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Embryo production in heifers with low or high dry matter intake submitted to superovulation

Marcos R. Mollo [a], Pedro L.J. Monteiro Jr. [b], Ricardo S. Surjus [b], Aline C. Martins [c], Alexandre F. Ramos [d], Gerson B. Mourão [b], Luiz H.D. Carrijo [e], Gláucio Lopes Jr. [f], Rodolfo Rumpf [g], Milo C. Wiltbank [h], Roberto Sartori [b, *]

[a] National Water Agency (ANA), Brasília, Federal District, Brazil
[b] Department of Animal Science, ESALQ, University of São Paulo, Piracicaba, São Paulo, Brazil
[c] Rio Verde University, Jataí, Goiás, Brazil
[d] Embrapa Genetic Resources and Biotechnology, Brasília, Federal District, Brazil
[e] Integral Animal Nutrition, Goiânia, Goiás, Brazil
[f] Alta Genetics Inc, Watertown, WI, USA
[g] Geneal Genetics and Animal Biotechnology, Uberaba, Minas Gerais, Brazil
[h] Department of Dairy Science, University of Wisconsin-Madison, Madison, WI, USA

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ABSTRACT

This study investigated the influence of feed intake on superovulatory response and embryo production of Nelore heifers. Pubertal heifers were kept in a feedlot and were submitted to the same diets, but with different levels of feed consumption: High (1.7 M; n = 20) or Low (0.7 M; n = 19) feed intake. Heifers in the 1.7 M treatment consumed 170% (2.6% of body weight [BW] in dry matter) and the 0.7 M heifers ate 70% (1.1% of BW in dry matter) of a maintenance diet. After 7 wk on these diets, heifers were treated with eight decreasing doses of follicle-stimulating hormone (FSH) given every 12 h, totaling 133 mg Folltropin (Folltropin-V; Bioniche Animal Health, Canada) per heifer. Seven d after AI, heifers had their uteri flushed and embryos were recovered and graded according to the International Embryo Technology Society standards. Data were analyzed using the GLIMMIX procedure of SAS and results are presented as least-squares means ± SEM ($P < 0.05$). At the onset of the FSH treatment (Day 0 of the protocol), 1.7 M heifers had greater body condition score (BCS), BW and serum insulin concentrations than 0.7 M heifers (4.1 ± 0.1 vs. 3.0 ± 0.1; 462.5 ± 10.1 vs. 382.7 ± 10.4 kg; and 14.3 ± 1.7 vs. 3.5 ± 0.8 μIU/mL, respectively). The 0.7 M heifers had more follicles ≥6 mm at the time of the last FSH (Day 7; 47.9 ± 6.4 vs. 23.5 ± 4.3 follicles), related to a better follicle superstimulatory response to FSH. Similarly, 0.7 M heifers had more corpora lutea at the time of embryo collection (33.6 ± 1.4 vs. 15.7 ± 0.9) than the 1.7 M heifers, which resulted in greater number of recovered embryos and ova (9.9 ± 0.7 vs. 6.7 ± 0.6) and viable embryos (5.3 ± 0.5 vs. 3.8 ± 0.4), despite having similar proportions of viable embryos (~62%). A negative correlation between circulating insulin and follicle superstimulatory response to FSH was observed ($r = -0.68$). Therefore, we conclude that high feed intake, for a long period of time, compromised the superovulatory response and embryo production potential of *Bos indicus* heifers possibly related to the elevation in circulating insulin.

1. Introduction

There continues to be substantial variability in the superovulatory response of cattle to FSH protocols and this represents a major limitation to the profitable and efficient implementation of embryo technology in cattle [1–3]. Approximately 20% of cows produce most of the viable embryos after multiple ovulation treatments [4]. Among the factors that influence the multiple ovulatory response and embryo production in cattle, nutritional management has a substantial impact [5,6]. Commonly, embryo donors are overweight and overfed due to inappropriate nutritional management, which may result in poor oocyte/embryo quality [7].

* Corresponding author.
E-mail address: robertosartori@usp.br (R. Sartori).

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The antral follicle count (AFC) at the beginning of a follicle-stimulating hormone (FSH) treatment is the most important factor affecting embryo production in cattle. The AFC has a high positive correlation with multiple ovulatory response [8–10] and embryo yield [10,11]. Nutritional flushing prior to multiple ovulation treatments has been related to increased number of small follicles in the ovaries, apparently through an increase in circulating insulin-like growth factor 1 (IGF1) [8,12] and insulin. It has been reported that these factors increase the sensitivity of granulosa cells to FSH [13,14]. However, high blood insulin concentration is also related to compromised AFC in cattle [15], lower multiple ovulatory responses [16], and poor embryo quality [15].

Data on the effect of dietary energy levels, or dry matter intake (DMI), on the multiple ovulatory response are controversial. Although some research has shown positive effects of DMI on multiple ovulatory response [8,12], most studies have shown negative effects [17–20]. Moreover, high-energy diets compromised *in vitro* and *in vivo* embryo production and changed expression of important genes related to embryo development [6,21,22].

Therefore, the objective of this study was to evaluate the chronic effect (9 wk) of distinct levels of DMI on multiple ovulatory response, and the quantity and quality of embryos produced in *Bos indicus* heifers.

