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Two experiments were conducted to evaluate the effects of gonadotropin releasing hormone (GnRH) on the function of the bovine corpus luteum during the estrous cycle. In Experiment 1, 10 heifers were assigned randomly into two groups with each heifer serving as her own control. Heifers in Group I (n=5) were injected intravenously (iv) with vehicle (saline) on day 2 of the cycle (day 0=day of estrus) followed by an iv injection of 100 ug GnRH on day 2 of the subsequent estrous cycle. Group II (n=5) heifers were similarly treated except injections were given on day 10 of the estrous cycle. All heifers were bled via the jugular vein at 15 minute intervals beginning 30 minutes prior to injection and for 3 hours after injection. Blood samples were also taken on alternate days after injection through day 16 of the cycle.

Gonadotropin releasing hormone caused a significant release of luteinizing hormone (LH) on both treatment days with the peak occurring at 15 to 30 minutes post-injection. Treatment with GnRH on

either day 2 or 10 of the cycle caused a reduction in serum progesterone levels on days 12, 14 and 16 of the cycle (Group I, control 3.99, 3.97, 4.07 vs treated 2.63, 3.45, 2.87; Group II, control 3.18, 3.82, 4.13 vs treated 2.50, 2.82, 3.17 ng/ml, respectively, $P < .03$). Length of the estrous cycle did not differ among the groups (Group I, control 20.7 vs treated 20.9; Group II, control 20.7 vs treated 21.1 days, respectively).

In Experiment 2, 20 heifers were assigned randomly into four groups (n=5) of 2 x 2 factorial design to examine the effects of GnRH on luteal LH receptors. Treatment consisted of an iv injection of vehicle or 100 ug GnRH on day 2 of the cycle. Heifers were sacrificed on day 8 or 14 of the same cycle at which time the ovaries were removed, corpora lutea enucleated, weighed and frozen until assayed for LH receptors. Concentrations of unoccupied LH receptors in the luteal plasma membranes were reduced at day 8 and 14 after treatment (control vs treated, day 8, 96.6 vs 54.7; day 14, 92.0 vs 40.7 pmol/ug protein, $P < .05$). Data from the present study suggests that exogenous GnRH may act indirectly via the released LH to cause down regulation of luteal LH receptors with a consequent attenuation of progesterone synthesis.

GONADOTROPIN RELEASING HORMONE-INDUCED ALTERATION OF CORPUS LUTEUM FUNCTION IN BEEF HEIFERS

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REVIEW OF LITERATURE

Introduction

Since the mid 1950's researchers have been actively investigating those factors that determine the functional life span of the corpus luteum in domestic animals during the estrous cycle. A wealth of information has been generated during this time with regard to pituitary, ovarian and uterine hormones that impinge on luteal function. For the most part, the mechanism of action of these hormones at the cellular level still remains an enigma. What follows is an attempt to review the current literature with respect to those factors that have been found to affect the life span of the corpus luteum in the cow, ewe and the sow during the estrous cycle.

Hormones Influencing the Lifespan of the Corpus Luteum in the Cow, Ewe and the Sow During the Estrous Cycle

Hypothalamic Hormones

Gonadotropin Releasing Hormone. Of all the hypothalamic hormones known to exist at the present time, only luteinizing hormone releasing hormone (LHRH) has been shown to effect ovarian function. Because of its ability to release both follicle-stimulating hormone (FSH) and luteinizing hormone (LH), this hypothalamic decapeptide will hereafter be referred to as gonadotropin releasing hormone (GnRH). Conflicting reports exist with regard to the effects of GnRH on bovine luteal function. This is probably due to the differences in dosages used, frequency of administration and whether the preparation was native GnRH or an analog of this hormone. To date, native GnRH administration has not been demonstrated to alter the length of the estrous cycle. However, a subsequent reduction in serum progesterone concentrations was observed when GnRH (100 ug) was administered iv during metestrus (Ford and Stormshak, 1978). Similarly, intramuscular (im) injection of GnRH into dairy cows 72 hours after treatment with prostaglandin $F_{2\alpha}$ to synchronize estrus has been shown to reduce serum concentrations of progesterone during the ensuing cycle (M.C. Lucy and J.S. Stevenson, unpublished observations). Yet, im administration of GnRH (100 ug) on day 10 or

11 did not affect corpus luteum function or duration of estrous cycles (Seguin et al., 1977). A single im injection of 200 ug GnRH given to heifers on day 15, 17 or 19 of the estrous cycle also did not alter cycle duration (Britt, 1975).

In a series of recent experiments by Milvae et al. (1984), twice daily intrauterine infusions of 100 ug GnRH on days 12, 13 and 14 of the bovine estrous cycle was without effect on plasma concentrations of progesterone and the functional life span of the corpus luteum. In addition, subcutaneous (sc) injection of 100 ug GnRH four times daily on days 9 through 12 of the cycle had no effect on serum concentrations of progesterone or estrous cycle length. In contrast, 10 ug of a highly potent GnRH agonist analog injected sc four times daily on days 9 through 12 of the cycle prolonged the estrous cycle (26.2 days) compared with that of control heifers (20.3 days). Plasma concentrations of progesterone were also increased in the GnRH agonist analog-treated group on days 9 through 13 and 15 through 22 of the estrous cycle. However, during the ensuing cycle, serum progesterone levels in the heifers previously treated with the GnRH analog were markedly reduced. The reason for this altered luteal function during the succeeding cycle is unknown.

More research is definitely needed to determine the precise role of GnRH, if any, on bovine luteal function. Research is also needed with respect to the effects of GnRH on luteal function in the ovine and porcine species. There have been many studies reporting the effects of GnRH on the release of gonadotropins in the ewe and the sow, but few studies have been conducted to determine the effects of

GnRH on luteal steroidogenesis in these latter species.

Pituitary Hormones

Luteinizing Hormone. Simmons and Hansel (1964) first suggested that LH is luteotropic in the cow by virtue of its ability to overcome the inhibitory effects of injected oxytocin on luteal life span. This was later confirmed by Donaldson et al. (1965). Donaldson and Hansel (1965) found that a single injection of bovine LH given to heifers on day 16 of the cycle prolonged the estrous cycle from an average of 20.0 days to 36.4 days. This effect was abolished after denaturation of the LH with urea. Similarly, daily administration of human chorionic gonadotropin (hCG), a hormone with predominantly LH activity, to beef heifers from day 15 of the cycle to day 26 caused maintenance of the corpus luteum and prolonged the estrous cycle from 17.7 to 32.4 days (Wiltbank et al., 1961b). Single injection of this hormone on day 10 or 15 of the cycle prolonged luteal function by 7 and 5 days, respectively (Seguin et al., 1977). Daily injections of equine LH (20 mg/day for 15 days) resulted in increased progesterone concentrations in corpora lutea of hysterectomized heifers (Brunner et al., 1969). However, because equine LH, as compared with bovine or ovine LH, is only slightly luteotropic in the cow, this result was attributed to the fact that the corpus luteum was not under the influence of a uterine luteolysin. Yet, in a later study, Carlson et al. (1971) demonstrated that bovine LH increased progesterone concentrations significantly in intact and hysterectomized heifers.

Using another approach, Snook et al. (1969) employed an antibovine LH preparation to demonstrate the in vivo luteotropic effects of LH in the cow. Administration of 50 ml of antiserum per day on days 2 through 6 of the cycle caused significant reductions in luteal weights and progesterone content in intact heifers. In keeping with the concept that LH is luteotropic in vivo, it was found that LH addition to bovine luteal cells in vitro caused an increase in progesterone synthesis (Mason et al., 1962; Savard et at., 1963; Mason and Savard, 1964; Savard and Casey, 1964; Hall and Koritz, 1965b; Armstrong and Black, 1966, 1968; Hansel, 1966). These aforementioned experiments clearly demonstrate that LH is luteotropic in the cycling cow.

Luteinizing hormone also has been established to be the major luteotropin in the ewe. Hypophysectomy of ewes on the day of ovulation (day 0) prevented corpora lutea formation and hypophysectomy on day 5 of the cycle caused luteal regression by day 12 (Kaltenbach et al., 1968b). Injection of LH or hCG into ewes hypophysectomized at mid-cycle significantly increased ovarian venous progesterone concentration and progesterone secretion rate (Hixon and Clegg, 1969). Continuous infusion of LH into ewes hypophysectomized on day 12 of the cycle also has been shown to cause luteal maintenance (Kaltenbach et al., 1968a). Likewise, infusion of LH (2.5 mg/day) beginning on day 12 and continuing to day 20 prolonged the life span of the corpus luteum in normal cycling ewes (Karsch et al., 1971). Smaller doses of infused LH (300 ug/day) also maintained corpora lutea to day 20, provided follicular growth was prevented by

X-irradiation (Karsch et al., 1970). This effect was attributed by the investigators to be due to competition between the follicles and corpora lutea for this gonadotropin. Furthermore, LH has been shown to increase the synthesis of progesterone in vivo (Niswender et al., 1976) or when incubated with ovine luteal tissue (Kaltenbach et al., 1966, 1967). Fuller and Hansel (1970) demonstrated that daily treatment of ewes with antibovine LH on day 2 through day 6 decreased luteal weight and progesterone concentrations.

Less is known about the luteotropic effect of LH in the sow. It was initially proposed that in cycling pigs a single pulse of a hormone, probably LH, of very short duration, was sufficient to cause the corpus luteum to form and to persist and function for its normal life span during the cycle (Sammelwitz et al., 1961; Brinkley et al., 1964a, 1964b). This concept was later confirmed. It was found that hypophysectomy of the pig on the day of ovulation was compatible with formation of corpora lutea that were normal in size and in their ability to synthesize progesterone (Nalbandov et al., 1967). Spies et al. (1967) were unable to cause a significant reduction in corpus luteum weight or luteal progesterone concentration upon daily treatment with antiovine LH serum from days 7 through 11 of the cycle. However, they were able to cause luteal regression and complete loss of embryos in pregnant gilts. In agreement with data on the cow and ewe, LH does have a stimulatory effect on progesterone production by porcine luteal tissue in vitro (Cook et al., 1967; Nalbandov et al., 1967; Watson and Wrigglesworth, 1975). Results of these in vitro studies, however, do not provide any indication as to

the ability of LH to prolong the life span of the corpus luteum. Collectively, these experiments suggest that in the sow, LH is probably not necessary for luteal development and function after the preovulatory LH surge has occurred.

Follicle Stimulating Hormone. In contrast to the effects of LH, FSH does not appear to be necessary for luteal maintenance and function in domestic animals. For instance, treatment of heifers with FSH during the estrous cycle failed to increase either luteal weight or progesterone content and concentration (Hansel, 1967). Malven and Hansel (1964) noted that 10 daily injections of urea-incubated aqueous extracts of bovine hypophysial tissue did not stimulate or inhibit luteal weight or progesterone content of hysterectomized heifers. However, administration of untreated aqueous pituitary extracts for a similar period increased the weight of the corpus luteum and the progesterone content. Because urea incubation inactivates LH, but not FSH, these investigators suggested that FSH had no luteotropic or luteolytic effects on the corpus luteum. The heifers treated with the urea-incubated pituitary extracts contained excessive numbers of medium-size (10 mm diameter) nonluteinized follicles on their ovaries, thus suggesting that the extracts contained FSH. In contrast, Romanoff (1966) noted an increased progesterone secretion from bovine ovaries perfused in vitro with FSH. However, it was believed that these stimulatory effects were due to the presence of LH as a contaminant. Other investigators also found that FSH addition to bovine luteal cells resulted in increased progesterone synthesis (Mason et al., 1962; Mason and Savard, 1964).

