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Impacts of body condition score at beginning of fixed-timed AI protocol and subsequent energy balance on ovarian structures, estrus expression, pregnancy rate and embryo size of *Bos indicus* beef cows

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HIGHLIGHTS

- Greater BCS and positive energy balance caused greater ovarian structures development.
- Greater BCS and positive energy balance caused greater estrus expression.
- Greater BCS and positive energy balance caused greater pregnancy rate.
- Greater BCS and positive energy balance caused greater embryo size.

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ABSTRACT

These two studies evaluated the effects of body condition score (BCS) at beginning of fixed-timed artificial insemination (FTAI) protocol and subsequent BCS change on ovarian structures, estrus expression, pregnancy rate and embryo size of multiparous Nellore beef cows. In Experiment 1, 240 cows, distributed over four years (*n* = 40, 20, 82 and 98 cows, for years 1, 2, 3 and 4, respectively) were assigned to FTAI protocol from d -11 to 0. Cow BCS (d -11 and 40), body weight (BW; d -11 and 40), dominant follicle (d 0) and corpus luteum (CL; d 7 and 14) size, plasma progesterone (d 7 and 14), pregnancy rate (d 40) and embryo size (d 40) were evaluated. In Experiment 2, 2443 cows (single year) were assigned to FTAI protocol from d -11 to 0. Cow BCS (d -11 and 30), BW (d -11 and 30), estrus expression (d 0), and pregnancy rate (d 30) were evaluated. BCS scale used ranged from 1 (extremely thin) to 9 (extremely fat) and the cows were categorized in groups (BCS $\geq$ 5 and $<$ 5 and in lost, maintained and gained BCS). Experiment 1, cows with BCS $\geq$ 5 at beginning FTAI protocol tended ($P \leq 0.10$) to have greater CL diameter (d 7), CL volume (d 7 and 14) and had greater ($P \leq 0.05$) plasma concentration of progesterone (d 14) and embryo size (d 40), compared to cows with BCS $<$ 5. Further, cows that gained BCS from d -11 to 40 tended ($P = 0.10$) to have greater dominant follicle size and cows that gained and maintained BCS had greater ($P = 0.02$) plasma concentration of progesterone (d 14), compared to cows that lost BCS. The embryo size was greater ($P < 0.01$) for cows that gained, intermediary to cows that maintained and lower to cows that lost BCS. In Experiment 2, cows with BCS $\geq$ 5 had greater ($P = 0.02$) estrus expression, compared to cows with BCS $<$ 5, and the pregnancy rate increased linearly with BCS ($P < 0.01$; 20.0 to 69.1% for BCS 2 and 9, respectively). Further, cows that gained had greater ($P < 0.01$) estrus expression compared to cows that maintained and lost BCS, and cows that gained and maintained BCS had greater pregnancy rate compared to cows that lost BCS ($P < 0.01$; 62.2, 59.7 and 49.3 $\pm$ 10.3%, respectively). Thus, cows with greater BCS at

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beginning of FTAI protocol or that gained or maintained BCS during synchronization and after AI had greater ovarian structures development, estrus expression, pregnancy rate and embryo size.

Introduction

Body condition score (BCS) is a subjective measure that estimates the body energy reserves of animals, and is one of main modulator of the reproductive performance of cows and overall productivity of cow-calf systems. Cows with greater BCS ($\geq$ 5 vs. $<$ 5; scale 1 – 9) at beginning of breeding season had greater pregnancy and calving rate, calving earlier and weaning heavier calves (Cooke et al., 2021). Also, BCS affects the physiology, and cows with greater BCS have greater blood concentrations of insulin-like growth factor 1 (IGF-1), leptin, insulin, thyroxine, glucose and non-esterified fat acids (NEFA; Ciccioli et al., 2003). Further, BCS also affects the ovary functions, and cows with greater BCS have greater dominant follicle size, corpus luteum area, estrus rate and ovulation rate (Lents et al., 2008; Nishimura et al., 2018).

In addition to the effects of BCS, the BCS change [indicator of energy balance (EB)] after calving affects cow fertility. Dairy cows that gained compared to cows that lost BCS from parturition to 3 wk postpartum have less concentration of NEFA and greater pregnancy rate. Further, EB affects embryo development in dairy cows (Carvalho et al., 2014). Also, dairy cows in positive compared to negative EB after calving have greater serum concentration of IGF-1, luteal-phase progesterone secretion and estrus expression (Spicer et al., 1990).

Little research has been conducted to evaluate the effects of EB during the fixed time-AI (FTAI) period on ovarian structures, estrus expression, and embryo size in *Bos indicus* beef cows. Further, we are not aware of study that have evaluated the effects of BCS at beginning of FTAI protocol on subsequent embryo size. It was hypothesized that cows with a BCS $\geq$ 5.0 and in positive EB will have enhanced fertility and embryo size, compared with cohorts with a BCS $<$ 5.0 or in a negative EB. Thus, two experiments evaluated the effects of BCS at beginning of FTAI protocol and subsequent BCS change on ovarian structures, estrus expression, pregnancy rate and embryo size of Nellore (*Bos indicus)* beef cows.

2. Material and methods

Experiments 1 and 2 were approved by the ethics committee on animal use of the Universidade Federal de Mato Grosso do Sul (UFMS) under the protocol n° 754/2016.

2.1. Animals, treatments and samples collection

Experiment 1

Table 1
Chemical composition of forages (Urochloa spp.) grazed during the experiments.

Items [a]	Experiment 1	Experiment 2			
	Year 1	Year 2	Year 3	Year 4	Year 1
Number of cows	40	20	82	98	2443
CP,%	6.88	4.60	5.08	5.50	8.70
NDF,%	72.77	71.7	71.90	72.0	66.3
TDN[b],%	57.20	53.6	54.5	57.6	55.5
NEm[c], Mcal/kg	1.22	1.09	1.12	1.11	1.16
NEg[c], Macal/kg	0.65	0.54	0.57	0.55	0.60

[a] CP, crude protein; NDF, neutral detergent fiber; TDN, total digestible nutrients; NEm, net energy for maintenance; NEg, net energy for gain.
[b] Calculated as described by Weiss et al. (1992).
[c] Calculated using the equations proposed by the NASEM (2016).

