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# GONADOTROPIN RELEASING HORMONE-INDUCED SECRETION OF LUTEINIZING HORMONE DURING THE MILK-EJECTION REFLEX IN THE POSTPARTUM BEEF COW<sup>1,2</sup>

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## ABSTRACT

A study was conducted to determine the effect of the milk-ejection reflex on exogenous gonadotropin releasing hormone (GnRH)-induced release of luteinizing hormone (LH) after short-term calf removal. Twenty-four postpartum multiparous beef cows were assigned randomly to groups arranged in a 2<sup>3</sup> factorial arrangement. Factors consisted of two levels of suckling [suckled (S) or nonsuckled (NS)], treatment with GnRH [saline (C) or 200 µg GnRH] and days postpartum (d 1 and 14). Dams were isolated from their calves for 4 h on d 1 and 14 postpartum. At the end of 4 h dams were reunited with their calves in S+C and S+GnRH groups, while dams of calves in NS+C and NS+GnRH groups remained separated an additional 2 h. Cows were injected iv with saline or GnRH following the 4-h isolation period, 5 min after calves had begun suckling or nuzzling the udder. Sera from jugular blood samples collected 15 min prior to the end of the 4-h isolation period, immediately prior to injection (0 h) and at 15 min intervals thereafter for 120 min were analyzed for LH. Serum concentrations of LH in control cows did not differ due to suckling or stage of the postpartum period and averaged  $2.3 \pm .1$  ng/ml. Pituitary response to GnRH was determined by computing the rate of LH release. Rate of LH release ( $\text{ng LH} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ ) in response to GnRH on d 14 was greater ( $P < .001$ ) than on d 1 in both suckled and nonsuckled groups (S+GnRH,  $37.1 \pm 3.9$  vs  $18.3 \pm 5.0$ ; NS+GnRH,  $34.7 \pm 5.9$  vs  $14.5 \pm 1.1$ ). However, GnRH-induced release of LH did not differ between suckled and nonsuckled cows on either d 1 or 14 postpartum. These data indicate that response of the bovine pituitary to GnRH during the postpartum period is not influenced by the act of suckling but is enhanced with time after parturition. (Key Words: Cows, Suckling, LH, Postpartum Interval, Gonadotropin Releasing Hormone.)

## Introduction

Length of the postpartum anestrus period has been recognized as the most critical variable affecting maintenance of an optimal calving in-

terval. Suckling intensity of the calf (Wettemann et al., 1978; Randel, 1981) has been demonstrated to be a major factor contributing to the length of this reproductive stage of the cow. Although the precise manner by which suckling prolongs the postpartum period is not clearly understood, some evidence suggests that ovarian inactivity may be due to reduced secretion of gonadotropins (Radford et al., 1978).

Secretion of luteinizing hormone (LH) in the postpartum cow appears to be dictated in part by frequency of suckling. Once-daily suckling as opposed to ad libitum suckling resulted in a shorter postpartum anestrus interval (Randel, 1981; Reeves and Gaskins, 1981) and an increased pituitary response to exogenous gonadotropin releasing hormone (Troxel et al., 1980). More drastic reductions in suckling frequency, such as 48-h calf removal (Smith et al., 1977) or early weaning (Bellows et al., 1974; Carruthers et al., 1980; Walters et al., 1982a) also

<sup>1</sup> Technical paper no. 7994, Oregon Agr. Exp. Sta.

<sup>2</sup> The authors thank Dr. Kenneth Rowe, Dept. of Statist., for assistance in the statistical analyses of the data and Charles Ballard and Steven Christensen, Union Exp. Sta., for their help with data collection. We also acknowledge Dr. Lloyd Swanson, Dr. Jack Rose and Lynn Rodger for their assistance with the radioimmunoassays. Appreciation is extended to Dr. Myron Brown of Ceva Lab., Overland Park, KS, for the donation of GnRH and Dr. Douglas Bolt of the USDA, Beltsville, MD, for the donation of bovine LH used in the radioimmunoassay of this hormone.

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Received September 15, 1986.

