http://dx.doi.org/10.1590/1678-4162-12470

Arq. Bras. Med. Vet. Zootec., v.74, n.3, p.390-398, 2022

Antral follicle count, productive and reproductive parameters in *Bos indicus* and *Bos indicus-taurus* prepubertal heifers with early puberty induction

[*Contagem de folículos antrais, parâmetros produtivos e reprodutivos em novilhas pré-púberes* Bos indicus *e* Bos indicus-taurus *com indução precoce de puberdade*]

S.B. *Delchiaro*[1], D.V. *Bonato*[1], P.V.L.F. *Oliveira*[2], R.J. *Paulossi Júnior*[1], F.G.C. *Bonato*[1], M.M. *Seneda*[1], F. *Morotti*[1*]

[1]Universidade Estadual de Londrina, Londrina, PR, Brasil
[2]Centro Universitário Filadélfia, Londrina, PR, Brasil

ABSTRACT

Prepubertal Nelore (G-N = 15) and crossbred Nelore x Aberdeen Angus heifers (G-NA = 15) were used for this study. AFC, live weight, body condition score (BCS), ovary and dominant follicle (DF) diameters were determined in each animal. Puberty induction was performed by insertion of a 4th use progesterone device (D0) which was removed on D12. Also, 1 mg estradiol benzoate was administered, and estrus intensity was classified (D12). At D21, the presence and diameter of the corpus luteum (CL) were registered. AFC was highly repeatable, regardless of hormone induction in both G-N (r=0.79) and G-NA (r=0.90). The mean AFC was greater in G-N compared to G-NA (24.2±8.5 *vs.* 17.7±9.0 follicles). A variation in BCS throughout the study occurred in G-NA, but not in G-N. The average weight gain (AWG) was greater in G-NA compared to G-N (0.69±0.33 *vs.* 0.40±0.29kg/day). The G-NA resulted in a larger diameter of DF at D12 than G-N (11.6±2.7 *vs.* 9.3±1.5mm). In conclusion, AFC was greater in Nelore heifers, although in both breeds this count was highly repeatable during puberty induction. Crossbred heifers had greater BCS and AWG with greater diameter of DF, indicating higher precocity when compared to Nelore heifers.

Keywords: heifers, puberty, antral follicle count, prepubertal, hormonal protocol

RESUMO

Novilhas pré-púberes Nelore (G-N=15) e mestiças Nelore x Aberdeen Angus (G-NA=15) foram utilizadas neste estudo. CFA, peso vivo, escore de condição corporal (ECC), diâmetros do ovário e folículo dominante (FD) foram determinados em cada animal. A indução da puberdade foi realizada pela inserção de um dispositivo de progesterona de quarto uso (D0), que foi retirado no D12. Além disso, 1mg de benzoato de estradiol foi administrado e a intensidade do estro foi classificada (D12). No D21, foram registrados a presença e o diâmetro do corpo lúteo (CL). A CFA foi altamente repetível, independentemente da indução hormonal em G-N (r=0,79) e G-NA (r=0,90). A CFA média foi maior em G-N em comparação com G-NA (24,2±8,5 vs. *17,7±9,0 folículos). Uma variação no ECC ao longo do estudo ocorreu em G-NA, mas não em G-N. O ganho de peso médio (GPM) foi maior em G-NA em comparação com G-N (0,69±0,33* vs. *0,40±0,29kg/dia). O G-NA resultou em um diâmetro maior de FD em D12 do que o G-N (11,6±2,7* vs. *9,3±1,5mm). Em conclusão, a CFA foi maior em novilhas Nelore, embora em ambas as raças essa contagem tenha sido altamente repetível durante a indução da puberdade. Novilhas mestiças apresentaram maior ECC e GPM com maior diâmetro de FD, indicando maior precocidade quando comparadas às novilhas Nelore.*

Palavras-chave: novilhas, puberdade, contagem de folículos antrais, pré-púbere, protocolo hormonal

*Corresponding author: fabiomorotti@uel.br
Submitted: July 6, 2021. Accepted: February 18, 2022.

INTRODUCTION

The ability of heifers to conceive early at the beginning of the breeding season is traditionally dependent on pubertal entry and onset of the estrous cycle (Diskin and Kenny, 2014). Studies look for ways to induce early puberty in heifers aiming a reduction of age at first calving, an increase of reproductive efficiency and genetic gain, which is an important goal to increase production per area and animal production capacity (Sá Filho *et al*., 2013). In this context, studies have investigated the relationship between antral follicle count (AFC) and reproductive performance in heifers and cows since AFC is related to female fertility (Ireland *et al*., 2007; Mossa *et al*., 2012; Morotti *et al*., 2017a, 2018; Zangirolamo *et al*., 2018; Seneda *et al*. 2019).

