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Effects of Body Condition and Composition on the Onset of Postpartum Ovarian Activity in Beef Cows

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Abstract: Beef cows were evaluated from 6.5±1.6 months of gestation until 4 months postpartum, to correlate postpartum ovarian activity with body characteristics. Cows with Body Condition Score (BCS) of 4 or 5 (9-point scale) were sorted into three groups (n = 7 each): High-High (H/H), fed to maintain adequate BCS; High-Low (H/L), adequately fed prepartum but restricted (80% of nutritional requirements) postpartum and Low-High (L/H), restricted before calving but overfed afterwards (80 and 120%, respectively). Body composition (Backfat and rib eye area), fetal and calf development were evaluated every 14 days using ultrasound; weight and BCS were also measured. Despite ration differences, body composition and fetal measurements were similar among groups. Group L/H exhibited lower body weight and BCS ($p<0.001$) and calf birth weight ($p<0.05$) than H/H and H/L. Postpartum ovarian function was similar among groups. However, cows with average postpartum BCS scores ≥4 showed larger maximum follicle diameters than those with <4 ($p<0.001$). After temporary weaning at 60-90 days postpartum, only four cows ovulated. In conclusion, cows' BCS affects fetal and early calf development, maximum follicle diameter and estrous cycle onset. Follicle waves of increasing diameter are present but adequate body condition is required to reach ovulation.

Key words: Body condition, postpartum ovarian function, ultrasound, body composition, beef cattle, Mexico

INTRODUCTION

To maintain a 12 months calving interval, cows must enter a breeding program approximately 2nd month after calving. Animals reaching this goal produce cheaper kilograms of weaned calf, compared to those conceiving after 80 days postpartum (Herd and Sprott, 1986).

The amount of body fat at specific stages of the productive cycle is an important factor that determines reproductive efficiency. Animals require adequate reserves to breed efficiently. The nutritional status of a cow can be monitored by observing body condition. Body Condition Scores (BCS) reliably reflect the status of body energy reserves in the cow (Braun *et al*., 1986; Schroder and Staufenbiel, 2006).

Body condition scores at calving are correlated to the length of the postpartum interval (The time from calving to the onset of a new estrous cycle), animals entering a breeding program with a low BCS show prolonged calving intervals (Ciccioli *et al*., 2003). Condition scores at calving also affect milk yield as well as the newborn calf's health and vigor (Lake *et al*., 2005). The main objective of the present investigation was to evaluate the onset of ovarian activity in postpartum beef cows at different levels of BCS. Another objective was to evaluate data on the cows' body composition (Weight, weight/height ratio, backfat and rib eye area), to identify variables that could give a more objective measure of body condition. Pre and postnatal data collected from the calves were also evaluated and correlated to data from the cows.

MATERIALS AND METHODS

The study was undertaken at the Veterinary College facilities in Ciudad Victoria, Tamaulipas in Northeastern Mexico, located at 23°44'North and 97°10'West, at an altitude of 340 m; the annual mean temperature is 25°C and annual precipitation reaches 900 mm. A total of 21 cows of mixed European breeds (Mainly Simmental, Brown Swiss and Charolais) was selected to start between 5 and 9 months of pregnancy (6.5±1.6 months,

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mean±SEM) with a BCS of 4 or 5 on a 9-point scale (Herd and Sprott, 1986) and was monitored up to an average of 4th month postpartum. Before starting the study, all animals were given a bolus injection of vitamins A, D_3 and E; the dose of vitamins was repeated after 3rd month. The cows were sorted at random in three groups (n = 7 each): High-High (H/H) which was fed to maintain a good body condition throughout the study (100% of nutritional requirements); High-Low (H/L) which maintained a good prepartum body condition but was restricted in feed after calving (80% of nutritional requirements) and Low-High (L/H) which lost body condition before calving and was overfed afterwards (80 and 120% of nutritional requirements, respectively). The rations included sorghum hay and a concentrate containing sorghum grain, soybean meal, molasses, urea and minerals. All rations were prepared according to nutritional requirements at the corresponding stages (NRC. (National Research Council), 2000). The prepartum high diet contained 1.15 Mcal kg^{-1} NEm and 6.5% crude protein; NEm in the prepartum low, postpartum high and postpartum low diets were 1.13, 1.19 and 1.15 Mcal kg^{-1}, respectively and the crude protein levels were 4.6, 6.2 and 5.6%, respectively. Feed was given twice a day and water was available *ad libitum*.

