LH and prolactin levels in postpartum beef cows and prolactin levels in cyclic beef cows subjected to various mating stimuli

by David Kendal Han

A thesis submitted to the Graduate Faculty in partial fulfullment of the requirements for the degree of MASTER OF SCIENCE in Animal Science
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Abstract:
Specific bovine radioimmunoassays (RIA) were utilized for quanitat-ing blood levels of LH and prolactin in beef cattle. Radioiodination damage was a problem with the prolactin assay and was characterized by the formation of molecular aggregates of prolactin-I 131. These aggregates exhibited abnormal binding affinity with the prolactin antibody and were not displaced from the antibody in a direct relationship with in-creasing concentrations of unlabeled prolactin. By using milder reaction conditions for preparation of the prolactin-I 131 and a double purification system on Sephadex G-75 columns, the prolactin-I-131 aggregates could be minimized. Following the second purification, the remaining prolactin-I 131 was suitable for use in the assay system and produced very desirable standard curves.

Four postpartum Hereford cows were randomly divided into a non-suckled and suckled group. Daily blood samples were collected through indwelling jugular cannulas for subsequent plasma LH and prolactin measurement. Calves from the non-suckled groups were removed from their dams on the day of parturition. Data were analyzed by the method of least-squares. Least-squares prolactin means were significantly different ($P<.01$) between non-suckled (94.9+13.6 ng/ml) and suckled (176.3+10.7 ng/ml) groups. A significant negative linear regression ($b= -6.795+0.985$) of prolactin on the day postpartum was observed in a combined group analysis. No significant differences in LH were detected between groups. The within subclass correlation coefficient ($r= -.29$) between LH and prolactin was significant ($P<.06$) for the combined group analysis.

Serum prolactin was measured in 16 mature estrous synchronized beef cows from the onset of estrus until ovulation. Blood samples were collected through indwelling jugular cannulas at hourly intervals for the first 24 hrs and at 2 hr intervals thereafter until ovulation.

Cannulas were inserted at the onset of estrus. All cows were divided into two treatment groups. The control groups (NS) received no clitoral stimulation while the treated group (S) received manual clitoral stimulation or natural mating to a surgically sterilized bull. Data were analyzed by the method of least-squares. LH data from this group of cows was previously reported by Randel et al. (1973) . The data was grouped and analyzed with the onset of estrus and the LH peak as distinct physiological events. A significant group X sample time interaction ($P<.01$) was observed when the data was aligned on the onset of estrus. When the data was regrouped and centered on the LH peak for the individual cows, a significant group difference ($P<.01$) was observed during the interval prior to the LH peak. The least-squares prolactin means for the interval before the LH peak were 39.7+3.6 ng/ml and 16.7+2.6 ng/ml, respectively, for the NS and S groups.

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by

DAVID KENDAL HAN

A thesis submitted to the Graduate Faculty in partial fulfullment of the requirements for the degree

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Approved:

R. V. Blackwell
Head, Major Department

Edward Moody
Chairman, Examining Committee

K. Greening
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

December, 1973

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ABSTRACT

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INTRODUCTION

The annual loss of income to U. S. beef producers, resulting from prolonged postpartum anestrus and infertility, has been conservatively estimated to be $621,000,000. This loss of income has been attributed to the reduced reproductive performance of cows that are not maintained on a 12-month calving interval. Calving intervals with a duration greater than 12 months, lead to extended breeding and calving seasons which produce economic losses due to increased labor requirements, reduction in management efficiency and lighter weight calves at weaning time from late calving cows. The actual physiological problem associated with postpartum anestrus and infertility seems to have increased in recent years due to selection practices designed to increase milk production. Studies designed to determine the relationship between milk production and fertility indicate that these events are negatively correlated. In the following study relating to postpartum anestrus and infertility, an attempt was made to assess some of the differences in endocrine physiology between non-suckled and suckled postpartum cows.

The economic benefits of artificial insemination (A.I.) in the beef industry are clearly established, however, A.I. conception rates have been shown to be lower in beef cattle than in dairy cattle. Increased conception rates resulting from improved A.I. techniques would be advantageous to both the beef and dairy industry. For this reason, a second study was designed to determine differences in the endocrine physiology of cows receiving various degrees of manual and natural mating stimulation.

LITERATURE REVIEW

Postpartum Interval

Reports in the literature indicate extreme variation in postpartum intervals of beef and dairy breeds ranging from 18 to 104 days (Casida, 1968; Wagner and Oxenreider, 1971). The following review will relate to the majority of factors contributing to the wide range in postpartum intervals.

The first factor to be discussed stems from the definition of the term postpartum interval. Postpartum interval refers to the period of time beginning with parturition and ending with a designated event such as first ovulation, first estrus, first breeding, conception or complete involution of the uterus (Casida, 1968). The lack of a standardized event marking the end of the postpartum interval has introduced much of the variation observed between individual studies in the literature.

The following factors have been reported to effect the duration of the postpartum interval to first ovulatory estrus in cattle.

Calving Season. Buch, Woehling and Casida (1955) observed that winter calving was accompanied by the longest postpartum intervals, while summer calving resulted in the shortest. Spring and fall calving were found to be intermediate between the two extremes. No differences were observed due to age or parity of the dams.

Disease. Morrow (1971) concluded that periparturient (pre- and post-partum) diseases consisting of abortion, dystocia, retained fetal membranes, metritis, milk fever, active mastitis, ketosis, displaced abomassum or other debilitating diseases, significantly retarded the

first postpartum ovulation. The differences being that ovulation occurred on the average of 18 days postpartum in normal dairy cows compared to 42 days postpartum in abnormal dairy cows.

Nutrition. Reduced energy levels prior to calving have been reported to increased postpartum intervals to first estrus (Wiltbank et al. 1962 and Dunn et al. 1969), while other reports indicate that increasing energy intake following parturition will decrease postpartum intervals to first estrus. Wiltbank et al. 1964 and Dunn et al. 1969). Wagner and Oxenreider (1971) found significant differences in the interval required to attain a 10 mm follicle, between high and low energy groups of postpartum cows (133% N.R.C. requirement vs. 66% N.R.C. requirement). The difference being a 10 mm follicle observed at day 10 postpartum in the high energy group and day 16 in the low energy group. Reduction of blood glucose levels were demonstrated in the low energy group and suggestions were made that hypoglycemia resulting from inadequate nutrition could be a primary factor affecting postpartum interval.

In the rat, underfeeding or starvation has been reported to decrease anterior pituitary and target organ function (as reviewed by Meites, 1970). Hypothalmic content of luteinizing hormone releasing hormone (LH-RH) in rats fed 50% of the normal food intake, showed a significant reduction to 25% of the level measured in the control ad libitum fed rats. Pituitary LH concentration was also significantly reduced, however, pituitary FSH did not change significantly (Meites, 1970).