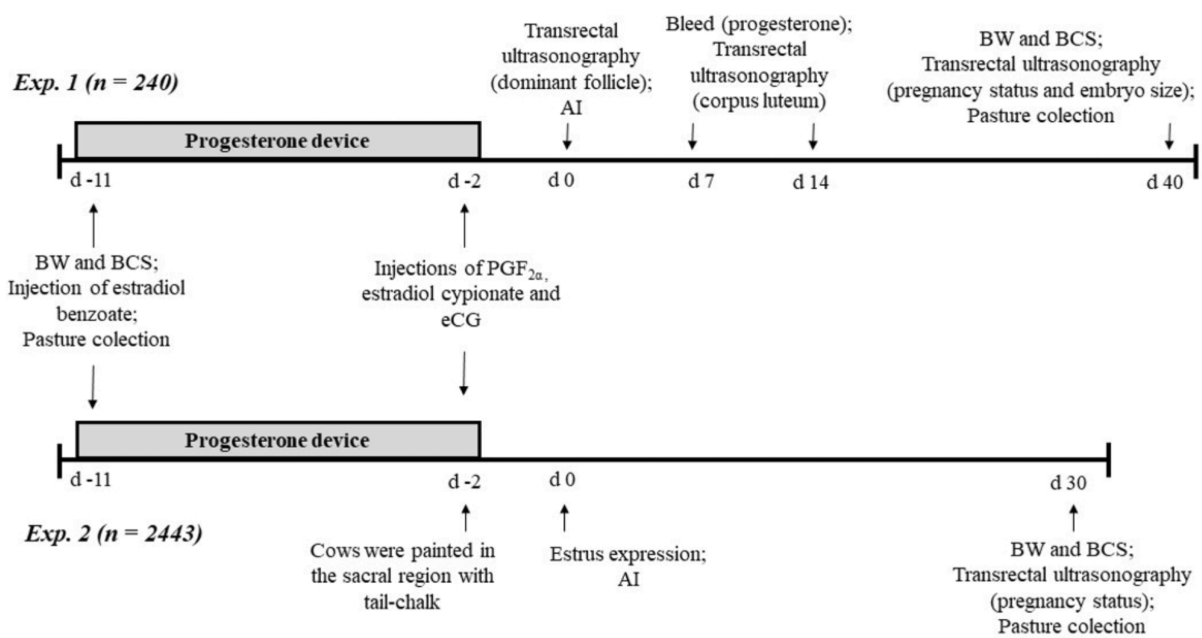

Fig. 1. Scheme of experimental design of experiments 1 and 2. All cows were assigned to a fixed time artificial inseminination (FTAI) protocol from d -11 to 0. On d -11, cows received a 2-mg intramuscular injection of estradiol benzoate (Gonadiol; Zoetis, São Paulo, Brazil) and inserted with an intravaginal progesterone-releasing device (CIDR; Zoetis) containing 1.9 g of progesterone (P4). On d -2, CIDR device was removed, and each cow received intramuscular injections of $PGF_{2\alpha}$ (12.5 mg of dinoprost trometamina/cow; Lutalyse; Zoetis), estradiol cypionate (1 mg/cow; ECP; Zoetis) and eCG (300 IU/cow; Novormon; Zoetis). On d 0, cows were timed artificial inseminated (AI). Body weight (BW) and body condition score (BCS; scale 1 – 9; evaluated according to Herd and Sprott, 1986) were evaluated on d -11 and d 40 (Exp. 1) or d 30 (Exp. 2). On d -2 (only on Exp. 2), all cows were painted in the sacral region with tail-chalk (RaidexMaxi; RAIDEX GmbH, Dettingen/Erms, Germany), and on d 0, estrus expression was evaluated and classified in scores according to paint removal: 1 – no paint removal = without estrus expression; 2 – poor paint removal = low estrus expression; 3 – total paint removal = high expression of estrus.

Table 2
Effects of body condition score category at beginning of fixed-time artificial insemination protocol (d – 11) on body variables and ovary structures, plasma progesterone, pregnancy rate and embryo size of multiparous cows (n = 240; Exp. 1).

Items	BCS category[a]	SEM	P-value	
	< 5	≥ 5		
Number of cows	136	104	–	–
Body variables				
BCS (d-11), 1 – 9	3.77[b]	5.37[a]	0.11	<0.01
Body weight (d -11), kg	367.29[b]	410.84[a]	13.65	<0.01
Ovary structures				
Dominant follicle (d 0), mm	12.55	12.72	0.57	0.73
Corpus luteum diameter, mm				
d 7	18.53[b]	19.23[a]	0.30	0.10
d 14	24.09	27.77	4.94	0.85
Corpus luteum volume, cm^3				
d 7	3.56[b]	3.95[a]	0.15	0.09
d 14	9.30[b]	11.56[a]	7.00	0.09
Plasma progesterone, ng/mL				
d 7	3.36[b]	4.74[a]	0.42	0.10
d 14	4.75[b]	6.86[a]	0.49	0.02
Pregnancy rate (d 40),%	57.36	57.55	8.00	0.97
Embryo size (d 40), mm				
Crown-rump	15.48[b]	18.19[a]	0.90	0.06
Thoracic	6.03[b]	7.29[a]	0.77	0.05

[a] Body condition score (BCS; scale 1 - 9) was evaluate on d -11 according to Herd and Sprott (1986) and the cows were categorized in BCS < 5 or ≥ 5.

Table 3
Effects of body condition score change (d -11 to d 40) category during fixed-time artificial insemination period on body variables and ovary structures, plasma progesterone, pregnancy rate and embryo size of multiparous cows (n = 240; Exp. 1).

Items	BCS change category[1]	SEM	P-value		
	Lost	Maintained	Gained		
Number of cows	63	90	87	–	–
Body variables					
BCS change (d -11 to d 40), 1 – 9	−1.10[c]	0.00[b]	1.13[a]	0.04	<0.01
Body weight change (d-11 to d 40), kg	−3.13[c]	6.52[b]	14.52[a]	3.12	<0.01
Ovary structures					
Dominant follicle (d 0), mm	11.89[b]	12.68[ab]	13.00[a]	0.59	0.10
Corpus luteum diameter, mm					
d 7	18.65	19.22	18.54	0.38	0.39
d 14	23.09	23.94	23.00	5.28	0.41
Corpus luteum volume, cm^3					
d 7	3.60	3.97	3.53	0.22	0.33
d 14	10.13	10.46	8.39	6.38	0.18
Plasma progesterone, ng/mL					
d 7	3.94[b]	2.41[b]	5.61[a]	0.91	0.01
d 14	3.60[b]	6.31[a]	7.14[a]	1.03	0.02
Pregnancy rate (d 40), %	50.82	62.25	63.20	6.53	0.38
Embryo size (d 40), mm					
Crown-rump	12.60[c]	15.56[b]	18.80[a]	1.89	<0.01
Thoracic	5.48[c]	6.53[b]	8.63[a]	0.67	<0.01

1Body condition score (BCS; scale 1 - 9) was evaluate on d -11 and d 40 according to Herd and Sprott (1986). The cows were categorized in lost, maintained and gained BCS from d -11 to d 40.
[a–c]Within a row, means without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).