The AFC is a remarkable ovarian characteristic in cattle, being highly variable among females, but highly repeatable in the same individual (Burns *et al*., 2005; Ireland *et al*., 2007). AFC can be defined as the estimated number of antral follicles with diameter ≥3mm during a follicular wave of estrous cycle. Therefore, through an ultrasound examination of the ovaries, it is possible to count all antral follicles and classify females in low, intermediate, or high AFC (Burns *et al*., 2005; Ireland *et al*., 2007; Morotti *et al*., 2018; Moraes *et al*., 2019). North American and European studies evaluated the correlation of AFC with the reproductive performance of *Bos taurus taurus* cows and concluded that high AFC show better reproductive and productive performance (Ireland *et al*., 2011; Evans *et al*., 2012) as well as studies have stated that low AFC demonstrated lower fertility (Mossa *et al*., 2012).

In contrast, studies performed with *Bos indicus* cattle in Brazil has revealed controversial data to those found in *Bos taurus* (Santos *et al*., 2016; Morotti *et al*., 2018; Moraes *et al*., 2019; Lima *et al*., 2020). For example, females with high AFC have better performance for *in vitro* embryo production; but had no better pregnancy rate to timed artificial insemination TAI (Santos *et al*., 2016). In addition, recent studies (Morotti *et al*., 2017a; 2018; Moraes *et al*., 2019; Seneda *et al*., 2019; Lima *et al*., 2020) have shown a positive influence of AFC on ovarian follicular dynamics and reproductive performance of Nelore females, contrary to the results found in taurine herds outside Brazil.

Crossbreeding heifers (F1; *Bos indicus-taurus*) have often been kept on the farm as a future breeder (Cardoso *et al*., 2018). In this context it is important to highlight that there are important reproductive differences between the subspecies (Sartori and Barros, 2011). In addition, there is evidence that suggests that the total number of ovarian follicles may influence response to hormone synchronization protocols (McNeel and Cushman, 2015) and in beef heifers, AFC can be positively associated with entry into puberty. Nevertheless, the relationship between AFC and reproductive performance in heifers is limited, especially to association with puberty induction.

Therefore, we hypothesized that even in animals as young as in this study, AFC is highly repeatable in both *Bos indicus* and *Bos indicus-taurus* heifers throughout the puberty induction protocol. In addition, we tested the hypothesis that animals with taurine blood show favorable results for productive aspects and sexual precocity. Therefore, this study aimed to evaluate the repeatability of AFC and to compare the productive and reproductive parameters in *Bos indicus* and *Bos indicus-taurus* heifers submitted to early puberty induction

MATERIAL AND METHODS

The present study was conducted according to the standards of the Ethics Committee for Animal Experimentation of the University of Londrina, based on Federal Law 11.794 of October 8, 2008, and was approved under number CEUA 19514.2018.86.

For this study a group (G) of pre-pubertal heifers (n=30), Nelore (G-N; n=15; *Bos indicus*) and Crossbred (Nelore x Angus; G-NA; n=15; *Bos indicus-taurus*) were evaluated to determine the number of antral follicle/AFC, body weight and body condition score (BCS) 15 days before the initiation of the puberty induction protocol (Day -15). The pre-pubertal condition was confirmed in two ultrasound exams (Day-15 and Day 0) and only heifers without CL and with follicles smaller than 8 mm in diameter were kept in the study. The animals from both groups were kept together in an area composed of *Brachiaria brizantha cv marandu* (15% of the area), *Brachiaria decubens* (35% of the area) and *Brachiaria humidicola* (50% of the area), besides receiving 0.8 to 1kg of concentrate per animal/day. Water and mineral salt (phosphorus content of 6.000 mg/kg) were kept *ad libitum*.

The live weight (kg) was measured by a scale without prior fasting. BCS was assessed using a scale from 1 to 5 as described by Machado *et al*. (2008). Each heifer was evaluated by transrectal palpation, followed by ultrasonography for uterine and ovarian examination. All evaluations were performed by a single veterinarian on Days -15, 0, 12 and 21, being that all variables were monitored on each assessment day.

