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The effect of exogenous follicle stimulating hormone (FSH) and endogenous plasma leptin concentrations on the pregnancy rate of beef heifers subjected to fixed-timed artificial insemination (FTAI)

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ABSTRACT

In this study, 53 crossbred beef heifers were used to test the hypotheses that administration of exogenous FSH 2 days following CIDR insertion and administration of estradiol would increase the pregnancy rate in heifers synchronized for FTAI and that plasma leptin concentrations in beef heifers would be higher for heifers that became pregnant to FTAI. The heifers used in this study had a median age of 440 days, an average weight of 324 kg, an average body condition score of 5.1 and a mean reproductive tract score of 3.1. Heifers were stratified by weight and BCS into two groups and then treatments were randomly allotted to each group: (1) control ($n = 28$) or (2) FSH ($n = 27$). Both groups were administered 200 mg estradiol benzoate (EB) and received an intravaginal controlled internal drug-releasing device (CIDR) on day 0. On day 2, females in the FSH treatment group were administered 20 mg of FSH, while the control group received 1 ml of saline. On day 7 all females were administered 25 μg $PGF_{2\alpha}$ and the CIDR was removed. Then 24 h following CIDR removal all females were administered 1 mg EB and 24 h later were subjected to FTAI. Pregnancy diagnosis was performed via transrectal ultrasonography 43 days following insemination. Blood samples were collected via jugular venipuncture on days 2, 6–10, 13 and 52 and plasma leptin concentrations were determined by radioimmunoassay. Pregnancy rates were higher ($P = 0.01$) for FSH-treated females (60%) compared with females not receiving FSH (25%). Circulating plasma leptin concentrations were higher ($P = 0.0051$) for pregnant females compared with females that did not become pregnant following FTAI during the experiment. Mean plasma leptin concentration was also higher ($P = 0.04$) from day 2 to day 9 during the synchronization protocol in heifers that became pregnant compared with heifers that did not become pregnant from FTAI. There was no difference ($P = 0.38$) in reproductive tract scores for heifers that became pregnant compared with heifers that did not become pregnant from FTAI. Circulating leptin concentrations were not different ($P = 0.11$) for females receiving FSH compared with females in the non FSH-treated group. Circulating leptin concentrations were affected by sampling day ($P < 0.0001$). However, there was no interaction between sampling day and pregnancy status ($P = 0.80$), treatment and pregnancy status ($P = 0.14$) or treatment and sampling day ($P = 0.12$). These results indicate that the administration of FSH on day 2 of the synchronization protocol may increase pregnancy rates in beef heifers and that increased circulating concentrations of plasma leptin during the synchronization protocol may be indicative of subsequent pregnancy outcome.

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1. Introduction

Follicular development occurring during the bovine estrous cycle has been characterized as having follicular growth culminating in a pattern of 2 or 3 distinct waves (Adams et al., 1992). These multiple waves are described as cohorts of 4–5 mm follicles emerging on the day of ovulation and growing throughout diestrus with a single follicle achieving dominance and ending in ovulation of that follicle in the 2nd or 3rd wave (Ginther et al., 1989). Increases of endogenous follicle stimulating hormone (FSH) precede wave emergence in each case (Adams et al., 1992; Sunderland et al., 1994). However, to date we have found no studies evaluating the effect of exogenous FSH on follicular turnover or initiating wave emergence.

The efficiency of any fixed-timed artificial insemination (FTAI) protocol is associated with the ability to control follicular wave emergence through initiation of follicular turnover and the subsequent regression of the corpus lutea in cattle (Bo et al., 1995; Fricke et al., 1998; Bó et al., 2003). In most FTAI protocols, either a gonadorelin or estrogens are utilized to control follicular development by either ovulation of the dominant follicle or directing atresia of the dominant follicle shortly after administration. Theoretically, this initiates a release of a cohort of follicles synchronous in development in treated females where one follicle achieves dominance and is scheduled to ovulate at a pre-determined time. However, timing of gonadorelin administration relative to stage of follicular development has been associated with reduced efficacy associated with follicular turnover and the initiation of a new follicular wave. Wave emergence following gonadorelin treatment typically occurs 2.5 days following administration when the affected follicle is dominant or in the growing stage (Twagiramungu et al., 1994). However, administration of estradiol does not exhibit the same stage dependent association on follicular turnover and wave emergence, which typically occurs 4.3 days after treatment in follicles that are growing, static or regressing (Bo et al., 1995).

