Seasonal differences in endocrine and ovarian patterns of *Bos taurus indicus* (Nelore) heifers estrous cycles

Diferenças sazonais no padrão endócrino e ovariano do ciclo estral de novilhas Bos taurus indicus (Nelore)

Anivaldo Olivio CORTE JUNIOR[1]; Bruno José Ferreira NAVARRETE[1]; Guilherme de Paula NOGUEIRA[1]

[1]Curso de Medicina Veterinária da Universidade Estadual Paulista Júlio de Mesquita Filho, Araçatuba-SP, Brazil

Abstract

Estrous cycle of eight Nelore heifers were evaluated during different seasons of the year (autumn n=11; winter n=8; spring n=9 and summer n=9) with daily count and measurement of follicles ≥3mm, blood was collected every 12h for LH and progesterone (P4), and after estrous every 3h for LH peak. Five ovariectomized heifers were injected with 17β-estradiol (2μg/kg) every season and blood samples collected every 3h (for 30h) thereafter for LH quantification. The monthly percent body weight difference (Δ%) did not vary among seasons. P4 concentration was higher ($p<0.01$) and follicle number lower during autumn and summer compared to winter and spring. During winter there were more estrous cycles with three and during summer only cycles with two follicular waves ($p<0.01$). As LH secretion did not vary despite P4 concentration and as there was negative correlation between higher P4 values and daily percentile variation of photoperiod (Δ%, $p<0.01$; $r= -0.45$) it is possible to suppose that there is seasonal variation on luteal cell sensitivity to LH. In the ovariectomized Nelore heifers, the LH basal concentration (without estradiol stimulus, $p=0.02$) and the LH response to estradiol ($p<0.01$) were lower during summer, leading to the hypothesis that there is seasonal variation of hypothalamic sensitivity to estradiol. According to the present experiment there are suggestions of seasonal reproduction in Nelore heifers.

Keywords: Seasonal. Estradiol. Luteinizing hormone. Ovarian follicle. Progesterone.

Resumo

Ciclos estrais em oito novilhas Nelore foram acompanhados durante diferentes estações (outono n=11; inverno n=8; primavera n=9; verão n=9) com contagem e diâmetro de folículos ≥ 3 mm diariamente, com colheitas de sangue a cada 12 h para LH e P4, e a cada 3 h para o pico de LH. Cinco novilhas ovariectomizadas receberam 17β estradiol (2μg/kg) em cada estação, com colheitas de sangue a cada 3 h para quantificar LH. A diferença percentual (Δ%) do peso entre os meses não variou entre as estações. Durante o ciclo estral, a concentração de P4 (média e máxima) foi maior ($p<0,01$), e o número de folículos menor ($p<0,01$), no outono e verão, se comparados ao inverno e primavera. No inverno houve mais ciclos com três ondas e no verão somente ciclos com duas ondas foliculares ($p<0,01$). Como as características da secreção de LH não foram diferentes apesar da variação na concentração de P4, e como houve correlação negativa entre os valores máximos de P4 e a variação percentual (Δ%) diária no fotoperíodo ($p<0,01$; $r= -0,45$), supõe-se que haja variação sazonal na sensibilidade das células luteínicas ao LH. Nas novilhas ovariectomizadas, a concentração basal (sem estradiol) circanual de LH foi menor no verão ($p=0,02$), assim como a sua secreção em resposta ao estradiol ($p<0,01$), o que permite supor que também haja uma variação sazonal na sensibilidade hipotalâmica ao estradiol. De acordo com o presente experimento, há indícios de sazonalidade reprodutiva em novilhas Nelore.

Palavras-chave: Estações do ano. Estradiol. Hormônio luteinizante. Folículo ovarian. Progesterona.

Introduction

Considering the Bovidae family there are several species that reproduces seasonally, such as sheep, goats and buffaloes. Although the *Bos taurus* species is considered yearly polyestrous, some reproductive characteristics varies throughout the year, such as a higher mounting frequency of beef cattle in winter compared to summer[1]. The literature generally presents conflicting results regarding the seasonal reproduction in cattle. In *Bos indicus* both the absence of

Correspondência para:
Guilherme de Paula Nogueira
R: Clóvis Pestana, 793, Araçatuba-SP
CEP.:16050-680
e-mail: gpn@fmva.unesp.br

