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Effect of lipid-enriched diet associated with bovine recombinant somatotropin on superovulation response and embryo recovery in heifers

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Abstract: The current study aimed to evaluate the effects of soybean oil supplementation associated with the administration of bovine recombinant somatotropin (rBST) on the population of ovarian follicles, standardization of both the superovulation response and embryo recovery in heifers. We used 40 Holstein heifers submitted to a 2 × 2 factorial, forming the experimental groups: DC-SL (control diet plus saline solution application); OL-SL (diet with soybean oil plus saline application); DC-rBST (control diet plus application of 320 mg rBST) and OL-rBST (diet with soybean oil plus the application of 320 mg rBST). The animals received two applications of 500 mg of PGF2α for estrus synchronization and were superovulated with 400 IU of FSH in decreasing daily doses for four consecutive days. On the day before the start of superovulation, the two largest follicles were punctured to quantify the intrafollicular concentration of high-density lipoprotein (HDL). Embryo collection took place seven days after the first insemination. There were no differences between the treatments adopted regarding follicular activity, P4 concentration, and superovulation response. The OL-rBST's corpus luteum (CL) area and HDL intrafollicular (4.67 ± 0.67 cm^2, 53.67 ± 10.35 mg/dL, respectively) were significantly higher than the OL-SL treatment. Soybean oil supplementation in the diet associated with the administration of rBST did not provide uniformity in the superovulation response and the embryonic recovery of heifers.

Key words: Superovulation, cattle, corpus luteum, HDL, embryo, IGF-1

1. Introduction

Superovulation aims to induce multiple ovulations, getting more transferable embryos with a greater possibility of developing into pregnancies [1,2]. However, many factors influence the superovulation response, which prevents the frequent use of embryo transfer (ET) in cattle [3,4]. This variability in response to super ovulatory treatment is mainly related to follicular populations of different sizes [5].

Using recombinant bovine somatotropin (rBST) increases follicular development since it modulates gonadotropins' action in the ovary, stimulating steroidogenesis, proliferation, and differentiation of granulosa and theca cells, increasing follicular growth [6]. The positive effects are the increase in the number of small follicles recruited before superovulation treatment, providing the growth of a greater number of follicles, and the embryonic quality [5,7].

According to data from in vitro studies, the most likely mechanism of rBST for increased follicular growth is the synergistic effect of insulin-like growth factor I (IGF-I) associated with gonadotropins and the expression of gonadotropin receptors [8]. Administration of rBST increases plasma concentrations of IGF-I that stimulate embryonic development by increasing mRNA expression of proteins that form the histotroph [9].

In this context, the energy density of diets can also affect the reproductive performance of females [10]. Castaneda-Gutierrez et al. [11] infer that cows with negative energy balance have lower plasma levels of glucose, insulin, and insulin-like growth factor-I (IGF-I); and show changes in ovarian activity [12, 13]. Improved fertility of dairy cows on high-energy diets has generally been associated with an increase in dominant follicle diameter, oocyte and embryonic quality, higher concentrations of progesterone produced by the corpus luteum (CL), and modulation of prostaglandin synthesis [12, 14], having linoleic acid as a precursor of the series two prostaglandins [15].

Thus, Fernandes and Madureira [16] report that linoleic acid (C18:2), found in abundance in soybean oil,

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effectively influences the reproduction of dairy cows by increasing the size of the dominant follicle and by being a precursor of some steroid hormone.

However, despite the information reported in the scientific literature, there is no information on lipid supplementation associated with rBST on ovarian follicular activity in superovulated bovine females.

Therefore, there is considerable potential for combining soybean oil and rBST administration to optimize superovulation in heifers. The aim was to evaluate the effects of rBST supplementation associated with soybean oil on the population of ovarian follicles, in increasing and standardizing the superovulation response and in embryonic recovery in Holstein heifers.

2. Material and methods

2.1. Location and experimental animals

The experiment was carried out at the Federal University of Viçosa (UFV), Viçosa, MG (Latitude: 20°45'14"S — Longitude: 42°52'53"W), for one year, starting in August. Forty Holstein heifers aged 13 to 18 months were used, with an average body weight of 352.13 ± 27.04 kg, regular cyclic activity, and no pathologies of the reproductive system detectable through gynecological examination.

