15	83	24
	16	82	23
	11	71	27
	12	89	23
	12	59	16
	12	72	28

EWE 16 CROSSBRED
LAMB NUMBER - TWINS

	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
Feed	6	64	20
	6	37	24
	6	80	15
	7	94	20
	7	53	14
	8	33	26
	6	33	29
	8	50	21
Feed	8	64	22
	8	52	26
	9	60	25
	5	61	23
	7	63	27
	6	65	25
	7	57	29
	7	85	20
	7	48	28
	6	58	24
	10	58	25
	10	40	21
	6	67	25
	8	73	23
	10	66	20
Feed	6	54	25
	5	29	24
	6	78	24
	5	70	21
	6	61	26

EWE 17 CROSSBRED
LAMB NUMBER - SINGLE

	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
Feed	29	55	25
	10	131	22
	9	159	29
	12	150	21
	12	124	25
	10	145	26
	13	105	23
	13	48	28
Feed	9	81	24
	12	110	22
	14	83	20
	11	115	25
	12	30	20
	14	61	22
	10	63	21
	12	47	27
	11	66	24
	13	96	28
	14	34	21
	11	48	22
Feed	12	67	24
	11	52	28
	25	87	24
	13	40	25

EWE 18 FINNISH LANDRACE
LAMB NUMBER - SINGLE

	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
Feed	27	28	24
	18	30	20
	17	37	28
	15	41	24
	16	19	26
	16	10	24
	18	23	28
Feed	18	30	29
	16	24	29
	22	14	28
	8	30	25
	12	29	26
	17	16	20
	17	23	20
	14	36	29
	16	37	25
	13	34	27
	21	11	24
	18	20	22
	18	23	29
Feed	21	41	29
	20	16	22
	23	22	25
	13	37	28
	23	15	27
	15	12	26
	15	55	25
	17	42	28
	15	15	29
	11	24	27

EWE 19 CROSSBRED
LAMB NUMBER - SINGLE

	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
Feed	23	118	23
	20	71	23
	17	59	25
	14	43	26
	20	47	23
	26	42	24
	17	75	21
	20	113	19
Feed	20	54	22
	23	46	34
	18	31	29
	20	59	30
	20	40	37
	17	37	23
	17	37	30
	18	88	34
	23	64	29
	20	45	30
	22	43	24
	21	100	24
Feed	20	44	37
	23	87	26
	24	69	30
	24	59	33
	27	78	17
	20	105	32
	20	41	29
	25	54	30

EWE 20 FINNISH LANDRACE
LAMB NUMBER - TRIPLETS

	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
Feed	27	67	32
	25	110	27
	25	77	33
	25	92	30
	30	69	19
	24	74	18
Feed	25	25	23
	17	23	21
	17	27	28
	36	31	22
	31	29	21
	30	32	20
	56	29	21
	30	30	27
	31	30	22
	30	30	20
	30	34	27
	31	29	21
	31	32	20
	30	24	28
Feed	27	30	28
	30	27	24
	23	27	24
	28	21	22
	30	35	22
	24	26	28
	30	30	26
	28	30	23
	24	48	29
	23	31	26

EWE 21 FINNISH LANDRACE
LAMB NUMBER - SINGLE

	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
Feed	11	18	24
	15	21	29
	12	32	20
	16	34	25
	14	18	25
	12	18	31
	12	13	21
Feed	27	19	25
	20	18	25
	13	29	27
	19	35	22
	20	42	19
	27	38	14
	20	25	14
	30	28	26
	18	35	19
	21	50	27
	21	32	22
	20	27	22
	13	32	20
Feed	20	44	25
	25	28	17
	10	39	19
	17	32	27
	18	31	13
	16	47	19
	17	20	18

EWE 22 FINNISH LANDRACE
LAMB NUMBER - TWINS

	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
Feed	41	48	26
	32	74	26
	33	62	25
	30	61	32
	28	47	27
	30	61	29
	35	66	24
	34	60	26
Feed	25	84	26
	25	69	31
	30	44	23
	26	46	23
	26	93	21
	32	49	22
	31	51	23
	26	92	25
	28	55	23
	31	56	22
	30	55	33
	28	67	31
	31	62	29
	31	43	26
	31	53	27
Feed	28	63	25
	26	67	23
	27	108	21
	27	47	21
	30	59	23
	33	41	31
	25	46	27

