ULTRASONOGRAPHIC EVALUATION OF REPRODUCTIVE TRACT MEASURES AND FAT THICKNESS TRAITS IN PRE-PUBERTAL NELLORE HEIFERS[1]

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ABSTRACT:The aim of this study was to evaluate the relationship between reproductive tract and fat thickness measures obtained by ultrasound in prepubertal Nellore heifers. A total of 128 Nellore heifers born in 2006 and 2007 were submitted to ultrasound evaluations (13, 16, 19 and 22 months of age) of reproductive tract measures and fat thickness traits. These animals were from a selection experiment (NeC: control line, and NeS: selection line) for yearling weight started in 1981. Mean values of ovary area, height of the right uterine horn (HU), maximum follicular diameter (FOL), backfat thickness (BF), rump fat thickness (RF), and body condition score were analyzed. Repeated records were modeled using the PROC MIXED procedure (SAS), fitting a model that included the selection line, year of birth, measurement as fixed effects, and interactions. Body weight differed between the selected (281.48 kg) and control (210.51 kg) lines. Only the least square means of FOL were lower in the NeC line compared to the NeS line ($P < 0.05$), although the difference in mean HU between the two lines was of only borderline significance ($P = 0.06$). The rate of growth for the three reproductive traits was similar in the two lines. Simple and residual correlations between the reproductive and subcutaneous fat traits ranged from low to medium. The highest correlations were observed between HU and RF (Pearson correlation = 0.71 and residual correlation = 0.34). The current results are consistent with the literature, indicating that fat thickness traits are not good predictors of prepubertal reproductive traits in heifers. Further studies are necessary to clarify the relationship between reproduction and body fat in Nellore heifers.

Key words: backfat thickness, *Bos indicus*, correlation, uterine horn, ultrasound.

AVALIAÇÃO ULTRASSONOGRÁFICA DO TRATO REPRODUTIVO E ESPESSURA DE GORDURA EM NOVILHAS NELORE PRÉ-PÚBERES

RESUMO: O objetivo deste estudo foi avaliar a relação entre medidas do trato reprodutivo com medidas de espessura de gordura subcutânea obtidas por ultrassom em novilhas Nelore. Um total de 128 novilhas Nelore, nascidas em 2006 e 2007, foi submetido a avaliações (13, 16, 19 e 22 meses de idade) ultrassonográficas do trato reprodutivo e da espessura de gordura subcutânea. Esses animais são provenientes de um experimento de seleção para peso ao sobreano (NeC: linha controle e NeS: linha selecionada) iniciada em 1981. As características analisadas foram: média da área dos ovários, altura do corno uterino direito (AU), diâmetro do maior folículo (FOL), espessura de gordura no lombo (EGL), espessura de gordura na garupa (EGG), e escore de condição corporal. Os registros repetidos foram analisados usando o PROC MIXED (SAS), ajustando um

modelo que incluiu os efeitos fixos de linha seleção, ano de nascimento, medida, e interações. O peso corporal diferiu entre as linhas seleção (281,48 kg) e controle (210,51 kg). Apenas as médias de FOL foram menores na linha NeC comparadas às da linha NeS (P < 0,05), apesar da diferença próxima da significância de AU entre as duas linhas (P = 0,06). A taxa de crescimento das três características reprodutivas foi similar nas duas linhas. Correlações simples e de resíduo entre as características reprodutivas e as características relativas à gordura subcutânea variaram de baixa **a** média. As correlações mais altas foram observadas entre AU e EGG (0,71 e 0,34 para correlação de Pearson e residual). Os resultados são consistentes com a literatura, indicando que as características relativas à gordura subcutânea não são bons preditores daquelas relativas às características reprodutivas em novilhas pré púberes. Mais estudos são necessários para esclarecer a relação entre reprodução e gordura corporal em novilhas Nelore.

Palavras-chave: espessura de gordura, *Bos indicus*, correlação, corno uterino, ultrassom.