Lactational Status. Several reports indicate that suckled cows have longer postpartum intervals than cows which are milked or non-suckled (Graves et al. 1968; Oxenreider, 1968; Saiduddin et al. 1968; Wagner and Oxenreider, 1971). Other evidence indicates that suckling retards the growth of early postpartum follicles, although it is suggested that follicles large enough to mature and ovulate are present in lactating cows by 1 to 2 weeks postpartum (Wagner and Oxenreider, 1971). Short et al. (1972) observed significant differences in the postpartum interval between groups of cows having different lactational status. The postpartum interval to first estrus for suckled, non-suckled and mastectomized cows was 65, 25 and 12 days, respectively. These effects were observed after the nutrient intake of the individual groups was adjusted for lactational status to minimized postpartum weight changes. Saiduddin et al. (1968) reported that the postpartum interval of dairy cows was 9 days longer for genetically high milk producers as compared to genetically low producers, regardless of the level of concentrate feeding. Menge et al. (1962) found a positive correlation ($r = .84$) between milk production and calving interval between sires lines for the first 90 days of lactation. This evidence suggests direct relationship between milk production and interval to first postpartum estrus.

Endocrine Physiology of the Postpartum and Cycling Female

In the following review, major emphasis will be placed on the

endocrine physiology of the cow, however, studies utilizing the female rat, rabbit, and ewe will also be included in areas where basic information is lacking for the cow.

Bovine FSH, LH and Prolactin Levels During the Postpartum Interval. Assessment of pituitary FSH during the postpartum interval, utilizing the HCG augmentation assay, indicates that FSH is high in parturition and declines as the postpartum interval progresses (Labhsetwar et al. 1964 and Saiduddin et al. 1968).

Several studies utilizing the ovarian ascorbic acid depletion assay (OAAD), have indicated that bovine pituitary LH exhibits an inverse relationship to FSH during the postpartum interval, being low at parturition and increasing gradually as the interval progresses. Saiduddin and Foote (1964) reported an increase in bovine pituitary LH from day 5 to day 30 postpartum in suckled cows. Similar results were observed by Saiduddin et al. (1966) indicating that pituitary LH increased progressively in suckled cows from days 10, 20 and 30 postpartum when compared to levels at parturition. Labhsetwar et al. (1964) observed an increase in pituitary LH from parturition to 20 days postpartum in lactating cows, while Sawhney (1966) reported that pituitary LH decreased during the 10-day interval before parturition and then increased until first ovulation. Wagner, Saatman and Hansel (1969) indicated that the rise in bovine pituitary LH was nonsignificant from day 7 to day 30 postpartum when LH was measured by the uptake of P^{32}

in chick testis.

Riesen et al. (1968) using the systemic pigeon crop sac assay, measured pituitary prolactin in suckled and non-suckled dairy cows and found significantly higher levels in the non-suckled group at 10, 20 and 30 days postpartum.

Arije, Wiltbank and Hopwood (1971) measured serum LH and prolactin by radioimmunoassay (RIA) in three multiparous suckled cows from 3 to 4 weeks prepartum until second estrus postpartum. Blood samples were collected through indwelling jugular cannulas. During the postpartum interval LH levels remained between 1 and 1.5 ng/ml with periodic peaks reaching 3 ng/ml after two weeks postpartum. During late gestation, prolactin levels remained below 50 ng/ml, increasing to 300 ng/ml two days before parturition until 20 days postpartum. Throughout the remainder of the postpartum interval, prolactin levels remained between 100 and 200 ng/ml.

In a similar study conducted by Ingalls, Hafs and Oxender (1971), serum LH and prolactin were measured in 32 heifers, from 30 days prepartum until first estrus postpartum. Blood samples were collected through indwelling jugular cannulas and analyzed by RIA. LH levels from 30 days prepartum until first estrus postpartum did not change significantly. Prepartum prolactin levels ranged from 50 to 100 ng/ml until two days before parturition when concentrations exceeded 200 ng/ml. By 60 hours postpartum, the levels had fallen to 50 ng/ml

and ranged between 50 and 100 ng/ml throughout the remainder of the postpartum interval.

Kaprowski, Tucker and Convey (1972) were able to detect a circadian pattern of prolactin secretion in lactating dairy cows. Serum prolactin averaged 28 ng/ml between 7 and 10 A.M. and increased to 58 ng/ml at 4 P.M., declining irregularly to 28 ng/ml by 4 A.M.

As seen from the previous studies, relating to postpartum pituitary and plasma hormone levels, prolactin tends to be the dominant pituitary secretion. For this reason, prolactin will be given special attention in a later section of this review.

Exogenous Hormone Treatment of Postpartum Cows. Modification of the postpartum interval by exogenous hormone therapy has been attempted in several studies. Results have shown that significant alterations can be achieved, however, a highly reliable method for shortening postpartum intervals to conception has not been demonstrated.

Foote and Hunter (1964) treated postpartum beef cows with ovarian steroids and found that a combined treatment of progesterone and estrogen resulted in a reduction in the interval to conception.

Foote (1971) injected 10 mg estradiol 17-β (I.V.) into beef cows early in their postpartum period (9 to 15 days postpartum). Average interval to first ovulation and first estrus was significantly ($P<0.05$) shorter in treated than untreated animals.

Brown, Peterson and Foote (1972) reported that exogenous hormone

therapy was most effective when treatment was initiated early in the postpartum period (5 to 25 days postpartum). Combination progesterone and estrogen treatments proved to be the most effective for initiation of estrus, ovulation and conception.

Bovine LH and Prolactin Levels During the Estrous Cycle as Determined by RIA. Circulating levels of LH throughout the bovine estrous cycle range between .4 and 4 ng/ml with preovulatory peaks of 3 to 100 ng/ml, at approximately the time of estrus (Schams and Karg, 1969; Hendricks, Dickey and Niswender, 1970; Arije, Wiltbank and Hopwood, 1971; Snook, Saatman and Hansel, 1971; Sprague et al. 1971). Other LH peaks occurring on day 8 or 9 of the cycle (Schams and Karg, 1969) and from 4 to 7 days before ovulation have been reported (Schams and Karg, 1969; Snook, Saatman and Hansel, 1971).

Geschwind (1972) summarized the observations of several investigators in regard to the time intervals recorded from the onset of estrus to the LH surge, from the LH surge to ovulation, and the duration of the LH surge in the cow (table 1).

Sinha and Tucker (1969) reported large increases in pituitary prolactin 3 days before ovulation, with minimum levels being detected at the time of ovulation in cycling heifers. A surge in plasma prolactin prior to or at the time of estrus has been reported in heifers (Hafs and Morrow, 1970) and cows (Raud, Kiddy and Odell, 1971). Raud, Kiddy and Odell (1971) found that diestrus serum prolactin

levels ranged between 31 and 64 ng/ml with a proestrus surge over 200 ng/ml in four cycling cow samples by jugular cannulas.

