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Development and validation of an objective method for the assessment of body condition scores and selection of beef cows for timed artificial insemination

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ABSTRACT

The objectives of this study were to: 1) evaluate the relationship of the angle formed between the left and right sides of the rump with body condition score (BCS) in cattle; and 2) develop an objective tool to select cows for timed artificial insemination (TAI) based on their BCS. In Experiment 1, 801 lactating Nelore cows, 3–12 years old and weighing 400–625 kg were enrolled. All females were evaluated according to BCS (scale 1–5). In addition, the angle formed between both sides of the rump was measured in all cows with a goniometer. The relationship between BCS and the rump angle was analyzed by linear regression. There was a positive relationship between BCS and rump angle ($P < 0.0001$). The linear regression equation was angle =77.76+9.94 x BCS; R^2=0.67. The aim of Experiment 2 was to evaluate BCS in a simple, direct and objective way using rump angle and related BCS to TAI performance. A device was developed called Vetscore® to determine BCS according to rump angle. Using the Vetscore, cows were classified into three different categories of BCS using a color-based method: red, BCS < 2.75; green, BCS between 2.75 and 4.5; and yellow, BCS > 4.5. A total of 429 Nelore suckling cows, 4–8 years old, were subjected to a TAI protocol based on estradiol benzoate, exogenous progesterone, prostaglandin F2α, equine chorionic gonadotropin and estradiol cypionate. At Day 0, all cows were evaluated with the Vetscore® and classified according to the device's BCS color scale. Pregnancy diagnosis was performed by ultrasonography 30 d after TAI. Pregnancy per AI (P/AI) was analyzed using the chi-square test. A good level of agreement was observed between Vetscore's scale and visual BCS (82.9%). Cows classified as "green" had higher P/AI than cows classified as "red" and "yellow" (60.4%, 168 of 278; 42.4%, 61 of 144; and 28.6%, 2 of 7; respectively; $P < 0.001$). These results demonstrate that Vetscore® is an efficient and low-cost methodology for the assessment of BCS and, indirectly, nutritional status of beef cows. Finally, cows classified as adequate according to Vetscore® color scale had higher P/AI at 30 d compared with those considered inadequate.

1. Introduction

Nutrition is one of the most important factors affecting fertility in cattle (Robinson et al., 2006), particularly in extensively managed Zebu (*Bos indicus*) cows raised under tropical conditions that rely on pastures (Jolly et al., 1995; Samadi et al., 2013). Visual evaluation of body condition score (BCS) is widely accepted as an important tool for subjective quantification of endogenous energy reserves in B. indicus cattle (Ayres et al., 2009; Silveira et al., 2015). Despite its subjective nature, BCS is an easy and inexpensive method to evaluate body tissue reserves in lactating cows independent of frame size and body weight (Edmonson et al., 1989). Nonetheless, only a small proportion of farmers and technicians use this tool routinely as part of cow nutritional management in beef herds in South America and elsewhere. One issue is that evaluation of BCS is subjective and distinct evaluators can score the same cow differently. The time spent to perform evaluation of BCS may also hamper its adoption, especially in large beef herds.

The profitability of beef farms is highly associated with the capacity of cows to resume ovarian activity early after parturition and to

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respond properly to timed artificial insemination (TAI) protocols to conceive within the first trimester postpartum. Additionally, BCS at calving and the puerperium period were associated with the likelihood of pregnancy early in the breeding season (Richards et al., 1986; Moraes et al., 2007). Evaluation of BCS may be an important tool to select cows eligible for activities related to the reproductive management of livestock, such as TAI. Previous studies involving BCS and reproduction in B. indicus cattle (Nelore cows) have used a BCS scoring system with a scale of 1–5 points (Ayres et al., 2009; Souza et al., 2009; Torres et al., 2014), and have demonstrated that BCS close to breeding is highly associated with reproductive performance. Others reported a positive influence of an adequate BCS on pregnancy outcomes after TAI in suckled beef cows (Sales et al., 2011; Sa Filho et al., 2013). For instance, Nelore cows with low BCS (< 2.75) had lower pregnancy per AI (P/AI) than cows with BCS > 2.75 at breeding (Sa Filho et al., 2010; Sales et al., 2011). Similarly, Nelore cows with BCS of 2.5 had lower P/AI than cows with a BCS of 3.5 (Meneghetti et al., 2009).