This effect was again due to LH contamination because urea treatment markedly reduced the stimulatory effect of FSH on luteal progesterone synthesis.

Failure of FSH to evoke changes in progesterone secretion also has been demonstrated in the ewe. Kaltenbach et al. (1967) incubated ovine luteal tissue in the presence of ovine FSH and found no effect on progesterone synthesis. Similarly, perfusion of FSH directly into the ovary of the intact ewe failed to stimulate progesterone secretion (Domanski et al., 1967). In addition, McCracken et al. (1971) infused FSH directly into the arterial supply of ovaries autotransplanted to the neck of the ewe in such a way that ovarian secretion rates of progesterone could be measured directly. Infusion of FSH failed to stimulate progesterone secretion rate in this experimental animal. Thus, similar to the cow, FSH does not appear to have a luteotropic or luteolytic effect on the ovine corpus luteum.

Considerably less research has been conducted to examine the effects of FSH on luteal function in the sow. Nalbandov et al. (1967) and Cook et al. (1967) did, however, report that addition of FSH to porcine luteal tissue failed to stimulate progesterone synthesis. Based upon these meager data and extrapolating from the data obtained with the cow and ewe, it seems highly unlikely that FSH has a luteolytic or luteotropic role in the sow.

Oxytocin. Several investigators have demonstrated that daily administration of oxytocin to heifers during the first week of the estrous cycle resulted in a marked shortening of the cycle from the

normal 21 days to 8 through 13 days (Armstrong and Hansel, 1959; Hansel and Wagner, 1960; Anderson and Bowerman, 1963; Anderson et al., 1965; Black and Duby, 1965; Ginther et al., 1967). In addition, oxytocin injections on days 0 through 6 significantly reduced luteal weight and progesterone content and concentration (Anderson and Bowerman, 1963; Labhsetwar et al., 1964; Donaldson et al., 1965; Harms and Malven, 1969). Conversely, administration of oxytocin beginning on day 14 or 15 of the cycle was ineffective in altering luteal function in intact heifers (Armstrong and Hansel, 1959; Lynn et al., 1965). Administration of oxytocin to hysterectomized heifers (Armstrong and Hansel, 1959; Malven and Hansel, 1964; Anderson et al., 1965; Brunner et al., 1969) and to heifers in which the uterine horn ipsilateral to the corpus luteum was removed (Anderson et al., 1965; Ginther et al., 1967) was also shown to be ineffective in causing luteal regression. Collectively, these data suggest that exogenous oxytocin acts via the uterus and that there exists a local utero-ovarian pathway by which the uterus regulates luteal function in the cow. The mechanism by which oxytocin is believed to promote luteal regression appears to involve uterine prostaglandins and will be addressed in a subsequent section of this review.

Oxytocin administered to ewes induced histological changes characteristic of luteolysis but did not shorten the estrous cycle (Milne, 1963). A role for endogenous oxytocin in controlling ovarian function is supported by the delay in luteal regression observed to occur in ewes that had been immunized against oxytocin (Sheldrick et al., 1980).

Few attempts have been made to demonstrate the effects of oxytocin on luteal function in swine. However, Duncan et al. (1961) found that oxytocin had no effect on *in vitro* synthesis of progesterone by porcine luteal tissue.

Ovarian Hormones

Estrogen. Results of several experiments have demonstrated that estrogen is luteolytic in the cow. In 1958 Greenstein et al. administered 1-2 mg estradiol-17β per day to dairy heifers beginning on day 2 of the cycle and continuing through day 12. These investigators reported a suppression of follicular development and an early regression of the corpus luteum. In verification of these observations, results of several trials were presented in which various forms and levels of estrogens were administered during mid-cycle (Wiltbank et al., 1961a). In this study, single injections of estradiol valerate (5-50 mg), estrone (25-100 mg) or 25 mg of a natural estrogenic product (95% estrone) all caused early regression of the corpus luteum as determined by daily rectal palpations. In subsequent studies the effect of various doses of estrogens on corpus luteum weight, progesterone content and concentration were compared in intact and hysterectomized heifers (Loy et al., 1960; Kaltenbach et al., 1964; Niswender et al., 1965; Brunner et al., 1969). In the first of these studies it was found that injection of 250 ug of estradiol-17β for 13 days beginning on the day after estrus exerted a marked depressing effect on corpus luteum weight and percentage of

functional luteal cells, but progesterone concentration was not significantly altered by this treatment. Kaltenbach et al. (1964) and Niswender et al. (1965) gave daily injections of various dosages and forms of estrogens to beef heifers from day 5 or 6 to days 12 or 18 of the cycle. Luteal weight, progestin content and concentration at day 12 and 18 was significantly lower in those heifers injected with the estrogens. Those heifers receiving the higher levels of estradiol showed the greatest reduction in luteal function. In addition, Brunner et al. (1969) administered estradiol (5 mg/day) on days 5 through 14 and on days 10 through 14 and caused complete luteal regression. However, similar treatment of hysterectomized heifers caused only partial luteal regression as indicated by higher luteal progesterone concentrations than those found in intact heifers. Kaltenbach et al. (1964) also noted higher progesterone levels in estrogen-treated hysterectomized heifers versus estrogen-treated intact heifers. The fact that large doses of estrogen caused only partial luteal regression in hysterectomized heifers, whereas similar doses caused essentially complete luteolysis in intact heifers clearly indicates that the primary mode of the luteolytic action of estrogen is via the uterus.

Data generated on studies with cycling ewes also indicates that estrogen has a luteolytic effect, but this depends on the stage of the cycle when the estrogen is administered. Several investigators have demonstrated that injection of estradiol-17β (750-1000 ug) into cycling ewes during mid-cycle (days 10-12) resulted in a reduction in corpus luteum weight, progesterone content and concentration on days

13 through 15 (Stormshak et al., 1969; Akbar et al., 1971; Bolt et al., 1971; Howland et al., 1971). Ginther (1970b) injected 0.5 mg of estradiol-17β on days 8 through 11 of the cycle and observed a decrease in cycle length (12.4 versus 17.0 days). Hawk and Bolt (1970) demonstrated that estradiol injections during the last week of the estrous cycle, but not the first week, initiated precocious regression of the corpus luteum. In this particular study, 250 or 750 ug of estradiol were administered to cycling ewes for 2 successive days, beginning on alternate days of the estrous cycle from day 1 through 11. Injection of estradiol on days 1 through 6 had no effect on luteal weight or morpohology whereas injection of estradiol on days 9 and 10 or days 11 and 12 decreased luteal weights significantly by day 14 or 15. However, Howland et al. (1968) administered estradiol-17β (2 mg) on day 5 of the cycle and showed a significant depression of the progesterone content of corpora lutea on day 7. Other studies, however, indicate that estrogen serves as a luteotropic hormone when given early in the cycle. Howland et al. (1971) injected 750 ug of estradiol-17β into ewes on day 4 of the cycle and observed an increase in corpus luteum weight. In addition, daily injections of estradiol-17 β (0.5 mg) increased the length of the estrous cycle in ewes treated on days 4 through 7 of the cycle (Ginther, 1970b). Denamur et al. (1970) administered twice-daily injections of 500 ug estradiol benzoate, starting on day 3 of the cycle, and were able to prolong the life of corpora lutea in sheep, as judged by their weight, DNA and RNA content and progesterone concentration in ovarian venous blood. Similar results were also

observed by Piper and Foote (1965, 1967, 1968, 1970). This observed dichotomy in the response of the corpus luteum to estrogen may reflect the ability of the injected steroid to induce an LH release early in the cycle but not later in the cycle when systemic progesterone levels are sufficiently high to block the estrogen-induced release of this luteotropin. In the study by Howland et al. (1971), estradiol elicited an LH release when administered on day 4 but failed to elicit an LH release on day 11. An alternative explanation for the ability of estrogen to cause regression during mid-cycle may be through its ability to reduce the sensitivity of the corpus luteum to LH by the release of $PGF_{2\alpha}$. The mechanism(s) by which estrogens are believed to induce luteal regression in the cow and the ewe will be discussed later.

The preponderance of experimental evidence seems to indicate that estrogen is primarily a luteotropic hormone in the sow. Kidder et al. (1955) administered 3 mg of diethylstilbestrol to gilts on either day 6, 11 or 16 of the cycle. Injections on day 11 significantly lengthened the estrous cycle by 6 days presumably due to luteinization of the follicles. Injections on day 6 and 16 produced variable results. In a similar study, daily injections of stilbestrol (1 mg), estradiol-17β and estrone (7.5 and 15 mg, respectively) beginning on day 11 of the estrous cycle also maintained corpora lutea (Gardner et al., 1963). These estrogen treatments resulted in a decrease in corpus luteum weight and an increase in progesterone concentration without altering total progesterone content of the corpora lutea. Estrone maintained

significantly greater size of corpora lutea than did estradiol-17β. Garbers and First (1969) extended these observations by demonstrating that daily injections of 3 mg of estradiol-17β starting on day 4 or day 14 of the cycle caused maintenance of corpora lutea when examined at necropsy 10 days after the start of the injection regimen. Daily injections of estradiol-17β (7 mg) beginning on day 11 through 16 of the estrous cycle did not affect luteal weight, but did increase luteal progesterone content and concentration when examined on day 17 (Chakraborty et al., 1972). More recently, Ford et al. (1982) conducted a study to determine if intrauterine infusions of estradiol-17β could maintain luteal function in cycling sows. Administration of 375 ng of this estrogen injected every 6 hours starting on day 11 to 15 resulted in an increase in luteal weight and progesterone concentrations in the utero-ovarian blood. Estradiol, however, did not result in increased in vitro progesterone synthesis by corpora lutea of cycling gilts (Cook et al., 1968) but did stimulate progesterone production by porcine granulosa cells (Goldenberg et al., 1972). Because these results indicate that estrogen is not luteolytic as it is in the cow and the ewe, this steroid must act by an entirely different mechanism in the sow. Most probably this involves an estrogen receptor mediated regulation of gene transcription in the luteal cell much in the same manner as demonstrated for the action of this steroid in other target tissues.