EWE 23 CROSSBRED
LAMB NUMBER - TWINS

	PROGEST NG/ML	CORTISOL NG/ML
Feed	47	51
	39	84
	42	62
	40	66
	35	67
	37	62
	37	83
Feed	36	100
	32	103
	32	97
	35	77
	36	100
	35	55
	40	62
	38	77
	38	96
	37	66
	42	78
	38	87
	38	82
Feed	40	99
	42	97
	41	79
	38	80
	36	61
	37	87
	35	76
	38	89
	40	78
	37	79

Appendix E

STEROID CONCENTRATIONS DURING THE LAST 7 DAYS OF PREGNANCY IN TRIAL 2

(Each value is a mean of two determinations)

EWE 14

HR BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML	HRS BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0	1	52	140	68	8	34	20
1	1	51	114	69	11	35	21
2	1	51	94	70	7	54	22
3	1	52	96	71	7	39	24
4	2	52	59	72	7	48	17
5	2	53	63	73	8	36	20
6	2	43	62	74	8	50	21
7	2	52	58	75	7	41	23
8	2	48	44	76	8	36	24
9	2	51	39	77	7	35	21
10	2	59	38	78	7	52	27
11	3	56	40	79	8	54	24
12	2	51	40	80	7	39	24
13	3	48	38	81	7	48	18
14	2	47	38	82	9	51	22
15	3	53	40	83	9	45	16
16	3	52	31	84	9	44	24
17	3	51	36	85	9	46	15
18	3	51	30	86	5	48	27
19	3	61	28	87	7	42	14
20	3	51	30	88	7	51	29
21	3	53	31	89	8	55	26
22	4	55	24	90	8	51	20
23	4	45	24	91	7	45	17
24	4	55	23	92	7	48	25
25	4	53	24	93	8	54	22
26	4	47	23	94	8	44	22
27	6	52	30	95	7	47	22
28	5	52	26	96	8	50	18
29	4	46	24	97	8	46	19
30	5	40	23	98	8	43	25
31	4	56	30	99	8	50	19
32	5	51	26	100	8	45	26
33	4	59	23	101	8	40	19
34	4	53	23	102	8	38	24
35	5	42	24	103	9	33	21
36	4	47	20	104	7	56	19
37	5	52	19	105	8	57	18
38	4	44	21	106	8	51	22
39	5	49	25	107	9	42	21
40	5	44	23	108	9	47	16
41	6	57	23	109	9	51	22
42	4	54	23	110	10	45	18
43	8	54	26	111	9	55	24
44	7	50	24	112	9	45	20
45	7	55	28	113	9	42	17
46	11	54	28	115	9	41	21
47	6	46	22	116	10	37	20
48	6	38	23	117	7	43	21
49	6	37	21	118	11	48	20
50	6	47	24	119	10	37	21
51	6	55	26	120	11	60	24
52	5	41	26	121	12	56	21
53	6	37	24	122	13	49	20
54	5	42	24	123	10	49	20
55	7	55	26	124	10	52	21
56	7	54	23	125	5	55	19
57	8	41	23	126	4	48	19
58	6	41	26	127	6	29	21
59	5	43	26	128	5	43	17
60	3	55	24	129	7	41	29
61	6	42	18	130	10	53	26
62	6	47	27	131	12	38	24
63	7	50	23	132	11	48	28
64	6	37	24	133	10	46	29
65	5	45	22	134	9	34	27
66	5	48	26				
67	7	44	23				

EWE 15

HR BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0	2	102	181
1	2	96	148
2	2	101	124
3	2	80	120
4	2	117	116
5	2	122	123
6	3	119	128
7	3	122	121
8	4	T70	35
9	4	117	45
10	4	86	15
11	6	79	16
12	10	71	15
45	12	90	15
46	12	85	14
47	11	135	17
48	10	147	18
49	11	98	20
50	10	104	16
51	10	108	19
52	12	83	19
53	14	121	17
54	14	78	14
55	14	81	14
56	14	71	12
57	14	110	12
58	17	117	10
59	14	142	12
60	14	139	11
61	16	125	10
62	16	165	14
63	14	146	12
64	15	159	15
65	16	154	12
66	16	133	13
67	14	145	12
68	19	161	13
69	17	157	13
70	12	159	12
71	12	149	12
72	8	130	16
73	13	76	18
74	17	68	22
75	12	54	16
76	11	109	27
77	11	86	22
102	7	147	22
108	10	151	24
109	13	150	23
110	13	82	25

