THE PATTERN OF ESTROGEN SECRETION DURING VARIOUS REPRODUCTIVE STATES OF THE COW

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ABSTRACT

THE PATTERN OF ESTROGEN SECRETION DURING VARIOUS REPRODUCTIVE STATES OF THE COW

The pattern of estrogen during four reproductive states of the cow was determined by utilizing a frequent blood sampling technique. Plasma samples from a Holstein heifer at thirty minute intervals were analyzed for estrone and estradiol-17β concentrations on day 218 of gestation, day 3 postpartum, day 12 postpartum, and day 10 of the first postpartum estrous cycle using a radioimmunoassay procedure. On day 218 of gestation two peaks in plasma estrone concentration occurred at a nine hour interval, while plasma estradiol-17β peaks were of smaller magnitude and greater frequency. On day 3 postpartum, mean plasma concentration of both steroids had decline and exhibited less variation. Mean plasma estradiol-17β concentration on day 12 postpartum was twice the concentration observed on day 10 of the estrous cycle. Mean concentration ratio of estrone to estradiol-17β was approximately 4:1 on day 218 of gestation and 25:1 on day 3 postpartum.

These results indicate that a freguent sampling interval is neccesary for determining the circulating estrogen status in the bovine. Also, the significance of episodic plasma estrogen peaks may be related to pituitary gonadotrophin release and the estrone:estradiol-17β ratio may be indicative of the reproductive status of the cow.

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INTRODUCTION

Reproductive effiency is of major economic importance to the dairy industry since milk production is maximized with a twelve month calving interval. The 283 day gestation period of the bovine requires the cow to conceive within ninety days of calving in order to maintain a yearly interval (Larson, 1970). However, a California study involving 16,000 dairy cows found that only 38% were rebred by ninety days (Pelissier, 1976). Fertility problems rank second only to low production as a reason for culling cows (Larson, 1970). Economic losses to the dairyman from reproductive inefficiency include milk losses, replacement costs, calf losses, veterinary services and added breeding fees, resulting in an annual loss per cow of approximately $100 (Pelissier, 1976).

Through basic knowledge of the hormonal involvement in bovine reproduction during pregnancy and postpartum, logical approaches can be made to prevent extended calving intervals and achieve maximum reproductive efficiency. This report is concerned with the determination

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The literature on the following pages follows the style of the Journal of Animal Science.

of the pattern of estrogen secretion into the peripheral circulation during various reproductive states of the cow in order to increase understanding of the physiological functions of the estrogens.

LITERATURE REVIEW

Sources of Estrogen

The estrogens are a major class of steroid hormones having many physiological functions in bovine reproduction. Estrogen production is under the master control of the hypothalamus of the brain. The hypothalamus monitors the animal's hormonal status and produces Gonadotrophin Releasing Hormone (GNRH). GNRH is transported from the hypothalamus via the hypothalamic-hypophyseal portal system to the anterior pituitary gland, subjecting the anterior pituitary to nervous control without direct innervation (Austin and Short, 1972). In response to GNRH, the anterior pituitary secretes Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). FSH and LH are glycoproteins with molecular weights of approximately 30,000 (Hafez , 1980). They are secreted into the efferent venous capillaries draining the anterior pituitary and circulate until they are taken up into organs containing receptor sites specific for each hormone (Austin and Short, 1972).

In the bovine, FSH promotes the growth and development of follicles in the ovary. LH stimulates estrogen synthesis by thecal, granulosa, and

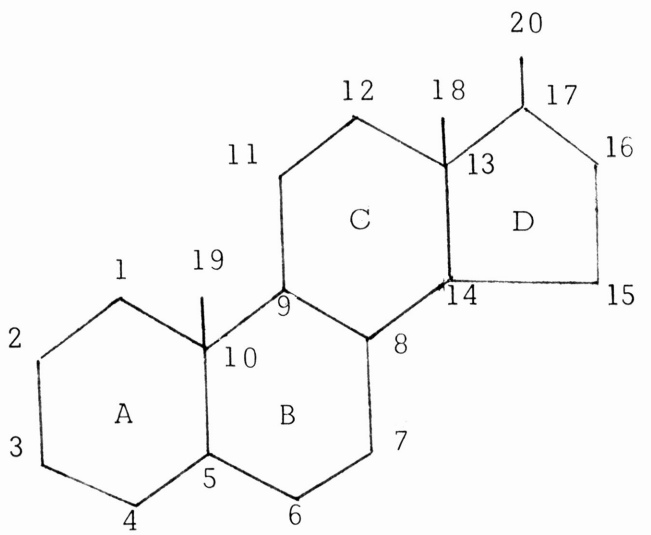
interstitial cells in the ovary, and induces ovulation in follicles primed with FSH (Austin and Short, 1972).

During pregnancy, estrogen is also produced by the feto-placental unit. The fetal adrenal glands provide the estrogen precursors and they are converted to the estrogens in the cotyledons of the fetal placenta (Austin and Short, 1972).