At the higher end of BCS, obese females can experience suboptimal reproductive performance (McCann and Reimers, 1986; Kawashima et al., 2008). Thus, it is generally accepted that cows with moderate to good BCS (2.75≤BCS≤4.5) are more likely to be fertile than thin or fat cows. Consequently, optimal pregnancy outcome could be achieved by evaluation of BCS by a well trained technician to determine which females are suitable for entering a TAI program. The inherent subjectivity of visual evaluations and the extra time spent to evaluate BCS, however, impairs widespread use of BCS determinations prior to TAI in large beef cattle operations.

One approach to evaluate body reserves is measurement of rump fat thickness (RFT; Schroder and Staufenbiel, 2006; Ayres et al., 2014). This trait is an accurate indication of subcutaneous fat (Silva et al., 2003) and body energy reserve (Ayres et al., 2009). The RFT is a measure of the accumulation of adipose tissue on both sides of the rump and is highly correlated with BCS (Ayres et al., 2014). Thus, it seems logical to investigate whether angle formed between both sides of the rump is associated with BCS in cows.

The primary objectives of this study were to determine the relationship between the internal angle formed between the left and right sides of the rump (IAR) and visual BCS; and to develop an objective tool for BCS evaluation in beef cows. Accordingly, we sought to determine the rate of agreement between IAR and the visual subjective evaluation of BCS; and whether a device to measure IAR would be useful to select cows for inclusion in TAI programs.

2. Material and methods

The Committee for Ethics in Animal Experimentation at Embrapa approved all experimental procedures described in this manuscript (Protocol F02.2014).

2.1. Experiment 1

Lactating beef cows (*B. indicus*, Nelore; n=801), 3–12 yr of age, maintained under a grazing system (pastures of *Brachiaria brizantha* grass) with *ad libitum* access to mineral salt and water were enrolled in this study. The experiment was conducted in State of Rondônia, Brazil, from June 2012 to March 2014. The descriptions of number of farms enrolled in the experiment and average BCS of cows at time of enrollment are depicted in Table 1.

All animals underwent a BCS evaluation at random stages of lactation. The BCS of each cow was determined, by a single observer (LFMP), using the visual technique developed for Nelore cows (Ayres et al., 2009), which in turn was adapted from a scoring system for dairy cattle (Edmonson et al., 1989). Cows were classified using a 1 (very thin) to 5 (very fat) scale with 0.25-unit increments, as described elsewhere (Ayres et al., 2009). Also, each cow was restrained in a chute to measure IAR. A goniometer was used to measure the angle between the two sides of the rump, as shown in Fig. 1A. The goniometer device was placed on the rump, one rod to each side of the rump, between the sacral bone and the first coccygeal vertebrae. The data for IAR were recorded directly from the goniometer device (Fig. 1A).

Table 1
Descriptive statistics of experiments 1 and 2.

Item	
Experiment 1	
Farms	4
N.of cows in study	801
Mean BCS (1–5 scale)	3.11
Experiment 2	
Farms	2
N. of primiparous cows	147
N. of multiparous cows	282
Mean BCS (1–5 scale)	
Primiparous	2.3
Multiparous	3.0
Number of sires used in TAI	8

2.2. Experiment 2

A device for IAR measurements in cattle was developed and named Vetscore® (Fig. 1B; patent n. BRA1020140049916). The device aims to evaluate BCS in a simple, direct and objective way. Experiment 2 was then designed to validate the use of Vetscore in beef cows. Lactating Nelore cows (n=429) from two commercial beef farms in the State of Rondônia, were used in this experiment, performed from October 2014 to April 2015. Cows were kept in pastures of *Brachiaria brizantha* grass and had *ad libitum* access to minerals and water. The experimental design is described in Fig. 2 and the number of farms, parity, average BCS at Day 0 of the TAI protocol, and number of sires used are shown in Table 1. The TAI protocol was initiated between 30 and 80 days postpartum. At Day 0, cows were given 2 mg of estradiol benzoate (Bioestrogen®, Biogénesis-Bagó, Curitiba, Brazil) i.m. and received an intravaginal progesterone insert (1.9 g progesterone, CIDR®, Pfizer Animal Health, São Paulo, Brazil) to synchronize follicular wave emergence. The CIDR was removed at Day 8 when all cows were treated with 150 µg i.m. of D-Cloprostenol (PGF2α-analogue; Croniben®, Biogénesis-Bagó, Curitiba, Brazil), 1 mg i.m. of estradiol cypionate (E.C.P.®, Pfizer, Cravinhos, Brazil), and 300 IU, i.m., of equine chorionic gonadotrophin (eCG; Novormon®, Syntex, Buenos Aires, Argentina). Timed artificial inseminations were performed 48 ± 2 h after CIDR removal by a single technician within each farm.

