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Ovarian responses in *Bos indicus* heifers treated to synchronise ovulation with intravaginal progesterone releasing devices, oestradiol benzoate, prostaglandin $F_{2\alpha}$ and equine chorionic gonadotrophin

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ABSTRACT

The objectives were: (i) improve understanding of the ovarian responses of *Bos indicus* heifers treated with different ovulation synchronisation protocols, (ii) compare ovarian responses of *B. indicus* heifers treated with intravaginal progesterone releasing device (IPRD) + oestradiol benzoate (ODB) versus a conventional prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) protocol and (iii) investigate whether reducing the amount of progesterone (P_4) in the IPRD, and treatment with equine chorionic gonadotrophin (eCG) would increase the proportion of heifers with normal ovarian function during the synchronised and return cycles. Two-year-old Brahman ($n = 30$) and Brahman-cross ($n = 34$) heifers were randomly allocated to three IPRD-treatment groups: (i) standard-dose IPRD (Cue-Mate® 1.56 g P_4; $n = 17$); (ii) half-dose IPRD (Cue-Mate® 0.78 g P_4; $n = 15$); (iii) half-dose IPRD + 300 IU eCG at IPRD removal ($n = 14$), and a non-IPRD control group (iv) 2 × $PGF_{2\alpha}$ (500 μg cloprostenol) on Days −16 and −2 ($n = 18$). IPRD-treated heifers received 250 μg cloprostenol at IPRD insertion (Day −10) and IPRD removal (Day −2) and 1 mg ODB on Days −10 and −1. Ovarian function was evaluated by ultrasonography and plasma P_4 throughout the synchronised and return cycles. The mean diameter of the dominant follicle observed at 54–56 h after IPRD removal, was greater for heifers which ovulated than heifers which did not ovulate ($P < 0.001$; 14.5 ± 1.1 vs. 9.3 ± 0.6 mm, respectively). The prevalence of IPRD-treated heifers with ovarian dysfunction (persistent CL, failure to re-ovulate, shortened luteal phase) was 39%. This relatively high prevalence of ovarian dysfunction may explain the commonly reported, lower than expected pregnancy rates to FTAI in *B. indicus* heifers treated to synchronise ovulation.

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1. Introduction

The potential benefits of AI have yet to be realised in extensively managed *Bos indicus* and *B. indicus* crossbred cattle herds in northern Australia. A major constraint to the adoption of AI in these herds is the difficulties in detecting oestrus in *B. indicus* genotypes which characteristically have reduced expression of oestrus (Galina et al., 1982) compared to *Bos taurus* genotypes. Treatment protocols to synchronise ovulation remove the need for oestrus detection and enable AI at a fixed-time (FTAI). However, variable responses to treatment protocols to synchronise ovulation, and lower than expected pregnancy rates, continue to limit the adoption of FTAI (Bo et al., 1995; Diskin et al., 2002). Further most ovulation synchronisation protocols have been developed for use in *B. taurus* genotypes and it has been assumed that these protocols will result in similar pregnancy rates to FTAI in *B. indicus* genotypes.

Typical FTAI protocols utilise combinations of progestin and progesterone (P_4) implants, oestradiol benzoate (ODB), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and equine chorionic gonadotrophin (eCG) (McGowan, 1999; Bo et al., 2003, 2007; Baruselli et al., 2004, 2008). However, the response of *B. indicus* heifers to these FTAI protocols is often quite variable in terms of the proportion of heifers that show oestrus within 2–3 days (44–98%) and the proportion that become pregnant (35–62%) (McGowan, 1999; Phillips et al., 2010). In a study of the ovarian response of Nelore and Gir (*B. indicus*), Nelore × Angus (*B. indicus* × *B. taurus*), Angus × Holstein (*B. taurus* × *B. taurus*) heifers (Carvalho et al., 2008), treatment with $PGF_{2\alpha}$ at the time of insertion of an IPRD containing 1.9 g P_4 lowered plasma P_4 concentrations during the period of IPRD insertion, and increased the maximum diameter of the dominant follicle (DF) and the proportion of heifers which ovulated. Further, this study also demonstrated that genotype significantly influenced the ovarian response to the IPRD + ODB ovulation synchronisation protocol; a significantly lower proportion of Nelore and Gir heifers ovulated (39%) compared with Nelore × Angus (84%) and Angus and Holstein (73%) heifers (Carvalho et al., 2008). In a preliminary study of the ovarian response of two-year-old cycling Brahman heifers treated with an IPRD + ODB protocol similar to that used by Carvalho et al. (2008), Butler et al. (2008) reported that although seven out of 10 treated heifers ovulated only 2 subsequently developed a normal functional corpus luteum (CL). Therefore, it may be concluded that treatment of *B. indicus* heifers with currently recommended IPRD + ODB ovulation synchronisation protocols might adversely affect follicular development, ovulation and subsequent CL development.

