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Effect of progesterone concentration and duration of proestrus on fertility in beef cattle after fixed-time artificial insemination

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ABSTRACT

The objective was to determine the effect of plasma progesterone concentration and the duration of proestrus during growth of the ovulatory follicle on fertility in beef cattle. Heifers (N = 61) and postpartum cows (N = 79) were assigned randomly to four groups in a two-by-two design involving luteal-phase versus subluteal-phase plasma progesterone concentrations and normal versus short proestrus. To synchronize follicular wave emergence, estradiol-17β was given im during the midluteal phase (Day 0) and concurrently, a once-used controlled intravaginal progesterone-releasing device was placed intravaginally. In the subluteal-phase progesterone groups, a luteolytic dose of $PGF_{2\alpha}$ was given on Day 0 and again 12 hours later. In the luteal-phase progesterone groups, $PGF_{2\alpha}$ was not given (so as to retain a functional CL). The controlled intravaginal progesterone-releasing device was removed and $PGF_{2\alpha}$ was given on Days 7 or 8 in the normal- and short-proestrus groups, respectively. Cattle were given lutropin im 12 or 36 hours later in the short- and normal-proestrus groups, respectively, with AI at 12 hours after lutropin treatment. Transrectal ultrasonography was used to monitor ovarian response during treatments and to diagnose pregnancy 60 days after AI. Cattle (heifers and cows combined) in the subluteal-phase progesterone groups and normal proestrus groups had a larger follicle at the time of AI, and a larger CL that secreted more progesterone 9 days after AI than cattle with luteal-phase progesterone concentrations or those with short proestrus ($P < 0.03$). There was a higher incidence of ovulation ($P < 0.01$) the day after AI in heifers (55/61; 90%) than in cows (44/79; 56%). Pregnancy rates ranged from 11% to 54%, and were higher in cattle (heifers and cows combined) in the subluteal-phase progesterone groups and normal proestrus groups than in the luteal-phase progesterone or short proestrus groups, respectively, ($P < 0.02$). In conclusion, a short proestrous interval reduced pregnancy rate after fixed-time AI in beef cattle. A low progesterone environment during growth of the ovulatory follicle increased the preovulatory follicle size and subsequent CL size and function, and compensated for the effect of a short proestrus on pregnancy rates.

1. Introduction

The purpose of fixed-time AI protocols is to permit insemination during a preplanned interval, thereby eliminating the necessity of estrus detection and minimizing time and labor costs. Fixed-time AI protocols involve synchronization of follicular wave emergence and ovulation. Synchronization of follicular wave emergence might be achieved by GnRH-induced ovulation [1], ultrasound-guided follicular ablation [2], or treatment with estradiol and progesterone [3–6]. Synchronization protocols often involve the use of an intravaginal progesterone-releasing device for 5 to 9 days, $PGF_{2\alpha}$ at the time of device removal, and treatment with LH, GnRH, or estradiol 24 to 72 hours later to induce ovulation, reviewed in other work [7].

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Progestin devices have been used for intervals up to 15 days [8] and might be used more than once (with appropriate treatments to ensure hygiene) to minimize cost. Previously-used intravaginal devices induce subluteal-phase plasma progesterone concentrations ranging from 0.5 to 2 ng/mL [9,10].

Progesterone has a negative feedback effect on LH secretion [11] and subluteal-phase plasma progesterone concentrations resulted in elevated LH pulse-frequency in cattle [12,13]. The effect of negative feedback on LH secretion was demonstrated in an earlier study in which progesterone suppressed the growing phase of the dominant follicle in a dose-dependent manner [14]. Administration of exogenous progesterone for 10 days resulted in lower fertility, possibly caused by maturational changes in the oocyte before a gonadotropin surge [15]. However, fertility did not differ between beef heifers in which an intravaginal progesterone device was in place for 6 versus 3 days [16]. In more recent reports [9,17] in which a previously used intravaginal progesterone device was in place for a shorter duration, no adverse effects on fertility were observed. Moreover, preovulatory follicle diameter was increased when a previously-used device was used [9]. In this regard, there was positive relationship between ovulatory follicle diameter and fertility [18]. We reported that maintaining subluteal-phase progesterone for a short interval during follicular growth resulted in an oocyte with improved *in vitro* fertilization capabilities compared with those growing in a high-progesterone environment [19]. Based on these findings, we propose using subluteal-phase progesterone concentrations during dominant follicle growth to attain a larger preovulatory follicle and greater fertility compared with normal luteal progesterone concentrations.

