Animal Reproduction Science 165 (2016) 17–21

Contents lists available at ScienceDirect

Animal Reproduction Science

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

High numbers of antral follicles are positively associated with *in vitro* embryo production but not the conception rate for FTAI in Nelore cattle

Gustavo Martins Gomes dos Santos[a], Katia Cristina Silva-Santos[a], Thales Ricardo Rigo Barreiros[b], Fábio Morotti[a], Bruno Valente Sanches[a], Fábio Lucas Zito de Moraes[a], Wanessa Blaschi[b], Marcelo Marcondes Seneda[a,*]

[a] *Laboratório de Biotecnologia da Reprodução Animal (ReproA), DCV-CCA-Universidade Estadual de Londrina (UEL), Londrina 86051-990, PR, Brazil*
[b] *Laboratório de Reprodução Animal, Universidade Estadual do Norte do Paraná (Uenp), Bandeirantes 86360-000, PR, Brazil*

ARTICLE INFO

Article history:
Received 31 July 2014
Received in revised form 7 November 2015
Accepted 30 November 2015
Available online 3 December 2015

Keywords:
Antral follicles
Conception rate
Oocytes
Embryos
Cattle

ABSTRACT

The objective was to compare the conception rates for FTAI and *in vitro* embryo production between Nelore cows with different antral follicle counts (AFC = number of follicles ≤3 mm in diameter in the ovaries). Nelore cows ($n = 547$) were subjected to ovulation synchronization. Randomly during the estrous cycle (D0), cows received an intravaginal device containing 1.9 g P4 (CIDR®) and 2 mg BE (Estrogin®), IM. When the device was removed (D8), the cows received 500 μg PGF2α (Ciosin®), 300 IU eCG (Novormon®) and 1 mg EC (ECP®), IM. All cows were inseminated 48 h after P4 device removal. Antral follicles ≥3 mm were counted using an intravaginal microconvex transducer (D0), and the cows were assigned to high (G-High, ≥25 follicles, $n = 183$), intermediate (G-Intermediate, 16–20 follicles, $n = 183$) or low AFC groups (G-Low, ≤10 follicles, $n = 181$). In another experiment, COCs were retrieved by OPU from Nelore cows ($n = 66$), which were assigned to groups according to oocyte production: G-High ($n = 22$, ≥40 oocytes), G-Intermediate ($n = 25$, 18–25 oocytes) or G-Low ($n = 19$, ≤7 oocytes). All COCs from the same cow were cultured individually (maximum of 25 COCs per drop) and then *in vitro* fertilized using thawed frozen sperm (2×10^8/dose) from a Nelore sire of known fertility. The data were analyzed using a Kruskal–Wallis and a Chi-square test ($P \leq 0.05$). There was no difference in the conception rates after FTAI between Nelore cows with high, intermediate or low AFC (51.9 *vs.* 48.6 *vs.* 58.6%). The number of viable embryos was 18.4 ± 6.7 (G-High), 6.1 ± 3.6 (G-Intermediate) and 0.6 ± 0.7 (G-Low; $P < 0.05$). Therefore, AFC had no influence on the conception rates for FTAI; however, Nelore cows with high oocyte production exhibited better *in vitro* embryo production.

1. Introduction

The large number of oocytes obtained from *Bos taurus indicus* donor cows has stimulated *in vitro* embryo production commerce in Brazil. In this context, Nelore cattle are reported to have high oocyte production, with reports of hundreds of oocytes collected from a single *ovum pick up* (OPU; Santos et al., 2005). In recent years, follicular aspiration procedure has been largely and successfully performed in cattle (Pontes et al., 2011; Sanches et al., 2013; Silva-Santos et al., 2014a) but high individual variability in the number of oocytes has influenced

* Corresponding author. Tel.: +55 43 99485394; fax: +55 43 33714063.
E-mail address: mseneda@uel.br (M.M. Seneda).

http://dx.doi.org/10.1016/j.anireprosci.2015.11.024
0378-4320/

both embryo production and pregnancy (Pontes et al., 2011).

Recently, there has been an attempt to minimize the high individual variation responses to reproductive biotechnologies and identify predictive tools for the early selection of high-producer cattle. Variability in the number of antral follicle numbers/oocytes recovered by OPU in cattle appears to be a limiting factor for large scale *in vitro* embryo programs.

High variability in the number of preantral and antral follicles has been described in *Bos indicus* and *Bos taurus* cattle (Burns et al., 2005; Erickson, 1966; Silva-Santos et al., 2014a, 2014b), although the number of antral follicles ≥3 mm in diameter during follicular waves (antral follicle count, AFC) is repeatable (0.85–0.95) within individuals on both beef and dairy cattle (Burns et al., 2005; Ireland et al., 2007; Mossa et al., 2012; Silva-Santos et al., 2014a, 2014b). Therefore, ultrasonography has been used to identify cattle with high or low numbers of antral follicles during follicular waves (Singh et al., 2004).