Accepted March 25, 1987.

elevated serum levels of LH and shortened the postpartum interval. Suckling-induced suppression of LH secretion may not only be provoked by stimulation of mammary glands but also may be due in part to a neuroendocrine mechanism triggered by the presence of the calf. Peters et al. (1981) reported that serum LH concentrations began to rise about 2 wk postpartum in milked cows, but remained low for an extended period in suckled cows.

Treatment of suckled cows with gonadotropin releasing hormone (GnRH) to cause release of LH and reduce postpartum anestrus has been attempted with limited success (Echternkamp, 1978; Fernandes et al., 1978; Carter et al., 1980; Troxel et al., 1980; Smith et al., 1983). Administration of small intermittent doses of GnRH to mimic endogenous pulsatile GnRH release and hence episodic release of LH caused suckled cows to cycle earlier than suckled controls, but later than weaned controls (Walters et al., 1982b). These observations suggest that inhibition of LH release in suckled cows may be due in part to suppression of GnRH secretion.

The act of suckling is known to have an immediate effect on the hypothalamo-hypophysial system by evoking the milk-ejection reflex. Research to determine whether the act of suckling or the milk-ejection reflex affects LH release through modification of function of higher centers in the brain has not yet been conducted. The purpose of this study was to measure the immediate effect of suckling or the milk-ejection reflex on exogenous GnRH-induced LH release following short-term calf removal on d 1 and 14 postpartum.

#### Materials and Methods

**Experimental Design.** Twenty-four postpartum multiparous Simmental  $\times$  Hereford cows (mean  $\pm$  SE: age,  $7.9 \pm .4$  yr; no. of parities,  $6.2 \pm .4$ ) were assigned randomly to four groups ( $n=6$ ) in a  $2^3$  factorial arrangement. Factors consisted of two levels of suckling [suckled (S) or nonsuckled (NS)], treatment with GnRH [saline (C) or  $200 \mu\text{g}$  GnRH] and days postpartum (d 1 and 14). Groups and treatments were as follows: group 1, suckled control (S+C); group 2, suckled+GnRH (S+GnRH); group 3, nonsuckled control (NS+C) and group 4, nonsuckled+GnRH (NS+GnRH). Dams were isolated from their calves for 4 h on d 1 and again on d 14 postpartum. After this isolation period each calf was reunited with its dam in S+C and S+GnRH groups and allowed to nurse for 5 min to permit stimulation of the milk-

ejection reflex. Cows in the S+C and S+GnRH groups then received an iv injection of either saline or GnRH, respectively. A dose of  $200 \mu\text{g}$  of GnRH was chosen based upon the results of Webb et al. (1977), Echternkamp (1978) and Fernandes et al. (1978). Animals in NS+C and NS+GnRH groups were treated similarly except calves were kept separated for an additional 2 h after the initial 4-h isolation period. To assess changes in serum LH, jugular blood samples were collected 15 min prior to the end of the 4-h calf isolation period, at the end of the isolation period (0 min), and at 15-min intervals for 120 min. All samples were collected between 1100 and 1700. Upon completion of the 2-h sampling period the cow and calf were placed in a lot and allowed to remain together until d 14 postpartum, when the treatment was repeated. After d 14, cows and their calves were moved to a pasture and observed twice daily for behavioral estrus.

**Radioimmunoassay.** Blood samples (10 ml) were stored at 4 C for 48 h and subsequently centrifuged at  $500 \times g$  for 10 min. The resulting serum was harvested and stored at  $-20$  C until assayed for LH by use of radioimmunoassay, as previously validated in our laboratory by McCarthy and Swanson (1976). Serum LH concentrations were determined for all samples in six assays. Intra- and inter-assay coefficients of variation were 4.5 and 10.8%, respectively. Sensitivity of the assay was .5 ng/tube.

**Statistical Analysis.** Pituitary response to GnRH was determined by computing the quantity of LH released with a linear interpolation between sampling times and subtraction of basal treatment levels of gonadotropin. Resulting values were divided by 120 min to yield the rate of LH release in response to GnRH ( $\text{ng LH} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ ). These data were subjected to split-plot analysis of variance (Steel and Torrie, 1980).