Fifteen days before (Day -15) the initiation of the puberty induction protocol, each animal was evaluated only once for AFC estimation. During this examination, each ovary (right and left) was observed in detail with an ultrasound (SonoScape® S2 equipped with a 5Mhz rectal linear frequency transducer) and all visible follicles (follicles≥3mm) were identified and counted as described by Morotti *et al*. (2018). Briefly, the entire ovarian surface was scanned starting from one face close to the pedicle to the other face. AFC was defined by the sum of follicles from both ovaries. Diameter of dominant follicle was calculated from two cross-sectional linear measurements of the follicular antrum captured on the ultrasound monitor. Ovary diameter was obtained from largest image of an ovary captured on the monitor, and the measurements of each ovary image were determined. The animal identification, follicle numbers, follicle diameters, ovulation status, BCS, and weight were recorded on individual data sheets.

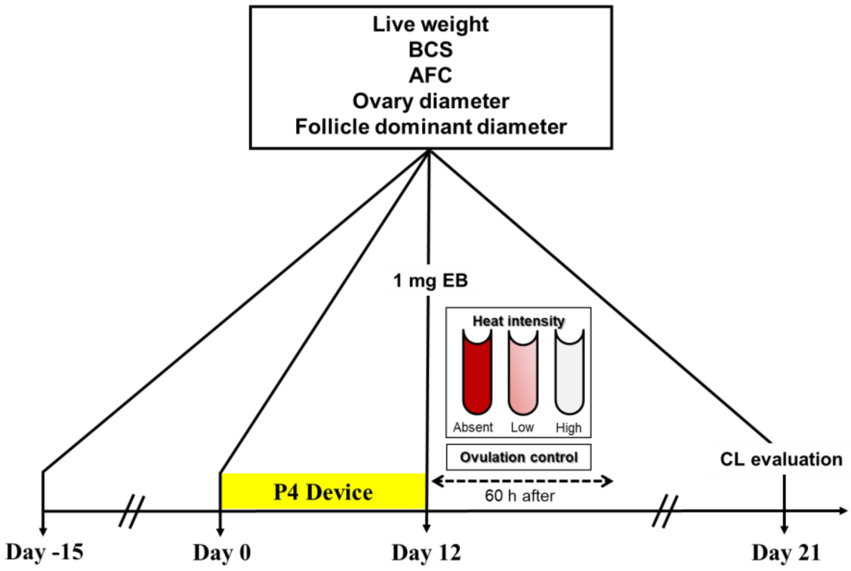
Weight, BCS and ovarian assessments were performed on Days -15, 0, 12 and 21. The heifers received a puberty induction protocol on Day 0, as described in Fig. 1. Hormone treatment was performed as suggested by González *et al*. (2020) that consisted of the insertion of a 4th use intravaginal progesterone (P4) device (CIDR®, Zoetis, Sao Paulo, Brazil) that remained until day 12, when the P4 source was removed. On the same day of the device withdrawal, 1mg estradiol benzoate (EB; Gonadiol®, Zoetis, Sao Paulo, Brazil) was applied in addition to the fixation of an adhesive (Estrotect ™, Spring Valley, United States) in the sacrococcygeal region to evaluate the estrus expression rate and perform the heat intensity classification (0 = absent, 1 = low and 2 = high intensity). The heat intensity was defined by the amount of the grey coating that was removed from the fixing adhesive similar to what was described by Nogueira *et al*. (2019).

To confirm ovulation, the heifers were evaluated between 48 and 60 hours after ovulation induction and the absence of the dominant follicle used to indicate that ovulation had occurred and reconfirmed by the presence of the corpus luteum (CL) in the same ovary on the 21st day after the onset of the puberty induction protocol.

Quantitative variables were analyzed by the generalized linear model (GLM), including the main effect of the genetic grouping as a fixed effect and all other sources of variation were included as covariates of the model. In the case of repeated variables, data were analyzed by repeated measures ANOVA using GLM, including the time effect as the main random effect and the other sources of variation as covariates.

In the presence of a significant effect, the averages were compared by the Tukey test. Differences in ovulation rate between genetic groups were analyzed by Fisher's exact test. Repeatability (range 0 - 1, where 1 = perfect) was defined as the proportion of the total variance that could be attributed to the animal variation and was calculated as follows: $\delta 2$ animal/($\delta 2$ animal + $\delta 2$ error).

For descriptive analysis, quantitative data are presented as mean (M) and standard deviation (SD) and qualitative data as percentage (%). All statistical analyzes were performed using the Minitab® statistical software version 18.1. To indicate an effect of categorical variables and their interactions, a significance level ≤ 0.05 was adopted.