TABLE 1. TEMPORAL RELATIONSHIPS AMONG THE ONSET OF ESTRUS, THE LH SURGE AND OVULATION IN THE COW*

	Onset of estrus to LH surge (hr)	LH peak to ovulation (hr)	Duration of LH surge (hr)	Reference
1.	10	15-22	$\leq$6;8	Schams and Karg (1969a,b)
2.	3-6	22-26	8-10	Henricks, Dickey and Niswender (1970a)
3.	-1.2±2.5	31±6	$\sim$6	Swanson and Hafs (1970)
4.	-4- +12		4-8	Garverick et al. (1971)
5.	9.2±8.2	24.0±2.5	12.4±1.6	Christensen, Wiltbank and Hopwood (1971)
6.	0-12		6-<12 .	I.I. Geschwind, P.T. Cupps and R. D. Dewey (Unpublished data)

* From Geschwind (1972)

Hormonal Response to Mating Stimuli. VanDemark and Hays (1952) measured uterine activity in reponse to mating stimuli in mature cows. Increase in uterine tone and contraction rate were observed due to various mating stimuli. Additional bovine studies demonstrated that stimulation of the vulva and cervix initiates the release of an oxytocin-like substance capable of increasing mammary pressure (Hays and VanDemark, 1953), while rectal massage of the cervix and vagina produced a short-term release of prolactin (Schams and Bohm, 1972). Natural mating to a bull has also been reported to elicit a small immediate increase in

plasma prolactin following each service (Cummins et al. 1973).

Half-Life and Metabolic Clearance Rate of Prolactin and Gonadotropins. Bryant, Greenwood and Linzell (1968) reported that the half-life of ovine prolactin-I^{131} injected i.v. into a single goat was 19 min. as determined by RIA.

Half-life determinations of prolactin in the rat have been extremely variable. Gay, Midgley and Niswender (1970) and Watson and Danhoff (1971) reported the half-life to be approximately 13 min., while Koch, Chow and Meites (1971) observed a half-life of approximately 5 min. The observed difference has been explained in terms of the interval between blood collections for the individual studies. In this situation, a greater intensity sampling scheme results in a more accurate estimate of the half-life, favoring the estimated 5 min. half-life calculated by Koch et al.(1971).

Half-life determinations of LH in the rat have been reported to range between 20 and 32 min., while FSH ranges between 107 and 149 min. (as reviewed by Geschwind, 1972).

Metabolic clearance rate (MCR), defined as the volume of blood cleared of hormone per unit time, has been estimated by single injection and constant infusion of labeled prolactin. Both techniques have been shown to yield comparable results. In the rat, MCR of prolactin has been calculated to be 1.26 ± 0.08 ml/min. (Koch, Chow and Meites, 1971), while in sheep, MCR was shown to be significantly higher in lactating

ewes (6.09±0.41) than in lambs (4.45±0.38). The MCR in ovariectomized ewes 2.93±0.13) was significantly lower than all other groups (Davis and Borger, 1973).

Metabolic clearance rate for LH has been measured in premenopausal women by constant infusion techniques and calculated to be 24.4±1.8 ml/min. (Kohler, Ross and Odell, 1968), while premenopausal MCR of FSH has been reported to be 14.2±1.1 ml/min. (Coble et al. 1969).

Ovulation and Luteal Function. The effect of FSH on the ovary is to initiate growth of graffian follicles to a point near maturity. FSH does not bring follicles to complete maturity, induce the formation of thecal or luteal tissue, or induce estrogen secretion. With a background of FSH activity, LH promotes complete maturation of follicles and thecal tissue, while initiating estrogen secretion. Following a preovulatory surge of gonadotropins, the follicle is ovulated and a functional C.L. develops secreting progestrone (as reviewed by Harris and Campbell, 1966).

Depletion of pituitary FSH stores has been reported to be associated with ovulation in the rat (Caligaris, Astrada and Teleisnik, 1967) and sheep (Santolucito, Clegg and Cole, 1960; and Robertson and Hutchinson, 1962). Recent studies indicate a synergistic role between LH and FSH in the induction of ovulation. These studies support the idea that ovulating hormone consists of more than one hormonal substance. Labhsetwar (1970) blocked ovulation in 4-day cyclic rats by

administration of a potent antiestrogen during diestrus. On the day of proestrus, treatment with sub-threshold doses of LH or FSH were only marginally active in restoring ovulation, while combination of the gonadotropins resulted in the incidence of ovulation being higher than the sum of the two groups receiving LH or FSH individually. Labhsetwar (1972) induced partial ovulations on the day of proestrus in 4-day cyclic rats by administration of either 4 ug LH or 15 ug FSH. When identifical doses of LH and FSH were administered in combination, normal ovulation occurred in 93% of the rats. Ovine prolactin alone, or in combination with either LH or FSH did not exert synergistic effects on ovulation. Similar evidence supports the synergistic concept of LH and FSH for the induction of ovulation in the rabbit (Jones and Nalbandov, 1972).

Blood levels of FSH have been shown to fluctuate in a similar pattern with blood levels of LH, throughout the estrous cycle of the rat. FSH begins to increase during proestrus, reaches a peak on the afternoon or evening of proestrus, and declines slowly for 3 days following proestrus. The magnitude of the FSH peak being considerably less than the LH peak, while the duration of the peak is much longer (Gay, Midgley and Niswender, 1970; Daane and Parlow, 1971). The differences in the duration of the LH and FSH peak are most likely attributed to the differences in the half-life of the two hormones (as reviewed by Geschwind, 1972).

The luteotropic substance which maintains progesterone secretion during the luteal phase of the cycle varies between species. LH is believed to be luteotropic in most species, while prolactin is generally accepted as being part of the luteotropic hormone complex in the rat (as reviewed by Harris and Campbell, 1966). Synergistic effects on luteal function have been reported for LH and prolactin in the rat by Armstrong and Greep (1962).

Malven and Sawyer (1966) reported that in the rat, prolactin exerts a luteotropic action on newly formed corpora lutea and a luteolytic effect on inactive corpora lutea from the previous cycle. A luteolytic effect on previously formed corpora lutea has been associated with the proestrus prolactin surge in the rat (Wuttke and Meites, 1971) and mouse (Grandison and Meites, 1972). Malven and Hoge (1971) reported that ergocornine acted at the pituitary level to suppress prolactin secretion in rats. Their work demonstrated that pituitary tissue transplanted to the kidney capsule released sufficient prolactin to initiate structural luteolysis of nonfunctional luteal tissue, however, continuous ergocornine treatment inhibited structural luteolysis. This evidence indicates that structural luteolysis occurs from tonic prolactin secretion rather than from the proestrus prolactin surge suggested by Wuttke and Meites (1971) and Grandison and Meites (1972).