Ultrasound examinations were performed at Day 10 to determine the diameter of the pre-ovulatory follicle (POF) and 30 d after TAI for pregnancy diagnosis. Visualization of the embryonic vesicle and detection of the embryonic heartbeat were the positive criteria for pregnancy.

2.3. Definitions

At Day 0, cows were evaluated using the Vetscore® and categorized according to the BCS observed on the device. Vetscore® was used to classify the BCS according to the IAR into three statuses: 1) red (n=144), cows with BCS < 2.75 and IAR < 102°; 2) green (n=278), cows with 2.75≤ BCS ≤4.5 and 102° ≤ IAR ≤124°; and 3) yellow (n=7), cows with BCS > 4.5 and IAR > 124°. The three classes of BCS (< 2.75, 2.75–4.5, and > 4.5) were adopted to classify cows according to the potential benefits on fertility that were reported by previous studies: Nelore cows with BCS < 2.75 had lower P/AI than cows with BCS > 2.75 (Sa Filho et al., 2010; Sales et al., 2011). Thus, such values were considered as representing adequate and inadequate BCS, respectively. Conversely, cows considered obese were of low fertility (Siddiqui et al., 2002; Kawashima et al., 2008). Therefore, cows in the study with BCS

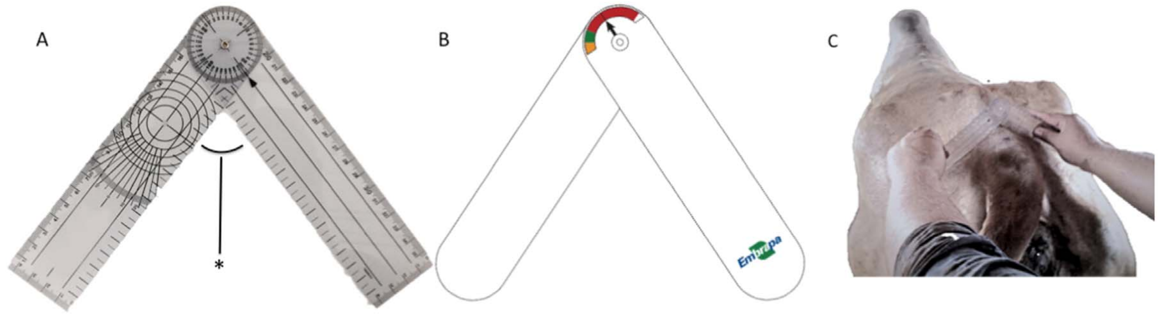

Fig. 1. A goniometer device (A) used to measure the internal angle of the rump (IAR) in Expt 1 and the Vetscore® device (B) developed and used for objective assessment of the energy reserves based on BCS of postpartum Nelore cows in Experiment 2, and (C) place of IAR evaluation of a cow (caudal view). The colors in the center of the Vetscore® refer to BCS status as follows: red=thin; green=adequate; and yellow=obese. * Internal angle of the rump (IAR).

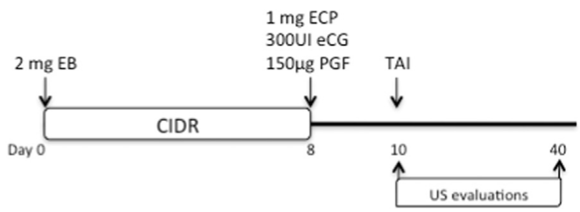

Fig. 2. Experimental design used for suckled cows treated with an estradiol-progesterone based TAI protocol in Experiment 2. Abbreviations are as follows: CIDR, intravaginal progesterone device; EB, estradiol benzoate; eCG, equine chorionic gonadotropin; ECP, estradiol cypionate; PGF, prostaglandin F2α; TAI, timed artificial insemination; US, ultrasound examinations.

