BOVINE SERUM PROTEIN HORMONE CONCENTRATION DURING LATE PREGNANCY, PARTURITION AND EARLY LACTATION

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ABSTRACT

BOVINE SERUM PROTEIN HORMONE CONCENTRATION DURING LATE PREGNANCY, PARTURITION AND EARLY LACTATION

By

Winston Ingalls

Jugular serum was collected from 32 heifers daily from 6 days before to 5 days after parturition, otherwise twice weekly from 30 days before parturition, until first estrus. Serum prolactin ranged from 50 to 100 ng/ml until 2 days before parturition, exceeded 200 ng/ml during the 2 days before parturition and declined to about 60 ng/ml by 60 hours postpartum. The values ranged between 50 and 100 ng/ml throughout the remainder of the postpartum period. Serum growth hormone varied from 4 to 7 ng/ml before parturition, increased to 12 ng/ml for about 36 hours beginning at parturition and then decreased to prepartum levels. Luteinizing hormone in blood serum did not change measurably from 30 days before parturition until 4 days postpartum when a gradual increase commenced. A peak of 1.7 ng/ml was noted on day 12 after calving. Changes in release of prolactin, growth hormone, and LH are asynchronous around parturition.

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Winston Ingalls

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To his student colleagues who assisted in all aspects of the research being reported he expresses his sincerest appreciation and thanks.

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INTRODUCTION

Knowledge of endocrine events that control conception, pregnancy and lactation could be of fundamental economic importance to the animal industry. Understanding endocrine involvement in these processes could provide the basis for therapeutic manipulation to optimize reproductive efficiency and lactation. In addition, understanding reproduction and lactation disorders requires data from normal animals for comparison.

Reproductive efficiency can be increased by shortening the interval from parturition to conception. Improvements in nutrition, health management and estrus detection have all been shown to reduce this interval. But in many cases it would appear that endocrine imbalance may cause a prolonged calving interval. Knowledge of normal endocrine parameters during the early post-partum period would allow us to appraise and possibly correct endocrine disfunction.

The obvious goal of research dealing with initiation of lactation is to bypass the necessity of pregnancy by using hormone therapy to induce udder growth and cause the onset of copious milk secretion. Attempts have been made

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to hormonally establish lactation but variability of response coupled with lesser milk yields than during a normal lactation has limited its use. Perhaps when we gain sufficient information concerning endocrine control of mammary growth and lactation we shall be able to simulate the biological changes that occur in the mammary gland during pregnancy and parturition and artificially produce lactation.

This thesis research was undertaken to define, in part, endocrine changes occurring during late pregnancy, parturition and early lactation in cattle. It is hoped that the changes in serum growth hormone, prolactin and luteinizing hormone concentrations described here-in will add to a growing body of knowledge which will eventually allow optimum control of reproductive and lactational performance.

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REVIEW OF LITERATURE

Gestation Period

The time interval between fertilization of an ovum and expulsion of a fully developed fetus is referred to as the gestation period, or gestation. Its duration is influenced by many factors and drastic alteration in length may be detrimental to both fetus and dam. The term parturition refers to the physical act of expelling the fetus.

Factors Affecting Length of Gestation

For most dairy cattle breeds the gestation period is 278-284 days (Salisbury and VanDemark, 1961). In Brown Swiss cattle it is 290 days. Herman and Spalding (1947) stated that gestation length was affected by breed differences. They also reported that: (1) Multiple fetuses reduced gestation length by approximately 8 days; (2) the gestation period was slightly shorter for heifers than for older cows; (3) cows calving in the fall and winter carried their young one to three days longer than animals calving during the spring and summer; and (4) male calves were carried one day longer than female calves.

Cases of abnormal length gestation are of interest when searching for clues as to what controls timing of parturition. Mead et al. (1949) observed prolonged gestation periods in cattle (310-350 days) and reported that most of the calves delivered were hypoglycemic and failed to survive more than a few hours. Subsequent, pedigree examination revealed the syndrome to be elicited by a single autosomal recessive gene and the dam of any fetus homozygous for this gene exhibited prolonged gestation. In addition, the fetal pituitary was absent and fetal adrenals were hypoplastic. This disorder provided evidence that a normal functioning fetal endocrine system is needed in cattle to insure a normal length gestation. A condition displaying the same trend and a similar genetic pattern was described by Stormont et al. (1956) wherein the fetus failed to grow after seven months and gestation was extended up to 500 days.

Ewes ingesting skunk cabbage (Veratum Californicum) produced lambs with cephalic abnormalities and displayed prolonged gestation (Binns et al., 1963). Similarly, feeding sheep the shrub Salsola tuberculata during the final 50 days of gestation resulted in lambs with small pituitaries and hypoplastic adrenals (Basson et al., 1969). These cases of prolonged gestation involve interference with a component of the fetal pituitary-hypothalmic-adrenal axis which apparently is essential for a normal duration of gestation in sheep and cattle.

Shortened rather than prolonged gestation is more common in farm animals. Bacterial agents are the most frequent cause of premature deliveries. Osburn et al. (1969) were able to induce abortions within a few days following intrauterine inoculation of pregnant cows with vibrio fetus. Plasma progesterone levels always dropped near the time of fetal expulsion but the timing of the progesterone decline was dependent on the stage of gestation. Abortions during the latter stage of pregnancy (7-8 months) followed 24 hours after a rapid decline in blood progesterone similar to the normal decline in maternal progesterone changes preceding normal births.

The examples cited demonstrate the importance of both the fetal and maternal endocrine systems for normal gestation and parturition in sheep and cattle.

Parturition

Non-Endocrine Factors Affecting Parturition

The size and weight of the near term fetus may cause uterine distension and irritability which in turn could cause increased uterine contractions and initiation of labor (McDonald, 1969). Severe uterine distension near parturition may cause decreased uterine blood flow resulting in waste product build up which may trigger parturition. Baird and McDonald (1964) reported a gradual and consistent decrease in oxygen consumption by the maturing

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bovine placenta from the fifth month until term, perhaps indicating placental aging which eventually may signal the onset of parturition.

Uterine Contractility Near Parturition

Gillette and Holm (1963) studied uterine and abdominal wall contractions in cattle by means of balloon implants attached to strain gauges. Uterine contractions 2-4 days prepartum were irregular, brief and uncoordinated. In contrast, uterine contractions were regular, propagative and longer in duration beginning 2 hrs before and continuing through parturition. In addition, abdominal muscle contractions during parturition were strong, rapid and appeared to commence with the movement of the allantoic sac through the cervix reaching a peak 10 minutes prepartum. Despite the strength of the abdominal contractions these workers considered uterine contractions to accomplish 90 percent of the work of fetal propulsion at parturition. Postpartum uterine contractions were expulsive and rapid for up to 24 hours.

Hindson *et al.* (1965) found no changes in intrauterine pressure in ewes due to myometrial contractions until 12 hours before fetal delivery and even then no consistent pattern was observed. Cervical dilation occurred most rapidly during the last hour prepartum and this appeared to initiate intense abdominal muscle contractions

which corresponded with the peak of uterine pressure waves. Immediate postpartum contractions were similar to those of the prepartum period.

Stimuli for uterine contractions are maximum near parturition but coordinated contractions will not result unless the uterus is responsive. Csapo (1950), working with rats and rabbits, reported that as pregnancy progressed uterine actomyosin content increases thereby increasing contractile capacity. Despite this increase in potential contractility the uterus remains quiescent until term. Csapo (1956) theorized that during most of pregnancy progesterone prevents the uterus from contracting when stimulated. He found that electrical stimulation of the uterus of early pregnancy resulted in local, nonpropagated contractions whereas similar stimuli applied to the uterus at term or early postpartum produced propagated contractions which moved over its entire surface. Based on these observations, Csapo and Woodbury (1963) suggested that somehow the intercellular conduction process is facilitated near parturition.

Phases of Parturition

Parturition or fetal expulsion has been separated into three phases by Marshall (1952) and reviewed by Salisbury and Vandemark (1961) and McDonald (1969).

During stage one gradually strengthening myometrial contractions begin and cervical dilation commences. The fluid filled fetal membranes are forced into the cervix

along with the forelimbs of the fetus resulting in even more strenuous uterine contractions. During this preparatory stage in monotocous species, the fetus which normally lays on its back is rotated into a dorsal position with its head and forelimbs forcing against the cervix. In cattle this stage is generally limited to a few hours.

The second or expulsive stage results in complete cervical dilation and extremely strong uterine and abdominal muscle contractions, which force the fetus through the birth canal. In cattle this is generally accomplished in approximately 20 minutes but may last two hours, especially in first calf heifers.

Following fetal delivery continuous, strong uterine contractions persist. During this third phase fetal membranes, blood and other fluids are expelled. If the fetal membranes have not been delivered by 12 hours in the cow, it is generally considered pathological and referred to as a "retained placenta." Postpartum uterine contractions aid in reducing uterine size and constricting blood vessels to prevent excess hemorrhaging.

Endocrine Pattern Near Parturition

Many factors are involved in parturition and some of the possible control mechanism have been alluded to in the previous discussion. Despite all of these contributing forces, control of parturition is probably dominated by the endocrine system.

Estrogens

Levin (1945) demonstrated increased concentrations of estrogens in the feces and urine of cattle during the final two weeks of pregnancy. Similar results have been reported for laboratory animals (Meites and Turner, 1948). Hunter et al. (1970) reported that urinary excretion of total estrogens and estradiol 17-α increased continually during the final 30 days prepartum and peaked either at parturition or 12 hours after. Timing of the estrogen peak was primarily dependent on the length of gestation. Estrone and estradiol 17-β were unchanged during the last month of pregnancy. Breed, twining and stage of gestation significantly affected prepartum urinary excretion rates. The greatest increase in estrogen excretion occurred during the final 40 hours prepartum, but estrogen excretion declined continuously up to 48-72 hours postpartum (Mellin et al., 1966). Estradiol 17-β in the urine continued to increase until 16 hours postpartum and declined thereafter.

Holm and Galligan (1966) observed a marked and steady increase in plasma estrone and total estradiol concentration during the final month of normal pregnancy, followed by a sharp postpartum decline. In contrast, cows exhibiting prolonged gestations showed elevated urinary estrogens 25-40 days before expected calving and a steady decline thereafter with no increase at expected term. The significance of this observation is unknown, but it does

suggest that proper serum estrogen concentration may be required to insure normal parturition.

Urinary estrogen concentration, although recognized to be of limited value as an estimator of ovarian function, does indicate a trend toward increasing estrogen secretion during the last month of pregnancy. Recent development of radioimmunoassays capable of detecting plasma levels of estrogens should provide more sensitive estimates of altered estrogen secretion.