A similar study has demonstrated that an ergot derivative

injected intramuscularly in cycling ewes depresses prolactin release and abolishes the proestrus prolactin surge without altering the proestrus LH and FSH surge. Estrous cycles of normal length and normal regression of corpora lutea (marked with carbon) were observed in both control and treated animals. These results have been interpreted to indicate that in sheep the proestrus prolactin surge is not required for normal cyclic behavior or for the regression of the previous crop of corpora lutea (Niswender, 1972). Blockage of the proestrus surge of prolactin with ergocornine does not interfere with ovulation or induce alterations in the estrous cycle of rats (Wuttke, Cassell and Meites, 1971).

Several studies have reported luteotropic properties of LH in cattle. Mason, Marsh and Savard (1962) were the first to demonstrate that purified preparations of both LH and FSH were capable of stimulating in vitro progesterone biosynthesis in bovine luteal tissue. The authors suggested that contamination of the FSH preparation with LH could have been responsible for the luteotropic action of FSH. Donaldson and Hansel (1965) injected pituitary extract and purified bovine LH into separate groups of cows and observed an extension of the estrous cycle from 20 days in control animals to 31 and 36 days, respectively, in the treated groups. Rectal palpatation and laparotomy revealed nonregressing corpora lutea in treated animals. Armstrong and Black (1966) demonstrated that LH would stimulate progesterone

biosynthesis in in vitro slices of bovine luteal tissue, if the C.L. was obtained before day 19 of the estrous cycle.

In contrast to earlier reports, prolactin has been reported to have luteotropic properties in both the ewe and cow. In the ewe, pituitary stalk section allows for the maintenance of progesterone secretion, which has been attributed to the continued secretion of prolactin from the pituitary (Denamur, Martinet and Short, 1966; and Bryant et al. 1971). In the cow, both prolactin and LH enhance the secretion of progesterone from bovine ovaries containing luteal tissue and perfused in vitro, while only LH stimulates progesterone secretion in the contralateral ovary containing only follicles (Bartosik et al. 1967). Under conditions of constant infusion of labeled acetate, increased progesterone secretion was induced by LH and accompanied by an increase in specific activity, while prolactin failed to increase the specific activity of progesterone. These results were interpreted to indicate that prolactin and LH operate through different mechanisms to exert their effect on progesterone biosynthesis.

Snook et al. (1969) injected antibovine LH into intact and hysterectomized heifers throughout the estrous cycle. Treated heifers exhibited decrease in C.L. weight, total progesterone, total progestins and 20-β-ol concentration; however, the progesterone concentration per unit weight of C. L. tissue was not affected. The suggestion was made that the inability of the antibovine LH to significantly reduce

progesterone concentration might indicate additional factor(s) involved in C.L. maintenance in the bovine.

Extrinsic Factors Affecting Prolactin Release. Suckling was first shown to reduce pituitary prolactin levels in the rat by Reece and Turner (1937). Ratner and Meites (1964) reported that suckling stimulus reduces the content of prolactin inhibiting factor (PIF) in the hypothalmi of lactating rats, resulting in increased prolactin release from anterior pituitary cultures. Grosvenor (1965a) observed that hypothalmic extracts of rat, bovine and ovine sources would block the suckling-induced release of prolactin from the anterior pituitary of lactating rats. Grosvenor, Mena and Schaefgen (1967) observed that 2 minutes of suckling reduced pituitary stores the same extent as 30 minutes of suckling in lactating rats. Shino, Rennels and Williams (1971) used the electron microscope to observe the ultrastructure of pituitary prolactin cells in rats. The absence of suckling during lactation produced an accumulation of secretory granules, while acute suckling resulted in their discharge.

Various types of stress in the rat have been reported to deplete pituitary prolactin stores (Grosvenor, 1965a; Grosvenor, McCann and Nallar, 1965) and increase serum prolactin levels (Neill, 1970). Exterioceptive stimuli arising from an interrelationship between the mother and the hungry young has also been reported to initiate a discharge of prolactin in lactating rats (Grosvenor, 1965b;

Grosvenor et al. 1967).

Raud, Kiddy and Odell (1971) evaluated the effect of stress on bovine serum prolactin levels by RIA. Detailed tests were conducted on 15 cycling dairy cows, 4 to 5 years of age. Nine animals were sampled by jugular puncture and four by indwelling cannulas. Mean diestrus prolactin concentrations of cows sampled by jugular puncture ranged from 113 to 485 ng/ml, while cows sampled by indwelling cannulas ranged between 31 and 64 ng/ml. Included in this study was an investigation to determine the effect of noise and restraint on serum prolactin levels. An increase from 64 to 206 ng/ml was observed within 30 minutes when a single cannulated cow as subjected to noise and restraint for a 10 minute period. Neither technique of bleeding (jugular puncture vs. indwelling cannula) had any apparent effect on LH concentration in any of the cows studied. Conclusion by the authors indicated that physiological studies measuring bovine prolactin should be conducted under carefully controlled environmental conditions.

Johke (1970) studied the effects of various stimuli on plasma prolactin levels in Holstein cows. Rapid increases in plasma prolactin were detected by RIA in cannulated animals arising from stimuli associated with needle puncture (pain, forced restraint, emotional disturbances), while no significant increases were detected during continuous sampling of cannulated animals receiving no external stress stimuli. Washing the udder with warm water, followed by machine milking, induced

prompt increase in plasma prolactin resulting in peaks occurring from 4 to 20 minutes after stimulation. Prolactin peaks resulting from milking were highest in the early stage of lactation and decreased throughout lactation. Lowest plasma prolactin levels were obtained 2 to 3 hours after milking.

Koprowski, Convey and Tucker (1971) reported that washing the brisket as well as washing the udder, stimulated significant increases in plasma prolactin in unbred heifers, dry cows and lactating cows.

Butler, Willett and Malven (1971) studied patterns of prolactin release in sheep and concluded that heat, surgical and psychological stress cause a release of pituitary prolactin,

Pharmaceutical Agents Effecting Prolactin Release. Several tranquilizing drugs are know to increase prolactin release. In the rat, reserpine, chloropromazine, meprobamate (as reviewed by Meites, Nicoll and Talwalker, 1963) and perphenazine (Ben-David et al. 1971), stimulate prolactin release while acepromazine (Bryant, Connan and Greenwood, 1968) and perphenazine (McNeilly and Lamming, 1971) are potent stimulators in the sheep.

Shelesnyak (1958) reported that the ergot drug ergotoxine could prevent prolactin secretion in the rat. Wuttke, Cassell and Meites (1971) demonstrated that ergocornine would completely block the proestrus surge of prolactin in the rat and suppress all fluctuations in serum prolactin during the estrus cycle without inhibiting ovulation

and normal cycling. Similar results were noted in the ewe (Niswender, 1972).

