Animal Reproduction Science 144 (2014) 1–5

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Prostaglandin treatment at the onset of norgestomet and estradiol-based synchronization protocols did not alter the ovarian follicular dynamics or pregnancy per timed artificial insemination in cyclic *Bos indicus* heifers

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ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form 6 November 2013
Accepted 22 November 2013
Available online 1 December 2013

Keywords:
Bovine
Hormonal therapy
Progesterone
Luteolysis
Nelore

ABSTRACT

The aim of the present study was to evaluate the effects of the $PGF_{2\alpha}$ treatment given at the onset of a synchronization of ovulation protocol using a norgestomet (NORG) ear implant on ovarian follicular dynamics (Experiment 1) and pregnancy per AI (*P*/AI; Experiment 2) in cyclic (CL present) *Bos indicus* heifers. In Experiment 1, a total of 46 heifers were presynchronized using two consecutive doses of $PGF_{2\alpha}$ 12 days apart. At first day of the synchronization protocol the heifers received implants containing 3 mg of NORG and 2 mg of estradiol benzoate (EB). At the same time, heifers were randomly assigned to receive 150 mg of D-cloprostenol ($n = 23$; $PGF_{2\alpha}$) or no additional treatment ($n = 23$; Control). When the ear implants were removed 8 days later, all heifers received a $PGF_{2\alpha}$ treatment and 1 mg of EB was given 24 h later. The follicular diameter and interval to ovulation were determined by transrectal ultrasonography. No effects of $PGF_{2\alpha}$ treatment on the diameter of the largest follicle present were observed at implant removal ($PGF_{2\alpha} = 9.8 \pm 0.4$ vs. Control = 10.0 ± 0.3 mm; $P = 0.73$) or after 24 h ($PGF_{2\alpha} = 11.1 \pm 0.4$ vs. Control = 11.0 ± 0.4 mm; $P = 0.83$). No differences in the time of ovulation after ear implant removal ($PGF_{2\alpha} = 70.8 \pm 1.2$ vs. Control = 73.3 ± 0.9 h; $P = 0.10$) or in the ovulation rate ($PGF_{2\alpha} = 87.0$ vs. Control = 82.6%; $P = 0.64$) between treatments were observed. In Experiment 2, 280 cyclic heifers were synchronized using the same experimental design described above ($PGF_{2\alpha}$; $n = 143$ and Control; $n = 137$), at random day of the estrous cycle. All heifers received 300 IU of equine chorionic gonadotropin (eCG) and 0.5 mg of estradiol cypionate (as ovulatory stimulus) when the NORG ear implants were removed. Timed artificial insemination (TAI) was performed 48 h after implant removal and the pregnancy diagnosis was conducted 30 days later. No effects on the *P*/AI due to $PGF_{2\alpha}$ treatment were observed ($PGF_{2\alpha} = 51.7$ vs. Control = 57.7%; $P = 0.29$). In conclusion, $PGF_{2\alpha}$ treatment at the onset of NORG-based protocols for the synchronization of ovulation did not alter the ovarian follicular responses or the *P*/AI in cyclic *Bos indicus* beef heifers synchronized for TAI.

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http://dx.doi.org/10.1016/j.anireprosci.2013.11.008

1. Introduction

Hormonal therapies have been to control both the follicular and the luteal phases of the estrous cycle in both *Bos taurus* and *Bos indicus* cattle (Pursley et al., 1995; Bó et al., 2003; Baruselli et al., 2004; Sá Filho et al., 2009; Sá Filho et al., 2011). The synchronization of ovulation protocols allow for the use of timed artificial insemination (TAI), without the need for estrus detection. However, the TAI programs in *Bos indicus* heifers lead to greater variation and lower pregnancy per AI (*P*/AI) in comparison to suckled *Bos indicus* cows (Peres et al., 2009; Sá Filho et al., 2011). This greater variability on the reproductive outcomes of *Bos indicus* heifers may be due to the cyclic status at the onset of the synchronization protocol (Sá Filho et al., 2010b) or the hormonal therapy applied (Sá Filho et al., 2005; Dias et al., 2009; Peres et al., 2009; Butler et al., 2012; Martins et al., 2013).