BCS – body condition score; AFC – antral follicle count; P4 – progesterone, device of 4th use; EB – estradiol benzoate; CL – corpus luteum.

Figure 1. Experimental scheme and puberty induction protocol in Nelore (*Bos taurus indicus*) and Crossbred (Nelore x Angus) heifers (*Bos taurus taurus*).

RESULTS AND DISCUSSION

Comparing Nelore (*Bos taurus indicus*) and Nelore x Angus (*Bos indicus-taurus*) heifers, AFC was highly repeatable during the study days and regardless of hormonal induction, both in G-N (r=0.79) and G-NA (r=0.90). The AFC proved to be a reproductive trait highly consistent in both genetic groups, being more constant in crossbred heifers than in Nelore. However, AFC was greater (P < 0.05) in G-N compared to G-NA at Day 0, 12 and 21, except for Day -15, as shown in Table 1. Furthermore, the BCS varied (P<0.001) in G-NA and was greater (P<0.05) in G-NA than G-N.

Table 1. Mean and standard deviation of body condition score (BCS), antral follicle count (AFC) and ovary and dominant follicle diameters between evaluation days in Nelore (n=15) and crossbred (Nelore x Angus, n=15) pre-pubertal heifers submitted to early puberty induction protocol

Variables		Day-15	Day 0	Day 12	Day 21	*P-value*
BCS	Nelore	2.6±0.2	2.6±0.2	2.6±0.2	2.6±0.2	0.84
	Nelore x Angus	2.7±0.3^{b}	3.0±0.2^{a}	3.1±0.1^{a}	3.2±0.2^{a}	<0.0001
	P-value	0.43	<0.0001	<0.0001	<0.0001	-
AFC	Nelore	23.4±10.3	24.5±7.2	21.7±5.3	27.1±10.2	0.37
	Nelore x Angus	17.9±12.9	17.3±6.6	17.7±6.3	18.2±9.6	0.99
	P-value	0.20	0.009	0.06	0.02	-
Ovary diameter (mm)	Nelore	17.4±2.3	18.3±1.6	19.0±2.0	19.2±2.8	0.12
	Nelore x Angus	16.5±1.4^{b}	17.9±2.3^{ab}	18.1±2.2^{ab}	20.0±2.9^{a}	0.001
	P-value	0.15	0.59	0.23	0.47	-
Diameter of dominant follicle (mm)	Nelore	8.9±1.5^{ab}	10.4±1.5^{a}	9.3±1.5^{ab}	7.6±2.4^{b}	0.001
	Nelore x Angus	9.8±1.7^{bc}	10.9±1.3^{ab}	11.6±2.7^{a}	9.0±2.6^{c}	0.008
	P-value	0.16	0.30	0.007	0.15	-

Superscript lowercase letters (a, b, c) on the same line means statistical difference between evaluation days.
BCS – body condition score; AFC – antral follicle count.

The average weight gain was greater (P=0.01) in G-NA compared to G-N, as well as crossbred heifers were heavier in Days -15 and 21 (Table 2). The dominant follicle diameter was similar (P>0.05) between G-NA and G-N at Day -15, 0 and 21, but G-NA resulted in a larger diameter of dominant follicle at Day 12 than G-N (11.6±2.7 *vs.* 9.3±1.5mm; P=0.007), as shown in Tab. 1. The estrus intensity (1.5±0.5 *vs.* 1.7±0.5), ovulation rate (33.33 *vs.* 46.67%) and CL diameter (11.2±2.4 *vs.* 11.2±5.5mm) were similar (P>0.1) between G-N and G-NA, respectively (Table 3).

Table 2. Mean and standard deviation of age, live weight, and average weight gain in Nelore and crossbred (Nelore x Angus) pre-pubertal heifers during the puberty induction protocol

Genetic group	Age (months)	Live weight D-15 (kg)	Live weight D21 (kg)	Average weight gain (kg/day)
Nelore	14.5 ± 0.4	220.0 ± 25.6	233.9 ± 30.8	0.39 ± 0.29
Nelore x Angus	12.0 ± 1.0	238.4 ± 28.9	262.9 ± 27.2	0.69 ± 0.33
P-value	< 0.0001	0.04	0.01	0.01

Table 3. Mean and standard deviation of estrus intensity, ovulatory rate, and corpus luteum (CL) diameter in Nelore and crossbred heifers (Nelore x Angus) after hormonal protocol for puberty induction