#### Results

Administration of GnRH to suckled and nonsuckled cows on d 1 or 14 postpartum provoked increased secretion of LH, with maximal serum concentrations occurring 105 to 120 min post-injection. Secretory patterns of serum LH for each treatment group on d 1 and 14 postpartum are displayed in figures 1 and 2, respectively. Regardless of suckling status of the cow, response of the pituitary to GnRH increased with time from parturition. As shown in figure 3, rate of LH release in response to GnRH on d 14

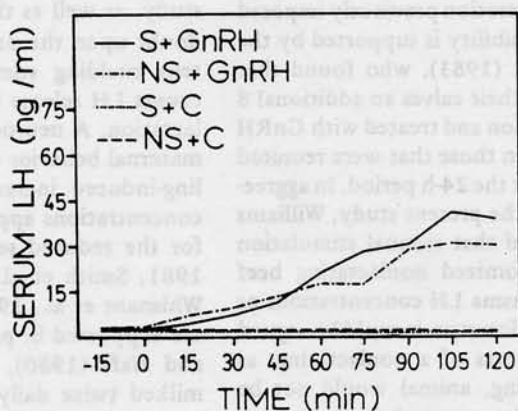


Figure 1. Pattern of LH release after iv injection of saline (C) or gonadotropin releasing hormone (GnRH) into suckled (S) and nonsuckled (NS) beef cows on d 1 postpartum.

postpartum was greater ( $P < .001$ ) than on d 1 in both suckled and nonsuckled cows (S+GnRH,  $37.1 \pm 3.9$  vs  $18.3 \pm 5.0$ ; NS+GnRH,  $34.7 \pm 5.9$  vs  $14.5 \pm 1.1$   $\text{ng} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ ).

Serum concentrations of LH in control cows did not differ due to suckling or stage of the postpartum period and averaged  $2.3 \pm .1$   $\text{ng}/\text{ml}$ . In addition, there was no significant difference in basal concentrations of LH between control or treated cows ( $2.3 \pm .1$  vs  $2.9 \pm .1$   $\text{ng}/\text{ml}$ , respectively).

On both d 1 and 14 postpartum only three of the six calves replaced with their dams in the S+GnRH group nursed during the sampling peri-

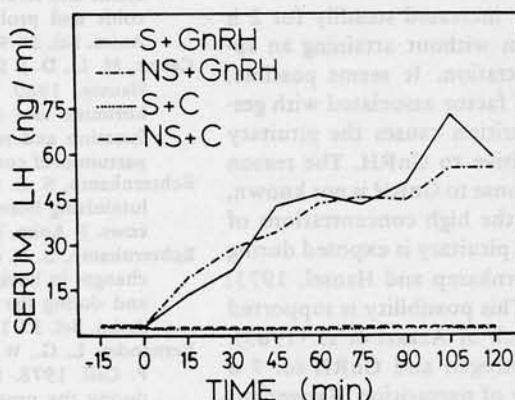


Figure 2. Pattern of LH release after iv injection of saline (C) or gonadotropin releasing hormone (GnRH) into suckled (S) and nonsuckled (NS) beef cows on d 14 postpartum.

od. Nevertheless, presence of the calf and its nuzzling of the udder did cause milk ejection to occur in a majority of these "nonsuckled" S+GnRH cows, as determined by appearance of milk at the opening of the streak canal.

Although a limited number of animals was utilized in this study, treatment had no effect on later reproductive performance. Administration of GnRH to suckled and nonsuckled cows did not affect the interval to first postpartum estrus [GnRH (S + NS),  $62 \pm 4$  vs control (S + NS),  $61 \pm 6$  d] or conception [GnRH (S + NS),  $82 \pm 3$  vs control (S + NS),  $84 \pm 4$  d]; the differences in days between mean intervals to first estrus and conception were approximately equal in duration to that of one cycle.