After calving, cows were routinely observed to detect signs of estrus. Starting at 60 days postpartum, the calves were temporarily removed for 48 h to induce estrus.

An ALOKA SSD-500 ultrasound equipment was used to study body composition, fetal development and ovarian activity. Throughout the study, body composition was examined every 14 days including BCS, body weight (Recorded in the morning before feeding), weight/height ratio (Height to the withers), backfat thickness and rib eye area; ultrasonographic images of rib eye and backfat were taken using a 3.5 MHz external probe over the 12th intercostal space (Hamlin *et al*., 1995).

The cows were examined to monitor pregnancy every 14 days, alternating weeks with the evaluation of body composition. Ultrasonographic images of the fetuses and the cows' uterine arteries were taken using a 7.5 MHz rectal probe. Only images of fetal structures that were considered repeatable were recorded. From birth to the end of the study, the calves were also monitored every 14 days to follow the development of body composition variables (Weight, weight/height ratio, backfat thickness and rib eye area).

Starting on day 30th postpartum, the cows' ovaries were scanned every 2 days using a 7.5 MHz rectal probe. The largest diameters of ovarian follicles and corpora lutea were measured and sequential changes in ovarian structures were assessed. Only follicles measuring ≥5 mm were included. Data from ovarian examinations were analyzed to determine the intervals between follicle waves, the maximum diameter attained by the dominant follicle in each wave interval from calving to first ovulation and the lifespan and size of the corpora lutea. Blood samples were also collected from the caudal vein during these postpartum evaluations and serum was separated and frozen; estradiol and progesterone levels were measured using standard radioimmunoassay techniques at the Reproduction Laboratory, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autonoma de Mexico.

In order to compare pre or postpartum data between animals and groups, all data were adjusted according to calving dates. Variables related to ovarian activity were correlated to those of body composition. Analysis of variance was used to compare mean values between experimental groups. Variables related to body composition were also correlated to determine which ones are more useful as objective measurements of BCS. Statistical analyses were performed using GraphPad Prism (San Diego, CA).

RESULTS AND DISCUSSION

Cows in group H/H did not maintain their anticipated postpartum condition and decreased together with those in group H/L whereas cows in group L/H did not recover as much as expected after calving. Mean values for the main variables in the study are shown in Table 1.

Table 1: Body composition and ovarian function for experimental groups during the pre and postpartum periods (Mean±SEM)