The use of intravaginal progesterone (P4) devices with estradiol administration is one of the most frequently used treatments for the synchronization of ovulation in South America (Bó et al., 2003; Baruselli et al., 2004; Bó et al., 2007; Meneghetti et al., 2009; Sá Filho et al., 2009). However, the high P4 concentrations during the synchronization of ovulation protocols could reduce the growth of the dominant follicle, which negatively affect the ovulation rate and pregnancy success of beef cattle (Adams et al., 1992; Carvalho et al., 2008; Dias et al., 2009; Peres et al., 2009; Cipriano et al., 2011; Dadarwal et al., 2013; Martins et al., 2013). Furthermore, it is already known that cyclic *Bos indicus* heifers treated with new P4 intravaginal devices, either containing 1.0 g (DIB®) (Cipriano et al., 2011) or 1.9 g (CIDR®) (Carvalho et al., 2008; Martins et al., 2013) of P4, present reduced dominant follicle growth and smaller preovulatory follicle diameter compared to heifers treated with a previously used P4 intravaginal device (Martins et al., 2013) or those receiving a $PGF_{2\alpha}$ at the insertion of the intravaginal P4 device (Carvalho et al., 2008; Cipriano et al., 2011). Additionally, the manipulation of the timing of the $PGF_{2\alpha}$ treatment relative to the insertion (Carvalho et al., 2008; Cipriano et al., 2011) and removal of the exogenous P4 source is a successful strategy to reduce the circulating P4 concentrations during the synchronization of ovulation protocol in *Bos indicus* heifers (Carvalho et al., 2008; Peres et al., 2009; Mantovani et al., 2010; Cipriano et al., 2011; Martins et al., 2013). Therefore, these hormonal strategies have increased fertility in *Bos indicus* (Peres et al., 2009; Martins et al., 2013) and *Bos taurus* (Dadarwal et al., 2013) beef heifers synchronized for TAI.

The norgestomet (NORG)-based synchronization protocols have been successfully used as hormonal therapies to control the estrous cycle and ovulation in cattle (Garcia and Salaheddine, 2001; Rathbone et al., 2001; Sá Filho et al., 2005; Sá Filho et al., 2010a; Sá Filho et al., 2010b; Sá Filho et al., 2013). The progestin treatments result in greater pulse frequency of LH compared to the endogenous P4 produced by corpus luteum (CL) (Kojima et al., 1992; Sanchez et al., 1995; Rathbone et al., 2001). When cyclic *Bos indicus* beef heifers were treated with NORG ear implants, they had greater follicular growth, their dominant follicle was larger in size, ovulation rates were greater and synchronization of ovulation was better than heifers treated with new intravaginal P4 devices (1.9 g of P4; CIDR®) (Sá Filho et al., 2005). Although this greater ovarian response following the use of NORG ear implant compared with the use of intravaginal P4 device, limited information are available regards the manipulation of the timing of the $PGF_{2\alpha}$ in *Bos indicus* heifers synchronized with NORG-based synchronization protocols for TAI.

Thus, the objective of the present study was to evaluate the effects of the $PGF_{2\alpha}$ treatment at the onset of the NORG and estradiol-based synchronization of ovulation protocol on ovarian follicular dynamics (Experiment 1) and pregnancy per AI (*P*/AI; Experiment 2) in cyclic *Bos indicus* heifers. Our hypothesis was that $PGF_{2\alpha}$ treatment at the onset of a NORG and estradiol-based TAI program has no effect of ovarian follicular response and *P*/AI in cyclic *Bos indicus* heifers.

2. Materials and methods

2.1. Experiment 1

2.1.1. Heifers used and animal management

Experiment 1 was conducted at two research centers located in São Paulo State, Brazil [São Paulo University (Pirassununga *Campus*) and the State Research farm (APTA–Vale do Paraiba Regional Center; Pindamonhangaba)] during the 2005 spring-summer breeding season. Regardless of the location of the farm, the heifers were kept on pasture with free access to mineralized-salt and water. A total of 46 [Pirassununga Campus ($n = 22$) and APTA ($n = 24$)] cyclic (CL present at the onset of the synchronization protocol determined by ultrasonography) Nelore (*Bos indicus*) heifers aged 22–28 months and weighting 371.6 ± 9.9 kg were used. Body condition scores (BCS) were recorded on the first day of the synchronization protocol using a 1–5 scale (1 = emaciated, 5 = obese) (Ayres et al., 2009).

2.1.2. Experimental design

To increase the proportion of heifers presenting with CL (equivalent to approximately 7–10 days of the estrous cycle) at the onset of the synchronization of ovulation protocol, heifers were presynchronized using two doses of $PGF_{2\alpha}$ (150 mg of D-cloprostenol; Preloban®, MSD Animal Health, Sao Paulo, Brazil); 12 days apart. At the first day of the synchronization of ovulation protocol, which was 12 days after the last $PGF_{2\alpha}$ treatment, all animals received a NORG ear implant (Crestar®, MSD Animal Health, Sao Paulo, Brazil) containing 3 mg of NORG and an intramuscular administration of 2 mg of estradiol benzoate (EB; Estrogin®, Farmavet, Sao Paulo, Brazil). At the insertion of the NORG ear implants, the heifers were randomly assigned to one of the two following treatments: $PGF_{2\alpha}$ [$n = 23$; 150 mg of D-cloprostenol (Preloban®, MSD Animal Health)] or no additional treatment (Control; $n = 23$). When the NORG ear implants were removed 8 days later, all heifers received an intramuscular administration of $PGF_{2\alpha}$. All heifers received 1 mg of EB 24 h after the implants were removed as an ovulatory stimulus.