Progesterone. Exogenous progesterone when given early in the cycle, acts as a luteolysin in the cow. One milligram of progesterone per pound of body weight given as a single injection on day 1 of the

cycle was demonstrated to cause significant reductions in corpus luteum weight, proportion of functional luteal cells and luteal progestin concentration on day 14 (Loy et al., 1960). This same treatment on day 5 of the cycle also caused significant reductions in proportions of functional cells and progestin concentration, but did not reduce corpus luteum weight significantly. Ray et al. (1961) administered a single injection of a synthetic progestogen (0.76 mg/lb body weight) to heifers on the day of estrus. The following estrus was observed 13.2 days later. Average corpus luteum diameter was also significantly smaller, thus indicating that exogenous progesterone interfered with the formation and(or) maintenance of the corpus luteum. Daily injections of progesterone also have caused reductions in estrous cycle lengths of heifers (Woody et al., 1967; Harms and Malven, 1969; Ginther, 1970a). In the first of these studies, 10 daily injections of 100 mg progesterone beginning on the day of estrus reduced estrous cycle length from an average of 20.7 to 16.7 days. A decrease in cycle length of approximately 5 days on the average was also observed when 100 mg progesterone per day was administered on three or four successive days beginning on day 0 to day 2 (Harms and Malven, 1969; Ginther 1970a). This progesterone treatment also reduced luteal weights significantly but did not affect luteal concentration or content of progesterone. Woody and Ginther (1968) performed a series of experiments to determine the effects of exogenous progesterone on corpus luteum weight and life span in intact and unilaterally hysterectomized heifers. It was found that progesterone treatment on days 1 through 10 (100 mg/day)

reduced estrous cycle length in intact and unilaterally hysterectomized heifers when the remaining uterine horns were ipsilateral but not contralateral to the ovary bearing the corpus luteum. Hence, the estrous cycle shortening effect of progesterone administered during the early part of the cycle requires the presence of the uterine horn adjacent to the corpus luteum. Most probably this action of progesterone results in the synthesis of $PGF_{2\alpha}$ by the uterine endometrium. This aspect of uterine function will be discussed further in a following section. In contrast to these luteolytic effects, a lengthening of the cycle was observed when progesterone was given as a single injection later in the cycle (day 8 or 16) or if daily injections were given from days 8 through 11, 12 through 15 or 16 through 19 (Ray et al., 1961; Ginther, 1970a). The endogenous production of progesterone by the corpus luteum at this time of the cycle together with the exogenous supply does not appear to prolong the life of the corpus luteum, but rather acts at the level of the central nervous system to suppress estrous behavior and release of gonadotropins necessary for ovulation.

Exogenous progesterone given during the early part of the cycle in the ewe also results in a reduction in estrous cycle length and inhibits corpus luteum development. Woody et al. (1967) reported a reduction of about 4 days in cycle length following 6 daily injections of 25 mg progesterone beginning at estrus. Ginther (1969) confirmed this effect and demonstrated that the reduction in cycle length became progressively less as the first progesterone injection was delayed from day 0 (9.9 days) to day 4 (15.6 days). Similar

results were obtained by Thwaites (1971). In this latter study a series of 4 daily injections of progesterone (10, 25 or 40 mg) beginning on day 0 or 1 significantly reduced estrous cycle length. There was a consistent trend for this reduction to be greatest in ewes treated first on day 0. In contrast, but in agreement with studies in the cow, progesterone treatment given on days 12 through 15 lengthened the cycle but did not alter the mean weight of the corpus luteum (Ginther, 1969). These contrasting results may reflect an increase in the pituitary sensitivity to the inhibitory effects of progesterone during the latter part of the cycle whereas during the early part of the cycle the effect of progesterone on the corpus luteum may be mediated through the uterus. The daily administration of progesterone to bilaterally hysterectomized ewes from day 0 to 10 of the cycle failed to significantly alter the life span and weight of the corpus luteum suggesting that the action of progesterone was mediated via the uterus (Woody et al., 1965; Woody et al., 1968). This premise is supported in part by the study of Ginther and Woody (1970) who found that progesterone administered to unilaterally hysterectomized ewes that had ovulated bilaterally lowered the weight of the corpus luteum adjacent to the intact ipsilateral horn but not that of the corpus luteum on the contralateral ovary.

In contrast to studies with the cow and ewe, a luteolytic role for exogenous progesterone has not been demonstrated in cycling gilts. Daily injections of progesterone beginning after estrus did not result in significant reductions in the number or in the average weight of corpora lutea (Sammelwitz and Nalbandov, 1958; Sammelwitz

et al., 1961). Likewise, Woody et al. (1967) were not able to demonstrate a difference between average estrous cycle length of control gilts and that of gilts treated from the day of detected estrus until day 10 of the cycle with 200 mg progesterone per day. However, corpus luteum regression did occur when hysterectomized gilts were treated with progesterone (1 mg/lb of body weight) daily for 15 days beginning 3 days after hysterectomy on day 7 of the cycle (Spies et al., 1960). The reason for the discrepancy between these studies in the action of progesterone is not known but may be a dosage effect.

Uterine Hormones

Prostaglandins. Total hysterectomy in the cow (Wiltbank and Casida, 1956; Anderson et al., 1962; Malven and Hansel, 1964), ewe (Wiltbank and Casida, 1956) and sow (Anderson et al., 1961; Duncan et al., 1961) results in prolonged maintenance of the corpus luteum. These findings suggested that the regression of the corpus luteum during the estrous cycle requires a stimulus from the uterus. Evidence indicates that the uterine luteolytic effect in all three species is local and requires the presence of the uterine horn ipsilateral to the corpus luteum. Unilateral regression of corpora lutea has been observed on the side of the conserved uterine horn in partially hysterectomized cows (Ginther et al., 1967), ewes (Inskeep and Butcher, 1966) and sows (Anderson et al., 1966; Anderson and Melampy, 1967).

Babcock (1966) first suggested that a prostaglandin might be the uterine luteolytic agent. Pharriss and Wyngarden (1969) later postulated that prostaglandin $F_2\alpha$ ($PGF_2\alpha$) a strong vasoconstrictor, and an abundant uterine prostaglandin (Eglinton et al., 1963), might produce luteolysis as a result of a reduction in blood flow through the corpus luteum. Pharriss and Wyngarden were the first investigators to demonstrate that administration of $PGF_2\alpha$ caused luteolysis, but as will be discussed later, this does not seem to be entirely due to vasoconstriction. Subsequently, extensive research finally culminated in the conclusion that $PGF_2\alpha$ is the uterine luteolysin in cattle (Lauderdale, 1972; Liehr et al., 1972; Rowson et al., 1972; Louis et al., 1972, 1973; Inskeep, 1973; Lamond et al., 1973; Stellflug et al., 1973; Hafs et al., 1974; Thatcher and Chenault, 1976), sheep (Barrett et al., 1971; Thorburn and Nicol, 1971; McCracken et al., 1971, 1972; Chamley et al., 1972, 1973; Goding et al., 1972) and swine (Diehl and Day, 1974; Hallford et al., 1975; Guthrie and Polge, 1976; Lindloff et al., 1976). In support of the premise that $PGF_2\alpha$ is the endogenous luteolysin, investigators have demonstrated that administration of this hormone to hysterectomized cows (LaVoie et al., 1975), ewes (Bolt, 1973) and gilts (Muljono et al., 1974) caused luteal regression. Thus no other uterine factor appears to be required for luteolysis. There are, however, a series of endocrinological events leading to the release of $PGF_2\alpha$ from the uterus involving the synthesis and release of estrogen, progesterone and oxytocin.

Several other prostaglandins have been implicated in regulating

luteal function, although less research has been conducted on their roles due to the intense interest in $PGF_2\alpha$. Evidence suggests that prostacyclin (PGI_2), a vasodilator, is luteotropic in the cow because injection of this prostaglandin directly into the corpus luteum at mid-cycle caused an increase in systemic plasma progesterone concentrations (Milvae and Hansel, 1980). In this same experiment, PGI_2 was shown to stimulate in vitro progesterone synthesis by dispersed luteal cells. Experiments need to be conducted to determine if in vivo injection of PGI_2 actually prolongs luteal life span and the duration of the estrous cycle.

In the ewe, most of the studies have primarily centered on the prostaglandins of the E series. Chronic intrauterine infusions of PGE_2 in ewes blocked natural luteolysis for 2 days (Pratt et al., 1979). In addition, PGE_1 (Huie et al., 1981) or PGE_2 (Magness et al., 1981) prevented luteolysis only when infused chronically into a uterine horn adjacent to an ovary with a corpus luteum but not when infused into the contralateral uterine horn. Prostaglandin E_1 (Hoyer et al., 1978) and PGE_2 (Colcord et al., 1978) also blocked estradiol-17β-induced luteolysis. Experiments have been conducted in which both $PGF_2\alpha$ and PGE_2 were given simultaneously. Mapletoft et al. (1977) demonstrated that PGE_2 prevented $PGF_2\alpha$-induced decreases in luteal weight and Henderson et al. (1977) demonstrated that PGE_2 prevented $PGF_2\alpha$-induced decreases in progesterone secretion. In the sow, Schneider et al. (1980) demonstrated that multiple intrauterine infusions of PGE_2 resulted in increases in systemic plasma progesterone concentrations and a delay of 2 days in the time at

which plasma progesterone concentrations declined. Estrous cycle lengths were not altered by this treatment.

These data indicate that the corpus luteum may be regulated by both luteolytic and luteotropic prostaglandins. However, more research is needed to determine how these prostaglandins act at the cellular level to regulate luteal function.

The Mechanism of Action of Hormones Regulating Luteal Function

The Luteotropic Mechanism. Regardless of the species, the mechanism by which LH stimulates progesterone synthesis in the luteal and granulosa cell is believed to be the same. The initial step is an interaction of LH with its receptor on the cell membrane. The LH receptor has recently been isolated and purified from bovine corpora lutea and was found to be an oligomer with an approximate molecular weight of 280,000 daltons (Dattatreyamurty et al., 1983). After interaction of LH with its membrane bound receptor, activation of the enzyme adenylate cyclase occurs, which in turn causes the production of cyclic AMP (Marsh, 1975). Hall and Koritz (1965a, 1965b) demonstrated that cyclic AMP, like LH, stimulated progesterone synthesis by bovine corpora lutea. Thus, cyclic AMP was denoted to serve as a "second messenger" in LH stimulation of steroidogenesis in the ovary. The next step is the binding of cyclic AMP to protein kinase causing its activation (Ling and Marsh, 1977) which in turn phosphorylates many steroidogenic enzymes (Caron et al., 1975; Caffrey et al., 1979) and enhances protein synthesis. The major effect of LH is in regulating the enzymatic conversion of cholesterol to pregnenolone which has been identified as the rate limiting step in the steroidogenic pathway leading to progesterone synthesis (Hall and Koritz, 1964; Koritz and Hall, 1965). Thus, LH controls the synthesis and secretion of progesterone from the corpus luteum upon binding with its receptor.

Two different luteal cell types, based on cell diameter, have been isolated from cows (Ursely and Leymarie, 1979; Koos and Hansel, 1981), sheep (Fitz et al., 1982) and pigs (Lemon and Loir, 1977). In the ovine corpus luteum, it has been demonstrated that small luteal cells have a greater number of LH receptors and a lower number of $PGF_2\alpha$ receptors compared with the large luteal cells (Fitz et al., 1982). However, the large cells appear to secrete most of the progesterone produced by the corpus luteum. This is also true for the cow (Ursely and Leymarie, 1979; Koos and Hansel, 1981) and the pig (Lemon and Loir, 1977). Only in the case of the ovine large luteal cell has this secretion been demonstrated to be independent of LH, yet these large cells outnumber the small cells when serum progesterone is maximal. In contrast, the small cells in all three species secrete minimal quantities of progesterone in the unstimulated state but do exhibit a significant response to LH or hCG stimulation compared with the large cells. Thus, it appears that progesterone secretion from the two types of cells is regulated by different mechanisms. Unfortunately, it is not yet known how or why this occurs.