Variables	Group H/H*	Group H/L*	Group L/H*
Prepartum BCS	4.4 ± 0.1^{a}	4.3 ± 0.1^{a}	3.7 ± 0.1^{b}
Postpartum BCS	3.8 ± 0.1^{a}	3.6 ± 0.1^{a}	3.1 ± 0.1^{b}
Prepartum body weight (kg)	514 ± 4.4^{a}	445 ± 6.7^{b}	398 ± 3.5^{c}
Postpartum body weight (kg)	442 ± 2.4^{a}	410 ± 2.0^{b}	357 ± 4.1^{c}
Prepartum backfat (cm)	0.62 ± 0.02^{a}	0.62 ± 0.01^{a}	0.61 ± 0.01^{a}
Postpartum backfat (cm)	0.58 ± 0.01^{a}	0.56 ± 0.01^{a}	0.56 ± 0.01^{a}
Prepartum rib eye area (cm^2)	41 ± 2.4^{a}	39 ± 1.5^{a}	37 ± 0.9^{a}
Postpartum rib eye area (cm^2)	36 ± 0.4^{a}	34 ± 0.5^{a}	34 ± 0.8^{a}
Prepartum weight/height ratio (kg cm^{-1})	3.8 ± 0.03^{a}	3.4 ± 0.05^{b}	3.0 ± 0.02^{c}
Postpartum weight/height ratio (kg cm^{-1})	3.3 ± 0.02^{a}	3.2 ± 0.04^{a}	2.7 ± 0.02^{b}
Maximum follicular diameter (mm)	12.8 ± 0.4^{a}	12.2 ± 0.3^{a}	11.4 ± 0.3^{a}
Follicle wave interval (days)	7.9 ± 0.4^{a}	8.3 ± 0.4^{a}	7.4 ± 0.7^{a}
Uterine artery diameter, pregnancy (mm)	11.3 ± 0.4^{a}	10.8 ± 0.4^{a}	10.1 ± 0.3^{a}
Calf birth weight (kg)	41 ± 2.9^{d}	41 ± 2.4^{d}	29 ± 3.1^{e}
Calf backfat (cm)	0.49 ± 0.1^{a}	0.48 ± 0.1^{ab}	0.44 ± 0.1^{b}

*Pre and postpartum rations: H/H (High/High), H/L (High/Low), L/H (Low/High), BCS = Body Condition Score within rows, values with different superscripts are significantly different: $^{a-c}$($p<0.001$), ^{d,e}($p<0.05$)

Table 2: Body composition values for cows according to Body Condition Scores (BCS) for each evaluation, regardless of experimental groups (Mean±SEM)

BCS	(n)	Backfat (cm)	Rib eye area (cm^2)	Rib eye/backfat ratio (cm^2 cm^{-1})	Body weight (kg)	Weight/height ratio (kg cm^{-1})
2	6	0.57±0.05[ab]	28±2.1[d]	51±6.3[a]	288±5.3[d]	2.2±0.05[d]
3	114	0.56±0.01[a]	34±0.4[d]	62±1.0[a]	399±4.8[e]	3.0±0.04[e]
4	194	0.59±0.01[a]	37±0.5[e]	64±1.0[a]	430±4.5[f]	3.3±0.03[f]
5	30	0.64±0.02[b]	41±1.5[f]	65±2.0[a]	479±11.6[g]	3.6±0.07[g]

Within columns, values with different superscripts are significantly different; [a-c]($p<0.05$); [d-g]($p<0.001$)

Table 3: Correlation coefficients for variables related to body composition in cows

	Body condition score	Weight	Weight/height ratio	Rib eye area	Backfat	Rib eye/backfat ratio
Weight	0.40**	-	-	-	-	-
Weight/height ratio	0.46**	0.96**	-	-	-	-
Rib eye area	0.33**	0.32**	0.33**	-	-	-
Backfat	0.22**	0.15*	0.15*	0.43**	-	-
Rib eye/backfat ratio	0.12*	0.20**	0.21**	0.59**	-0.44**	-
Days in study	-0.37**	-0.03	-0.03	-0.21**	-0.25**	0.03

*($p<0.05$); **($p<0.01$)

Body composition variables in cows: Body condition scores were similar in cows from groups H/H and H/L and were significantly higher than those from group L/H ($p<0.001$). For example, the last evaluation before calving gave a mean BCS of 4.1±0.1, 4.1±0.4 and 3.4±0.2 for cows in groups H/H, H/L and L/H, respectively while the corresponding values for the sixth evaluation postpartum (Approx. 80 days after calving) were 4.0±0.2, 3.7±0.2 and 3.0±0.3, respectively. Similar patterns were also observed in body weight. Individual measurements of backfat and rib eye area varied greatly and despite ration differences, both variables showed similar means among groups. When data for composition variables were grouped according to individual BCS scores (Regardless of gestational status or experimental groups), values increased with condition scores (Table 2). There were few data for cows with a score of 2 (n = 6) but the differences between other scores were significant in most variables. Linear correlations between weight/height ratio, body condition and rib eye area were significant ($p<0.001$) with coefficients ranging from 0.32-0.46 (Table 3). The weight/height ratio was better correlated to other variables than weight alone.