Synthesis of Estrogen

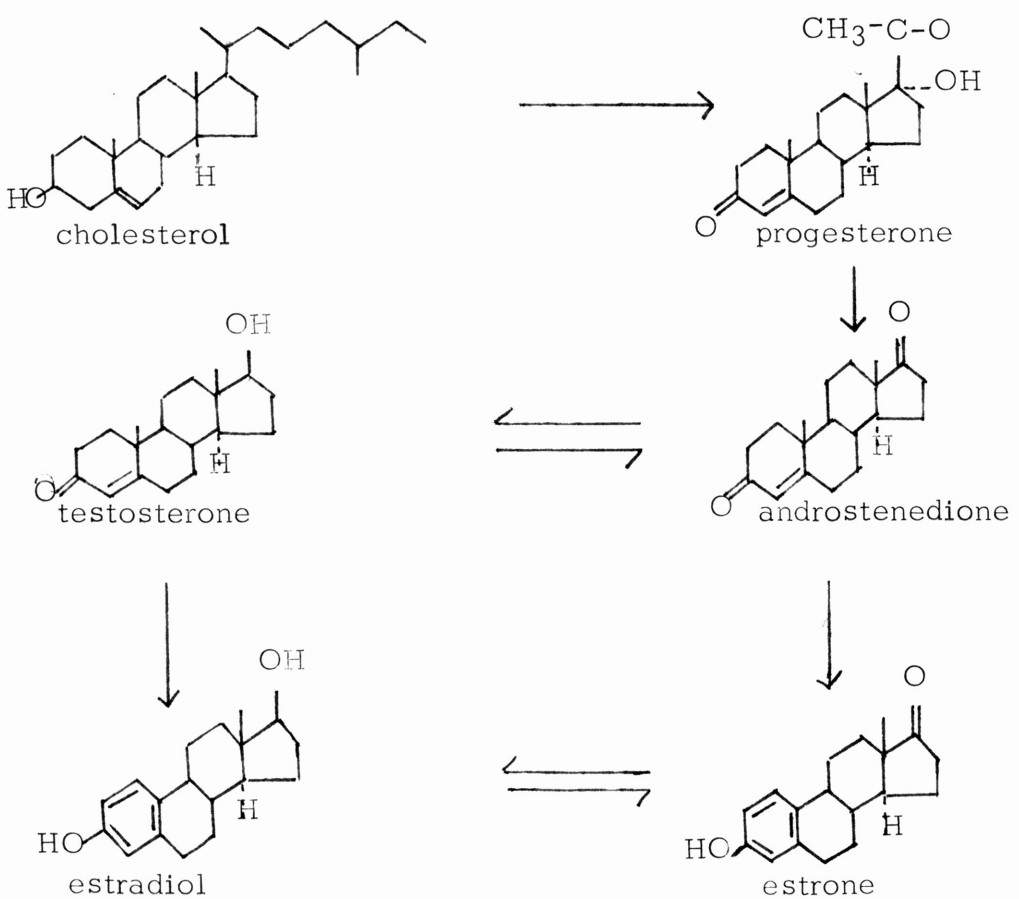
Estrogens are classified as steroid hormones which are lipid compounds having a common basic structure. The steroid nucleus is composed of a cyclopentanophenanthrene ring; the separate hormones differ in the nature of the attached side chains (Hafez, 1980). (Refer to Figure 1)

FIGURE 1
Conventional Representation of the Steroid Ring System (Hafez, 1980)



The enzymes responsible for catalyzing the chemical steps involved in the biosynthesis of reproductive hormones are present in cells of the adrenal glands and the ovaries. All steroid hormones are derived from cholesterol; cholesterol is converted to pregneolone, then pregneolone is oxidized to progesterone. Progesterone is converted to the androgens such as testosterone and androstenedione by hydroxylation at position 17 followed by removal of the C-21 side chain. The androgens are further metabolized to the estrogens (Austin and Short, 1972). (Refer to Figure 2)

FIGURE 2
Biosynthesis of Steroid Hormones (Haefez 197)



The major forms of estrogen in the bovine are estradiol and estrone. The phenolic A ring of the compounds is essential for estrogenic activity and conveys many of the characteristic properties of the estrogens. Two isomers of estradiol exist; estradiol-17α has the hydroxyl group at position 17 extending below the molecular plane, while estradiol-17β has the hydroxyl group extending above the plane. Estradiol-17β is the most biologically potent estrogen and is the major estrogen produced by the ovarian cells. Estradiol and estrone differ in the functional group at position 17 where estrone has a keto group in place of the hydroxyl group. Estrone is intermediate in potency and is the major estrogen produced by the feto-placenta unit. Estradiol-17α is very weak in biological activity. The minor differences in structure between the estrogens have major physiological consequences (Forrest, 1982).

Biological Actions of Estrogen

After synthesis by ovarian cells or the feto-placental unit, the estrogens are released into the peripheral circulation by exocytosis and are transported to target organs bound to plasma proteins. The binding proteins increase the solubility of the steroids in aqueous media such as blood and protects them from metabolism by the liver (Hafez, 1980). The hormones are biologically inactive while associated with the protein, but the steriod-protein complex is in equilibrium with free steroid, which is the form in which the hormone is taken up by tissues (Austin and Short, 1972).

The uptake of estrogen from the blood stream and its retention within the cells of the target organ is dependent on a receptor site specific for the particular estrogen. The mechanism of estrogen interaction with intracellular receptor sites consists of:

1. passive diffusion of hormone across cell membrane;
2. binding of hormone to a cytoplasmic "receptor" protein;
3. changes in the size and shape of the receptor induced by the hormone;
4. penetration of hormone-receptor complex into the cell nucleus;
5. interaction of the complex with the cell's chromosomes.

This interaction is believed to regulate the cell's genomic activity by increasing the number of initiation sites for messenger RNA formation. Secondary changes in cellular protein synthesis, directed by the altered population of mRNA molecules, are responsible for hormonal affects on cellular structure and function (Alder, 1981).

Estrogen Concentration During the Reproductive Cycle

The estrous cycle of the cow is 21 days in length, with behavioral estrus (heat) marking the beginning of the cycle and lasting approximately 16 hours. During the estrous cycle, plasma levels of estradiol-17β are higher than plasma levels of estrone. Peripheral levels of both estrogens peak several hours before the onset of behavioral estrus and the surge of pituitary LH release that results in follicle rupture and ovulation. The delay between the peak in estrogen secretion and the appearance of

FIGURE 3

Hormonal Control of the Bovine Estrous Cycle (Forrest, 1982)