The Luteolytic Mechanism. Despite considerable efforts in this field, the precise sequence of events that culminate in luteolysis still remains elusive. Steroid hormones secreted by the ovary seem to be involved in the physiological stimulus for increasing the synthesis and(or) release of $PGF_2\alpha$ from the uterus and eventually causing luteolysis. In ovariectomized ewes, exogenous progesterone for several days increased the uterine release of $PGF_2\alpha$ (Scaramuzzi

et al., 1977). Baird et al. (1976) and Ottobre et al. (1980) proposed that a period of progestational influence regulates the timing of the initial rises in the release of $PGF_2\alpha$. Ottobre and colleagues demonstrated that the administration of progesterone to ewes on days 0 and 1 advanced the time of occurrence of the initial release of $PGF_2\alpha$ to day 8, compared with the usual time of day 12 in control ewes. McCracken (1980), utilizing ewes with utero-ovarian autotransplants, also demonstrated that intravenous infusion of progesterone for 10 days beginning immediately after estrus promoted a spontaneous release of uterine $PGF_2\alpha$ after withdrawal of the steroid.

Administration of estradiol-17β resulted in an increase in the uterine secretion or endometrial concentration of $PGF_2\alpha$ only after several days of exogenous or endogenous progesterone (Barcikowski et al., 1974; Ford et al., 1975; Louis et al., 1977; Scaramuzzi et al., 1977). Endogenous concentrations of estradiol-17β have been demonstrated to be associated with the maximal utero-ovarian concentrations of $PGF_2\alpha$ during luteal regression in the ewe (Baird et al., 1976; Scaramuzzi et al., 1977; Ottobre et al., 1980). These results have led authors to suggest that endogenous estrogen stimulates $PGF_2\alpha$ production by the uterus, after a prior period of progestational influence (Barcikowski et al., 1974; Horton and Poyser, 1976).

Oxytocin also has been demonstrated to play a role in luteal regression. Roberts et al. (1975) infused physiological amounts of oxytocin into the arterial supply of the uterus of the ewe and found

an increase in $PGF_2\alpha$ release; this response being present only late in the cycle. It was subsequently shown that the concentration of oxytocin receptors in the ovine uterine endometrium varied cyclically with the highest concentration occurring late in the estrous cycle (Roberts et al., 1976). McCracken (1980) found that continuous infusion of estradiol-17β into the autotransplanted uterus of ovariectomized ewes induced the formation of oxytocin receptors in 6 hours as determined by the ability of this exogenous neuropeptide to stimulate the uterine secretion of $PGF_2\alpha$. McCracken also infused estrogen intravenously into progesterone-treated ovariectomized ewes bearing autotransplanted uteri. Estradiol-17β administered to ewes treated with progesterone for 2 or 6 days failed to facilitate oxytocin-induced secretion of $PGF_2\alpha$. However, after 10 days of progesterone treatment the infusion of estradiol did promote oxytocin-induced secretion of this prostaglandin. McCracken hypothesized that progesterone treatment after 2 or 6 days suppressed the levels of estrogen receptor in the endometrial cells thus precluding the ability of estradiol to stimulate the synthesis of oxytocin receptor. The ability of progesterone to suppress cellular levels of estrogen receptor in the ewe had been previously demonstrated by Koligian and Stormshak (1977). It was also postulated that continuous exposure of the uterus to progesterone, such as for a period of 10 days, resulted in a reduction of progesterone receptors through autoregulation (Milgrom et al., 1973) thus allowing estradiol to stimulate increased synthesis of its own receptor and hence, the oxytocin receptor. An alternative

explanation may be that estradiol was effective in stimulating the synthesis of oxytocin receptors at days 2 and 6 of progesterone treatment but that a longer period of progesterone dominance of the uterus is required for the accumulation or synthesis of some cellular component that permits oxytocin to be effective in provoking a release of $PGF_{2}\alpha$.

The posterior pituitary was initially believed to be the sole source of oxytocin until Wathes and Swann (1982) demonstrated that the ovine corpus luteum contains high concentrations of oxytocin. In addition, Flint and Sheldrick (1982) found that the concentration of oxytocin in ovine ovarian venous blood exceeds that in arterial blood. These investigators also demonstrated that administration of an analog of $PGF_{2}\alpha$ to ewes on days 11 to 14 of the cycle caused the release of oxytocin from the ovary. Subsequently, Flint and Sheldrick (1983) found that endogenous uterine venous levels of $PGF_{2}\alpha$ were positively correlated with ovarian venous levels of oxytocin in the ewe.

In summary, it appears that the luteolytic mechanism involves: 1) induction of oxytocin receptors in the progesterone-primed uterus by estradiol; 2) response of oxytocin receptors to the neuropeptide released from the posterior pituitary and(or) the corpus luteum to evoke uterine $PGF_{2}\alpha$ secretion. However, it is quite likely that this is an oversimplification and future research will probably demonstrate further complexities.

Prostaglandin $F_{2}\alpha$ is secreted by the uterus into the uterine vein at the time of luteolysis and reaches the ovary directly via a

counter-current transfer mechanism in the utero-ovarian pedicle (McCracken et al., 1971; Hixon and Hansel, 1974). The mechanism by which $PGF_2\alpha$ acts at the level of the luteal cell to cause its regression is not entirely understood. It has been demonstrated that $PGF_2\alpha$ does not cause a reduction in luteal LH receptors at the time of luteal regression (Diekman et al., 1978b; Fitz et al., 1980; Spicer et al., 1981). This is consistent with previously conducted research by Thatcher and Chenault (1976). These latter investigators observed a transient increase in plasma progesterone concentrations at 12, 24 and 48 hours after $PGF_2\alpha$ treatment in response to GnRH-induced LH release. However, $PGF_2\alpha$ has been demonstrated to cause a reduction in adenylate cyclase activity (Fitz et al., 1980; Agudo et al., 1984) and an increase in phosphodiesterase activity (Agudo et al., 1984). It was proposed by Silvia et al. (1984) that $PGF_2\alpha$ acts upon the large luteal cells, since the small cells have few receptors for $PGF_2\alpha$, provoking them to secrete a cytotoxic factor that interacts with the small luteal cells to cause the observed reduction in adenylate cyclase. Further studies are needed, however, before the nature of these interactions can be defined. The resultant reduction in adenylate cyclase activity with the increase in phosphodiesterase activity may reduce intracellular cyclic AMP concentrations resulting in lowered luteal progesterone concentrations observed at the time of luteolysis (Marsh, 1976). Alternativley and(or) in addition, luteolysis may involve a suppression of blood flow to the corpus luteum (Niswender et al., 1976).

STATEMENT OF THE PRESENT PROBLEM

In order to maximize the use of artificial insemination, efforts have been made to develop effective methods of regulating the life span of the corpus luteum, for the purpose of developing more effective methods of synchronizing estrus. A wide variety of substances (progestogens, prostaglandin $F_2\alpha$ and analogs of this lipid) have been employed to regulate luteal life span, with some having more success than others. Unfortunately, problems remain with the present methods and the available commercial products have not been widely adopted by the livestock industries.

Since the isolation and synthesis of GnRH, researchers have been actively investigating the effects of this hypothalamic hormone with great fervor. It was first assumed that GnRH, through its ability to release LH, would act as a luteotropic hormone. Surprisingly, many researchers have disproved this assumption and have observed luteolytic effects, especially in the rat and the human. However, the effects of GnRH on luteal steroidogenesis and function in other species remains quite controversial. Existing data are equivocal because GnRH has been shown to have a positive, negative or, in some instances, no effect on luteal function in domestic animals. The objectives of the following experiments were to examine further the effects of GnRH on luteal function in the beef heifer.

EXPERIMENT 1 AND 2: GONADOTROPIN RELEASING HORMONE-INDUCED ALTERATION OF CORPUS LUTEUM FUNCTION IN BEEF HEIFERS

INTRODUCTION

It is well established that administration of GnRH or its agonist analogs to rats (Kledzik et al., 1978; Harwood et al., 1980a; Jones and Hsueh, 1980) or humans (Koyama et al., 1978; Casper and Yen, 1979) interferes with luteal progesterone synthesis. The effects of GnRH on luteal steroidogenesis in cattle, though studied extensively, have been equivocal. Treatment of dairy heifers with GnRH during the mid or late stages of the cycle did not alter serum levels of progesterone nor the duration of the estrous cycle (Britt, 1975; Seguin et al., 1977; Milvae et al., 1984). However, repetitive injections of GnRH or a GnRH agonist analog during the midluteal phase of the estrous cycle of dairy heifers increased serum progesterone concentrations (Kittok et al., 1973; Milvae et al., 1984). In contrast to the above reports, Ford and Stormshak (1978) found that injection of GnRH into beef heifers during metestrus reduced serum progesterone levels beginning on day 8 of the cycle. Similarly, GnRH administered to dairy cows on the day of detected estrus (induced by treatment with prostaglandin $F_2\alpha$) reduced serum

progesterone concentration during the ensuing 18 days in pregnant and nonpregnant animals (M.C. Lucy and J.S. Stevenson, unpublished data). Quantity of GnRH administered does not appear to be a cause for the variable responses of cattle because in most studies the dosages of hormone have been comparable.

Exogenous GnRH or GnRH agonist analogs may act directly on the ovary or indirectly via the released gonadotropins to alter luteal steroidogenesis. Receptors for GnRH have been detected in rat luteal cells (Clayton et al., 1979; Harwood et al., 1980a, 1980b). These data coupled with those of various in vivo and in vitro experiments demonstrating an inhibitory effect of GnRH or its agonist analogs on luteal and granulosa cell steroidogenesis (Hsueh and Erickson, 1979; Behrman et al., 1980; Harwood et al., 1980b; Jones and Hsueh, 1980) provide rather convincing evidence that this peptide hormone acts directly on the ovary of the rat. However, it is unlikely that exogenous GnRH or agonist analogs of this hormone act directly on the bovine ovary because GnRH receptors have not been detected in luteal or follicular tissues of the cow (Brown and Reeves, 1983). In this species the observed effects of GnRH on luteal steroidogenesis may be mediated indirectly via the increased release of LH. Although LH is luteotropic in the cow (Donaldson et al., 1965), brief exposure of the corpus luteum to large amounts of this gonadotropin may cause down regulation of the LH receptor as has been shown to occur in the rat (Conti et al., 1976). Thus, in the cow, luteal response evoked by exogenous GnRH may depend upon such variables as frequency and(or) route of injection of the decapeptide, quantity of LH released

and(or) stage of development of the corpus luteum.

The present experiments were conducted to further assess the effects of GnRH on bovine luteal function during the estrous cycle. Experiment 1 was conducted to determine the effects of GnRH administered during various stages of the cycle on serum concentrations of LH and progesterone and the duration of the cycle. The objective of Experiment 2 was to assess the effect of GnRH on the luteal concentration of unoccupied LH receptors.

