

Endocrine physiology of the puerperal sow 151

by

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INTRODUCTION

Several studies have been done on the levels of reproductive steroids in various domestic species, such as the cow, ewe and sow, at various stages of gestation and during the puerperal period. However, the corticoid level in the sow during this period has not been studied, and studies involving estrogen levels have been limited to measurement of urinary estrogen.

A concurrent analysis of plasma corticoids, progesterone and estrogens is necessary in order to know how the levels of these steroids behave during the puerperal period in the sow. This knowledge would help elucidate the possible role that these hormones play in the initiation of parturition in this species. More importantly, since these hormones are incriminated as playing an important role in lactogenesis, a knowledge of their levels under physiological conditions would help lay the groundwork for the study of their possible roles in the pathologic conditions, such as the Metritis-Mastitis-Agalactia (MMA) complex, affecting lactogenesis or maintenance of lactation during the puerperal period. A study of the changes in the level of thyroxine is also necessary because of the metabolic role of this hormone which suggests a possible effect on the intensity of lactation.

LITERATURE REVIEW

Hormonal Changes During the Periparturient Period

Periparturient alteration in plasma or urinary levels of some reproductive steroids is a characteristic feature that has been observed in several species (cow, sheep, sow, mare and human). The degree and timing of these changes may vary from species to species, but the general pattern is that parturition is preceded by a concomitant rise in plasma (or urinary) estrogens at a time when the plasma progesterone level is beginning to fall. Lagging somewhat behind this event is the rise in plasma corticoid levels.

Corticoids

Smith, Smith, Convey and Ingalls (1971) found that in the cow the total serum corticoids averaged 4.7 ng/ml from the day 26 to day 1 before parturition, increasing to 10.9 ng/ml and 11.7 ng/ml at 12 hours prepartum and at parturition respectively. They noticed a decrease to 4.8 ng/ml by 12 hours following calving after which the level averaged 5.5 ng/ml. Adams and Wagner (1970) observed a significant rise in corticoids in the cow during the last 4 days prior to parturition when compared to the values they obtained on days 5-7 prepartum and 3-7 days postpartum. Hoffmann, Schams and Karg (1972) found increasing values of corticoids that were almost coincidental with the decrease of progesterone two days prior to calving.

Bassett and Thorburn (1969) noted a rise in the ovine fetal plasma corticosteroid level just before parturition. They failed to detect any corresponding rise in the maternal plasma.

Progesterone

Stabenfeldt, Osburn and Ewing (1970) found that, in the cow, peripheral progesterone levels decreased from 6.8 ng/ml on day 250 of gestation to approximately 4 ng/ml during the final week of gestation. This was followed by a further abrupt decrease to less than 1 ng/ml about 24 hours prior to parturition. The decline continued following parturition. The result obtained by Henricks, Dickey, Hill and Johnston (1972) showed similar pattern and value. Smith et al. (1971) found a similar general prepartum pattern of decline. However, their values were slightly higher and, in addition, they noted a sudden unexplained increase at 3.5 days prepartum before a final rapid decrease to parturition.

In the sheep, Stabenfeldt, Drost and Franti (1972) found that the peripheral plasma progesterone level starts to decline two weeks prior to parturition in both single and twin pregnancies, and that this decline continued to the day of parturition. Progesterone levels were about 2 ng/ml at the time of delivery of the fetus. Bassett, Oxborrow, Smith and Thorburn (1969) found that changes in the peripheral progesterone levels during the last week prior to lambing were variable. They observed that the levels in some animals did not show a decline until

less than 24 hours before parturition. Fylling (1970) found a mean value of 14.4 ng/ml at eight days before parturition. There was a sharp decline in the days that followed, reaching a mean of 2.1 ng/ml at lambing. The level remained almost unchanged at 1.8 ng/ml during the first 2 days postpartum.

Information on peripheral plasma values of progesterone in the sow during the puerperal period is lacking. Short (1960) published the values that he obtained for sows at different stages of pregnancy up to day 114 of gestation. The concentrations were 10.4 ng/ml and 12.0 ng/ml on day 99 and day 102 of pregnancy respectively. By day 112 the value had fallen to 3.4 ng/ml. The levels were higher on day 113 and day 114, 6.7 ng/ml and 4.9 ng/ml, respectively. The value for each day came from a different animal or animals. The method employed for obtaining his plasma samples (from blood collected at slaughter), the small number of animals used in this study and his failure to correct for extraction losses, make it difficult to rely on the absolute values of progesterone levels obtained in this study. Edgerton and Erb (1971) found that the total metabolites of progesterone in the urine hardly decreased one week prior to farrowing. Indeed, they noted an increase from about 2800 ng/mg urinary creatinine on day 13 pre-farrowing to about 3600 ng/mg on day 7 before farrowing. There was a marked decline during the last 7 days of gestation, and by 2 to 3 days postpartum the concentration had fallen from 3100 ng/mg 2 days prepartum to 1200 ng/mg urinary creatinine.

Estrogen

The measurement of circulating plasma levels of estrogens in the domestic species, for a very long time, proved a difficult task, so that until recently all determinations of estrogen levels in these species had been limited to measurements of urinary estrogens.

Urinary estrogens in cattle, measured by chemical methods, increase gradually throughout pregnancy. They peak immediately before parturition and fall rapidly after parturition (Hansel and McEntee, 1970). Hunter, Erb, Randel, Garverick, Callahan and Harrington (1970), using three groups of cows of different gestational lengths (less than 280 days, 280-284 days and above 284 days), found that total urinary estrogen excretion increased in each of the three groups during the last month of pregnancy. However, the rate of increase was markedly more rapid for the group that had a 280-284 day gestation period.

Bowerman, Anderson and Melampy (1964) found that urinary estrogens in two sows, determined by chemical methods, increased from 0.227 and 0.769 mg per 24 hours at day 43 and day 40 prepartum respectively to peaks of 16.52 and 26.77 mg per 24 hours 2 and 3 days prior to parturition. These values closely support the trend reported by Rombauts, Fèvre and Terqui (1971) who noted rapid increase in urinary estrogens from the 75th or 80th day of gestation, attaining a maximum concentration of 15 to 20 mg per 24 hours around parturition. The value fell rapidly after farrowing, and by the 4th day postpartum the level

had returned to normal cyclic values.

The values obtained in these two studies in the sow are well above that of an earlier study by Rombauts (1962) in which the urinary estrogen level started a rapid rise at day 80 of pregnancy and peaked at 4 to 6 mg per 24 hours prior to parturition. The reason for this is not clear.

Development of radioimmunoassay methods for estrogens has made it possible to measure blood levels of estrogens in domestic animals rather than relying on urinary measurements. Using this method Challis (1971) measured the levels of total unconjugated estrogens throughout seven pregnancies in five sheep. He found the concentration of estrogens to be less than 5 pg/ml until day 31 prepartum, followed by a slow increase to 20-40 pg/ml by day 5 prepartum. Samples taken one day prior to lambing or on the day of lambing ranged from 75-411 pg/ml. By day 1 postpartum only one animal showed any detectable estrogen. Bedford, Challis, Harrison and Heap (1972) obtained comparable results.

Henricks et al. (1972), also employing the radioimmunoassay method, measured total plasma estrogen concentration in cows from two weeks pre-calving to 60 days post-calving. They found that total estrogen concentrations rose consistently from a mean of 510 pg/ml on day 14 pre-partum to 2660 pg/ml at parturition with a rate increase of 248 pg/ml per day during the last 5 days. There were nondetectable levels during the immediate postpartum period.

Robinson, Baker, Anastassiadis and Common (1970) and

Robinson, Anastassiadis and Common (1971) used chemical methods to measure whole-blood levels of estrone in cows before and after parturition. In the former study, average estrone levels rose rapidly from 4.8 ng/ml at 4-2 weeks to a peak of 8.3 ng/ml at 5 days prepartum. The level was 0.7 ng/ml at day 5 postpartum. No samples were taken closer than 5 days preceding or following calving. In the latter study they noted a similar peak of 8.2 ng/ml at day 5 prepartum. This peak was followed by a decline to day 1 prepartum, and by a further steep decline from day 1 prepartum to day 1 postpartum. The reason and the significance of this prepartum decline are not clear when viewed in the light of other studies mentioned earlier. However, more studies on the periparturient level of estrone alone are needed to confirm or oppose this finding since other studies have involved total estrogens.

Role of Hormones in Lactogenesis

A possible scheme for the role played by estrogen, progesterone and the glucocorticoids in lactogenesis has been proposed by Meites (1966) as follows.

1. During pregnancy, there is insufficient prolactin, adrenal glucocorticoids, or both to initiate lactation. Adequate amounts of these two hormones are absolutely essential for initiation of milk secretion.
2. Estrogens and progesterone, which are secreted in large quantities during pregnancy, render the mammary glands relatively refractory to stimulation by prolactin and glucocorticoid hormones. This is believed to be of much less importance than the lack of adequate amounts of the lactation-stimulating hormones.

3. At about the time of parturition, there is a rise in circulating prolactin and glucocorticoids and a fall in estrogen and progesterone, permitting the onset of lactation.