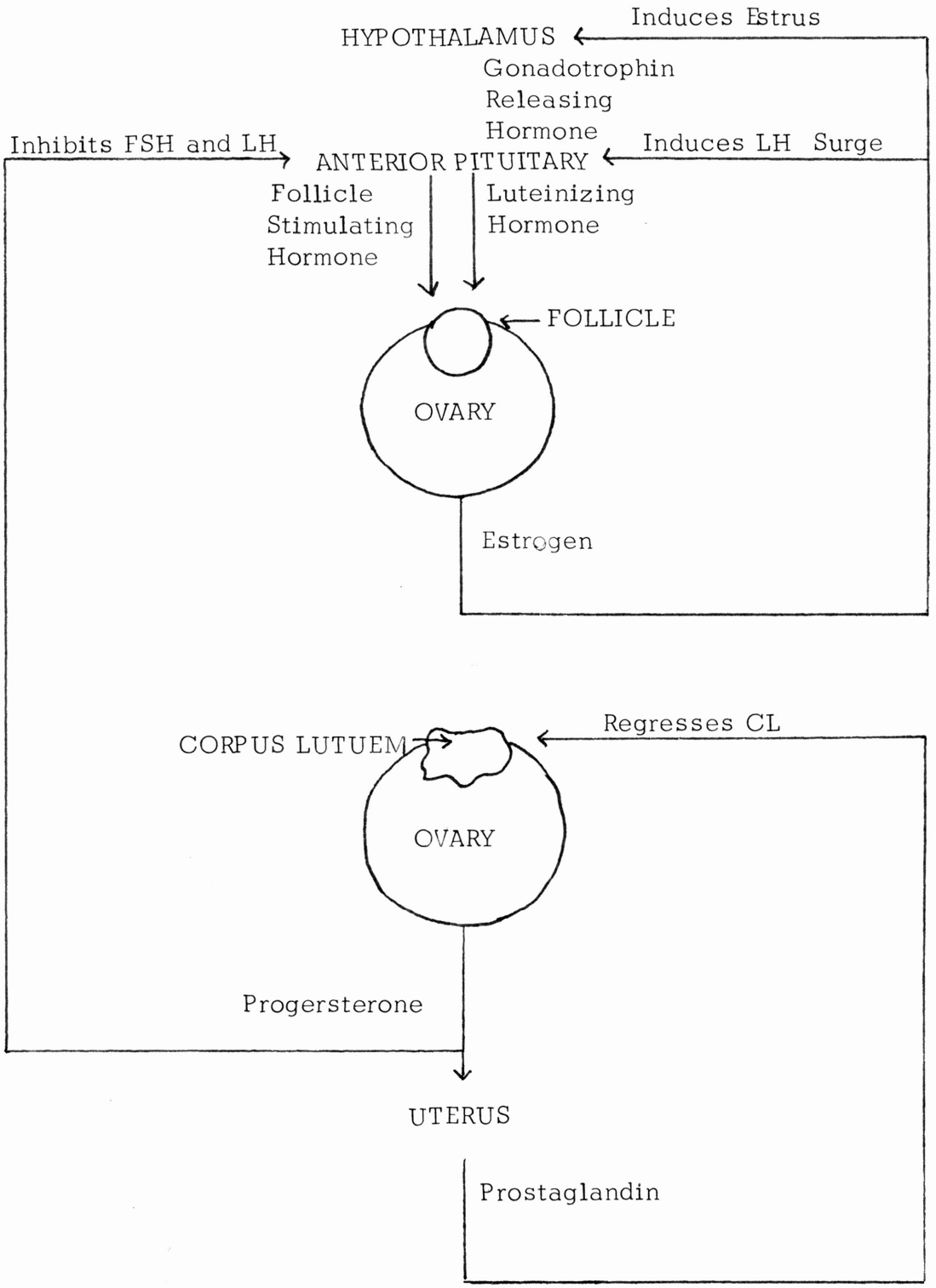
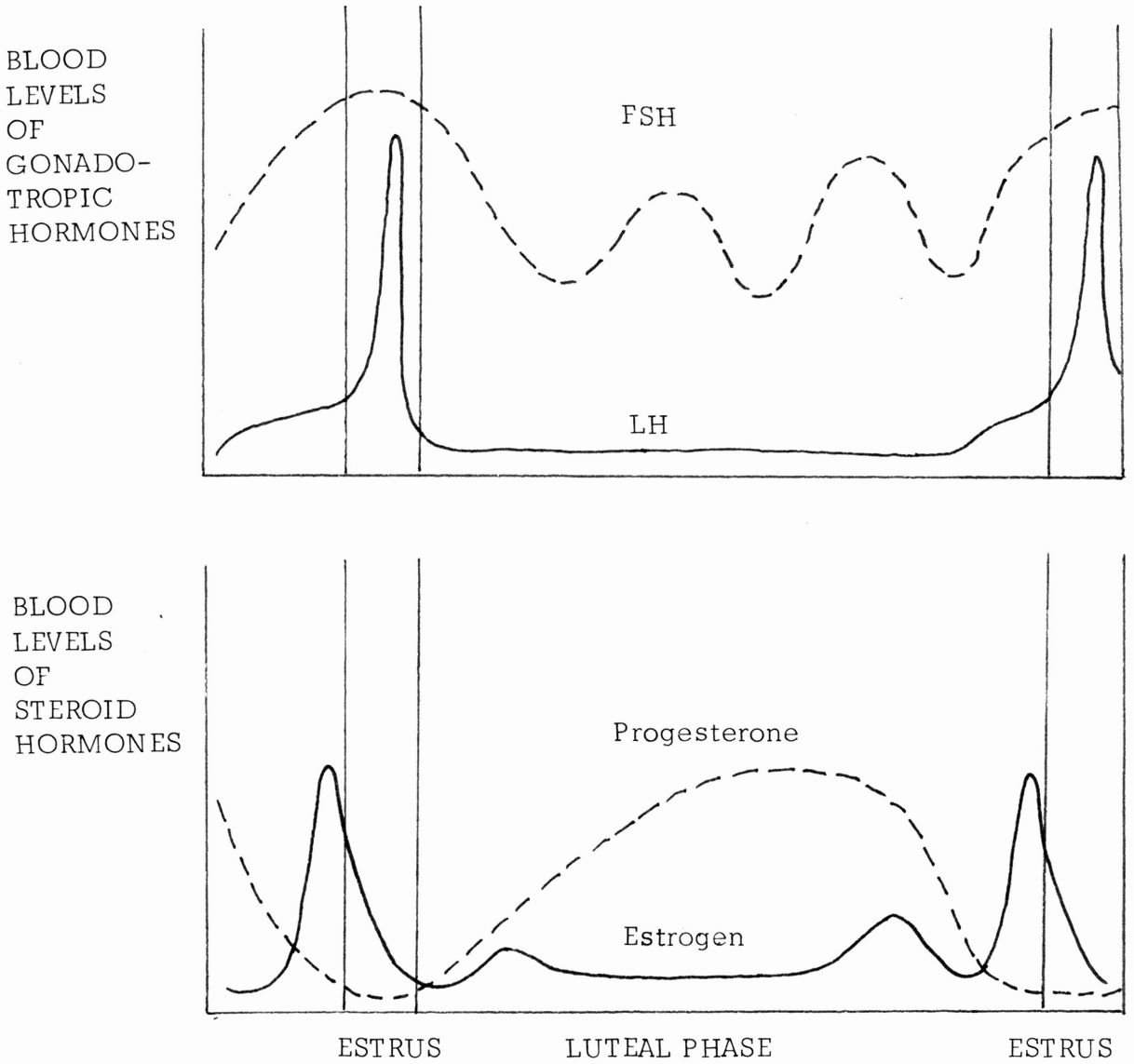