2.2. Animal management and experimental design

The animals were kept in individual pens (40 m^2), without bedding, being fed twice a day (7:00 am and 3:00 pm). We adjusted the roughage and concentrated daily to maintain leftovers of 5%–10% dry matter (DM). The amount of roughage and concentrate offered to each animal and the leftovers from the previous day were weighed daily. All animals were weighed once a week.

The experiment was in a completely randomized design in a 2 × 2 factorial arrangement, and the factors the diet provided, and the application or not of rBST (Lactotropin, Elanco, EUA), with 10 replicates per treatment and one heifer as an experimental unit. Thus, the animals were balanced in terms of age and body weight, being distributed into four groups: DC-SL (control diet with 3% ether extract (EE) based on dry matter (DM) plus saline application); OL-SL (diet supplemented with soybean oil calculated to provide approximately 6% EE in DM plus saline application); DC-rBST (control diet with 3% ether extract (EE) based on dry matter (DM) plus the application of 320 mg of rBST) and OL-rBST (diet supplemented with soybean oil calculated to provide approximately 6% EE in the DM plus the application of 320 mg of rBST).

The beginning of treatments with the application of rBST or saline solution (control) was seven days after the detection of estrus (day zero - D0), and the superovulation protocols with follicle-stimulating hormone (FSH) started ten days later of estrus (D10), according to Gong et al. [17].

Diets were formulated as recommended by the National Research Council [18]. Chemical-bromatological analyzes of the diet ingredients were performed according to Association of Official Analytical Chemists (AOAC) [19] (Table 1).

The diets were similar in composition except for the ether extract and net energy for live weight gain because of the addition of soybean oil. The energy of the OL diet presented 0.10 Mcal/kg of DM more than the DC diet.

The heifers were adapted to the management conditions and fed with DC for two weeks before the experimental period, with partial replacement of the control diet during the third week in the proportion of 20% until reaching 100% of the OL in the respective treatments. The respective diets, water, and mineral salt were provided ad libitum.

Samples of silage and leftovers were collected daily and stored in plastic bags at –20 °C until completing seven days, composing a weekly sample. This procedure was repeated until the end of the experimental period for further chemical analysis. The concentrate samples were collected fortnightly and stored in plastic bags at –20 °C.

During the first three weeks, all animals received two injections of 500 mg of cloprostenol (Ciosin, Coopers, Brazil) intramuscularly, 12 days apart, so that after the second application, the manifestation of estrus coincided with the last day of the period of food change. Estrus was monitored twice a day for 1 h (at 6:30 am and 5:30 pm) with the help of a ruffian, and females that accepted the ruffian or herd mates were considered in estrus.

2.3. Ovarian ultrasound

We monitored follicular dynamics and cyclic luteal activity of the treatments only during the first two nonsuperovulated estrous cycles after the beginning (day zero) of complete administration of diets and SL or rBST applications and throughout the first superovulated estrous cycle.

For this purpose, ultrasound evaluation (Aloka, mod. SSD - 500) was used with a 5 MHz linear transducer. Ultrasound was performed transrectally, daily, in the morning, before providing saline or rBST applications. The variables studied were: the number of follicular growth waves, duration of the interovulatory period, day of emergence of follicular growth waves, and the number of follicles within the classes: *I* (0.3–0.59 cm in diameter), *II* (0.6–0.9 cm in diameter), *III* (1–1.5 cm in diameter) and *IV* (> 1.5 cm in diameter), according to Rincón et al. [20], the maximum diameter of dominant follicles, follicular growth rate and the number of follicular growth waves, as well as the CL area. The numbers of total, viable, and degenerated embryos were evaluated for superovulation response.

2.4. Superovulation and follicular puncture protocols

From the 42nd day (1st superovulated estrous cycle) and the 84th day (2nd superovulated estrous cycle) of

Table 1. Ingredients and nutritional composition of experimental diets as a percentage of dry matter (DM).