Genetic group	Estrus intensity (M ± SD)	Ovulatory rate % (n/N)	CL diameter (M ± SD)
Nelore	1.5 ± 0.5	33.33 (5/15)	11.2 ± 2.4
Nelore x Angus	1.7 ± 0.5	46.67 (7/15)	11.2 ± 5.5
P-value	0.14	0.71	0.93

CL – corpus luteum

In this study the AFC was highly repeatable between genetic groups and highly constant among the evaluated heifers, proving to be a reliable evaluation parameter. The number of antral follicles was greater in Nelore than crossbred heifers. However, the dominant follicle from crossbred animals showed a larger diameter on the day that the P4 device was removed, indicating a better response to the puberty induction protocol of this genetic group. In addition, Nelore x Angus heifers also showed a better productivity parameter, resulting in superior weight gain and BCS during all days of the experiment. The innovative and determining factor of this study was the precocity of the studied animals, since there are few studies comparing the reproductive and productive parameters in Nelore and Nelore x Angus pre-pubertal heifers.

Regarding physiological aspects, ovarian functions are regulated by endocrine hormones secreted by the hypothalamus (gonadotropin-releasing hormone - GnRH), anterior pituitary (follicle stimulating hormone - FSH and luteinizing hormone - LH), ovaries (progesterone, estradiol, and inhibin) and uterus (prostaglandin F2α - $PGF_{2\alpha}$) (Aungier *et al.*, 2015). Elevated concentrations of estradiol secreted by the pre-ovulatory follicle in turn promote a GnRH surge and allow – when progesterone levels are low – the expression of behavioral estrus and the release of LH to cause ovulation.

Classically, a prepubertal heifers reach puberty when age and weight are satisfactory, being characterized by classical estrus signs, ovulation occurrence followed by luteal phase (Perry, 2016). Estrus behavior is characterized by mounting or attempting to mount other cattle, standing to be mounted by other cattle, smelling other females, trailing other females, bell owing, depressed appetite, nervous and excitable behavior, roughed up tail hair, vulva swelling and reddening and clear vaginal mucous discharge (Reith and Roy, 2017).

First ovulation is usually characterized by early regression of the CL because the heifer is not yet sexually mature (Garverick *et al.*, 1992). In the present study, a progesterone device was used to induce puberty, since exposure to progesterone allows the secretion of LH at the end of the treatment, inducing ovulation of the dominant follicle and contributing to the formation of functional CL in the first estrous cycle (Vrisman *et al*., 2018; González *et al*., 2020). Physiologically, in prepubertal heifers, the low concentration of estradiol produced in the ovaries due to an intense negative feedback mechanism prevents the hypothalamic preovulatory center from secreting adequate GnRH pulses (Amstalden *et al*., 2011). Therefore, this process also inhibits the release of FSH and LH from the anterior pituitary, causing absence of follicular waves and ovulation. On the other hand, when the hypothalamus initiates GnRH neuron function (influenced primarily by nutritional control), there is decreased negative feedback from estradiol, which leads to an increase in the frequency of GnRH pulse release (Kinder *et al*., 1995; Amstalden *et al*., 2011). Thus, the treatment with exogenous progesterone reduces negative feedback caused by estradiol receptors in the hypothalamus when they are in excess, increasing the release of gonadotropins (FSH and LH) by the pituitary gland and, consequently, stimulating the growth rate and ovulation of the dominant follicle (Vrisman *et al*., 2018).

The response to cyclicity induction in the present study was low in both groups (Table 3). In this context, two main aspects that may justify this low response are suggested. First, it should be noted that the heifers used are very young (12 to 14 months), which may suggest the absence of minimum sexual maturity for the hypothalamus-pituitary-gonad axis to be responsive to hormonal treatment (Sá Filho *et al*., 2015). Second, it can be hypothesized that the residual amount of P4 in the 4th use device had suboptimal concentrations, which may have compromised LH secretion at the end of cyclic induction hormone treatment (Vrisman *et al*., 2018), as well as Brunoro *et al*. (2017) presented in their study, where pregnancy rates decreased by reusing P4 devices.

In addition to the effect of P4, other substances and hormones may influence the pattern of LH secretion. The study by Macedo *et al*. (2019), who evaluated the effect of intramuscular administration of 25 different doses of kisspeptin (Kp) on LH release pattern in pre-pubertal *Bos taurus* and *Bos indicus* heifers, demonstrated that treatment was more efficient in *Bos taurus* that presented higher LH release. This shows that animals of taurine origin respond more efficiently to puberty induction protocols, as well as the crossbred heifers of the present study, which were found to be earlier than zebu heifers.