Recebido: 01/09/2009
Aprovado: 29/02/2012

seasonal effect on serum progesterone concentrations (P4)[2], greater P4 during decreasing photoperiod[3,4] or even the opposite[5] were described. Also, the *Bos indicus* corpus luteum (CL) showed larger area and volume during the summer compared to winter[5], associated to an increased capacity of P4 production in response to luteinizing hormone (LH)[6,7]. There was no seasonal influence on plasma LH concentration in intact Brahman cows (*Bos indicus*), but in ovariectomized cows there was a marked LH decrease during summer[8]. In ovariectomized *Bos taurus* cows, there was a higher LH concentration and greater pulse amplitude in response to estradiol around the spring equinox, compared to other year seasons[9,10]. Higher LH concentration was found in spring and summer compared to winter, in intact *Bos indicus* cows[6].

Winter starts after the winter solstice, after which the photoperiod gradually increases until the summer solstice when the photoperiod begins to regress. A reproductive seasonal animal uses photoperiod as a cue, aiming a more favorable period for offspring birth. Mechanism may include altering the hypothalamic gonadotropin-releasing-hormone (GnRH) secretion, and, consequently LH and follicle stimulating hormone (FSH) release from the pituitary, so that ovulation in concentrated in a more successful season. Throughout evolution, animals were selected by environmental pressure to give birth in seasons when food supply was greatest. For example, the Indian domestic buffalo (*Bubalus bubalis*) from the same geographic region of *Bos indicus*, belongs to the same Bovidae family and has a gestation lenght not very different from cattle. That species, has a defined breeding season concentrated in the lower photoperiod days[11]. Analyzing the Indian climatic conditions, the pasture supply increases with the onset of summer, when the monsoon winds increases rainfall[12]. According to Darwinian evolutionary theory, animals with gestation period of 9 (cattle) and 10 (buffalo) months, shall have reproductive activity between late summer and autumn (diminishing photoperiod), to give birth in late spring and early summer, at favorable environmental conditions for the offspring's survival.

In the bovine estrous cycle, follicle growth exhibits a wave pattern that occurs periodically in both the taurine (*Bos taurus*)[13] and in the zebu (*Bos indicus*)[14,15] breeds. Each follicular wave is composed by three distinct phases: recruitment, selection and dominance[16,17,18]; the developing dominant follicle requires LH[19] and restraints the growth of other follicles through the suppression of FSH concentrations. This event depends on inhibin, produced by the growing follicles and also on estradiol produced by the dominant follicle[16,20]. The hypothalamic estradiol sensitivity is stimulated by a previous exposure to P4, that is also needed to stimulate the release of the LH preovulatory peak[21]. Treatment with P4 failed to suppress the surge of FSH in cattle[22,23], however plays an important role in the LH suppression[19,22].

The motivation of the present experiment came from results of a preliminary study, conducted at the Laboratory of Animal Endocrinology at the Universidade Estadual Paulista (UNESP) Campus Araçatuba-SP, Brazil, in which seasonal variation was observed in serum LH concentrations in ovariectomized Nelore heifers. In that study, there was a negative correlation with the 24h photoperiod variation.

In the presentstudy, we used intact Nelore heifers to test the hypothesis that Nelore females have different LH concentration during the estrous cycle in different seasons. We also used ovariectomized heifers to test whether such seasonal variation in LH concentration occurs due to a variation in hypothalamic sensitivity to estradiol.

Material and Method

This study was approved by the ethics committee on animal experimentation from the local institution (2007/003614). We used 11 Nelore heifers at 36

months of age, born with in an interval of 30 days. Five heifers were ovariectomized for more than two years and six were intact (not-ovariectomized). Animals were kept at the Veterinary Medicine College-UNESP Campus Araçatuba -SP (South 21°12'32"West and 50°25'58"), with free access to water and mineral supplementation. Heifers were kept in grazing conditions (*Brachiaria brizantha)* during the summer months. During the dry season, heifers were fed on semi-feedlot system with a mixture of sugar cane bagasse supplemented with 2 kg/day/animal of concentrate (soybean, corn and mineral and vitamins). Heifers were weighed monthly.

Climatic variables were collected at the CATI (Integrated Technical Assistance Coordination) in Araçatuba, and local photoperiod variation was obtained from the Apollo 1125 web site.

