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Ovarian follicular growth suppression by long-term treatment with a GnRH agonist and impact on small follicle number, oocyte yield, and *in vitro* embryo production in Zebu beef cows

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ABSTRACT

The aim of the present study was to evaluate small follicle number, oocyte yield, and *in vitro* embryo production (IVEP) in Zebu beef cows treated long term with a GnRH agonist to suppress ovarian follicular growth. Nelore (*Bos indicus*) cows (n = 20) showing regular estrous cycles were randomly assigned to one of two groups: control (n = 10, placebo ear implant without a GnRH agonist); GnRH agonist (n = 10, GnRH agonist ear implant containing 9.4-mg deslorelin). All cows underwent an ovum pick-up (OPU) session 14 days (Day 14) before the start of treatments (Day 0) followed by seven OPU–IVEP procedures at 30-day intervals (Days 0, 30, 60, 90, 120, 150, and 180). Semen from a single batch of a previously tested bull was used for all the IVEP. Cows treated with agonist reported a decrease over time in the proportion of animals with a (CL; $P \leq 0.05$) and large follicles (>10 mm, $P \leq 0.05$). These cows had a lesser number of medium + large follicles (>5 mm; 1.74 ± 0.5 vs. 4.13 ± 0.5; $P \leq 0.05$), greater number of small follicles (2–5 mm; 44.3 ± 2.8 vs. 30.8 ± 1.8; $P \leq 0.05$), greater yield of cumulus-oocyte complexes (COCs; 21.0 ± 2.3 vs. 15.6 ± 1.9; $P \leq 0.05$), greater proportion of COCs cultured (79.2 vs. 73.9%; $P \leq 0.05$), COCs cleaved (10.6 ± 1.5 vs. 6.8 ± 1.1, $P \leq 0.05$), and cleaved rate (52.8 vs. 44.3%; $P \leq 0.05$) compared with control cows. The number (3.4 ± 0.7 vs. 3.0 ± 0.6; $P > 0.05$) and proportion (16.5 vs. 19.1%; $P > 0.05$) of blastocysts produced were similar between agonist and control cows, respectively. The study has shown that Zebu beef cows treated long term with a GnRH agonist had follicular growth restricted to small follicles. This did not compromise the ability of oocytes to undergo IVF and embryonic development.

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1. Introduction

The use of assisted reproductive technologies such as *in vitro* embryo production (IVEP) has increased rapidly in recent years and is now applied worldwide for genetic improvement in beef and dairy cattle. Factors that influence the efficiency of ovum pick-up (OPU) and IVEP include ovarian follicular population [1], follicle size [2,3], donor category (breed, age, lactation status) [3–5], environment [5–7], and stage of the estrous cycle [8–12] Concentrations of circulating steroid hormones such as progesterone (P4) also have been implicated in the OPU–IVEP outcome [13,14].

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In cyclic cows, circulating concentrations of P4 regulate the pulsatile secretion of GnRH which, in turn, determines LH pulse frequency [15]. Low concentrations of P4 result in increased LH pulse frequency and prolonged follicular dominance, which is deleterious to oocyte quality [16]. High concentrations of P4 during the later stages of follicular growth was associated with improved embryo quality in dairy cows presumably because of lower exposure of developing oocytes to excessive LH pulsatility [17]. Concentrations of P4 can therefore indirectly affect oocyte quality through LH pulsatility [13]. Evidence of a direct role for P4 in determining oocyte quality was provided by reduced IVEP after the inhibition of P4 synthesis by cumulus cells and by blocking nuclear P4 receptor activity during IVM[13].

Agonists of GnRH have undergone extensive evaluation in cattle and are used routinely in estrus synchronization [18–20] and superovulation [21,22]. Treatment with a GnRH agonist is associated with acute phase and chronic phase responses in gonadotropin secretion [23]. During the acute phase which lasts for several days, the secretion of LH and FSH is increased [24]. The chronic phase is characterized by a downregulation of gonadotrope cells in the anterior pituitary gland [25]. There are a lack of pulsatile secretion of LH and FSH [24,26] and preovulatory surge releases of LH do not occur [21]. Transient increases in FSH required to recruit follicles into a follicular wave also do not occur and follicular growth is arrested at small follicles [26–29]. Suppressed follicular growth is maintained long term by continued treatment with a GnRH agonist [28]. Although female cattle treated with a GnRH agonist do not have pulsatile LH or transient rises in FSH, there is some constitutive release which produces low concentrations of LH and FSH [24]. Treatment with a GnRH agonist provides a model to investigate the effect of follicular and endocrine suppression on oocyte yield, oocyte quality, and IVF and embryonic development.