Prolactin Release in Response to Milking. Tucker (1971) devised a series of experiments to test the patterns of prolactin release associated with milking procedures. Blood samples were obtained through indwelling cannulas and analyzed for prolactin by RIA. Baseline prolactin levels obtained 2 minutes before udder washing from four first-calf heifers averaged 18 ng/ml. Stimulation of the udder by washing, followed by 4 minutes of machine milking, increased plasma prolactin to 39 ng/ml. Five minutes after removal of the milking machine, plasma levels reached 44 ng/ml and decreased to 23 ng/ml by 6 hours post-milking.

A second experiment, using four multiparous cows, was designed to measure the time interval necessary for plasma prolactin levels to return to baseline after milking. Average baseline levels from samples obtained at 10 and 4 minute intervals prior to washing were 45 and 38 ng/ml, respectively. Five minutes after washing the levels increased slightly to 52 ng/ml. At this time, the milking machine was attached and followed by a 5 minute milking interval. Plasma prolactin levels obtained immediately after the removal of the milking machine averaged 119 ng/ml and decreased to 51 ng/ml by 30 minutes post-milking. Both experiments 1 and 2 demonstrated extreme biological variation between animals in the degree of response to the milking stimulus.

A third experiment was conducted to determine if continuous stimulation of the udder by machine milking could maintain elevated prolactin levels. Tests were carried out on two cows during the latter stage of lactation by 40 minute machine milkings. Prolonged milking failed to maintain elevated prolactin levels with baseline values being detected 20 minutes after the initiation of milking.

Koprowski and Tucker (1973) measured serum prolactin by RIA in postpartum Holstein cows at 4-week intervals throughout lactation. Samples were collected by venipuncture of the tail vein 2 to 4 hours before the PM milking, 5 minutes after the removal of the milking machine and 1 hour after the PM milking. The magnitude of the serum prolactin release in response to milking and the milk production were greatest during the 8th week of lactation. As lactation advanced past the 12th week, serum prolactin release in response to milking declined until the 32nd week when prolactin was no longer released in response to milking. Serum prolactin levels 2 to 4 hours before milking and 1 hour after milking were greater for nonpregnant cows than pregnant cows. In contrast, serum prolactin levels 5 minutes after milking did not differ between nonpregnant and pregnant cows.

Fell et al. (1973) measured serum prolactin in response to milking in dairy cattle subject to different time intervals between milkings. Blood samples were collected by a continuous sampling device inserted into the jugular vein. Sampling began immediately before milking and

continued for a 30-minute period. Deletion of one or two consecutive afternoon milkings produced a significant decrease in the amount of prolactin released in response to the following milking.

In a second experiment, a significant drop was noted in serum prolactin released in response to milking when cows were milked at 30-hour intervals as compared to 22-hour intervals. A 30 to 50% drop in milk yield was observed during the 30-hour interval.

Hypothalmic Control of Gonadotropins and Prolactin

Recent advances in neuroendocrinology have clearly established the concept of hypothalmic control over the pituitary gland.

Hypophysitropic Hormones. Luetinizing hormone releasing hormone (LH-RH) has recently been isolated from porcine hypothalmi and physiological studies indicate that it stimulates the release of both LH and FSH (Schally et al. 1971a). Artificial synthesis of LH-RH has been accomplished and both in vivo and in vitro studies indicate it has equal biological potency with nuatural LH-RH of porcine origin (Schally et al. 1972). From these studies, it has been suggested that a single hypothalmic hormone designated as gonadotropin releasing hormone (Gn-RH) is responsible for the secretion and release of both LH and FSH from the anterior pituitary in several species (Schally et al. 1971b; Schally, et al. 1972).

Observations from several studies have developed the concept that hypothalmic control of prolactin secretion and release is due to an

inhibiting factor rather than a stimulating factor associated with the secretion and release of other anterior pituitary hormones. Supportive evidence for this idea stems from the following studies. Increased prolactin secretion accompanied by decrease secretion of other anterior pituitary hormones results from the following experimental procedures: (1) transection of pituitary stalk, (2) transplantation of the anterior pituitary to noncranial sites, (3) administration of central nervous system depressants, (4) properly oriented lesions in the hypothamlus, (5) and in vitro cultures of anterior pituitary glands (Meites, Nicoll and Talwalker, 1963). The increase in prolactin secretion and release observed in these type of experiments has established the presence of an unidentified prolactin inhibiting factor (PIF).

A second hypothalmic factor controlling prolactin secretion and release has been suggested in the following studies. This factor, tentatively known as prolactin releasing factor (PRF), has been shown to be present in hypothalmic extracts of lactating rats.

Meites, Talwalker and Nicoll (1960) reported that hypothalmic extracts from postpartum rats would stimulate mammary secretion in estrogen primed mature rats.

In a similar experiment, Mishkinsky, Khazen and Sulman (1968) observed that either hypothalmic or anterior pituitary extracts from postpartum lactating rats would increase lactogenic activity in estrogen primed virgin female rats. Their results indicate that

hypothalmic extracts obtained on the 10th day postpartum and anterior pituitary extracts obtained on the 14th day postpartum, stimulated peak lactogenic activity in virgin estrogen primed rats.

Desclin and Flament-Durand (1969) tested the effects of reserpine (stimulates prolactin release) on the morphology of pituitaries in situ and of pituitaries grafted into the hypothalmus and to the kidney capsule of rats. Pituitary grafts located in a definite region of the hypothalmus including the anterior hypothalmic area up to the level of the paraventricular nuclei, the ventromedial nuclei and the arcuate nuclei were strikingly different from grafts located in other areas of the hypothalmus or in the kidney capsule. In the designated areas of the hypothalmus, pituitary grafts showed numerous prolactin secreting cells with a high degree of stimulation resembling prolactin cells seen during lactation in the in situ pituitary. Pituitary grafts located outside the designated areas contained small oval cells comparable to untreated controls. These results were interpreted to indicate the presence of a prolactin stimulating factor located in the previously designated hypothalmic areas.

Mishkinsky and Sulman, as reviewed by Sulman (1970), demonstrated that hypothalmic extracts from suckled rats would enhance the lactogenic effect observed by perphenazine suppression of PIF in nonsuckled estrogen primed rats. The enhanced lactogenic effect was suggested to be the result of a prolactin releasing factor (PRF).

Valverde-R, Chieffo and Reichlin (1972) extracted porcine and rat hypothalmic tissue with methanol and obtained a factor that stimulated prolactin release in estrogen-progesterone primed male rats. Identical treatment of cerebral cortex tissue failed to initiate a measureable response. In the experimental rats, plasma prolactin rose significantly from the pre-injection level of 11.8 ng/ml to 106 ng/ml within 10 minutes. Baseline values were re-established by 20 minute post-injection. Similar effects were observed in estrogen primed rats, while progesterone primed rats showed no response.