INTRODUCTION

In Brazil there are about 170 million head of cattle and most of them (about 80%) are Zebu cattle and their crossbreeds. The primary objective of Nellore cattle breeding is to improve the growth rate of the animals (ALBUQUERQUE *et al*., 2006). However, selection for increased subcutaneous fat deposition adds value to the animals and improves the return to the producer since some slaughterhouses already pay on average an additional 2% for carcasses of Zebu animals aged up to 36 months (4 teeth), animals with a subcutaneous fat thickness of 3-6 mm, and animals weighing 270 kg.

The average age at first calving of tropical heifers is 36 months. This fact is due to the stressful environment in which the animals are reared and because puberty occurs at an older age in *Bos indicus* than in *Bos taurus* cattle (BAKER *et al*., 1989). Evaluation of the reproductive tract by transrectal ultrasonography is used for the indirect determination of age at puberty and reproductive efficiency. The parameters evaluated include size and number of ovarian follicles, ovary size, and diameter of the uterus, cervix and vagina, or the establishment of a reproductive tract score comprising ovarian and uterine measures (HONARAMOOZ *et al*., 2004; HOLM *et al*., 2009). These traits, when measured before the beginning of the breeding season are predictors of heifer reproductive performance, even after adjustment for age, body weight and body condition score (BCS). These traits better predict fertility than other commonly used traits such as body weight and BCS, and are likely to be a predictor of lifetime production of the cow (HOLM *et al*., 2009). In addition, these traits may also provide a relatively inexpensive indirect genetic measure of puberty (JOHNSTON *et al*., 2009).

There is evidence for and against the hypothesis that ovulation is regulated by the amount of body fat. In general, the evidence from human studies does not support the hypothesis, while the results of animal studies argue against it. Studies have shown little relationship between fatness and ovulation in both adult and peripubertal females of several species (BRONSON and MANNING, 1991). In beef cattle, quantitative genetic studies involving a large number of records have reported low phenotypic correlations between fat thickness and days to calving (-0.06 to -0.01), and medium negative correlations between fat thickness and presence of the corpus luteum measured by ultrasound (-0.16 to -0.28), although the magnitude of the genetic correlations tended to be higher (Meyer *et al*., 2004; JOHNSTON *et al*., 2009). These results suggest that sexually precocious heifers are likely to have lower fatness; however, when fat is measured at a standard age, fatter females show a better reproductive performance. Using scrotal circumference as a sexual precocity trait, YOKOO *et al*. (2010) reported a low genetic correlation between this trait and fat thickness in young bulls, suggesting few pleiotropic gene effects affecting these traits in Nellore cattle.

The aim of the present study was to evaluate the relationship between reproductive tract and fat thickness measures obtained by ultrasound in prepubertal Nellore heifers from two lines selected for yearling weight (control and selection line) since 1981 (MERCADANTE *et al*., 2003; BONILHA *et al*., 2008).

MATERIAL AND METHODS

Humane animal care and handling procedures of the State of Sao Paulo (Brazil) law number 11.977 were followed for all experiments.

The Animal Science Experimental Station of Sertãozinho is located in the State of São Paulo, Brazil (21°10' south latitude and 48°5' west longitude). The region is characterized by a wet tropical climate with a rainy season in the summer (November to April) and a dry season in the winter (May to October). The predominant pastures are *Panicum maximum* and *Brachiaria brizantha*, the most common grasses in Brazil.

The animals were obtained from two Nellore selection lines established in 1981. Bulls and heifers were selected based on their yearling weight performance. Animals presenting selection differentials close to zero were selected from the control line (NeC) and animals with the highest selection differentials were selected from the selection line (NeS) (MERCADANTE *et al*., 2003). All females 128 heifers (45 from the control line and 83 from the selection line) were included in the experiment. The mean body weights of NeC and NeS heifers at the beginning of the experiment were 159.6 and 194.6 kg, respectively. The heifers were kept on pasture since weaning.