FIGURE 4
BLOOD LEVELS OF HORMONES DURING THE ESTROUS CYCLE (Hafez, 1980)

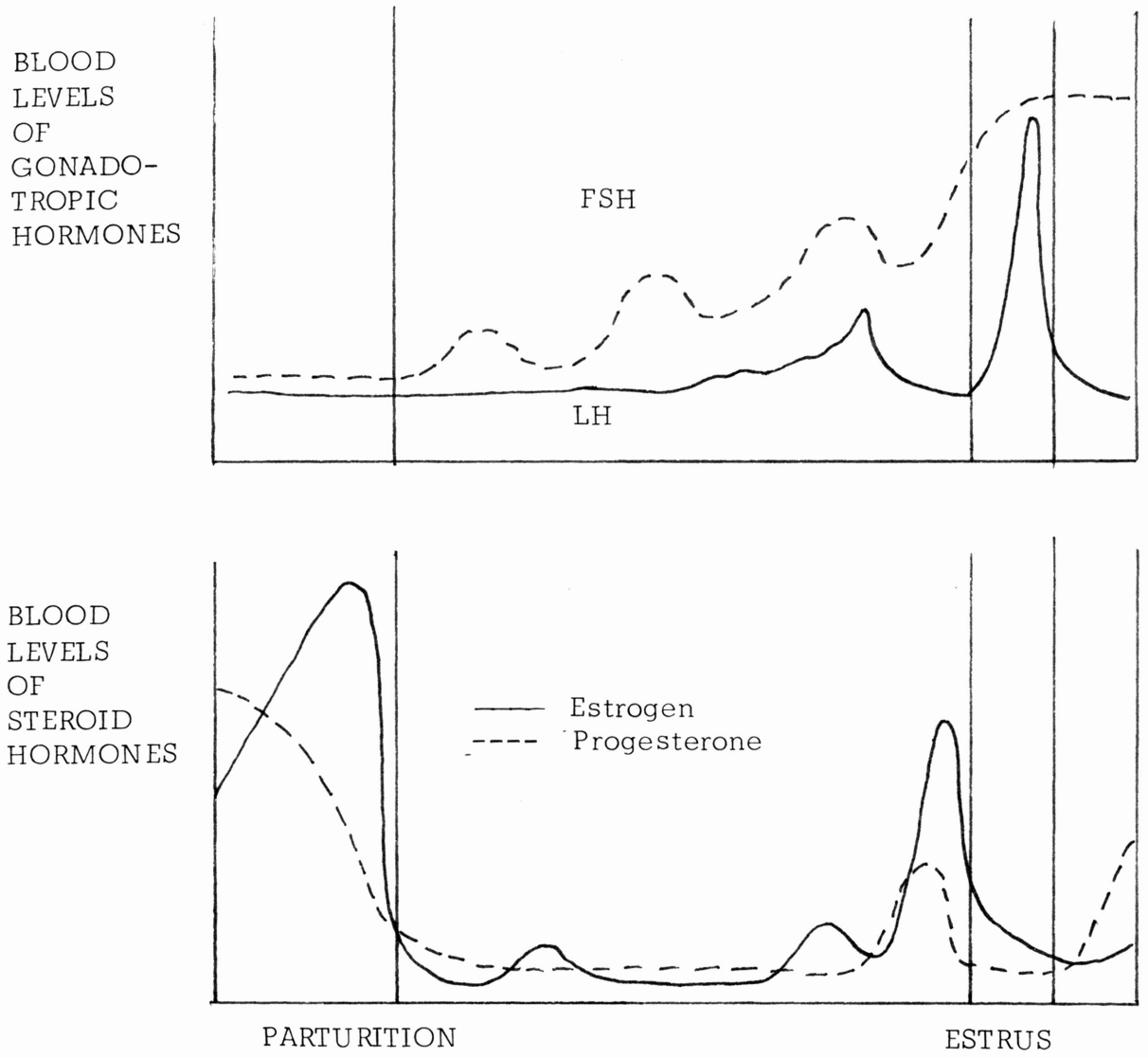


estrogenic effects supports the proposed mechanism of estrogen interaction with intracellular receptor sites. Therefore, estrogen's effect should be conceptualized as a "permissive agent" (increasing the probability that appropriate stimuli occurring after an induction period will elicit the response) rather than as a "stimulus" (required at the time that the response occurs (Alder, 1981). The high estrogen levels result from the maturation of the follicle under the influence of FSH and cause the hypothalamus to initiate estrus and act as a "permissive agent" on the anterior pituitary by increasing its sensitivity to the action of GNRH, resulting in the LH surge. Ovulation occurs approximately 30 hours after the onset of estrus; the cells of the ruptured follicle are transformed into the Corpus Luteum (CL) and produce the steroid progesterone which functions to prepare the reproductive system for pregnancy and to maintain pregnancy. Progesterone secretion has an inhibitory effect on FSH and estrogen production, but if pregnancy does not occur the uterus produces prostaglandins and causes the regression of the CL and progesterone levels decline on day 15 of the estrous cycle. The preceeding events allow FSH to stimulate follicular growth and estrogen production by the ovarian cells which initiates the start of the next estrous cycle. (Refer to Figures 3 and 4).

If pregnancy does occur, estrogen levels increase throughout gestation with plasma estrone levels exceeding plasma estradiol-17β. Smith et al. (1973) and Robertson (1974) have established that normal calving by the cow is preceded by a 10-fold increase in plasma estrogens during the

month before parturition. By cannulation of maternal utero-ovarian and jugular veins, Peterson et al. (1974) determined that this increase in peripheral estrogen was primarily of feto-placental origin. (Refer to Figure 5).

FIGURE 5
BLOOD LEVELS OF HORMONES
FROM PARTURITION THROUGH THE FIRST POSTPARTUM ESTRUS (Hafez, 1980)



The placental and ovarian estrogens promote the growth and development of the mammary glands and uterus during pregnancy as well as inducing changes in maternal metabolism by altering carbohydrate metabolism, protein synthesis, and thyroid and adrenal functions (Austin and Short, 1972).

Plasma levels of estrogen peak and decline rapidly at parturition, but the role of estrogen in the parturition process is not clearly defined. Smith et al. (1973) suggests that the altered estrogen metabolism may actively initiate the onset of parturition. Stellflug et al. (1978) proposed that the rapid decline in plasma estrogen may be due to a loss of circulation between maternal and fetal placental units since estrogen levels remain high after parturition in cows that have retained placentas.