> 4.5 were also considered as having inadequate condition. Finally, we examined the distribution of our data to confirm that the chosen thresholds were supported by the results of Experiment 2 (Supplementary Figure).

2.4. Statistical analysis

All statistical analyses were performed using the SAS 9.0 software. In Experiment 1, the relationship between BCS and IAR was determined by linear regression.

In Experiment 2, the initial analysis of the maximum diameter of the ovulatory follicle at TAI included the effect of sire, age, parity, BCS, farm, and days postpartum in the statistical model. The variables sire, age, days postpartum, and farm had no significant effect and were, therefore, excluded from the final statistical model. Thus, maximum diameter of the ovulatory follicle at TAI was analyzed using a two-way ANOVA to test the effects of Vetscore classification (red, green, and yellow) and parity (primiparous and multiparous). Means were compared among groups using the Tukey's post hoc test. Logistic regression analysis was used to examine the effects of Vetscore classification and parity on P/AI. A second analysis was performed to determine separately the effect of Vetscore scale and Parity on P/AI. Data were analyzed using the chi-square test.

The relationship between the diameter of the dominant follicle at TAI and P/AI was determined using logistic regression curves created using the coefficients provided by the interactive data analysis from SAS and the formula $\pi = 1/1 - exp^{-(\beta_0+\beta_1 x)}$.

Kappa values were calculated to quantify the degree of agreement between visual BCS and the Vetscore classification. Formulas used to calculate kappa values are described elsewhere (Noordhuizen et al., 2001) and considered the number of identical results, the proportion of genuine agreement, and corrections for agreement due to chance (expected values for chance alone). If kappa =0, no agreement exists other than what would be expected by chance. A kappa value of 1 indicates perfect agreement (Martin et al., 1987; Noordhuizen et al., 2001). In the current study, agreements between visual BCS and the Vetscore scale was considered moderate if kappa values were 0.4–0.5, good if they were 0.5–0.6, and high is kappa > 0.6 (Martin et al., 1987). Analysis was performed using GraphPad InStat software (GraphPad Software Inc., La Jolla, CA), and statistical significance was determined based on a P-value of 0.05.

3. Results

Amongst cows enrolled in Experiment 1, five were considered outliers and excluded from statistical analysis. Thus, of the 801 animals, data from 796 were used in this study. The linear regression model demonstrated that cows with higher BCS had higher IAR (Fig. 3; y=9.94x +77.76; R^2=0.67; $P < 0.001$).

Reproductive responses (fertility and follicle diameter) observed in Experiment 2 are summarized in Table 2 and P/AI of cows within different BCS categories and parity are shown in Fig. 4. Overall, P/AI was greater for cows classified as green using the Vetscore system (60.4%, 168 of 278) compared with cows classified as red (42.4%, 61 of 144) or yellow (28.6%, 2 of 7; $P < 0.001$). Cows with higher BCS had larger POF at TAI ($P < 0.05$; Table 2). In addition, multiparous females had larger POF at TAI than primiparous ($P < 0.05$). An increase in the diameter of the POF was accompanied by increases in P/AI (Fig. 5).

Kappa value demonstrated a good level of agreement between Vetscore's scale and visual BCS (Kappa =0.58). The number of observed agreements between the techniques was 660, which corresponded to 82.9% of all evaluations.

4. Discussion

Linear regression analysis demonstrated a positive relationship between IAR and BCS. Subsequently, we developed a device named Vetscore® as a tool to assess nutritional status of suckled beef cows. It was shown that Vetscore® was a useful tool for detecting cows with

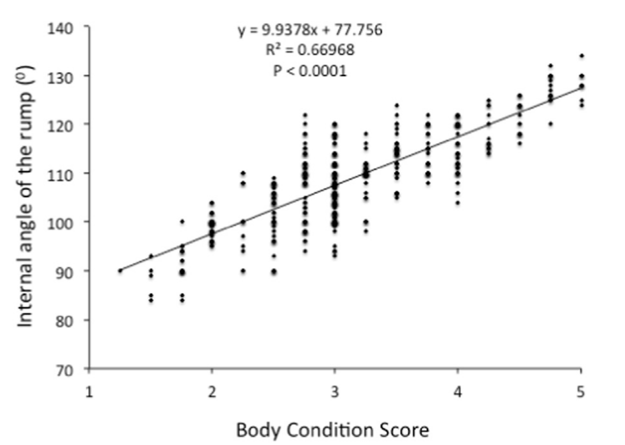

Fig. 3. Relationship between internal angle formed between both sides of the rump (IAR) and body condition score (BCS) in Nelore cows.

