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Stimulation of a pulse of LH and reduction in PRL concentration by a physiologic dose of GnRH before, during, and after luteolysis in heifers

O.J. Ginther[a,b,*], M.A. Beg[b]

[a] Eutheria Foundation, Cross Plains, WI 53528, USA
[b] Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, 1656 Linden Drive, Madison, WI 53706, USA

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ABSTRACT

A single physiologic dose (5.0 μg) of GnRH was given to 9 heifers each day (Hour 0) beginning on Day 15 postovulation until regression of the corpus luteum. Blood samples were taken each day for Hours −3, −2, −1, 0, 0.25, 0.5, 0.75, 1, 1.25, 1.50, 1.75, 2, 3, 4, and 5. Based on daily progesterone concentrations, data were grouped into phases of before ($n = 4$), during ($n = 8$), and after ($n = 7$) luteolysis. The number of LH pulses with a peak at pretreatment Hours −2 or −1 (0.35 ± 0.12 pulses/sampling session) was less ($P < 0.0001$) than for a pulse peak at posttreatment Hours 1 or 2 (1.0 ± 0.0 pulses/session). The characteristics and effects of LH pulses on progesterone and estradiol were similar between natural (pretreatment) and primarily induced (posttreatment) LH pulses. The same dose of GnRH stimulated an LH pulse with greater ($P < 0.05$) amplitude after luteolysis than during luteolysis. Concentrations of PRL and number and prominence of PRL pulses decreased ($P < 0.05$) between Hours 0 and 2 within each of the phases of before, during, and after luteolysis. The hypothesis that a physiologic dose of GnRH increases the concentration of PRL was not supported; instead, GnRH reduced the concentration of PRL. Results supported the hypotheses that an appropriate dose of GnRH stimulates an LH pulse during luteolysis that is similar to a natural pulse in characteristics and in the effects on progesterone and estradiol.

1. Introduction

Gonadotropin-releasing hormone (GnRH) is produced by the hypothalamus and is delivered to the gonadotropes of the anterior pituitary for the synthesis and release of the gonadotropins LH, FSH (Thatcher et al., 1993; Conn and Crowley, 1994). The GnRH secretory neurons are regulated by feedback effects of gonadal steroids (Jadav et al., 2010; Kinder et al., 1996). The gonadotropins have physiologic effects associated with ovulation, luteinization, and follicular and luteal function. Thus, GnRH and its analogues are used for managerial manipulation of reproductive events (Jadav et al., 2010; Peters, 2005).

A single dose of 100 μg of GnRH is used commonly to induce a preovulatory LH surge in cattle (Haughian et al., 2004; Lucy and Stevenson, 1986; Pursley et al., 1995). However, a dose of 50 μg also induces ovulation of the preovulatory follicle (Fricke et al., 1998), and a 25 μg dose induces ovulation of the anovulatory follicle of the first follicular wave (Sato et al., 2005). Apparently, the minimal effective dose for inducing ovulation in cattle has not been established. Preovulatory treatment with 100 μg GnRH induces concomitant LH and FSH surges beginning within 1 h of treatment, and the induced surges are not different from control surges in area under the curve (Haughian et al., 2004). Doses as small as 2.5 or 5.0 μg, especially when infused or injected every 2 h, induce the pulsatile release of LH in prepubertal cattle (McLeod et al., 1984) and in

* Corresponding author at: Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, 1656 Linden Drive, Madison, WI 53706, USA. Tel.: +1 6082621037; fax: +1 6082627420.
E-mail address: ginther@vetmed.wisc.edu (O.J. Ginther).

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anovulatory postpartum cattle (Jagger et al., 1987; Riley et al., 1981; Edwards et al., 1983). Apparently, stimulation of an LH pulse by GnRH treatment on the days before, during, and after luteolysis with similarity between the induced and natural pulses has not been reported in any species.

In cattle, the peak of an LH pulse occurs about every 4 h during mid-diestrus (Procknor et al., 1986) and preceding the beginning of luteolysis (Ginther et al., 2011c), and an LH pulse has a transient positive effect on a temporally associated progesterone fluctuation (Ginther et al., 2011a,b,c; Pugliesi et al., 2011). Pulses of PGF2α occur during luteolysis and can be assessed by assay of its metabolite (PGFM). A decrease in progesterone occurs within the hours of a PGFM pulse, followed by a progesterone rebound (Ginther et al., 2010b). A pulse of LH accounts for the progesterone rebound, as indicated by a temporality study (Ginther et al., 2011c) and by reducing LH concentrations and pulses with an antagonist of GnRH receptors, acyline (Ginther et al., 2011b). The complete progesterone rebound that occurs during the infusion of PGF2α to simulate a PGFM pulse is incomplete when acyline is given (Shrestha et al., 2011). On the basis of these results, it is believed that luteolysis in cattle initially involves balancing between a negative effect of PGF2α pulses and a positive effect of LH pulses (Ginther et al., 2011c).

