

Gonadotropin-Releasing Hormone-Induced Secretion of Luteinizing Hormone in Postpartum Beef Heifers Maintained on Two Planes of Nutrition Before and After Breeding¹

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ABSTRACT: An experiment was conducted to examine the effect of pre- and postbreeding nutrition on GnRH-induced LH release in beef heifers on d 3 and 14 of the subsequent postpartum period. Treatment groups consisted of heifers fed high (H; n = 12) and low (L; n = 12) planes of nutrition for 204 d before breeding. Each group was further subdivided to receive either high or low planes of nutrition after breeding in a 2 × 2 factorial arrangement of treatments (H-H, H-L, L-H, and L-L). On d 3 and 14 postpartum, heifers were injected with 100 µg of GnRH (i.v.), and blood was collected via jugular venipuncture at 15-min intervals for 2.5 h and at 30-min intervals for an additional 2.5 h for LH analysis. Heifers fed a high level of nutrition through-

out gestation (H-H and L-H) had a greater ($P < .05$) mean cumulative serum concentration of LH (ng LH·mL⁻¹·min) in response to GnRH on d 3 than did those fed a lower level of nutrition. On d 14, mean cumulative serum concentration of LH in the H-H group was greater ($P < .05$) than that of the other three groups. These data indicate that postbreeding nutritional status significantly influenced pituitary responsiveness to GnRH on d 3 and that response to GnRH on d 14 was greatly enhanced by maintaining heifers on a high plane of nutrition both before and after breeding. In addition, the negative effect of low prebreeding nutrition on GnRH-induced LH secretion on d 14 was not overcome by increasing the level of nutrition after breeding.

Key Words: Nutrition, GnRH, LH, Heifers, Postpartum, Beef Cattle

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Introduction

Factors such as nutrition (Randel, 1990), suckling (Williams, 1990), and season of calving (Hansen and Hauser, 1983; King and Macleod, 1984) influence the duration of postpartum anestrus in beef cows. Restricting total energy (Wiltbank et al., 1962) or crude protein (Sasser et al., 1988) before calving reduced

the occurrence of estrus, prolonged the interval from calving to first estrus, and reduced conception and(or) pregnancy rate in beef cows. The mechanism by which undernutrition impairs postpartum reproductive function likely involves the regulation of LH secretion. Feeding beef cows a diet deficient in crude protein before parturition reduced pituitary content of gonadotropin and responsiveness to exogenous GnRH (Nolan et al., 1988). Similarly, reducing dietary energy during gestation decreased pituitary response to estradiol (Echternkamp et al., 1982) and GnRH (Killen et al., 1989) in postpartum heifers.

Previous research investigating the effect of diet on reproductive performance and gonadotropin secretion in postpartum cows concentrated on feeding various levels of nutrition before and(or) after parturition. There is a paucity of information concerning the influence of nutritional status before pregnancy on reproductive function after calving. Therefore, the objective of this experiment was to determine the effect of pre- and postbreeding nutrition on GnRH-induced LH release in beef heifers on d 3 and 14 postpartum.

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Materials and Methods

Animals and Experimental Diets. Hereford \times Angus heifers of comparable age ($\bar{x} \pm \text{SE}$, 160 ± 1.79 d) and weight (163 ± 1.52 kg) were assigned at weaning (October 10) to one of two treatment groups. Treatment regimens were begun 4 wk after weaning and consisted of heifers fed high (**H**; $n = 12$) or low (**L**; $n = 12$) planes of nutrition before breeding. Each group was further subdivided after breeding (May 15 to August 1) to receive either high or low planes of nutrition in a 2×2 factorial arrangement of treatments (H-H, H-L, L-H, and L-L). Throughout the experiment, all heifers had ad libitum access to rake-bunched hay and meadow aftermath daily or rangeland pastures with supplement provided at communal feeders that provided ample room for all heifers in a group to feed simultaneously. From weaning to parturition, heifers were weighed and scored for body condition (**BCS**; scoring 1 to 9 with 1 = thin, 9 = obese) every 28 d, and supplement levels were adjusted as necessary to attain desired target weights at the 1st- and 2nd-yr breeding periods. Initial H and L target weights represented 65 and 60% of eventual mature weight of the cow, whereas L-L, H-L, L-H, and H-H treatment groups were expected to range from 75 (L-L) to 90% (H-H) of eventual mature weight after calving. In this herd, mature BW is approximately 454 kg at a BCS of 5. Body weights were recorded at the start of the experiment (November 7), breeding (May 13), midwinter precalving (January 21), and within 24 h after calving (February 19 to March 16).