2. Materials and methods

2.1. Animals and experimental diets

Thirty nine pubertal Nelore heifers, age ranging from 24 to 36 mo, BW of 390 ± 6.8 kg (ranging from 330 to 459 kg) and BCS of 3.5 ± 0.04 (ranging from 3.0 to 3.8; on a 1 to 5 scale; [23]) were kept on a feedlot system with *ad libitum* access to water and mineral mix. The experiment was performed at the Sucupira Experimental Station of Embrapa Genetic Resources and Biotechnology in Brasília, DF, Brazil.

Before the start of the experiment, heifers were kept together and were fed a maintenance diet for 21 d according to the National Research Council [24]. After, this 21-d period, heifers were randomly divided into two treatment groups: 1.7 M, in which they consumed 170% of the necessary maintenance diet (2.62% of BW in DM) or 0.7 M, in which heifers received and ate 70% of the necessary maintenance diet (1.08% of BW in DM). Groups were fed once a day at 7:00 a.m. on the same schedule in two pens (one for each treatment group with a feeding area with 70 cm of length per heifer for a period of 9 wk (Table 1). Body weight was evaluated weekly and the data were used to adjust the diet. The BCS was also evaluated weekly by two experienced technicians, and the mean was calculated.

Table 1
Diet composition and percentages of dry matter (DM), total digestible nutrients (TDN) and crude protein (CP).

	% of diet in DM
Coast-cross hay	40.8
Corn silage	51.9
Energy and protein supplement[a]	7.3
Total of digestible nutrients	62.4
Crude protein	11.5

[a] Boião PPU, Integral Animal Nutrition, Goiânia, GO, Brazil (Quantities per kg of product): antioxidant (320.0 mg), calcium (39.4 g), cobalt (32.0 mg), copper (240.0 mg), sulfur (14.4 g), iron (320.0 mg), fluorine (max; 542.0 mg), phosphorus (32.8 g), iodine (48.0 mg), magnesium (99.6 g), manganese (160.0 mg), Non-protein nitrogen (NPN; 80.9 g), NPN equiv. protein (max; 50.5%), flavor agent (200.0 g), selenium (4.8 mg), sodium (118.8 g), vitamin A (4000.0 IU), vitamin E (40.0 IU), zinc (960.0 mg).

2.2. Multiple ovulation protocol and embryo collection

After 7 wk on treatment diets, heifers were submitted to a superovulation treatment using the following protocol (Fig. 1): On Day 0, all embryo donors received an intravaginal device (IVD) impregnated with 1 g of progesterone (DIB, Syntex S.A., Argentina) and 2 mg of estradiol benzoate (EB; Ric-Be, Syntex S.A., Argentina) for synchronization of follicle wave emergence. Although EB is used in South American and other countries, we acknowledge there are countries that prohibit its use. On Days 4–7, heifers received eight decreasing doses of FSH (133 mg total, i.m., Folltropin-V, Bioniche, Ontario, Canada), and concomitant with the sixth treatment of FSH (Day 6), all heifers received d-cloprostenol (PGF2α, 0.150 mg, i.m., Prolise, ARSA S.L.R., Argentina) and the IVD was removed. Estrus detection was observed twice-daily for 1 h and heifers detected in estrus were inseminated twice 12 and 24 h later by the same technician using semen from a single sire of proven fertility. Embryo collection was performed 7 d after estrus using the double uterine flushing technique [25]. All embryos were classified for quality (1 = good, 2 = fair, 3 = poor and 4 = degenerate) according to the International Embryo Technology Society (IETS) guidelines [26]. Embryos grading 1 and 2 were defined as viable embryos.

2.3. Ultrasound evaluations

Ovarian structures were evaluated using a 7.5-MHz linear-array transducer (Aloka SSD-500 V; Corometrics Medical Systems Inc., Wallingford, CT, USA). On Day 0, the ovaries were evaluated and structures recorded. On the first d of treatment with FSH (Day 4) all follicles ≥ 3 mm were counted. Similarly, on the fourth d of treatment with FSH (Day 7) all follicles ≥ 6 mm were counted. The follicle size of 6 mm in diameter was chosen because follicle deviation occurs when the largest growing follicle of the wave reaches between 5.7 and 7.0 mm in diameter and becomes dominant in *Bos indicus* cattle [27]. Follicle superstimulatory response to FSH was defined and calculated by subtracting the number of antral follicles ≥ 6 mm at the time of the last FSH treatment (Day 7 of the protocol) from the number of antral follicles ≥ 3 mm at the time of the first FSH treatment (Day 4 of the protocol) in each heifer. The superstimulatory response was also evaluated by dividing the number of antral follicles ≥ 6 mm at the time of the last FSH treatment by the number of antral follicles ≥ 3 mm at the time of the first FSH treatment in each heifer. On Day 10, all follicles ≥ 6 mm still present in the ovaries were considered anovulatory follicles. On Day 15, the number of CL were evaluated by ultrasound to estimate the superovulatory response.

2.4. Insulin assay

Blood samples were collected on Day 4 of the protocol from the coccygeal vein or artery into evacuated tubes (Becton Dickinson Co., Franklin Lakes, NJ, USA), stored at 4 °C for 24 h, and then centrifuged at 1700 × g for 15 min. The overnight blood storage before centrifugation was done to minimize post-centrifugation fibrin in serum samples [R. Sartori, personal observation] without compromising circulating insulin concentrations measurement [28]. Serum was kept at −20 °C until the hormone assay was performed. Insulin concentrations were determined using a single solid-phase radioimmunoassay (Coat-a-Count; Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay CV was 1.9%.

2.5. Statistical analyses

The GLIMMIX procedure (ANOVA) of SAS was used with generalized linear models methodology. For BW, BCS and