Animal Reproduction Science 118 (2010) 182–187

Contents lists available at ScienceDirect

Animal Reproduction Science

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

Equine chorionic gonadotropin improves the efficacy of a progestin-based fixed-time artificial insemination protocol in Nelore (*Bos indicus*) heifers

M.F. Sá Filho[a], J.R.S. Torres-Júnior[b], L. Penteado[c], L.U. Gimenes[a], R.M. Ferreira[a], H. Ayres[a], L.A. Castro e Paula[a], J.N.S. Sales[a], P.S. Baruselli[a,*]

[a] Departamento de Reprodução Animal, FMVZ-USP, Rua Prof. Orlando Marques de Paiva, 87, CEP 05508-000, São Paulo, SP, Brazil
[b] Universidade Federal do Maranhão, Centro de Ciências Agrárias e Ambientais, Boa Vista, CEP 65500-000, Chapadinha, MA, Brazil
[c] FIRMASA-IATF, Rua Antonio Maria Coelho, 3443, CEP 790020-210, Campo Grande, MS, Brazil

ARTICLE INFO

Article history:
Received 30 May 2009
Received in revised form 5 October 2009
Accepted 16 October 2009
Available online 29 October 2009

Keywords:
Corpus luteum
eCG
Follicular diameter
Heifers
Norgestomet
Progesterone

ABSTRACT

A total of 177 Nelore heifers were examined by ultrasonography to determine the presence or absence of a corpus luteum (CL) and received a 3 mg norgestomet ear implant plus 2 mg of estradiol benzoate i.m. On Day 8, implants were removed and 150 μg of D-cloprostenol i.m. was administered. At the time of norgestomet implant removal, heifers with or without CL at the time of initiating treatment were assigned equally and by replicate to be treated with 0 IU ($n = 87$) or 400 IU ($n = 90$) eCG i.m. All heifers received 1 mg of EB i.m. on Day 9 and were submitted to fixed-time artificial insemination (FTAI) 30–34 h later. The addition of eCG increased the diameter of the largest follicle (LF) at FTAI (10.6 ± 0.2 mm vs. 9.5 ± 0.2 mm; $P = 0.003$; mean ± SEM), the final growth rate of the LF (1.14 ± 0.1 mm/day vs. 0.64 ± 0.1 mm/day; $P = 0.0009$), ovulation rate [94.4% (85/90) vs. 73.6% (64/87); $P = 0.0006$], the diameter of the CL at Day 15 (15.5 ± 0.3 mm vs. 13.8 ± 0.3 mm; $P = 0.0002$), serum concentrations of progesterone 5 days after FTAI (6.6 ± 1.0 ng/ml vs. 3.6 ± 0.7 ng/ml; $P = 0.0009$), and pregnancy per AI [P/AI; 50.0% (45/90) vs. 36.8% (32/87); $P = 0.04$]. The absence of a CL at the beginning of the treatment negatively influenced the P/AI [30.2% (16/53) vs. 49.2% (61/124); $P = 0.01$]. Therefore, the presence of a CL (and/or onset of puberty) must be considered in setting up FTAI programs in heifers. In addition, eCG may be an important tool for the enhancement of follicular growth, ovulation, size and function of the subsequent CL, and pregnancy rates in progestin-based FTAI protocols in *Bos indicus* heifers.

1. Introduction

Fixed-time artificial insemination (FTAI) programs using estradiol esters associated with intravaginal progesterone (P4) devices or norgestomet ear implants have resulted in satisfactory pregnancy outcomes (around 50% pregnancy rate) in suckled *Bos indicus* cows (Baruselli et al., 2004a; Maraña et al., 2006; Meneghetti et al., 2009). However, *Bos indicus* heifers do not respond as effectively to an intravaginal P4 device compared to *Bos taurus* or crossbred heifers (Carvalho et al., 2008). The poor fertility may be due to their poor ovulation response (around 30–50%) at the end of the protocol (Baruselli et al., 2004b; Carvalho et al., 2008; Sá Filho et al., 2005a), resulting in less desirable FTAI pregnancy outcomes (Dias et al., 2009; Marques et al., 2005; Meneghetti and Miguel, 2008).

LH pulses are less frequent when greater concentrations of P4 are present, regardless of the source (endogenous or exogenous; Adams et al., 1992; Kojima et al., 1992; Rathbone et al., 2001). However, exogenous progestins are

* Corresponding author. Tel.: +55 11 3091 7674; fax: +55 11 3091 7412.
E-mail address: barusell@usp.br (P.S. Baruselli).

0378-4320/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.anireprosci.2009.10.004

less suppressive of LH pulse frequency than endogenous P4 (Kojima et al., 1992; Rathbone et al., 2001). Sá Filho et al. (2005a) compared ovarian responses to norgestomet implants and intravaginal P4 devices during protocols for FTAI in *Bos indicus* heifers. Growth rate of the dominant follicle was more rapid in heifers treated with norgestomet implants than those treated with intravaginal P4 devices, and this resulted in a larger ovulatory follicle size and greater ovulation rates. Two other experiments confirmed greater ovulation rates in both peripubertal (Sá Filho et al., 2005b) and estrous cyclic (Torres-Júnior et al., 2005) *Bos indicus* heifers treated with norgestomet implants.