Table 4
Pearson correlation coefficients between the variables evaluated (n = 240; Exp. 1).

Variables[a]	Pearson correlation coefficients	P–value[b]
BCS (d-11) ×		
BCS change (d-11 to d 40)	−0.51	<0.01
Body weight (d-11)	0.45	<0.01
Body weight change (d-11 to d 40)	−0.16	0.07
Dominant follicle (d 0)	0.06	0.39
Corpus luteum diameter (d 7)	0.18	0.03
Corpus luteum diameter (d 14)	0.11	0.13
Corpus luteum volume (d 7)	0.17	0.04
Corpus luteum volume (d 14)	0.11	0.14
Plasma progesterone concentration (d 7)	−0.11	0.53
Plasma progesterone concentration (d 14)	−0.17	0.30
Embryo size – crown-rump (d 40)	0.22	0.04
Embryo size – thoracic (d 40)	0.21	0.04
BCS change (d -11 to d 40) ×		
Body weight (d -11)	0.28	<0.01
Body weigh change (d -11 to d 40)	0.27	<0.01
Dominant follicle (d 0)	−0.15	0.03
Corpus luteum diameter (d 7)	−0.17	0.04
Corpus luteum diameter (d 14)	−0.07	0.35
Corpus luteum volume (d 7)	−0.08	0.32
Corpus luteum volume (d 14)	−0.02	0.77
Plasma progesterone concentration (d 7)	0.50	<0.01
Plasma progesterone concentration (d 14)	0.36	0.02
Embryo size – crown-rump (d 40)	−0.19	0.05
Embryo size – thoracic (d 40)	−0.20	0.05

[a] Body condition score (BCS; scale 1 - 9) was evaluate on d -11 and d 40 according to Herd and Sprott (1986).
[b] Only significant correlations ($P \leq 0.05$) or tendencies ($P \leq 0.10$) were presented.

Experiment 1 (Fig. 1) was conducted at the Faculdade de Medicina Veterinária e Zootecnia of UFMS in Terenos, MS, Brazil (20°26′50.8″S 54°50′21.5″W). A total of 240 multiparous, lactating Nellore cows [n = 40, 20, 82 e 98 cows in year 1, 2, 3 and 4, respectively; BCS = 4.4 ± 0.78, scale 1–9; body weight (BW) = 393 ± 36.8 kg, and 5.4 ± 2.5 yr of age] were enrolled in the experiment. The experiment started 11 days before and ended 40 days after FTAI (d -11 to 40). All cows were kept in a single group per year in pasture of marandu-grass [*Urochloa brizantha* (Hochst. ex A. Rich) R. D. Webster, cv. Marandu; Table 1] and had free-choice access to water and a complete trace mineral/vitamin mix (Mega Fós 90 Milk, AgroMega Indústria de Alimentos Animal, Tamboara, PR, Brazil; Ca: 196 g / kg, P: 90 g / kg, Na: 99 g / kg, Mg: 20 g / kg, S: 14 g / kg, Fe: 2.400 mg / kg, Zn: 3000 mg / kg, Mn, 1670 mg / kg, Se: 40 mg / kg, Cu:1200 mg / kg, F: 900 mg / kg, Co: 200 mg / kg, I: 180 mg / kg, vitamin A: 150,000 UI / kg, vitamin D3: 30,000 UI / kg and vitamin E: 1.500 UI / kg; target intake of 100 g / d).

All cows were assigned to a FTAI protocol from d -11 to 0. On d-11, cows received a 2-mg intramuscular injection of estradiol benzoate (Gonadiol; Zoetis, São Paulo, Brazil) and inserted with an intravaginal progesterone-releasing (CIDR; Zoetis) device containing 1.9 g of progesterone (P4). On d -2, CIDR device was removed, and each cow received intramuscular injections of $PGF_{2\alpha}$ (12.5 mg of dinoprost trometamina/cow; Lutalyse; Zoetis), estradiol cypionate (1 mg/cow; ECP; Zoetis) and eCG (300 IU/cow; Novormon; Zoetis). On d 0, cows were timed-AI by a single technician using semen from a single Nellore bull.

The dominant follicle diameter (d 0), corpus luteum (CL; d 7 and 14) diameter, pregnancy status (d 40) and embryo size (crown-rump and thoracic; d 40) were assessed by transrectal ultrasonography (7.5-MHz transducer; Mindray DP 2200 VET, Shenzhen, China). The CL volume (cm^3) was calculated using the formula for the volume of the sphere [$V = 4/3\pi(D/2)^3$ where D is the maximum diameter (mm) of the CL (Cooke et al., 2009). Cow BW and BCS were collected on d −11, and 40. Cow BCS was evaluated by a single technician during the four years, according to Herd and Sprott (1986). No manipulation of BCS was conducted in this study and cows were managed according to the farm

