Lack of LH Response to Exogenous Estradiol in Heifers with ACTH-Induced Ovarian Follicular Cysts

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(Received 14 October 1998/Accepted 5 April 1999)

ABSTRACT. The aim of the present study was to examine the LH response to exogenous estradiol in 4 heifers with ACTH-induced ovarian follicular cysts. During the control experiment, administration of estradiol 24 hr after PGF$_{2\alpha}$ in luteal phase heifers resulted in a LH response in all 4 heifers. The LH response was obtained between 16–20 hr after estradiol administration. The peak LH concentration (Mean ± SEM; 5.1 ± 0.8 ng/ml) during the control study was significantly different (P<0.05) from the concentration after cyst formation. None of the 4 heifers responded to estradiol after ovarian cyst formation. This result suggests that heifers with ACTH-induced ovarian follicular cysts may have a defective hypothalamo-pituitary response to exogenous estradiol similar to cows with spontaneous ovarian cysts.—KEY WORDS: heifer, luteinizing hormone, ovarian cyst.

In normal cows, administration of exogenous estradiol, in the absence of high levels of progesterone, results in a luteinizing hormone (LH) response 18–24 hr later [1]. Kesner et al. [13] suggested that both gonadotropin releasing hormone (GnRH) secretion and increased pituitary responsiveness are necessary for the estradiol-induced preovulatory LH surge in cows.

The feedback regulation of estradiol on the hypothalamo-pituitary axis is altered in cows with spontaneous ovarian cysts [3–5, 23]. Nanda et al. [17] reported failure of LH surge in response to estradiol in 20 out of 38 cows with ovarian cysts. It is not clear why some cows with ovarian cysts respond to estradiol while others do not. In some cows, ovarian cysts may change their course of development and recover spontaneously [21]; cystic cows that respond to estradiol may be in this category. Some confounding factors with studies on spontaneous ovarian cysts include the fact that as the cases are already established, it is difficult to ascertain for how long the cyst had existed and the morphological and endocrine profile at the time of diagnosis may be different to the circumstances at the time of initial cyst formation.

Kesler et al. [12] hypothesized that postpartum ovarian cysts developed when the hypothalamus and pituitary appeared to be less responsive in releasing pituitary LH under the influence of endogenous estradiol-17β. A high incidence of ovarian dysfunction including ovarian cysts has been reported in cows with reproductive disorders at calving [10, 15]. Gruenert [6] indicated that ovarian cysts, among other ovarian disorders, were a sign that fertility was impaired by stress. Furthermore, the use of adrenocorticotropic hormone (ACTH) to experimentally induce ovarian cysts [14, 19] has provided an excellent model to closely study this disorder.

While several studies have documented the hypothalamo-pituitary response to estradiol in cows with spontaneous ovarian cysts [3–5, 17, 23], there is a paucity of information on the response to estradiol in cattle with experimentally-induced ovarian follicular cysts. The objective of the present study was, therefore, to characterize the feedback effect of estradiol on the hypothalamo-pituitary axis in heifers with ACTH-induced ovarian follicular cysts.

Animals and treatments: Four Holstein-Friesian heifers (15–18 months old) were used for this study. The heifers were housed in tied stalls and fed hay and concentrates twice a day. Water was provided ad libitum. To characterize a control LH response, heifers with functional corpora lutea, as determined by ultrasonography and plasma progesterone concentration, were injected with 25 mg tromethamine dinoprost (prostaglandin F$_{2\alpha}$; Pronalgon F®; Takeda Pharmaceutical Co., Ltd., Osaka, Japan) intramuscularly. Twenty four hours later, 1 mg of estradiol benzoate (Gynandol®, Sankyo Ltd., Tokyo,) in 2 ml peanut oil was injected intramuscularly. The same heifers were used for the control study as well as for the experimental cyst induction using ACTH. At least one normal estrous cycle had elapsed between the control study and the experimental cyst induction in the heifers.

Experimental induction of ovarian follicular cysts consisted of the administration of 1 mg ACTH (Cotrosyn® Z; Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan) subcutaneously every 12 hr for 7 days beginning on day 15 of the estrous cycle. Cyst formation was monitored by ultrasonography and plasma progesterone determination. A cyst was defined as a follicular structure ≥ 20 mm that had existed for 10 days or more in the absence of CL [2]. Ten days after cyst formation, 1 mg of estradiol in 2 ml peanut oil was injected intramuscularly.

Blood sampling: Blood was sampled by tail venepuncture at the time of Dinoprost injection in control heifers. Further blood samples were obtained through an indwelling jugular vein catheter at the time of estradiol injection (0 hr) and...
every 2 hr from 16 to 32 hr after estradiol administration during the control study and after cyst formation. Catheters were maintained patent by infusion of heparinized saline (10 iu heparin/ml). Blood samples were immediately centrifuged at 1700 × g for 20 min and stored at −30°C until hormone analysis.