Gestational variables: Measurements were taken from several fetal structures including the eye (Transverse and longitudinal diameters), vertebrae, trachea, scrotum, hooves and umbilical cord. However, some of these organs were observed only occasionally, giving few data for analysis. Placental structures (Cotyledons) were not measured due to variability in size within the same animal. Fetuses were easily observed and measured from 176-279 days of pregnancy but very seldom in second-trimester pregnancies due to the position of the uterus in the cow's abdominal cavity. Transverse ocular diameter was observed most frequently, giving the following non-linear regression equation (Fig. 1):

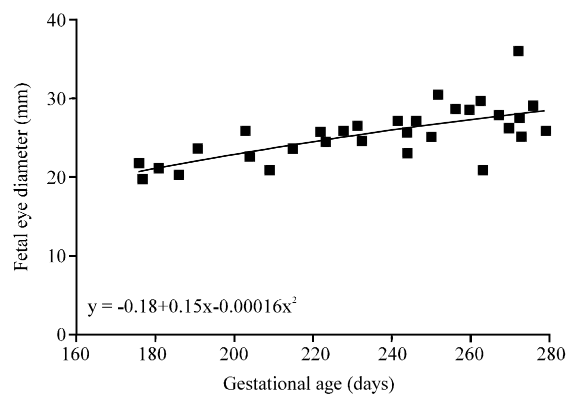

Fig. 1: Relationship between the cross-sectional diameter of the fetal eye and gestational age in bovine fetuses. The non-linear regression equation is valid to predict fetal age (days) from eye measurements (mm) between days 176 and 279

$$y = -0.18+0.15x-0.00016x^2$$

Where:

y = Transverse ocular diameter (mm)

x = Fetal age (Days)

This equation allows a prediction of gestational age from eye measurements. All measurements taken from fetuses were similar among groups. The diameters of maternal uterine arteries tended to be smaller in cows from group L/H, compared to the other groups (p = 0.08) particularly during the last 5 observations. The diameters of the arteries increased throughout pregnancy but were variable between animals and did not give a reliable regression curve.

Calf variables: Birth weight was significantly lower ($p<0.05$) in calves from group L/H (29.1±3.1 kg), compared to groups H/H (41.0±2.9 kg) and H/L (40.9±2.4 kg) and this

Table 4: Correlation coefficients for variables related to body composition in calves

	Weight	Weight/ height ratio	Rib eye area	Backfat	Rib eye/ backfat ratio
Weight/height ratio	0.99**	-	-	-	-
Rib eye area	0.83**	0.91**	-	-	-
Backfat	0.30**	0.61**	0.40**	-	-
Rib eye/backfat ratio	0.67**	0.91**	0.75**	-0.25*	-
Age	0.87**	0.90**	0.68**	0.17	0.59**

*(p<0.05); **(p<0.01)

difference remained significant up to 4th month of age. Two calves from group L/H weighed 20 and 21.5 kg at birth; the former was weak at birth and died 9 days later while the latter weighed only 40 kg at 4th month of age. A calf from group H/H died at 60 days of age due to an abomasal ulcer caused by excessive ingestion of concentrate. The first measurements of rib eye area were significantly larger (p<0.001) in calves from group H/H (17.0±1.4 cm^2) and H/L (15.3±1.2 cm^2) compared to those from group L/H (11.1±1.3 cm^2); this difference was also maintained throughout the study. The layer of backfat was thinner in group-L/H calves during the first 5 measurement periods (p<0.05) but became similar to that of other groups from the sixth observation onwards. The correlation coefficients for calves' variables are shown in Table 4. Most variables showed higher correlation coefficients than did those of cows' variables and again backfat showed a low correlation with other variables.

