# Effect of Days Postpartum and Endogenous Plasma Progesterone Concentrations on Estradiol Benzoate Induced LH Release in Dairy Cattle

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## ABSTRACT

Fifty Holstein cows were divided into 5 groups (10 cows/group) and received a single injection of 2 mg EB (IM) 0–3, 10–13, 20–23, 30–33 or 40–43 days post-partum (PP), groups 1–5, respectively. Blood samples were collected prior to EB administration (0 hr), and every 2 hr from 9–43 hr., posttreatment. The objective of this experiment was to determine the time interval postpartum when the hypothalamo-hypophysial axis regains responsiveness to estrogen by releasing a surge of LH. Plasma LH and progesterone concentrations, prior to treatment increased with days PP. The number of animals that responded to EB treatment was significantly lower in group 1 than groups 2–5. The mean peak LH concentrations for groups 1–5 significantly increased with increasing days PP. These data suggest that diary cows can respond to EB and release LH by 10–13 days following calving and this response increases as plasma progesterone decreases and time postpartum increases.

## INTRODUCTION

In normal suckled beef cows the mechanisms controlling the release of LH from the anterior pituitary seem not to be functioning normally during the early postpartum period (9, 11). However, by the third week postpartum beef cows regain their ability to respond to Estradiol Benzoate (EB) by releasing significant levels of LH (9). Furthermore, it was reported that at times when plasma progesterone is high, EB fails to induce an LH release (7, 12). We previously reported (15) that none of the dairy cows with plasma progesterone $>1$ ng/ml prior to EB administration released LH in response to a 2 mg injection of EB. The nature of positive feedback mechanisms controlling LH release, and the time PP when dairy cows release LH in response to exogenous EB have not been determined. Therefore, the objective of this study was to

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determine when following calving in dairy cows the hypothalamo-hypophysial axis can regain its responsiveness to estrogen as evidenced by a release of a surge of LH.

## MATERIALS AND METHODS

Fifty Holstein cows from Al-Nasre Dairy Project in Tripoli were divided into 5 groups (10 cows/group) and received 2 mg Estradiol Benzoate (EB) intramuscularly (IM) on days 0–3, 10–13, 20–23, 30–33, or 40–43 postpartum (day 0 = day of calving), groups 1–5, respectively. Blood samples for hormonal analysis were collected via jugular vein puncture immediately prior to EB administration (0 hr) and every 2 hr from 9–43 hr, posttreatment. Samples were immediately centrifuged at 4,000 rpm for 12 minutes and plasma was stored at $-20^{\circ}$C until assayed. The data were analyzed by one way analysis of variance. Comparisons between means were made using the least significant difference (LSD). Simple correlations among LH release, days PP, and progesterone concentrations were computed. The enumerative data were compared using Chi-square analysis (13).

*Hormone Assays:* Plasma concentrations of LH were measured by double antibody radioimmunoassay (RIA, 10) as previously validated in our laboratory (16). Plasma progesterone was determined by RIA as described by Cantley *et al.* (3).

## RESULTS AND DISCUSSION

The mean plasma LH and progesterone concentrations prior to EB treatment in groups 1–5 are summarized in Table 1. Plasma LH concentrations increased quadratically ($r = 0.44$, $P < .05$) with days PP. Plasma progesterone concentrations, however, showed a linear relationship (increase, $r = 0.47$, $P < .05$) with days PP. In cows that released LH in response to EB the mean peak concentrations of LH released after treatment and the mean concentrations of progesterone prior to treatment in groups 1–5 are summarized in Table 2. The proporation of animals with plasma progesterone less than 1.0 ng/ml were 10/10, 10/10, 9/10, 6/10 and 5/10 (groups 1–5, respectively). None of the cows with plasma progesterone concentrations $> 1.0$ ng/ml prior to treatment released LH in response to EB. The proportion of animals with plasma progesterone $< 1.0$ ng/ml that released LH in response to EB (fig. 1) was 4/10, 8/10, 7/9, 5/6 and 4/5 (groups 1–5, respectively). The response of EB induced LH release in groups 1–5 is illustrated in Fig. 1. Group 1 had significantly fewer animals ($P < .05$) responding to EB treatment than groups 2–5. The mean peak LH concentrations of responding animals increased with increasing days PP ($r = .54$; $P < .05$). Furthermore,

Table 1. Mean plasma concentrations ($\bar{x} \pm$SEM) of LH and progesterone prior to EB treatment in groups 1–5.

| | Groups | | | | |
|---|---|---|---|---|---|
| Item | 1 | 2 | 3 | 4 | 5 |
| Days postpartum (PP) | (0–3) | (10–13) | (20–23) | (30–33) | (40–43) |
| LH (ng/ml) | $2.5 \pm .3^{a}$ | $3.1 \pm .3^{ab}$ | $3.7 \pm .3^{b}$ | $3.0 \pm .2^{ab}$ | $2.6 \pm .3^{a}$ |
| Progesterone (ng/ml) | $.6 \pm .1^{a}$ | $.4 \pm .1^{a}$ | $.8 \pm .3^{ab}$ | $1.3 \pm .3^{b}$ | $1.7 \pm .3^{b}$ |

Means with different superscripts are significantly different at ($P < .05$) between groups.