Progesterone

Progesterone secretion patterns during pregnancy have been well documented for many species. In the bovine, plasma progesterone concentration fluctuates a great deal during the course of gestation (Randel and Erb, 1971). Erb et al. (1968a) demonstrated no significant change in corpus luteum content and concentration of progesterone during pregnancy. Short (1960) reported that blood progesterone concentration in cattle was elevated until the last month of pregnancy declining thereafter until parturition. Hunter et al. (1970) confirmed this gradual decline in progesterone during this final month. Pope et al. (1969) and Stabenfeldt et al. (1970) indicated a more rapid decrease in progesterone concentration about 24 hours prepartum in cattle. Smith et al. (1971) indicate that the rapid prepartum progesterone decline may commence as early as 3.5 days prepartum in Holstein heifers.

Although the corpus luteum of pregnancy is not required in several species (Hafez, 1968) during the third trimester of pregnancy, most studies indicate that gestation is not normal following ovariectomy at 200 days gestation in cattle (Estergreen et al., 1967). Generally if exogenous progesterone is not administered gestation is shortened, calving difficulties occur and fetal membranes are nearly always retained (Tanabe, 1970). Evidently extraovarian sources of progesterone are insufficient to support normal gestation and parturition.

Using in vitro methods, Ainsworth and Ryan (1967) demonstrated the ability of placenta from late pregnant cattle and sheep to convert pregnenolone-7-^{3}H to radioactive progesterone. Many metabolites of progesterone were also recovered with the pregnane derivatives predominating. These results indicate a placental capacity to both synthesize and metabolize progesterone.

The low blood progesterone during late pregnancy may only be necessary for proper parturition and deciduation of the placenta. Why ovarian progesterone production decreases as parturition approaches is unknown. Mills and Morrissette (1970) demonstrated that progesterone syntheses by ovaries from cattle during early and late pregnancy responded equally well to luteinizing hormone stimulation. They postulated that decreased progesterone secretion during late pregnancy may be due to a decreased

concentration of circulating gonadotropin. This is doubtful in light of data reported by Randel and Erb (1971) which demonstrated no change in plasma LH from day 7 to day 260 of pregnancy.

Adrenal Corticoids

Glucocorticoids are steroid hormones secreted by the adrenal cortex which are necessary for normal parturition in sheep and cattle.

Adams and Wagner (1970) observed a significant increase in bovine plasma corticoid concentrations to a plateau at 4 days prepartum which continued through normal parturition and declined thereafter. Smith et al. (1971) found that serum glucocorticoid concentration increased 12 hours prepartum, remained increased at parturition and declined to prepartum levels by 12 hours after calving. Inactivation of cortisol by protein binding in serum is apparently of limited physiological significance in domestic ruminants. In sheep, cortisol binding to specific globular protein is low relative to non-ruminant species (Linder, 1964).

Synthetic glucocorticoids have been used successfully (Adams and Wagner, 1970) to induce fetal expulsion in sheep and cattle, i.e. a single 20 mg injection of 9-α F Prednisolone within a range of 250-293 days was effective. More recently Jochle et al. (1971) induced parturition in the bovine with a single 10 mg intramuscular

injection of Flumethasone at day 270 of pregnancy and 100 mg progesterone daily could block this effect. Both investigators reported a high incidence of dystosia and retained fetal membranes.

Prolactin

Pituitary prolactin concentration remains relatively unchanged throughout pregnancy then increases markedly near parturition in rats (Reece et al., 1939), rabbits and guinea-pigs (Holst and Turner, 1939) and mice (Hurst and Turner, 1942). The increase of prolactin occurs within hours before or after delivery of young in all cases. This pattern established with bioassay of pituitary prolactin has recently been confirmed by radioimmunoassay of serum prolactin. Amenomori et al. (1970) reported prolactin concentration to average 8.3 ng/ml during pregnancy, increased to 29.2 ng/ml one day prepartum and peaked at 65.5 ng/ml on the day of parturition. Thereafter prolactin was maintained at 50 ng/ml presumably by the suckling stimulus. Everett and Baker (1945) observed a constant pituitary acidophil population in rats during pregnancy which increased 100% by the third day postpartum.

Schams and Karg (1970) using radioimmunoassay detected a rapid increase in plasma prolactin beginning 12 hours prepartum and continuing through day 2 in the bovine. Peak plasma prolactin concentration at parturition exceeded 300 ng/ml. But, average serum prolactin values during late

pregnancy (dry period) and lactation were not significantly different. These results have been confirmed by Arije and Wiltbank (1971) with beef cows. Plasma prolactin measured at 90, 180 and 260 days of pregnancy in dairy cows averaged 220, 145 and 365 ng/ml, respectively (Oxender, 1971) but differences between these values were not significant.

The physiological significance of increased pituitary and plasma prolactin at parturition is equivocal. A possible role in the multiplicity of endocrine events which culminate in initiation of lactation has been postulated by Meites (1966). This hypothesis holds that: (a) during pregnancy there is insufficient prolactin and adrenocortical hormones or both to initiate lactation. Both hormones are required to initiate lactation in the guinea pig (Folley, 1956), rat (Lyons et al., 1958) and goat (Cowie et al., 1964b); (b) estrogens and progestins, which are secreted in large quantities during pregnancy prevent the stimulatory action of prolactin and corticoids on the mammary cells; and (c) at the time of parturition, prolactin and corticoids increase concurrently with a decline in estrogen and progesterone resulting in the onset of lactation during pregnancy. Because multiple stimuli will evoke prolactin release in ruminants (Bryant et al., 1970; Tucker, 1971 and Raud et al., 1970) the possibility that increased serum prolactin at parturition may be a non-specific response to massive stimuli associated with parturition must be considered.

During early lactation (day 1-8) serum prolactin concentration remains elevated in rats actively nursing litters (Amenomori et al., 1971). In contrast, average serum prolactin concentration during early lactation in cows is not different than during pregnancy (Schams and Karg, 1970; Arije and Wiltbank, 1971) but prolactin increases in cows and goats in response to milking stimuli (Bryant, Linzell and Greenwood, 1970; Tucker, 1971; and Raud et al., 1971). The difference in response of rats and large animals may reflect differences in suckling or milking frequency.

Growth Hormone

There is a puacity of information concerning serum and pituitary growth hormone (GH) concentration during gestation and parturition and the physiological role of GH at this time is equivocal. Bassett et al. (1970) observed a prepartum increase in GH in three ewes, but the time of increase was variable ranging from 10-1 days prepartum. Oxender (1971) reported serum GH concentration to be 6,8 and 10 ng/ml at 90, 180 and 260 days gestation in cows, but differences between means were not significant. Similarly, Grumbach et al. (1968) reported pregnancy concentration of serum GH to average 7.0 ng/ml in women with no apparent fluctuations during gestation.

Trenkle (1970) working with beef cattle found that feeding diethylstilbestrol at 10 mg per day significantly

increased plasma GH. Increasing serum estrogen near term (Hunter et al., 1970; Smith et al., 1972) may stimulate GH release from the pituitary of cattle nearing parturition.

The paucity of information on the role of GH during pregnancy and lactation probably reflects the fact that GH has not been reported to be a limiting factor to normal gestation. Yet the well documented role of GH in protein, carbohydrate and lipid metabolism (Evans et al., 1966) favors the view that this hormone, although not limiting, importantly contributes to normalcy of pregnancy.

An adequate supply of GH is required for normal mammary growth and lactation (Meites, 1966). A lactational requirement for GH was established by Lyons (1958) and Lyons et al. (1958) using hypophysectomized, gonadectomized and adrenalectomized rats. They determined that GH along with estrogen was required for mammary duct growth whereas GH plus estrogen, progesterone and prolactin was required for lobuloalveolar growth. Results obtained with mice (Elias, 1957; Rivera, 1967) or guinea pig (Gemtsen, 1960) mammary explants in vitro confirmed results obtained in vivo.

After parturition, GH is required to obtain maximum milk production. Cowie et al. (1964) reported that GH could increase and maintain milk production in hypophysectomized goats when administered with prolactin, insulin, glucocorticoids and thyroid hormone. The important

contribution of GH in increasing lactation performances in these goats was shown by an immediate drop in milk production following GH withdrawal even though therapy with the other hormone was continued.

Wrenn and Sykes (1953) reported GH administration markedly improved milk production in heifers in which lactation had been induced by estrogen and progesterone therapy. The increase in milk production was much greater than that obtained with prolactin or crude pituitary extract.

In reviewing the work concerning the galactopoetic effect of GH, Meites (1961) cited the data of Shaw (1955) and Chung (1955) indicating GH administration for 9 days prepartum until 16 days after calving resulted in increased production during the entire lactation period. Brumby (1956) as cited by Meites (1961) was unable to confirm these observations but found an increase until day 7 post-treatment.

Luteinizing Hormone

Limited information is available concerning the LH pattern during pregnancy and around parturition. Labhsetwar et al. (1964) demonstrated pituitary LH concentration to be lower at parturition than at days 260 or 265 of pregnancy or day 21 postpartum. Using radioimmunological techniques Randel and Erb (1971) reported that LH decreased significantly from day 0 to day 7 of pregnancy and changed

very little thereafter. Using serum LH values in a multiple regression equation for predicting progesterone concentration indicated a negative partial regression for LH which they interpreted to mean that LH may exert tonic regulation of luteal function during pregnancy.

Postpartum Period

Reproductive Tract Changes

Uterus and Cervix.--The economic merits of a short calving interval has spirited a great deal of research on the postpartum period. Palpation via the rectum has allowed researchers to monitor changes in uterine horn and cervical diameter as well as ovarian activity. According to Morrow (1969), the uterine horns of cattle are palpable by 4 to 7 days postpartum. A slow decrease in diameter was witnessed from days 4 to 9 followed by a more rapid decrease during days 10 to 14. The period of greatest involution occurred at the time of first estrus in normal cows and was concomitant with uterine lochia discharge. Cows with abnormal parturitions required 3 to 5 days longer to attain uterine size comparable to normal cows. Cervical involution appeared to continue gradually until day 30 in normal cows and day 35 in problem animals after which time no further decrease was discernible by palpation. Wagner and Hansel (1970) have reported similar findings. They also indicated that in most normal cows the uterine mucosal epithelium was reestablished by 30 days postpartum.

In sheep uterine involution is completed by about 30 days postpartum (Uren, 1935; Basset, 1963 as cited by Wagner and Oxenreider, 1971).

Ovarian Activity

Casida and Venzke (1936) and Labhsetwar (1964) indicated mature follicles were present around 30 days postpartum in cattle. According to Morrow et al. (1969), ovarian follicular activity commences 7 to 10 days postpartum at which time follicles averaged 0.5 to 1.5 cm in diameter. Enlargement of such follicles continued until first estrus at approximately 15 days. The corpus luteum of pregnancy had regressed to a small elevated mass on the ovarian surface at 4 to 7 days postpartum and by 14 days it was usually undetectable.