MATERIALS AND METHODS

Experimental Protocol. In Experiment 1, 10 Hereford and Hereford x Angus heifers (2-year-old; 350-400 kg) were observed twice daily for estrus using a vasectomized bull. After exhibiting at least two consecutive estrous cycles of normal duration (20.6 $\pm$ 0.3 days), heifers were assigned randomly to two groups of five animals each (Figure 1). Group I heifers were injected with saline on day 2 of the cycle followed by an injection of 100 ug GnRH on day 2 of the subsequent estrous cycle. Heifers in Group II were injected with saline on day 10 of the estrous cycle followed by an injection of 100 ug GnRH on day 10 of the next estrous cycle. All injections were administered by jugular venipuncture.

To characterize changes in serum LH, jugular blood samples were collected at 30 and 15 minutes prior to injection, immediately after injection (designated as time 0), at 15 minute intervals for 60 minutes and at half-hour intervals from 60 to 180 minutes. To assess

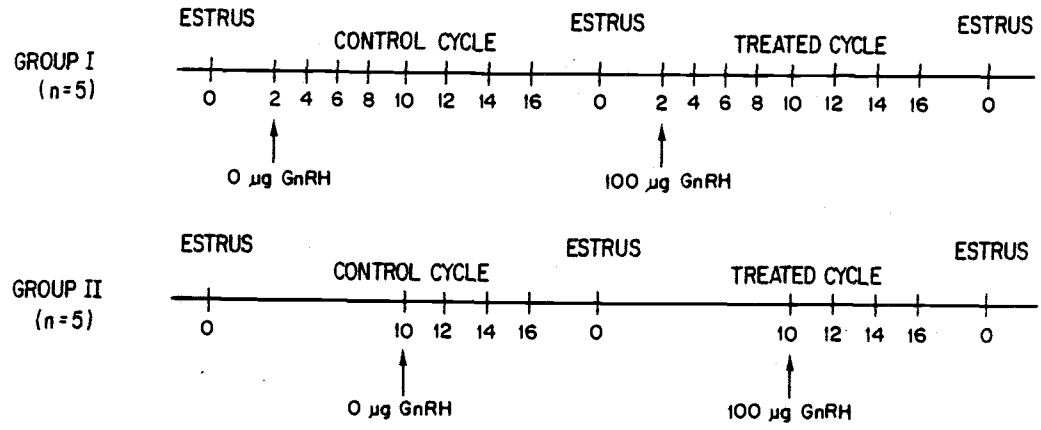

Figure 1. Design of Experiment 1. On the day of injection (day 2 or 10 of the cycle) heifers were bled at 15 to 30 minute intervals for a 3 hour period to permit characterization of serum LH levels. Samples were collected on alternate days thereafter through day 16 to determine serum progesterone concentrations.

changes in serum progesterone concentrations, heifers were bled on alternate days following injection through day 16 of the cycle.

In Experiment 2, 20 Hereford and Hereford x Angus heifers (2-year-old; 350-400 kg) were assigned randomly into four groups (n=5) of 2 x 2 factorial design to examine the effects of GnRH on the concentration of unoccupied luteal LH receptors (Figure 2). Treatment consisted of an intravenous injection of saline or 100 ug GnRH on day 2 of the cycle and heifers were sacrificed on day 8 or 14 of the same cycle.

Radioimmunoassays. Blood samples (10 ml) were stored at 4°C for 48 hours and then centrifuged at 500 x g for 10 minutes at 4°C. The serum was decanted and stored at -20°C until assayed for progesterone (Koligian and Stormshak, 1977) and LH (McCarthy and Swanson, 1976) by use of radioimmunoassay as previously validated in our laboratory.

The intraassay and interassay coefficients of variation for the progesterone assay were 8.36% and 17.58%, respectively. Serum LH concentrations were determined for all samples in two assays and the intraassay coefficient of variation was 3.50%. The sensitivities of the progesterone and LH assays were 10 pg/tube ($P < .001$, N=10 assays) and 0.5 ng/tube ($P < .001$, N=2 assays), respectively.

Tissue Preparation and Protein Determination. Following slaughter of heifers on day 8 or 14 in Experiment 2, the ovaries were removed, placed in ice-cold phosphate buffered saline (PBS; 0.01 M, pH 7.0) and transported to the laboratory. Corpora lutea were excised, trimmed of excess connective tissue, quartered, weighed and rapidly frozen in PBS-20% glycerol at -70°C until assayed for LH

GnRH (μg)[a]	DAY OF ESTROUS CYCLE	
	8	14
0	5[b]	5
100	5	5

[a]GnRH WAS INJECTED iv ON DAY 2 OF THE ESTROUS CYCLE.

[b]NUMBER OF HEIFERS/GROUP

Figure 2. Design of Experiment 2. Heifers were injected iv with GnRH or saline on day 2 of the cycle and subsequently slaughtered on day 8 or 14 to assess the concentration of unoccupied LH receptors.

receptors.

On the day of each assay luteal tissue was thawed, sliced and homogenized over ice using a 30-ml capacity Potter-Elvehjem homogenizer with a Teflon pestle. Tissue was homogenized in 0.25M Sucrose, 25mM Tris-HCl, 1mM $CaCl_2$ (pH 7.4; 10 ml/g tissue). The homogenate was filtered over ice through two layers of cheesecloth and centrifuged at 800 x g at 4°C for 10 minutes. The nuclear pellet was discarded and the supernatant recentrifuged at 20,000 x g at 4°C for 20 minutes. The resulting supernatant was discarded and the membrane pellet resuspended in 0.05M Tris-HCl (pH 7.4; 3 ml/g tissue). A 50 ul fraction of this luteal membrane preparation was assayed for protein (Bio-Rad Protein Assay Kit; Bio-Rad Laboratories, Richmond, CA). Following the protein determination, membrane preparations were diluted in 25mM Tris-HCl, 1mM $CaCl_2$ (pH 7.4) to provide a final concentration of 1 ug protein/ul.

Radioiodination of hCG. Human chorionic gonadotropin (CR-121; 13,450 IU/mg) was iodinated with $Na^{125}I$ (IMS.30; Amersham Corporation, Arlington Heights, IL) using the lactoperoxidase method (Catt et al., 1976; Rose et al., 1983). Labeled hCG was separated from the free iodine by gel filtration through a column of Sephadex G-75 (0.7 x 24 cm) equilibrated with 0.05M Tris-HCl (pH 7.4)-0.1% bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO). Following the collection of 32 fractions (0.5 ml each), two peaks of radioactivity were observed with the ^{125}I-hCG eluting in the first peak (fractions 10-13) which also included the void volume. To separate damaged and aggregated proteins, further purification was

performed by passing the iodinated hormone through a column of Sephadex G-100 (0.9 x 50 cm) equilibrated with 0.05M Tris-HCl(pH 7.4)-0.1%BSA. After collecting forty-five 1 ml fractions, two distinct peaks of radioactivity were again observed. The first peak of radioactivity included the labeled hCG which eluted in fractions 14-18.

Maximum Bindability. To determine maximum bindability of the hormone after iodination, a constant amount of ^{125}I-hCG (25,000 cpm/tube) was incubated at 25°C for 16 hours with increasing concentrations of luteal plasma membrane protein (0-200 ug/tube) in the presence or absence of a constant quantity (250 ng) of unlabeled hCG (Pregnyl; 1500 IU/mg; Organon Inc., West Orange, NJ). After incubation, separation of bound and free hormone was achieved as described by Bramley and Ryan (1978) by precipitation of the bound fraction with 20% polyethylene glycol (J.T. Baker Chemicals, NJ). This procedure involved adding 0.5 ml bovine-γ-globulin (0.5% in 25 mM Tris-HCl, 1mM $CaCl_2$; pH 7.4; Sigma Chemical Co.) to each tube after which the tubes were vortexed. An equal amount of polyethylene glycol was added and the tubes were again vortexed and then centrifuged at 500 x g for 10 minutes at 4°C. The supernatants were aspirated and the pellets were resuspended by vortexing in 25 mM Tris-HCl (1.0 ml; pH 7.4). An equal volume of polyethylene glycol was again added and the precipitate was collected as above. Radioactivity was determined in a Packard Gamma Spectrometer with 66% counting efficiency.

Subsequent to separation of bound from free hormone, specific

binding was determined by subtracting the quantity of labeled hCG bound to membranes incubated in the presence of unlabeled hCG (nonspecific binding) from the quantity of labeled hormone bound to membranes in the absence of unlabeled hCG (total binding). Specifically bound hormone was then plotted as a function of increasing luteal plasma membrane protein as depicted in Figure 3. Maximum bindability was determined after each iodination and averaged 36.6% in the presence of 200 ug membrane protein. Specific binding of ^{125}I-hCG was linear up to 100 ug protein and 50 ug protein were routinely used in all subsequent assays. All calculations were corrected for maximum bindability during Scatchard (1949) analyses, because the ligand specifically bound to luteal tissue is thought to represent only intact hCG (Ketelslegers et al., 1975).

Specific Activity. The specific activity of the labeled hormone was determined in a self-displacement radioligand-receptor assay (Ketelslegers et al., 1975; Diekman et al., 1978a; Rose et al., 1983). Increasing concentrations of hCG (0.1-100 ng) were added to tubes containing a constant quantity of luteal plasma membranes (50 ug protein/tube) and ^{125}I-hCG (25,000 cpm/tube) to generate curve A (Figure 4). Increasing concentrations of ^{125}I-hCG (2×10^4- 20×10^4 cpm/tube) were added to tubes containing a constant amount of protein to generate curve B. Specific activity was calculated by dividing the cpm (converted to uCi) obtained at a bound/free ratio of 50% (curve B) by the quantity of hCG (converted to ug) that displaced 50% of the ^{125}I-hCG in curve A. Two iodinations were used in this experiment with specific activities of 25.8 (Figure 4) and 20.6

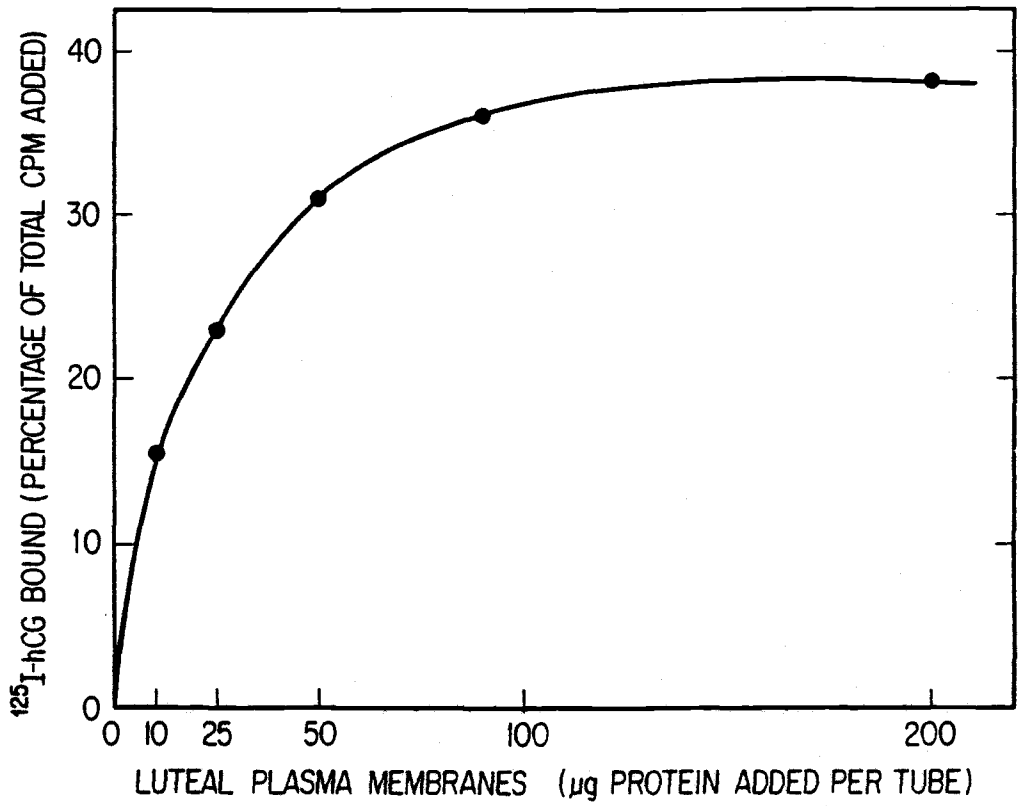

Figure 3. A constant amount of labeled hCG (25,000 cpm/tube) was incubated at 25°C for 16 hours with increasing concentrations of luteal plasma membrane protein (0-200 ug protein/tube) in the presence or absence of 250 ng unlabeled hCG to determine maximum bindability of the iodinated hCG. Maximum bindability averaged 36.6% in the presence of 200 ug membrane protein for all iodinations.