Ingredients	Diets	
	Control (DC)	Oil (OL)
% Dry matter		
Corn silage	81.90	81.68
Corn meal	13.05	9.9
Soybean meal (48% of CP)	2.83	2.82
Soy oil	-	3.13
Urea	1.28	1.55
Mineral core	0.57	0.56
Ammonium sulfate	0.28	0.28
Vitamin premix	0.09	0.08
Chemical composition		
CP[1] % DM	12.4	12.9
NEg[2] Mcal/kg DM	1.11	1.21
SFND[3] % DM	41.4	40.9
FDN % DM (forage)	66.0	65.0
$TDN^{4}_{(1x)}$, % DM	73.0	77.0
EE[5], % DM	3.0	6.1
NFC[6], % DM	43.0	40.0

[1]CP: crude protein; [2]NEg: net energy for gain; [3]SFND: soluble fiber in neutral detergent; [4]TDN: total digestible nutrients (maintenance level); [5]EE: ethereal extract; [6]NFC: nonfibrous carbohydrate.

treatment, days of estrus manifestation, all heifers were superovulated with 400 IU of FSH (Pluset, Calier, Brazil) in decreasing daily doses for four consecutive days (35, 30, 20 and 15%), starting on the 10th day after the onset of estrus, administered every 12 h, intramuscularly. In the fifth application of FSH, the donors received 500 mg of cloprostenol (Ciosin, Coopers, Brazil) intramuscularly for estrus induction (Figure 1).

Inseminations were performed at the time of estrus detection and subsequent inseminations 12 and 24 h later, with cryopreserved semen, from a single bull, from a single batch, and with proven fertility.

On the day before starting the first FSH application of each superovulated cycle, the largest (dominant) follicle and the second largest follicle were aspirated transvaginally to optimize the superovulation response. The follicular fluids from the two follicles were stored in tubes at a temperature of –20 ºC for further high-density lipoprotein (HDL) analysis using commercial kits (HDL LD, Labtest, Brazil).

Prior to follicular puncture, the animals were contained in individual chutes. They underwent anesthesia by applying 3 to 5 mL of 2% lidocaine hydrochloride with a vasoconstrictor (Anestt, Syntec, Brazil) in the sacrococcygeal intervertebral space in order to achieve low epidural anesthesia, which would facilitate the manipulation of the genitalia during the process of follicular puncture and embryo collection.

Subsequently, the vulva and adjacent region were cleaned with running water and neutral soap. The follicular puncture was performed during the two superovulated cycles.

A 50 × 12 diameter needle (21 gauge) coupled to a polyethylene tube was used for follicular aspiration. The tube was connected to a 1 mL (13 × 4 mm) insulin syringe with a 20 µL. A 5.0/7.5 MHz transvaginal transducer guided the needle path, coupled with the ultrasound device (Aloka, mod. SSD-500). Because of the echogenicity of the needle after penetrating the vaginal wall and peritoneum, its tip was positioned in the follicle's antrum, and the syringe connected to the other end of the polyethylene tube, the follicular fluid was suctioned.

2.5. Embryo collection and evaluation

Embryos were collected during the two superovulated cycles by the nonsurgical method seven days after the first artificial insemination of the corresponding superovulated estrous cycle. The Foley catheter (# 18 or 20) was fixed in each uterine horn after inflating its balloon. The mandrel was removed, and each horn was washed individually, using 500 mL of commercial changed PBS solution (phosphate buffer saline) (DMPBS, Biodux) heated to 37 ºC in the collector with a filter (Millipore).

After the collection, measured by the volume of liquid recovered in a 1000 mL Erlenmeyer flask, the filtrate was taken to the laboratory, transferred to a 9 cm diameter Petri dish with a grid bottom, and the embryos were located with the aid of a stereomicroscope (Coleman, mod. XTB-2B) (40× magnification).

Subsequently, the collected structures were transferred to a 5-cm diameter Petri dish containing the changed PBS solution (phosphate buffer saline) enriched with 10% inactivated and sterile fetal bovine serum, proceeding to count and classify them according to the stage of development and the quality [21].

2.6. Plasma progesterone measurement

Blood was sampled on days 7, 14, and 21 of the first two nonsuperovulated estrous cycles for plasma progesterone measurement using commercial solid-phase radioimmunoassay (RIA) kits (Coat-a-count progesterone kit, DPC, Diagnostic Products Co., USA). Blood was collected before the ultrasound examination, in the preprandial state, with a vacuum tube with EDTA, by puncturing the coccygeal artery or vein.