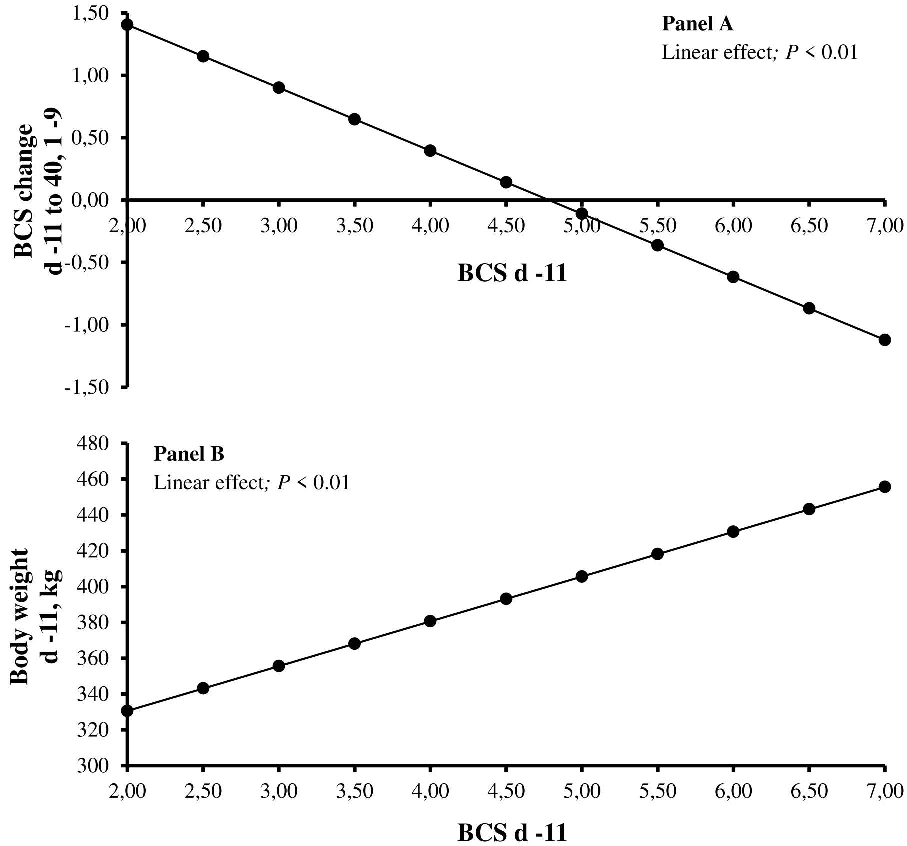

Fig. 2. Effects of body condition score (BCS; d-11) at beginning of fixed-time artificial insemination protocol on BCS change (d-11 to 40; Panel A) and body weight (d-11; Panel B). The BCS (scale 1 - 9) was evaluate according to Herd and Sprott (1986). $N = 240$; Exp. 1.

management practices. The cows were divided into two categories according to BCS on d -11 (BCS < 5.0 and BCS ≥ 5.0) and also, into three categories according to BCS change from d-11 to 40 (lost, maintained, or gained BCS).

Blood samples were collected from the coccygeal vein on d 7 and 14 into 10-mL blood collection tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) with sodium heparin. Immediately after collection, blood samples were stored on ice and then centrifuged at 1200 × g for 30 min at 4 °C for plasma harvest. Plasma samples were stored at −20 °C for further analysis of the plasma concentrations of progesterone. Hand plucked samples of pastures were collected on d -11 and 40, and then dried at 60 °C for 5 days, ground to 1 mm, made a composite sample, and analyzed for chemical composition.

Experiment 2

In Experiment 2 (Fig. 1), 2443 multiparous, lactating Nellore cows (BCS = 5.0 ± 0.52; BW = 399 ± 58 kg; approximately 6 yr of age) from a commercial cow-calf operation, located in Miranda, MS, Brazil (20°24′02.0″S 56°18′11.2″W) were enrolled in this study. Cows were maintained in 20 groups (approximately 122 cows/group) and each group was rotated within its respective pasture pair every 14 days to maintain pasture quantity and quality between two pastures (40 to 60 ha/pasture) containing decumbens-grass [*Urochloa decumbens* (Stapf) R. D. Webster]. All cows had free-choice access to water and a complete trace mineral/vitamin mixture (Probeef 800, Nutron Nutrição Animal, Itapira, SP, Brazil; Ca: 140 - 190 g / kg, P: 80 g / kg, Na: 110 g / kg, S: 16 g / kg, Mg: 10 g/kg, Zn: 3272.7 mg / kg, Mn: 4681.8 mg / kg, Se: 11.8 mg / kg, Cu: 1091 mg / kg, F: 880 mg / kg, Co: 61 mg / kg, I: 55 mg / kg; target intake of 200 g / d).

Cow BW and BCS were collected on d -11 and 30. Cow BCS was evaluated by a single technician, according to Herd and Sprott (1986). No manipulation of BCS was conducted in this study and cows were managed according to the farms management practices. The cows were divided into two categories according to BCS on d -11 (BCS < 5.0 and BCS ≥ 5.0) and also, into three categories according to BCS change from d-11 to 30 (Exp. 2; lost, maintained, or gained BCS).

All cows were submitted to a FTAI protocol from d -11 to 0, as described for Experiment 1. However, in this study, at the time of progesterone dispositive removal (d -2), all cows were painted in the sacral region with tail-chalk (RaidexMaxi; RAIDEX GmbH, Dettingen/Erms, Germany), and in the insemination moment (d 0), estrus expression was evaluated and classified in scores according to paint removal on sacral region: 1 – no paint removal = without estrus expression; 2 – poor paint removal = low estrus expression; 3 – total paint removal = high expression of estrus (Rodrigues et al., 2019). Also, cows that had scores 1 were classified as negative and scores 2 and 3, as positive for mating occurrence, respectively.

Cows were inseminated by three technicians using semen from seven Nellore bulls. Pregnancy status was assessed on d 30 by transrectal ultrasonography (7.5-MHz transducer; Mindray DP 2200 VET, Shenzhen, China). Hand plucked samples of pastures were collected on d -11 and 30, and then dried at 60 °C for 5 d, ground to 1 mm, made a composite sample, and analyzed for chemical composition.

2.2. Laboratory analysis

Forage samples (Exp. 1 and 2) were analyzed for crude protein (CP) concentration, according to AOAC (2000; method 976.05) and neutral detergent fiber (NDF) concentration according to Van Soest et al. (1991). The total digestible nutrients (TDN) concentrations were calculated as described by Weiss et al. (1992), and net energy for maintenance (NEm) and gain (NEg) by the equations proposed by the NASEM (2016).