EWE 16

HR BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0	1	155	15
1	1	74	20
2	1	119	12
3	1	130	11
4	1	96	13
5	1	89	14
6	1	68	12
7	1	129	14
8	1	69	14
9	1	109	18
10	1	75	11
11	1	74	15
12	1	105	14
13	1	70	14
14	1	80	15
15	1	141	13
17	1	135	25
18	1	71	19
19	1	42	21
20	1	80	22
21	1	108	15
22	1	123	15
23	1	96	15
24	2	74	12
25	3	104	14
26	3	95	16
27	2	93	11
28	3	77	14
29	2	84	15
30	3	43	18
31	4	38	16
32	4	36	18
33	2	46	17
34	2	34	19
35	2	69	16
36	3	49	14
37	2	86	16
38	3	43	13
39	3	96	11
40	2	67	13
41	2	64	13
42	3	55	17
43	2	79	14
44	3	27	16
45	4	28	.
46	4	80	18
47	3	97	11
48	3	111	20
49	4	122	10
50	6		.
51	4	17	10

EWE 17

HR BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML	HR BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0	1	97	124	68	5	72	17
1	1	112	117	69	5	68	22
2	2	82	81	70	7	104	21
3	3	123	95	71	6	71	16
4	3	105	74	72	6	67	19
5	4	98	52	73	7	54	17
6	3	113	53	74	7	49	17
7	3	128	53	75	6	81	18
8	4	97	48	76	6	45	23
9	4	71	63	77	6	93	24
10	5	65	43	78	7	58	22
11	3	100	50	79	7	48	20
12	3	72	43	80	7	39	20
13	3	106	45	81	7	56	18
14	3	111	39	82	7	84	20
15	4	90	44	83	12	51	17
16	4	66	45	84	6	111	16
17	3	66	37	85	7	94	19
18	4	76	34	86	7	115	26
19	4	78	32	87	5	88	23
20	3	79	36	88	7	114	22
21	4	109	34	89	5	78	23
22	4	92	41	90	8	63	22
23	4	98	35	91	7	95	21
24	5	101	32	92	8	64	27
26	5	52	30	93	11	97	22
27	6	52	35	94	8	60	38
28	5	105	27	95	7	114	19
29	5	98	37	96	8	71	24
30	4	96	17	97	8	69	25
31	5	85	25	98	8	52	24
32	5	77	28	99	10	108	26
33	5	64	27	100	7	103	22
34	5	70	22	101	9	110	27
35	5	95	23	102	8	23	28
36	5	88	26	103	7	103	24
37	6	46	24	104	9	75	23
38	4	65	23	105	8	80	21
39	5	23	26	106	7	49	21
40	5	44	26	107	8	90	27
41	5	45	27	108	10	125	28
42	6	95	22	109	11	56	22
43	6	102	24	110	7	67	28
44	5	45	27	111	9	61	28
45	5	70	21	112	12	61	24
46	6	66	23	113	10	116	26
47	6	61	28	114	8	105	28
48	6	47	24	115	9	72	20
49	6	100	24	116	.	43	22
50	6	52	24	117	9	79	21
51	6	111	39	118	9	81	27
52	6	67	27	119	10	76	24
53	8	35	25	120	8	106	29
54	6	108	24	121	9	65	23
55	5	113	23	122	9	56	23
56	5	66	22	123	9	69	29
57	6	56	19	124	9	112	29
58	5	50	20	125	9	94	24
59	6	50	22	126	9	97	21
60	6	47	21	127	8	86	20
61	6	75	17	128	7	54	21
62	6	90	16	129	9	100	21
63	7	98	22	130	7	101	24
64	5	85	22	131	8	100	24
65	6	.	26	132	9	71	24
66	6	75	19	133	11	32	20
67	5	121	21	134	8	88	22