The AFC, a reproductive parameter that has been widely studied, was analyzed in this study to assess its influence on the puberty of prepubertal heifers. The mechanisms involving AFC and fertility in bovine females are not yet fully elucidated and there are challenges regarding their influence on the biotechnology used, pregnancy rate, oocyte quality, embryonic development, and *in vivo* and *in vitro* embryo production (Ireland *et al*., 2011; Evans *et al*., 2012; Morotti *et al*., 2017a, 2017b). However, AFC already proves to be promising as a marker of reproductive activity and productive efficiency in beef herds (McNeel and Cushman, 2015; Seneda *et al*., 2019).

A study performed by McNeel and Cushman (2015) confirmed that AFC is related to the time of conception of cows, and those who give birth at the beginning of the breeding season have more antral follicles. Thus, the ovarian characteristics of beef cattle females can be used as parameters for commercial meat production (Morotti *et al*., 2017b). For example, the high AFC seem to respond better to embryo production biotechnology (*in vivo* and *in vitro*) (Silva-Santos *et al*., 2014; Santos *et al*., 2016; Morotti *et al*., 2017a, 2017b; Garcia *et al*., 2020), although the low AFC seems to be more efficient in the TAI technique (Morotti *et al*., 2018; Moraes *et al*., 2019; Lima *et al*., 2020).

As is well known, AFC is greater in Nelore females (Pontes *et al*., 2011) and the present study confirmed this fact in prepuberal and pubertal heifers, even with a limited number of animals studied. During the hormonal induction protocol, AFC remained highly constant because of its high repeatability in both Nelore and crossbred heifer group, being a reliable marker for evaluating reproductive performance (Ireland *et al*., 2007; Santos *et al*., 2016; Morotti *et al*., 2017b). In this context, the present study proving that a single ultrasound evaluation would be reliable for determining the AFC of prepubertal heifers, as has been reported by Burns *et al*. (2005) and Silva-Santos *et al*. (2014) in heifers and by Burns *et al*. (2005) and Morotti *et al*. (2018) in cows.

Throughout the puberty induction protocol of prepubertal heifers, a highest productive performance of crossbred (Nelore x Angus) heifers was remarkable, both in BCS and average weight gain evaluation. The explanation for crossbreed animals having shown a better performance is due to the relationship between puberty and body condition (Day and Nogueira, 2013) and possibly the inherent characteristics of the subspecies that tend to be more precocious in taurine herds than indicus (Sartori and Barros, 2011). However, the nutrition has been identified as one of the factors that positively influence the fully functioning of the hypothalamus-pituitary-gonad axis. On the other hand, heifers with unfavorable genetic merit failed to reach puberty early, even when receiving adequate nutritional supplementation (Ferraz *et al*., 2018).

In addition, the diameter of dominant follicle from crossbred females was greater than the Nelore heifers, showing that in the condition of super early induction, as in the present study, crossbred animals can respond better reproductively possibly due to their sexual precocity and superior productive performance, similarly to the study shown by Paim *et al*. (2018). In this context, the data of the present study highlights the importance of using the crossbreed as a matrix, contributing to the beef production chain, precisely due to the advantages related to heterosis coming from the crossbreeding of the Nelore and Angus breeds.

It is important to emphasize that the relationship between AFC, reproductive characteristics and genetic merit is an essential selection factor in beef cattle herds (Morotti *et al*., 2017b), but little is known, and few studies have been conducted on puberty induction in prepubertal *Bos taurus indicus* animals relating the precocity factor, genetic merit, and AFC. Furthermore, we predict that AFC may be another tool for improving reproductive efficiency in cattle in a puberty induction protocol, especially when genetics and parameters related to puberty in heifers are also considered.

CONCLUSION

The super-early induction of puberty resulted in low cyclicity rate in *Bos indicus* (Nelore) and crossbred *Bos indicus-taurus* (Nelore x Angus) heifers. AFC was greater in Nelore heifers, although in both groups this count was highly repeatable throughout puberty induction. Crossbred heifers, however, had greater body condition score and average weight gain with greater dominant follicle diameter, indicating earlier precocity when compared to Nelore heifers.

REFERENCES

AMSTALDEN, M.; ALVES, B.R.; LIU, S. *et al*. Neuroendocrine pathways mediating nutritional acceleration of puberty: insights from ruminant models. *Front. Endocrinol*., v.2, p.109, 2011.

AUNGIER, S.P.M.; ROCHE, J.F.; DUFFY, P. *et al*. The relationship between activity clusters detected by an automatic activity monitor and endocrine changes during the periestrous period in lactating dairy cows. *J. Dairy Sci.*, v.98, p.1666-1684, 2015.