Each progeny was submitted to four noninvasive ultrasound evaluations (at about 13, 16, 19 and 22 months of age, s.d = 0.90 months) of reproductive tissues and fat thickness traits. For reproductive tissues assessment, images for ovary area (OV, mean of the right and left; cm^2), height of the right uterine horn (HU, mm), maximum follicular diameter (FOL, mm), and presence of a corpus luteum was measured ultrassonographically (Pie Medical Aquila equipped with 6.0-MHz linear probe) as described by SOUZA *et al*. (2011). The right uterine horn was measured placing the probe in the middle uterine horn. Minimum pressure was applied with the ultrasound probe on the top of the uterine horn, to avoid deformation of the uterine horn. Height of the right uterine horn was defined as distance between the edge of the endometrial lumen to the visualized interface between the endometrium and myometrium (SOUZA *et al*., 2011). For fat thickness measurement, images were obtained between the 12th and 13th ribs (backfat thickness, BF) and at the intersection of the gluteus medius and biceps femoris muscles (rump fat thickness, RF) using a 3.5-MHz carcass linear probe as described by YOKOO *et al*. (2010). The images were captured and stored with a Pie Medical 401347-Aquila ultrasound equipment (Esaote Europe B.V.). The images were then interpreted using the Echo Image Viewer 1.0 software (Pie Medical Equipment B.V., 1996). There were differences of up to 15 days between the date of reproductive and fat thickness traits evaluation.

Repeated measurements (n=507) of OV, HU, FOL, BF, RF, and BCS were analyzed. The least square means were estimated and used to fit a model that included selection line (NeC and NeS), year of birth (2006 and 2007), measurement (1 to 4), and the interactions of selection line x year of birth and selection line x measurement. The effects of BF, RF and corpus luteum class (absence or presence, 0 or 1) were tested separately using the model described above.

Statistical analysis was performed with the PROC MIXED procedure of the SAS program (SAS Inst., Inc., Cary, NC) using the REPEATED statement to model the covariance structure within animals with a first-order autoregressive structure that decreases the correlations with increasing lag between measures. The residual correlations were estimated using the same procedure and the general model.

RESULTS AND DISCUSSION

Mean OV, HU and FOL increased with age ($P < 0.01$; Figure 1). The OV, HU and FOL were 2.48 ± 0.05 cm^2, 8.75 ± 0.07 mm and 8.07 ± 0.17 mm, respectively, at the first measurement (13 months of age), and 3.52 ± 0.05 cm^2, 10.81 ± 0.07 mm and 9.24 ± 0.16 mm at the last evaluation (22 months of age). Johnston et al. (2009) reported mean HU of 13.5 ± 3.8 and 16.3 ± 4.8 mm for Brahman and tropical crossbred heifers, respectively, at 24 months of age. A mean FOL similar to that observed in the present study was reported by CLARO JÚNIOR *et al*. (2010) for prepubertal *Bos indicus* heifers at 24 months of age (9.45 ± 0.24 mm).

Only the least square means of FOL were lower in the NeC line compared to the NeS line ($P < 0.05$), although the difference in mean HU between the two lines was of borderline significance ($P = 0.06$). The pattern of change and rate of change for the three reproductive traits was similar in the two lines. Considering as the mean breeding value for yearling weight of animals born between 2004 and 2007 (equating to 5.5 generations of selection for growth), was 0.2 and 49.5 kg for NeC and NeS, respectively (RAZOOK and MERCADANTE, 2007), these results suggest no correlated change in the reproductive traits studied. MERCADANTE *et al*. (2003), analyzing data from these selection lines, did not observe significant differences in pregnancy rates between NeC and NeS heifers mated at 24 months of age.