Postpartum Estrus Activity

Morrow et al. (1966) reported the interval from calving to first postpartum estrus to be 15.0 days in normal cows and 34.4 days for abnormal cows. Abnormal cows in this study were animals that experienced dystocia, ketosis or other disease conditions shortly after parturition. A 14.0 day interval was reported by Wagner and Hansel (1969). Other reports have indicated that the first postpartum ovulation occurred somewhat later (Saiduddin et al., 1967a; Tennant et al., 1967). In most reports indicating an interval longer than 15.0 days

palpations were initiated to detect an early ovulation. Morrow et al. (1966) found 79% of the first ovulations were accompanied by silent heats (ovulation without clinical signs of estrus). Menge et al. (1962) and Saiduddin et al. (1967b) have reported similar observations. In comparing data on this subject, many of the discrepancies witnessed are dependent on the quality and thoroughness of estrus detection and estrus classification criteria.

First postpartum estrus cycle length (first ovulation to second ovulation) was 16 to 17 days in normal and 19.7 days in abnormal cows compared to a second cycle length of 21 days (Morrow et al., 1966; Marion and Gier, 1967). Morrow et al. (1969) have speculated that the short cycle is due to failure of the corpus luteum earlier than usual. Oxytocin injections, uterine dilation and intrauterine infusions of contaminated seminal plasma early in the cycle all cause similar reductions in cycle length (Hansel and Wagner, 1960). This implicates the uterus in corpus luteum maintenance, perhaps by decreasing LH production by the anterior pituitary.

Wagner and Oxenreider (1970) reviewed the data from several other species. Horses apparently exhibit an ovulatory estrus within 18 days of foaling and pigs have an anovulatory estrus 1 to 3 days postpartum.

Postpartum suckling and low energy diets appear to be two factors which can increase the postpartum

interval to first estrus. Both may exert their influence via disturbed hypothalamic control of gonadotropin secretion. McClure (1968a,b) postulated poor nutrition might result in hypoglycemia and this in turn might disturb hypothalamic control of the anterior pituitary.

Endocrine Pattern

The endocrine profile during this time has been very poorly defined. Most work has been limited to pituitary or ovarian levels of hormones but blood levels are now measurable with radioimmunoassay procedures.

Luteinizing hormone concentrations of bovine pituitaries increase from parturition to day 21 postpartum and to first estrus (Labhsetwar et al., 1963; Saiduddin et al., 1964). Saiduddin et al. (1966) showed pituitary LH activity to be lowest at parturition, increased to day 10 and then continued a gradual increase until day 30. Pituitary FSH levels were highest at parturition and shortly after but then declined somewhat. In humans, plasma FSH postpartum was quite constant from parturition until day 30 but these levels were about one-half of those found during the follicular phase of the cycle (Crystle et al., 1970). Luteinizing hormone activity was highest near parturition and decreased thereafter but the assay procedure used cross reacted with human chorionic gonadotropin (HCG) thus explaining the high levels of LH activity during the early postpartum period.

MATERIALS AND METHODS

Experimental Design

This thesis was designed to quantify changes in blood hormone concentrations of the bovine during the last month of gestation and the interval between calving and first estrus.

Jugular blood (40 ml) was collected via venipuncture from each of 34 pregnant Holstein heifers twice weekly from 30 to 6 days prepartum, twice daily (8:00 AM and 5:00 PM) from 6 days before to 5 days after parturition then twice weekly until first estrus or day 25 postpartum whichever occurred first.

All animals were in loose housing with access to pasture ad libidum, supplemented with corn silage, hay and grain concentrate. Animals were placed in individual maternity pens (when prepartum twice daily bleedings were started) where they remained until 48 hours post-calving.

Estrus Detection

Following parturition, the heifers were observed twice daily for signs of estrus. In addition, beginning 1 week postpartum twice weekly rectal palpations were

initiated to monitor ovarian activity and changes in reproductive tract size. This allowed us to detect both animals displaying signs of estrus and those with silent heats.

Blood Handling Procedure

Blood was placed in 50 ml polypropylene centrifuge tubes (Ivan Sorvall, Inc., Norwalk, Conn.) containing 31.7 mg oxalic acid crystals, centrifuged (6500 xg, 20 min. 4°C) and plasma transferred to similar centrifuge tubes containing 27.8 mg $CaCl_2$ to promote clot formation. After 48 hours at 5°C, samples were centrifuged as before to remove the fibrin clots and serum was transferred to 7-dram plastic vials and stored at -20°C until assayed for hormones.

Radioimmunoassays

A double antibody radioimmunoassay (RIA) was employed to quantify serum LH, GH and prolactin. The procedures for these assays were similar to those reported by Niswender et al. (1969) for bovine LH. Descriptions of the prolactin (Tucker, 1971 and Koprowski and Tucker, 1971) and GH (Purchas, 1970) and LH (Swanson, 1970) assay methodology has been previously described.

General Principles of Radioimmunoassay

As opposed to steroid hormones which possess distinct chemical groupings and solubility characteristics allowing separation from other plasma components, protein

or peptide hormones are much less distinguishable and not easily isolated from other plasma proteins. These hormones are however antigenic and this phenomena has allowed development of immunoassays which are sensitive and precise even in the presence of a multiplicity of proteins.

The production of an antiserum specific for one hormone is the first phase of the RIA procedure. This antibody must combine specifically with the hormone antigen to produce an antigen-antibody complex. Displacement of a radioactively labeled antigen (hormone) by unlabeled or "cold" antigen in proportion to its concentration is used to quantify hormone concentrations in unknowns by comparison with the displacing capacities of standard amounts of unlabeled hormone. Finally, by equating unknown values to standard values the quantity of unknown can be calculated.

Antibodies

Antibodies against LH, GH and prolactin were induced in guinea pigs by repeated injections of purified NIH preparations of these hormones in Freunds adjuvant (Appendix I.C.1). Such antibodies are referred to as the first antibody. According to Yalow and Berson (1968) maximum assay sensitivity is obtained when approximately 33% of the labeled hormone is bound by the antibody preparation. This was generally obtained when first antibody was diluted 1:200,000 for LH; 1:3,200 for GH and 1:30,000 for prolactin.

The second antibody was a sheep or goat preparation of anti-guinea pig gamma globulin obtained by a method similar to that used to induce the first antibody (Appendix I. C.2). The purpose of the second antibody was to precipitate the first antibody-hormone complex and was used at a dilution to yield maximum precipitation, i.e., usually 1:4 - 1:6.

Labeled Hormone Preparation

Mixing a purified preparation of the protein hormone (5 ug of GH and prolactin; 2.5 ug LH) with approximately 1 mc of ^{125}I (50 mc/ml, Iso-Serve Division of Cambridge Nuclear Corporation, Cambridge, Mass.) in the presence of chloramine-T resulted in labeling of the ortho positions of tyrosyl residues (Appendix I. A.3). Reaction time was confined to 2 minutes and was stopped by adding sodium metabisulfate (Appendix I. A.4). A transfer solution (Appendix I. A.5) was added to the reaction mixture and it was layered on top of a column of Bio-Gel-P-60 (Bio-Rad Labs., Richmond, Calif.) (Appendix I. 3.2) and 15 1.0 ml fractions were collected in 12 x 75 mm disposable glass culture tubes containing 1 ml egg white albumin in phosphate buffered saline (2% EWA-PBS) for LH and bovine serum albumin in phosphate buffered saline (2% BSA-PBS) for GH and prolactin. This column allowed separation of the labeled hormone from the free iodine. A typical elution profile using LH as the example is represented in

Figure 1. The labeled hormone was diluted to yield a solution containing approximately 25,000 cpm per 100 ul. The diluent for LH was 1% EWA-PBS (Appendix I. B.3) and 1% BSA-PBS for GH and prolactin (Appendix I. B.3).

Radioimmunoassay

Each serum sample was assayed in duplicate. Growth hormone and LH concentrations were estimated in undiluted serum aliquots whereas serum was diluted 1:2 to 1:10 with 1% bovine serum albumen in BSA-PBS for prolactin quantitation. The serum sample volume used ranged from 300 to 500 ul. Samples were added to 12 x 75 mm disposable glass tubes containing either 1% eggwwhite albumin in phosphate buffered saline (LH) or 1% BSA-PBS for GH and prolactin so that the final volume of serum plus diluent constituted 500 ul. Four sets of standards (Appendix I. B.4) were evenly distributed throughout the assay and each tube contained 500 ul of the standard. Table 1 illustrates the mechanics of the radioimmunoassay procedure.

The radioactivity of the precipitate was determined by counting in an automatic gamma counter for 10 minutes or 10,000 counts whichever accumulated first. Unknown samples were quantified by comparing with the standard.

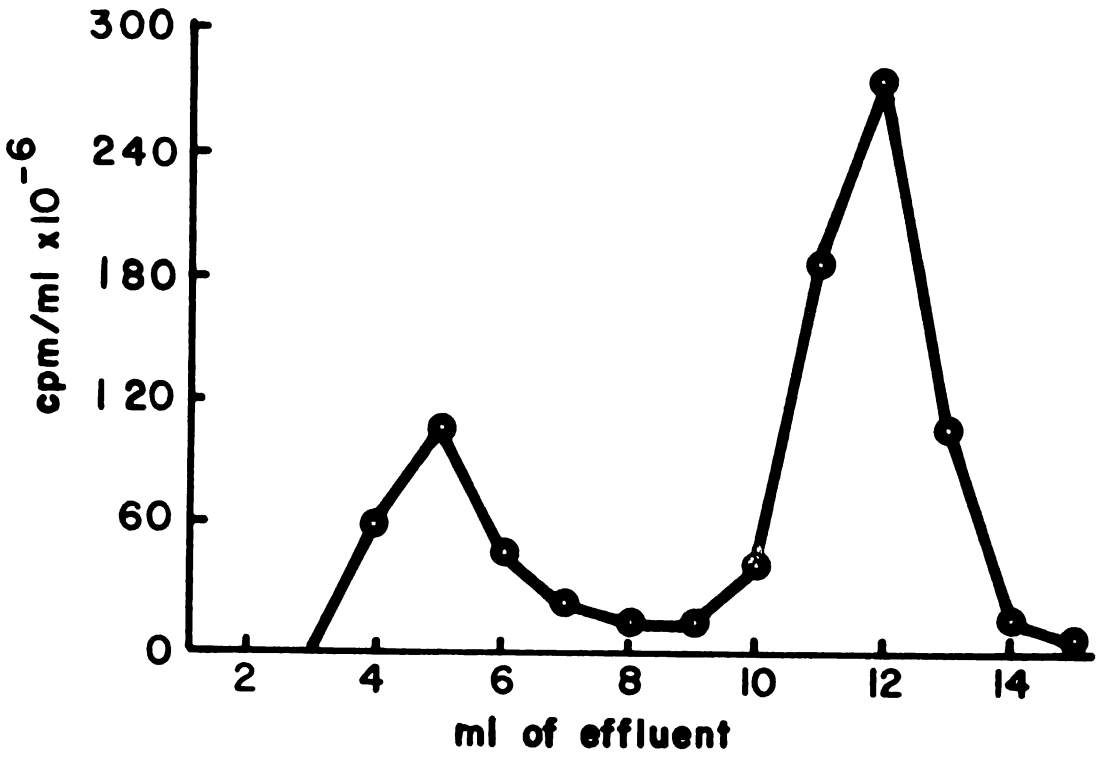

Figure 1.--Radioactivity elution profile for Bio-Gel P-60 chromatography of iodinated luteinizing hormone (LH). The first peak represents iodinated LH and the second peak represents free iodine.