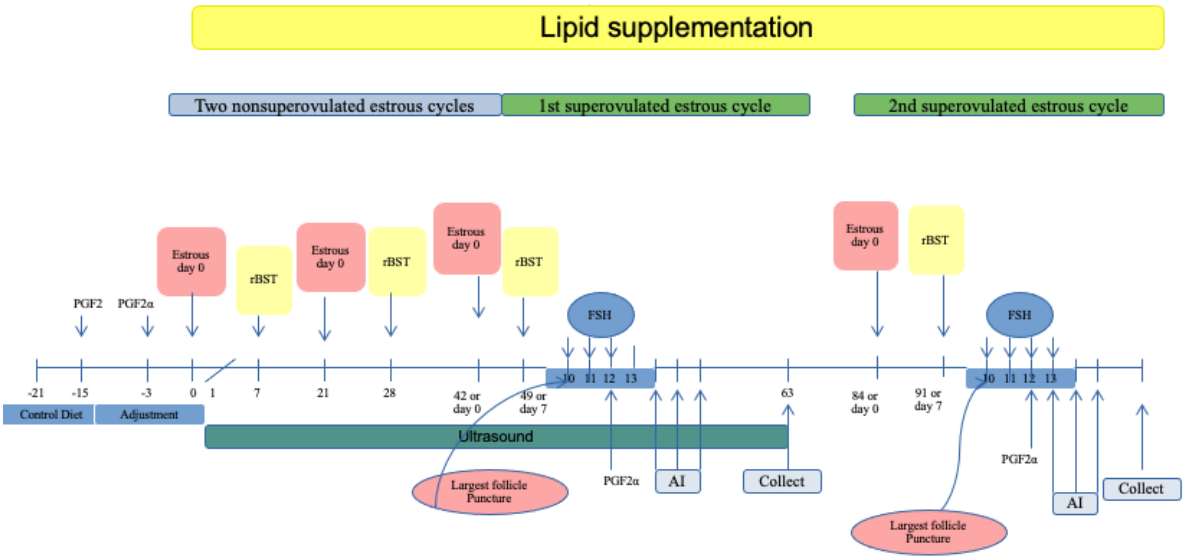

Figure 1. Outlined protocol of the OL-rBST group (diet with soybean oil and application of recombinant bovine somatotropin-rBST). On day 0, adapted to their respective diets, heifers were monitored using ovarian ultrasound daily during the first 42 days (or two consecutive estruses) without superovulation and a further 21-day cycle with superovulation. Superovulation started on day 10, when the two largest follicles present in the ovaries were, and continued until day 13. An injection of PGF2α (Prostaglandin 2α- Cloprostenol) was administered in the fifth application of FSH (stimulating follicle hormone), and inseminations were performed three times: at estrus detection, 12 and 24 h later. Embryo collection was performed on the seventh day after the first artificial insemination (AI), and a second collection was performed 42 days after the first, with the same animals kept within their respective group until the end of the experiment.

The tubes with the samples were centrifuged at 3000 rpm for 15 min for the complete separation of the plasma, which was recovered with Pasteur pipettes and transferred to a sterilized vial, identified, and stored at a temperature of –20 ºC until the analyses were performed.

2.7. Statistical analysis

The analyzes were processed by the statistical software Action R version 3.0.2 (2014), according to the following model:

$y_{ijk} = \mu + D_i + S_j + DS_{ij} + e_{ijk}$;

y_{ijk} = observation on the animal *k* on the diet *j* in the applied solution *i*;

μ = overall average;

D_i= diet effect *i*, *i* = 1,2;

S_j= effect of saline or somatotropin application *j*, *j* = 1,2;

DS_{ij} = effect of diet x somatotropin interaction;

e_{ijk} = error associated with each observation *ijk*.

The means of the characteristics were tested by Fisher's test (Test F; two comparisons), processed by the R with a probability level of 5%.

3. Results

There was no difference in intake in the different treatments for dry matter (DMC), crude protein (CP), net energy for body weight gain (NEg), neutral detergent insoluble fiber (NDF), nonfibrous carbohydrates (NFC), and body weight. An increase in ether extract (EE) consumption was observed in the OL-SL and OL-rBST groups (Table 2). These results show treatments with different consumption of EE, not altering the consumption of the other components of the diet.