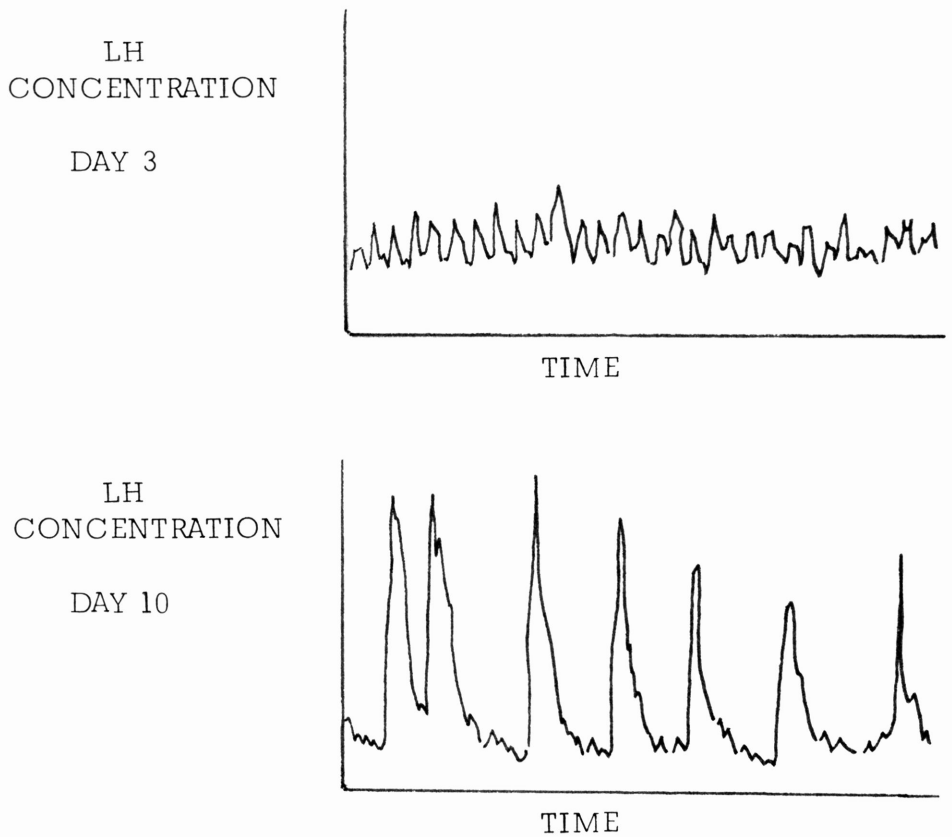
With the expulsion of the placent at parturition, ovarian production of estrogen remains low until resumption of the estrous cycles. Through the estrous cycle, concentrations of estradiol-17β are greater than those of estrone. According to Echternkamp and Hansel (1973) erratic changes in estradiol-17β concentrations postpartum may be due to FSH release and its effect on follicular growth and degeneration. Resumption of the estrous cycle occurs when estrogen levels peak and induce the pituitary LH surge to cause ovulation. Estrogen concentrations then remain low during the luteal phase, increasing again when progesterone levels decline.

Frequent Blood Sampling Techniques

Most of the research previously conducted on determining hormonal

levels during different states of reproduction utilized infrequent blood sampling techniques. By utilizing a 10 minute sampling interval, Rahe et al. (1979) found that the concentration of plasma LH in cows during the estrous cycle was not maintained at a constant level but fluctuated in a pulsatile manner depending upon the period of the cycle. (Refer to Figure 6)

FIGURE 6
PLASMA LH LEVELS ON DAY3 AND DAY 10 OF THE ESTROUS CYCLE (Rahe et al., 1979)



This research suggested that a frequent sampling interval needed to be utilized to determine if estrogen is secreted in an eposodic pattern during various reproductive states of the cow.

MATERIALS AND METHODS

Heifer Selection

Six pregnant Holstein heifers from the Texas A&M University Dairy Center herd were selected on the basis of:

1. an expected calving date between August 25 and September 25;
2. good disposition for easy handling;
3. similar genetic potentials for milk production.

The cow is an ideal experimental animal because its large blood volume of blood allows for frequent blood sampling over prolonged periods.

Heifer Management

The cows were trained to lead and gentled prior to the start of the collections to minimize stress. During the collection periods they were tied in individual stalls and offered hay, silage, and water. On the day preceding each collection, the jugular vein of each heifer was cannulated using a medical grade plastic tubing and the cannula was held in place with gray duct tape.

Sample Collection

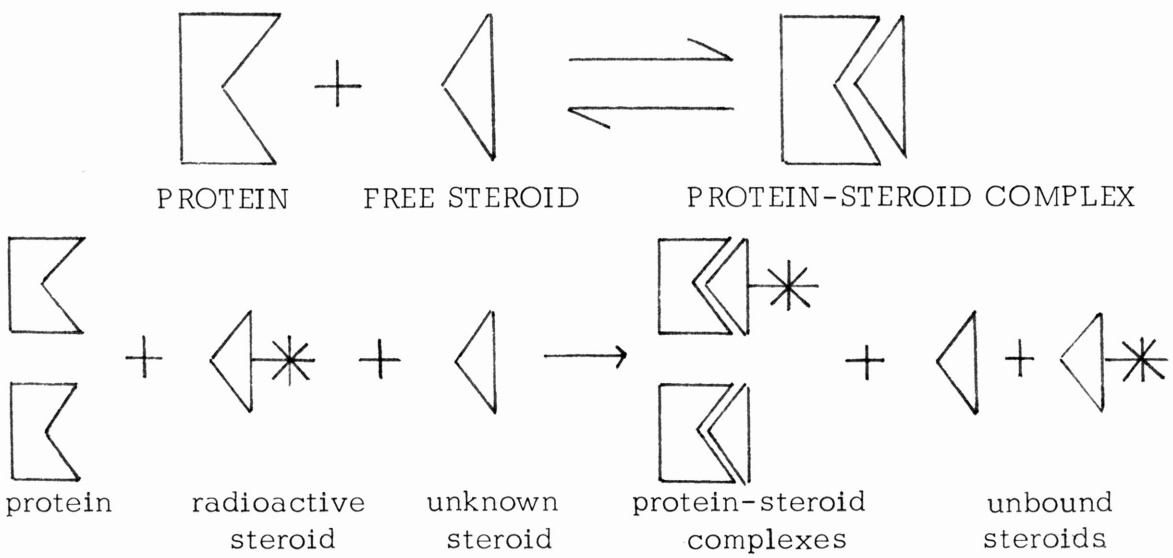
Blood samples were collected from each cow at ten minute intervals over a ten hour period on or near day 218 of gestation, day 279 of gestation, day 3 postpartum, day 12 postpartum, and day 10 of the first postpartum

estrous cycle. Individual plastic syringes were used to withdraw 10 ml samples of blood from the cannula; following sample collection, sterile physiological saline solution (PSS) and heparinized saline were flushed through the cannula. The samples were immediately transferred from the syringes to 12 x 75 mm test tubes containing two drops of heparinized saline. The samples were then centrifuged at 4° C and the plasma drawn off and transferred to 12 x 75 mm test tubes and stored at -20° C until assayed.