EWE 18

HR BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML	HR BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0	5	70	66	112	20	22	21
1	5	45	54	113	21	19	26
2	8	46	75	114	24	45	18
3	8	41	56	115	19	16	23
4	7	63	41	116	22	28	20
5	9	18	20	117	21	24	19
6	6	40	48	118	24	29	16
40	14	26	28	119	16	52	23
41	12	61	20	120	16	44	21
42	11	59	20	121	18	60	21
43	15	40	16	122	18	58	25
44	15	34	20	123	18	39	25
45	14	46	17	124	18	44	19
46	13	68	17	125	22	77	17
47	11	41	20	126	19	69	23
48	13	79	17	127	21	63	24
49	19	40	17	128	21	72	19
50	19	87	18	129	25	73	24
52	17	94	19	130	24	83	18
53	16	64	16	131	21	87	28
54	16	90	14	132	23	86	26
55	18	71	15	133	25	78	28
56	16	62	14	134	29	25	27
57	15	84	15				
58	15	79	14				
59	19	78	15				
60	18	70	19				
61	16	74	15				
62	16	81	14				
63	19	77	18				
64	19	86	16				
65	18	86	22				
66	18	83	25				
67	20	88	26				
68	14	84	25				
69	14	86	25				
70	14	79	19				
71	16	78	19				
72	17	34	26				
102	14	51	20				
103	16	20	25				
104	14	27	20				
105	20	21	22				
106	18	28	25				
107	18	30	23				
108	18	45	19				
109	14	25	25				
110	14	13	21				
111	14	52	21				

EWE 19

HR BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0	5	89	100
1	4	119	95
2	5	109	88
3	5	83	79
4	5	105	89
5	5	125	85
6	6	132	70
7	6	100	94
8	5	62	53
9	7	110	57
10	8	116	49
11	9	93	50
12	8	96	43
13	7	89	36
14	7	104	37
15	9	61	41
16	8	63	35
17	7	90	40
18	9	97	33
19	9	65	40
20	9	81	41
21	8	97	38
22	9	98	42
23	9	74	35
24	9	104	40
25	9	113	31
26	9	108	31
27	8	73	33
28	9	68	37
29	9	96	37
30	10	101	36
31	9	111	61
32	9	101	31
33	11	66	31
34	9	74	32
35	11	66	21
36	10	57	20
37	10	51	22
38	12	95	27
39	13	102	26
40	10	82	28
41	12	71	29
42	10	64	28
43	11	68	27
44	11	83	17
45	13	70	18
46	16	54	24
47	17	50	15
50	11	44	17
51	14	77	23
52	12	67	24
53	10	114	23
54	14	82	32
55	27	66	29
56	34	100	26
57	20	78	32
58	14	47	28
59	17	56	25
60	18	48	27
61	17	80	29
62	15	106	28
63	15	75	28
64	15	45	28
65	15	75	26
66	18	65	26
67	15	43	29
68	13	82	27
69	16	56	33
70	17	50	29
71	16	50	31
73	20	53	23
74	15	79	25
75	15	54	26
76	24	59	31
77	16	76	22
78	19	65	26
79	16	111	26
80	18	98	26
81	18	73	27
82	20	71	26
83	18	52	31
85	17	80	27
86	23	91	30
87	15	65	35
88	19	91	31
89	21	80	24
90	18	90	25
91	17	47	29
92	22	104	26
93	18	67	28
94	23	36	32
95	21	62	28
96	19	57	27
97	18	57	28
98	19	77	25
99	17	47	29
100	19	86	29
101	22	45	32
102	22	72	33
103	19	45	33
104	20	100	28
105	20	106	29
106	21	50	27
107	21	104	31
109	18	84	29
110	18	99	33
111	16	69	35
112	18	40	35
113	18	96	40
114	17	57	26
115	16		33
116	16	95	29
117	18	104	34
118	20	64	27
119	18	97	39
120	19	93	33
121	26	83	34
122	23	76	29
123	17	50	35
124	17	74	34
125	22	74	30
126	21	95	28
127	20	75	37
128	26	53	26
129	18	104	32
130	19	71	28
131	21	49	33
132	19	94	31
133	24	86	31
134	18	48	31