At weaning, heifers were gradually acclimated to protein supplementation by feeding increasing concentrations of barley and biuret for 30 d, until supplement levels reached 1.35 kg of barley and .05 kg of biuret per heifer, at which time heifers were separated into H and L treatment groups. Prebreeding diets consisted of 1.40 (L) and 2.25 kg (H) of total barley and biuret supplement·heifer⁻¹·d⁻¹ in addition to rake-bunched hay and meadow aftermath. Heifers were fed to achieve target weights of 272 to 295 kg (L) and 295 to 319 kg (H) by the time of breeding.

At breeding, heifers were placed with a larger group of cattle and exposed to Hereford \times Angus bulls (one bull per 21 heifers) on 81-ha ranges from May 15 to August 1. During this period, all heifers were on native range and received no additional supplementation. Although the heifers were managed as one group throughout the breeding season the difference in BCS between H and L heifers was maintained. Immediately after the breeding season, the prebreeding treatment groups were further divided into high and low postbreeding groups ($n = 6$ per group), and heifers on a low nutritional plane (L-L and H-L) continued to receive no supplementation, whereas heifers on a high nutritional plane (H-H and L-H) received .9 kg of barley and .04 kg of biuret daily. After parturition, daily supplement level in the H-H

and L-H groups was increased to 1.35 kg of barley and .05 kg of biuret to compensate for lactational demands. Level of supplementation in H-H heifers was adjusted as needed to keep condition scores under 7 and to attain a target weight of 498 kg by the 2nd-yr breeding. We anticipated that the L-L heifers receiving no supplement would achieve a weight of 340 kg over the same time period and that the weights of L-H and H-L heifers would fall between those of the H-H and L-L groups.

Cows and calves were brought in from pasture within 24 h after parturition and on d 13 after calving. On d 3 and 14 postpartum, dams were separated from their calves and restrained in squeeze chutes. On both days, all cows were injected (i.v.) with 100 μg of GnRH (Cystorelin[®], Sanofi Animal Health, Overland Park, KS), and blood samples (10 mL) were collected via jugular venipuncture at 15-min intervals beginning 30 min before and for 2.5 h after GnRH for LH analysis. At 2.5 h after GnRH, samples were collected at 30-min intervals for an additional 2.5 h. After the sampling period, cow and calf were reunited and returned to pasture.

Radioimmunoassay. Blood samples were allowed to clot at room temperature and then stored for 24 h at 4°C. Sera were separated by centrifugation ($500 \times g$) for 15 min at room temperature and stored at -20°C until they were assayed for LH.

Serum LH was quantified with a RIA following the method of McCarthy and Swanson (1976) with some modification. Purified bLH (USDA-bLH-B-5, AFP 5500) was iodinated by reacting the gonadotropin (5 $\mu\text{g}/25 \mu\text{L}$ of double-distilled H₂O) with [¹²⁵I]sodium iodide (1 mCi; Amersham) and chloramine-T (10 μL ; .5 mg/mL) for 1 min followed by the addition of sodium metabisulfite (10 μL ; 1 mg/mL) to terminate the reaction. Radiolabeled LH was separated from free ¹²⁵I by adding the mixture to an anion exchange column (3-mL syringe with 2.54 cm of Tygon tubing attached to the hub) containing AG 2 \times 8 resin (chloride form, 100 to 200 mesh; Bio-Rad, Richmond, CA) that had been sequentially rinsed with .5 M sodium phosphate buffer (**PB**; pH 7.6, 4 to 5 mL), .05 M PB-5% BSA (pH 7.5, 1 mL), and .05 M PB (pH 7.5, 4 to 5 mL) before use. After depositing the reaction mixture on the resin bed, the column was rinsed twice with .05 M PB (1 mL) and the eluate was collected in a culture tube (12 mm \times 75 mm; borosilicate glass) containing .01 M PBS-1% gelatin (pH 7.2, 1 mL; Knox gelatin). The tube containing the radiolabeled LH was capped, stored undiluted at 4°C, and used without further purification.