Estrogen Radioimmunoassay

The assay used to determine estrone and estradiol-17β concentrations in the plasma samples was previously described by Korenman et al. (1974). The radioimmunoassay (RIA) procedure is based on the observation that steriod hormones are transported in the blood stream bound to proteins. The RIA procedure is a form of saturation analysis in which a limited quantity of antibody (protein) is added to an excess of unknown antigen and radioactively labeled antigen (steroid). The antigens compete for binding sites on the antibody according to the law of mass action. (Refer to Figure 7) When increasing amounts of unknown antigen are added to the assay, the limited binding sites of the antibody are progressively saturated and can bind less of the radioactively labeled antigen. The antibody solution is diluted to allow about 50% of the tracer dose of radioactive antigen to be bound in the absence of unknown antigen. After incubation of the three

FIGURE 7
COMPONENTS OF RADIOIMMUNOASSAY (Austin and Short, 1972)

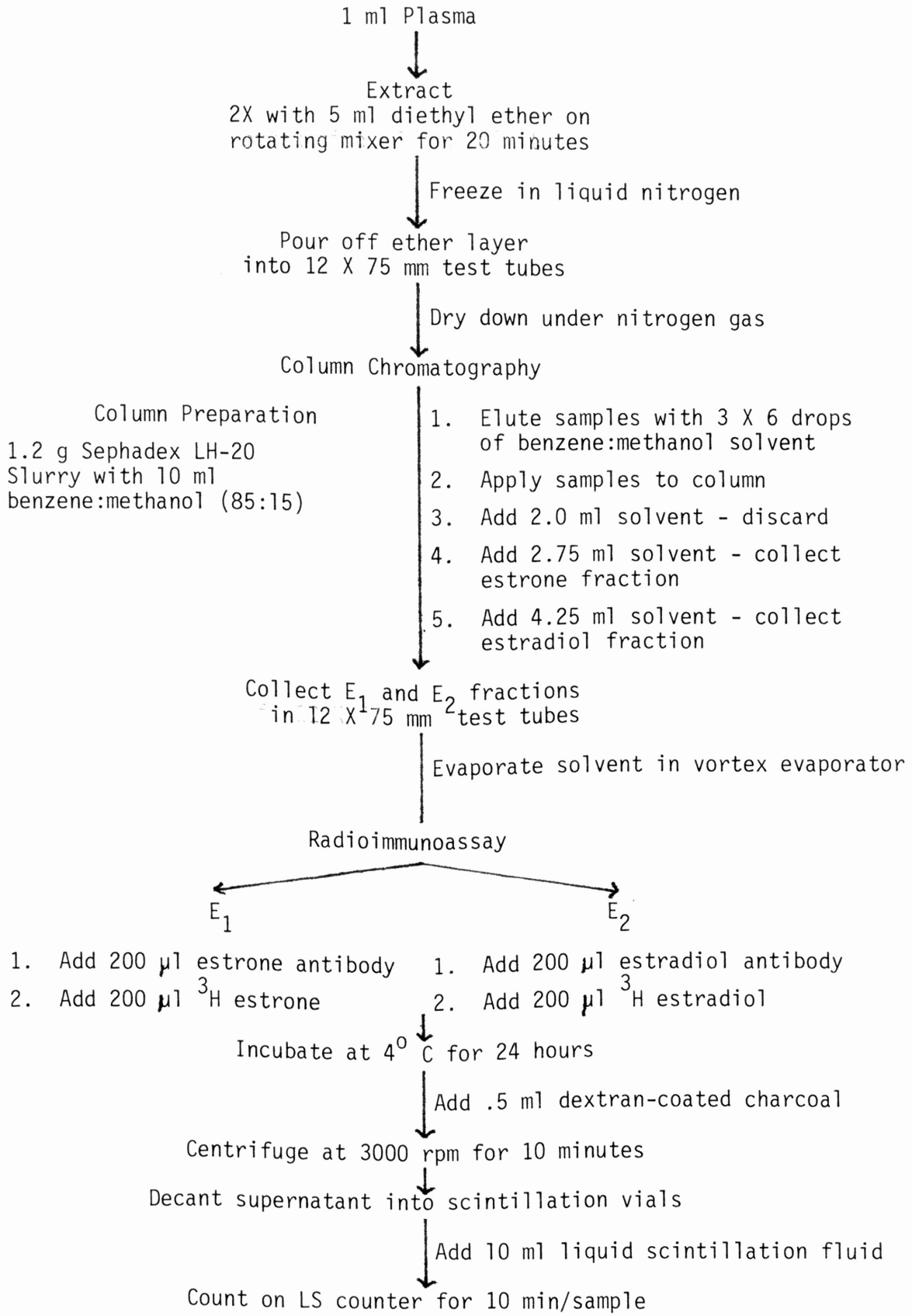


components, the antigen-antibody complexes are seperated from the free antigens and the radioactivity is measured. A diminished binding of radioacively labeled antigen indicates the presence of unknown antigen (Skelley et al., 1973).

The first step in the assay procedure is separation of the steriod fraction from aqueous plasma. 1 ml portions of plasma are thawed and extracted twice with 5 ml of diethyl ether, each time the aqueous layer is frozen and the ether layer is poured off into 12 x 75 mm test tubes and dried under nitrogen gas. (Refer to Figure 8). Polarity differences between estrone and estradiol-17β allow them to be separated by column chromatography on Sephadex LH-20 columns with a benzene:methanol solvent. The estrone (E_1) and estradiol-17β (E_2) fractions are collected in separate test tubes and the solvent is evaporated off.