EWE 20

HR BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0	8	88	
1	7	57	106
2	8	71	122
3	8	112	131
4	9	102	95
5	10	85	103
6	12	95	99
7	11	102	43
8	9	103	75
9	9	62	59
10	11	80	70
11	13	60	71
12	13	106	28
13	13	97	62
15	10	69	65
16		82	54
17	10	117	52
18	7	97	39
19	11	108	34
20	13	66	43
21	11	107	44
22	15	104	40
23	14	80	52
24	13	109	47
25	13	89	44
27	14	62	38
28	16	72	35
29	15	123	27
30	14	87	25
31	14	84	24
32	19	101	25
33	22	88	26
34	18	93	44
35	16	64	29
36	12	104	25
37	17	86	29
38	22	120	35
39	18	77	31
40	23	45	29
41	24	100	28
42	21	61	23
43	14	78	25
44	22	84	23
45	17	40	21
46	19	70	23
47	22	46	27
48	19	89	26
49	17	60	26
51	18	53	25
52	21	60	23
53	20	86	23
54	20	69	26
55	22	46	20
56	22	95	26
57	25	57	19
58	22	82	22
59	19	53	23
60	20	71	28
61	24	46	27
62	19	105	27
63	24	51	26
64	22	50	26
65	29	70	24
66	25	54	25
67	21	61	28
68	22	80	27
69	23	81	25
70	28	78	23
71	25	71	29
72	20	68	26
73	21	77	32
74		46	27
75	22	72	27
76	27	79	25
77	21	45	24
78	24	78	22
79	21	51	21
80	20	65	25
81	24	99	25
82	22	44	27
83	23	86	33
84	20	43	29
85	24	83	32
86	21	74	24
87	26	66	31
88	24	48	27
89	28	82	29
90	21	79	25
91	26	76	24
92	20	71	27
93	24	79	25
94	23	58	24
95	25	55	26
96	22	108	22
97	21	97	33
98	21	86	25
100	25	58	28
101	24	102	20
102	24	85	25
103	25	55	22
104	26	72	27
105	23	50	23
106	21	85	25
107	24	68	21
108	25		27
109	26	84	23
110	24	84	22
111	22	69	27
114	23	103	23
115	25	62	27
116	23	90	23
117	25	67	22
118	29	94	23
120	22	44	25
121	24	95	26
122	23	46	31
123	23	71	20
124	25	67	25
125	25	113	24
126	28	83	24
127	22	46	19

EWE 21

HR BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML	HR BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0	3	66	261	50	38	49	19
1	3	64	240	51	24	60	20
2	3	60	79	52	29	49	21
3	.	55		53	28	67	17
4	5	46	79	54	34	56	16
5	4	50	65	55	32	36	23
6	4	56	61	56	30	48	18
7	5	55	50	57	35	72	16
8	4	46	57	58	36	48	15
9	3	47	58	59	29	96	14
10	5	56	52	61	27	46	15
11	4	48	67	62	28	94	16
12	4	56	54	63	29	88	17
13	5	40	42	64	30	48	16
14	4	46	34	65	27	73	15
15		40		66	28	63	16
16	4	37	43	67	25	78	20
17	5	51	35	68	26	58	22
18	4	42	52	69			
19	5	32	38	70	33	61	26
20	6	28	33	79	38	50	26
21	3	16	31	85	39	58	20
22	7	43	33	86	37	81	24
23	8	25	35	87	36	70	30
24	8	19	27	88	39	39	26
25	8	49	34				
26	6	35	28				
27	5	36	30				
28	5	31	31				
29	4	50	32				
30	7	32	29				
31	5	31	31				
32	7	46	25				
33	10	33	26				
34	11	46	30				
35	9	33	25				
36	17	35	37				
37	11	52	27				
38	11	41	23				
40	12	31	26				
41	10	46	24				
42	9	50	23				
43	11	32	19				
44	10	27	22				
45	14	44	18				
46		48	16				
47	9	33	18				
48	12	28	20				
49	11	46	15				