Table 2
Diameter of the dominant follicle 48 h after CIDR removal (mean ± S.E.M.) and pregnancy per AI after TAI according to the Vetscore classification and parity.

	Vetscore scale			Parity		P-Values*		
	Red	Green	Yellow**	Primiparous	Multiparous	Vetscore	Parity	Vet*Parity
Diameter of POF***, mm	11.6 ± 0.3^{A}	13.3 ± 0.2^{B}	15.5 ± 1.2^{B}	11.2 ± 0.2^{a}	13.6 ± 0.2^{b}	< 0.001	< 0.001	< 0.001
Pregnancy per AI, % (n/n)	42.4%^{A} (61/144)	60.4%^{B} (168/278)	28.6%^{A} (2/7)	37.4%^{a} (55/147)	62.4%^{b} (176/282)	0.13	< 0.002	0.47

[AB]Means with different superscripts differ within the Vetscore scale. [ab]Means with different superscripts differ within parity.
* P values from statistical analyses for Vetscore's scales (red vs. green vs. yellow), Parity (primiparous vs. multiparous), and Vetscore by parity interaction (vet by parity) are provided in last three columns.
** Only multiparous cows.
*** Pre-ovulatory follicle at TAI (Day 10).

increased probability to become pregnant in TAI programs. Cows considered in adequate BCS by Vetscore (green) had an increase of 18% points in P/AI compared to cows with low BCS (red) and of 31.8% points compared to cows considered obese (yellow).

The methods of estimating body energy reserve have been well described elsewhere (Schroder and Staufenbiel, 2006). However, there is still no consensus in the literature about the best approach to determine this trait in cows. Respiration calorimetry is considered the gold standard method to determine energetic metabolism (Schroder and Staufenbiel, 2006), but other methods such as body water estimate (Panaretto and Till, 1963) and the measurement of the mean diameter of fat cells (Waltner et al., 1994) have also been used. Even though these methods may be accurate, the requirement for laboratory facilities makes their use under field conditions unsuitable. An objective and yet practical method to measure fat is the determination of the tickness of subcutaneous backfat, which can be assessed by ultrasound (Ayres et al., 2009). Rump fat thickness measured by ultrasonography in beef (Ayres et al., 2009) and dairy cows (Domecq et al., 1995) were highly correlated with visual BCS. Moreover, it has been demonstrated that greater BCS and RFT at parturition were associated with increased probability of pregnancy rate in Nelore cows (Ayres et al., 2014). Ultrasonographic method represents a rapid and non-invasive approach but requires technical knowledge and a portable ultrasound machine, which may not be accessible in most beef farms. Visual evaluation of BCS, however, is an easy and inexpensive method to estimate body tissue reserve in cows independent of the frame size and body weight (Edmonson et al., 1989). Its major disadvantage is the subjective nature of the method, which implies considerable variation among technicians. The subjectivity of the visual method and the limited access to the whole body (especially rib cage and hooks) makes visual BCS determination a relatively difficult task when examining cows restrained in a chute. As an alternative, the Vetscore® device provides a simple and objective method to evaluate body reserve. Indeed, the Vetscore® device may be the optimal method for determining body condition among available techniques because it is objective, inexpensive, easily available, and not time consuming.

Standardizing a new method for BCS evaluation is a complex task, unless respiration calorimetry is used as a gold standard to confirm accuracy. Thus, in Experiment 2, we decided to use the rate of agreement between Vetscore colors and the subjective visual BCS evaluation to verify the efficiency of the Vetscore method. The rate of agreement was high (~80%) and hence the Vetscore® device is a reliable method to evaluate the energy reserve of cows to assist reproductive management procedures.