The LH assay was validated using rabbit anti-bovine LH (PKC-242; 1:80,000) and sheep anti-rabbit gamma globulin (PKC-pool C; 1:60) as the primary and secondary antibodies, respectively. Recovery of LH standard (.125 to 2.0 ng/tube) added to 200 μL of calf serum averaged $108 \pm 3.9\%$, and standard dilutions of serum (50 to 300 μL) from ovariectomized

heifers were parallel to the standard curve. Assay sensitivity was .125 ng/tube ($P < .01$), and sample volume assayed was 200 μL per tube except after GnRH injection (50 to 100 μL of serum/tube). Intra- and interassay CV were 8.6 and 8.2%, respectively ($n = 6$ assays). Cross-reactivity of the primary anti-serum with bFSH and bGH was .3 and 2.9%, respectively. Samples were assayed in duplicate and concentrations of LH are expressed as nanogram equivalents of NIH-bLH-B10/milliliter of serum.

Statistical Analysis. Differences in BW and BCS among the treatment groups were analyzed with ANOVA (Snedecor and Cochran, 1980), and differences among individual group means were tested for significance with Fisher's Protected Least Significant Difference (FPLSD) test following a significant F -test. Basal LH secretion was determined for individual cows calculating the mean LH concentration from samples collected -30 min, -15 min, and immediately before injection of GnRH (0 min). After subtraction of basal LH concentration from each LH value, area under the LH response curve was determined for each cow on both days by integration (i.e., summation of the area of trapezoids). Because there was extreme animal-to-animal variation in LH response during the final 90 min of the sampling period, only LH data from 0 to 210 min were used. The resultant areas (nanograms of LH-milliliter⁻¹-min) for each day (d 3 and 14 postpartum) were subjected to ANOVA in which pre- and postbreeding diet were the factorial variables.

Results and Discussion

Mean BW did not differ among H and L treatment groups at the start of the experiment (184 vs 183 kg, SEM = 5). However, by the beginning of the breeding period the desired target BW had been attained and heifers receiving a high plane of nutrition averaged 24 kg heavier ($P < .05$) than those receiving a diet low in energy (298 vs 274 kg, SEM = 6). Prebreeding level of nutrition also affected body condition at breeding. Heifers in the H treatment group had greater ($P < .05$) BCS than those in the L treatment group (5.5 vs 5.1, SEM = .1). Differences in mean BW after breeding and the subsequent division into postbreeding H and L nutrition groups are shown in Figure 1. Heifers in the H-H group were heavier during the midwinter of their pregnancy and at calving (422 ± 11 and 398 ± 13 kg), respectively, than those in the H-L (386 ± 11 , $P < .05$ and 339 ± 13 kg, $P < .01$) and L-L (372 ± 11 and 335 ± 13 kg, $P < .001$) groups. Body condition scores 2 mo after the end of the breeding period (October 1) did not differ among the four treatment groups (mean = $5.7 \pm .1$). However, heifers in the H-H group tended ($P = .09$) to have greater BCS ($5 \pm .3$) than those in the H-L ($4 \pm .3$) and L-L ($4.2 \pm .3$) groups after calving (May 5). Furthermore, heifers receiving a high plane of nutrition postbreeding (H-H and L-H)

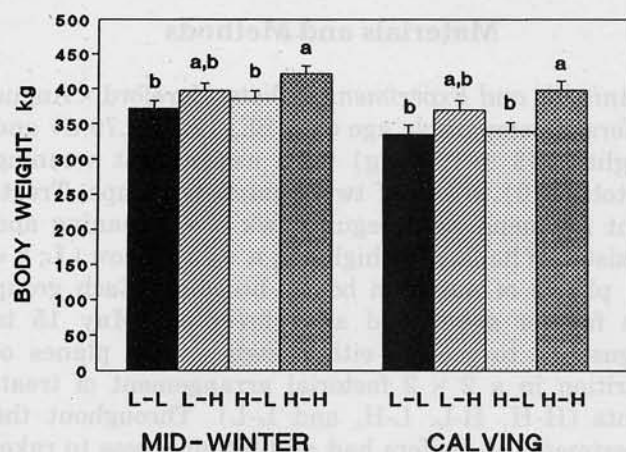


Figure 1. Mean BW at midwinter precalving and within 24 h after calving in heifers maintained on high (H) or low (L) planes of nutrition before and after breeding. Means (a,b) within a weighing date without a common superscript differ ($P < .05$).

were heavier at midwinter (409 vs 379 kg, SEM = 8; $P < .05$) and calving (384 vs 337 kg, SEM = 9; $P < .01$) and averaged greater BCS late spring after calving (4.8 vs 4.1, SEM = .2; $P < .05$) than those receiving a low plane of nutrition after breeding (H-L and L-L). Overall, BW increased from weaning to late gestation and decreased at calving in all heifers. However, BW of heifers in the H-L and L-L groups were consistently lower than those in the H-H and L-H groups.