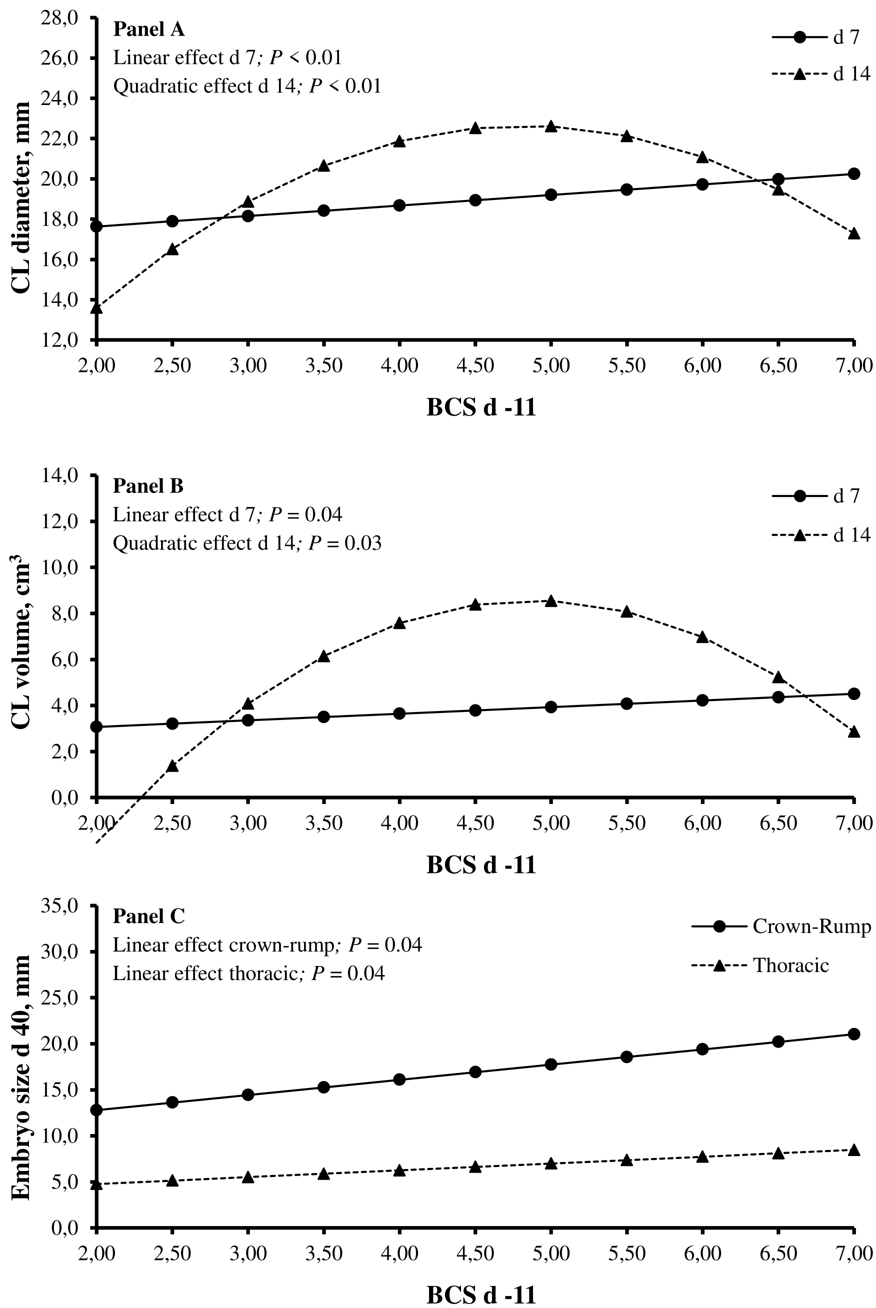

Fig. 3. Effects of body condition score (BCS; d-11) at beginning of fixed-time artificial insemination protocol on corpus luteum (CL) diameter (Panel A) CL volume (Panel B) and embryo size (Panel C). The BCS (scale 1 - 9) was evaluate according to Herd and Sprott (1986). $N = 240$; Exp. 1.

Plasma progesterone concentrations (Exp. 1) were determined using a solid-phase, competitive, chemiluminescent enzyme immunoassay (IMMULITE 1000, Diagnostics Products Corp. USA) previously validated for cattle samples (Martin et al., 2007). Detectable range and intra-assay CV for plasma progesterone concentrations were 0.2 to 9.9 ng/mL and 4.7%, respectively.

2.3. Statistical analyzes

All results were analyzed using SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4) with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. In Experiment 1, body variables, ovarian structures, plasma progesterone and embryo size were analyzed using MIXED procedure and pregnancy rate using GLIMMIX procedure of SAS. These variables were tested for fixed effect of BCS category, BCS change category, year, and all resulting interactions using cow(BCS category) or cow(BCS change category) as random effects. Means were separated using PDIFF and all results were reported as LSMEANS followed by SEM. Significance was defined when $P \leq 0.05$, and tendency when $P > 0.05$ and ≤ 0.10. To evaluate the correlation between the variables the CORR procedure of SAS was used. Further, polynomial regressions were made between BCS obtained on d -11 or BCS change from d -11 to 40 on body variables, ovarian structures, plasma progesterone and embryo size using the GLM procedure of SAS and tested for linear and quadratic effects. In Exp. 2, body variables and estrus score were analyzed using MIXED procedure and mating rate, pregnancy rate and pregnancy lost using GLIMMIX procedure of SAS. These variables were tested for fixed effect of BCS category or BCS change category, sire, technician that inseminate and all resulting interactions using cow(BCS category) or cow(BCS change category) and

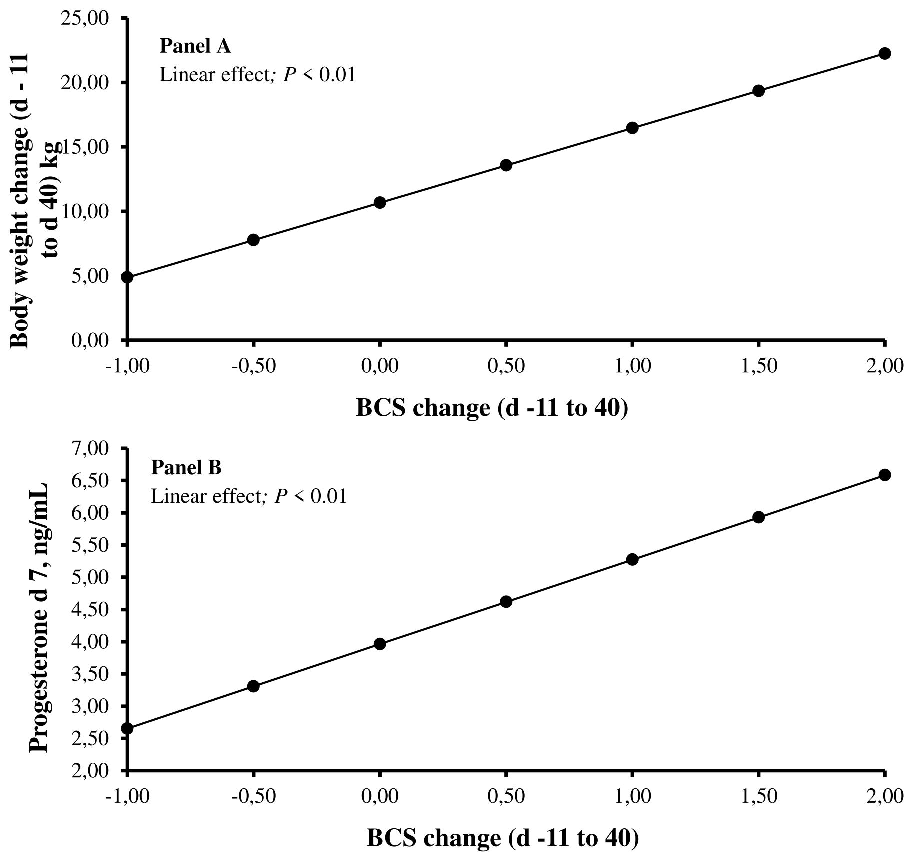

Fig. 4. Effects of body condition score (BCS) change (d-11 to 40) during the fixed-time artificial insemination period on body weight change (d-11 to 40; Panel A) and plasma progesterone concentration (d 7; Panel B). The BCS (scale 1 - 9) was evaluate according to Herd and Sprott (1986). $N = 240$; Exp. 1.

