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Follicle stimulating hormone secretion and dominant follicle growth during treatment of *Bos indicus* heifers with intra-vaginal progesterone releasing devices, oestradiol benzoate, equine chorionic gonadotrophin and prostaglandin $F_{2\alpha}$

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ABSTRACT

The aim of this study was to investigate the effects on follicle stimulating hormone (FSH) secretion and dominant follicle (DF) growth, of treatment of *Bos indicus* heifers with different combinations of intra-vaginal progesterone releasing devices (IPRD), oestradiol benzoate (ODB), $PGF_{2\alpha}$ and eCG. Two-year-old Brahman (BN; $n = 30$) and Brahman-cross (BNX; $n = 34$) heifers were randomly allocated to three IPRD-treatments: (i) standard-dose IPRD [CM 1.56 g; 1.56 g progesterone (P_4); $n = 17$]; (ii) half-dose IPRD (CM 0.78 g; 0.78 g P_4; $n = 15$); (iii) half-dose IPRD + 300 IU eCG at IPRD removal (CM 0.78 g + G; $n = 14$); and, (iv) non-IPRD control ($2 \times PGF_{2\alpha}$; $n = 18$) 500 μg cloprostenol on Days −16 and −2. IPRD-treated heifers received 250 μg $PGF_{2\alpha}$ at IPRD insertion (Day −10) and IPRD removal (Day −2) and 1 mg ODB on Day −10 and Day −1. Follicular dynamics were monitored daily by trans-rectal ultrasonography from Day −10 to Day 1. Blood samples for determination of P_4 were collected daily and samples for FSH determination were collected at 12 h intervals from Day −9 to Day −2. A significant surge in concentrations of FSH was observed in the $2 \times PGF_{2\alpha}$ treatment 12 h prior and 48 h after follicular wave emergence, but not in the IPRD-treated heifers. Estimated mean concentrations of total plasma P_4 during the 8 days of IPRD insertion was greater ($P < 0.001$) in the CM 1.56 g P_4 treated heifers compared to the CM 0.78 g P_4 treated heifers (18.38 ng/ml compared with 11.09 ng/ml, respectively). A treatment by genotype interaction ($P = 0.036$) was observed in the mean plasma P_4 concentration in heifers with no CL during IPRD insertion, whereby BN heifers in the CM 1.56 g treatment had greater plasma P_4 than the BNX heifers on Days−9, −7, −6, −5, and −4. However, there was no genotype effect in the CM 0.78 g ± G or the $2 \times PGF_{2\alpha}$ treatment. Treatment had no effect on the DF growth from either day of wave emergence ($P = 0.378$) or day of IPRD removal ($P = 0.780$) to ovulation. This study demonstrates that FSH secretion in *B. indicus* heifers treated with a combination of IPRD's and ODB to synchronise ovulation was suppressed during the period of IPRD insertion but no significant effect on growth of the DF was observed.

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1. Introduction

The development of effective ovulation synchronisation protocols to enable fixed-time artificial insemination (FTAI) of extensively managed *Bos indicus* heifers in the north Australian rangelands is highly desirable as a means of accelerating the rate of genetic improvement in these herds. However, less than expected pregnancy rates after FTAI have been reported (Bo et al., 1995; Diskin et al., 2002) in *B. indicus* heifers treated to synchronise time of ovulation among heifers with intravaginal progesterone releasing device (IPRD) + oestradiol benzoate (ODB) protocols. More recently, a moderate prevalence of ovarian dysfunction has been observed in *B. indicus* heifers treated with similar IPRD + ODB protocols (Butler et al., 2011).

Synchronous ovulation of normally developed dominant follicles (DF) is required to achieve acceptable pregnancy rates to FTAI. A combination of progesterone (P_4) or progestagen and ODB treatments are commonly used to induce a new follicular wave (Bo et al., 2003) and development of a DF capable of ovulating and subsequently developing into a normal CL (Diskin et al., 2002). A significant surge in FSH secretion initiates the emergence of a follicular wave and growth of a new cohort of follicles in *Bos taurus* heifers (Adams, 1998). However, a significant surge in circulating FSH prior to wave emergence has not been reported in normally oestrous cycling *B. indicus* heifers (Castilho et al., 2007), and there are very few reports describing the pattern of FSH secretion in these genotypes. The follicular wave dynamics are similar in *B. indicus* and *B. taurus* heifers (Rhodes et al., 1995; Bo et al., 2003). The prevalence of Brahman (*B. indicus*) heifers with two, three and four DFs emerging through the oestrous cycle is reported to be 10%, 61% and 29%, respectively. The maximum diameter of the DF has been reported to be smaller in *B. indicus* than *B. taurus* genotypes (Bo et al., 2003), with the mean maximum diameter observed to be 9.5 ± 0.5 mm and 11.6 ± 0.5 mm ($P < 0.01$), respectively in a recent study of the two genotypes (Carvalho et al., 2008).