The objectives of this study were to: (i) improve our understanding of the ovarian responses of *B. indicus* heifers treated with different ovulation synchronisation protocols, (ii) compare the ovarian responses of *B. indicus* heifers treated with IPRD + ODB protocols versus the conventional double $PGF_{2\alpha}$ protocol and (iii) investigate whether reducing the amount of P_4 in the IPRD, and treatment with eCG would increase the proportion of heifers showing evidence of normal ovarian function during the synchronised and return cycles. The underpinning concept in this study was that *B. indicus* heifers which have a normal ovarian response following treatment to synchronise ovulation will have a normal probability of conceiving to FTAI, whereas heifers which have an abnormal ovarian response will have a reduced probability of conceiving.

2. Materials and methods

2.1. Experimental design and animals

The study was conducted in the spring at Agri-Science Queensland's, Brigalow Research Station Feedlot, Theodore, central QLD (24°50′13″S 149°47′33″E). Ethical approval for this animal research was granted by The University of Queensland's Animal Ethics Committee – approval number: SVS/605/07/CRC-APA. All heifers used in the study were considered typical replacement heifers which would be normally mated during that breeding season in northern Australia. Two-year-old heifers were sourced from two commercial beef cattle properties: (i) Brahman crossbred (BNX) heifers (3/4 to 7/8 Brahman content; $n = 34$) with an average liveweight (LW) of 299 kg (range 250–363 kg) at study induction were sourced from north east of Rockhampton, QLD (23°15′S 150°65′E) (ii) Brahman (BN) heifers ($n = 30$) with an average LW of 297 kg (range 248–323 kg) were sourced from south east of St Lawrence, QLD (22°45′S 149°65′E). 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. All heifers were vaccinated against bovine ephemeral fever (Websters Bovine Ephemeral Fever vaccine; Fort Dodge Animal Health; Baulkham Hills, Australia), botulism (Longrange®; Pfizer Animal Health, West Ryde, Australia) and the common clostridial diseases (Ultravac® 5 in 1; Pfizer Animal Health, West Ryde, Australia). At the start of the study a random sample of 10 heifers from each source were tested for antibodies to bovine viral diarrhoea virus (BVDV) and all tested negative, indicating that it was very unlikely that either group of selected heifers contained an animal persistently infected with BVDV.

The heifers were inducted into the research feedlot and fed three different barley based rations (i) starter ration (ME of 12.1 MJ/kg, 13.9% protein, 13% fibre) for 7 days after induction, (ii) intermediate ration (ME 12.8 MJ/kg, 13.1% protein and 12.7% fibre) for the next 9 days, and (iii) a finisher ration until study completion (ME 12.9 MJ/kg, 13.5% protein and 14.6% fibre). The heifers were managed in a research feedlot to (a) facilitate intensive monitoring of ovarian function, and (b) to ensure adequate nutrition throughout the study.

2.2. Ovulation synchronisation protocols

The heifers were allocated to one of three IPRD-treatment groups: (i) standard-dose IPRD (Cue-Mate®; Bioniche Animal Health, Aust/Asia and Bayer Animal Health Australia, Sydney, NSW, Australia) 1.56 g P_4; $n = 17$); (ii) half-dose IPRD (Cue-Mate® 0.78 g P_4; $n = 15$); (iii) half-dose IPRD (Cue-Mate® 0.78 g P_4) + 300 IU eCG (Pregnecol™, Bioniche Animal Health, Aust/Asia and Bayer Animal Health Australia, Sydney, NSW, Australia) at IPRD removal ($n = 14$),