Plasma progesterone and LH concentration were determined using a validated double antibody assay as described by Kaneko et al. [11]. The inter- and intra-assay coefficients of variation were, respectively, 8.3 and 4.2 percent for progesterone and 14.4 and 8.1 percent for LH. The sensitivities of the assays were 0.2 and 0.1 ng/ml for progesterone and LH, respectively.

A response to estradiol treatment was defined as an increase in LH concentration four fold greater than the pretreatment LH values [1]. Basal progesterone (0 hr; pretreatment) and LH concentrations in heifers during control and after cyst formation were compared by Student’s t-test. One-way ANOVA was used to determine the effect of estradiol treatment on control and cystic heifers. Values are presented as Mean ± S.E.M.

Ovarian follicular cysts were found in the 4 heifers after completion of ACTH treatment. The mean day of initial cyst detection was 24.8 ± 1.9 (day 0=day of reference estrus). The mean maximum cyst size was 26.8 ± 3.7 mm in diameter. The cysts remained for 12–16 days before cyst regression and/or ovulation occurred. By ultrasonography, ovarian follicular cysts appeared as uniformly non echogenic structures ≥ 20 mm in diameter with a wall < 3 mm thick. The mean progesterone concentration during cyst existence was 0.4 ± 0.1 ng/ml.

The basal levels of LH at the time of estradiol treatment (0 hr) during the control study was 0.67 ± 0.07 ng/ml and was not significantly different (P>0.05) from the basal concentrations after cyst formation, 1.18 ± 0.51 ng/ml (n=4). A positive response to estradiol treatment was obtained in all 4 heifers during the control study. The LH response was elicited between 16–20 hr after estradiol treatment. Mean peak LH concentration (5.1 ± 0.8 ng/ml) was obtained at 16 hr after estradiol administration. In contrast, estradiol treatment completely failed to elicit an LH response in all the 4 heifers with follicular cysts. The responses of the heifers to estradiol treatment during the control and after cyst formation are presented in Fig. 1.

As intensive blood sampling for LH began 16 hr after estradiol injection, it was not possible to precisely establish whether LH peaked earlier than 16 hr. However, the highest mean concentrations (5.1 ± 0.8 ng/ml) were observed at this time. In one heifer, however, the peak concentrations (8.13 ng/ml) was observed at 20 hr after estradiol administration. There was no difference (P>0.05) in mean progesterone concentration at the time of estradiol injection (0 hr) between the control study and after cyst formation in the heifers.

During the control study, LH response to estradiol was elicited in all heifers. This is consistent with previous studies in postpartum cows [1, 4, 22]. The mean peak concentration of LH (5.1 ± 0.8 ng/ml) was, however, lower than in previous studies. This may be attributed to the difference in the methods of RIA used for measuring LH in previous reports and the present study. The LH peak in the present study occurred earlier (16 hr) compared to 25 hr reported in a previous study [1]. The difference between the two studies may be attributed to differences in sample sizes and the fact that heifers were used in the present study while cows were used in the former study.

After cyst formation, estradiol administration completely failed to elicit an LH response. This agreed with the results of Dobson and Alam [4] and Refsal et al. [20] who have reported a lack of response in 4 out of 4 cows, and 11 out of 12 cows with spontaneous ovarian follicular cysts, respectively. In another study, Nanda et al. [17] reported a failure to exhibit an LH response in 20 out of 38 cystic cows studied. Zaied et al. [23] reported a delayed LH response to estradiol in cows with ovarian cysts.

High circulating levels of progesterone are known to suppress the release of LH through the negative feed back effect on the hypothalamo-pituitary axis [7–9, 16, 18]. The lack of response to estradiol after cyst formation in the present study was not due to high levels of progesterone as mean progesterone concentration at the time of estradiol administration was low (0.4 ± 0.1 ng/ml) and was not significantly different (P>0.05) from control heifers which showed LH response to estradiol. The reason for the lack of response to exogenous estradiol in the heifers after cyst formation may be attributed to a decrease in the sensitivity of the hypothalamo-pituitary axis to the positive feed back effect of estradiol.

In conclusion, this result indicates that heifers with ACTH-induced ovarian follicular cysts may have a defective
hypothalamic-pituitary functions similar to cows with spontaneous ovarian cysts.

ACKNOWLEDGEMENTS. A.Y. Ribadu was supported by a postdoctoral fellowship from Japan Society for the Promotion of Science (JSPS). The authors are grateful to Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan and Takeda Chemical Co., Ltd., Osaka, Japan for the kind donation of Cortrosyn® Z and Pronalgon F®, respectively.

REFERENCES