Table 1.--Radioimmunoassay Procedure.

	SET UP DAY VOL. OF RAW OR DILUTED SERUM ADDED	DAY 1 VOLUME OF FIRST ANTIBODY	DAY 2 VOLUME OF IODINATED HORMONE	DAY 3 VOLUME OF SECOND ANTIBODY	DAY 4	DAY 5	DAY 6 VOLUME OF COLD PBS[5] ADDED
LH	200 ul AND 100 ul WHOLE SERUM	GPABLH[1] 200 ul	LH^{125} 100 ul	SAGPγG[4] 200 ul	INCUBATE 4°C		3 ml CENTRIFUGE 30 MIN AT 2510xg POUR OFF SUPERNATANT, DRY TUBES, COUNT PPT.
PROLACTIN	200 ul AND 100 ul SERUM DILUTED 1:2 - 1:10	GPABP[2] 100 ul	$PROL.^{125}$ 100 ul	SAGPγG 100 ul	INCUBATE 4°C		SAME
GH	200 ul AND 100 ul WHOLE SERUM	GPABGH[3] 200 ul	GH^{125} 100 ul	SAGPγG 200 ul	INCUBATE 4°C		SAME

[1]Guinea pig anti-bovine luteinizing hormone. [2]Guinea pig anti-bovine prolactin.

[3]Guinea pig anti-bovine growth hormone. [4]Sheep anti guinea pig gamma globulin.

[5]Phosphate buffered saline.

RESULTS

Pre and Postpartum Serum Prolactin, GH and LH Concentration in Heifers

Serum Prolactin

Serum prolactin concentration of heifers from 26 days prepartum through 26 days postpartum are shown in Figure 2 excluding details of the period from prepartum day 9 through postpartum day 9 which are shown on Figure 3. From day 26 to 2 days before calving, prolactin concentrations varied between 80 and 110 ng/ml. But, from day 2, serum prolactin concentration increased rapidly reaching a peak of 285 ng/ml 1 day before calving with a gradual decline thereafter. Serum concentrations were 217 ng/ml at parturition and 93 ng/ml on postpartum day 2. From day 2 until day 9 serum prolactin values fluctuated between 93 and 86 ng/ml (Figure 2). Following day 9 a gradual decrease continued until day 26 when the concentration was 36 ng/ml. Postpartum concentrations (Figure 2) were generally lower than those found during the final month of gestation. In contrast to GH which peaks at parturition, prolactin values were maximum at 24 hours prior to calving and declined thereafter.

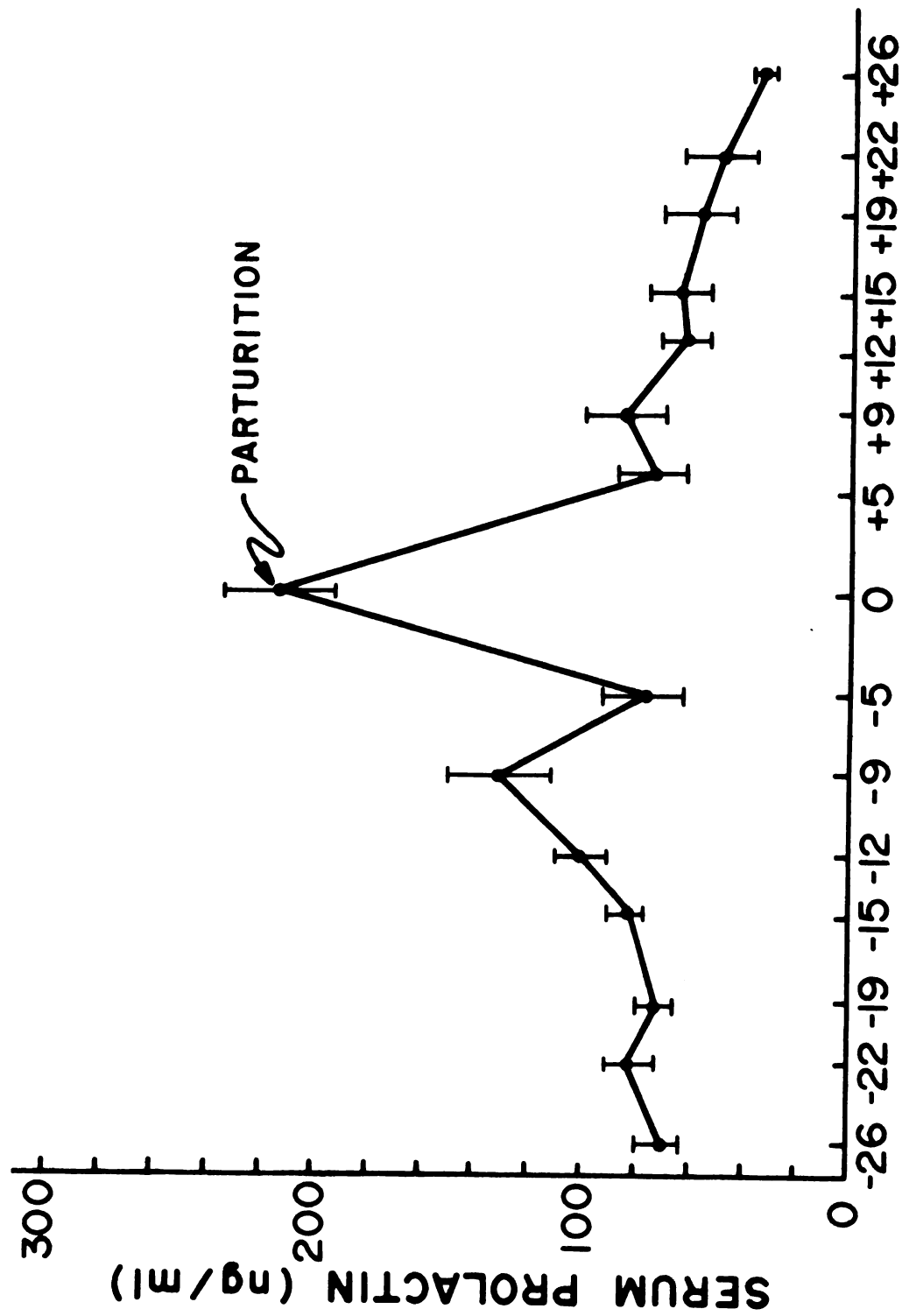

Figure 2.--Serum Prolactin from 26 days prepartum through 26 days postpartum.

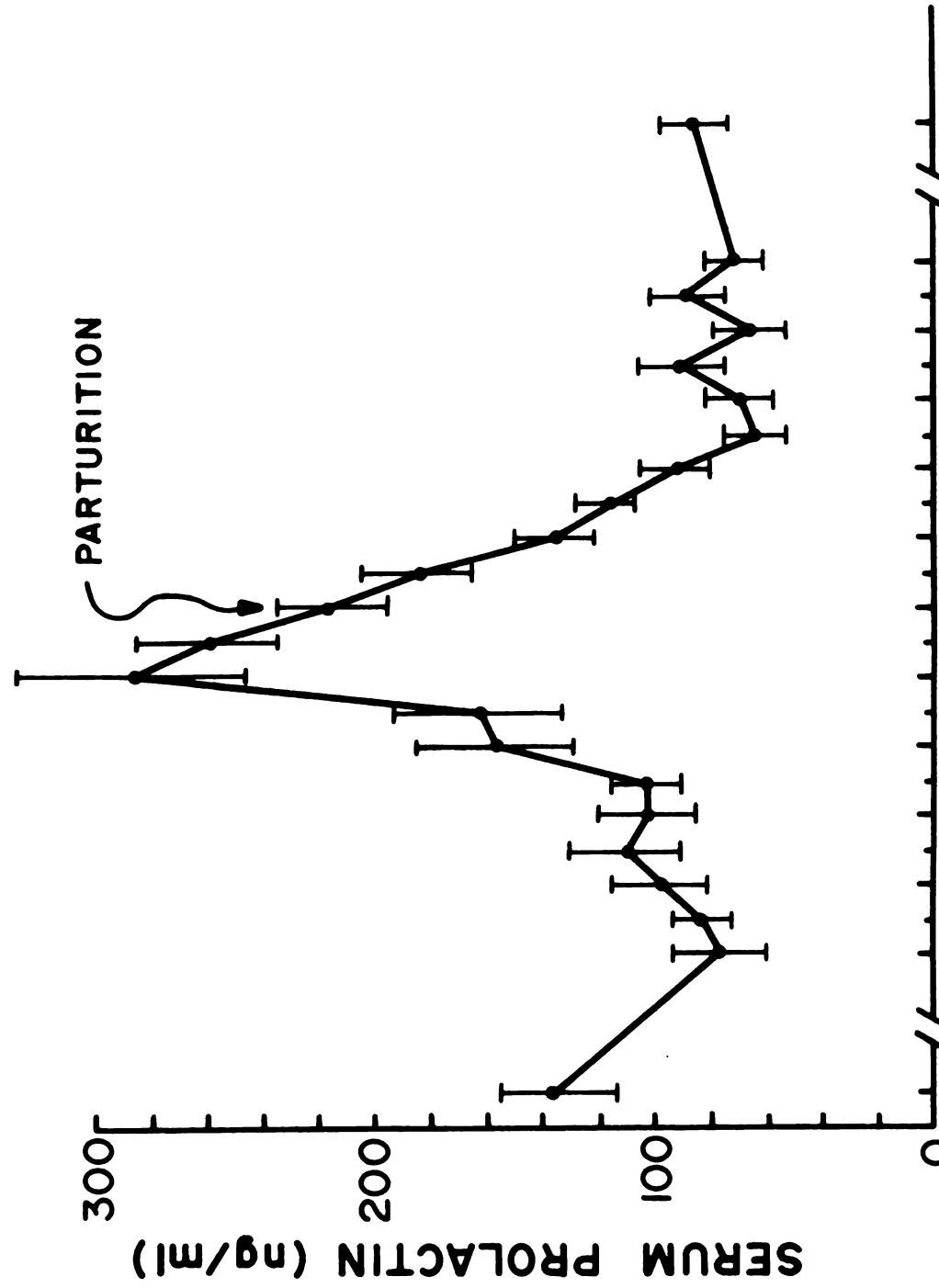

Figure 3.--Serum Prolactin from 9 days postpartum through 9 days postpartum.

Orthogonal contrasts showed average serum prolactin concentration from day -2.5 to calving to be significantly greater ($p<0.01$) than the comparable average for days -5 to -2.5. Similarly, average serum prolactin concentrations from day 26 to day 9 prepartum were significantly greater ($p<0.01$) than from day 9 to day 26 postpartum.