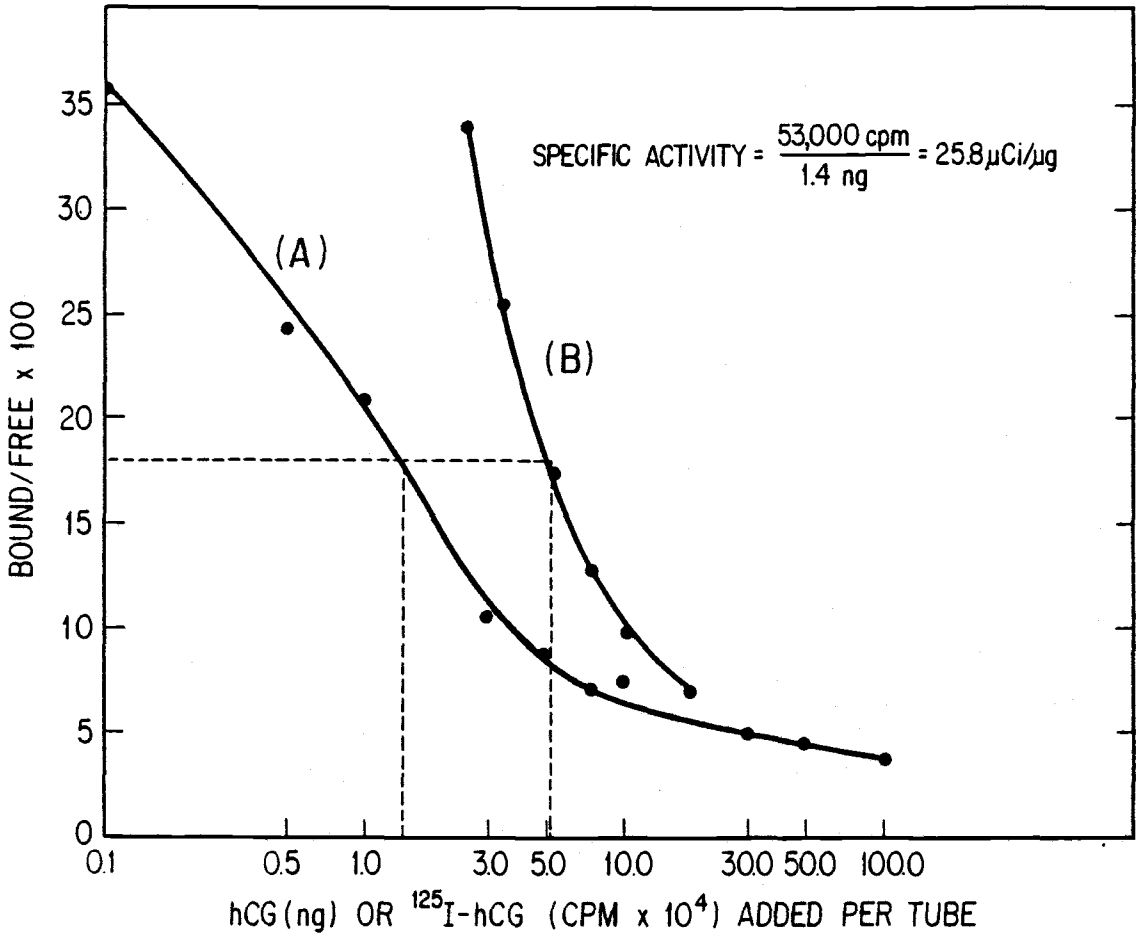

Figure 4. Increasing concentrations of hCG (0.1–100 ng) were added to tubes containing a constant quantity of luteal plasma membranes (50 ug protein/tube) and ^{125}I-hCG (2 x 10^4–20 x 10^4 cpm/tube) were added to tubes containing a constant amount of protein to generate curve B. Specific activity was calculated by dividing the cpm (converted to uCi) obtained at a bound/free ratio of 50% (curve B) by the quantity of hCG (converted to ug) that displaced 50% of the ^{125}I-hCG in curve A.

uCi/ug. These values are comparable to those reported by Diekman et al. (1978a).

Saturation Analysis. The amount of labeled hCG needed to saturate all LH binding sites in 50 ug luteal plasma membrane protein was determined by a procedure similar to that described by Diekman et al. (1978a). A 50 ug aliquot of membrane protein was incubated in triplicate with increasing concentrations of labeled hCG in the presence or absence of a 1000-fold excess of unlabeled hCG (Pregnyl) for 16 hours at 25°C. Bound and free hormone was separated as previously described. Saturation analysis was performed on luteal tissue from three heifers in each group and the characteristics of the saturation curves were similar in all four groups. The curve depicted in Figure 5 represents an average of data from three heifers of the control group slaughtered on day 14. A concentration of approximately 75.0 fmol ^{125}I-hCG(100,000 cpm) per tube saturated the LH receptor in luteal tissue from heifers in all four groups. Nonspecific binding averaged 17.8 $\pm$ 1.0, 31.4 $\pm$ 1.9, 16.1 $\pm$ 2.7, and 35.5 $\pm$ 7.5% for the control and treated groups on days 8 and 14, respectively.

Data from the saturation curves were used to construct Scatchard (1949) plots for the determination of dissociation constants (K_d) and total unoccupied LH binding sites (R_t; pmol/ug protein). Based upon a molecular weight of 46,000 daltons for hCG (Morgan and Canfield, 1971), all calculations in the Scatchard analyses were corrected for molecular weight, maximum bindability and specific activity. The Scatchard plot from three heifers in the control group slaughtered on

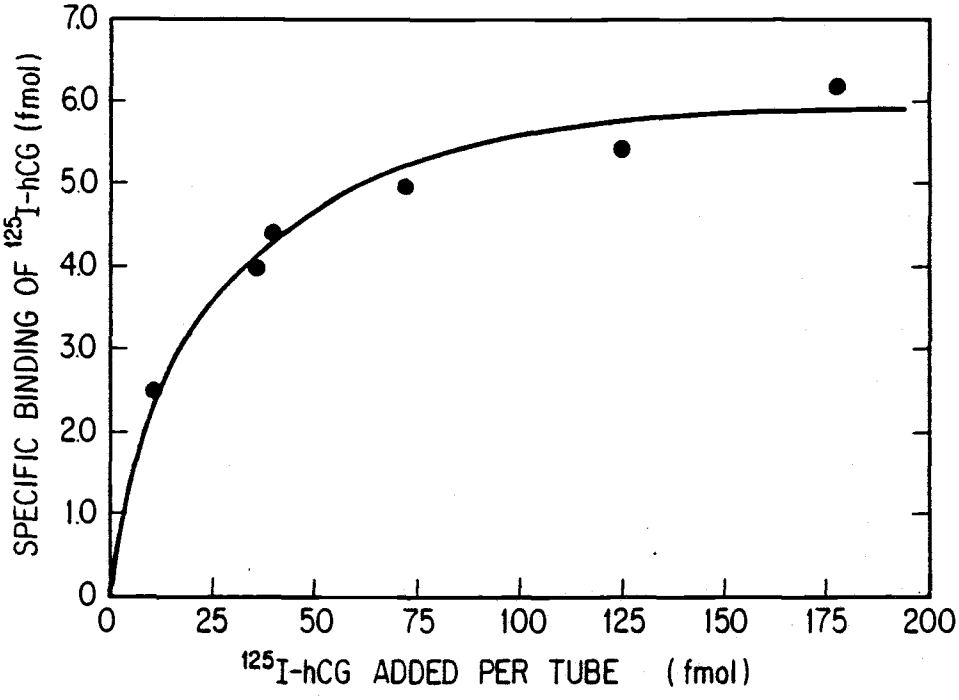

Figure 5. An average of the specifc binding of ^{125}I-hCG to luteal plasma membranes from each of three heifers in the control group slaughtered on day 14. A concentration of approximately 75.0 fmol ^{125}I-hCG (100,000 cpm) per tube saturated the LH receptor using 50 ug protein in all four groups.