Growth of the DF prior to ovulation is an important determinant of the quality of the resulting corpus luteum (CL; Robinson et al., 2005). In an attempt to improve pregnancy rates to FTAI in *B. indicus* heifers, different strategies have been investigated to increase the likelihood that during the period of DF recruitment and growth, plasma P_4 concentrations are similar to those in normal cycling females. This has been achieved by luteolysis of any CL present at the time of IPRD insertion or insertion of an IPRD containing a lesser amount of P_4; both reported to increase DF diameter (Carvalho et al., 2008; Dias et al., 2009; Peres et al., 2009) and growth rate of the DF (Carvalho et al., 2008). Further, the plasma concentration of P_4 resulting from treatment with an IPRD (1.9 g P_4) is dependent on genotype, with *B. indicus* heifers having 42% and 30% greater plasma P_4 concentrations than *B. taurus* and *B. taurus* × *B. indicus* heifers, respectively (Carvalho et al., 2008). In addition, genotype also had an effect ($P = 0.002$) on the ovulation rate after IPRD (1.9 g P_4) treatment, with 39.1%, 72.7% and 84.0% of *B. indicus*, *B. taurus* and *B. taurus* × *B. indicus* heifers ovulating, respectively (Carvalho et al., 2008). In conclusion, it is hypothesised that treatment of *B. indicus* heifers with IPRD + ODB synchronisation protocols developed for use in *B. taurus* cattle, may negatively affect FSH secretion increasing the risk of sub-optimal development of the DF, ovulation and subsequent development of the CL.

The primary objectives of this study were to compare the patterns of FSH secretion and DF dynamics in *B. indicus* heifers treated with different IPRD + ODB protocols designed to synchronise ovulation, and to compare these with that observed in heifers treated with a standard double $PGF_{2\alpha}$ (non-IPRD) protocol.

2. Materials and methods

2.1. Heifers

Heifers were selected and maintained during the trial according to (Butler et al., 2011). Two-year-old heifers were sourced from two commercial beef cattle properties: (i) 34 Brahman crossbred heifers (BNX; 3/4 to 7/8 Brahman content) with an average body weight (BW) of 299 kg (range 250–363 kg) at experiment induction and (ii) 30 Brahman (BN) heifers with an average BW of 297 kg (248–323 kg). The heifers enrolled in the study were considered typical of Brahman heifers which would be mated at this time in northern Australia. More BNX heifers (16/34; 47.1%) than BN heifers (4/30; 13.3%) had a CL detected by ultrasonography at either Day −32 or Day −20 (Day 0 = synchronised oestrus).

Heifers were managed in the Brigalow Research Station Feedlot located in central Queensland (24°50′13″S and 149°47′33″E) for the duration of the trial. Heifers were fed once a day in the afternoon at 3:00 pm with a barley based starter, intermediate and finisher ration for seven, nine and 55 days, respectively in a stepwise manner after trial induction.

2.2. Ovulation synchronisation protocols

The treatment and data collection schedule is outlined in Fig. 1 The *B. indicus* content (BN or BNX), LW, body condition score (BCS) and presence or absence of a CL on Day −32 were used in a randomised block design to allocate heifers to eight feedlot pens and one of four synchronisation protocols: (i) full-dose IPRD (Cue-Mate®; Bioniche Animal Health Aust/Asia; two P_4-impregnated pods each containing 0.78 g P_4; CM 1.56 g; $n = 17$); (ii) half-dose IPRD (Cue-Mate®; Bioniche Animal Health Aust/Asia; one P_4-impregnated pod containing 0.78 g P_4; CM 0.78 g; $n = 15$); (iii) half-dose IPRD + 300 IU eCG (Pregnecol®; Bioniche Animal Health, Aust/Asia; CM 0.78 g + G; $n = 14$) and (iv) $2 \times PGF_{2\alpha}$ (500 μg cloprostenol; Estromil, Ilium Veterinary Products, Smithfield, Australia; $n = 18$). The half-dose IPRD was prepared by removing one of the two P_4-impregnated silicone pods from the standard Cue-Mate® device and replacing it with a silicone pod that contained no progesterone. Heifers in the CM 1.56 g, CM 0.78 g and CM 0.78 g + G treatments received 1 mg ODB (Cidirol; Genetics Australia, Bacchus Marsh, Victoria, Australia) at IPRD insertion (Day −10) and 24 h after IPRD removal (Day −1) and 250 μg cloprostenol im. at IPRD insertion (Day −10 (Carvalho et al., 2008)) and removal (Day −2). Heifers in the $2 \times PGF_{2\alpha}$