FIGURE 8

FLOW SHEET FOR ESTRONE (E_1) AND ESTRADIOL (E_2) RADIOIMMUNOASSAY



E_1 and E_2 standards are prepared from stock solutions diluted from concentrations of 200 pg/200µl down to 0.78 pg/200 µl plus total bound standards that contain only methanol. 200 µl of estrone antibody at a 1:15,000 dilution with normal sheep serum (NSS) is added to the E_1 standards and unknowns, while 200 µl of estradiol-17β antibody at a 1:100,000 dilution with NSS is added to the E_2 standards and unknowns. Tritiated estrone (2,4,6 7- ^{3}H-estrone) and estradiol-17β (2,4,6,7,16,17-^{3}H-estradiol-17β) are added at approximately 10,000 counts per minute (cpm) to their respective standards and unknowns. The samples are allowed to incubate at 4° C for 24 hours, then the antibody-antigen complexes are separated from the free hormone by using dextran coated charcoal. 500 ul of the charcoal suspension is added to each sample; the synthetic glucose polymer forms a matrix that allows only the small molecular weight unbound antigen to be absorbed by the charcoal. The tubes are centifuged to form a charcoal pellet and the supernatant is poured off into scintillation vials and counted on the Liquid Scintillation Counter for 10 minute per sample.

Linearization of the curve obtained from the cpm of the standards allows for interpolation and calculation of the original concentrations of estone and estradiol-17β in the plasma samples.

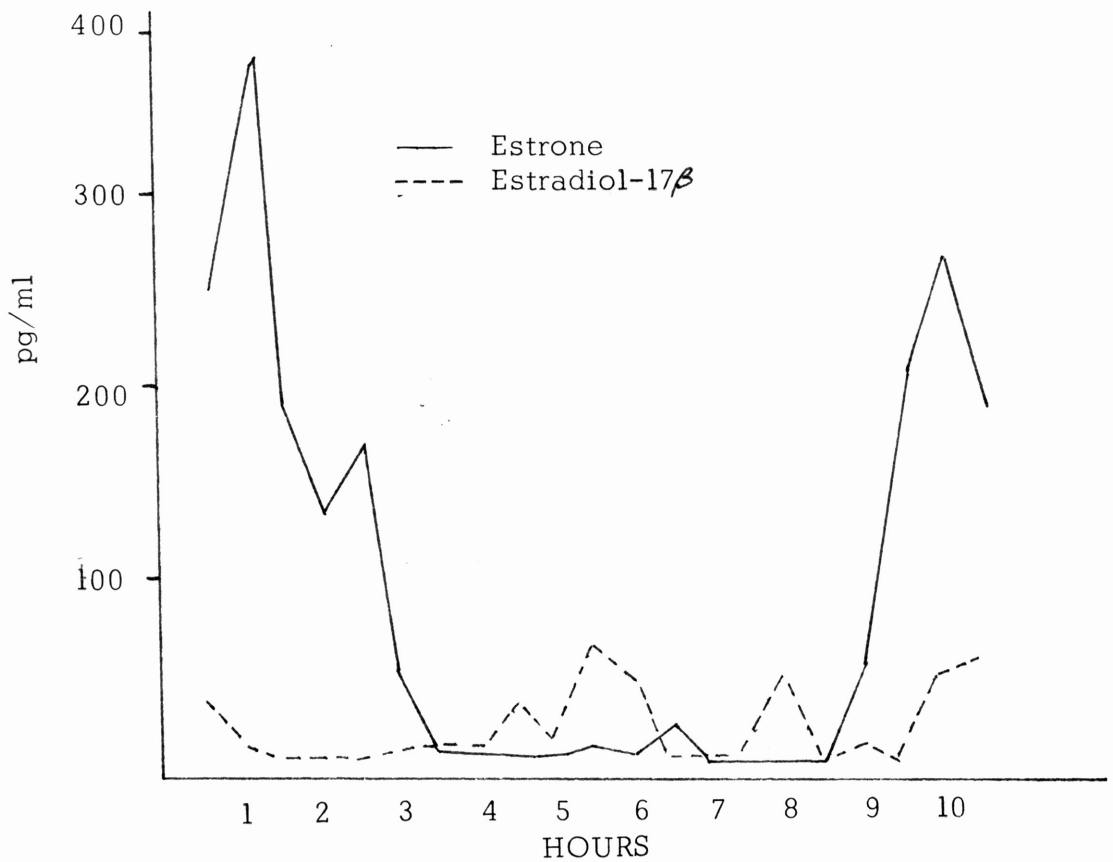
RESULTS AND DISCUSSION

The pattern of estrogen secretion in one Holstein heifer was determined for four different reproductive states utilizing 30 minute interval samples. The concentrations obtained were plotted in pg/ml against time.

On day 218 of gestation, plasma estrone levels peaked at 383 pg/ml, within four hours had dropped to 10 to 20 pg/ml, then peaked again after five hours at 270 pg/ml. Estradiol-17β concentrations varied from 10 to 60 pg/ml with a greater frequency of peaks than estrone. (Refer to Figure 9).

FIGURE 9

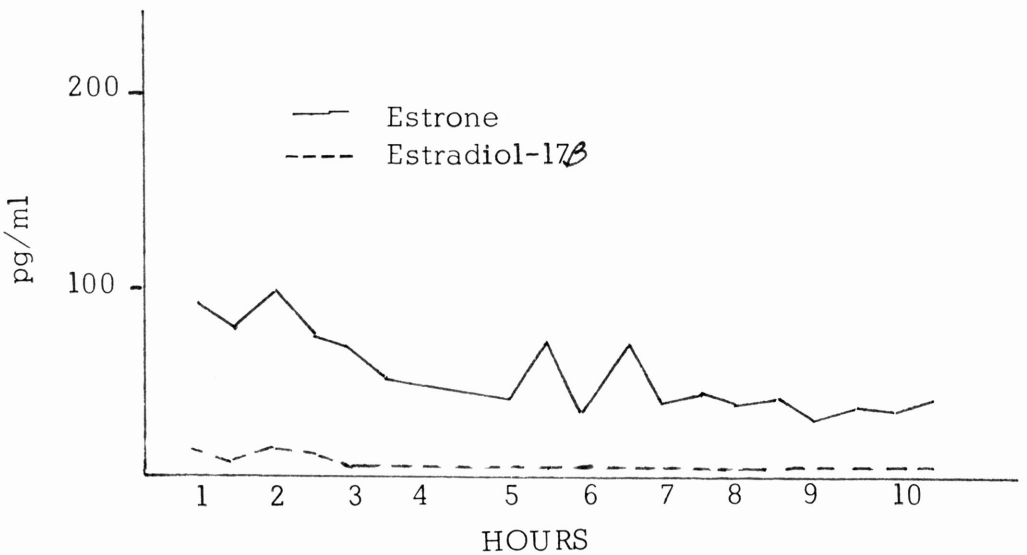
PLASMA ESTRONE AND ESTRADIOL-17β ON DAY 218 OF GESTATION



By day 3 postpartum, both estrone and estradiol-17β levels had declined. Estrone concentration ranged from 32 to 96 pg/ml with several small peaks. Estradiol-17β concentration reached 10 pg/ml in the first three hours then declined below the limits of the standard curve for the remainder of the sampling period. (Refer to Figure 10).

