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Characteristics and retention of luteal structures, extended postinsemination cycle, progesterone, and pregnancy-specific protein B in serum after human chorionic gonadotropin treatment of dairy cows[1]

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ABSTRACT

Our objectives were to determine characteristics (size, number, and stayability) of luteal structures formed in response to human chorionic gonadotropin (hCG) administered on d 7 after timed artificial insemination (AI) and the influence of hCG on returns to estrus and pregnancy outcome. Holstein cows (n = 328), milked 3 times daily, previously inseminated at first service were assigned randomly to a completely randomized design consisting of 2 treatments when at least 1 corpus luteum (CL) was detected on d 7 after AI. Treatment consisted of 1,000 IU hCG or 1 mL of saline (control) administered i.m. Blood was collected and luteal structures were mapped and sized by transrectal ultrasonography on d 7, 14, 21, 28, and 32 after AI. Blood also was collected on d 60 in all pregnant cows. Treatment with hCG induced new luteal structures in 70% of cows, regardless of pregnancy status or number of pretreatment CL. Cows producing greater than the median 46 kg of energy-corrected milk per day were less likely to respond to hCG. The number of total luteal structures per cow, original CL volume, and total luteal volume (original CL + new luteal structures) were increased by hCG. Progesterone concentration was greater in pregnant than nonpregnant cows on d 14 unless cows responded to hCG by forming new luteal structures. Concentrations of progesterone were greatest in pregnant, hCG-treated cows. Pregnancy per AI at d 32 or 60 after first AI was less in hCG- than saline-treated cows because pregnancy outcome for hCG cows that had only 1 pretreatment CL and failed to respond to hCG was only 55 to 61% of that observed in controls. Proportions of cows returning to estrus from 18 to 25 d after AI were less in hCG than control cows but greater for cows returning >25 d. Regardless of treatment, 25% of cows in both treatments retained at least 1 original CL to d 28 after AI and were not pregnant on d 32. Progesterone concentrations in these nonpregnant cows with retained CL between d 14 and 28 after AI were intermediate between nonpregnant cows that returned to estrus by d 25 and all pregnant cows. Concentrations of pregnancy-specific protein B were elevated in some of these nonpregnant, CL-retained cows, indicating early pregnancy loss. Retention of original luteal tissue in nonpregnant cows to d 28 after AI indicated that pregnancy had been initiated but failed, as verified by concentrations of progesterone and pregnancy-specific protein B.

Key words: human chorionic gonadotropin, corpus luteum, progesterone, pregnancy-specific protein B

INTRODUCTION

Luteal structure(s) (corpus luteum or corpora lutea; **CL**) that form after ovulation of bovine ovarian follicles are critical for facilitating pregnancy because they secrete progesterone, which is essential to development of the conceptus and future pregnancy recognition (Mann and Lamming, 1999). Conception failure is coincident with less-than-normal concentrations of progesterone as early as d 6 after insemination (Thatcher et al., 2001). In general, blood concentrations of progesterone rise earlier and achieve greater concentrations in pregnant than in nonpregnant cows (Thatcher et al., 2001). Progesterone stimulates normal embryo development (Thatcher et al., 1994) and the ability of the conceptus to secrete the antiluteolytic signal IFN-τ (Mann et al., 1999).

A meta-analysis of 17 progesterone-supplementation studies indicated that treatment during the first week after AI enhanced conception rates but had little or no effect when administered during the second or third weeks after AI (Mann and Lamming, 1999). Several treatments have been used to increase peripheral concentrations of progesterone after AI, including those that increase endogenous function of the existing CL,

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induce accessory luteal structures, or supplement progestin or progesterone directly (Stevenson et al., 2007).