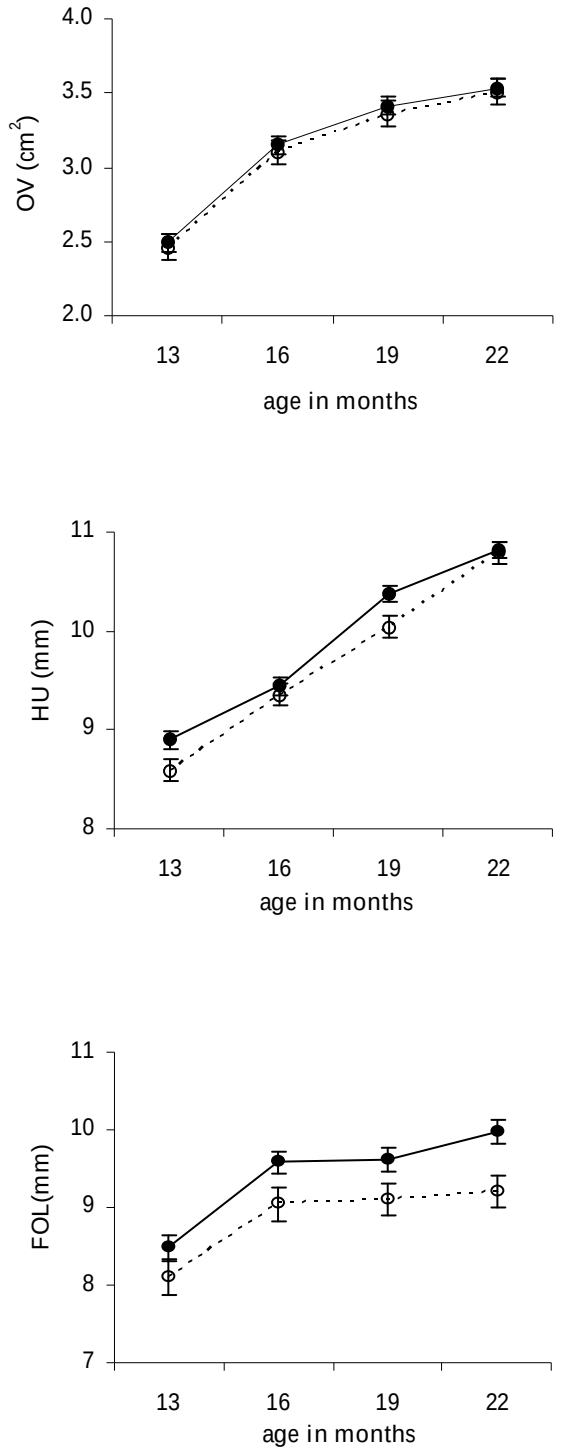

Figure 1. Least squares means (± standard error) of ovaries area (OV), height of the right uterine horn (HU), maximum follicular diameter (FOL) for NeC (○) and NeS (●)

The diameter of the dominant follicle has been used over recent years as an indicator of fertility in cattle. PERRY *et al*. (2005) reported a reduction in the pregnancy rate of *Bos taurus* cows in which ovulation was induced with gonadotropin-releasing hormone when the diameter of the largest follicle was less than 11 mm. However, the authors observed no influence on pregnancy rate when ovulation was spontaneous. Studying *Bos taurus* heifers, PERRY *et al*. (2007) observed a reduction in the pregnancy rate of both heifers synchronized with gonadotropin-releasing hormone and spontaneously ovulating heifers when the largest follicle was smaller than 10.7 mm or larger than 15.7 mm.

Morphological differences exist between the preovulatory follicles of *Bos taurus* and *Bos indicus*. GIMENES *et al*. (2005) treated heifers exhibiting follicles of different sizes with 25 mg LH in order to determine the diameter at which the follicles of *Bos indicus* heifers (Nellore, Gyr and Nellore x Gyr crossbreed) acquire ovulatory capacity. The authors observed that 33% of *Bos indicus* heifers ovulated when the follicles had a diameter of 7.0 to 8.4 mm, and this responsiveness to LH increased when the follicle diameter reached 8.5 to 10.0 mm (80%) or exceeded 10.0 mm (90%). These results suggest that in *Bos indicus*, ovulatory capacity is acquired at smaller follicular diameters than in *Bos taurus*. In the present study, Nellore heifers exhibited follicles with ovulatory capacity (diameter of 8.04 ± 1.51 mm) as early as 14 months of age. However, these animals did not ovulate, probably because of the strong negative feedback exerted by estradiol on the secretion of LH (DAY *et al*., 1987). In the present study, a corpus luteum was detected by ultrasound in only 2% and 6% of the heifers at 19 and 22 months of age, respectively. These animals were reared on pasture after weaning and had experienced two periods of food restriction (a natural consequence of the dry season) since births. The mean body weight at the four evaluations was 184.46 ± 2.80, 227.28 ± 2.66, 273.56 ± 2.67 and 299.83±2.76 kg, respectively.