The theory proposed by Folley (1956) is similar but in addition he considers the inhibitory effect that the high level of estrogen during pregnancy might have on the anterior pituitary to be important. According to Folley, this high level of estrogen might be acting at the level of the pituitary or at the level of the mammary gland or both, to hold lactogenesis in check.

Cowie (1969) points out that species differences exist as to what constitutes the "lactogenic hormone complex", e.g., components such as the adrenal corticoids. Based on studies on hypophysectomized animals, the minimum components in the rat, mouse and guinea-pig were found to be sheep prolactin + adrenal corticoids. In the goat, a combination of sheep prolactin + corticoids, or bovine growth hormone + corticoids is able to induce traces of milk secretion, but an induction of copious milk flow required all three hormones as well as thyroid hormones. In the rabbit, sheep prolactin or human growth hormone is all that is required to initiate lactation. Cowie suggested a cautious interpretation of these studies since the pituitary hormones used were of ruminant origin.

Tucker and Meites (1965) were able to initiate lactation in heifers during early, mid- and late pregnancy by injecting an adrenal corticoid steroid for 15, 7 or 6 days respectively.

This would suggest that adrenal corticoid is the limiting factor in this species. Delouis and Denamur (1967) found that from day 120 of gestation in the sheep, injection of hydrocortisone would induce lactation. The quantity of milk produced following an injection of the same amount of hydrocortisone (25 mg or 50 mg) became more copious the later in the course of pregnancy the hydrocortisone was given. Ovine prolactin injected at the same stages of pregnancy gave little or no milk yield. On the basis of this it seems that the sheep, like the cow, requires only an adequate level of glucocorticoid to trigger lactation during pregnancy. The studies in these intact animals would lend support to Meites' (1966) contention that high levels of estrogen and progesterone are not as important as lack of lactation-stimulating hormones in being the inhibitory factor to lactogenesis during pregnancy.

The thyroid does not seem essential for lactogenesis, but in its absence the intensity and duration of milk secretion are reduced (Sulman, 1970).

Role of Hormones in Parturition

There is a general agreement that the onset of parturition is regulated by a complex mechanism which involves an interaction between several factors, endocrine, neural and mechanical (Reynolds, 1959; Catchpole, 1969; Csapo, 1969; Bedford et al., 1972). The studies of Liggins, Kennedy and Holm (1967) and Liggins (1968) in the sheep point to the important role

that the fetus might play in the initiation of labor.

Bedford et al. (1972) recently summarized the current theories advanced in connection with the initiation of parturition:

1. Withdrawal of progesterone block on myometrium
2. A change in the progesterone-estrogen ratio
3. An increase in uterine volume
4. A rise in estrogen level
5. A release of pharmacologically active substances (oxytocin, prostaglandins, catecholamines, etc.)
6. An activation of neural mechanisms

Direct evidence is lacking which would support the role that the maternal prepartum rise in plasma corticosteroids plays in the initiation of parturition. However, results obtained from studies in which exogenous corticosteroid was used to induce parturition or precipitate abortion suggest that there is an interaction between corticosteroids, progesterone and estrogens. Schams, Hoffmann, Fischer, Marz and Karg (1972) observed that the pattern of progesterone decline following glucocorticoid-induced parturition in cows resembled that seen in normal parturition. They suggested that this similarity gives an indication of a possible mechanism involved in luteolytic action near term. Evans, Wagner and Adams (1971), after dexamethasone injection to induce parturition, found that jugular progesterone levels decreased abruptly from an average of 4.9 ng/ml to 2.6 ng/ml during 24 hours post-treatment in four cows that calved

within 48 hours after the injection. Edqvist, Ekman, Gustafsson, Jacobsson, Johansson and Lindell (1971) noted a marked decline in peripheral progesterone when dexamethasone was used to precipitate abortion in cows 75 or 150 days pregnant, or to induce parturition at 250 days of pregnancy. They further noted that there was a marked increase in estrone level following the dexamethasone injection and before the decline in progesterone level in the latter class of cows. Based on these findings they proposed that the parenteral administration of dexamethasone alters the activities of enzyme systems resulting in increased estrone and decreased progesterone production.

The relative importance of the roles played by progesterone and estrogens in initiation of parturition seems to differ from species to species.

In the species that have an extra-placental source of progesterone during pregnancy, such as the cow and the sow, there is a decided progesterone decline a day or two before parturition. This event supports the theory of progesterone-block removal at parturition. The situation in the animals in which the ovaries are dispensable at a certain stage of pregnancy and the progesterone level remains high in maternal or fetal blood until birth does not support a simple theory of parturition (Catchpole, 1969). These species include the human, sheep, mare and monkey. Stabenfeldt et al. (1970) postulate that since progesterone is of placental origin in these animals, there should be no progesterone withdrawal prior to parturition

since progesterone production by the placenta cannot be separated from general placental support of the fetus which must continue until the moment of delivery.

The levels of 2.1 ng/ml and 2 ng/ml of progesterone obtained in the ewe by Fylling (1970) and Stabenfeldt et al. (1972), respectively, at lambing are about the luteal phase values and therefore represent a substantial amount of progesterone which does not support the simple progesterone-withdrawal theory as a trigger mechanism for parturition in this species.

Csapo (1961, 1969) suggests that parturition occurs as a result of a gradual failure of the placenta to adequately compensate for the increasing uterine volume by progesterone production. This, he argues, leads to the uterine volume:progesterone ratio increasing beyond a critical value thus triggering parturition. It seems that the sheep would fit into this modified progesterone-block theory. Still, Bedford et al. (1972) question the likelihood that uterine volume:progesterone ratio change alone can account for the precise events associated with the onset of parturition. They contend that in view of the gradual nature of this change in ratio, it probably should be viewed only as playing a permissive role in allowing the release, or stimulating the production of other agents that trigger parturition directly.

Catchpole (1969) proposes that the changing levels of estrogen and progesterone, and especially an increase in the estrogen:progesterone ratio sensitizes the uterus to the effect

of oxytocin which through its contractile effect on the uterus assists parturition.

There is a general agreement among authorities that in all species there is a prepartum rise in urinary or plasma estrogens. This rise is accompanied by a concomitant decrease in progesterone levels though there are conflicting data in some species such as the human (Yannone, Mueller and Osborn, 1969) and the guinea-pig (Heap and Deanesly, 1966; Challis, Heap and Illingworth, 1971). An increase in estrogen:progesterone ratio prior to parturition is also a feature consistently observed in all species. The problem seems to lie in the interpretation of the significance of these changes and how they relate to the mechanisms that bring about parturition. Whether the changes in the levels of the steroids, or in their ratio, play a primary, secondary or permissive role in the initiation of parturition is a matter yet to be resolved.

MATERIALS AND METHODS

Animals used in this study consisted of 23 sows (11 Hampshire, 7 Duroc, 4 Poland China and 1 Yorkshire) located in three swine herds owned by Iowa State University. Daily blood samples were collected beginning 5 to 7 days prior to expected farrowing date and continuing through day 7 post-farrowing. A gestation length of 115 days was used and the day 1 post-farrowing sample was defined as the first sampling period after farrowing.

The sows were prepared for blood collection by placing indwelling vinyl catheters (Becton-Dickinson and Co., ID .058" X OD .080") in the anterior vena cava one day prior to start of blood sampling. Five 10 ml blood samples were collected from each sow per day between 0800 to 0900 at 15 min. intervals. Samples were immediately cooled, centrifuged at 4C and plasma separated within an hour. The plasma samples were stored at -20C until needed for hormone assay. The plasma samples were assayed for corticoids, progesterone, estrone, estradiol and thyroxine.

Hormone Assay Procedures

Corticoids

Each of the five samples collected each day was assayed for total plasma corticoid content using the protein-binding method of Murphy (1967) as modified by Whipp and Lyon (1970). Aliquots

of plasma or cortisol standard (0.2 ml) were pipetted into 16 X 125 screw cap disposable glass culture tubes. The concentrations of standards used were 0, 7.5, 15, 30, 60, 90 and 120 ng/ml prepared in distilled water. Five ml nanograde hexane (Mallinckrodt Chemical Works, St. Louis, Mo.) were added to each tube, the tubes shaken manually for 30 sec. and centrifuged for 10 min. to separate the contents into two phases. The tubes were placed in a dry ice-alcohol bath until the plasma was frozen at the bottom of the tubes. The hexane was poured off and discarded. The purpose of the hexane treatment was to remove the progesterone present in the plasma. The tubes were then placed in a 45C water bath to thaw the plasma. Nanograde methylene chloride (4.5 ml) (Mallinckrodt) was added to each tube and the tubes were shaken and centrifuged in the same manner as above. The plasma or water phase remained on top of the solvent and was removed by aspiration using a water syphon attached to a disposable Pasteur pipet. A 1 ml aliquot of the methylene chloride extract was then transferred to each of two 12 X 75 mm disposable glass culture tubes (unknowns) or each of three tubes (standards). The methylene chloride was then evaporated in a 45 C water bath. A single set of standards was placed in each rack of unknown samples. One ml of the corticoid-binding globulin (CBG)-³H-cortisol solution (Appendix Table 1) was added to each tube. The tubes were incubated for 5 min. in 45C water bath and for 15 min. in a 10C water bath. Forty mg florisil (60-100mesh) (Fisher Scientific Co.) were added sequentially

to each tube, the tube mixed slowly on a Vortex mixer for 30 sec. and returned to the 10C water bath for 5 min. A 0.5 ml aliquot of the supernatant was transferred to a scintillation vial for counting. Ten ml of scintillation fluid A (Appendix Table 2) were added, mixed and counted in a Beckman LS-100 counter.