There were no differences in the number of follicular waves in each estrous cycle, interovulatory period, and days of follicular wave emergence (Table 3). In the OL-rBST, we observed animals with three follicular waves, which may have numerically increased the interovulatory period (24.15 ± 7.89 days).

There was no effect between treatments on follicle number, dominant follicle diameter, and follicular growth rate. The follicles were classified by size, and treatments did not affect the number of follicles in the different classes (Table 3).

The OL-rBST group had a larger CL area (4.67 ± 0.67 cm^2, $p < 0.05$, Table 3). There was an effect ($p < 0.05$) for the intrafollicular concentration of HDL (mg/dL), with higher means in the OL-SL (40.86 ± 9.8 mg/dL) and OL-rBST (53. 67 ± 10.35 mg/d).

Plasma progesterone concentration (P4) did not differ for the different treatment groups on day 1 (DC-SL 0.35; OL-SL 0.38; DC-rBST 0.33 and OL-rBST 0.22 ng/mL, respectively), day 7 (DC-SL 3.92; OL-SL 3.92; DC-rBST

Table 2. Body weight (BW) and daily consumption of Holstein heifers treated with dietary fat associated with recombinant bovine somatotropin.

Parameters	DC-SL	OL-SL	DC-rBST	OL-rBST	*p
BW (kg)	358.56 ± 27.14	362.53 ± 29.81	337.13 ± 21.5	350.28 ± 23.57	0.81
DM	7.09 ± 1.14	6.49 ± 0.50	6.67 ± 0.73	6.52 ± 0.49	0.79
DM (% BW)	1.98 ± 0.20	1.82 ± 0.19	1.98 ± 0.17	1.88 ± 0.13	0.91
CP[1]	0.96 ± 0.15	0.90 ± 0.07	0.91 ± 0.09	0.90 ± 0.06	0.88
NEg[2]	7.85 ± 0.59	7.94 ± 0.60	7.36 ± 1.21	7.91 ± 1.29	0.78
FND[3]	3.26 ± 0.53	3.03 ± 0.22	3.05 ± 0.34	3.04 ± 0.22	0.85
EE[4]	0.20 ± 0.03[b]	0.42 ± 0.03[a]	0.20 ± 0.02[b]	0.43 ± 0.03[a]	0.01
CNF[5]	3.49 ± 0.52	2.94 ± 0.21	3.30 ± 0.33	2.96 ± 0.21	0.18

*p-value;[1]CP: crude protein (kg/day); [2]NEg: net energy for gain (Mcal/day); [3]FND: insoluble fiber in neutral detergent (kg/day); [4]EE: ethereal extract (kg/day); [5]CNF: nonfibrous carbohydrate (kg/day). [a,b] Means followed by different letters within the same line differ from each other by Fisher's test ($p < 0.05$). DC-SL: control diet with an application of the saline solution; OL-SL: diet of soybean oil with the application of the saline solution; DC-rBST: control diet with the application of recombinant bovine somatotropin; OL-rBST: diet of soybean oil with the application of bovine recombinant somatotropin.

Table 3. Parameters evaluated in ovarian follicular dynamics and intrafollicular high-density lipoprotein concentration of heifers treated with dietary fat associated with recombinant bovine somatotropin.

Parameters	DC-SL	OL-SL	DC-rBST	OL-rBST	p*
No. of follicular waves/cycle	2	2	2.2	2.2	-
Interovulatory period (days)	21.14 ± 2.7	21.56 ± 3.2	22.33 ± 3.9	24.15 ± 7.8	0.40
First-wave emergency (days)	0.57 ± 0.7	1.11 ± 1.0	0.83 ± 0.7	1.23 ± 1.7	0.98
Second wave emergency	8.14 ± 4.3	9.00 ± 2.0	9.5 ± 2.3	10.31 ± 1.3	0.61
Third wave emergency	-	-	-	16.00 ± 1.4	-
	Follicle				
Class I (0.3–0.59 cm)	4.97 ± 2.1	3.26 ± 0.5	3.99 ± 3.6	3.10 ± 2.0	0.49
Class II (0.6–0.9 cm)	2.51 ± 0.7	2.12 ± 0.5	1.85 ± 0.5	1.88 ± 1.0	0.37
Class III (1.0–1.5 cm)	1.03 ± 0.2	0.99 ± 0.3	0.88 ± 0.1	0.77 ± 0.3	0.92
Class IV (>1.5 cm)	0.30 ± 0.4	0.11 ± 0.1	0.08 ± 0.09	0.19 ± 0.3	0.16
Diameter of the dominant follicle(cm)	1.49 ± 0.15	1.53 ± 1.14	1.62 ± 0.2	1.60 ± 0.3	0.66
Follicular growth rate (cm/day)	0.11 ± 0.03	0.13 ± 0.03	0.15 ± 0.03	0.15 ± 0.09	0.80
CL area (cm^2)	4.23 ± 0.5[b]	4.21 ± 1.0[b]	3.62 ± 2.1[b]	4.67 ± 0.6[a]	0.04
HDL intrafollicular (mg/dL)	34.83 ± 13.7[b]	40.86 ± 9.8[a]	29.8 ± 15.9[b]	53.67 ± 10.3[a]	0.01