Fat thickness traits (BF and RF) increased between 13 and 19 months ($P < 0.01$; Figure 2), a period coinciding with the onset and the end of the rainy period (summer in the southern hemisphere) and characterized by higher forage availability. Fat thickness decreased from 19 to 25 months with the beginning of the dry season. The BCS showed the same increasing and decreasing trend as BF and RF (Figure 2), but with less variation between measures, and is therefore likely to be a less accurate parameter for the determination of fat thickness.

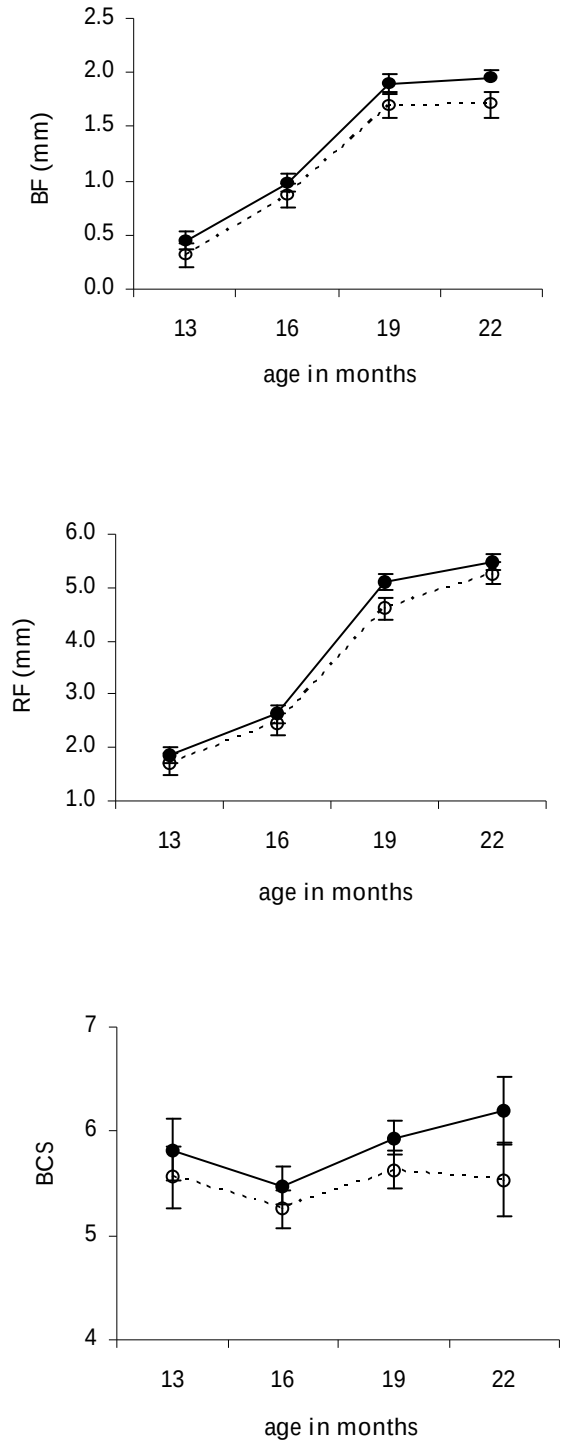

Figure 2. Least squares means (± standard error) of backfat thickness (BF), rump fat thickness (RF) and body condition score (BCC) for NeC (○) and NeS (●)