The average time required to count each standard and unknown to a preset total count was determined. Using the data for the standards, the slope of the regression line was calculated and utilized in a regression equation to calculate the mean concentration of corticoids in the unknown samples. The following equation was used.

$$Y_i - \bar{y} = b(X_i - \bar{x})$$

This equation was solved for X_i (concentration of corticoid in unknown sample) as follows.

$$X_i = \frac{Y_i - \bar{y} + b\bar{x}}{b}$$

The terms used were defined as follows.

Y_i = Time required to count the unknown

b = Slope of the standard curve

X_i = Corticoid (ng/ml) in unknown

\bar{x} = Mean corticoid (ng/ml) for all standards

\bar{y} = Mean time required to count standards

Progesterone

Plasma samples were assayed using the competitive protein-binding method for progesterone as described by Neill, Johansson, Datta and Knobil (1967) with slight modifications. Only one sample per day per animal was assayed (the third sample).

Two ml of each plasma sample were pipetted into a 16 X 125 mm screw cap tube. In addition, 2 tubes each containing 2 ml of plasma of known progesterone concentrations (0.5 ng/ml and 3 ng/ml) and 3 tubes containing 2 ml of triple distilled water were used to determine accuracy of the assay system and background values for the assay. Two μ l of 14 C-progesterone, equivalent to 170 counts per min. (cpm) were added to each tube to calculate procedural losses. All samples and control tubes were extracted three times with 10 ml nanograde petroleum ether (Mallinckrodt). Each time the tubes were shaken manually for 30 sec. and centrifuged for 15 min. to obtain separation into plasma and petroleum ether phases. The plasma was then frozen in a dry ice-alcohol bath and the ether poured into 15 ml conical centrifuge tubes and evaporated by placing the tubes in a 45C water bath. After the ether extract had evaporated to dryness, the sides of the tubes were rinsed with 1 ml petroleum ether and evaporated. The samples were spotted on 20 X 20 cm thin-layer silica gel chromatogram sheets (N-HR/UV254, Machery-Nagel). Each sheet was divided into 6 lanes. Progesterone standards were spotted on the outside lanes and four samples on the inner lanes. The dried extract of each unknown was picked

up and spotted in 0.2, 0.1 and 0.1 ml of benzene-methylene chloride (1:1). The sheets were developed by ascending chromatography in diethyl ether-benzene (2:1). The progesterone standards were located on the plate under ultraviolet light, and the areas in each lane corresponding to these spots were marked. The sample spots were scraped and eluted in 3.0 ml absolute methanol in disposable Pasteur pipets previously prepared by packing with acid-washed glass wool. The glass wool was washed in a sulfuric-nitric (2:1) acid bath and rinsed in distilled water. The prepared pipets were rinsed with absolute methanol before use. For each sample, 0.9 ml of the alcohol eluate was placed in a scintillation vial to be counted for ^{14}C for correction of procedural losses. The eluate in the tubes was evaporated to dryness in a 45C water bath.

Progesterone standards were prepared from stock progesterone solution in absolute methyl alcohol (100 ng/ml). The standards were placed in 12 X 75 mm culture tubes in triplicate. These standards corresponded to 0, .25, .5, 1.0, 3.0, 5.0 and 10.0 ng of progesterone. The tubes were evaporated to dryness. One ml of CBG- ^3H -corticosterone (Appendix Table 1) was added to each tube. The test tube rack was shaken briefly and then transferred to a 10C water bath for 10 min. At the end of the 10 min. each tube, in turn, received 75 mg florisil. The tube was shaken slowly on a Vortex mixer and then returned to the water bath for 5 min. A 0.5 ml aliquot of the supernatant was transferred to a scintillation counting vial. Ten ml of

scintillation fluid A (Appendix Table 2) was added to each vial and the contents mixed thoroughly. Samples were then counted in a Beckman LS-100 counter. The scintillation vials that received the 0.9 ml alcohol eluate and the three ^{14}C -progesterone standards were evaporated to dryness at room temperature by leaving them partially uncapped. Ten ml of scintillation fluid B (Appendix Table 2) were added to each vial and they were counted in a Beckman LS-100 counter. The values (cpm) for each standard were plotted on semi-log paper and the curve drawn. The amount of progesterone present in each unknown was read from this graph. The value obtained from the graph for each sample was corrected for procedural losses and for the dilution factors involved at points in the assay. The final value gave ng progesterone/ml plasma for each sample.

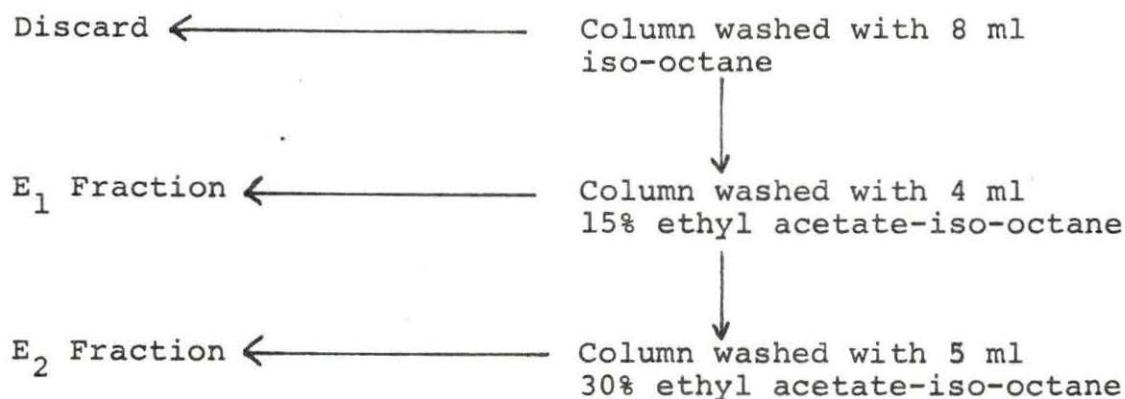
Estrogens

The plasma samples were assayed for estrone and estradiol using the second sample for each day from each sow. The assay used was the radio-ligand assay technique described by Korenman, Tulchinsky and Eaton (1970).

Plasma samples were extracted twice with 10 ml anhydrous diethyl ether (from a freshly opened can). The amount of plasma assayed varied from 0.5 to 3.0 ml depending on the relationship to the time of farrowing, and therefore, the expected content of estrogens. Two distilled water samples were assayed as blanks in each assay. After addition of ether to the plasma, they were shaken, centrifuged, the plasma frozen in a dry ice-

alcohol bath and the ether phase decanted into conical centrifuge tubes. The extracts were dried in a 45C water bath. Each tube was washed down with ether (1 ml) using a Pasteur pipet, and dried again. The dried extract in each tube was then transferred to a prepared celite column (Appendix Table 3) with 3 rinses (0.3 ml) of iso-Octane. The columns were run under air pressure of 2 psi. The subsequent steps involved in separating the estrone (E_1) and estradiol (E_2) are summarized in the following flow-sheet diagram.

Celite Separation of Estrogens



The tubes' contents (the E_1 and E_2 fractions) were evaporated in a 45C water bath. The sides of the tubes were washed down with 1 ml of ethyl acetate and the solvent was completely evaporated.

For the rest of the procedure E_1 and E_2 fractions were treated in the same manner except for some minor differences. Each sample was run in duplicate for both E_1 and E_2 in 12 x 75 mm tubes. Tris-HCl-ethylene glycol buffer (Appendix Table 4)

(0.25 ml) was put into each tube (0.24 ml for E₂). Tris-HCl-buffer (Appendix Table 4) (0.6 ml) was added to each tube containing dried E₁ and E₂ extract and mixed on a Vortex mixer. A 0.2 ml aliquot was placed in each of the corresponding 12 X 75 mm assay tubes. ³H-estrogen (0.02 ml) was added to each tube. Rabbit cytosol solution (Appendix Table 5) was then added to each tube (0.03 ml for E₁ and 0.04 ml for E₂).

A series of standards were prepared in duplicate for E₁ and E₂. The standards used for E₁ were 0, 10, 25, 50, 100, 200, 300, 400, 500 and 600 pg estrone and for E₂ 0, 5, 10, 20, 40, 100 and 200 pg estradiol. The stock solutions of the standards were prepared such that 0.2 ml would give the corresponding amount of estrogen. This amount (0.2 ml) was added to each of 2 assay tubes for each level of E₁ and E₂ and the other reagents added as for the unknowns. All tubes were mixed by gentle rocking of the tube rack. The samples and standards were incubated over night (about 24 hrs.) at 4C.