For estrous synchronization protocols to be effective heifers must have reached puberty. Increased luteinizing hormone (LH) pulse frequency has been associated with the attainment of puberty in heifers (Day et al., 1986) and exogenous administration of leptin has been reported to increase release of LH in domestic ruminants (Amstalden et al., 2002; Henry et al., 2004). Similarly, increased endogenous concentrations of the adipose derived hormone leptin have been negatively associated with length of postpartum intervals in beef cattle (Strauch et al., 2003). Therefore, it appears as though circulating concentrations of leptin may be an indicator or a predictor of pregnancy outcome in cattle.

Therefore, the objectives of this study were to first determine if the administration of exogenous FSH 2 days following CIDR insertion and administration of estradiol would increase the pregnancy rate in beef heifers synchronized for FTAI. The second objective was to characterize plasma leptin concentrations in beef heifers synchronized for FTAI to determine if levels of this hormone could be utilized to predict the odds of becoming pregnant following synchronization of estrus for FTAI.

2. Materials and methods

2.1. Animals

Yearling crossbred (British XBrahman) beef heifers ($n = 53$) maintained at the Louisiana Agricultural Center's Reproductive Biology Center, Saint Gabriel, LA, USA (30°N and 91°W) were used in this experiment. The age of the heifers at the start of the breeding season ranged from 398 to 457 days (median 440). Seven days prior to the breeding season the heifers were weighed and body condition scored (BCS) using a 9-point scale (Whitman, 1975) and their reproductive tract scored (Anderson et al., 1991) (Table 1). Heifers weighed an average of 324 kg (range: 281–415 kg), exhibited an average BCS of 5.1 (range: 4.5–6.0) and a mean reproductive tract score of 3.1 (range 1–5). Heifers were stratified by weight and BCS into two groups and then treatments were randomly allotted to each group, either control ($n = 28$) or FSH ($n = 27$). Starting 101 days prior to estrus synchronization and throughout the study heifers were allowed ad libitum access to winter ryegrass and mixed grass hay. Two animals were removed from the FSH treated group, one due to a health problem and the second was identified to be a freemartin twin.

2.2. Synchronization of estrus

Both groups were synchronized for FTAI using the co-synch protocol (Fig. 1) and all injections were administered intramuscularly. Briefly, on day 0 all females were administered 200 mg estradiol benzoate (EB) and received a controlled internal drug releasing device (CIDR). On day 2, females in the FSH treatment group were administered 20 mg FSH (1 ml), while the control group received 1 ml of saline. This FSH dose was chosen to decrease the instance of multiple ovulations. On day 7 all females were administered 25 μg $PGF_{2\alpha}$ and the CIDR was removed. 24 h following CIDR removal all females were administered 1 mg EB and 24 h later were subjected to FTAI. All heifers were inseminated using frozen-thawed semen from a single fertile beef bull. Pregnancy diagnosis was performed via trans-rectal ultrasonography on day 52 (43 days post-insemination) and the resulting fetuses were aged using the procedure described by White et al. (1985).

2.3. Blood sampling

Blood samples were collected via jugular venipuncture on days 2, 6–10, 13 and 52 (CIDR in = day 0). Plasma leptin concentrations were determined by radioimmunoassay using a primary antiserum generated against a highly conserved segment of leptin encompassing the connecting loop between the A and B α-helices (Richards et al., 2000). It was used with radio-iodinated human recombinant leptin resulting in an assay specific for leptin in samples from horses (Cartmill et al., 2003), cattle, sheep, and pigs. Serial dilutions of bovine plasma run in conjunction with serial dilutions of recombinant bovine leptin standard (100 ng/mL) produced parallel inhibition curves. The minimum detection limit of leptin was 0.2 ng/ml and