Growth Hormone

Serum GH concentrations of heifers from 26 days prepartum through 26 days postpartum are in Figure 4 with details of prepartum day 9 through postpartum day 9 on Figure 5. Serum GH concentrations fluctuated between 4.5 and 7.0 ng/ml from day 26 to 12 hours before parturition then increased significantly ($p<0.005$) to 10.0 ng/ml at parturition. There appeared to be a gradual rise in serum GH concentration commencing about 9 days prepartum and reaching a peak at calving. Orthogonal contrasts revealed mean GH concentrations during the period from -2.5 days to calving to be significantly greater ($p<0.01$) than the comparable mean from -5 to -3 days prepartum (Figure 4).

The prepartum increase in serum concentration of GH at parturition was rapid; rising from 6.1 ng/ml 12 hours prior to calving to 10.0 ng/ml at parturition ($p<0.01$), whereas the slope of the decline was much more gradual. Twelve hours after calving, GH concentrations were 9.4 ng/ml; then GH continued a slow linear ($p<0.05$) decline until day 4 postpartum when concentrations were 5.0 ng/ml.

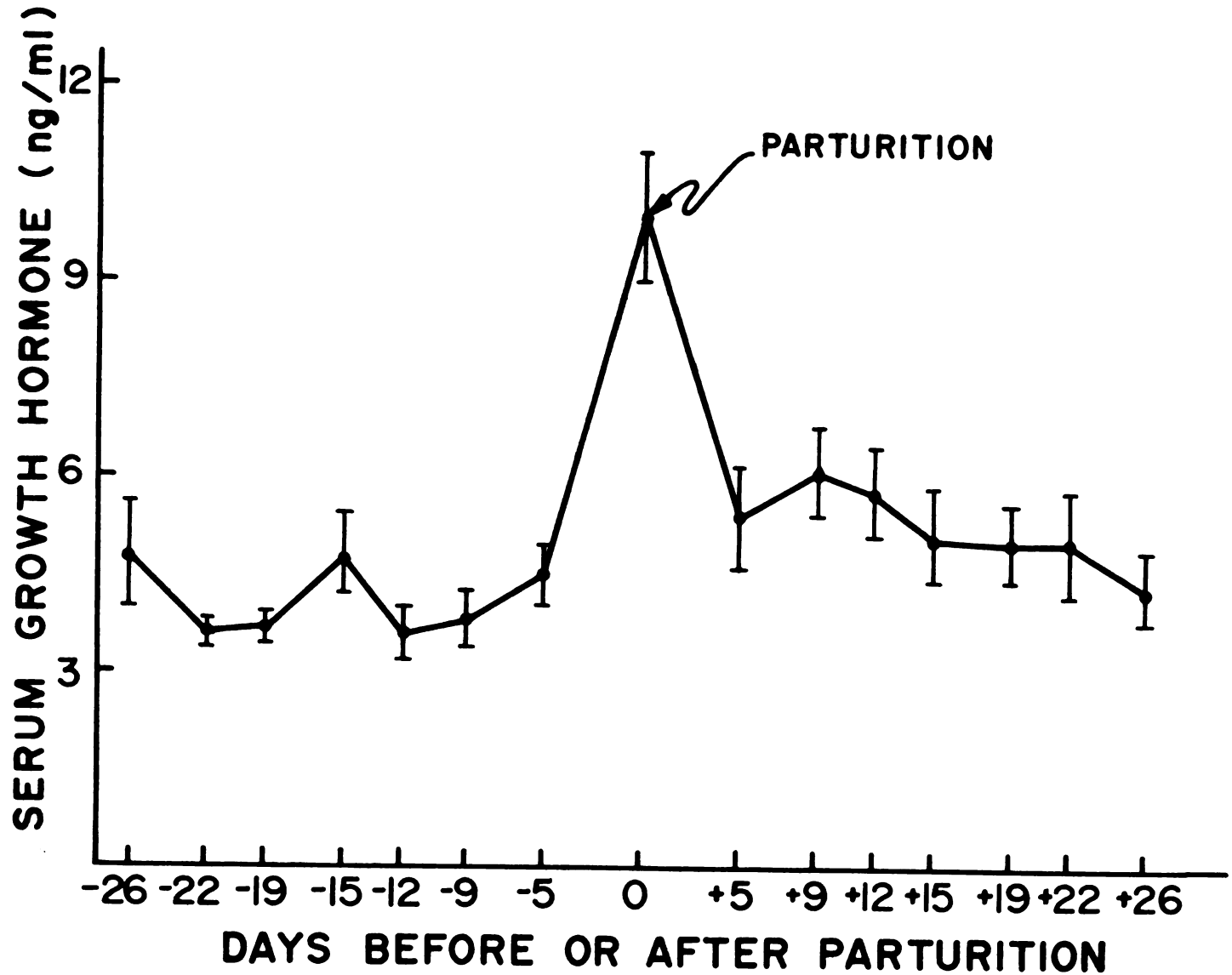

Figure 4.--Serum growth hormone from 26 days postpartum through 26 days postpartum.

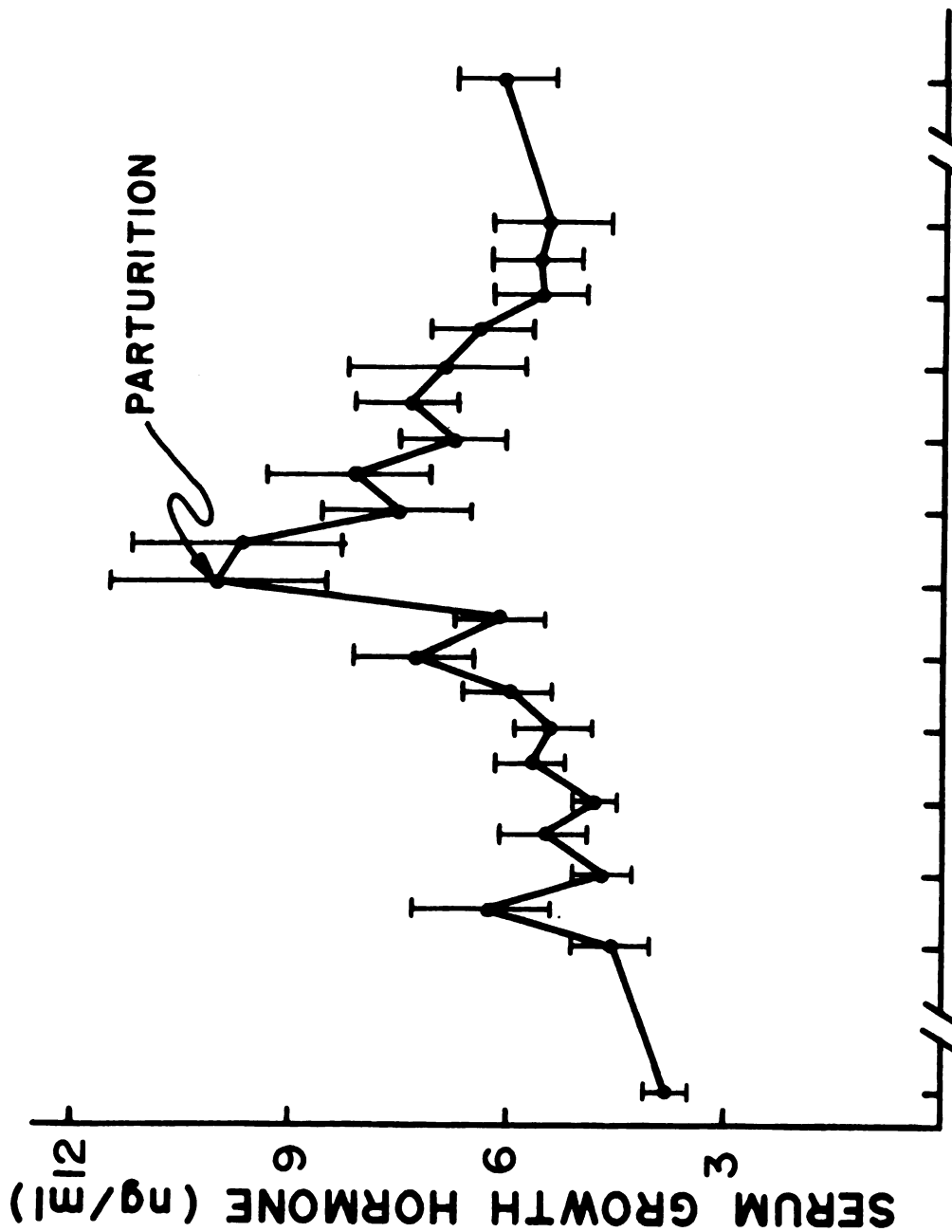

Figure 5.--Serum growth hormone from 9 days prepartum through 9 days postpartum.

From day 4 to day 26 postpartum GH values remained between 5.0 and 7.0 ng/ml (Figure 3).

Serum LH

The pattern for serum LH during the final month of gestation and first 26 days of lactation is shown in Figure 6. Very little change is seen throughout the sampling period but an increase was noted on day 4 which continued until day 22.

From 26 days before parturition until 3.5 days after calving LH concentrations ranged between 0.4 and 0.7 ng/ml with no large deviations from this range. At the time of calving the LH concentration was 0.6 ng/ml. Commencing on day 4, the LH concentration rose gradually to a maximum of 1.7 ng/ml on day 12 postpartum.

Orthogonal contrasts indicated LH concentrations from day 9 to day 26 postpartum to be significantly higher ($p<0.01$) than those during the period from day 26 to day 9 prepartum.

Generally the pattern for LH is one of very low values which are quite stable during the final month of pregnancy and no demonstrable increases occurred until about day 9 postpartum.

Appendix II indicates this more accurately as the mean LH values are based on 32 observations per day rather than 16 per day as in Figure 6.