Table 5
Effects of body condition score category (d-11) at beginning of fixed-time artificial insemination protocol on body variables, estrous expression and pregnancy rate of multiparous cows ($n = 2443$; Exp. 2).

Items	BCS category[a] < 5	≥ 5	SEM	*P*-value
Number of cows	858	1585	–	–
Body variables				
BCS (d-11), 1 – 9	4.44[b]	5.36[a]	0.04	<0.01
Body weight (d -11), kg	397[b]	423[a]	15.4	<0.01
Estrus expression[b]				
Estrus score (d 0), 1 – 3	2.59[b]	2.66[a]	0.04	0.02
Mating (d 0),%	89.7	91.0	1.59	0.38
Pregnancy rate (d 30),%	57.8	60.7	0.02	0.22
Pregnancy lost (d 30 to 90),%	8.04	7.63	1.43	0.80

[a] Body condition score (BCS; scale 1 - 9) was evaluate on d -11 according to Herd and Sprott (1986) and the cows were categorized in BCS < 5 or ≥ 5.
[b] In fixed-time AI protocols, at the time of progesterone dispositive removal (d -2), all cows were painted in the sacral region with tail-chalk (RaidexMaxi; RAIDEX GmbH, Dettingen/Erms, Germany), and in the insemination moment (d 0), estrus expression was evaluated and classified in scores according to paint removal on sacral region: 1 – no paint removal = without estrus expression; 2 – poor paint removal = low estrus expression; 3 – total paint removal = high expression of estrus (Rodrigues et al., 2019).). Also, cows that had scores 1 were classified as negative and scores 2 and 3, as positive for mating occurrence, respectively.

group as random effects. As no effects of sire or technicians, or interactions with them were detected ($P \geq 0.33$) for any variable, they were moved to random effects. Means were separated using PDIFF and all results were reported as LSMEANS followed by SEM. Significance was defined when $P \leq 0.05$, and tendency when $P > 0.05$ and ≤ 0.10. Further, polynomial regressions were made between BCS obtained on d -11 or BCS change from d -11 to 30 on body variables and estrus score using the GLM procedure of SAS and tested for linear and quadratic effects. Also, logistic regressions were made between BCS obtained on d -11 or BCS change from d -11 to 30 on mating rate, pregnancy rate and pregnancy lost using the LOGISTIC procedure of SAS using link logit function.

3. Results

Experiment 1

Effects of year or interaction of year × BCS category × BCS change category were not detected ($P > 0.44$) for any variables. Cows with BCS ≥ 5 had greater ($P < 0.01$) BCS and BW, compared to cows with BCS < 5 (Table 2). No effects of BCS category were detected ($P \geq 0.73$) for dominant follicle size; however, cows with BCS ≥ 5 tended ($P \leq 0.10$) to have greater CL diameter on d 7 and CL volume on d 7 and 14, compared to cows with BCS < 5. Further, the plasma concentration of progesterone was affected by BCS and cows with BCS ≥ 5 tended ($P = 0.10$) to have greater concentration on d 7 and had greater ($P = 0.02$) concentration on d 14, compared to cows with BCS < 5. Although cows with BCS ≥ 5 vs. < 5 had greater ($P = 0.05$) embryo size on d 40, no differences in pregnancy rate ($P = 0.97$) were observed.

The BCS change category from d -11 to 40 had different ($P < 0.01$) BCS change and BW change (Table 3). Further, cows that gained tended ($P = 0.10$) to have greater dominant follicle size, compared to cows that lost BCS. Although no effects ($P \geq 0.18$) of BCS change category were detected for CL diameter and volume, cows that gained BCS had greater ($P = 0.01$) plasma concentration of progesterone on d 7, compared to cows that maintained and lost BCS. Cows that maintained and gained BCS had greater ($P = 0.02$) plasma concentration of progesterone on

Table 6
Effects of body condition score change (d-11 to d 30) category during fixed-time artificial insemination period on body variables, estrous expression and pregnancy rate of multiparous cows (n = 2443; Exp. 2).

Items	BCS change category[a]			SEM	P-value
	Lost	Maintained	Gained		
Number of cows	410	767	1266	–	–
Body variables					
BCS change (d -11 to d 30), 1 – 9	−0.51[a]	0.00[b]	0.70[c]	0.04	<0.01
Body weight change (d -11 to d 30), kg	4.04[a]	7.36[b]	11.5[c]	4.07	<0.01
Estrus expression[b]					
Estrus score (d 0), 1 – 3	2.56[b]	2.59[b]	2.70[a]	0.04	<0.01
Mating (d 0),%	88.7[b]	88.3[b]	92.6[a]	1.73	0.01
Pregnancy rate (d 30),%	49.3[b]	59.7[a]	62.2[a]	10.3	<0.01
Pregnancy lost (d 30 to 90),%	7.11	7.20	8.43	1.48	0.72

[a–c]Within a row, means without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).

[a] Body condition score (BCS; scale 1 - 9) was evaluate on d -11 and d 40 according to Herd and Sprott (1986). The cows were categorized in lost, maintained and gained BCS from d -11 to 40.

[b] In fixed-time AI protocols, at the time of progesterone dispositive removal (d -2), all cows were painted in the sacral region with tail-chalk (RaidexMaxi; RAIDEX GmbH, Dettingen/Erms, Germany), and in the insemination moment (d 0), estrus expression was evaluated and classified in scores according to paint removal on sacral region: 1 – no paint removal = without estrus expression; 2 – poor paint removal = low estrus expression; 3 – total paint removal = high expression of estrus (Rodrigues et al., 2019). Also, cows that had scores 1 were classified as negative and scores 2 and 3, as positive for mating occurrence, respectively.

d 14, compared to cows that lost BCS. Although BCS change category did not affect (P = 0.38) pregnancy rate, BCS change category impacted ($P \leq 0.01$) the embryo size (greater for cows that gained, intermediary for cows that maintained and lower for cows that lost BCS, respectively).