After incubation, 0.5 ml of charcoal-dextran-tris suspension (Appendix Table 4) was added to each tube. Samples were shaken, incubated in a 15C water bath for 15 min. and then centrifuged for 15 min., after which the supernatant of each tube was poured off into a counting vial and 10 ml of scintillation fluid C (Appendix Table 2) were added and mixed. Samples and standards were then counted in a Beckman LS-100 counter. The percentage of tritiated estrogen (³H-estrone or ³H-estradiol) bound to cytosol for each standard was computed

and these values were used to draw a standard curve. Percentage binding for the unknown samples was calculated in the same manner, but the water blank cpm replaced the standard blank cpm. The amount of estrogen in each sample was read off from the standard curve on the basis of percentage binding for that sample. The concentration of estrogen (E_c), expressed as pg/ml plasma, was calculated according to the following formula:

$$E_c = \left[\frac{T_t}{T_a} (E) \right] \frac{1}{V}$$

T_t = Total amount of tris-HCl used for each sample (0.6ml)

T_a = Amount of tris-HCl used per assay tube (0.2 ml)

E = Picogram of estrogen read from standard curve

V = Volume (in ml) of plasma extracted

Thyroid hormones

The thyroxine assay involved determination of the Effective Thyroxine Ratio (ETR) which is a reflection of free plasma thyroxine rather than total plasma thyroxine.

The Res-O-Mat ETR Diagnostic Kit (Mallinckrodt Nuclear) was used in the assay. Since the standard serum provided for this test is for use for human samples, the test as performed in this study was intended to compare the relative changes in ETR value during the period preceding and following farrowing.

The standard serum was reconstituted in 2 ml of triple distilled water, and was allowed to stand for 5 to 10 min. with

periodic gentle mixing. Two ml of extraction alcohol were placed in centrifuge tubes, and 1 ml of plasma or standard was added to each tube. At the same time about 0.1 ml of plasma was placed into a labeled plastic serum cup to be used later. The tubes were mixed on a Vortex mixer and centrifuged at 2500 rpm for 5 min. A 0.3 ml aliquot of the alcohol supernatant was transferred to a Res-0-Mat ETR vial. Serum micropipets were filled from corresponding plastic cups and each was placed in an appropriate solution vial. Using forceps, a Res-0-Mat strip was added to each vial and the vial was tightly capped. The vials were then turned on a rotator (Mallinckrodt Nuclear Constant Speed Rotator) for 1 hour. Using an Eppendorf pipet, 0.5 ml of solution was taken out of the vial and was placed in a scintillation vial. Ten ml of scintillation fluid A (Appendix Table 2) were added to each scintillation vial. The contents of the vial were mixed and counted for radioactivity in a Beckman LS-100 counter.

The Effective Thyroxine Ratio was determined in the following way:

$$\text{ETR} = \frac{\text{Mean CPM of the 2 standards}}{\text{CPM of unknown}}$$

RESULTS

Hormone Analyses

Corticoids

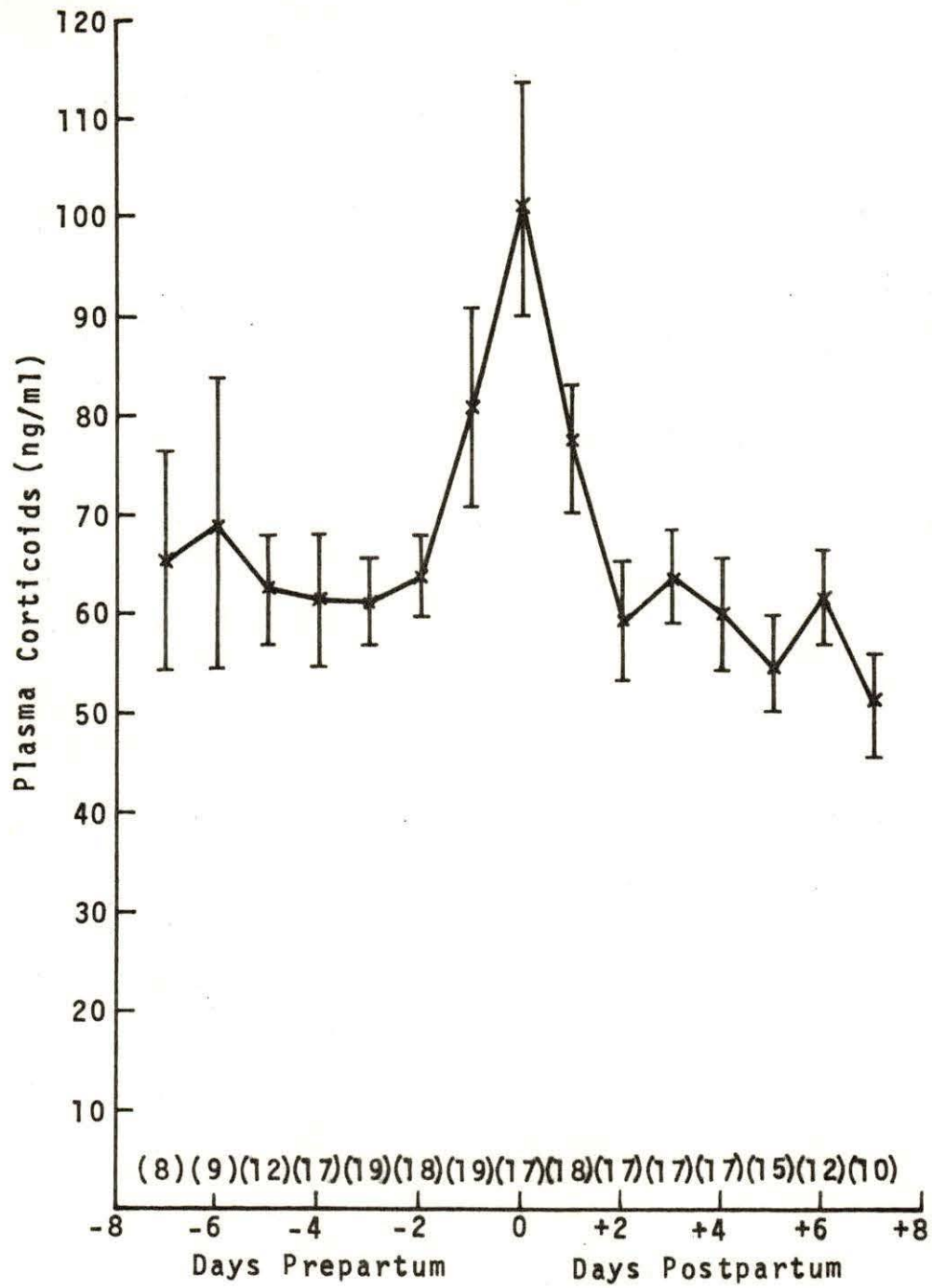
The mean plasma corticoid levels are shown in Table 1 and Figure 1. The corticoid level began to rise on day 3 prepartum. The rate of rise became very rapid during the last 48 hours prepartum. The rate of increase exceeded 20 ng per 24 hr. during this period, compared to an increase of only 2.5 ng during the preceding 24 hr. The level peaked at 101.8 ng/ml on day 0. During the 48 hr. immediately postpartum, the corticoid level fell as abruptly as it had risen, returning to pre-farrowing levels by day +2.

Plasma corticoid levels on days -1, 0 and +1 were significantly higher ($P < 0.001$) than the levels for all other days sampled.

Progesterone

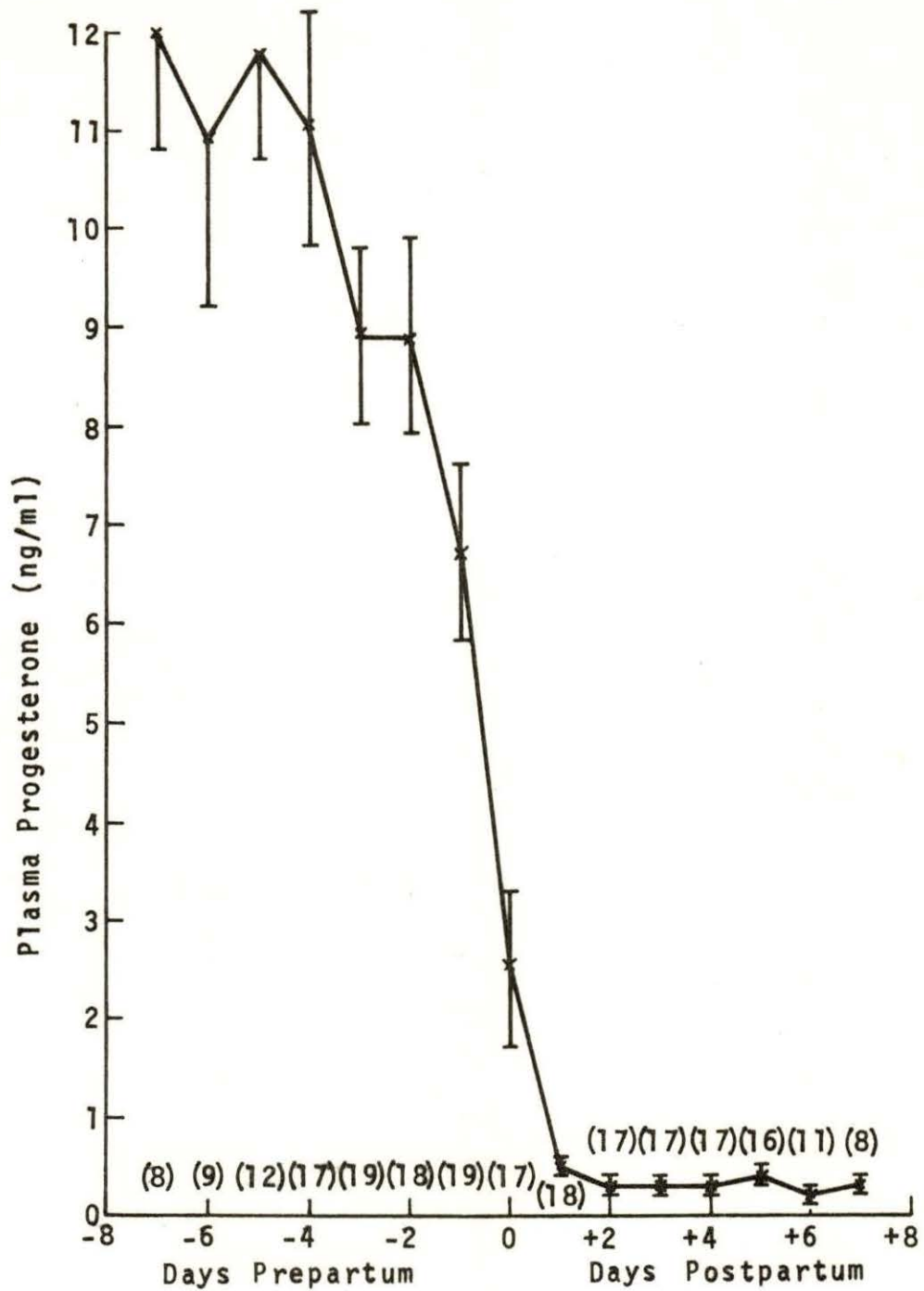
The mean progesterone level (Table 1 and Figure 2) started to decline on day 5 prepartum. This decline was very rapid from day 4 prepartum (11 ng/ml) to day 1 postpartum (0.5 ng/ml). The greatest rate of decline (4.2 ng/ml/day) occurred in the last 24 hr. prior to farrowing. The plasma level remained fairly constant at about 0.3 ng/ml from day 2 postpartum to the end of the experimental period.

