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Progesterone concentration, estradiol pretreatment, and dose of gonadotropin-releasing hormone affect gonadotropin-releasing hormone-mediated luteinizing hormone release in beef heifers

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Abstract

We examined whether progesterone (P_4)-induced suppression of LH release in cattle can be overcome by an increased dose of exogenous gonadotropin-releasing hormone (GnRH) or pretreatment with estradiol (E_2). In Experiment 1, postpubertal Angus-cross heifers (N = 32) had their 2 largest ovarian follicles ablated 5 d after ovulation. Concurrently, these heifers were all given a once-used, intravaginal P_4-releasing insert (CIDR), and they were randomly assigned to be given either prostaglandin $F_{2\alpha}$ (Low-P_4) or no treatment (High-P_4) at follicle ablation, and 12 h later. Six days after emergence of a new follicular wave, half of the heifers in each group (n = 8) were given either 100 or 200 μg of GnRH i.m. Plasma luteinizing hormone (LH) concentrations were higher in the Low- vs High-P_4 groups, and in heifers given 200 vs 100 μg of GnRH (mean ± SEM 15.4 ± 2.2 vs 9.1 ± 1.2, and 14.8 ± 2.1 vs 9.8 ± 1.4 ng/mL, respectively; $P \leq 0.01$). Ovulation rate was higher ($P = 0.002$) in the Low-P_4 group (15/16) than in the High-P_4 group (6/16), but it was not affected by GnRH dose ($P = 0.4$). In Experiment 2, heifers (n = 22) were treated similarly, except that 5.5 d after wave emergence, half of the heifers in each group were further allocated to be given either 0.25 mg estradiol benzoate i.m. or no treatment, and 8 h later, all heifers were given 100 μg GnRH i.m. Both groups treated with E_2 (Low- and High-P_4) and the Low-P_4 group without E_2 had higher peak plasma LH concentrations compared to the group with high P_4 without E_2 (12.6 ± 1.8, 10.4 ± 1.8, 8.7 ± 1.3, and 3.9 ± 1.2 ng/mL, respectively; ($P < 0.04$)). However, E_2 pretreatment did not increase ovulation rates in response to GnRH ($P = 0.6$). In summary, the hypotheses that higher doses of GnRH will be more efficacious in inducing LH release and that exogenous E_2 will increase LH release following treatment with GnRH were supported, but neither significantly increased ovulation rate.

Keywords: Estradiol; Progesterone; GnRH; LH; Cattle

1. Introduction

Gonadotropin-releasing hormone (GnRH) is a decapeptide, synthesized and released by the hypothalamus, which acts on the anterior pituitary to induce the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [1]. Steroid hormones regulate gonadotropin release by both positive and negative feedback on the hypothalamus, anterior pituitary, or

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both [2,3]. Progesterone (P_4) suppresses LH release, whereas estradiol (E_2) enhances the LH surge [2,3]. The response of pituitary cells in culture to GnRH was associated with the number of GnRH receptors on the surface of the gonadotropes [4]. Increased peripheral P_4 concentrations decreased the frequency of GnRH pulses secreted by the hypothalamus and the number of GnRH receptors in the anterior pituitary [2,5]. In this regard, we recently reported that high plasma P_4 concentrations suppressed pituitary release of LH and reduced the ovulatory response following the administration of 100 μg GnRH in both beef heifers and cows [6].

Pituitary LH release was increased with higher doses of GnRH in anestrous, diestrous, and ovariectomized ewes [7], and in ovariectomized cows [8]. Although the magnitude of plasma LH response increased with increasing doses of GnRH in diestrous heifers, the greatest increase occurred between 50 and 100 μg [9], suggesting that P_4 concentrations may influence the pituitary response to GnRH doses exceeding 100 μg. However, Mihm et al [10] reported that 250 μg GnRH was more effective than 100 μg GnRH in inducing ovulation in diestrous beef heifers. Hence, whether the pituitary response of ovary-intact cattle to a dose of GnRH $>$100 μg can be modulated by peripheral P_4 concentrations remains to be investigated.