Cow BCS obtained on d -11 had negative correlations ($P \leq 0.05$) with BCS change and BW change, and positive correlations ($P \leq 0.04$) with BW, CL and embryo size (Table 4). Further, BCS change from d -11 to 40 had negative correlations ($P \leq 0.05$) with dominant follicle size, CL diameter (only on d 7) and embryo size, and positive correlations ($P \leq 0.02$) with BW change and plasma concentration of progesterone. No correlations ($P \geq 0.11$) of BCS or BCS change with any other variable were detected.

In the regressions analysis, the BCS change linearly decreased ($P < 0.01$) as BCS increased (Fig. 2; Panel A) whereas BW increased linearly ($P < 0.01$) as BCS increased (Fig. 2; Panel B). As BCS increased, CL diameter and volume increased linearly ($P \leq 0.04$) on d 7 and quadratically ($P \leq 0.04$) on d 14 (Fig. 3; Panel A and B). Further, the embryo size increased linearly ($P \leq 0.04$) with the BCS (Fig. 3; Panel C). The cows linearly ($P \leq 0.01$) gained BW and increased the plasma concentration of progesterone as cows gained more BCS (Fig. 4; Panel A and B).

Experiment 2

Cows with BCS ≥ 5 (Table 5) had greater ($P \leq 0.01$) BCS, BW and estrus score, compared to cows with BCS < 5 and no effects of BCS category were detected ($P \geq 0.22$) for mating rate, pregnancy rate and pregnancy lost. The BCS change category from d -11 to 30 had different ($P < 0.01$) BCS change and BW change (greater for cows that gained, intermediary for cows that maintained, and lower to cows that lost BCS, respectively; Table 6). Further, cows that gained BCS had greater ($P < 0.01$) estrus score and mating rate, compared to cows that maintained and lost BCS. However, cows that gained and maintained had greater pregnancy rate ($P < 0.01$), compared to cows that lost BCS. No effects (P

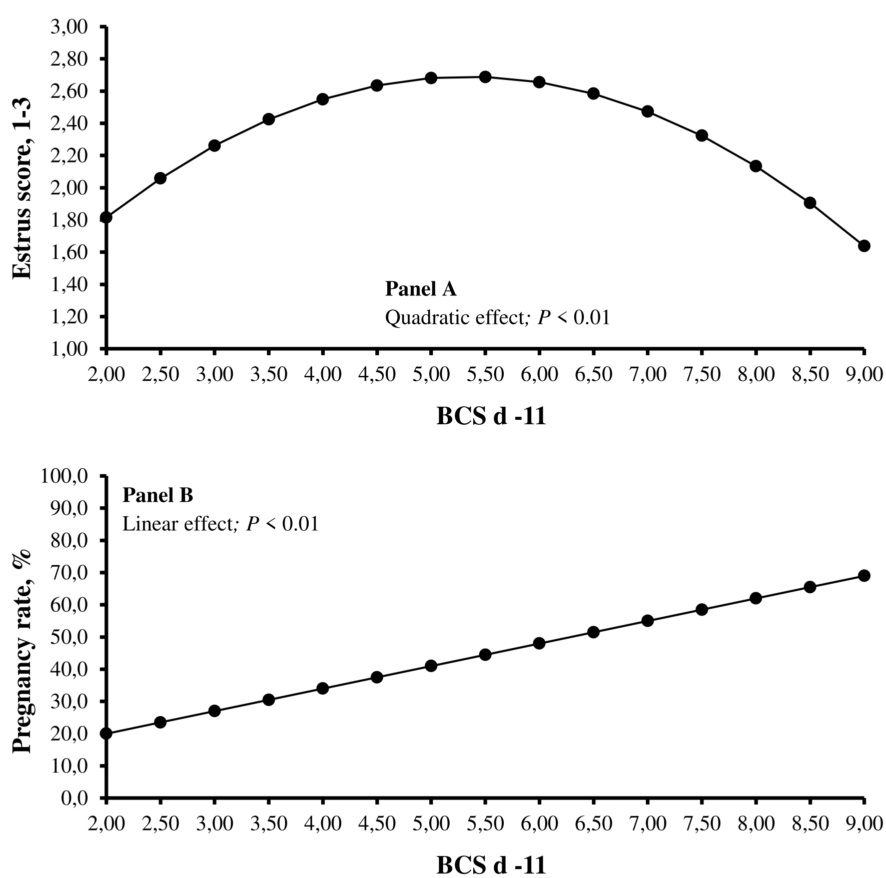

Fig. 5. Effects of body condition score (BCS; d-11) at beginning of fixed-time artificial insemination protocol on estrus score (Panel A) and pregnancy rate (Panel B) The BCS (scale 1 - 9) was evaluate according to Herd and Sprott (1986). In fixed-time AI protocols, at the time of progesterone dispositive removal (d-2), all cows were painted in the sacral region with tail-chalk (RaidexMaxi; RAIDEX GmbH, Dettingen/Erms, Germany), and in the insemination moment (d 0), estrus expression was evaluated and classified in scores according to paint removal on sacral region: 1 – no paint removal = without estrus expression; 2 – poor paint removal = low estrus expression; 3 – total paint removal = high expression of estrus (Rodrigues et al., 2019). N = 2443; Exp. 2.

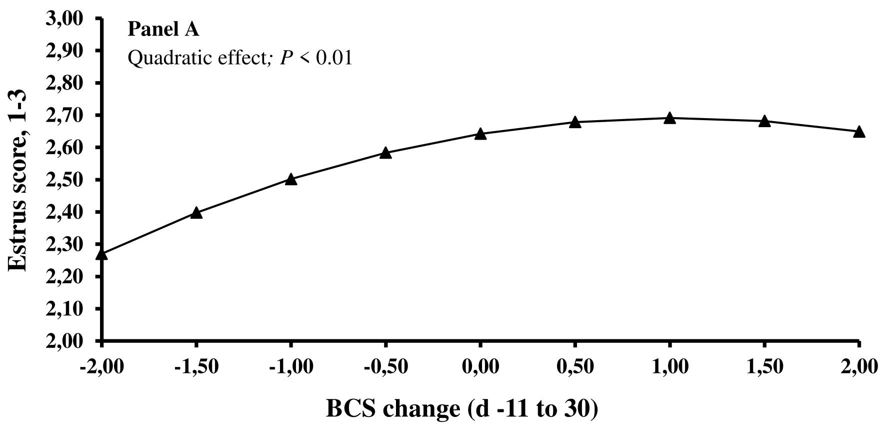

Fig. 6. Effects of body condition score (BCS) change (d-11 to 30) during the fixed-time artificial insemination period on estrus score (Panel A). The BCS (scale 1 - 9) was evaluate according to Herd and Sprott (1986). In fixed-time AI protocols, at the time of progesterone dispositive removal (d-2), all cows were painted in the sacral region with tail-chalk (RaidexMaxi; RAIDEX GmbH, Dettingen/Erms, Germany), and in the insemination moment (d 0), estrus expression was evaluated and classified in scores according to paint removal on sacral region: 1 – no paint removal = without estrus expression; 2 – poor paint removal = low estrus expression; 3 – total paint removal = high expression of estrus (Rodrigues et al., 2019). $N = 2443$; Exp. 2.