*p-value; [a, b] Means followed by different letters within the same line differ from each other by Fisher's test ($p < 0.05$). DC-SL: control diet with an application of the saline solution; OL-SL: diet of soybean oil with the application of the saline solution; DC-rBST: control diet with the application of recombinant bovine somatotropin; OL-rBST: diet of soybean oil with the application of bovine recombinant somatotropin.

3.55 and OL-rBST 3.79 ng/mL, p = 0.94), and on 14th day (DC-SL 5.03; OL-SL 5.73; DC-rBST 6.13 and OL-rBST 6.50 ng/mL, p = 0.68) (Figure 2).

There was no effect of treatments on the total number of viable and degenerated embryos (Table 4).

4. Discussion

In this study, there were no changes in the consumption of the components of the diet in the different experimental treatments, except for the EE, which was higher in the treatments supplemented with soybean oil. Doyle et al.

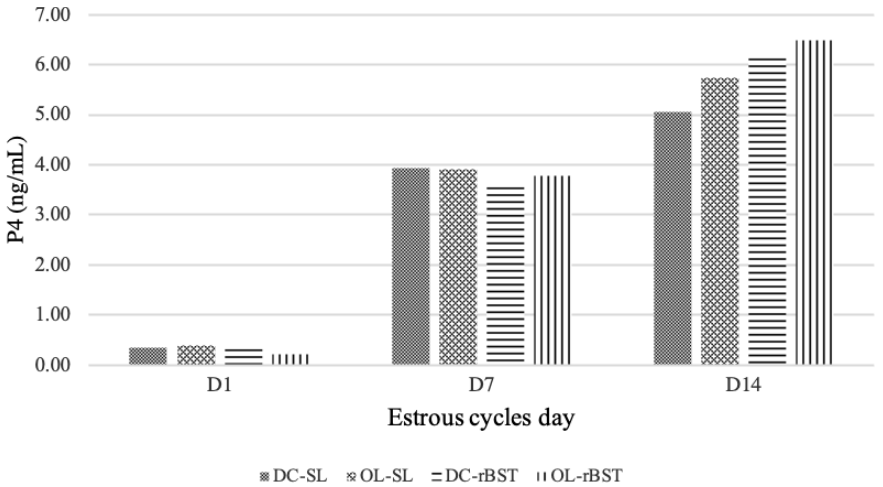

Figure 2. Plasma concentration of progesterone (P_4) in Holstein heifers treated with dietary fat associated with recombinant bovine somatotropin.

Table 4. Superovulatory response in Holstein heifers treated with dietary fat associated with recombinant bovine somatotropin.

	DC-SL	OL-SL	DC-rBST	OL-rBST	*p
Total No. of Embryos	10.83 ± 7.31	6.00 ± 3.32	11.50 ± 8.74	10.13 ± 8.58	0.63
No. of viable embryos	5.17 ± 2.22	3.50 ± 3.67	9.00 ± 8.19	7.00 ± 6.955	0.91
No. Embryos degenerates	5.67 ± 5.57	2.50 ± 1.60	2.50 ± 0.58	3.13 ± 3.50	0.22

p-value; [a, b] Fisher's test ($p < 0.05$). DC-SL: control diet with an application of the saline solution; OL-SL: diet of soybean oil with the application of the saline solution; DC-rBST: control diet with the application of recombinant bovine somatotropin; OL-rBST: diet of soybean oil with the application of bovine recombinant somatotropin.