#### Discussion

Results of the present experiment indicate that the act of suckling or induction of the milk-ejection reflex had no immediate effect on GnRH-induced LH release in cows on d 1 and 14 postpartum. Failure to detect an effect of the suckling stimulus may have been due to the

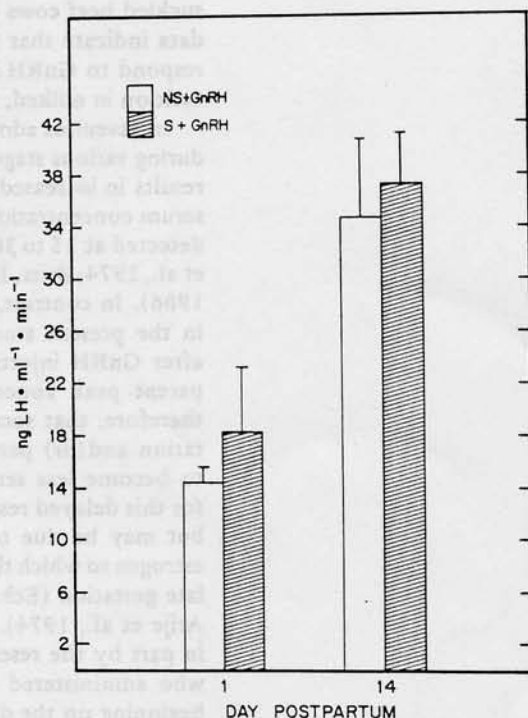


Figure 3. Serum LH release ( $\text{ng LH} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ ) in suckled (S) and nonsuckled (NS) cows after iv injection of gonadotropin releasing hormone (GnRH) on d 1 and 14 postpartum.



short duration of isolation of the calf from its mother. The 4-h isolation utilized in this experiment may not have been sufficient to release the "brake" on LH secretion previously imposed by the calf. This possibility is supported by the data of Smith et al. (1983), who found that cows separated from their calves an additional 8 h after 24 h calf isolation and treated with GnRH released more LH than those that were reunited with their young after the 24-h period. In agreement with results of the present study, Williams et al. (1984) reported that manual stimulation of teats of ovariectomized nonlactating beef cows did not alter plasma LH concentrations or LH pulse frequency. However, it could be argued that stimulation of teats of a nonlactating, as opposed to a lactating, animal would not be expected to elicit the same physiological response.

Rate of LH release in response to GnRH in this study was greater on d 14 than on d 1 postpartum. In addition, the pattern of induced LH release in this experiment was similar to those reported to occur after im injection of GnRH into postpartum cows at similar stages postpartum (Fernandes et al., 1978; Carter et al., 1980; Williams et al., 1982). Increased response to GnRH with increasing time from parturition also occurred in dairy cows (Kesler et al., 1977; Fernandes et al., 1978) and in suckled and non-suckled beef cows (Williams et al., 1982). These data indicate that the ability of the pituitary to respond to GnRH increases with time from parturition in milked, suckled and weaned cows.

Intravenous administration of GnRH to cows during various stages of the estrous cycle usually results in increased release of LH, with maximal serum concentrations of the gonadotropin being detected at 15 to 30 min after injection (Schams et al., 1974; Britt, 1975; Rodger and Stormshak, 1986). In contrast, serum concentrations of LH in the present study increased steadily for 2 h after GnRH injection without attaining an apparent peak concentration. It seems possible, therefore, that some factor associated with gestation and (or) parturition causes the pituitary to become less sensitive to GnRH. The reason for this delayed response to GnRH is not known, but may be due to the high concentrations of estrogen to which the pituitary is exposed during late gestation (Echternkamp and Hansel, 1973; Arije et al., 1974). This possibility is supported in part by the research of Azzazi et al. (1983), who administered estrogen and GnRH for 7 d beginning on the day of parturition. Exogenous

estrogen first stimulated, then inhibited GnRH-induced secretion of LH until d 16 postpartum.

In conclusion, the data from the present study, as well as those from other studies, cast doubt upon the premise that it is the intermittent suckling stimulus per se that indirectly causes LH release to be suppressed during early lactation. A neuroendocrine block triggered by maternal behavior toward the calf and (or) suckling-induced increases in hypothalamic opioid concentrations appear to be more likely causes for the reduced secretion of LH (Peters et al., 1981; Smith et al., 1983; Malven et al., 1986; Whisnant et al., 1986). These latter possibilities are supported in part by the data of Carruthers and Hafs (1980), who found that dairy cows milked twice daily and suckled ad libitum released less LH and had an extended postpartum interval to ovulation with nonsuckled cows milked two or four times daily.

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