Table 2. Mean peak concentrations ($\bar{x} \pm$SEM) of LH (post-EB treatment) and progesterone concentrations (pre-EB treatment) in animals responding to EB.

| Item | Groups | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Days postpartum (PP) | (0–3) | (10–13) | (20–23) | (30–33) | (40–43) |
| LH (ng/ml) | $11.9 \pm 1.8^{a}$ | $16.7 \pm 1.6^{ab}$ | $20.9 \pm 2.0^{bc}$ | $16.8 \pm 2.7^{ab}$ | $25.0 \pm 1.9^{c}$ |
| Progesterone (ng/ml) | $.6 \pm .1$ | $.4 \pm .1$ | $.5 \pm .1$ | $.7 \pm .1$ | $.6 \pm .1$ |

Means with different superscripts are significantly different at ($P < .05$) between groups.

the mean time from treatment with EB until peak of LH release averaged $20.6 \pm 1.0$ hr, among the five groups.

Mechanisms controlling the LH release in postpartum cows are influenced by endogenous estrogen and progesterone concentrations. Estrogen may be responsible for the preovulatory surge in cattle (4, 6). Moreover, the decline in progesterone levels to less than 0.5 ng/ml prior to the preovulatory surge in cattle was suggested to be a factor for eliciting the preovulatory LH surge (14). Estrogen when administered alone in ovariectomized cows induced an LH release (2, 7, 12). However, when a combination of estrogen and progesterone was administered, a suppression of the LH release resulted (1). Data presented in this study have shown that cows with plasma progesterone concentrations greater than 1.0 ng/ml prior to EB treatment did not respond by an LH release, while the majority of those animals exhibiting low progesterone concentrations ($< 1.0$ ng/ml) and are more than 13 days PP released LH in response to EB. These findings are in agreement with other reports (5, 8, 12, 15). The

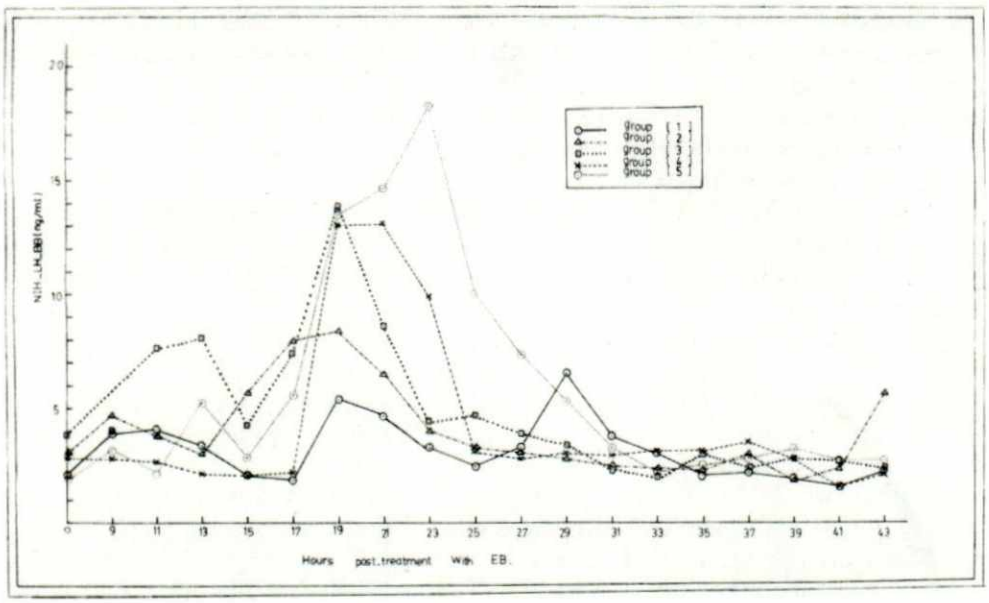


Fig. 1. Mean concentrations (ng/ml) of LH in plasma of dairy cows following administration of 2 mg EB (time 0). Only cows which released LH > 5 ng/ml are presented. Numbers of cows shown are 4, 8, 7, 5 and 4 for groups 1, 2, 3, 4 and 5, respectively.

negative feedback caused by high progesterone concentrations at the hypothalamo-hypophysial axis may be necessary to allow organization of the LH secretory mechanism in order for endogenous estradiol to induce a LH release.