BRUNORO, R.; FRANCISCO, F.F.; PINHO, R.O. *et al*. Reutilização de implantes de progesterona em vacas Nelore de diferentes categorias submetidas a IATF. *Rev. Bras. Reprod. Anim.*, v.41, p.716-722, 2017.

BURNS, D.; JIMENEZ-KRASSEL, F.; IRELAND, J. *et al*. Numbers of antral follicles during follicular waves in cattle: evidence for high variation among animals, very high repeatability in individuals, and an inverse association with serum follicle-stimulating hormone concentrations. *Biol. Reprod.*, v.73, p.64-72, 2005.

CARDOSO, R. C.; ALVES, B. R. C. and WILLIAMS, G. L. Neuroendocrine signaling pathways and the nutritional control of puberty in heifers. *Anim. Reprod.*, v. 15, p. 868-878, 2018.

DAY, M. L. and NOGUEIRA, G. P. Management of age at puberty in beef heifers to optimize efficiency of beef production. *Anim. Front.*, v. 3, p. 6-11, 2013.

DISKIN, M. G. and KENNY, D. A. Optimising reproductive performance of beef cows and replacement heifers. *Animal*, v. 1, p. 27-39, 2014.

EVANS, A.C.O.; MOSSA, F.; WALSH, S.W. Effects of maternal environment during gestation on ovarian folliculogenesis and consequences for fertility in bovine offspring. *Reprod. Domest. Anim*., v.47, p.31-37, 2012.

FERRAZ, J.R.M.V.; PIRES, A.V.; SANTOS, M.H. *et al*. A combination of nutrition and genetics is able to reduce age at puberty in Nelore heifers to below 18 months. *Animal*, v. 12, p.569-574, 2018.

GARCIA, S.M.; MOROTTI, F.; CAVALIERI, F.L.B. *et al.* Synchronization of stage of follicle development before OPU improves embryo production in cows with large antral follicle counts. *Anim. Reprod. Sci.,* v. 221, p.106601, 2020.

GARVERICK, H.A.; ZOLLERS, J.R.W.G.; SMITH, M.F. Mechanisms associated with corpus luteum lifespan in animals having normal or subnormal luteal function. *Anim. Reprod. Sci.*, v.28, p.111-24, 1992.

GONZÁLEZ, S.M.; ASSUNÇÃO, I.E.G.; DUARTE, J. *et al*. Induction of puberty in Bos indicus heifers in the western Amazon region. *Semin. Ciênc. Agrár.*, v.41, p.2153-2162, 2020.

IRELAND, J.J.; SMITH, G.W.; SCHEETZ, D. *et al.* Does size matter in females? An overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti-müllerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in cattle. *Reprod. Fertil. Dev*., v.23, p.1-14, 2011.

IRELAND, J.J.; WARD, F.; JIMENEZ-KRASSEL, F. *et al*. Follicle numbers are highly repeatable within individual animals but are inversely correlated with FSH concentrations and the proportion of good-quality embryos after ovarian stimulation in cattle. *Hum. Reprod.*, v.22, p.1687-1695, 2007.

KINDER, J. E.; BERGFELD, E.G.M.; WEHRMAN, M.E. *et al*. Endocrine basis for puberty in heifers and ewes. *J. Reprod. Fertil.*, v.49, p.393-407, 1995.

LIMA, M.A.; MOROTTI, F.; BAYEUX, B.M. *et al*. Ovarian follicular dynamics, progesterone concentrations, pregnancy rates and transcriptional patterns in *Bos indicus* females with a high or low antral follicle count. *Sci. Rep*., v.10, p.19557, 2020.

MACEDO, G.G.; MINGOTI, R.D.; BATISTA, E.O.S. *et al.* Profile of LH release in response to intramuscular treatment with kisspeptin in *Bos indicus* e *Bos taurus* prepubertal heifers. *Theriogenology*, v.125, p.64-70, 2019.

MACHADO, R.; CORRÊA, R.; BARBOSA, R. *et al*. *Escore da condição corporal e sua aplicação no manejo reprodutivo de ruminantes*. São Carlos: Embrapa, 2008. 16p. (Circular Técnica, n.57).

MCNEEL, A.; CUSHMAN, R. Influence of puberty and antral follicle count on calving day in crossbred beef heifers. *Theriogenology*, v.84, p.1061-1066, 2015.