Ovarian activity: Despite group differences in maternal or calf body composition, maternal ovarian function was similar among groups. Most cows showed follicle wave activity from the first postpartum examinations (30 days) onwards. Only one cow from group L/H extended the range >69 days. Other ovarian variables were also similar between groups and follicles reached a maximum diameter of 12.4±0.2 mm (Range: 7-20) while the interval between follicle waves was 7.8±0.3 days (Range: 4-23). After sorting data according to average postpartum body condition (Regardless of diet), cows with average BCS ≥4 reached a significantly larger maximum follicle diameter than those with <4 (14.6±0.3 vs. 11.9±0.3 mm; p<0.001). Of all the cows in the study, 77% showed at least one follicle wave reaching a maximum diameter of ≥15 mm. One cow from group H/H presented follicles measuring 15-20 mm during ten consecutive follicle waves from 67-128 days postpartum however, none of these follicles ovulated.

Despite temporarily weaning the calves at 60-90 days after calving only three cows from group H/H and one from group L/H (The one whose calf died) ovulated and began to exhibit estrous cycles with a minimum of 2 or 3 ovulations per cow. Only the 2 cows which lost their calves ovulated before 3rd month postpartum. However,

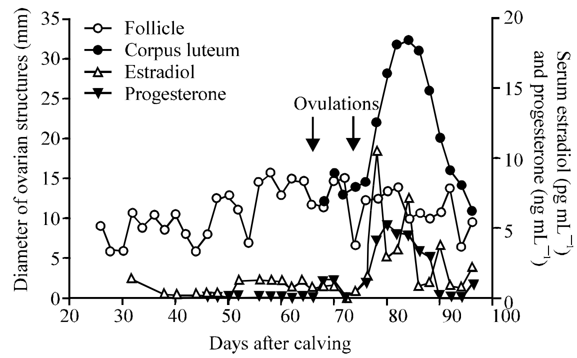

Fig. 2: Postpartum patterns of follicular and luteal development and serum levels of estradiol and progesterone, observed in one of the cows in group H/H. Note the increasing diameter of follicles previous to the first ovulation and the presence of a short-lived corpus luteum before the second ovulation

none of these cows exhibited signs of estrus before these ovulations. Two cows exhibited a short-lasting (2-4 days) corpus luteum before a second ovulation that formed a corpus luteum with longer duration (10-18 days).

Serum estradiol and progesterone patterns were followed in the cows and exhibited variations that paralleled follicle waves and luteal growth and regression. Figure 2 shows the patterns of follicular and luteal development as well as serum levels of estradiol and progesterone in one of the cows in group H/H. Some of the expected diet-related changes of body composition in the experimental groups were not observed. Groups H/H and H/L followed similar patterns for most variables despite receiving different postpartum diets. This was due in part to heat stress which appeared in the summer months (Coinciding with the postpartum period) and induced a reduction in feed intakes (Ealy *et al*., 1997).

Cows in groups H/H and H/L calved at the limit of acceptable BCS of 4 those in group L/H were below this limit. Other researchers recommend a score of at least 5 to ensure adequate fertility (Rae *et al*., 1993; Spitzer *et al*., 1995; Crowe, 2008).

Reproduction is low in the list of priorities for survival if an animal exhibits low BCS then maternal survival, milk production and accumulation of body reserves become more important than reproduction, thus inducing a state of anestrus (DeRouen *et al*., 1994; Freetly *et al*., 2006). Restricted prepartum nutrition leads to reduced body reserves at calving and affects postpartum performance including lactation and fertility (Spitzer *et al*., 1995; Lake *et al*., 2006; Castaneda-Gutierrez *et al*., 2009).