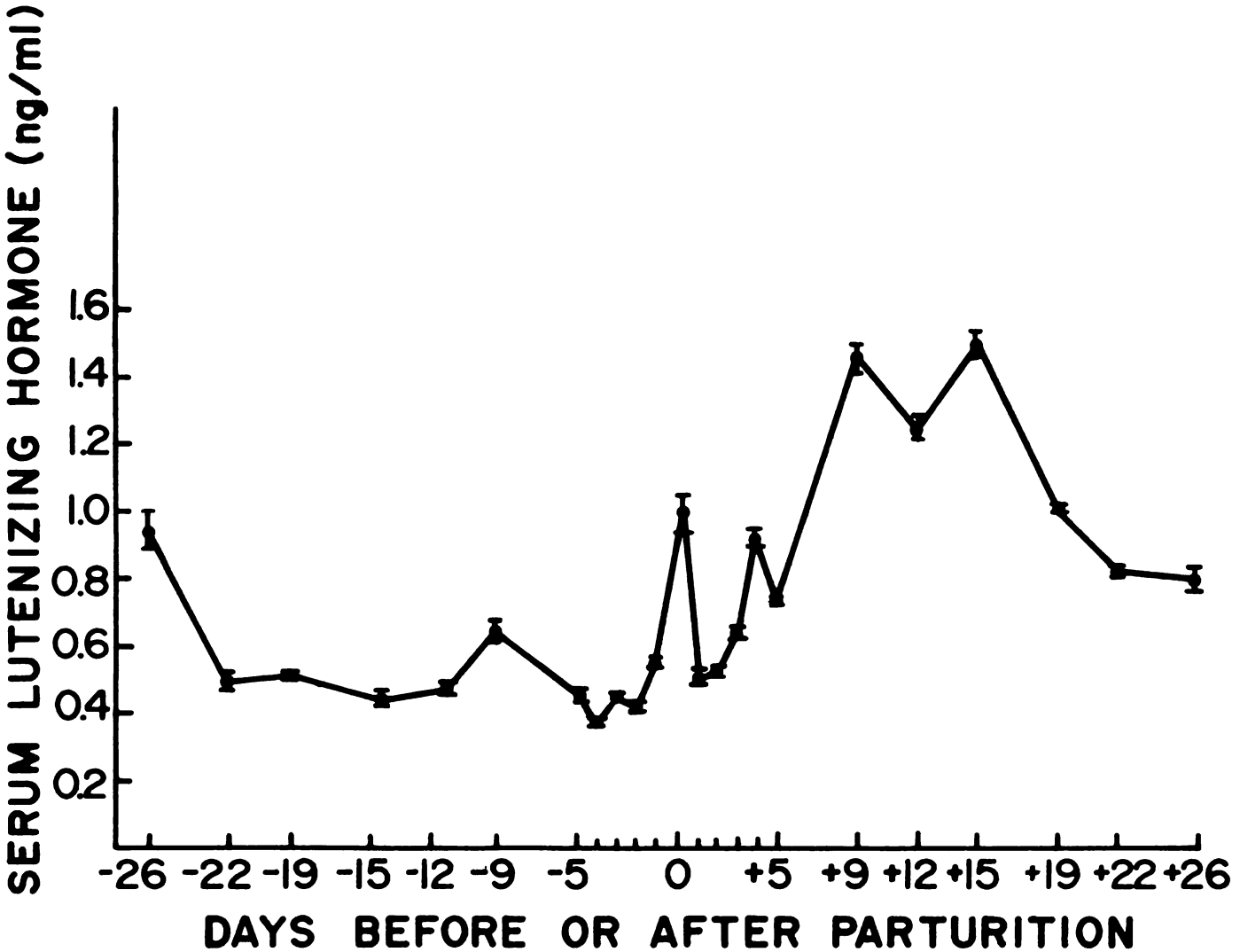

Figure 6.--Serum luteinizing hormone from 26 days prepartum through 26 days postpartum.

DISCUSSION

The data reported here are in agreement with limited data reported by Schams and Karg (1970); they demonstrated an increase in plasma prolactin concentration to over 300 ng/ml 12 hours before parturition. Similar results were reported by Arije and Wiltbank (1971) on beef cattle.

The physiological significance of increased serum prolactin is not clear. A partial explanation may be based on evidence that estrogen causes prolactin release by a direct action on the pituitary and be decreasing hypothalamic prolactin inhibiting factor (PIF). Smith et al. (1972) reported increased estradiol and estrone concentration just prior to parturition in the same sera used in this report. Similarly, total estrogens increased dramatically during the last eight days prepartum (Henricks et al. 1972) and Mellin et al. (1966) and Holm and Galligan (1966) reported maximum urinary estrogen secretion during the final 40 hours prepartum in cattle. If estrogen does release prolactin by direct action on the pituitary and be decreasing PIF, then this may trigger a massive release of prolactin shortly before parturition.

Our results indicate a continuous decline in serum prolactin concentration from 1 day prepartum until 26 days postpartum with postpartum values lower than those found during late gestation. Amenomori et al. (1970) reported prolactin concentration in rats to be 8.3 ng/ml during pregnancy, 29.2 ng/ml 1 day prepartum and at 65.5 ng/ml at parturition. Thus, the time of prolactin increase prepartum is similar in rats and cows. But, during the first 8 days of lactation, rat serum prolactin concentration remains increased relative to pregnancy values. This difference may be the direct result of differences in nursing intensity and frequency, since rat pups suckle almost continually during this period.

In light of reports by Raud et al. (1970) and Tucker (1971) it is entirely possible that partum increase in serum prolactin is the result of pain associated with the stress of parturition. But, the elevation in prolactin concentration during 24 hr before parturition is much greater than the response associated with the suckling stimulus or fright. The possibility that a combination of steroid hormone sensitization and massive stimuli associated with parturition causes this large prolactin increase must be considered.

Meites (1966) proposed a need for both prolactin and glucocorticoids in initiation of lactation in several species. Only at parturition, when the inhibitory effect

of progesterone and estrogen at the mammary cell level was declining did increased serum prolactin and glucocorticoids become effective in initiating lactation. Recent evidence by Adams and Wagner (1970) and Jochle et al. (1971) demonstrate the ability of synthetic glucocorticoids to initiate parturition in cattle during the latter part of pregnancy. Also Adams and Wagner (1970) and Smith et al. (1972) observed a marked increase in plasma glucocorticoids near normal parturition in cattle. The hypothesis may be correct in cows as well as rats; and a surge in both corticoids and prolactin may be necessary to initiate a normal parturition.

The physiological importance of increased serum GH concentration at parturition is difficult to explain. Growth hormone is generally considered to be much less influenced by physical stimuli than is prolactin (Tucker, 1971). Trenkle (1970) observed a significant increase in serum GH concentration in animals fed diethylstilbestrol at 10 mg per day. A partial explanation may be based on the data of Smith et al. (1972) and Hunter et al. (1970) who demonstrated an increase in serum estrogens near term which may have triggered a short lived but significant release of GH.

The physiological role of GH during lactation is not well defined but nonetheless it appears to be essential for normal lactation (Meites, 1966). Cowie (1964)

reported that hypophysectomized goats need GH to maintain normal lactation even when administered prolactin, insulin, corticoids and thyroid hormone. Removal of GH from this hormone therapy resulted in an immediate drop in milk production.

The slight elevation of GH postpartum relative to prepartum concentration may be the direct result of lactation imposing a strain on the general metabolic system, thereby causing a general elevation in GH concentrations to meet this increased demand for nutrients.

Luteinizing hormone is generally released following a decrease in progesterone and an increase in estrogen concentration. Yet no increase was noted near parturition in these heifers when rising estrogen and decreasing progesterone concentrations were observed (Smith et al., 1972). In fact, during the majority of this sampling period, LH remained at the constant low level similar to that reported by Swanson (1970) in prepubertal heifers approaching first estrus.

A possible explanation of a rise in LH beginning 6-9 days postpartum may be due to early follicular development giving rising to a silent estrus. The average time for returning to the first visible estrus was 20 days. However undetected estrus with rising LH levels causing ovulation may have occurred around 9 days in a sufficient

number of cases to have caused this elevation in serum LH concentrations.

Rectal palpation of the heifers commenced about day 10 postpartum but in many cases it was difficult to accurately assess ovarian function until about day 15.

SUMMARY AND CONCLUSION

Jugular blood was collected from 34 first calf Holstein heifers commencing 30 days prepartum and ending at first estrus or day 26 postpartum whichever occurred first. Serum was analyzed for prolactin, growth hormone and luteinizing hormone concentration by radioimmunoassay.

Serum prolactin varied between 80 and 110 ng/ml until day 2 prepartum when it started rising rapidly and peaked at 285 ng/ml 24 hours prior to calving. This coincides with an increase in uterine contractile activity prior to calving and this fact along with the high concentrations of plasma estrogen at this time may help explain this peak in prolactin. A continuous decline was noted from this time until 2 days post-calving when prolactin stabilized at 90 ng/ml and remained near this value until day 9. Following this, a gradual decline continued until day 36 when concentrations were 36 ng/ml. These postpartum values are lower than those of late pregnancy also demonstrate a trend opposite that in rats perhaps due to the difference in suckling intensities for these two species.

Growth hormone followed a pattern similar to prolactin but peaked at parturition. Pre- and postpartum concentrations for GH were nearly identical. The GH increase at parturition coincided with a peak in plasma estrogen and this may in fact be responsible for the increase.

Serum LH remained near 0.5 ng/ml throughout the majority of the sampling period. A rise in LH was noted beginning about day 4, and LH peaked at 1.7 ng/ml on day 12 postpartum. It appears that during late pregnancy, pituitary release and/or production of LH is minimal. This may be partially due to high circulating levels of progesterone. Following calving, the pituitary is released from this inhibitory action of progesterone and starts to release more LH.

In general, prolactin and GH peak at or near calving, possibly due to increased serum estrogen concentrations, increased uterine contractile activity and the general stress of calving. Increased prolactin and GH at parturition is consistent with the need for prolactin and GH in the initiation and maintenance of milk production. Luteinizing hormone remained at basal levels until day 4 postpartum when a gradual increase was noted. These low LH values indicate a general suppression of the releasing and/or synthesis of LH possibly by such high concentrations of plasma steroid.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adams, W. M. and W. C. Wagner. 1970. The role of corticoids in parturition. Bio. Reprod. 3: 223.

Ainsworth, L. and K. J. Ryan. 1967. Steroid transformation by endocrine organs from pregnant mammals II. Formation and metabolism of progesterone by bovine and sheep. Placental preparations in vitro. Endocinol. 81: 1349.

Amenomori, Y. C. L. and J. Meites. 1970. Serum prolactin levels in rats during different reproductive states. Endocrinol. 86: 506.

Anje, G. F., J. N. Wiltbank and M. L. Hopwood. 1971. Hormone levels in pre- and postparturient beef cows. J. Animal Sci. 33: 247.

Baird, E. R. and L. E. McDonald. 1964. In vitro oxygen uptake by the maturing bovine placenta. Am. J. Vet. Res. 25: 367.

Bassett, E. G. 1963. Some effects of endogenous hormones on muscles and connective tissue, with special reference to the ewe. Proc. N. Z. Soc. Animal Prod. 23: 107.

Bassett, J. M., G. D. Thorburn and A. L. C. Wallace. 1970. The plasma growth hormone concentration of the fetal lamb. J. Endocrinol. 48: 251.

Basson, P. A., J. C. Morgenthal, R. B. Billrough, J. L. Marais, S. P. Kruger and J. L. de B. VanDer Merwe. 1969. Grootlamsiekte, a specific syndrome of prolonged gestation in sheep caused by a shrub, Salsola tuberculata. Onderstepoort J. Vet. Res. 36: 59.

Binns, W., J. F. Lynn, J. L. Shupe and G. A. Everett. 1963. A congenital cyclopian-type malformation in lambs induced by maternal ingestion of Veratum Californicum. Am. J. Vet. Res. 24: 1164.

Bryant, G. D., J. L. Linzell and F. C. Greenwood. 1970. Plasma prolactin in goats measured by radiimmuno-assay: The effects of teat stimulation, mating behavior, stress, fasting and of oxytocin, insulin and glucose injections. Hormones 1: 26.