Figure 1. Plasma Corticoid Levels in Sows During the Puerperal Period. (Mean concentrations for the five daily samples. Vertical bars represent standard error of the mean. Numbers in parentheses indicate the number of animals sampled for each day.)



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Figure 2. Plasma Progesterone Levels in Sows During the Puerperal Period. (Vertical bars represent standard error of the mean. Numbers in parentheses indicate the number of animals sampled on the corresponding day.)



Estrogens

The mean levels of estrone and estradiol are shown in Table 1 and Figure 3 (E_1) and Figure 4 (E_2). These two estrogens showed similar patterns of change. A steady increase in concentration was observed from day 6 (day 5 for estradiol) prepartum until day 2 prepartum. Estrone then steadily decreased through farrowing, finally reaching a plateau of 6 pg/ml on day +6. Estradiol showed a brief rise on day 0 and then declined steadily thereafter reaching a low level of 3.6 pg/ml on day +7.

Estrogen:Progesterone Ratio

The changes in estrogen:progesterone ($E_1 + E_2:P$) ratio are shown in Figure 5. This ratio showed an increase which became increasingly rapid as the farrowing day was approached. This ratio decreased postpartum, first abruptly, and then more gradually. There was a significant positive correlation ($r = .798$, $P < 0.05$) between this ratio and day prefarrowing.

Thyroxine

The Effective Thyroxine Ratio (ETR) increased from 0.858 on day 1 prepartum to a peak of 0.899 on day 1 postpartum. During all the postpartum period covered by the study, the ETR values remained above the prepartum period. The ETR value for the period from day -6 to day 0 was significantly lower ($P < 0.001$) than the value for the period day +1 to day +7 postpartum.

Figure 3. Plasma Estrone Levels in Sows During the Puerperal Period. (Vertical bars represent standard error of the mean. Numbers in parentheses indicate the number of animals sampled on each day.)

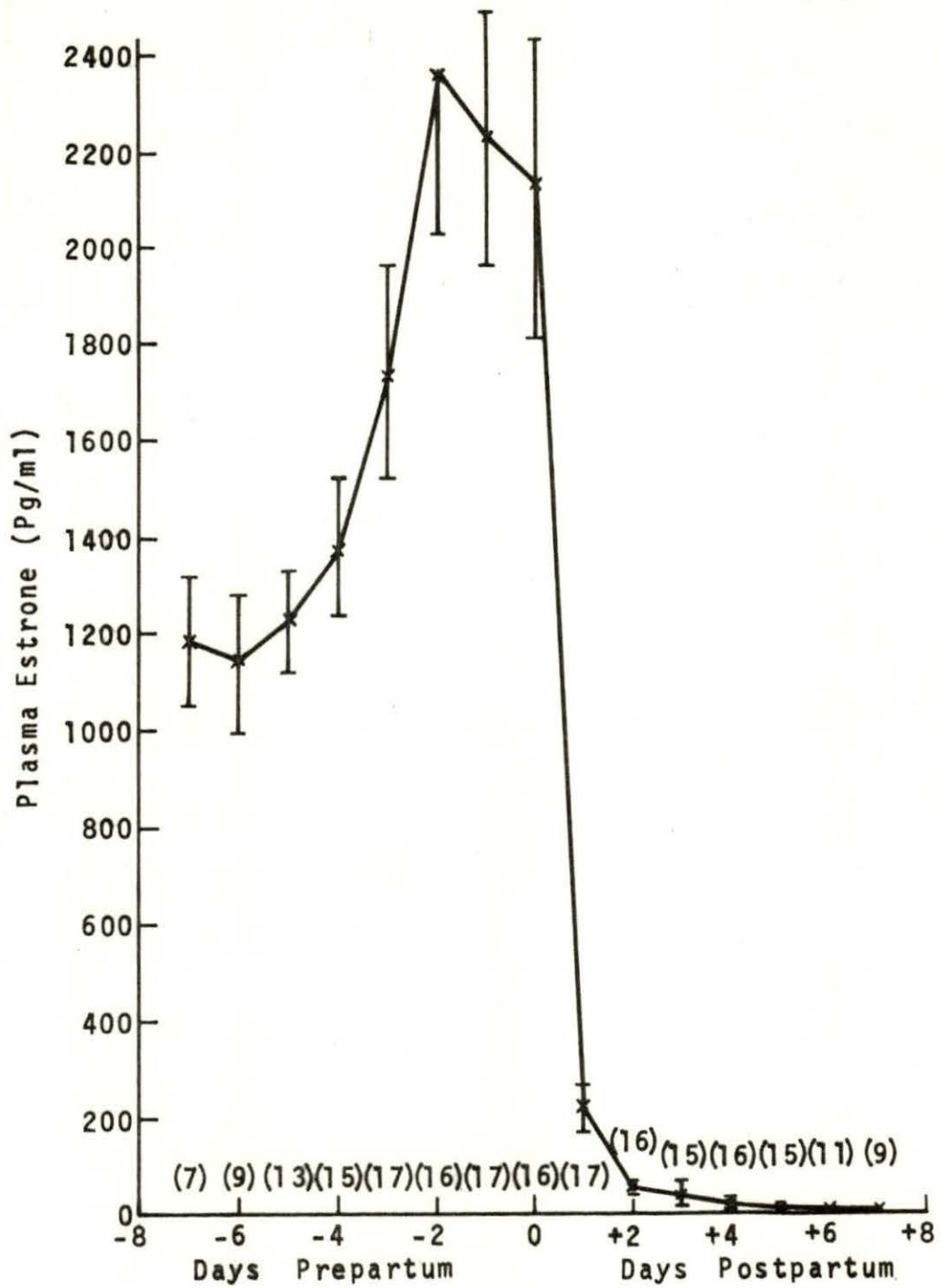


Figure 4. Plasma Estradiol Levels in Sows During the Puerperal Period. (Vertical bars represent standard error of the mean. Numbers in parentheses indicate the number of animals sampled on each day.)