Measurements of backfat are correlated with the amount of fat deposits in cows (Herd and Sprott, 1986; Hamlin *et al*., 1995; Schroder and Staufenbiel, 2006). However, in the present study, these measurements were similar between groups, despite the differences in body weight and BCS. Backfat thickness also showed low correlations with other variables. Nevertheless when data were sorted by BCS instead of experimental groups, backfat values were significantly higher in cows with scores of 5, compared to those with 3 or 4 (Table 2).

The weight/height ratio was better correlated to other variables than weight alone. This could be due to the fact that compound variables reflect the combined effects of two variables (Hamlin *et al*., 1995; Schroder and Staufenbiel, 2006). This combined effect was not observed with the rib eye/backfat ratio, probably due to the variation in measurements of backfat. It is thus possible to use the weight/height ratio as a supplementary tool to estimate BCS in cattle; however, animals with a certain value of weight/height ratio could exhibit different BCS scores which may explain why the correlation coefficient between both variables was not as high as expected.

It was possible to follow fetal development through most stages of advanced pregnancy. Some of the earlier observations, between 135 and 175 days of pregnancy were hindered by the relatively far location of the pregnant uterus, making it difficult to locate the fetus (Kahn, 1989, 1990). The regression curve established in the present study allows a prediction of fetal age based on measurements of the transverse diameter of the fetal eye, from 176-279 days of pregnancy. For example, fetuses at 180, 200, 220, 240 and 260 days of development would exhibit ocular diameters of 21.6, 23.4, 25.1, 26.6 and 28.0 mm, respectively. This equation is comparable to that described by Kahn (1990). Other fetal structures such as vertebrae, trachea, hooves and umbilical cord were measured only on few occasions because they were less accessible (Compared to the eye). Therefore, these data could not be analyzed to compare groups or through fetal development.

Although, the diameter of the uterine arteries was similar between experimental groups, it tended to be smaller in group L/H, especially during the last 5 observations (Approx. the last 70 days of pregnancy). A reduced uterine artery would be expected to lead to a decreased blood supply during late pregnancy (Buczinski *et al*., 2007) if this slightly-reduced amount of blood was also of lower quality due to the reduced nutritional level of the diets these cows were receiving this compound effect would have contributed to the reduced birth weight of the calves in this group. Indeed, the birth weight of calves in group L/H was significantly lower than that of the other groups; one calf was very weak and survived only 9 days after birth. Even though nutrient intake may affect final fetal development and thus, modify birth weight, these effects are variable because the amount of nutrients a fetus receives from maternal circulation depends on maternal intake but also on the amount of maternal body fat reserves mobilized to maintain pregnancy (Belkacemi *et al*., 2010).

The differences in calf birth weights paralleled the differences in maternal BCS and in both variables, group L/H showed lower values than the other groups. Other researchers have also described the effects of maternal BCS on calf birth weight and development (Spitzer *et al*., 1995; Belkacemi *et al*., 2010). This difference was maintained during the following 3 or 4 months because the calf's nutrition during these early stages derives mostly from maternal milk and the quality of this milk also depends on the cow's BCS and diet (Lake *et al*., 2005).

Ultrasonography is a very useful technique for the evaluation of body composition, pregnancy and ovarian function, allowing frequent, non-invasive examination of internal organs (Rajamahendran *et al*., 1994; Hamlin *et al*., 1995; DesCoteaux *et al*., 2009). The accuracy of ultrasound for detecting small changes in backfat has been described (Brethour, 1992) as well as high correlations between backfat, body condition and weight (Herd and Sprott, 1986; Schroder and Staufenbiel, 2006). In the present study, backfat exhibited a low correlation with other variables, suggesting technical difficulties in determining the limits of the thin layer of backfat. However, other structures such as rib eye, ovarian structures and fetal organs were readily identified and reliably measured.