In a previous study, we found that mature Brahman (Zebu, *Bos indicus*) heifers treated with the GnRH agonist deslorelin for 6 months had chronically suppressed follicular growth but were able to undergo an immediate follicular response to treatment with FSH [28]. The follicular response to FSH in heifers treated with a GnRH agonist was similar to the follicular response in heifers showing regular estrous cycles. In the present study, we have extended our earlier observation by undertaking sequential OPU and IVEP in Nelore (Zebu, *B indicus*) cows that were downregulated with the agonist deslorelin. The findings would report whether exposure to transient increases in FSH, pulsatile LH, and cycles of P4, are required for oocytes to undergo IVM, IVF, and embryonic development. The hypothesis tested was that oocytes obtained from cows with suppressed follicular growth induced by treatment with a GnRH agonist, and lacking P4, would have a reduced capacity to undergo fertilization and embryonic development *in vitro*.

2. Material and methods

Humane animal care and handling procedures of the State of São Paulo (Brazil) law number 11.977 were followed for all experimentation.

2.1. Animals and management

The study was conducted at an experimental station (Instituto de Zootecnia Sertãozinho) located in Sertãozinho, São Paulo, Brazil. Nelore (Zebu, *B indicus*) cows (n = 20) weighing 485 ± 18 kg, aged 47.4 ± 4.1 months, and with body condition score 5.8 ± 0.31 (1–9 scale; [30]) were used in the study. During the study, all cows received the same total mixed ration with free access to mineralized salt and water.

2.2. Experimental design

Cyclic cows were randomly assigned (Day 0) to one of two groups: control (n = 10, placebo ear implant without a GnRH agonist); GnRH agonist (n = 10, GnRH agonist ear implant containing 9.4-mg deslorelin; Suprelorin 12, Virbac; Milperra, New South Wales, Australia). Implants were placed subcutaneous on the dorsal surface of the ear using aseptic conditions. Cows underwent seven consecutive OPU procedures at 30-day intervals (Day 0, Day 30, Day 60, Day 90, Day 120, Day 150, Day 180; Fig. 1).

2.3. Ultrasonography

Cows were considered to be cycling if a CL was identified in at least one of two ultrasound examinations performed 14 days apart before the first OPU procedure (Fig. 1). Immediately before each OPU, all visible ovarian follicles (≥2 mm diameter) were quantified and classified according to their diameter by ultrasound evaluation (Mindray DP - 2200 Vet, China; with 5-MHz convex array transducer). Follicle classifications were: small, 2 to 5 mm; medium, 6 to 10 mm, large, greater than 10 mm.

OPU–IVEP procedures were undertaken without previous synchronization of the ovarian follicular wave.

2.4. OPU procedure

To facilitate the transvaginal OPU, cows were restrained in a chute and epidural anesthesia was administered using 2% lidocaine hydrochloride (Lidovet, Bravet, Brazil). All follicles 2 mm or more were aspirated using a portable scanner with a 5-MHz convex array transducer (Mindray DP - 2200 Vet, China). The latter was assembled into a vaginal-handle equipped with a stainless steel needle guide (20 G; 0.9 × 50 mm; Terume Europe NV, Belgium) connected to a vacuum pump system (85–90 mmHg of negative pressure; V-MAR 5000, Cook Australia, Queensland, Australia). Follicular contents were recovered using a 1.1 mm inner diameter by 120 cm length tubing (Watanabe Tecnologia Aplicada Ltda, Cravinhos, São Paulo, Brazil) and connected to a 50-mL conical tube containing 15 mL of Dulbecco phosphate – buffered saline (DPBS; Nutricell Nutrientes Celulares, Campinas, São Paulo, Brazil) and 5000 IU/mL sodium heparin (Parinex, Hipolabor, Belo Horizonte, Minas Gerais, Brazil) at 35 °C to 37 °C. All oocyte recovery procedures were performed by the same operator.

The conical tube containing follicular contents was transported to a laboratory and cumulus-oocyte complexes (COCs) were recovered using a 75-μm filter (Watanabe

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