EWE 22

HR BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML	ESTROGEN PG/ML
0	9	114	118
1	9	99	119
2	9	96	118
3	10	79	97
4	10	85	93
5	10	55	94
6	11	87	106
7	10	85	93
8	13	97	82
9	12	58	80
10	12	82	73
12	12	86	71
13	15	88	60
14	14	83	53
15	15	63	44
16	15	91	43
17	13	71	30
18	13	72	34
19	14	87	23
20	17	89	28
21	17	92	17
22	18	86	17
23	20	58	10
24	20	52	12
25	20	107	16
26	19	94	16
27	19	72	21
28	25	86	17
29	27	66	14
30	20	70	16
31	22	61	16
32	21	55	15
33	27	64	17
34	19	85	13
35	23	74	12
36	28	40	15
37	23	50	13
38	31	87	17
39	25	52	15
40	27	52	17
41	24	75	18
42	25	67	19
43	24	37	21
44	31	63	9
45	28	57	26
46	22	62	18
47	23	77	19
48	31	47	19
49	30	70	18
50	12	27	26
51	13	25	17
52	12	39	21
53	13	23	20
54	16	42	17
55	15	45	18
56		38	20
57	12	32	23
58	17	51	21
59	12	42	17
60	13	38	14
61	14	29	20
62	16	31	23
63	11	29	18
64	18	27	19
65	15	21	16
66	11	18	19
67	14	50	20
68	13	23	22
69	16	27	18
70	13	38	19
71	11	43	17
72	18	23	21
73	19	39	19
74	19	34	18
75	15	17	18
76	15	36	15
77	15	26	19
78	16	45	17
79	16	29	19
80	19	34	20
81	13	37	20
82	18	25	16
83	15	36	15
84	11	20	23
85	21	36	23
86	24	48	15
87	19	27	16
89	20	29	15
90	18	24	15
91	21	23	18
92	19	21	14
93	23	34	15
95	17	17	17
96	17	32	16
98	17	27	14
99	14	24	17
100		36	17
103		26	13
104	17	28	13
105	16	32	17
106	14	27	20
107	11	47	16
109	15	37	18
110	14	39	16
111	21	26	17
112	15	29	15
113	13	24	14
114	16	13	17
115	23	40	15
116	25	41	13

EWE 23

HR BEFORE LAMBING	PROGEST NG/ML	CORTISOL NG/ML
0	6	111
1	5	112
2	5	94
3	13	70
4	13	93
5	14	103
6	14	118
7	12	104
8	14	116
9	11	107
10	11	84
11	10	78
12	10	113
13	9	125
14	10	84
17	16	83
18	18	116
19	22	113
20	17	102
21	16	117
22	20	113
23	18	105
24	16	101
25	18	85
26	18	100
27	19	109
28	20	83
29	19	84
30	23	92
31	30	89
32	25	85
33	25	68
34	22	62
35	26	84
36	23	55
37	22	64
38	30	74
39	29	64
40	32	83
41	31	67
42	26	59
43	30	64
44	28	71
45	25	71
46	32	64
47	32	59
48	30	63
49	34	59
50	29	57
51	33	61
52	35	41
53	32	41
54	35	51
55	29	48
56	30	41
57	30	43
60	33	40
61	34	56
62	38	49
63	33	41
64	36	66
65	33	63
66	34	51
67	33	42
68	38	56
69	31	56
70	37	51
71	40	54
72	29	85
73	37	59
74	31	58
75	32	80
76	26	64
77	27	54
78	32	57
79	28	67
80	33	66
81	31	61
83	35	81
84	43	81
85	35	71
86	37	49
87	34	84
88	35	75
89	31	72
90	31	79
91	33	67
92	39	69
93	39	
95	41	58
96	31	96
97	36	79
98	35	85
99	38	64
100	42	53
101	37	95
102	36	106
103	37	76
104	37	85
105	38	64
106	38	88
107	40	79
108	38	84
109	40	69
110	42	95
111	37	105
112	37	84
113	35	101
114	39	81
115	39	103
116	30	69
117	34	77
119	36	80
120	32	88
121	42	71
122	39	71
123	37	70
124	40	61
125	47	73
126	36	89
127	38	81
128	35	83
129	39	116
130		111
131	49	99
132	38	73
133	40	99
134	46	68