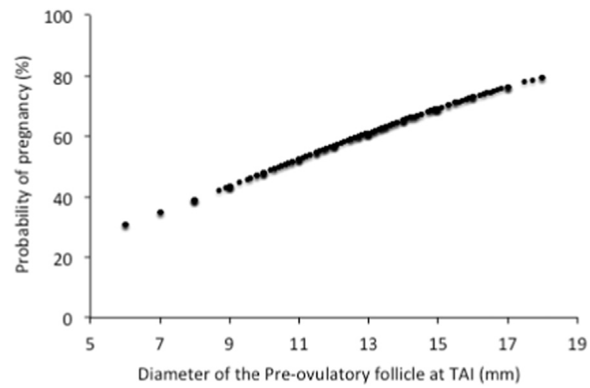

Fig. 5. Probability of pregnancy after timed artificial insemination (TAI) of suckled Bos indicus cows (n=429) according to the diameter of the pre-ovulatory follicle at TAI (Day 10 of the protocol) [probability of ovulation $=1/1\text{-}\exp^{-(-1.87+0.18x)}$; (P < 0.0001)].

In Experiment 2, cows with higher BCS had a larger dominant follicle at TAI compared with low BCS cows, which corroborates results described previously (Burke et al., 2001; Peralta-Torres et al., 2010). It is known that dominant follicles in cows with low BCS have less steroidogenic activity. This could be due to inadequate circulating stimulatory hormones, lack of substracts for steroidogenesis, or impaired intracellular steroidogenic pathways (Wiltbank et al., 2006). Previous studies demonstrated the detrimental effect of nutritional deficiency on the growth rate and maximum diameter reached by the dominant follicle in cattle (Rhodes et al., 1996; Mackey et al., 1999). Moreover, changes in the level of nutrition can affect follicular growth (Gutierrez et al., 1997; Gong et al., 2002) by inducing changes in

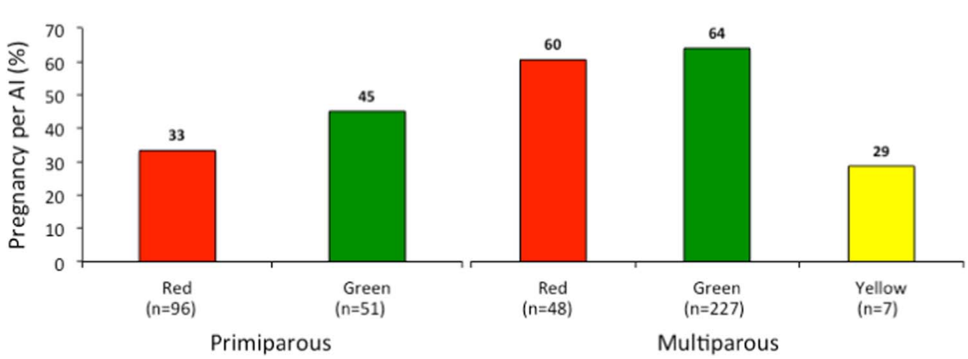

Fig. 4. Pregnancy per AI after TAI in suckled beef cows according to parity (primiparous vs. multiparous) and BCS status (red, green, or yellow) in Experiment 2. The chi-square test was used for comparisons between each group to examine the effect of BCS status on P/AI in primiparous (P=0.16) and multiparous cows (P=0.15).

plasma metabolites and metabolic hormones, such as insulin and IGF1 (Armstrong et al., 2002) and/or changes in hormones and growth factors within the follicular fluid (Matoba et al., 2014). In contrast, other studies failed to detect differences in the diameter of follicles in cows with low vs high body condition scores (Burke et al., 1998; Silva et al., 2013). Whether or not BCS affects reproductive performance and the degree at which BCS exerts these effects has been a controversial matter.

Some studies reported no effect of BCS on reproductive indices (Waltner et al., 1993; Ruegg and Milton, 1995; Meneghetti et al., 2009), whereas other authors reported small (Sa Filho et al., 2009, 2010) or even significant effects (Selk et al., 1988; Sales et al., 2011; Sa Filho et al., 2013). In the present study, cows with an adequate BCS developed a larger POF and had higher pregnancy rates compared with thin cows (low BCS). The association of BCS with fertility may be related to effects on POF diameter because probability of pregnancy establishment increased when diameter of the ovulatory follicle was increased (Perry et al., 2005) and when post-ovulatory progesterone concentrations were higher in cattle (Dadarwal et al., 2013; Pereira et al., 2013). Taking into consideration previous reports on the negative effects of low BCS on fertility of beef cattle after TAI (Randel, 1990; Baruselli et al., 2004; Sa Filho et al., 2013), we speculate that impaired metabolic status during negative energy balance may reduce oocyte quality as described elsewhere (Leroy et al., 2005; Velazquez, 2015).