Smaller ovaries, diminished ovarian reserves, lower responsiveness to superovulation and transferable embryos, lower concentrations of anti-Müllerian hormone (AMH) and other phenotypic characteristics associated with aging and fertility have been described in cattle with lower AFC (Evans et al., 2012; Ireland et al., 2007, 2011; Mossa et al., 2007; Singh et al., 2004).

Therefore, although the association between markers associated with low fertility has been reported in dairy cattle, few studies have reported the influence of AFC on conception rates resulting from artificial insemination in cattle (Mossa et al., 2012), and there has been no reports for Nelore cows. We hypothesized that Nelore cows with low or intermediate AFC had lower conception rates to fixed timed artificial insemination (FTAI) as well as lower *in vitro* embryo production compared to Nelore cows with high AFC. Therefore, two experiments were designed to compare (1) the conception rates after a hormonal protocol was administered for ovulation synchronization and (2) the *in vitro* embryo production between Nelore cows with high, intermediate or low AFC/oocyte production.

2. Materials and methods

2.1. Experiment I: Hormonal protocol for ovulation synchronization

2.1.1. Animals

Multiparous anestrus Nelore cows (*Bos taurus indicus*, $n = 701$) aged 72 ± 12 months and maintained in *Brachiaria brizantha* pasture supplemented with mineral salt *ad libitum* were submitted to a hormonal protocol for ovulation synchronization. The mean postpartum interval was 45 ± 15 days, the mean body weight was 450 ± 15 kg, and the average body condition score (BCS) was 3.0 ± 0.5 (scale 1–5; Lowman et al., 1976).

2.1.2. Hormonal protocol

The cows were inserted with a new intravaginal progesterone-releasing device containing 1.9 g of P4 (CIDR®, Zoetis, Brazil) and 2 mg of estradiol benzoate (EB, im, Estrogin®, Farmavet, Brazil) on day zero (D0). At device removal (D8), the cows were injected with 500 μg sodium cloprostenol (PGF2α, Ciosin®, Intervet-Schering Plough, Brazil), 300 IU equine chorionic gonadotropin (eCG, Novormon®, Syntex SA, Argentina), and 1 mg estradiol cipionate (EC, ECP®, Pfizer, Brazil), IM. After the device had been removed for 48–52 h, the cows were artificially inseminated using frozen-thawed semen from a single Aberdeen Angus sire with known fertility (Fig. 1). A pregnancy test was determined with a 5-linear transrectal array transducer (Aquila PRO, Pie Medical, Maastricht, Holanda), 45 days after FTAI.

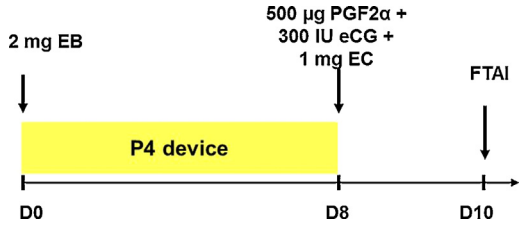

Fig. 1. The hormone protocol for ovulation synchronization in Nelore cows with high (G-High, ≥25 follicles), intermediate (G-Intermediate 16–20 follicles) or low AFC (G-Low, ≤10 follicles). EB: estradiol benzoate, eCG: equine chorionic gonadotropin, EC: estradiol cipionate, PGF2α: prostaglandin, P4: progesterone, FTAI: fixed time artificial insemination.

2.1.3. Antral follicular counts

When the progesterone device was inserted (D0), the ovaries of each animal were ultrasonographically monitored with a 7.5-convex intravaginal array transducer (Aquila PRO, Pie Medical, Maastricht, Holanda), and the antral follicles were counted as previously described (Burns et al., 2005; Ireland et al., 2008; Silva-Santos et al., 2014a, 2014b). Each ovary was systematically scanned from end-to-end and the AFC was determined for each animal, as well as the mean number of antral follicles and the standard deviation (SD) for the whole population ($n = 701$). After an ultrasound evaluation, the females were assigned to three groups according to the number of antral follicles ≥3 mm and the SD: females with a high (mean number of follicles of all the 701 cows plus 1 SD; G-High AFC, ≥25 follicles; $n = 183$), intermediate (25% of cows with AFC closest to the mean number of follicles of all the 701 cows; G-Intermediate AFC, 16–20 follicles, $n = 183$) or low AFC (mean number of follicles of all the 701 cows minus 1 SD; G-Low AFC, ≤10 follicles; $n = 181$) in all ultrasound scans.

2.2. Experiment II: In vitro embryo production

2.2.1. Animals

Multiparous Nelore cyclic cows ($n = 101$, *Bos taurus indicus*) aged 84 ± 12 months, mean postpartum interval was 45 ± 15 days, with a mean BCS of 3.5 ± 0.5 (scale 1–5; Lowman et al., 1976), mean body weight of 480 ± 20 kg, and maintained in *B. brizantha* pasture supplemented with mineral salt *ad libitum* were submitted to follicular aspiration without hormonal stimulation (Pontes et al., 2009, 2011). Each cow was aspirated once.

Before each procedure, feces were removed from the rectum, and the perineal area was cleaned with tap water