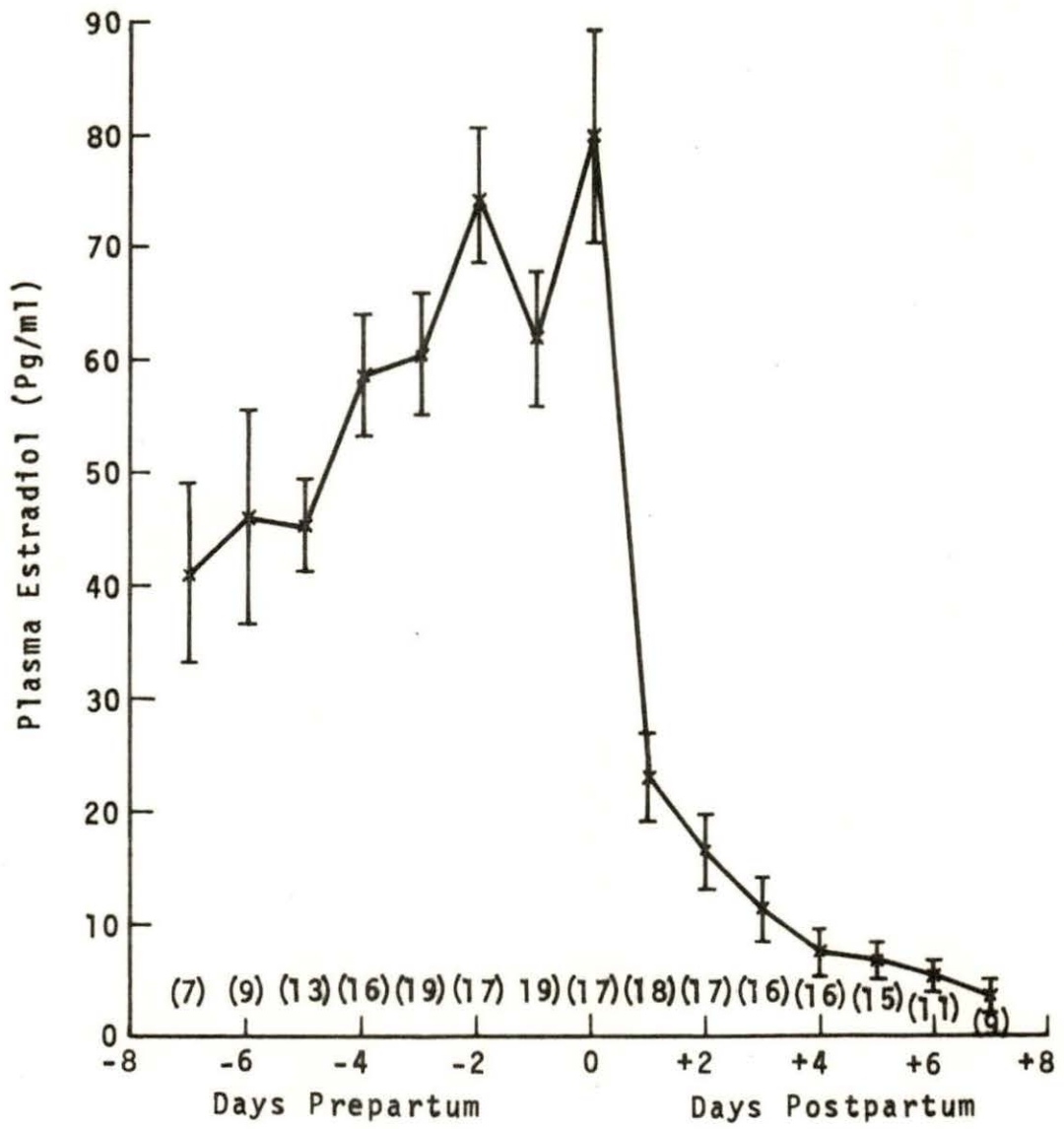


Figure 5. (Estrone + Estradiol):Progesterone Ratios in
Sow Plasma During the Puerperal Period.

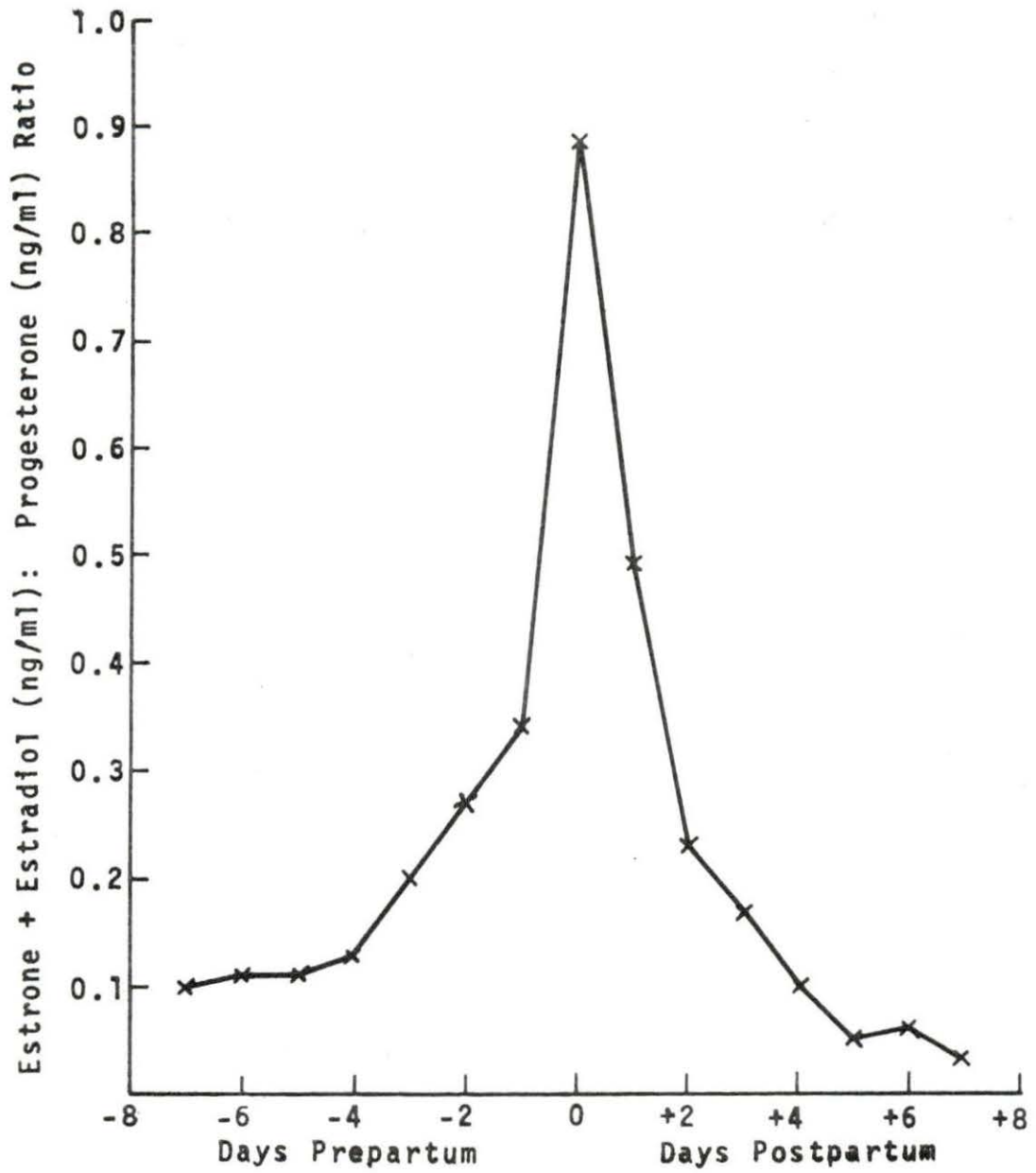


Figure 6. Plasma Effective Thyroxine Ratio in Sows During the Puerperal Period. (Vertical bars represent the standard error of the mean. Numbers in parentheses indicate the number of animals on each day.)