Another related aspect of dominant follicle growth in the preovulatory wave is the progesterone-free interval (i.e., proestrus) between spontaneous luteolysis and onset of estrus. In natural estrous cycles, the duration of proestrus is 3 to 4 days, whereas induction of ovulation in most fixed-time AI protocols involves administration of an ovulation-inducing agent much earlier after progestin device removal; i.e., estradiol at 24 hours, lutropin (pLH) at 48 hours, or GnRH at 48 to 72 hours [20]. Therefore, fixed-time AI protocols usually involve a shortened proestrus interval, with a potential effect on fertility. This notion has been addressed in a recent synchronization study [21] in which placement of an intravaginal progesterone device for 5 days with longer proestrus (72 hours) resulted in an increased pregnancy rate compared with 7-day placement with shorter proestrus (60 hours).

We hypothesized that: (1) exposure of the growing dominant follicle to luteal-phase plasma progesterone concentrations followed by a short duration of proestrus results in lower fertility than longer proestrus; and (2) exposure of the growing dominant follicle to subluteal-phase progesterone concentrations overcomes the effects of short proestrus. The experiment was designed to compare the effects of changes in duration of proestrus and progesterone concentration on follicular development and fertility after fixed-time AI in beef cows and heifers.

2. Materials and methods

Hereford-cross cows (N = 85) and pubertal heifers (N = 62) were used during the months of May to August. The cows were between 2 and 8 years of age, weighed 400 to 750 kg, and were ≥35 days postpartum and lactating. The heifers were between 14 to 24 months of age and weighed 280 to 425 kg. The cattle were maintained on pasture, except on days of examination or treatment when they were housed in corrals and fed alfalfa hay and barley silage with free access to fresh water and mineral blocks. The experimental protocol was approved by the University Committee on Animal Care and Supply and conducted in accordance with the guidelines of the Canadian Council on Animal Care.

2.1. Animal groups and treatment protocol

Cattle were assigned randomly in four groups based on a two-by-two design with two levels of progesterone (luteal- vs. subluteal-phase) and durations of proestrus (normal vs. short; Fig. 1). The experiment was done in two replicates and all groups and parities were represented in both replicates. The level of progesterone was classified as luteal-phase (>1.5 ng/mL) and subluteal-phase (0.5–1.5 ng/mL) during the growing phase of the ovulatory follicle. This cutoff limit was based on progesterone concentrations in cattle 4 days after placement of a once-used controlled intravaginal progesterone-releasing device (CIDR, Pfizer Canada Inc., Montreal, Quebec, Canada) in previous studies [9,10]. The duration of proestrus was classified as normal (36 hours) and short (12 hours). Thus, the groups formed were referred to as: luteal-phase progesterone with normal proestrus (LN), luteal-phase progesterone with short proestrus (LS), subluteal-phase progesterone with normal proestrus (SuN), and subluteal-phase progesterone with short proestrus (SuS).

At the beginning of the treatment protocol, all cattle were given 500 μg cloprostenol im ($PGF_{2\alpha}$; Estrumate; Schering-Plough Animal Health, Pointe-Claire, Quebec, Canada) twice, 11 days apart, to synchronize estrus. At the time of the second $PGF_{2\alpha}$ treatment, ovaries were examined by transrectal ultrasonography using a 7.5-MHz linear-array transducer (Aloka SSD-900; Tokyo, Japan) and cattle were assigned randomly to the four treatment groups (Fig. 1). Those in which a CL was not detected (N = 6) were distributed evenly among treatment groups.

Nine days later (5–8 days after expected ovulations), cattle were given 1 mg (heifers) or 1.5 mg (cows) im estradiol-17β (Catalog #E8875, Sigma-Aldrich; St. Louis, MO, USA) dissolved in canola oil (0.5 mg estradiol-17β per mL of canola oil) to synchronize follicular wave emergence [5]. A once-used CIDR (uCIDR, previously used for 7 days) was placed in the vagina to maintain subluteal-phase plasma progesterone concentrations [9]. Concurrently (Day 0), cattle in the subluteal-phase progesterone groups (SuN, SuS) were given a luteolytic dose of $PGF_{2\alpha}$ and another 12 hours later, and those in the luteal-phase progesterone groups (LN, LS) were allowed to retain their functional CL. The uCIDR was removed on Day 7 (LN, SuN) or Day 8 (LS, SuS). Cattle were given $PGF_{2\alpha}$ on the day of