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Effect of treatment of *Bos indicus* heifers with progesterone 0, 3 and 6 days after follicular aspiration on follicular dynamics and the timing of oestrus and ovulation

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ABSTRACT

Synchronisation of wave emergence is used to synchronise oestrus in cattle. The aim of this study was to determine if treatment with high concentrations of progesterone in *Bos indicus* heifers for 3 days would synchronise new wave emergence when treatment commenced at early, mid and late stages of follicular development. Heifers were treated with a sc silicone implant containing norgestomet from Days -7 to 9 and cloprostenol (IM) on Days -7 and -2. All follicles > 4 mm in diameter were removed by transvaginal follicular aspiration either on Days 0 (Experiment 1), 3 (Experiment 2) or 6 (Experiment 3). From Days 6 to 9 every heifer was treated with two intravaginal progesterone releasing inserts that each contained either no progesterone (Control, n = 8/experiment) or 3.12 g of progesterone (n = 8/experiment). Ovarian follicular development was monitored at least once daily following aspiration until oestrus and ovulation. In each experiment, treatment with progesterone significantly increased concentrations of progesterone in plasma from Days 6 to 9 compared to Control heifers. It also significantly delayed the day of emergence of the ovulatory follicle (1.6 ± 0.6 vs 8.6 ± 0.3; 4.1 ± 0.1 vs 8.6 ± 0.2; 7.0 ± 0.0 vs 9.3 ± 0.4, for Control vs progesterone treated heifers, respectively in Experiments 1 to 3) and the interval from implant removal to oestrus and ovulation. In conclusion, treatment with high concentrations of progesterone can synchronise wave emergence in *Bos indicus* heifers when administered at early, mid and late stages of follicular development.

1. Introduction

Precise control of the timing of the onset of oestrus and ovulation in cattle is necessary to facilitate artificial insemination (AI) in extensively managed beef cattle herds where daily detection of oestrus is impractical or not economic. As a result, treatments have been developed that synchronise oestrus and ovulation and enable artificial insemination of all cows on a single day (Bó et al., 2007; Sa Filho et al., 2013). Optimum fertility to AI occurs when cows are inseminated within 4 to 12 h from the onset of oestrus and before ovulation (Dransfield et al., 1998). Pregnancy rates to a timed insemination in cattle are frequently less when females are not in oestrus (Perry et al., 2007; Sa Filho et al., 2010; Dorsey et al., 2011; Echternkamp and Thallman, 2011) and have immature follicles (Perry et al., 2007; Sa Filho et al., 2010) at the time of AI, and when females do not ovulate close to the time of AI (Hockey et al., 2010). Fertility could be improved by developing protocols that help enable the majority of potential ovulatory follicles to be of adequate maturity before a timed AI occurs. This requires several physiological requirements to be met, one of which is that the timing of the emergence of pre-ovulatory follicles is synchronised among treated cattle (Cavalieri et al., 2006).

Experimentally, synchronisation of new wave emergence in cattle has been achieved by aspirating dominant follicles (Berfelt

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et al., 1994), inducing ovulation or luteinisation of dominant follicles with either hCG (Rajamahendran and Sianangama, 1992) or GnRH (Twagiramungu et al., 1995), inducing atresia of dominant follicles by administering oestrogen in combination with progesterone (Bo et al., 1995), or by administering progesterone (Anderson and Day, 1994; Cavalieri et al., 1998a; Cavalieri et al., 1998b; Cavalieri et al., 1998d). Administration of oestrogen in combination with progesterone or a progestogen has been widely used since the 1970′s to induce new wave emergence. It is economical and effective with new wave emergence expected 3–4 days after treatment (Bo et al., 1995). Since 2006, the use of oestradiol-17β and its derivatives within food producing cattle in Europe has been phased out (Lane et al., 2008). Use of oestradiol-17β and its derivatives is also prohibited in lactating dairy cows in Australia and in beef producing herds that are accredited to supply beef to the European Union. Derivatives of oestradiol-17β are also no longer available in North America for use in synchronisation programs. This has resulted in GnRH being used as the predominant means of synchronising new wave emergence. The ability of GnRH to induce new wave emergence is variable resulting in reductions in pregnancy rates when timed insemination strategies are used, particularly in heifers (Pursley et al., 1997).