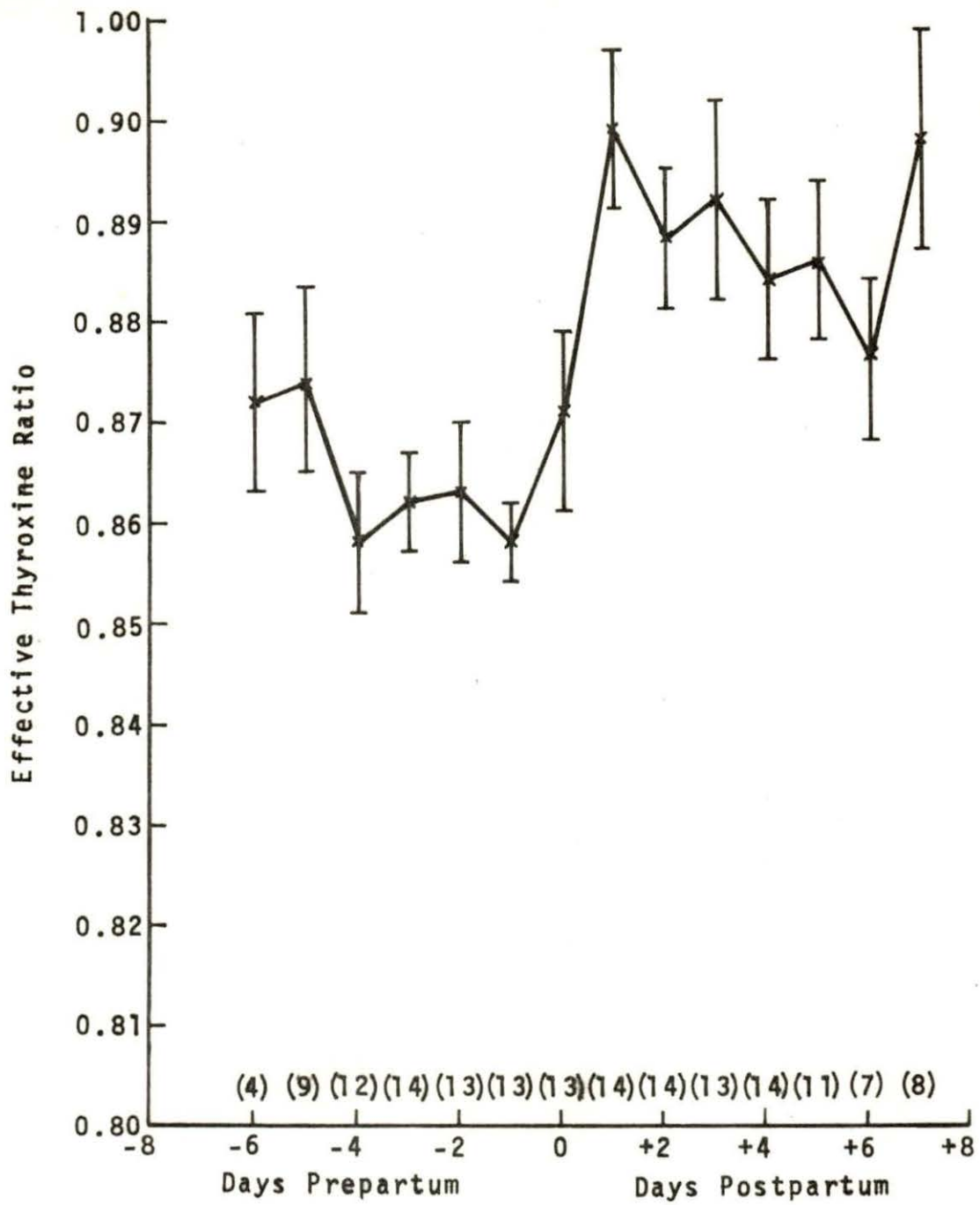


Table 1. Plasma Hormone Levels in Sows During the Periparturient Period (Mean[±]S.E.)^a

| No. of days to farrowing | Corticoids (ng/ml) | Progesterone (ng/ml) | Estrone (pg/ml) | Estradiol (pg/ml) | Thyroxine (ETR) ^b |
|--------------------------|-------------------------|-----------------------|---------------------------|-----------------------|------------------------------|
| -7 | 65.4 [±] 11.1 | 12.0 [±] 1.2 | 1187.4 [±] 134.7 | 41.0 [±] 8.1 | - |
| -6 | 69.0 [±] 14.5 | 10.9 [±] 1.7 | 1139.0 [±] 143.7 | 46.1 [±] 9.5 | 0.872 [±] 0.009 |
| -5 | 62.3 [±] 5.7 | 11.8 [±] 1.1 | 1224.2 [±] 106.0 | 45.3 [±] 4.3 | 0.874 [±] 0.009 |
| -4 | 61.4 [±] 6.8 | 11.0 [±] 1.2 | 1376.0 [±] 141.0 | 58.7 [±] 5.5 | 0.858 [±] 0.007 |
| -3 | 61.3 [±] 4.5 | 8.9 [±] 0.9 | 1740.0 [±] 218.2 | 60.4 [±] 5.5 | 0.862 [±] 0.005 |
| -2 | 63.8 [±] 4.1 | 8.9 [±] 1.0 | 2368.1 [±] 342.2 | 74.7 [±] 6.0 | 0.863 [±] 0.007 |
| -1 | 80.9 [±] 10.2 | 6.7 [±] 0.9 | 2224.1 [±] 266.3 | 61.8 [±] 6.0 | 0.858 [±] 0.004 |
| 0 | 101.8 [±] 11.7 | 2.5 [±] 0.8 | 2124.4 [±] 310.0 | 80.2 [±] 9.4 | 0.870 [±] 0.009 |
| +1 | 76.6 [±] 6.5 | 0.5 [±] 0.1 | 221.4 [±] 52.1 | 23.1 [±] 4.1 | 0.899 [±] 0.008 |
| +2 | 59.2 [±] 6.1 | 0.3 [±] 0.1 | 51.6 [±] 10.8 | 16.6 [±] 3.3 | 0.888 [±] 0.007 |
| +3 | 63.8 [±] 4.7 | 0.3 [±] 0.1 | 38.1 [±] 22.2 | 11.4 [±] 3.0 | 0.892 [±] 0.010 |
| +4 | 60.0 [±] 5.7 | 0.3 [±] 0.1 | 20.9 [±] 13.1 | 7.7 [±] 2.4 | 0.884 [±] 0.008 |
| +5 | 54.8 [±] 4.9 | 0.4 [±] 0.1 | 12.0 [±] 5.4 | 6.8 [±] 1.7 | 0.886 [±] 0.008 |
| +6 | 61.7 [±] 4.8 | 0.2 [±] 0.1 | 5.9 [±] 3.4 | 5.5 [±] 1.6 | 0.876 [±] 0.008 |
| +7 | 50.8 [±] 5.0 | 0.3 [±] 0.1 | 6.8 [±] 3.5 | 3.6 [±] 1.3 | 0.898 [±] 0.011 |

^aMean[±]Standard Error.

^bEffective Thyroxine Ratio.

DISCUSSION

The data from this study show that as in the cow, there is a prepartum rise in plasma corticoid level in the sow. The corticoid rise began 3 days prepartum which was intermediate between the findings of Adams and Wagner (1970) (4 days prepartum) and Smith et al. (1971) (12 hr. prepartum) in cattle. However, the concentration of plasma corticoids during the periparturient period is higher in the sow than in the cow (100 ng/ml vs 10-15 ng/ml).

An important question is whether the high periparturient corticoid level in the sow is of fetal or maternal origin. Beitins, Kowarski, Shermeta, de Lemos and Migeon (1970) indicated that the fetal lamb cortisol secretion rate was too low compared with the maternal rate (1.6 vs 73.5 ng/24 hr.) to contribute substantially to maternal plasma levels. If the same holds true for the sow, it would be safe to assume that the high level observed in the present study was coming from the dam rather than from the fetus.

The pattern of changes in progesterone levels observed in this study is in general agreement with the patterns noted by Short (1960) for plasma progesterone and by Edgerton and Erb (1971) for total urinary progesterone metabolites. It is difficult, however, to compare the absolute values of Short's results with those from the present study because of the inadequacies in Short's experiments, as previously noted. With respect to

the study by Edgerton and Erb, the concentration of 1200 ng progesterone metabolites per mg of urinary creatinine recorded 2 to 3 days postpartum seems to show insufficient postpartum fall when compared with the plasma progesterone level of 0.3 ng/ml obtained in this study during a comparable postpartum period. Their results showed approximately a 3-fold decrease between day 3 prepartum and day 2 postpartum, while ours show a nearly 30-fold decrease during the same period.

Many studies have indicated that the corpora lutea (CL) remain the main physiological source of progesterone all through pregnancy, and that the ovary is indispensable during gestation in the sow. Short (1956) was unable to detect any progesterone in the late-term placenta of the sow. Kimura and Cornwell (1938) found that the progestin content of the corpora lutea remained high and apparently rose, even into the fourth month of gestation. Rombauts, Pupin and Terqui (1965) observed that between the 10th day of gestation and 5 days prepartum the concentration of progesterone of the CL remained always above 40 $\mu\text{g/g}$ of luteal tissue. Masuda, Anderson, Henricks and Melampy (1967) noticed that total luteal tissue progesterone content at day 102 of pregnancy was still the same as at the early stages of pregnancy. Rombauts et al. (1971) found that both CL weight and the progesterone concentration per gram of CL remained about the same from day 27 to day 110 of gestation. They pointed out that the progesterone of ovarian origin is indispensable for the maintenance of pregnancy in the sow and that

the placenta does not secrete progesterone. Du Mesnil du Buisson and Dauzier (1957) obtained abortion within three days after ovariectomy in all eight sows that were ovariectomized between days 89 and 106 of gestation.

The abrupt postpartum decline of plasma progesterone observed in this study coincides with the rapid degeneration of the CL of pregnancy. Palmer, Teague and Venzke (1965), in a microscopic study of the CL of pregnancy, noticed that by day 1 after farrowing the luteal cells were already showing degenerative changes and that immediately after farrowing the luteal cells were much smaller than just prior to parturition (25 vs 40-60 μ in diameter). Thus, it appears that the changes observed in plasma progesterone in the present study would be consistent with the view that the CL are the major source of progesterone in the pregnant sow.

The pattern of prepartum changes in plasma estrogens is similar to that found in other species and to the pattern that Rombauts (1962) found in urinary estrogens in the sow.

Estrone was found to be the principal estrogen in the urine of pregnant sows (Terqui, 1971) and in the placenta (Rombauts, 1964). This study indicates that it is also the principal estrogen in the peripheral plasma of pregnant sows. We failed to observe a significant prepartum decline in estrone level as reported by Robinson *et al.* (1971).

Several studies have demonstrated that swine placentae

produce estrogens. Bowerman et al. (1964) found 102.0 mcg free estrone, 17.8 mcg conjugated estrone, 4.7 mcg free estradiol and less than 1 mcg of conjugated estradiol per 100 g of placenta delivered at term. Rombauts (1964) found that concentrations of estrogens in the placenta rose as pregnancy advanced and he argued that since the rise is in the form of non-conjugated steroid, its synthesis would be in the placenta. He was also able to reduce urinary levels of estrone during pregnancy by removal of portions of the uterus at 12 days of gestation thereby reducing the amount of placenta available for estrogen synthesis. He admitted a possible synergism of estrogens during pregnancy.