Synthetic thyrotropin-releasing hormone (pyro-glutamyl-histidyl-proline) has been shown to initiate a release of prolactin in cattle (Convey, et al. 1973). Single i.v. infusion via jugular cannulae, produced a significant peak in plasma prolactin occurring from 8 to 10 minutes post-infusion. The authors concluded that synthetic TRH is not the proposed prolactin releasing factor because in vivo studies failed to show a dose-related response to TRH and in vitro studies failed to initiate a significant release of prolactin.

Autofeedback Mechanism: Torok (1964) observed the existence of a two-directional blood flow in the hypophysial portal system from the hypothalmus to the pituitary and vice versa. This observation was conducted in live dogs and cats and uncovered an anatomical pathway by which pituitary hormones may pass directly from the pituitary to the hypothalmus and regulate their own production. Regulation of this

type is referred to as internal, direct, short or autofeedback (Flerkó, 1966; Sulman, 1970).

The existence of an autofeedback mechanism for LH (Corbin and Cohen, 1966) and FSH (Corbin and Storey, 1967) have been reported in the rat. Median emminence implants of LH and FSH were found to significantly reduce the pituitary content of the corresponding gonadotropin.

A similar autofeedback mechanism for prolactin regulation has also been demonstrated in the rat (Clemens and Meites, 1968). Median eminence implants of prolactin significantly reduced prolactin secretion, mammary growth and luteal function. Additional studies by Clemens, Sar and Meites (1968, 1969) indicate that median eminence implants of prolactin will inhibit lactation and luteal function in rats.

Chen, Minaguchi and Meites (1967) measured pituitary prolactin by the pigeon crop sac method in ovariectomized-adrenalectomized rats bearing pituitary tumors capable of prolactin and GH secretion. Anterior pituitary weight and prolactin content of tumor-bearing rats were significantly lower than control rats. Measurement of hypothalmic PIF content showed a significant increase in tumor-bearing rats over controls. Intense lobuloalveolar development of the mammary gland was also noted in tumor-bearing rats.

Chen, Voogt and Meites (1968) demonstrated that prolactin implants in the median eminence of rats on day 1, 4 or 8 of pseudopregnancy,

significantly shortened the duration of pseudopregnancy, while implants of FSH and LH had no significant effect.

Hypothalmic Areas Controlling Ovulation and Pseudopregnancy. Differentiation of the hypothalmic areas controlling ovulation and pseudopregnancy have been investigated in several studies by sterotaxic lesions and electrical or electrochemical stimulation.

Lesions placed rostral to the paraventricular nuclei, medial in the plane of the paraventricular nuclei, or in the ventromedial nucleus, produce periods of prolonged diestrus indicative of pseudopregnancy in some experimental rats (as reviewed by Everret, 1966). Lesions in the thalmohypothalmic border initiate prolonged periods of diestrus and hyperluteinized ovaries indicative of prolactin secretion (as reviewed by Flerkó, 1966).

Electrical stimulation of the preoptic area (POA) induces ovulation while stimulation of the basal medial hypothalmus (BMH) results in ovulation accompanied by pseudopregnancy (Everret and Quinn, 1966). Measurement of plasma LH and prolactin before and after electrical stimulation of the POA in rats, results in increased plasma LH and decreased prolactin, while stimulation of the BMH results in increased LH and prolactin (Clemens et al. 1971a).

Clemens et al. (1970) measured plasma LH and FSH in female rats before and after electrochemical stimulation of the preoptic area (POA). A significant increase in LH was detected within 30 minutes

post-stimulation while FSH remained constant. In a similar study by Clemens et al. (1971a), electrochemical stimulation of the medial septum, preoptic area (POA), anterior hypothalmic area (AHA) and median eminence arcuate complex (MEAC), was applied to determine the effect on plasma LH and FSH. Approximately 80% of the stimulation sites in the POA, AHA and MEAC which were found to be stimulatory to LH, were also stimulatory to FSH secretion. Plasma LH peaked .5 hour after stimulation while FSH did not increase until 3 hours post-stimulation.

Sex Differentiation of the Hypothalmus. Early work in 1936 by Pfeiffer (as reviewed by Harris and Campbell, 1966 and Barraclough, 1967) has contributed greatly to the present understanding of the hypothalmic control mechanisms regulating gonadotropins in both male and female rats. In a series of experiments, Pfeiffer observed that male rats castrated at birth and implanted with an ovary when adult show normal follicular and corpora lutea development, while females ovariectomized at birth and implanted with an ovary when adult show normal vaginal cycles accompanied by follicular growth and corpora lutea development. In contrast, when testis were implanted into intact females at birth, the ovaries of the adult females show only follicular activity with no C. L. development accompanied by persistent vaginal cornification after puberty. From these observations, Pfeiffer concluded that the mechanisms regulating gonadotropin secretion are undifferentiated

in both males and females at birth and that gonadal steriods secreted early in life initiate hypothalmic differentiation.

Barraclough (1961) observed an androgen sensitive period in neonatal female rats from birth to the 10th day of age. Treatment of female rats with 1.25 mg testostoerone propionate at 2 to 5 days of age initiates permanent sterility, while treatment after ten days has no affect on reproductive processes.

Flerkó and Bardos (as reviewed by Barraclough and Gorski, 1961) observed that small specific lesions in the suprachiasmatic nucleus of the hypothalmus produce an anovulatory condition in normal rats similar to that observed in androgen sterilized rats.

Lesions placed in the anterior hypothalmic-preoptic region, located topographically dorsal and adjacent to the suprachiasmatic nucleus, induce an anovulatory syndrome in normal female rats (Van Dyke et al. 1957; Taleisnik and McCann, 1961).

Flerkó and Bardos reported that stimulation of the suprachiasmatic nucleus in normal rats initiates ovulation, while similar stimulation in androgen sterilized rats has no observable effects (as reviewed by Barraclough and Gorski, 1961).

Segal and Johnson (as reviewed by Gorski, 1966) transplanted pituitaries of androgen sterilized females into normal hypophysectomized females and observed normal cyclic activity in the recipient rats.

Barraclough and Gorski (1961) electrically stimulated anterior

hypothalmic areas in progesterone primed androgen sterilized rats causing sufficient release of gonadotropins to initiate ovulation.