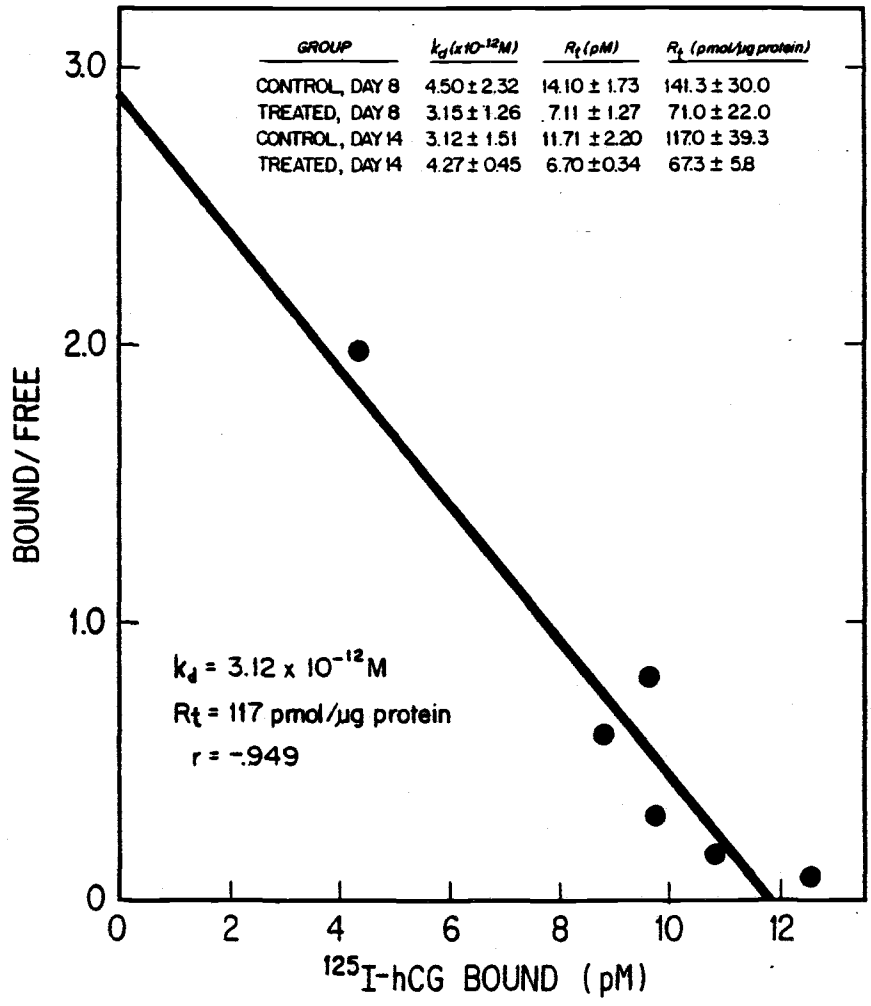

Figure 6. Scatchard analysis was used to estimate the number of LH receptor sites (R_t) and dissociation constants (K_d) from each of three heifers per group. Scatchard plot depicted above is an average of 3 heifers from the control group slaughtered on day 14. Inset lists the K_d and R_t values for all four groups.

day 14 is presented in Figure 6. Depicted in the inset of Figure 6 are the $K_d \pm SE$ and $R_t \pm SE$ values for the three heifers from each group.

Statistical Analyses. Data on the effect of treatment on serum levels of progesterone and LH were analyzed by use of split-split-plot analysis of variance (Steel and Torrie, 1980). Due to heterogeneity of variance, data on serum LH concentrations were subjected to log transformations for analysis but are presented using nontransformed values. Data on the length of the estrous cycle, weight of corpora lutea and the LH receptor levels were subjected to analysis of variance.

RESULTS

Administration of GnRH to heifers in Exp. 1 on day 2 or 10 of the cycle caused an increase in serum levels of LH with peak concentrations occurring at 15 to 30 minutes post-injection (treatment x time interaction, $P < .001$; Figure 7). The pattern of GnRH-induced release of LH, however, differed between days 2 and 10 of the cycle (day x time interaction, $P < .001$). During metestrus (day 2) the release of LH was characterized by a return to basal levels by 150 minutes post-injection but on day 10 levels of LH were still elevated above baseline at 180 minutes after treatment. Serum levels of LH on subsequent days of the cycle after treatment are presented in Table 1. There was no significant difference in basal levels of LH between control or treated animals during the remaining

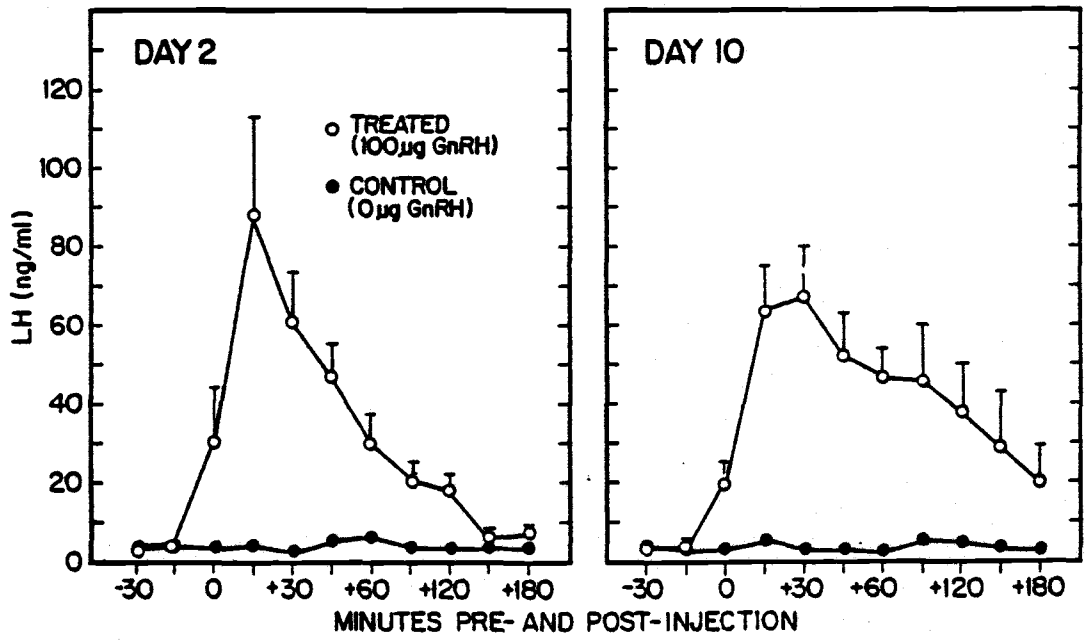

Figure 7. Serum LH concentrations of heifers treated on day 2 or 10 of the cycle. The estimate of the common SE=0.10.

Table 1. Serum levels of LH(ng/ml) on alternate days of the estrous cycle following the 3 hour sampling period.

Day of Treatment[a]	Treatment	Day of estrous cycle after treatment[a] 4	6	8	10	12	14	16	SE[b]
2	0 ug GnRH	3.53	2.08	3.30	3.73	3.83	2.28	2.91	0.31
	100 ug GnRH	3.58	3.25	2.72	3.42	3.61	4.41	2.86	
10	0 ug GnRH	–	–	–	–	4.06	3.50	5.17	0.42
	100 ug GnRH	–	–	–	–	3.71	4.42	5.70	

[a]N=5 heifers/group.
[b]Estimate of the common SE.

days of the cycle studied.

Treatment of heifers with GnRH on day 2 resulted in a reduction ($P < .06$) in serum concentration of progesterone beginning on day 8 of the cycle (Figure 8) compared with levels of progesterone present in the same heifers during the control cycle. Serum levels of progesterone on days 12, 14 and 16 of the cycle were evaluated in heifers treated with GnRH on days 2 and 10 of the cycle (Figure 9). Administration of GnRH on day 10 was as effective as on day 2 of the cycle in causing a subsequent reduction in serum progesterone levels during the midluteal phase of the cycle ($P < .03$). It should be noted, however, that the immediate effect of GnRH when injected on day 10 was to enhance progesterone secretion. Serum levels of progesterone increased during the 3 hour period after injection of GnRH on day 10 of the cycle (time x treatment interaction, $P < .01$; Table 2).

Although treatment of heifers with GnRH on days 2 or 10 reduced serum levels of progesterone during subsequent days of the cycle there was no effect of treatment on the duration of the cycle (Group I, control 20.7 vs treated 20.9 days; Group II, control 20.7 vs treated 21.2 days). The estimate of the common SE for estrous cycle lengths was 0.25 days.

In Exp. 2, treatment of heifers with GnRH on day 2 reduced the weight of corpora lutea at day 8 but not at day 14 of the cycle (control vs treatment, day 8, 4.01 vs 3.38 g; day 14, 4.39 vs 4.79 g, estimate of the common SE = 0.20; treatment x day interaction, $P < .05$). However, these results are attributed to one treated heifer on

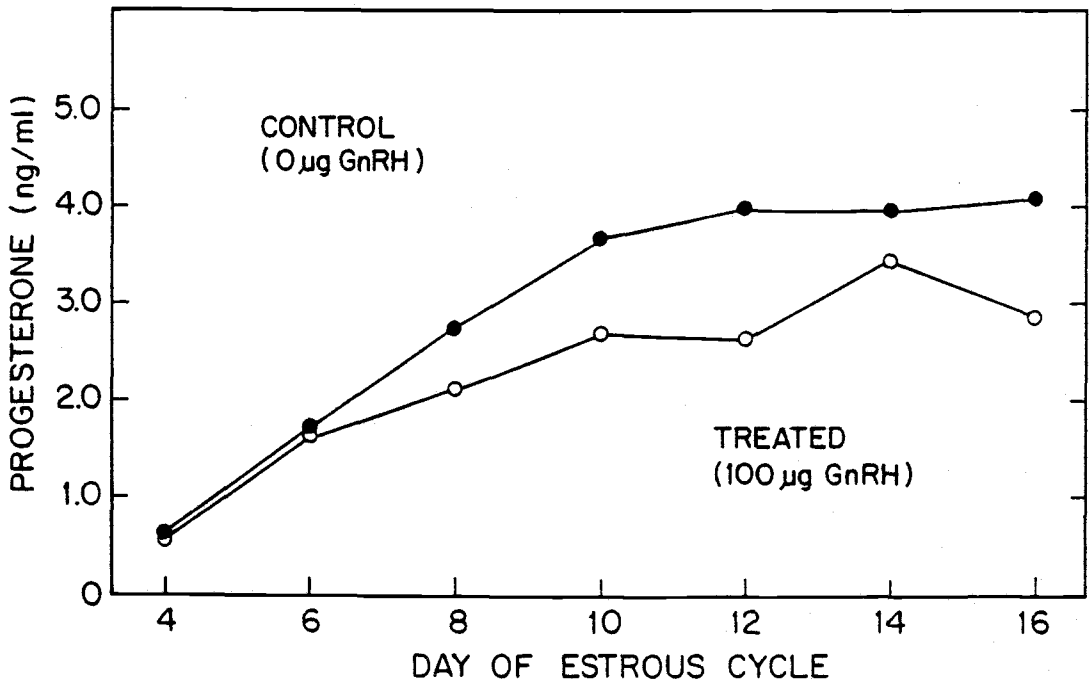

Figure 8. Serum progesterone concentrations after administration of GnRH to heifers on day 2 of the cycle. The estimate of the common SE=0.24.