Serum LH profiles for the four treatment groups on d 3 and 14 postpartum are depicted in Figures 2 and 3, respectively. All cows, on both days, responded to GnRH injection with increased release of LH that approached or returned to basal concentrations by the end of the 5.5-h sampling period. Basal concentrations of LH did not differ significantly among treatment groups or between sampling days and averaged $3.6 \pm .1$ ng/mL. Others have reported no effect of pre- or postpartum nutrition on basal LH secretion in intact postpartum cows (Echternkamp et al., 1982; Nolan et al., 1988; Killen et al., 1989). Postbreeding, but not prebreeding, level of nutrition significantly altered pituitary response to GnRH on d 3 postpartum. Heifers maintained on a high plane of nutrition after breeding (H-H and L-H) had greater ($P < .05$) mean cumulative serum concentrations of LH than those fed a lower (H-L and L-L) level of nutrition (1,956 vs 1,053 ng of LH-mL⁻¹-min; SEM = 138).

Results of this experiment indicate that both pre- and postbreeding level of nutrition influence pituitary response to GnRH in first-calf cows during the early stages of the postpartum interval. Feeding heifers a low plane of nutrition postbreeding reduced the amount of LH released in response to GnRH on d 3 postpartum. Similarly, release of LH in response to

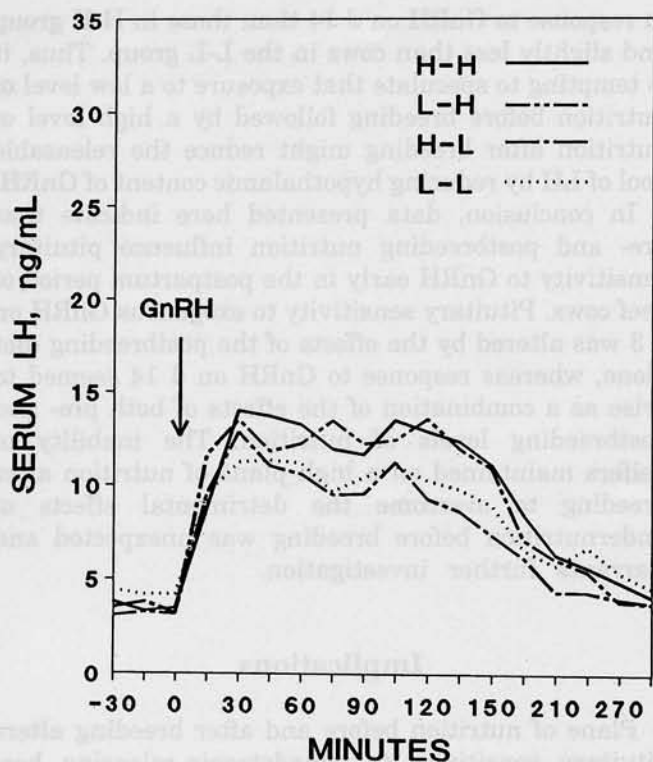


Figure 2. Pattern of luteinizing hormone (LH) release after injection of gonadotropin-releasing hormone (GnRH; 100 μ g, i.v.) on d 3 postpartum in heifers maintained on high (H) or low (L) planes of nutrition before and after breeding.

exogenous estradiol on d 14 and 28 postpartum was lower in heifers maintained on a low plane of nutrition beginning the last trimester of pregnancy (Echternkamp et al., 1982), and the quantity of LH released after injection of GnRH between d 8 and 21 after calving was 50% lower in heifers nutritionally restricted during the final two trimesters of gestation (Killen et al., 1989). Collectively, these data demonstrate that feeding low levels of nutrition after breeding is sufficient to suppress the ability of the pituitary to respond to exogenous hormonal stimuli during the early stages of the postpartum period.