With the information obtained from several previous studies, Barraclough and Gorski (1961) postulated a model with dual function for the regulation of hypothalmic gonadotropins in the female rat. In their proposed scheme, the first area of gonadotropin control, located in the arcuate-ventromedial nuclei of the median eminence, regulates tonic gonadotropin secretion in sufficient quantity to maintain estrogen production. Electrical stimulation of this area in androgen sterilized females initiates gonadotropin secretion while lesions result in inhibition of estrogen production, ovarian atrophy and anestrus (Flerkó and Bardos, 1959; as reviewed by Barraclough and Gorski, 1961). The second area of gonadotropin control located in the anterior hypothalmus (anterior hypothalmic-preoptic-suprachiasmatic region) initiates rhythmic secretion necessary for ovulation. This area is believed to be undifferentiated at birth and will differentiate normally in the female to form the second area of control, however, if subjected to androgen treatment before differentiation occurs, as in the case of the androgen sterilized neonatal female and normal male, the area becomes refractory to both intrinsic and extrinsic stimuli. With the absence of the second area of control, only baseline gonadotropin levels prevail which results in anovulatory sterility in females due to the lack of cyclic gonadotropin surges.

A similar hypothalmic control mechanism for regulating prolactin has been postulated by Neil (1972). This mechanism is identical with the scheme proposed by Barraclough and Gorski (1961) for gonadotropin regulation. The first area of control regulates tonic prolactin release and is located in the arcuate-ventromedial nuclei, while the second area of control regulates rhythmic prolactin release and is located in the anterior hypothalmus (anterior hypothalmic-preoptic-suprachiasmatic region). The following studies have provided the basis for the proposed dual mechanism of hypothalmic control over prolactin secretion and release.

Several investigators observed a major prolactin peak during proestrus in the absence of extrinsic stimuli (Kwa and Verhofstad, 1967; Gay, Midgley and Niswender, 1970; Wuttke and Meites, 1970). The obervation that spontaneous prolactin release occurs during proestrus, introduced the idea that some unknown intrinsic factor could elicit a major prolactin surge similar to the rhythmic discharge of gonadotropins previously discussed.

Additional studies have demonstrated that a single intrinsic factor is responsible for the initiation of the rhythmic peak of gonadotropins and prolactin. Ferin et al. (1969) reported that 17β-estradiol secretion during diestrus is a prerequisite for the preovulatory LH surge. Administration of a specific antibody to 17β-estradiol on diestrus-2 abolished the LH surge and ovulation in female rats.

In a similar study by Neill, Freeman and Tillson (1971) injection of a specific antibody to 17β- estradiol blocked both the LH and prolactin surge.

Chen and Meites (1970) injected ovariectomized rats with 1, 1, and 5 ug of estradiol bonzoate daily for six days. Serum prolactin concentrations increased 2, 3 and 10-fold, respectively, over ovariectomized controls.

Sharr and Clemens (1971) measured plasma prolactin levels before and after estrogen infusion in the rat and concluded that estrogen is necessary for the activation of neurons which either inhibit the secretion of PIF or stimulate an unidentified prolactin releasing factor.

Several investigators have reported that the anterior hypothalmic-preoptic-suprachiasmatic region concentrates estradiol (Kato and Villee, 1967; Pfaff, 1968), while other evidence indicates that intramuscular injections of estrogen alter the electrical activity of this area when subjected to extrinsic stimuli (Lincoln and Cross, 1967).

In a study conducted by Neill (1972), estrogen was injected into female rats ovariectomized when adult, adult neonatally castrated males, androgen sterilized females and males castrated when adult. On the third day of the estrogen treatment, blood samples were collected through arotic catheters at two-hour intervals from 1100-0100 hours on the following morning for LH and prolactin analysis. Female rats ovariectomized when adult and adult neonatally castrated male rats

showed surges of LH and prolactin on the afternoon and evening of the third day. Androgen sterilized females and males castrated when adult showed no surge of LH or prolactin in response to the estrogen treatment. In an additional experiment, surgical cuts were made in the hypothalmus of females ovariectomized when adult and treated with estrogen. The surgical cuts were made directly behind the optic chiasma (retrochiasmatic) with a modified Halasz knife. The retrochiasmatic cut inhibited the surge of LH and prolactin, while sham operated animals showed the characteristic surge of LH and prolactin. These results were interpreted to indicate fundamental differences between male and female rats in the hypothalmic regulation of LH and prolactin. The differences resulting from neonatal steriod feedback from the gonads, which stimulate the hypothalmic surge center to differentiate into male or female components.

Evidence For Antagonism Between Prolactin and Gonadotropin Production

Several studies have attempted to demonstrate that antagonism exists between prolactin and gonadotropin production at the hypothalmic level. Compete understanding of the control mechanisms in this area would tentatively aid in understanding the mechanism responsible for postpartum anestrus and associated infertility in the bovine species, however, most of the available information in this particular area stems from studies conducted primarily with the laboratory rat.

Studies with ovariectomized rats indicate that the removal of gonadal steroids results in increased gonadotropin secretion (as reviewed by Harris and Campbell, 1966) and decreased prolactin secretion (Amenomori, Chen and Meites, 1970). Administration of 5 ug of estradiol benzoate to ovariectomized rats, significantly increases pituitary and serum prolactin (Amenomori, Chen and Meites, 1970). Estrogen has been reported to promote prolactin secretion in the rat by direct action on the pituitary (Nicoll and Meites, 1964) and by reducing the PIF content of the hypothalmus (Ratner and Meites, 1964). Estrogen implants into the pituitary or median eminence of rats, have resulted in reduced gonadotropin (Ramirez, Abrams and McCann, 1964) and increased prolactin secretion (Ramirez and McCann, 1964).

Ajika et al. (1972) measured plasma and pituitary FSH, LH and prolactin after subcutaneous injection of varying doses of estradiol benzoate (.2-5 ug) in ovariectomized rats. Single injection of all

doses of estradiol produced a significant decrease in plasma LH and FSH at 48 hrs post-treatment, while plasma prolactin increased significantly. At the 5 ug doses of estradiol, pituitary content of LH, FSH and prolactin increased significantly by 48 hrs post-treatment.

As previously stated, Clemens and Meites (1968), demonstrated that median eminance implants of prolactin into mature intact and ovariectomized rats, resulted in increased hypothalmic content of PIF and reduced anterior pituitary prolactin content. These findings have been utilized to establish the existence of an autofeedback loop for prolactin regulation.

Clemens, Sar, and Meites (1968) reported that prolactin implants in the median eminence of postpartum lactating rats caused regression of the corpora lutea of lactation and resumption of estrous cycles. Voogt, Clemens and Meites (1969) reported that implants of prolactin into the median eminence of immature female rats stimulated the release of pituitary FSH, as indicated by a fall in pituitary FSH and growth of follicles. These observations indicate that when endogenous prolactin secretion is suppressed by median eminence implants of prolactin through autofeedback mechanisms, the pituitary responds by increasing gonadotropin secretion.

Sinha and Tucker (1968) observed that exogenous prolactin administration or additional pituitary's transplanted to the kidney capsule, decrease anterior pituitary prolactin contents in mature

virgin female rats and increase the content of mammary DNA and RNA.