Administration of equine chorionic gonadotropin (eCG) can improve the efficacy of FTAI protocols (Baruselli et al., 2004a,b; Dias et al., 2009; Marques et al., 2005; Meneghetti and Miguel, 2008). The use of eCG at the time of P4-releasing intravaginal device removal has been an alternative to increase ovulatory responses (Baruselli et al., 2004b) and pregnancy rates (Dias et al., 2009; Marques et al., 2005; Meneghetti and Miguel, 2008) in *Bos indicus* heifers submitted to FTAI programs. However, no studies have been conduced aiming to evaluate the effect of eCG treatment in Nelore heifers treated with norgestomet auricular implant. Therefore, the aim of the present study was to evaluate the effect of eCG on ovarian response, luteal function and pregnancy outcomes in Nelore (*Bos indicus*) heifers treated with norgestomet ear implant plus estradiol benzoate in a FTAI program. Our hypothesis was that eCG treatment would effectively increase ovulation rates, circulating concentrations of P4 in the subsequent diestrus and pregnancy per AI.

2. Material and methods

2.1. Animals and management

The experiment was conducted at a commercial beef cattle farm in west-central Brazil (19°57′30″S/54°01′04″W) on January, 2005. The heifers were maintained on *Brachiaria brizantha* pasture with free access to mineralized-salt and water. Nelore (*Bos indicus*; $n = 180$) heifers ranging between 24 and 32 months of age were examined by transrectal ultrasonography for presence or absence of a corpus luteum (CL). All procedures, including injections, FTAI, and ovarian ultrasonography were performed preserving the animal welfare state.

2.2. Experimental design

The FTAI protocol was initiated on random days of the estrous cycle, designated as Day 0, when all animals received a 3 mg norgestomet ear implant (Crestar®, Intervet, Netherlands) plus 2 mg of estradiol benzoate i.m. (EB; Estrogin®, Farmavet, Brazil). On Day 8, the implant was removed and 150 μg of D-cloprostenol i.m. (Preloban®, Intervet, Netherlands) was administered. On Day 0, heifers with ($n = 124$) or without CL ($n = 53$) at the beginning of the estrous synchronization protocol were randomly assigned to treatments with 0 IU ($n = 87$) or 400 IU ($n = 90$) of eCG i.m. (Folligon®, Intervet, The Netherlands). Thus, half of the heifers with CL on Day 0 were treated with either 0 IU ($n = 61$) or 400 IU ($n = 63$) of eCG and half of the heifers without CL received 0 IU ($n = 26$) or 400 UI ($n = 27$) of eCG. All heifers received 1 mg of EB 24 h after implant removal and were fixed-time artificial inseminated 30–34 h later. A single batch of semen from a single sire with proved fertility (satisfactory pregnancy outcomes in previous FTAI programs) was used in all inseminations.

2.3. Ultrasonographic examinations

Transrectal ultrasonography of both ovaries was first performed on the day of norgestomet ear implant insert to determine the presence or absence of a CL. Then, ovaries were evaluated ultrasonically at the time of implant removal (Day 8) and immediately before FTAI (Day 10) in order to measure the diameter of the largest follicle (LF), and 5 days later (Day 15) to determine the presence and the diameter of the resulting CL. A real-time ultrasonic scanner equipped with a 6.0 MHz/8.0 MHz linear transducer (Falco Vet, Pie Medical, The Netherlands) was used.

The growth rate of the LF from the time of implant removal until FTAI (mm/day) was defined as the difference between the diameter of the LF on Days 10 and 8, divided by the number of days (two). Ovulation was considered as the appearance of a CL on the same ovary where the LF was detected on Day 10. Pregnancy diagnosis was performed by ultrasonography 30 days after FTAI. Pregnancy per AI (P/AI) was defined as the number of pregnant heifers divided by the total number of heifers submitted to FTAI in each treatment (eCG or no eCG).

2.4. Blood sampling and progesterone assay

Blood samples were collected by jugular venipuncture 5 days after FTAI (Day 15). The samples were refrigerated (4 °C) during the first 4–5 h and then were centrifuged (3000 × *g* for 20 min) and stored at −20° C until assayed for concentrations of P4. Serum concentrations of P4 were evaluated from unextracted sera using an antibody-coated-tube RIA kit (Coat-A-Count®, Diagnostic Products Corporation, Los Angeles, CA, USA), previously validated by Garbarino et al. (2004). Intra-assay coefficient of variation was 2.8% and the assay sensitivity was 0.006 ng/ml.

2.5. Statistical analyses

Statistical analyses were performed using the SAS System for Windows (SAS Institute Inc., Cary, NC, USA, 2000). Explanatory variables considered for inclusion in the statistical models were eCG, presence of a CL at norgestomet implant insert, and interactions. Dependent variables (i.e. diameter of the LF on Day 8, diameter of the LF at FTAI, growth rate of the LF from the time of implant removal (D8) until FTAI (D10), diameter of the CL 5 days after FTAI, and serum concentrations of P4 5 days after FTAI) were analyzed by two-way ANOVA using PROC GLM. Response variables were tested accordingly to their homogeneity and normality of variances using Guide Data Analysis from SAS. The concentration of P4 and the diameter of the LF at FTAI went through log and exponential transformation, respectively. The effect of the diameter of the LF on Day 8 (x) on