In conclusion, the aforementioned findings suggest that the hypothalamic-hypophysial axis of dairy cows regains responsiveness to EB, as evidenced by LH release by 10–13 days PP. In addition this response increases in magnitude as time PP increases.

Key Words: LH surge, Estradiol Benzoate, Progesterone, Postpartum, Dairy cows.

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## LITERATURE CITED

1. Beck, T. W. and E. M. Convey. 1974. LH after estradiol and GnRH in spayed heifers. J. Ani. Sci. 39:198. (abstract).
2. Beck, T. W., V. G. Smith, B. E. Seguin and E. M. Convey. 1976. Bovine serum LH, GH and prolactin following chronic implantation of ovarian steroids and subsequent ovariectomy. J. Ani. Sci. 42:461.
3. Cantley, T. C., H. A. Garverick, C. J. Bierschwal, C. E. Martin and R. S. Youngquist. 1975. Hormonal response of dairy cows with ovarian cysts to GnRH. J. Ani. Sci. 41:1666.
4. Ecternkamp, S. E. and W. Hansel. 1973. Concurrent changes in bovine plasma hormonal levels prior to and during the first postpartum estrous cycle. J. Ani. Sci. 37:1362.
5. Forrest, P. K., H. J. Irvin and H. A. Garverick. 1981. Effect of time postpartum on estrogen and GnRH induced LH release in dairy cows. 73rd Annual Meeting of ASAS. (abstract).
6. Henricks, D. M., D. R. Lamond, J. R. Hill and J. F. Dickey. 1971. Plasma total estrogen and progesterone concentrations during proestrus and after mating in beef heifers. Abstract 34. Fourth Annual Meeting. Soc. Study Reproduction. p. 13.
7. Hobson, W. C. and W. Hansel. 1972. Plasma LH levels after ovariectomy, corpus luteum removal and estradiol administration in cattle. Endocrinol. 91:105.
8. Nancarrow, C. D. and H. M. Radford. 1976. Responses in ovariectomized cows to repeated injections of thyrotrophin releasing hormone (TRH) and to estradiol benzoate (EB). Therio. 6:642.
9. Nancarrow, C. D., H. M. Radford, R. J. Scaramuzzi and T. B. Post. 1977. Responses to induced estrogen in suckled beef cows. Therio. 8:192.
10. Niswender, G. D., L. E. Reichert, A. B. Midgley and A. V. Nalbandov. 1969. Radioimmunoassay of bovine and ovine luteinizing hormone. Endocrinol. 84:1166.
11. Radford, H. M., C. D. Nancarrow and P. E. Mattner. 1976. Evidence for hypothalamic dysfunction in suckled beef cows. Therio. 6:41.

12. Short, R. E., R. D. Randel, D. S. Christenson, and R. A. Bellows. 1974. Effects of $E_2\beta$ induced LH release in the bovine. (abstract). J. Ani. Sci. 39:226.
13. Snedecor, G. W. and W. G. Cochran. 1973. Statistical methods. Sixth Ed., Iowa State University Press.
14. Snook, R., R. Saatman and W. Hansel. 1971. Serum progesterone and luteinizing hormone levels during the bovine estrous cycle. Endocrinol. 88:678.
15. Zaied, A. A., H. A. Garverick, D. J. Kesler, C. J. Bierschwal, R. G. Elmore and R. S. Youngquist. 1981. Luteinizing hormone response to estradiol benzoate in cows postpartum and cows with ovarian cysts. Therio. 16:349.
16. Zaied, A. A., H. A. Garverick, C. J. Bierschwal, R. G. Elmore, R. S. Youngquist and A. J. Sharp. 1980. Effects of ovarian activity and endogenous reproductive hormones on GnRH induced ovarian cycles in postpartum dairy cows. J. Ani. Sci. 50:508.

## تأثير طول فترة ما بعد الولادة
## وكذلك هرمون البروجستيرون في الدم على حساسيـة
## الغدة النخامية لهرمون(Gn–RH)

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### المستخلـــص

تم إجراء هذا البحث بمزرعة النصر — طرابلس — ليبيا . وقد استخدم في هذه التجربة خمسون بقرة من نوع هولستين فريزيان تتراوح فترة ما بعد الولادة في هذه الأبقار ما بين اليوم الأول واليوم الأربعين . وقد كان الهدف من إجراء هذا البحث هو دراسة تأثير طول فترة ما بعد الولادة وكذلك هرمون البروجستيرون في الدم على حساسية الغدة النخامية لهرمون (Gn–RH) وقد أثبتت نتائج هذا البحث أن هذه الغدة لا تستجيب لتأثير هرمون (Gn–RH) إلا بعد اليوم العاشر بعد الولادة وكذلك بشرط أن يكون معدل هرمون البروجستيرون في الدم أقل من واحد نانوجرام لكل ملیلتر (ng/ml) وقت حقن هرمون الـ (Gn–RH) .