Differences in fertility of multiparous and primiparous Nelore postpartum cows were not observed in previous studies (Meneghetti et al., 2009; Sa Filho et al., 2010). In this study, however, primiparous cows had lower BCS than multiparous cows, which negatively impacted fertility. Primiparous beef cows in pasture-based systems often have a prolonged postpartum anestrus (Wiltbank, 1970; Randel, 1990) and reduced pregnancy rates in TAI programs compared with multiparous cows (Sa Filho et al., 2009). The energy requirements for growth, in addition to the requirements for maintenance and lactation, are believed to account for the greater negative effects on cyclicity and reproductive performance of primiparous cows under pasture conditions (Wiltbank, 1970; Randel, 1990). Similar conditions are observed in postpartum buffaloes, in which having an average adequate BCS (2.5–3.5) increased the likelihood of pregnancy compared with thin (≤2.0) or overconditioned (> 3.5) females (Rahman et al., 2012). Thus, maintaining an adequate BCS during the postpartum period is a crucial aspect for successful reproduction in Nelore cows.

Another aspect of cow reproduction is that ovulation early in the postpartum period represents increased probability of becoming pregnant after TAI than cows that ovulate late. Beef cows with good BCS ovulate earlier in the postpartum than cows with poor BCS (Murphy et al., 1990; Stagg et al., 1995). Although the percentage of cows in anestrus at the beggining of the TAI protocol was not recorded in this study, one could speculate that parity (primiparous vs multiparous) and BCS (low vs adequate) play a key role to determine resumption of ovulation during the postpartum period. A primiparous cow with low BCS would be the most likely to ovulate late, whereas a multiparous cow with good BCS would most likely ovulate early in the postpartum. These postpartum events can explain the higher P/AI observed in multiparous and also in cows with higher BCS.

In the present study, obese cows were a very restricted class (only 7 cows with a BCS between 4.5 and 5). This low number of cows was due to the particular condition of suckled cows raised in pastures under tropical conditions. These cows normally calve in the period that the growth of pastures is reduced (dry season). Thus, it is rare to detect a suckled beef cow between 30 and 60 days postpartum with such high BCS under these conditions. In this study cows in yellow (BCS > 4.5) were among the ones having lower fertillity. Beef cows with a BCS of 2.5–3 yielded more transferable embryos than cows with a BCS≥4 in a 1–5 scale (Siddiqui et al., 2002). Hence, we confirmed that apparently there is a point at which increasing BCS can lead to a reduction in fertility. Although obese cows had larger POF, these cows also had lower P/AI than cows with adequate BCS (green). Overfeeding has been shown to be detrimental to the oocyte quality and embryo development *in vivo* (Freret et al., 2006). Additionally, obese cows tend to have higher plasma insulin concentrations, which may disrupt ovarian steroidogenesis (Armstrong et al., 2002; Butler et al., 2004) and, therefore, impair ovulation or conception per se (Kawashima et al., 2008).

Finally, understanding the endocrine regulation and patterns of follicular development and growth during the anestrous period in beef cows is required for the development of better methods to reduce days open and, consequently, reduce calving intervals (Stagg et al., 1995). Based on these considerations, incorporating Vetscore® as a tool for reproductive and nutritional management of beef cows may benefit owners in the process of decision-making to define among multiple strategies to maintain the herd in an adequate BCS, which may include forage quality, supplementation, and other managerial options.

5. Conclusions

In summary, IAR has a high positive relationship with BCS, which allowed the development of the Vetscore® device for objective assessment of BCS in cows. Moreover, the use of Vetscore® to select suitable cows prior to TAI based on their BCS was considered a reliable and successful method. Thus, we suggest that monitoring BCS using the Vetscore prior to TAI may increase pregnancy per AI at 30 d in beef cattle.

Conflict of interest statement

We declare that there are no conflicts of interests in this study.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.livsci.2017.01.011.

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