[22] reported similar results in the administration of diets with fish oil, in which there was no reduction in dry matter intake.

The lipid sources in ruminant diets have been used to increase the energy of the diet, especially in categories of higher nutritional requirements. However, fat can cause changes in rumen metabolism, which can change microbial flora and digestibility [23].

Leroy et al. [24] advise that the lipid content in the diet does not exceed 5%, avoiding a negative effect on the digestibility of dietary fiber, consumption, and changes in the cellulolytic population, lower availability of energy. However, the NRC [18] and Ibrahim et al. [25] infer that including a lipid source above 8% in DM can have negative and inhibitory effects on ruminal fermentation.

After ingestion, polyunsaturated fatty acids (PUFAs) are hydrolyzed, and bio hydrogenated [24]. Biohydrogenation acts as a detoxifier, protecting rumen microorganisms from the deleterious effect of PUFAs since they are toxic to many rumen bacteria [15].

High-fat levels can still exceed the biohydrogenation capacity, causing an accumulation of PUFAs in the rumen and interfering with fermentation [25]. Despite the increase in the concentration of lipids above 5%, there were no deleterious effects on the consumption of dietary components in the present study.

The days of the emergence of follicular waves and the number of waves per cycle did not differ from the pattern found for Holstein heifers. We observed animals with three waves of follicular growth in OL-rBST, which numerically increased the interovulatory period. However, the estrous cycle length verified with rBST and soybean oil is within the physiological standards for the species, showing no difference since the estrous cycle in cattle can vary from 19 to 24 days for cycles with two to three waves of follicular growth [26, 27].

In this study, no effect of rBST was associated or not with soybean oil on the number of small follicles. Rincón et al. [20] got similar effects, in which rBST in a single application (500 mg) could not increase the total number

of follicles and the size of small follicles. Ribeiro and Júnior [28] reported no differences in the number of ovarian follicles when tested at doses of 250 and 500 mg of rBST for Sindhi cows.

Based on the reports by Lucy [8], an explanation for the results found in the present study is that most of the effects of rBST appear to be systemic rather than local actions in the follicle. Therefore, the rBST receptor is expressed on granulosa cells, cumulus cells, and oocytes but does not appear to control the local expression of IGF-I in the ovary. rBST binds to its receptors in the liver, activating them and stimulating the production and secretion of IGF-I, which is released into the bloodstream, acting in the ovary on the receptors present in the follicles and stimulating the production of estradiol [29]. Although rBST receptor mRNA is found in bovine ovarian follicles, the amount in the follicle is extremely low compared to the amount observed in the CL.

Lucy [8] clarifies that the number of medium-sized follicles recruited increased in cows or heifers treated with rBST but would need daily applications or sustained-release treatment, which would promote indirect effects mediated by changes in IGF-1 concentration.

Heifers treated with rBST may even increase the number of medium-sized follicles. However, these follicles do not seem to respond to superovulation, as they do not become ovulatory. Based on data in vitro, Lucy [8] suggests that the increased activity of gonadotropin receptors by the synergistic effect with IGF-1 delays the process of atresia in the population of recruited follicles, explaining the increase in the number of average follicles, while the number of selected and ovulatory follicles remains the same.

The proportion of different PUFAs can explain the increased CL area supplied in the diet [14]. Linoleic acid (Omega 6) can cause competitive inhibition of regulatory enzymes involved in PGF2α synthesis [16]. Because of the inhibition of PGF2α secretion, there is a delay in the CL's regression and an increase in its half-life [12]. Omega-6 has also been linked to more luteal cells and corpus luteum volume in cattle [25, 30].

Despite the potential positive effects related to dietary fat, no effect on CL was observed in the OL-SL group. However, when fat was associated with rBST, it was possible to notice a positively greater effect regarding the CL area. The increase in CL in this group may be related to a synergistic action between rBST and fat since there is a large amount of mRNA for rBST receptors in the CL [31].