Casida, L. E. and W. G. Venzke. 1936. Observation on reproductive processes in dairy cattle and their relation to breeding efficiency. Proc. Amer. Soc. Anim. Prod. 29: 221.

Cowie, A. T., G. S. Knaggs and J. S. Tindal. 1964b. Complete restoration of lactation in the goat after hypophysectomy. J. Endocrinol. 28: 267.

Crystle, C. D., J. Powell and V. C. Stevens. 1970. Plasma Gonadotropins during the postpartum period. Obstet. Gynec. 36: 887.

Csapo, A. 1950. Actomyosin of the uterus. Am. J. Physiol. 160: 46.

Csapo, A. 1956. Progesterone "block." Am. Jour. Anat. 98: 273.

Elias, J. J. 1957. Cultivation of adult mouse mammary gland in hormone enriched synthetic medium. Science 126: 842.

Erb, R. E., V. L. Estergreen Jr., W. R. Gomes, E. D. Plotka and O. L. Frost. 1968a. Progestin levels in corpora lutea and progesterone in ovarian venous and jugular vein blood plasma of the pregnant bovine. J. Dairy Sci. 51: 401.

Estergreen, V. L. Jr., O. L. Frost, W. R. Gomes, R. E. Erb and J. F. Bullard. 1967. The effect of ovariectomy on pregnancy maintenance and parturition in dairy cows. J. Dairy Sci. 50: 1293.

Evans, H. M., J. H. Briggs and J. S. Dixon. 1966. The physiology and chemistry of growth hormone. In: The Pituitary Gland, eds. G. W. Harris and B. T. Donovan, Vol., pp. 439-491. Univ. Calif. Press, Los Angeles.

Everett, N. B. and B. L. Baker. 1945. The distribution of cell types in the anterior hypophysis during late pregnancy and lactation. Endocrinol. 37: 83.

Folley, S. J. 1956. The physiology and biochemistry of lactation. Oliver and Boyd, Edinburgh.

Gillette, D. D. and L. Holm. 1963. Prepartum to postpartum uterine and abdominal contractions in cows. Am. J. Physiol. 204: 1115.

Gorski, J. and R. E. Erb. 1959. Characterization of estrogens in the bovine. Endocrinol. 64: 707.

Grumbach, M. M., S. L. Kaplan, J. J. Sciarra and I. M. Burr. 1968. Chorionic growth hormone-prolactin (CGP): secretion disposition, biologic activity in man, and postulated function as the "growth hormone" of the second half of pregnancy. Ann. N. Y. Acad. Sci. 148: 501.

Hafez, E. S. E. 1968. Reproduction in Farm Animals. Lea & Febiger, Philadelphia.

Hansel, W. and W. C. Wagner. 1960. Luteal inhibition in the bovine as a result of oxytocin injections, uterine dilation, and intrauterine infusions of seminal and preputial fluids. J. Dairy Sci. 43: 796.

Henricks, D. M., J. F. Dickey, J. R. Hill and W. E. Johnston. 1972. Plasma estrogen and progesterone levels after mating and during late pregnancy and postpartum in cows. Endocrinol. 89: 1343.

Herman, H. A. and R. W. Spalding. 1947. A study of factors affecting the length of gestation in dairy cattle. J. Dairy Sci. 30: 545.

Hindson, J. C., B. M. Schofield, C. B. Turner and H. S. Wolff. 1965. Parturition in sheep. J. Physiol. 181: 560.

Holm, L. W. and S. J. Galligan. 1966. Estrogens in the peripheral plasma of cows during normal and prolonged gestations. Am. J. Obstet. Gynecol. 95: 887.

Holst, S. and C. W. Turner. 1939. Lactogen content of pituitary of pregnant and lactating rabbits and guinea pigs. Proc. Soc. Exp. Biol. N. Y. 42: 479.

Hunter, D. L., R. E. Erb, R. D. Randel, H. A. Garverick, C. J. Callahan, and R. B. Harrington. 1970. Reproductive steroids in the bovine. I. Relationships during late gestation. J. Animal Sci. 30: 47.

Hurst, V. and C. W. Turner. 1942. Lactogenic hormone content of anterior pituitary gland of albino mouse as compared to other species. Endocrinol. 31: 334.

Jochle, W. 1971. Corticosteroid induced parturition domestic animals: mechanism of action and economic importance. Folia Vet. Lat. 1: 229.

Koprowski, J. A., and H. A. Tucker. 1971. Failure of oxytocin to initiate prolactin or luteinizing hormone release in lactating dairy cows. J. Dairy Sci. 54: 1675.

Labhsetwar, A. P., W. E. Collins, J. W. Tyler and L. E. Casida. 1964. Some pituitary ovarian relationships in the periparturient cow. J. Reprod. Fert. 8: 85.

Levin, L. 1945. The fecal secretion of estrogens by pregnant cows. J. Biol. Chem. 157: 407.

Lindner, H. R. 1964. Comparative aspects of cortisol transport: lack of firm binding in domestic ruminants. J. Endocrinol. 28: 301.

Lyons, W. R. 1958. Hormonal synergism in mammary growth. Proc. Royal Soc. Biol. Sci. (B). 149: 303.

Lyons, W. R., C. H. Li and R. E. Johnson. 1958. The hormonal control of mammary growth and lactation. Recent Progr. Hormone Res. 14: 129.

Marion, G. B., J. S. Norwood and H. T. Gier. 1968. Uterus of the cow after parturition: Factors affecting regression. Amer. J. Vet. Res. 29: 71.

Marshall's Physiology of Reproduction. 1952. Edited by A. S. Parkes, Vol. II. Parturition--F. H. A. Marshall and J. Chassar Moir. 496.

McClure, T. J. 1968a. Malnutrition and infertility of cattle in Australia and New Zealand. Australian Vet. Jour. 44: 134.

McClure, T. J. 1968b. Hypoglycemia, an apparent cause of infertility of lactating cows. Br. Vet. Jr. 124: 126.

McDonald, L. E. 1969. Pregnancy and parturition. In Veterinary Endocrinology and Reproduction. pp. 389-403. Lea & Febiger Publishers, Philadelphia.

Mead, S. W., P. W. Gregory and W. M. Regan. 1949. Prolonged gestation of genetic origin in cattle. J. Dairy Sci. 32: 705.

Meites, J. and C. W. Turner. 1948. Studies concerning the induction and maintenance of lactation. 1. The mechanism controlling the initiation of lactation at parturition. Missouri Univ. Agri. Exp. Sta. Res. Bull. 415.

Meites, J. 1961. Farm animals: Hormonal induction of lactation and galactopoesis. In S. K. Kon and A. T. Cowie (eds.) Milk: The Mammary Gland and Its Secretion. 1: 321. Academic Press, New York and London.

Meites, J. 1966. Control of mammary growth and lactation. In Neuroendocrinology, Vol. 1. L. Martini and W. F. Ganong Eds., p. 669. Academic Press, New York and London.

Mellin, T. N., R. E. Erb and V. L. Estergreen, Jr. 1966. Urinary excretion of estrogen by the bovine before and after parturition. J. Animal Sci. 25: 955.

Menge, A. C., S. E. Mores, W. J. Tyler and L. E. Casida. 1962. Variation and association among postpartum reproductive and production characteristics in Holstein-Friesian cattle. J. Dairy Sci. 45: 233.

Mills, R. C. and M. C. Morrissette. 1970. Progesterone synthesis by perfused bovine ovaries of early and late pregnancy. J. Reprod. Fert. 22: 435.

Morrow, D. A., S. J. Roberts, K. McEntee and H. G. Gray. 1966. Postpartum ovarian activity and uterine involution in dairy cattle. J. Am. Vet. Med. Ass. 149: 1596.

Morrow, D. A., S. J. Roberts and K. McEntee. 1969. Review of postpartum ovarian activity and involution of the uterus and cervix in cattle. Cornell Vet. 59: 134.

Niswender, G. D., L. E. Reichart Jr., A. R. Midgley Jr., and A. V. Nalbandor. 1969b. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinol. 84: 1166.

Osburn, B. I., G. H. Stabelfeldt and L. L. Ewing. 1969. Relation of plasma progesterone to mid and late term bovine abortions due to Vibrio fetus infection. J. Reprod. Fert. 20: 77.

Oxender, W. D. 1971. Comparison of bovine fetal and maternal growth hormone, luteinizing hormone and prolactin at 90, 180 and 260 days gestation. Ph.D. Thesis, Michigan State University.

Pope, G. S., S. K. Gupta and I. B. Munro. 1969. Progesterone levels in the systemic plasma of pregnant, cycling and ovariectomized cows. J. Reprod. Fert. 20: 369.

Purchas, R. W. 1970. Some relationships of growth hormone levels to bovine growth and carcass quality. Ph.D. Thesis, Michigan State University.

Randel, R. D. and R. E. Erb. 1971. Reproductive steroids in the bovine. VI Changes and interrelationships from 0 to 260 days of pregnancy. J. Animal Sci. 33: 115.

Raud, H. R., C. A. Kiddy and W. D. Odell. 1971. The effect of stress upon the determination of serum prolactin by radiimmunoassay. Proc. Soc. Exptl. Biol. Med. 136: 689.

Reece, R. P., I. L. Hathaway and H. P. Davis. 1939. The lactogen content of the pituitary gland of the lactating rat. J. Dairy Sci. 22: 1.

Rivera, E. M., I. A. Forsyth and S. J. Folley. 1967. Lactogenic activity of mammalian growth hormones. Proc. Soc. Expt. Biol. Med. 124: 859.

Saiduddin, S. and W. D. Foote. 1964. Pituitary luteinizing hormone activity of postpartum bovine. J. Animal Sci. 25: 930 (Abstr.).

Saiduddin, S., J. W. Riesen, W. E. Graves. W. J. Tyler and L. E. Casida. 1966. Pituitary luteinizing hormone activity in the postpartum cow. J. Animal Sci. 25: 930.

Saiduddin, S., J. W. Riesen, W. E. Graves, W. J. Tyler and L. E. Casida. 1967a. Effect of suckling on the interval from parturition to first estrus in dairy cows. J. Animal Sci. 26: 950.

Saiduddin, S., J. W. Riesen, W. J. Tyler and L. E. Casida. 1967b. First estrus and ovulation in the post-partum dairy cow. J. Animal Sci. 26: 1494.

Salisbury, G. W. and N. L. VanDermark. 1961. Physiology of Reproduction and Artificial Insemination of Cattle. W. H. Freeman, San Francisco. p. 639.

Schams, D. and H. Karg. 1970. Untersuchungen uber Prolaktin im rinderblut mit einer radiomunnologischen bestimmungsmethode. Zbl. Vet. Med. A. 17: 193.

Short, R. V. 1960. Blood progesterone levels in relation to parturition. J. Reprod. Fert. 1: 61.