From the six intact heifers, two estrous cycles per season per animal were analyzed, heifers were examined by transrectal ultrasonography and blood samples were collected. Before each evaluated cycle, estrus was synchronized with two luteolytic agent (cloprostenol sodium - Ciosin ® or d-cloprostenol - Prolise ®; 530 µg dose, I.M.) injections given at a 12 days interval. After the second injection, heifers were evaluated daily by ultrasound, to indentify ovulations induced by the luteolytic agent (D0). Ultrasound examinations were conducted there after throughout the estrous cycle either until the following ovulation, defined as the disappearance of the dominant or until D24. Estrous cycle data from heifers that did not ovulate until the D24 were not included in the statistical analysis. A GE ultrasound (General Electric ®) Model Logic-100, with a 5 MHz linear array transducer was used; ultrasound examinations were performed by a single operator, always around 6 pm.Both ovaries were evaluated, all antral follicles ≥ 3 mm were counted and the diameter measured (average of follicle height and width). The follicles were then sorted into the following size categories and counted between ≤ 3 and ≤ 5 mm – pre-follicular deviation, between 5 < and ≤ 9 mm and between 9 < and ≤ 14 mm.

During the estrous cycle evaluation, two blood samples were collected daily from all intact heifers, the first sample before performing the ultrasound examination (around 6 am), and the second at 6 pm. Upon the detection of estrus signs (vaginal mucus, heat behavior and large dominant follicle diameter) blood samples were collected every three hours until ovulation, to characterize the LH surge. For P4 quantification, morning samples from every three days in each cycle, were used.

Once during each season, five ovariectomized heifers were injected with 17β estradiol (2µg/kg) and had blood samples collected (once before injection) every three hours (for 30 h) to evaluate the LH concentration.

Both intact and ovariectomized heifers had blood samples collected every four days to assess the circannual plasma LH profile. For this analysis all LH peaks were excluded from intact heifers and all estradiol-induced LH peaks were excluded from ovariectomized heifers.

The hormone measurements in plasma samples were performed by radioimmunoassay (RIA) at the Animal Endocrinology Laboratory at Unesp Araçatuba. For P4 the DPC® kit was used, with 4.88% of intra-assay CV for 20.67 ng/mL and 7.92% for 0.11 ng/mL plasma standards, sensitivity was 0.0191 ng/mL. The LH quantification was performed by RIA as described by Bolt et al.[23], intra-assay CV was 9.36% for 7.26 ng/mL and 13.31% for 0.34 ng/mL plasma standards, the inter-assay CV was 12.37% for the high and 13.74% for low standards and the sensitivity was0.048 ng / mL.

Daily photoperiod percentile change (Δ%) and climatic variables (temperature, rainfall and photoperiod) between different seasons were compared using Kruskal-Wallis and Dunn as post-hoc test, and the number of cycles with two and three follicular waves in each season evaluated with the exact Fisher test.

Average of the dominant follicle diameter from first and second follicular wave in cycles with two waves were compared using the paired t test, and dominant follicle diameter between three waves with repeated measures analysis of variance (ANOVA) with Tukey as post-hoc test.

Among the seasons, both for intact and ovariectomized heifers, the mean weight and weight percentile change (Δ%) were compared by repeated measures ANOVA with Tukey post-hoc test. The same test was used for estrous cycles length and the data obtained from follicular ultrasound examinations. Some of these variables were transformed into logarithm (LOG10 (X +1)) or square root (SQRT (X +1)), when the assumption of normality of residues was not fulfilled.

To identify LH peaks and to calculate LH secretion variables (total area, area under the peak, amplitude between the first and last peak point and highest concentration) from ovariectomized and intact heifers samples, the program GrafPad 3:00 PRISM® was used. Variables analyzed in intact heifers were: LH secretion characteristics, the basal LH plasma concentration (without peaks) during the cycles, circannual plasma LH baseline profile, P4 plasma concentrations, the maximum P4 concentration in each cycle and LH concentration in four samples (D6, D9, D12 and D15) during each cycle were compared between the different seasons. Variables analyzed in ovariectomized heifers were: the LH secretion variables, the LH concentration (with peaks) after estradiol treatment and circannual baseline of plasma LH profile were compared among the different seasons. Original data were also transformed when the assumption of normality of residues was not fulfilled. For clarity, data on tables and figures were shown untransformed.

The maximum P4 values obtained from intact heifers, were correlated to the weight percentage change by Pearson correlation, and daily photoperiod change (Δ%) by Spearman correlation.

A 5% significance level was considered and the data were presented as mean and standard error of mean.