= 0.72) of BCS change category were detected for pregnancy lost.

In the regressions analysis, the estrus score increased quadratically ($P < 0.01$; Fig. 5; Panel A) and pregnancy rate increased linearly ($P < 0.01$; Fig. 5; Panel B) as BCS increased. Further, the estrus score increased quadratically ($P < 0.01$; Fig. 6; Panel A) as cows gained BCS. In the regression analysis, no effects ($P \geq 0.15$) of BCS or BCS change were detected for any other variable (data not shown).

4. Discussion

Cows with greater BCS at the beginning of the FTAI protocol, lost more BCS throughout the study, indicating a greater negative EB. Cows with BCS $\geq$ 5 were heavier than cows with BCS <5, and consequently had greater energy requirements (NASEN, 2016), which was not met by the low-quality forage available, leading to greater BCS compared to cows of BCS < 5. In experiment 1, the CP concentration in forage ranged from 4.60% to 6.88% and TDN ranged from 53.6% to 57.6%.

Cows with greater BCS or in positive EB had better development of ovarian structures. In another study, Lents et al. (2008) also describe that cows with BCS $\geq$ 5 had larger dominant follicles size than cows with BCS <5. Further, Nishimura et al. (2018) also observed that cows with greater BCS have greater dominant follicle size, CL area, and ovulation rate. Cows with greater BCS may have greater circulating concentrations of IGF-1, leptin, insulin, thyroxine, glucose and NEFA, and these changes in physiological parameters could affect follicular development (Ciccioli et al., 2003). In addition, cows with lower BCS may have consumed less forage. According to Murphy et al. (1990), the lower nutrient intake leads to higher turnover rate of cells and the increase in turnover rate in follicles and CL, and consequently, decreasing follicles and CL sizes. In other study, Holstein heifers in negative EB have smaller CL size and serum concentration of IGF-1 (Yung et al., 1996). These differences in physiology between females in positive compared to negative EB could explain the differences on ovary structures size in the current study.

Cows with greater BCS or that gained BCS had higher progesterone production. Others studies have reported that beef and dairy cows with greater BCS (Lents et al., 2008; Carvalho et al., 2014) or dairy heifers in positive EB (Yung et al., 1996) had greater progesterone production. It is known that progesterone is produced by CL and the CL size is positively correlated with the synthesized and secreted amount of progesterone (Kastelic et al., 1990). As the BCS affected CL size, this effect on progesterone concentration was expected. In addition, cows with greater BCS or that gained BCS may have greater concentration of substrates available to the CL, such as glucose and cholesterol, thus also affecting the concentration of progesterone by this way too.

Cows with greater BCS or that gained BCS had greater embryo size in the current study, which could be partially explained by the greater plasma concentrations of progesterone, compared to cows with less BCS and that lost BCS during the experiment, respectively. Reduced circulating concentrations of progesterone results in an alteration in endometrial transcriptome and retarded embryonic development (Forde et al., 2012). Further, cows with greater BCS or that gained BCS may had greater concentrations of IGF-1 (Ciccioli et al., 2003; Yung et al., 1996), and this hormone stimulates embryonic growth (Moreira et al., 2001). In addition, cows with greater BCS or that gained BCS may have greater concentration of substrates available to the embryo, thus also affecting the embryo growth by this way too.

Cows with greater BCS or that gained BCS had greater estrus expression in our study. Beef cows with greater BCS (Nishimura et al., 2018) and dairy heifers that gained BCS (Villa-Godoy et al., 1990) had greater estrus expression. As well known, the dominant follicle size is positively correlated with serum concentration estradiol (Perry et al., 2014) that have action on sexual behavior. In our study, cows gained BCS had greater dominant follicle size, and it could explain the greater estrus expression. However, the BCS at beginning of FTAI protocol have no effect on dominant follicle size, but affected the estrus expression. The greater differences in hormones and substrates from cows with BCS $\geq$ 5 vs. < 5 (Ciccioli et al., 2003) could affect the concentration of estradiol produced by follicle, even if the follicle have the size not increased.

Greater BCS at beginning of FTAI resulted in greater pregnancy rate in *Bos indicus* and *Bos taurus* beef cows in several studies (Nishimura et al., 2018; Vedovatto et al., 2019a, 2019b; Cooke et al., 2021) as was observed in our study. The results from the current study that explain why cows with BCS $\geq$ 5 had greater pregnancy rate were greater CL size, progesterone concentration, estrus expression, mating rate and embryo size compared to cows with BCS < 5. Further, dairy cows in negative EB from parturition to 3 wk postpartum have lower greater pregnancy rate (Carvalho et al., 2014) as observed in our study with beef cows during FTAI period. The results from the current study that explain why cows in negative EB had lower pregnancy rate were lower dominant follicle size, progesterone concentration, estrus expression, mating rate and embryo size. All these results together help to better understand the main effects of two main variables (BCS and EB) that most affect the reproduction in beef cattle systems.

5. Conclusions

Cows with greater body condition score (BCS $\geq$ 5; scale 1 to 9) or in positive energy balance during synchronization and immediately after AI had greater development of ovarian structures, progesterone concentration, estrus expression, pregnancy rate and embryo size. To our knowledge, this is the first study to demonstrate that the body condition score and energy balance affects the embryo size in beef cows, then, studies evaluating the subsequent performance of the offspring are warranted.

CRediT authorship contribution statement

Marcelo Vedovatto: Conceptualization, Methodology, Formal analysis, Writing – review & editing. **Renan Barbosa Lecciolli:** Writing – original draft. **Eduardo de Assis Lima:** Methodology. **Raizza Fátima Abadia Tulux Rocha:** Methodology. **Rafaela Nunes Coelho:** Methodology. **Philipe Moriel:** Writing – review & editing. **Luana Gomes da Silva:** Methodology. **Luiz Carlos Louzada Ferreira:** Methodology. **Aldair Félix da Silva:** Methodology. **Willian Vaniel Alves dos Reis:** Methodology. **Dalton Mendes de Oliveira:** Writing – original draft. **Gumercindo Loriano Franco:** Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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