The biochemical characteristics and regulation of prolactin (PRL) secretion (Gregerson, 2006) and the role of PRL and other luteotropic factors in the regulation of the corpus luteum (CL) in various species have been reviewed (McNeilly, 1980; Stouffer, 2006). Prolactin increases the production of progesterone in vitro in perfused bovine ovaries (Bartosik et al., 1967). However, other studies did not implicate PRL in luteal function in cattle (Smith et al., 1957; Hoffmann et al., 1974). Binding sites for PRL are present in the CL (Poindexter et al., 1979). The expression of PRL and PRL-receptor mRNA in bovine CL throughout the luteal phase has been demonstrated, but expression of PRL mRNA is greatest 15–17 days after ovulation (Shibaya et al., 2006). The co-expression of PRL and PRL-receptor mRNA suggests that PRL is a local regulator in the bovine CL. In a recent study, changes in gene expression of PRL receptors in bovine CL also were consistent with the possible involvement of a form of PRL receptors in the regulation of progesterone secretion and metabolism during the estrous cycle and pregnancy in cattle (Thompson et al., 2011). The PRL in the CL of cattle is cleaved into antiangiogenic fragments that speculatively might mediate regression of luteal vessels during luteolysis (Erdmann et al., 2007).

The base (nadir to nadir) of PRL pulses is 4 or 5 h in heifers (Ginther and Beg, 2011) and mares (Ginther et al., 2012). In cattle, the ending nadir of one pulse usually occurs at the same hour as the beginning nadir of the next pulse, whereas in mares the interval between the ending nadir of a pulse and the beginning nadir of the next pulse is about 7 h. Pulses of PRL in heifers are most prominent and rhythmic during the last 12 h of luteolysis and thereafter. Pulse peaks of PRL and PGFM are synchronized for most PRL pulses during and after luteolysis in both heifers (Ginther and Beg, 2011) and mares (Ginther et al., 2012).

High concentration of PRL has a negative effect on LH secretion and reproductive function in several species (Milenkovic et al., 1994) and is mediated at least in part by a direct negative action of PRL on GnRH neurons (Grattan et al., 2007). These PRL/GnRH/LH relationships are represented during the induction of high concentrations of PRL by suckling in postpartum cows (McNeilly, 1980; Chang et al., 1981; Montiel and Ahuja, 2005). An example of a positive effect of PRL on LH and presumably on GnRH is the temporal association between an increase in PRL and LH preceding the onset of the ovulatory season in horses (Ginther, 1992). An effect of GnRH on PRL has also been shown. In sheep, GnRH stimulates the release of PRL in pituitary cell cultures obtained during the breeding season but not when obtained during the nonbreeding season (Henderson et al., 2008). A high dose of GnRH (200 μg) induces an increase in PRL in postpartum cows (Holt et al., 1991). In contrast, a high dose of GnRH (115–385 μg) induces a decrease in PRL concentrations in ovariectomized mares pretreated with various steroids, including estradiol, progesterone, or androgens (Thompson et al., 1991). Apparently, neither a positive nor negative effect of a physiologic dose of GnRH on PRL during the estrous cycle has been reported for any species.

The present study considered the temporal interrelationships among GnRH, LH, PRL, FSH, PGFM, progesterone, and estradiol before, during, and after luteolysis in heifers. Specific hypotheses were: (1) an appropriate dose of GnRH stimulates an LH pulse before, during, and after luteolysis that is similar in characteristics to a natural LH pulse; (2) a GnRH-stimulated pulse of LH is temporally associated with an increase in progesterone, except after luteolysis; (3) a dose of GnRH that stimulates an increase in LH also stimulates an increase in estradiol; and (4) a physiologic dose of GnRH stimulates an increase in PRL.

2. Materials and methods

2.1. Animals, GnRH product, and days of treatment

Dairy heifers (Holsteins) weighing 400–550 kg were used during August and September in the northern temperate zone. The management of heifers, including housing and feeding (Palhao et al., 2009) and ultrasonographic monitoring for ovulation detection (Ginther, 1998), has been described. Heifers had free access to hay (grass and alfalfa mixture), trace-mineralized salt, and water. Animals were selected with docile temperament and no apparent abnormalities of the reproductive tract, as determined by ultrasonographic examinations (Ginther, 1998). A CL area of <2.5 cm^2 on Day 14 (Day 0 = ovulation) was used as an indicator that early luteolysis may be underway (Kastelic et al., 1990), and the heifer was not used.

An indwelling jugular venous catheter for frequent collection of blood samples was inserted as described (Ginther et al., 2011a) about 12 h before the beginning of an experiment. Head restraint by either a stanchion or halter was not used during collection of samples. Heifers were given free access to water and hay between collection of samples. Fertagyl (gonadorelin; Intervet Inc., Millsboro, DE, USA) was used for the GnRH treatment. According to the