Exogenous treatment with progesterone offers some potential to synchronise new wave emergence in cattle, but variable responses in inducing new wave emergence have been encountered in some studies. For example, in one study undertaken in *Bos indicus* heifers, following treatment with progesterone for 24 h, a smaller percentage of follicles in the growing phase of follicular development became atretic compared to follicles that had reached a plateau phase of development (Cavalieri et al., 1998d). In dairy cows treated with intravaginal progesterone releasing inserts, and either progesterone or oestradiol benzoate at the start of treatment, variability in the mean day of emergence of ovulatory follicles was greater in cows treated with progesterone at the start of treatment than those treated with oestradiol benzoate. This indicated that oestradiol benzoate was more effective than progesterone alone in synchronising new wave emergence in cows with follicles at different stages of development at the time of treatment (Cavalieri et al., 2003). In these studies, treatment with progesterone resulted in elevated concentrations for only 24 hours. It remains uncertain whether altering the dose or duration of treatment with progesterone could induce new wave emergence in the presence of growing or newly emerged follicles. The aim of this study was to test the hypothesis that treatment with a high concentration of progesterone in *Bos indicus* heifers for 3 days would synchronise new wave emergence after follicular aspiration and when treatment was imposed at times that coincide with early, mid and late stages of follicular development. Treatment with supraphysiological concentrations of progesterone were applied over a 3-day period to determine if there was any merit in exploring relationships between dose and duration of treatment with progesterone in future studies to synchronise new wave emergence.

2. Materials and methods

2.1. Animals and treatments

Three experiments were conducted to examine the effects of administration of progesterone for three days on the pattern of wave emergence following treatment. Each experiment was conducted at James Cook University, Townsville 19.3278 °S, 146.7583 °E. The experimental site was located within a dry tropical region characterised by warm, moist summers and dry winters. Heifers had ad lib access to pasture, hay and a supplement containing molasses, urea and copra meal.

An outline of the treatment protocol is illustrated in Fig. 1. On the first day of each experiment (Day – 7) nulliparous Brahman heifers, 2–3 years of age were first examined using transrectal ultrasonogrpahy with a 7.5 MHz transducer (Mylab 30, Medical Plus Australia Pty Ltd, Crows Nest NSW). Heifers in which a corpus luteum was observed in at least one ovary were selected for the study to ensure that heifers had ovulated before commencing treatments. Each animal was treated with a silicone implant containing 3 mg of norgestomet (Crestar, Intervet Australia Pty Ltd, Bendigo Australia) that was inserted subcutaneously in the dorsal surface of the ear to prevent ovulation (Cavalieri et al., 1998c). Heifers were treated with 0.5 mg of cloprostenol IM (Estroplan, Parnell Laboratories Pty LTD, Alexandria NSW) at the time of insertion of implants (Day -7) and again 5 days later (Day -2) to induce luteolysis. All follicles $>$ 4 mm in diameter were removed by transvaginal follicular aspiration either on Days 0 (Experiment 1), 3 (Experiment 2) or 6 (Experiment 3) of the study. On Day 6 cows were treated with two intravaginal progesterone releasing inserts each containing either no progesterone (Asp-0, Asp-3 and Asp-6, for Experiments 1–3, respectively) or 3.12 g of progesterone (total dose 6.24 g of progesterone; Asp-0-P4, Asp-3-P4 and Asp-6-P4, for Experiments 1–3, respectively). Each intravaginal inserts was fitted with four silicone, but progesterone free pods (Asp-0, Asp-3 and Asp-6 treatments) or four progesterone impregnated silicone pods (Asp-0-P4, Asp-3-P4 and Asp-6-P4 treatments, Bioniche Animal Health Australia/Asia Pty Ltd, Caulfield). Each progesterone impregnated pod contained 0.78 g of progesterone providing a total dose of 6.24 g of progesterone per heifer.

Transvaginal follicular follicular aspiration was performed using a 7.5 MHz curvilinear transducer and 19 g x 3 inch disposable needle. Epidural anaesthesia was applied by injection of 4 to 5 mL of lignocaine hydrochloride (Lignocaine 20, Troy laboratories Pty Ltd, Smithfield, NSW) into the first or second intercoccygeal space using a 20 G needle. Subcutaneous norgestomet releasing implants and all intravaginal inserts were removed from every heifer on Day 9 of the study to synchronise the onset of pro-oestrus and aids for the detection of oestrus (Estrotect, Genetics Australia, Bacchus Marsh, VIC) were applied over the sacral region just cranial to the base of the tail.

Experiments 1 and 2 were run concurrently. Experiment 3 commenced 86 days after the start of Experiments 1 and 2. In each experiment heifers were stratified by liveweight and age and then randomly assigned to intravaginal treatment without progesterone (n = 8/treatment) and intravaginal treatments with progesterone (n = 8/treatment). The mean weight of heifers in Experiments 1, 2 and 3 was 366.6 ± 8.64 kg, 361.9 ± 10.07 kg, and 429.4 ± 5.22 kg, respectively. Within each experiment the weight of heifers did not differ between treatments ($P > 0.690$).