FIGURE 10

PLASMA ESTRONE AND ESTRADIOL-17β ON DAY 3 POSTPARTUM



Estimates of the concentration of estrone on day 12 postpartum and day 10 of the first estrous cycle were not obtained because of assay failure. Estradiol-17β concentration had increased by day 12 postpartum, varying from 10 to 60 pg/ml with frequent small peaks. (Refer to Figure 11) On day 10 of the first estrous cycle, samples were collected only for a 3 hour period, but estradiol-17β concentration exhibited a few peaks ranging from 4 to 26 pg/ml. (Refer to Figure 12)

FIGURE 11

PLASMA ESTRADIOL-17β ON DAY 12 POSTPARTUM

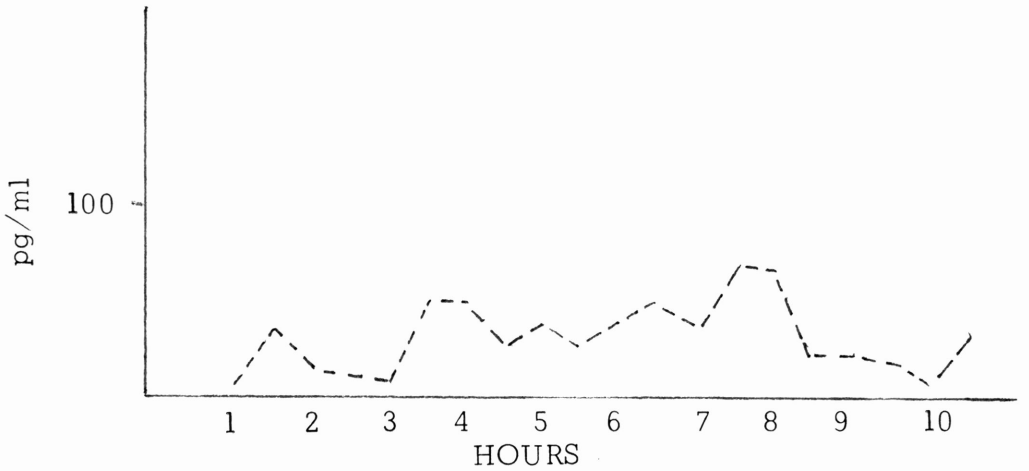
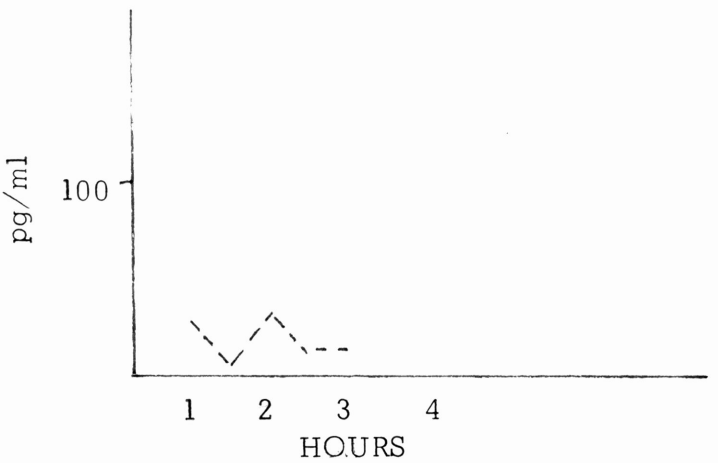


FIGURE 12

PLASMA ESTRADIOL-17β ON DAY 10 OF THE ESTROUS CYCLE



The mean concentration values for each period are listed in Table 1. The large standard errors for estrone indicate the fluctuation in the plasma estrone levels. The similar standard errors for estradiol-17β at day 218, day 12, and day 10 indicate consistent variability in estradiol-17β levels.

TABLE 1

MEAN CONCENTRATIONS OF PLASMA ESTRONE AND ESTRADIOL-17β

	Estradiol-17β ($\bar{X} \pm$ S.E.)	Estrone ($\bar{X} \pm$ S.E.)
Day 218 of gestation	24.7 ± 4.2	98.5 ± 24.3
Day 3 postpartum	2.1 ± 0.6	53.3 ± 4.1
Day 12 postpartum	28.9 ± 3.4	
Day 10 of estrous cycle	15.9 ± 4.3	

Summary

To summarize the results of the estrogen RIA:

1. On day 218 of gestation, two peaks in plasma estone concentration occurred at a nine hour interval; episodic increases of plasma estradiol-17β levels were of a smaller magnitude and greater frequency.

2. On day 3 postpartum, mean plasma concentration of estrone and estradiol-17β had declined, and the variation in the concentration of each steroid hormone also decreased during this period.

3. Mean plasma estradiol-17β concentration on day 12 postpartum was approximately twice the concentration observed on day 10 of the estrous cycle.

4. Mean concentration ratio of estrone to estradiol-17β was approximately 4:1 on day 218 of gestation and 25:1 on day 3 postpartum.

The greater estradiol-17β concentration on day 12 postpartum than day 10 of the estrous cycle could be indicative of ovarian follicular development preparatory to the first estrous cycle. The 25:1 ratio of estrone to estradiol-17β

concentration on day 3 postpartum may be due to residual estrone production by the maternal uterus or immediate postpartum development of follicles on the ovaries.

Conclusions

Several conclusions can be drawn from this research work. First, the maximum variation of up to 30-fold and 5-fold in plasma estrone and estradiol-17β, respectively, during a ten hour sampling period suggests the need for frequent sample collection to characterize the circulating estrogen status in the bovine. The significance of episodic plasma estrogen peaks may be related to pituitary gonadotrophin release. Finally, the estrone:estradiol-17β ratio may offer an indication of the reproductive status of the cow.

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