In contrast to the significant effect of postbreeding diet on GnRH-induced LH release on d 3, response to GnRH on d 14 (Figure 3) could not be attributed to the action of either pre- or postbreeding planes of nutrition alone. Most probably the observed effect resulted from a combination of the two factors as suggested by the significant prebreeding \times postbreeding diet interaction from the ANOVA. Maintaining heifers on a high plane of nutrition both before and after breeding increased ($P < .05$) the cumulative serum concentration of LH (nanograms of LH-milliliter⁻¹-minutes) after GnRH on d 14 (H-H, 4,190) compared with heifers in the H-L (1,526), L-H (1,904), and L-L (2,176) groups (pooled SEM = 676).

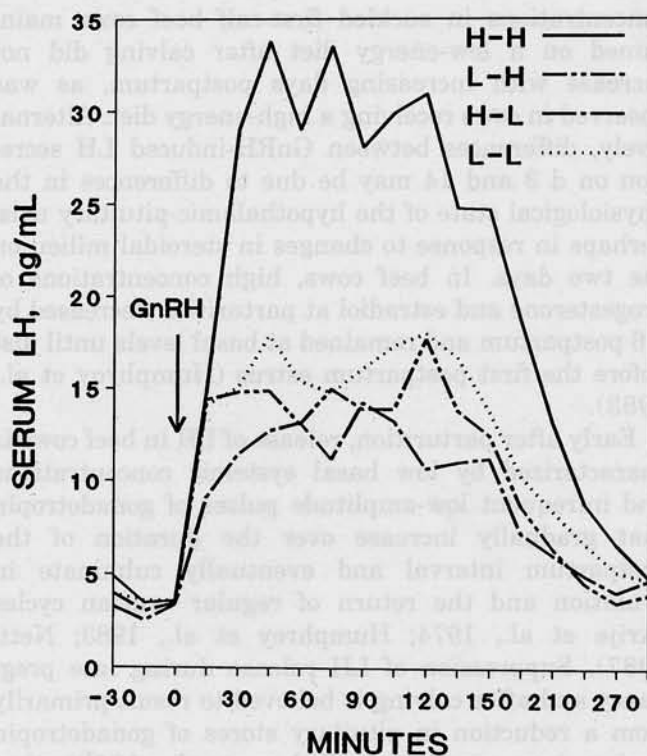


Figure 3. Pattern of luteinizing hormone (LH) release after injection of gonadotropin-releasing hormone (GnRH; 100 μ g, i.v.) on d 14 postpartum in heifers maintained on high (H) or low (L) planes of nutrition before and after breeding.

In contrast, feeding a low plane of nutrition either before or after breeding reduced pituitary response to GnRH regardless of the level of nutrition that preceded or followed it. These data indicate that on d 14 postpartum, the negative effect of feeding a low plane of nutrition before breeding could not be overcome by feeding a higher level of nutrition after breeding and that any benefit from feeding a high level of nutrition before breeding was subsequently suppressed by feeding a low plane of nutrition after breeding.

The differential effects of pre- and postbreeding nutrition on LH release in response to GnRH between d 3 and 14 are not readily explainable but may be related to the metabolic demands of lactation. Reduced response to GnRH in cows in the L-L and H-L groups on d 14 was not unexpected because these cows received no dietary compensation for lactation and had the lowest BCS several months after calving. In contrast, reduced pituitary response in the L-H group was somewhat surprising because these cows received additional supplement to meet lactational demands and mean BCS after calving did not differ significantly from that of the H-H group. This may indicate that these cows were not nutritionally stressed after calving. Hall et al. (1991) reported that systemic LH

concentrations in suckled first-calf beef cows maintained on a low-energy diet after calving did not increase with increasing days postpartum, as was observed in cows receiving a high-energy diet. Alternatively, differences between GnRH-induced LH secretion on d 3 and 14 may be due to differences in the physiological state of the hypothalamic-pituitary axis, perhaps in response to changes in steroidal milieu on the two days. In beef cows, high concentrations of progesterone and estradiol at parturition decreased by d 6 postpartum and remained at basal levels until just before the first postpartum estrus (Humphrey et al., 1983).

Early after parturition, release of LH in beef cows is characterized by low basal systemic concentrations and infrequent low-amplitude pulses of gonadotropin that gradually increase over the duration of the postpartum interval and eventually culminate in ovulation and the return of regular ovarian cycles (Arije et al., 1974; Humphrey et al., 1983; Nett, 1987). Suppression of LH release during late pregnancy and after calving is believed to result primarily from a reduction in pituitary stores of gonadotropin during late pregnancy (Rahe et al., 1988) that persists for several weeks after parturition (Moss et al., 1985; Nett et al., 1988), but it may also involve reduced secretion of GnRH from the hypothalamus (Allrich et al., 1985; Leshin et al., 1992) or reduced pituitary sensitivity to the decapeptide (Nett et al., 1988). Increased systemic concentrations of gonadal steroids during the latter stages of pregnancy and first week postpartum (Arije et al., 1974; Humphrey et al., 1983) are believed to inhibit LH synthesis, thus depleting pituitary content of the gonadotropin (Nett, 1987).