Diets rich in omega-6 are associated with higher concentrations of cholesterol and steroidogenic regulatory protein (StAR), which can stimulate P4 production and improve oocyte quality and CL function [24, 30]. However, plasma progesterone concentration remained within the physiological patterns of the species in the different groups (Figure 2). During the follicular phase, P4 remained lower than 0.40 ng/mL, increasing in the luteal phase, reaching concentrations lower than 3.92 ng/mL on day seven and higher than 5.03 ng/mL on the 14th day. Corroborating, Kozicki et al. [32] observed the physiological estrous cycle of cows and got a mean concentration of P4 lower than 5.48 ng/mL in the follicular phase and greater than 5.48 ng/mL in the luteal phase.

Hass et al. [33] clarify that the increase in the concentration of P4 in vitro and in vivo is dose-dependent. The increase in P4 concentration can be achieved with a higher dose of rBST which causes an increase in the concentration of IGF-1 capable of stimulating P4 synthesis by the CL. The animal category also must be considered since heifers treated with rBST during estrus do not have a satisfactory increase in P4 concentration, with higher P4 concentrations being found with rBST in lactating cows, and steroidogenic capacities are compromised by lactation. Thus, a higher dose of rBST increases the concentration of IGF-1 by increasing the uptake, absorption of lipoproteins, and steroidogenesis of granulosa cells and luteal tissue, stimulating P4 synthesis in heifers [33].

Hypercholesterolemia may increase plasma P4 concentration in cattle. However, in the present study, dietary fat did not increase P4 [24]. The relationship between the effects of PUFA supplementation on systemic P4 concentrations in the literature is inconsistent with reports of increase, decrease, or no change.

Cows treated with a soybean oil emulsion exhibited a reduction in hepatic P4 clearance [22]. Indicating that cholesterol availability may not be the only limiting factor in P4 synthesis and that there may have been changes in the mechanisms responsible for the observed variations in plasma P4 concentrations. These data may explain why there was no increase in P4, despite the increase in intrafollicular HDL, which reflects plasma cholesterol but did not change P4 concentration [12].

Despite the increase in the luteal CL area observed in the OL-rBST treatment, there was no difference in the plasma concentration of P4. These results suggest that there may be a greater number of granulosa cells undergoing differentiation at ovulation or an increase in the survival of these cells after CL formation [12]. Ovarian luteal cells use cholesterol for P4 synthesis through the reserve of lipoproteins conjugated with cholesterol.

High-density lipoproteins in ruminants carry cholesterol to steroidogenic tissues, such as the liver, ovaries, adrenal gland, and testes [16].

Although there was no difference in P4 concentration, these findings are positively correlated with the concentration of HDL cholesterol found in the intrafollicular fluid in the DC-rBST and OL-SL groups (Table 1). Serum changes in PUFA concentration also

reflect on the fatty acid composition of the follicular environment, i.e. plasma PUFA levels correlate with follicular fluid levels [24]. By these mechanisms, soybean oil associated with rBST possibly provided an increase in the corpus luteum area and an increase in intrafollicular HDL.

The groups treated with rBST and dietary fat did not increase embryo production or quality. Lucy [8] suggests that, although initially, the use of rBST in superovulation protocols showed promise, treatment with rBST has not increased the number of ovulation or the number of retrieved and transferable embryos after superovulation. In contrast, when pulsatile doses of rBST were administered for 63 days, there was an increase in the number of dominant follicles and the ovulation rate.

In animals subjected to dietary fat, lipids stored within oocytes and early-stage embryos represent an important source of energy [34]. Dietary polyunsaturated fatty acid supplementation benefits oocyte and embryo quality [15]. According to Haggarty et al. [35], embryos containing a higher proportion of unsaturated fatty acids are more likely to progress in embryonic development. However, Leroy et al. [24] reported that the supply of high levels of fatty acids in the oocyte microenvironment in cows that were in negative energy balance could lead to reduced fertility due to compromised quality of the early embryo.

However, it is still unclear to what extent the lipid concentration in the oocyte and embryo in vivo in response to supplementing diet affects embryonic quality. In addition, the time effect on the administration of dietary fat and the amount and nature of the dietary supplement must be considered.

Soybean oil supplementation (6% ethereal extract based on dry matter) associated with the application of 320 mg recombinant bovine somatotropin (rBST) during superovulation did not increase and standardize the superovulatory response and embryonic recovery of Holstein heifers.

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