Smith, V. G., L. A. Edgerton, H. D. Hafs and E. M. Convey. 1972. Bovine serum steroid hormone concentration during late pregnancy, parturition and early lactation. In Press.

Smith, V. G. 1971. Corticoid response to pregnancy, parturition, milking and nonspecific stimuli. M. S. Thesis, Michigan State University.

Stabenfeldt, G. H., B. I. Osburn and L. L. Ewing. 1970. Peripheral plasma progesterone levels in the cow during pregnancy and parturition. Am. J. Physiol. 218: 571.

Stormont, C., J. W. Kendrick and P. C. Kennedy. 1956. A "new" syndrome of inherited lethal defects associated with abnormal gestation in Guernsey cattle. Genetics 4: 663.

Swanson, L. 1970. Endocrine behavioral and ovarian changes in Holstein heifers from puberty to breeding size. Ph.D. Thesis, Michigan State University.

Tanabe, T. Y. 1970. The role of progesterone during pregnancy in dairy cows. Pennsylvania State University, Bulletin No. 774.

Tennant, B., J. W. Kendrick and R. G. Peddicord. 1967. Uterine involution and ovarian function in the postpartum cow. A retrospective analysis of 2,388 genital organ examinations. Cornell Vet. LVII: 543.

Trenkle, A. 1970. Plasma levels of growth hormone insulin and protein bound iodine in finishing cattle. J. Animal Sci. 2: 389.

Tucker, H. A. 1971. Hormonal response to milking. J. Animal Sci. 32: Suppl. 1,137.

Uren, A. W. 1935. Involution of the uterine mucosa in the ewe. Tech. Bull. No. 144, Mich. St. Agr. Exp. Sta.

Veenhuizen, E. L., R. E. Erb and J. Gorski. 1960. Quantitative determination of free estrone, estradiol-17B and estradiol. 17- in bovine fetal cotyledons. J. Dairy Sci. 43: 270.

Wagner, W. C., R. Saatman and W. Hansel. 1969. Reproductive physiology of the postpartum cow. II. Pituitary Adrenal and Thyroid function. J. Reprod. Fert. 18: 501.

Wagner, W. C. and S. L. Oxenreider. 1971. Endocrine physiology following parturition. J. Animal Sci. 32: Suppl. I, 1.

Woodbury, W. J., G. vanWagenen, A. I. Csapo. J. M. Marshall and H. Jung. 1963. Physiological principles of contraction in uterine muscle. In Initiation of Labor. J. M. Marshall and W. M. Burnett. Eds. Public Health Service Publication No. 1390.

Wrenn, T. R. and J. F. Sykes. 1953. The response of hormonally induced bovine mammary tissue to anterior pituitary hormones. J. Dairy Sci. 36: 1313.

Yalow, R. S. and S. A. Benson. 1968. General principles of radioimmunoassay. In Radioisotopes in Medicine: In Vitro Studies. R. L. Hayes, F. A. Goswitz and B. E. Pearson Murphy, Eds., pp. 7-39. U.S. Atomic Energy Commission.

APPENDICES

APPENDIX I

COMPOSITION OF REAGENTS USED IN RADIOIMMUNOASSAY

APPENDIX I

COMPOSITION OF REAGENTS USED IN RADIOIMMUNOASSAY

A. Reagents for radioiodination

1. 0.5 M sodium phosphate buffer, pH 7.5

Monobasic (0.5 M)

Add 69.005 g $NaH_2PO_4.H_20$ to distilled water. Dissolve, dilute to 1 liter.

Dibasic (0.5 M)

Add 70.98 g Na_2HPO_4 to distilled water. Heat to dissolve, then dilute to 1 liter.

Mix monobasic and dibasic to give pH 7.5.

Dispense in 1 ml portions, store at -20°C.

Store the monobasic and dibasic buffers at 4°C.

2. 0.05 M sodium phosphate buffer, pH 7.5

Solution A

$NaH_2PO_4.H_20$	2.78 g
Merthiolate	0.01 g

Dilute to 100 ml with distilled water.

Solution B

$NaHPO_4/7H_20$	26.825 g
Merthiolate	0.05 g

Dilute to 500 ml with distilled water.

Use 16 ml Solution A, 84 ml Solution B, dilute to 400 ml with distilled water.

Adjust pH to 7.5 with NaOH, if necessary.

Store at 4°C.

3. Chloramine-T

Upon receiving chloramine-T, dispense into small, tightly sealed vials, cover with foil, and store at -20°C.

Dilute 10 mg* chloramine-T to 10 ml with 0.05 M $NaPO_4$, pH 7.5 buffer. Use within 30 minutes of preparation. Discard chloramine-T remaining in vial.

*30 mg for GH

4. Sodium metabisulfite, 2.5
   Dilute 25 mg $Na_2S_2O_5$ to 10 ml with 0.05 M $NaPO_4$, pH 7.5 buffer. Use within 30 minutes of preparation.

5. Transfer solution
   Sucrose---------- 1.6 g
   KI---------- 0.1 g
   Dilute to 10 ml with distilled water.
   Dispense in 1 ml portions, store at -20°C.

6. Rinse solution
   Sucrose---------- 0.8 g
   KI---------- 0.1 g
   Bromphenol blue---------- 0.001 g
   Dilute to 10 ml with distilled water.
   Dispense in 1 ml portions, store at -20°C.

B. Reagents for Radioimmunoassay

1. 0.01 M phosphate buffered saline, pH 7.0 (PBS)
   NaCl----------143 g
   Monobasic phosphate----------100 ml (see Appendix A.1)
   Dibasic phosphate----------260 ml (see Appendix A.1)
   Merthiolate----------1.75 g
   Dissolve in distilled water and transfer to a large container.
   Dilute to 17.5 liters with distilled water.
   Adjust pH to 7.0, if necessary, store at 4°C.

2. 0.05 M EDTA - PBS, pH 7.0
   disodium EDTA----------18.612 g
   Add approximately 950 ml PBS.
   Adjust pH to 7.0 with 5 NaOH while stirring.
   Dilute to 1 liter,sstore at 4°C.

3. PBS - 1% egg white albumin (PBS - 1% EWA) or PBS - 1% bovine serum albumin (PBS - 1% BSA).
   Add 990 ml PBS to beaker.
   Add 10 g EWA (Sigma Chemical Corp.) or 10 g BSA.
   Mix over magnetic mixer.
   Filter through Whatman No. 1 filter paper.
   Store at 4°C.

4. Hormone standards (LH, GH and prolactin)
   PBS - 1% EWA is used for LH and PBS - 1% BSA is used for GH and prolactin; hereafter they will be referred to as buffers.
   Rinse a small screw-cap vial with buffer, dry.
   Weigh 200-400 ug NIH-LH-B5, NIH-GH-B12 or NIH-P-32 on Cahn Electrobalance and transfer hormone to the screw-cap vial.
   Add 0.85% saline to 1 mg/ml.
   Add buffer to 9 volumetric flasks.
   Using Hamilton microliter syringes, add appropriate volumes of the 1 mg/ml stock hormone to volumetric flasks to obtain the following concentrations:
   LH - 0.16, 0.32, 0.64, 1.28, 2.56, 5.12, 10.24, 20.48 and 40.96 ng/ml.
   GH - 0.2, 0.6, 1.0, 1.6, 2.0, 3.0, 4.0, 6.0, 8.0 and 10.0 ng/ml.
   Prolactin - 0.2, 0.4, 1.0, 1.6, 2.0, 3.0, 4.0, 6.0 and 8.0 ng/ml.
   Add buffer to final volume in each volumetric flask.
   Dispense each standard in quantities suitable for one assay.
   Freeze in Dry Ice - ethanol, store at -20°C.
   For use, thaw rapidly with 38°C. water.

5. 1:400 normal guinea pig serum (NGPS).
   Obtain blood from guinea pig that has not been used to develop antibodies.
   Allow blood to clot, recover serum and store the serum in convenient quantities at -20°C.
   Add 2.5 ml of appropriate serum to a 1 liter volumetric flask, dilute to 1 liter with 0.05 M PBS-EDTA, pH 7.0
   Divide into 100-ml portions and store at -20°C.

6. Guinea pig anti-bovine LH (GPABLH, identified in our laboratory as antibody I), guinea pig anti-bovine GH (GPABGH), or guinea pig anti-bovine prolactin (GPABP).
   Dilute the antisera to 1:400 with 0.05 M PBS-EDTA, pH 7.0.
   Dispense in small quantities, store at -20°C.
   On day of use, dilute the 1:400 antisera to the required concentration using 1:400 NGPS as diluent.

7. Anti-gamma globulin
   Use goat anti-guinea pig gamma globulin (GAGPGG) in LH assay and sheep anti-guinea pig gamma globulin (SAGPGG) in GH and prolactin assay.
   Dilute antisera to required concentration with 0.05 M PBS-EDTA, pH 7.0.
   Store at 4°C. or at -20°C.

C. Antibody and anti-gamma globulin production

1. Guinea pig anti-LH
   0.5 or 1.0 mg NIH-LH-B5 was dissolved in water and Freund's complete adjuvant added (1:1 ratio).
   1.1 or 0.6 ml of the emulsion per guinea pig was injected subcutaneously in 4 scapular region sites.
   The above procedure was repeated 15 and 30 days later substituting Freund's incomplete adjuvant for adjuvant.
   Antisera was collected by cardic puncture 46 and 78 days after the initial injection.

2. Goat anti-guinea pig gamma globulin
   Guinea pig gamma globulin (Fraction 11, Pentex, Inc., Kankakee, Illinois) (40 mg), streptomycin (100 mg) and penicillin (1000 I.U.) was emulsified in 5 ml of water plus 5 ml Freund's complete adjuvant.
   10 ml was subcutaneously injected in 8 scapular sites of a 75 kg goat.
   The above procedure repeated 15 days later substituting Freund's incomplete adjuvant for adjuvant.
   Antisera was collected 30 days after the second antigen injection by jugular vein puncture.

APPENDIX II

MEAN LH VALUES BASED ON 32 OBSERVATIONS PER DAY

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MEAN LH VALUES BASED ON 32 OBSERVATIONS PER DAY

Sample Days Pre- and Postpartum	Mean LH Values ug/ml
-26	0.7
-22	0.5
-19	0.5
-15	0.5
-12	0.5
- 9	0.6
- 5.5	0.5
- 5	0.4
- 4.5	0.5
- 4.0	0.4
- 3.5	0.6
- 3.0	0.4
- 2.5	0.4
- 2.0	0.4
- 1.5	0.5
- 1.0	0.5
- 0.5	0.5
0	0.7
+ 0.5	0.5
+ 1.0	0.6
+ 1.5	0.4
+ 2.0	0.5
+ 2.5	0.6
+ 3.0	0.6
+ 3.5	0.6
+ 4.0	0.8
+ 4.5	0.8
+ 5.0	0.7
+ 9.0	1.5
+12.0	1.7
+16.0	1.6
+19.0	1.4
+22.0	1.4
+26.0	1.0