Rothchild (1960) summarized his studies on the postpartum lactating rat, reporting that follicular quiescence during lactation results from the suppression of folliculotrophins (LH and FSH) and that the factor responsible for folliculotrophin suppression is also responsible for increased prolactin secretion. The factor being the intensity of the suckling stimulus from the nursing young. The more intense suckling stimulus having a greater suppressing effect on folliculotrophin production than a less intense suckling stimulus. In this study, the intensity of the suckling stimulus was dependent only upon the number of suckling young, not on the frequency of nursing. Rats nursing small litters (1 to 2 pups) were capable of conceiving during lactation while rats nursing large litters (6 and over) did not conceive until after weaning of the previous litter.

Ben-David, Danon and Sulman (1971) devised an experiment to test the possibility of antagonism between prolactin and gonadotropin secretion in rats. Their techniques utilized perphenazine, a compound that promotes prolactin secretion and release, and methallibure, a non-steroidal gonadotropin suppressor. Throughout a series of experiments utilizing perphanzaine and/or methallibure, the authors reported evidence that when the pituitary is secreting elevated levels of gonadotropin, as is the case in ovarectomized rats, its ability to release prolactin in response to perphenazine stimulation is severely

reduced. However, when subthreshold dose of methallibure were administered in combination with perphenazine to ovariectomized animals, serum prolactin increased to levels comparable with those measured in perphenazine treated intact rats. Conclusion; by the authors suggest that when gonadotropin levels in the pituitary are high, the ability of the pituitary to produce prolactin is reduced.

Veomett and Daniel (1971) studied the effect of the degree of suckling on the ability of lactating rats to maintain pregnancy. Reduction of the litter size to 2 or 3 pups at parturition, resulted in resumption of cyclic ovarian activity, mating and implantation in the majority of rats by 9 days postpartum. Implantation was diagnosed by laparotomy in both control and experimental animals. Following implantation, the litter size of the experimental animals was increased to 11 to 14 pups while controls remained constant. The increased suckling stimulus following implantation resulted in termination of pregnancy in all experimental animals, while 90% of the controls gave birth to normal young.

Hammons, Velasco and Rothchild (1973) devised a study to test the effect of suckling stimulus on serum LH levels in intact and ovariectomized postpartum rats. Their results deomonstrated that in ovariectomized females from day 6 to 18 postpartum, 9 pups had a greater suppressing effect on serum LH. than a suckling stimulus from 2 pups. No observable differences in LH were detected between intact females

nursing 2 or 9 pups. Additional studies determined that removal of the 9 pup litters from ovariectomized females on day 6 or 11 postpartum, induced prompt increases in serum LH. In contrast, increasing the litter size from 2 to 9 pups on days 6 or 11 postpartum, initiated only minor decreases in serum LH. The results were interpreted to reinforce previous finds that suckling supresses and castration stimulates gonadotropin secretion. Failure of the intact females to respond in a similar fashion to ovariectomized females has been attributed to the ovarian activity characteristic of pseudopregnancy, resulting from functional corpora lutea of lactation.

Nakayama and Nickerson (1973) observed the pituitary histology of female rats implanted with the MtT-WIO pituitary tumors capable of prolactin and growth hormone secretion. Tumor-bearing animals showed significant reductions in anterior pituitary weight in comparison to non-tumor-bearing controls. Pituitary mammotrophs in tumor-bearing animals exibited a gradual reduction in diameter of the secretory granules throughout a five week interval, while pituitary somatotrophs in tumor-bearing animals demonstrated a remarkable reduction in cell size, diameter and number of secretory granules. Pituitary gonadotrophs of tumor-bearing animals progressively increased in size until they became the largest cell in the anterior pituitary. During enlargement of the gonadotrophs, there was a progressive arrangement of cytoplasmic organelles characteristic of

a highly secretory state.

Schwartz and McCormack (1972) have summarized the work of Kamberi, Schneider and McCann, dealing with the potential neurotransmitters and associated anterior pituitary function in the rat. Table 2 is taken directly from their review and illustrates an interesting point, i.e., that all of the potential neurotransmitters when injected into the third ventricle of the brain at one dose or the other, produce inverse effects on the secretion of gonadotropins (LH and FSH) and prolactin.

TABLE 2. THE EFFECT OF POSSIBLE NEUROTRANSMITTERS ON THE PLASMA LEVELS OF FSH, LH AND PROLACTIN* **

Substance	Dose in µg	LH	FSH	Prolactin
Dopamine HCl (DA)	1.25, 2.5	Inc(103)	Inc(106)	Dec(107)
	100	NC (103)	NC (106)	NC (107)
Norepinephrine (NE)	2.5, 5.0	NC (103)	NC (106)	NC (107)
	100	Inc(103)	Inc(106)	Dec(107)
Epinephrine (E) bitartrate	2.5,5.0	NC (103)	NC (106)	NC (107)
	100	Inc(103)	Inc(106)	Dec(107)
Serotonin creatinine sulfate (5HT)	1.0,5.0	Dec(103)	Dec(108)	Inc(108)
	50	---	Dec(108)	Inc(108)
Melatonin (M)	1,0,5.0	Dec(103	Dec(108)	Inc(108)
	50	Dec(103)	Dec(108)	Inc(108)

Inc=Significant increase
Dec=Significant decrease
NC = No significant change
* Test animals were adult male Sprague Dawley rats; volume injected into third vetricle was 2.5 or 5 µl; FSH, LH and prolactin were measured by specific radioimmunoassay procedures.
**From Schwartz and McCormack (1972).

Assay Procedures for Reproductive Hormones

HCG Augmentation Assay for FSH. Steelman and Pohley (1953) described a technique for measuring folicle stimulating hormone (FSH) activity in pituitary tissue. The method is based on the observation that human chorionic gonadotropic (HCG) will augment the action of FSH on the ovary of the intact immature female rat. Relatively high doses of HCG (10-50 IU) make the ovary extremely sensitive to exogenous FSH, and a linear relationship exists between ovarian weight and the amount of FSH administered. The techniques of the HCG augmentation assay have been utilized to measure pituitary FSH contents of several species, although the sensitivity is relatively low.

Ovarian Ascorbic Acid Depletion Assay (OAAD) for LH. Parlow (1961), as reviewed by Apostolakis and Doraine (1967), described a procedure for measuring luteinizing hormone (LH) activity in pituitary tissue, based on the ability of LH to deplete the ascorbic acid content in the ovary of the intact immature female rat. Pretreatment with pregnant mare serum (PMS) and human chorionic gonadotropin (HCG) prepared the ovaries for the bioassay which is conducted 5 to 9 days later. Prior to bioassay, a control group is selected while LH standards and unknowns are administered intravenously to the remaining animals. Three hours after administration of the LH standards and unknowns, a single ovary is removed from LH treated and control rats and analyzed for ascorbic acid content. A linear negative regression in ascorbic acid content