The precise mechanism whereby nutrition alters reproductive function in postpartum cows is not known. However, increasing evidence indicates that the effects of undernutrition may be mediated at the level of the hypothalamic-pituitary axis to suppress LH secretion (Schillo, 1992), and data from the present study are consistent with this hypothesis. Reduced pituitary sensitivity to exogenous estradiol or GnRH after calving in nutrient-restricted heifers and cows seems to result from a decrease in the releasable pool of LH in the anterior pituitary (Echternkamp et al., 1982; Nolan et al., 1988; Killen et al., 1989) but not from a reduction in the number of receptors for GnRH (Nolan et al., 1988). Restricted dietary energy also negatively influenced the content of GnRH in the preoptic area of beef cows (Connor et al., 1990). Cows receiving a low-energy diet prepartum followed by a high-energy diet postpartum had less GnRH in the preoptic area on d 30 postpartum than did cows maintained on a low-energy diet pre- and postpartum or maintenance energy diet prepartum followed by high-energy diet postpartum. In the present study, cows in the L-H group released significantly less LH

in response to GnRH on d 14 than those in H-H group and slightly less than cows in the L-L group. Thus, it is tempting to speculate that exposure to a low level of nutrition before breeding followed by a high level of nutrition after breeding might reduce the releasable pool of LH by reducing hypothalamic content of GnRH.

In conclusion, data presented here indicate that pre- and postbreeding nutrition influence pituitary sensitivity to GnRH early in the postpartum period of beef cows. Pituitary sensitivity to exogenous GnRH on d 3 was altered by the effects of the postbreeding diet alone, whereas response to GnRH on d 14 seemed to arise as a combination of the effects of both pre- and postbreeding levels of nutrition. The inability of heifers maintained on a high plane of nutrition after breeding to overcome the detrimental effects of undernutrition before breeding was unexpected and warrants further investigation.

Implications

Plane of nutrition before and after breeding alters pituitary sensitivity to gonadotropin-releasing hormone during the early stages of the postpartum interval. Maintaining heifers on a low plane of nutrition after breeding reduced pituitary response to exogenous gonadotropin-releasing hormone on d 3 postpartum. The negative effects of feeding a low plane of nutrition before breeding on gonadotropin-releasing hormone-induced luteinizing hormone release on d 14 postpartum were not overcome by increasing the level of nutrition after breeding. This indicates that prebreeding level of nutrition can influence hypothalamic-pituitary function in first-calf beef cows.

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had immunological and biological properties similar to those of tissue inhibitor of metalloproteinases-1 (TIMP-1). In summary, luteinizing hormone-releasing hormone (LHRH) which may be involved in regulating pituitary gonadotropin secretion (post-hypophysial) preceding ovulation and in regulating cellular remodeling during the luteal phase.

Proteinase Inhibitors, Metalloproteinases, Proteinase

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not been thoroughly investigated. It is likely that ovarian proteinase have a role in the endocrine, paracrine, and autocrine regulation of important ovarian processes including gonadotropin binding, granulosa cell proliferation, follicular rupture, angiogenesis, and luteinization (Tealiff, 1988).

The experiments described in this paper are the result of our interest in identifying proteinase that have an important role in cell signaling and tissue remodeling during the postovulatory period and also during the luteal phase. The specific objectives were: (1) to characterize the pattern of protein secretion by granulosa follicles before and after the postovulatory gonadotropin surge and by corpus luteum whether after estrus (d 0 = estrus), (2) to determine whether luteolysis is increased during the follicular phase following hCG treatment, and (3) to determine whether the M_r 30,000 protein has immunological and biological properties similar to a tissue inhibitor of metalloproteinases-1 (TIMP-1).

Introduction

Differences in the responsiveness of follicles within the same ovary to gonadotropins indicates that intra-ovarian regulation may have an important role in regulating ovarian function (Abado and Roban, 1992). The postovulatory gonadotropin surge affects granulosa cells, however, the effect on gene expression and subsequent protein synthesis and secretion have

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