In recent years, human chorionic gonadotropin (**hCG**) has been evaluated for its ability to improve bovine fertility by altering ovarian function. Human chorionic gonadotropin has activity similar to LH, is able to bind to tissue LH receptors, and mimics the effects of LH by causing small luteal cells to increase progesterone synthesis (Niswender et al., 1985; 2000). When given early in the estrous cycle (d 3 to 5; d 0 = estrus), hCG can alter the proportions of small and large luteal cells in ewes (Farin et al., 1988), thus potentially changing total progesterone production by the CL, because approximately 80% of luteal progesterone is derived from large luteal cells (Niswender et al., 2000). When administered during the mid-luteal phase (d 10 to 15), hCG alters the duration of the estrous cycle (Eduvie and Seguin, 1982; Howard and Britt, 1990) and enhances endogenous concentrations of progesterone (Schmitt et al., 1996a; Santos et al., 2001; Stevenson et al., 2007) through luteotropic effects on the existing CL or by increasing the incidence of ovulation and accessory CL formation (Rajamahendran and Sianangama, 1992; Santos et al., 2001; Stevenson et al., 2007). In addition, luteal phase treatment with hCG after AI has increased conception rates in beef heifers (Breuel et al., 1989) and lactating dairy cows (Santos et al., 2001; Stevenson et al., 2007). In the latter most extensive study (Stevenson et al., 2007) of more than 1,500 cows, no positive effect of hCG in first-service cows was detected, but only in repeat-service cows and second-lactation cows when hCG was administered after AI.

Inducing accessory CL with GnRH or its agonists is well documented and forms the basis for the first GnRH injection of the Ovsynch protocol (Pursley et al., 1998). Subsequent work demonstrated that incidence of ovulation was greatest when GnRH was injected between d 5 and 12 of the estrous cycle (Vasconcelos et al., 1999). When injected on d 5 or 6, a GnRH agonist (8 μg of buserelin) was equally effective as 3,000 IU of hCG for inducing accessory CL, but the subsequent increase in concentrations of progesterone were greater in hCG-treated heifers (Schmitt et al., 1996a).

Lifespan or stayability of induced luteal structures is not known. We demonstrated that 1,000 IU of hCG induced accessory luteal structures in 50% of pregnant lactating cows between d 26 and 71 of pregnancy (Stevenson et al., 2008). Cows with new luteal structures had greater serum concentrations of progesterone at 1 and 2 wk after treatment than those without induced luteal structures, but approximately one-third of the new luteal structures regressed sometime during the 4-wk post-hCG study period. The majority of those disappearing luteal structures were gone by 2 wk after their induction, and regression occurred more often on the left ovary and contralateral to the CL of pregnancy.

We hypothesized that hCG would increase the number of post-AI luteal structures and progesterone concentration, thereby reducing embryonic loss between AI and the first diagnosis of pregnancy. The objective of the present study was to determine characteristics of luteal structures formed in response to hCG administered on d 7 after AI (first service); specifically, we wanted to find the frequency and characteristics of hCG-induced structures, including size, number, and stayability. Furthermore, we wished to determine whether duration of the estrous cycle or returns to estrus after AI were altered by hCG treatment as well as whether hCG improved pregnancy rate by increasing embryo survival between hCG treatment and d 32 after AI when pregnancy was first diagnosed.

MATERIALS AND METHODS

Experimental Approach

Lactating Holstein cows (n = 328) that previously received a timed AI (estrous cycles were presynchronized followed in 10 d by the Ovsynch protocol; Stevenson et al., 2012) at first service between 61 and 73 DIM were sorted by lactation number (1 vs. ≥2) and assigned to a completely randomized design consisting of 2 treatments (Figure 1; 164 cows per treatment) when at least 1 CL (visible cavity) was detected on d 7 after AI. More than 96% of the cows ovulated after the second GnRH injection of Ovsynch before first AI (Stevenson et al., 2012).

Treatment consisted of 1,000 IU of hCG (1 mL of Chorulon; Intervet Schering Plough Animal Health, Millsboro, DE) or 1 mL of physiological saline administered i.m. In this report, new or induced luteal tissue is referred to as luteal structure(s), rather than CL, because we have no evidence that ovulation (release of ovum) actually occurred even though ultrasonic appearance of these structures resembled other spontaneously formed or GnRH-induced CL.

The current studies were approved by the Kansas State University Institutional Animal Care and Use Committee. Cows were housed in covered freestalls and fed twice or three times (summer) daily a TMR calculated to meet nutrient requirements for lactating dairy cows producing 50 kg of 3.5% milk (NRC, 2001). The diet consisted of alfalfa hay, corn silage, soybean meal, whole cottonseed, corn or milo grain, corn gluten feed, vitamins, and minerals. Cows were milked every 8 h in a double 6 Herringbone milking parlor.