In contrast to the effects of P_4, E_2 increases pituitary responsiveness to GnRH by increasing the expression of the gene encoding the GnRH receptor [3]. Furthermore, exposure of pituitary cells to E_2 for 24 h caused a 3-fold increase in the secretion of LH into the incubation medium [11], indicating that E_2 can act directly on the hypophysis. However, E_2 treatment during the luteal phase failed to increase the number of GnRH receptors on pituitary cells in ewes [12]. Hence, the role of E_2 in amplifying LH secretion in P_4-treated animals is not well established.

The objectives of the present study were to further determine (1) whether LH release and ovulation in response to GnRH treatment in heifers is influenced by peripheral P_4 concentrations, and (2) whether P_4-induced suppression of LH release can be overcome by an increased dose of exogenous GnRH or pretreatment with E_2. We hypothesized that higher doses of GnRH or pretreatment with exogenous E_2 will enhance LH release and ovulatory response to exogenous GnRH in cattle with elevated peripheral P_4 concentrations.

2. Materials and methods

2.1. Cattle

Two experiments were conducted using nulliparous crossbred Angus heifers (14-18 mo) in which a corpus luteum (CL) had been previously detected by transrectal ultrasonography. Heifers were maintained in outdoor pens at the University of Saskatchewan Goodale Research Farm (52° north and 106° west). All procedures were performed in accordance with the Canadian Council on Animal Care and were approved by the University of Saskatchewan Protocol Review Committee.

2.2. Experiment 1

Heifers (N = 32) at random stages of the estrous cycle were given 2 luteolytic doses of 500 μg cloprostenol (Estrumate, Schering-Plough Animal Health, Pointe-Claire, Québec, Canada) i.m., 13 d apart, and assigned randomly to 4 treatment groups (n = 8 per group) in a 2 $\times$ 2 factorial design to determine the effects of P_4 concentration (high vs low) and the dose of GnRH (100 vs 200 μg) on ovulatory response. Starting 4 d after the second cloprostenol treatment, heifers were examined daily by transrectal ultrasonography, using a 7.5 MHz linear-array transducer (Aloka SSD-900; Tokyo, Japan) to detect ovulation. Five days after ovulation, the 2 largest ovarian follicles were ablated by transvaginal ultrasound-guided follicle aspiration [13] to induce emergence of a new ovarian follicular wave (expected 1.5 d later [13]). Heifers were given a previously used CIDR (once-used CIDR) (7 d) intravaginal P_4-releasing insert (CIDR; Pfizer Canada, Inc., Montréal, Québec, Canada), and half the heifers were randomly assigned to be given 2 treatments (concurrent with follicle ablation and repeated 12 h later) of 25 mg dinoprost (PGF; Lutalyse; Pfizer Canada, Inc.) i.m., (Low-P_4 group; n = 16) or no treatment (High-P_4 group; n = 16), that is, heifers in the High-P_4 group were allowed to retain their CL. The Low- and High-P_4 groups were expected to have sub-physiological (ie, $<$2.5 ng/mL [14]) and typical luteal phase plasma P_4 concentrations, respectively, during dominant follicle development. Six days after follicle wave emergence (ie, 7 d after CIDR insertion), heifers in each group were randomly allocated to receive either 100 or 200 μg GnRH i.m. (gonadorelin; Cystorelin, Mérial Canada, Inc., Victoriaville, Québec, Canada). To detect ovulation, heifers were examined by transrectal ultrasonography once daily for 4 d after GnRH treatment. The CIDR inserts were removed either on the day of ovulation, or in the absence of ovulation, 4 d after GnRH treatment.

Blood samples were collected by coccygeal venipuncture into 10 mL heparinized, evacuated tubes (Becton Dickinson Vacutainer Systems, Franklin