Fèvre, Léglise and Rombauts (1968) demonstrated that urinary estrogens present from the end of the first month of pregnancy to termination of gestation were not of ovarian origin nor under maternal hypophyseal control, since neither ovariectomy nor hypophysectomy altered the normal pattern of urinary estrone excretion. They concluded that, in the pregnant sow, the feto-placental unit possesses all the necessary enzymes for estrogen synthesis.

Fèvre, Léglise and Reynaud (1972), by adrenalectomizing sows at various stages of pregnancy, demonstrated that, unlike humans, estrogen synthesis in the pregnant sow was independent of the maternal adrenal. They found that feto-placental synthesis of estrogen occurred normally in the absence of maternal steroid precursors.

The experiment by Fèvre (1970) with labeled C-19 steroid strongly supported fetal participation in estrogen synthesis during pregnancy in the sow. This experiment not only showed fetal involvement in estrogen synthesis but also showed that conversion of C-19 steroids to estrone was higher when these steroids were administered directly into the fetal compartment than when administered into the uterine artery of the dam.

These various studies would lead one to conclude that the estrogens measured in the present study were of feto-placental source without any contribution from the maternal ovaries, adrenal glands or hypophysis. This would account for the abrupt fall in plasma estrogen levels when this feto-placental source was removed at parturition.

The highly significant increase in ETR immediately following parturition and continuing to the end of the experimental period would suggest that thyroid hormone plays a role in galactopoiesis or even lactogenesis in the sow. This periparturient elevation of ETR draws attention to the possible significance of the difference in thyroid cell heights that Wagner (1972) observed between normal postpartum sows and agalactic sows. He noted that the normal sows had significantly taller thyroid cells ($P < .05$) than agalactic sows.

Our $E_1 + E_2$:progesterone changes are only in partial agreement with urinary estrogen:progesterone changes observed by Edgerton and Erb (1971). Our result indicates a continued increase in this ratio up until day 0, whereas theirs indicated

lack of change during the last 4 days prepartum.

The prepartum elevation of the estrogens and decline in progesterone preceded the prepartum rise observed for the corticoid by 4 and 3 days respectively. This finding does not support any possible luteolytic role near term for corticoids in this species as suggested by Schams et al. (1972) in the cow.

Our data indicate that in the sow, before parturition occurs, the plasma estrogen and corticoid levels rise well above the gestation levels, and the progesterone levels show a substantial decline. A decrease in progesterone level seems to be a prerequisite to parturition in the sow since Nellor (1963) and Minar and Schilling (1970) were able to delay parturition in the sow by oral administration of progestagens or by parenteral administration of progesterone. Nellor (1963) delayed parturition for as long as 23 days beyond the expected farrowing date. The results of Nellor (1963) and Minar and Schilling (1970) agree with our findings in suggesting that progesterone-block withdrawal is important in initiating parturition in the pig.

Nellor (1963) observed that the systemic level of progesterone which was enough to block parturition and maintain the ovaries in an inactive state did not hinder lactogenesis. A copious milk letdown occurred near expected term. This indicates that a low systemic level of progesterone is not a prerequisite for lactogenesis in the sow and supports the

secondary role in lactogenesis that Meites (1966) assigns to this hormone.

It is reported that the mammary gland of MMA-affected sows are usually well developed (Hogg, 1952). This would suggest that pregnancy levels of progesterone and estrogen were sufficient for preparing the mammary gland for lactation. Ringarp (1960) reported that animals which developed this syndrome had suckled their piglets for the first 12-24 hours after farrowing and argued that this would mean that the condition is not a primary disturbance in milk secretion or ejection. Therefore, one may hypothesize that neither the hormones concerned with mammary gland development during gestation (estrogen and progesterone) nor the "lactogenic hormone complex" are involved in the MMA syndrome. The views on the possible involvement of the adrenal or adrenal hormones in this condition are so divergent that no conclusions can be drawn at present. Nachreiner, Ginther, Ribelin and Carlson (1971) noted hyperadrenalism in affected sows. Contrary to their results, Wagner (1972) did not find any significant difference between the adrenal cortisol contents of normal and affected sows. However, Ringarp (1960) was able to significantly reduce piglet mortality from 30.6% to 23.0% when other treatments for the affected sows were combined with prednisolone. Thus, it is difficult to say whether or not adrenal dysfunction is involved in MMA.

Wagner (1972) found that thyroid cell heights were significantly less in agalactic sows than in normal postpartum sows.

He found that thyroid cell heights in agalactic sows were identical to those of cyclic, non-lactating sows. Using cell height as a measure of thyroid function, it appears that the thyroid glands of agalactic sows are not able to function beyond the level of the non-lactating animal. Our study indicates a significant postpartum increase in thyroid secretion. Since the intensity and duration of milk secretion are reduced in the absence of adequate levels of thyroid hormone (Sulman, 1970), one may hypothesize that agalactia, which is the most important component of MMA complex, is a reflection of the failure of normal thyroid gland function needed to maintain the lactation that was initiated following parturition.

CONCLUSION

The present study involved simultaneous determination of plasma corticoids, progesterone, estrone, estradiol and Effective Thyroxine Ratio (ETR) in 23 puerperal sows. The corticoids, progesterone and estrogens were assayed by competitive protein-binding methods, while the ETR was determined by the Res-O-Mat ETR Test.

The results of the study indicate the following:

1. Parturition in the sow is preceded by a rise in plasma levels of corticoids and estrogens, but a decline in progesterone level.
2. Following parturition, the progesterone level continues to decline and remains very low all through the first week after parturition. The estrogens rapidly fall, becoming barely measurable by day 7 postpartum. The corticoids return to the prepartum level.
3. Estrone is the major plasma estrogen during late pregnancy in the sow.
4. Thyroid activity increases during the postpartum period as indicated by an increased ETR value. It is suggested that this increase could play a role in lactogenesis and or galactopoiesis and may be involved in the pathogenesis of the agalactia observed in the MMA complex.

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APPENDIX

Appendix Table 1: Preparation of Corticoid-Binding Globulin (CBG) Solution

- A. CBG Solution for Corticoid Assay. (for 100 ml CBG)
1. Place 4 ml of stock solution of ^3H -cortisol in a round bottom flask (RBF) and evaporate the ethyl alcohol solvent on a rotary evaporator in a 45C water bath.
 2. Rinse the flask with 1 ml of ethyl alcohol to resuspend the labelled corticoid.
 3. Add 96 ml of triple distilled water.
 4. Finally, add 3 ml of dog plasma. (Plasma is from a dog adrenalectomized 36-48 hours prior to blood collection).
 5. Mix by gentle manual rotation.
 6. Store at 4C for at least 1 hour before use.
- B. CBG Solution for Progesterone Assay (for 100 ml CBG)
1. Measure 0.03 of ^3H -corticosterone in RBF.
 2. Add 99 ml of triple distilled water.
 3. Add 1 ml dog plasma.
 4. Mix gently and store at 4C for 1 hour before use.
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Appendix Table 2: Preparation of Scintillation Fluid

Scintillation Fluid A.

1. Place 5 g of diphenyloxazole (PPO) into a large bottle.
2. Add 1 liter Toluene.
3. Add 100 ml Beckman Biosolv #3.
4. Mix thoroughly.

Scintillation Fluid B.

1. 4 g PPO.
2. 1 liter Toluene. Mix thoroughly.

Scintillation Fluid C.

1. 5 g of PPO.
 2. 1 liter Toluene.
 3. 200 ml Beckman Biosolv #3
 4. Mix thoroughly.
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Appendix Table 3: Preparation of Celite Column.

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1. Wash celite in 6N HCl.
 2. Wash with triple distilled water until pH 7.0 is attained.
 3. Wash with methyl alcohol.
 4. Wash with ether, then dry and store in a muffle furnace at 540 C.
 5. Prepare the celite column by thoroughly mixing some of the celite with ethylene glycol 2:1 (w/v) and packing the mixture into 5 ml disposable pipettes containing iso-octane to a height of 5 cm. Wash each column with 3 ml iso-octane before use.
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Appendix Table 4: Preparation of Estrogen Assay Reagents

1. Tris-HCl Buffer.

Dissolve 1.21 g of THAM (Tris (Hydroxymethyl) Aminomethane) in 1 liter of distilled water. Adjust pH to 8.0 with hydrochloric acid.

2. Tris-Ethylene Glycol.

To prepare 100 ml, mix 80 ml Tris-HCl Buffer with 20 ml ethylene glycol.

3. Tris-Charcoal-Dextran.

To prepare 200 ml, add 1 g of Norit A charcoal and 0.1 g dextran to 200 ml Tris-HCl Buffer. Mix thoroughly and maintain the charcoal in suspension during use by a magnetic stirrer.

Appendix Table 5: Preparation of Cytosol

1. Preparation is with uteri previously obtained from 6 day pregnant rabbits, frozen on dry ice and stored at -20C until needed.
 2. Homogenize the uteri in Tris-HCl Buffer (pH 8.0) using a Sorvall tissue grinder. Use tissue and Tris-HCl Buffer in the ratio of 1:3 (w/v). The buffer should contain 0.25 M sucrose and 0.001 M EDTA (ethylene-diaminetetraacetate).
 3. Centrifuge the homogenate at 3500 X g for 15 min.
 4. Recentrifuge the supernatant at 105,000 X g for 90 min.
 5. Place in small vials and store in liquid N₂ until used.
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