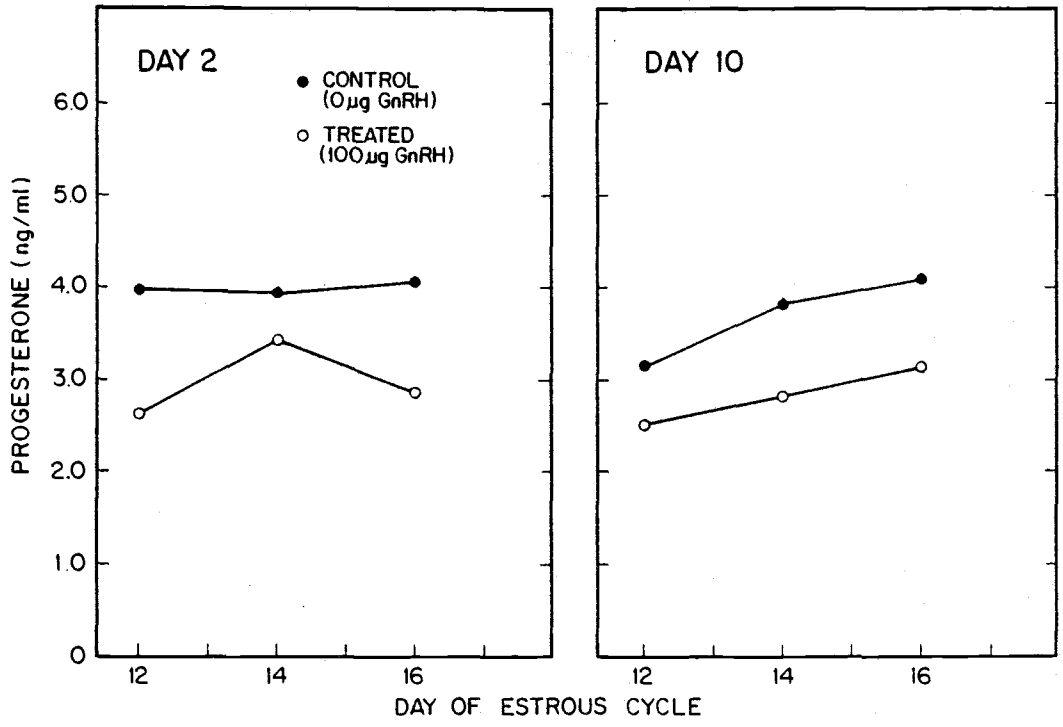

Figure 9. Effect of GnRH on serum progesterone concentrations on days 12, 14 and 16 when administered on day 2 or 10 of the cycle. The estimate of the common SE=0.24.

Table 2. Serum progesterone levels (ng/ml) immediately after treatment with GnRH on day 10 of the cycle.[a]

Treatment	Minutes post-injection								
	0	+15	+30	+45	+60	+90	+120	+150	+180
0 ug GnRH	1.74	1.70	2.20	3.32	3.01	2.56	2.47	3.48	2.41
100 ug GnRH	1.59	2.99	3.64	3.63	2.83	3.23	3.44	2.99	3.31

[a]Estimate of the common SE=0.18. N=5 heifers/group.

day 8 having an extremely small corpus luteum; 1.85 g compared with 3.80 g as an average for the remaining heifers in the same group. If the luteal weight of this heifer was deleted from the analysis there was no effect of GnRH on the weights of the corpora lutea.

Concentrations of unoccupied LH receptors in the luteal plasma membranes were reduced at days 8 and 14 after treatment with GnRH on day 2 ($P < .005$; Figure 10). Furthermore, there was no statistically significant difference between the numbers of luteal LH receptors in treated heifers on days 8 and 14. Similar binding affinities of the receptors of heifers of all four groups (see inset of Figure 6) indicate that the integrity of the receptor was not affected by treatment with GnRH.

DISCUSSION

Results of Experiment 1 indicate that administration of GnRH to heifers during the early or midluteal phase of the estrous cycle interferes with the secretion of progesterone without affecting the duration of the cycle. In part, these data confirm those of Ford and Stormshak (1978) and more recently those of Kansas State University investigators (M.C. Lucy and J.S. Stevenson, unpublished data) who found that iv or im injection of 100 ug GnRH into beef heifers during metestrus or $PGF_{2\alpha}$-treated dairy cows on the day of anticipated estrus, respectively, resulted in subsequent suppression of progesterone secretion in nonpregnant and pregnant animals. In contrast, the ability of GnRH to interfere with luteal function when

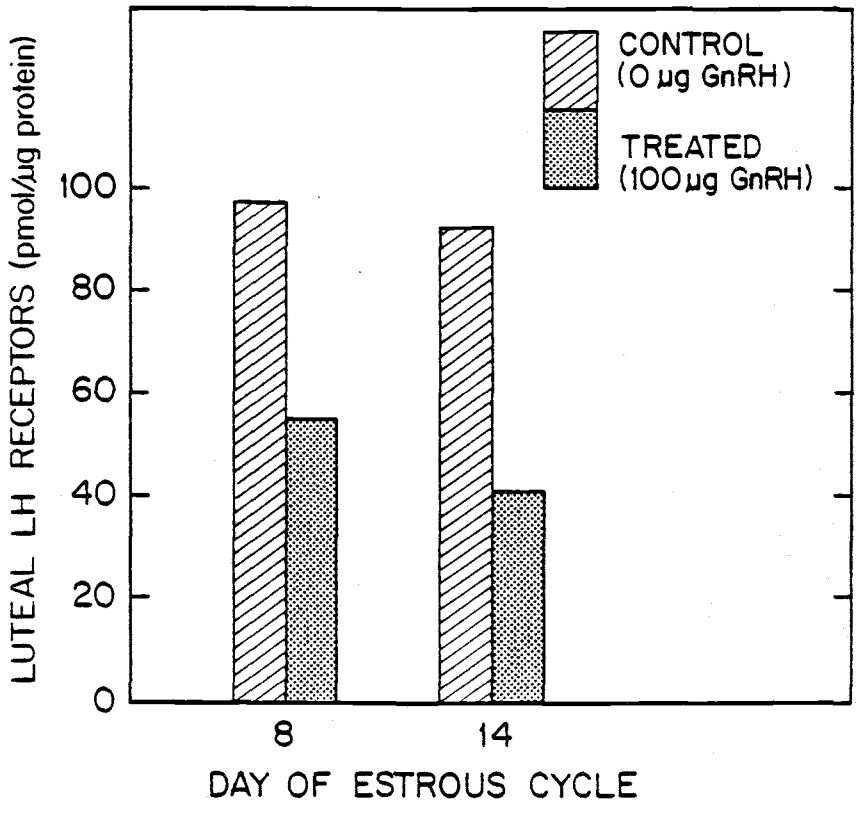

Figure 10. Effect of GnRH on the luteal concentration of unoccupied LH receptors on day 8 and 14. The estimate of the common SE=3.22.

injected on day 10 of the cycle was not anticipated because reports in the literature generally suggested it to be without effect on progesterone secretion or cycle length when given as single (100-200 ug) or daily repetitive injections (400 ug/day) during the mid or late stages of the cycle (Britt, 1975; Seguin et al., 1977; Milvae et al., 1984). Failure of these investigators to detect an effect of the hormone at these times may have been due to the intramuscular or subcutaneous route of administration as opposed to the intravenous route employed in the present study. An exception to the above cited studies is the report by Kittok et al. (1973) who found that three consecutive intravenous injections of GnRH into heifers during the midluteal phase of the cycle caused a transient increase in serum concentrations of progesterone. From this standpoint, the data of the present experiment indicate a similar response because serum progesterone levels in GnRH-treated heifers on day 10 did increase significantly during the 3 hour period immediately after injection of the hormone. The acute increase in serum progesterone after treatment with GnRH is consistent with the ability of LH to stimulate bovine luteal progesterone synthesis in vitro (Mason et al., 1962). It is also noteworthy that repeated subcutaneous injections of a GnRH agonist analog on days 9 to 12 of the cycle has recently been reported to markedly increase serum levels of progesterone and prolong the length of the cycle (Milvae et al., 1984).

In the present study, as in others involving the administration of GnRH to heifers during various stages of the cycle (Seguin et al., 1977; Milvae et al., 1984) injection of this hormone on days 2 and 10

of the cycle caused a significant release of LH. Stage of the cycle did not affect the quantity of LH released which is consistent with the data of Hooley et al. (1974) who reported that the quantity of LH released in response to GnRH administered to ewes on days 2 to 4 did not differ from that released on days 5 to 12 of the cycle. Stage of the cycle did, however, affect the pattern of LH release in the present study as evidenced by the fact that serum LH levels did not return to basal levels by the end of the 3 hour sampling period on day 10. This altered pattern of LH secretion in response to GnRH on day 10 may have been due to exposure of the pituitary to greater quantities of progesterone during this stage of the cycle. Kittok et al. (1973) found the LH released in response to three consecutive injections of 100 ug GnRH into cows during the midluteal phase of the cycle to be markedly less than in cows with follicular cysts and lacking corpora lutea. In addition, the pattern of LH release, at least after the first injection of GnRH, was characterized by the absence of a distinct peak with elevated serum levels of hormone present by the time of the second injection of GnRH 2 hours later. In the present study, secretion of LH apparently returned to normal after injection of GnRH during metestrus or the luteal phase because serum levels of LH from days 8 to 16 of the treatment cycle did not differ from those present during the control cycle.

Based upon the data of Experiment 2, it appears that the mechanism by which exogenous GnRH caused suppression of progesterone synthesis and(or) secretion involved a reduction in the number of unoccupied LH receptors in the luteal cell membrane. Luteinizing

hormone is luteotropic in the cow (Donaldson et al., 1965) by virtue of the fact that binding to its receptor in the target cell membrane stimulates adenylate cyclase resulting in the production of the second messenger cyclic adenosine-3',5'-monophosphate. It has been demonstrated that injection of hCG into rats, a hormone that binds with high affinity to the LH receptor (Lee and Ryan, 1973), causes down regulation of the luteal cell receptor with a concomitant reduction in response of adenylate cyclase to LH that persists for several days (Conti et al., 1976, 1977). In these latter studies the reduction in luteal LH receptors was detectable by 6 hours post-injection. Thus it is conceivable that the LH released in response to GnRH on day 2 of the cycle caused a down regulation of the LH receptor that was detectable on days 8 and 14 of the cycle. It is possible that a reduction in the number of receptors below a critical level could result in a decreased response to the gonadotropin reflected by a decreased synthesis and(or) secretion of progesterone. If this indeed represents the mode action of exogenous GnRH in the cow there are certain facets of the data that are perplexing. For example, a GnRH-induced reduction in serum progesterone levels was not detected prior to day 8 after treatment. It is not known whether this lag period to response was caused by a corresponding delay in the onset of down regulation of the LH receptor or to ability of the cells of the developing corpus luteum to respond to systemic LH even though receptor numbers may have been reduced. Along a related line, if GnRH-induced release of LH caused down regulation of the luteal cell receptor then the sensitivity of

the corpus luteum to this phenomenon would have to be greater on day 10 than on day 2 of the cycle. Injection of GnRH on day 10 of the cycle caused a reduction in serum progesterone without a delay period as occurred after treatment of heifers on day 2 of the cycle. The possibility that GnRH-induced release of LH on day 10 caused comparatively rapid down regulation of luteal LH receptors is supported by the data of Suter et al. (1980). These investigators demonstrated that intravenous injection of 1 mg of ovine LH into ewes on day 10 of the cycle caused a down regulation of the unoccupied receptors that was significant by 12 hours after treatment.

It is possible that GnRH acts directly on the bovine ovary to result in attenuation of serum levels of progesterone but such a mechanism is not supported by existing data. Not only are receptors for this decapeptide absent in bovine ovarian tissue (Brown and Reeves, 1983) but it has also been demonstrated that this hormone does not compete with LH for binding to the LH receptor in target tissues (Clayton et al., 1979; Harwood et al., 1980a). The mechanism by which GnRH interferes with luteal steroidogenesis in the bovine and human (Koyama et al., 1978; Casper and Yen, 1979) appear to be similar because luteal cells of the human, like those of the cow, are also devoid of receptors for GnRH (Clayton and Huhtaniemi, 1982). Thus, in conclusion, the results of the present study, as supported by those of investigators cited above, suggest that GnRH acts indirectly in the bovine to suppress luteal function.

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