MORAES, F.L.Z.; MOROTTI, F.; COSTA, C.B. *et al.* Relationships between antral follicle count, body condition, and pregnancy rates after timed-AI *in Bos indicus* cattle. *Theriogenology*, v.136, p.10-14, 2019.

MOROTTI, F.; MORETTI, R.; SANTOS, G.M.G. *et al.* Ovarian follicular dynamics and conception rate in Bos indicus cows with different antral follicle counts subjected to timed artificial insemination. *Anim. Reprod. Sci.*, v.188, p.170-77, 2018.

MOROTTI, F.; SANTOS, G.M.G.; KOETZ JÚNIOR, C. *et al*. Correlation between phenotype, genotype and antral follicle population in beef heifers. *Theriogenology*, v.91, p.21-26, 2017b.

MOROTTI, F.; ZANGIROLAMO, A.F.; SILVA, N.C. *et al*. Antral follicle count in cattle: advantages, challenges, and controversy. *Anim. Reprod*., v.14, p.514-520, 2017a.

MOSSA, F.; WALSH, S.W.; BUTLER, S.T. *et al.* Low numbers of ovarian follicles ≥3 mm in diameter are associated with low fertility in dairy cows. *J. Dairy. Sci.*, v.95, p.2355-2361, 2012.

NOGUEIRA, E.; SILVA, M.; SILVA, J. *et al*. Timed artificial insemination plus heat I: effect of estrus expression scores on pregnancy of cows subjected to progesterone–estradiol-based protocols. *Animal*, v.13, p.2305-2312, 2019.

PAIM, T.P.; GOULART, R.O.; SILVA, D.A.L.D. *et al*. Relationship of weight gain with infrared temperatures in Nelore and F1 (Nelore x Angus) heifers reared in two forage production systems. *J. Anim. Sci.*, v.96, p.4002-4011, 2018.

PERRY, G.A. Factors affecting puberty in replacement beef heifers. *Theriogenology*, v.86, p.373-378, 2016.

PONTES, J.H.F.; MELO-STERZA, F.A.; BASSO, A.C. *et al.* Ovum pick up, in vitro embryo production, and pregnancy rates from a large-scale commercial program using Nelore cattle (Bos indicus) donors. *Theriogenology*, v.75, p.1640-1646, 2011.

REITH, S.; HOY, S. Review: behavioral signs of estrus and the potential of fully automated systems for detection of estrus in dairy cattle. *Animal*, v.12, p.398-407, 2017.

SÁ FILHO, M.; NASSER, L.F.T.; PENTEADO, L. *et al*. Impact of progesterone and stradiol treatment before the onset of the breeding period on reproductive performance of *Bos indicus* beef heifers. *Anim. Reprod. Sci*., v.160, p.30-39, 2015.

SÁ FILHO, M.; PENTEADO, L.; SIQUEIRA, G.R. *et al*. Timed artificial insemination should be performed early when used norgestomet ear implants are applied for synchronizing ovulation in beef heifers. *Theriogenology*, v.80, p.642-647, 2013.

SANTOS, M.G.; SILVA-SANTOS, K.C.; BARREIROS, T.R.R. *et al.* High nmbers of antral follicles are positively associated with in vitro embryo production but not the conception rate for FTAI in Nelore cattle. *Anim. Reprod. Sci*., v.165, p.17-21, 2016.

SARTORI, R.; BARROS, C.M. Reproductive cycles in Bos indicus cattle. *Anim. Reprod. Sci.*, v.124, p.244-250, 2011.

SENEDA, M.M.; MOROTTI, F.; ZANGIROLAMO, A.F. *et al.* Antral follicle population in prepubertal and pubertal heifers. *Reprod. Fert. Dev.*, v.31, p.10-16, 2019.

SILVA-SANTOS, K.C.; SANTOS, G.M.G.; SILOTO, L.S. *et al.* The correlation between the number of antral follicles and ovarian reserves (preantral follicles) in purebred *Bos indicus* and *Bos taurus* cows. *Anim. Reprod. Sci*., v.151, p.119-25, 2014.

VRISMAN, D.P.; BASTOS, N.M.; ROSSI, G.F. *et al.* Corpus luteum dynamics after ovulation induction with or without previous exposure to progesterone in prepubertal Nellore heifers. *Theriogenology*, v.106, p.60-68, 2018.

ZANGIROLAMO, A.F.; MOROTTI, F.; DA SILVA, N.C. *et al.* Ovarian antral follicle populations and embryo production in cattle. *Anim. Reprod*., v.15, p.310-315, 2018.