Regardless of group differences in body composition, all three groups of cows showed similar ovarian activity. However, even though follicle waves were detected in most cases the follicles were not able to continue development into a mature follicle that could induce a surge of luteinizing hormone and thus ovulate. The lack of final follicular maturation and the subsequent onset of estrous cycles could be related to nutritional deficiencies in cows with low body condition scores. Low energy and protein levels may affect hormonal secretion, restricting the patterns necessary for final follicle maturation and ovulation (Jolly *et al*., 1995; Lake *et al*., 2006; Flores *et al*., 2008; Lents *et al*., 2008; Cassady *et al*., 2009; Castaneda-Gutierrez *et al*., 2009; Rubio *et al*., 2010). Occasionally, some of the animals were found to have only follicles smaller than 5 mm indicating a lack of adequate stimuli even to continue follicle development beyond 5 mm which could be related to a nutritional deficiency (Jolly *et al*., 1995; Lents *et al*., 2008; Martin *et al*., 2010). The patterns of estradiol and progesterone secretion examined in the present study were similar to those described by others (Grimard *et al*., 1995; Flores *et al*., 2008). Therefore, the secretion of these ovarian steroids did not seem to be affected by nutritional status. Nevertheless, estrogen levels produced by these developing follicles were not able to induce the preovulatory surge of luteinizing hormone necessary for ovulation to occur (Martin *et al*., 2010).

When cows were sorted according to individual average postpartum BCS and not by the diets received (Table 2) there were significant differences between cows with BCS $\geq$4 and those with BCS <4, the former exhibiting larger follicles. This finding agrees with the effect of BCS on the number of large, medium or small follicles present in the ovaries (Dominguez, 1995). Of all the cows in the present study, 77% showed at least one follicle wave reaching a maximum diameter of $\geq$15 mm. Follicles are able to ovulate after reaching a diameter of 9-23 mm (Edmondson *et al*., 1986). Therefore, the ability of a follicle to ovulate depends not only on its size but also on the presence of adequate hormonal patterns these patterns may be altered by the combined effects of low BCS and a nursing calf (Jolly *et al*., 1995; Ciccioli *et al*., 2003); heat stress may also impair ovarian function and fertility (Roth *et al*., 2001; Roth, 2008).

Only after several weeks postpartum could four of the cows ovulate and continue their estrous cycles. The two cows that did ovulate before 3rd month postpartum were the ones that did not have a suckling calf; one of them was from group L/H and despite her low BCS, the lack of suckling inhibition allowed full follicle development. Most cows in groups H/H and H/L were in better body condition but did not ovulate, supporting the inhibiting effect of a suckling calf on the onset of the estrous cycle (Kawashima *et al*., 2008).

Even though some of the cows started to exhibit their estrous cycles, none presented any sign of estrus that would have been followed by breeding. Therefore, fertility could not be evaluated in the present study. Lack of estrus accompanying the first postpartum ovulation is common in cattle (Murphy *et al*., 1990) but these animals continued to cycle and showed no signs of estrus in a total of 7 subsequent ovulations. The presence of a nursing calf may inhibit estrus in the cow despite the fact that she is cycling (Perry *et al*., 1991). Of the few cycles that were monitored in two cases the corpora lutea that developed after the first ovulation exhibited a short life span (2-4 days), a fact that has also been reported elsewhere (Looper *et al*., 2003). All other corpora lutea were observed for 10-18 days.

CONCLUSION

Ultrasonography has an excellent practical application for the evaluation of reproductive function and body composition in cattle. The weight/height ratio may be a useful, objective complement to the estimation of BCS. Body condition in the postpartum period may influence the maximum follicle diameter, thus affecting the onset of the estrous cycle in beef cows. Although, follicle waves begin regardless of body condition, an adequate body condition is required to reach ovulation in the presence of the calf. Furthermore, the presentation of estrus is also impeded in animals in low body condition and under heat stress.

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