The mean RF (Table 1) was close to that reported by Yokoo *et al*. (2008) for Nellore females at 15 to 20 months of age, whereas the mean BF was lower than that reported by these authors. The selection line effect was significant for BF ($P < 0.05$) and BCS ($P < 0.01$), but not for RF. Correlations (residual and Pearson) between BCS and fat thickness suggested that attribution of BCS may be confounded with animal structure and body weight. Moreover, the current studies have shown little difference in fat thickness between animals selected for growth performance and animals of the control line. In a meta-analysis of data from these selection lines, BONILHA *et al*. (2008) concluded that selection for yearling weight does not affect body fat. YOKOO *et al*. (2008), also analyzing Nellore data, concluded that there is no genetic antagonism between muscle growth (measured by longissimus muscle area) and fat thickness.

It has been hypothesized that ovulation in mammals depends on body fat (FRISCH, 1987). According to this hypothesis, prepubertal females do not ovulate for the first time until they have accumulated a critical amount of fat relative to their lean body mass. However, YELICH *et al*. (1995) observed that the percentage of body fat is not the only regulator of puberty and suggested that age might also be an important modulatory factor for the determination of the onset of puberty in heifers. In the present study, simple and residual correlations between reproductive and subcutaneous fat traits ranged from low to medium (Table 1). The highest correlations were observed between HU and RF (0.34 and 0.71). A significant increase in uterine diameter (SALES *et al*., 2008) and uterine tone (CLARO JÚNIOR *et al*., 2010) was observed in heifers with an intravaginal progesterone device for the induction of cyclicity, suggesting that these measures are indicators of puberty. This hypothesis was confirmed in other studies. For example, HOLM *et al*. (2009) showed that the reproductive tract score, which involves the evaluation of uterine and ovarian structures, is associated with fertility outcomes such as days to calving and pregnancy rate. Moreover, JOHNSTON *et al*. (2009) reported medium and high phenotypic (0.30) and genetic (0.70) correlations between uterine horn diameter and the presence of the corpus luteum before first breeding. In the present study, the correlation of BCS, a subjective measure of subcutaneous fat, with BF and RF was of medium magnitude, in agreement with the results discussed previously.

There are no studies in the literature investigating the correlation between reproductive tract measures and BF in prepubertal *Bos indicus* females. SHIOTSUKI *et al*. (2009) reported a low genetic correlation (0.18 ± 0.03) between sexual precocity of Nellore heifers and finishing score, an indicator of fat deposition. The present results are consistent with data already published, and indicate that fat thickness traits are not good predictors of prepubertal reproductive traits in heifers. Further studies are necessary to clarify the relationship between reproduction and body fat in Nellore heifers.

Table 1. Means ± standard deviation and residual (above) and phenotypic Pearson (below) correlations between ovaries area (OV), height of the right uterine horn (HU), maximum follicular diameter (FOL), backfat thickness (BF), rump fat thickness (RF) and body condition score (BCC)

Trait	OV, cm^2	FOL, mm	HU, mm	BF, mm	RF, mm	BCC, scores
mean±sd	3.15±0.71	9.32±1.41	9.86±2.17	1.26±0.98	4.68±2.17	5.07±0.98
OV		0.15*	0.28*	0.08	0.24*	0.15*
FOL	0.36*		0.18*	0.05	0.10*	0.03
HU	0.54*	0.33*		0.23*	0.34*	0.16*
BF	0.40*	0.22*	0.57*		0.61*	0.33*
RF	0.47*	0.26*	0.71*	0.75*		0.34*
BCC	0.52*	0.37*	0.54*	0.62*	0.59*	

* $P < 0.05$

CONCLUSION

The results of this longitudinal study suggest that follicles with ovulatory capacity (diameter of 7.02 mm) are present in Nellore heifers as early as at 14 months of age. No differences in fat thickness were observed between the control and selection lines. The low correlations between reproductive and fat thickness traits suggest that the latter traits are not good predictors of prepubertal reproductive traits.

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