Results and Discussion

Local tropical climate was well characterized by high temperatures throughout the year, with higher summer rainfall and dry season in winter. There were differences in mean percentage delta (Δ%) between seasons in the daily photoperiod (Table 1), showing difference of 2 h and 36 min after the solstices although it is an inter-tropical zone.

Table 1 - Environmental variables (mean ± SEM) in different seasons during the experiment (South 21 ° 12 '32"West and 50 ° 25' 58") - Araçatuba-SP - 2009

Variables	2007			2008	Seasonal effect
	Autumn	Winter	Spring	Summer	
Maximum Temperature (oC)	29.27±0.6^{c}	30.60±0.5^{bc}	33.32±0.4^{a}	31.81±0.3^{b1}	p<0.01
Minimun Temperature (oC)	15.32±0.5^{b}	14.19±0.3^{b}	19.74±0.2^{a}	20.26±0.1^{a}	p<0.01
Average Temperature (oC)	22.29±0.4^{b}	22.40±0.4^{b}	26.53±0.3^{a}	26.03±0.2^{a}	p<0.01
Rainfall mm/day	2.65±1.1^{b}	0.98±0.5^{b}	2.76±0.8^{ab}	5.69±1.5^{a}	p<0.01
Photoperiod (h:min)	11:20	11:20	12:55	12:55	-
Daily Photoperiod variation (%)	-0.12±0.0^{b}	0.12±0.0^{a}	0.12±0.0^{a}	-0.12±0.0^{b}	p<0.01

Values followed by different letters on the same line, differ (p <0.01) by Dunn's test
1 - Different letters differ average number (p <0.05)

On total, 37 estrous cycles were considered, 11 in the fall, 8 in winter, 9 in spring and 9 in summer. Another 11 estrous cycles were excluded for exceeding 24 days: one in the fall, four in winter, three in spring and three in the summer.

During the experimental period 25 cycles with two follicular waves (67.6%) and 12 cycles with three waves (32.4%) were observed. In cattle, more than 95% of estrous cycles have two or three follicular waves[16] and there may be variations in the same animal[14,15], although the majority of the heifers kept the same number of follicular waves in 16 consecutive cycles. Nelore cows have a predominance of cycles with two[14,26] or three follicular waves[27], while heifers with three follicular waves[14,26]. Considering that the heifers from this experiment were 36 months old, the 67.6% frequency of two waves cycles was similar to previously described for cows.

In the winter there was a greater number of three follicular waves estrous cycles, different ($p < 0.01$) from the summer when two follicular wave cycles prevailed (Table 2). However, the estrous cycles length average did not differ among seasons, lasting for 21±0.2 days throughout the experiment (Table 2), agreeing with previous report[14]. Normally the duration of the luteal phase of the estrous cycle determines the number of follicular waves and the estrous cycle length[13,14,28]. However, factors that influence the development of the dominant follicle in the first follicular wave may also be responsible for wave pattern regulation[16]. There are reports associating poor quality diet and heat stress to the increase in the proportion of cycles

Table 2 – Effect of season (mean ± SEM) on the number of follicles in different categories, the preovulatory follicle diameter, the estrous cycles length, numbers of estrous cycles with two or three follicular waves, average body weight and body weight percent changes in 8 intact Nelore heifers and body weight and percentile body weight change in the months of estradiol treatment in ovariectomized Nelore heifers (n=5) - Araçatuba-SP, 2009

Variable	2007		2008		Seasonal effect
	Autumn (n=13)	Winter (n=8)	Spring (n=9)	Summer (n=9)	
Total follicles	7.8±0.1^{b}	9.6±0.3^{a}	9.7±0.3^{a}	6.8±0.2^{b}	p<0.01
3mm ≤ Follicles ≤ 5mm	6.2±0.1^{ab}	7.8±0.3^{a}	7.7±0.3^{a}	4.9±0.2^{b}	p=0.02
5mm < Follicles ≤ 9mm	1.0±0.0^{a}	1.2±0.1^{a}	1.4±0.1^{a}	1.2±0.1^{a}	p=0.22
9mm < Follicles ≤ 14mm	0.6±0.0^{a}	0.6±0.0^{a}	0.6±0.0^{a}	0.6±0.0^{a}	p=0.93
Pre-ovulatory follicle diameter (mm)[1]	12.0±0.5^{ab}	11.6±0.4^{b}	12.1±0.3^{ab}	13.1±0.5^{a}	p=0.06
Number of cycles with 2 and 3 follicular waves[2]	7 and 4^{ab}	3 and 5^{a}	6 and 3^{ab}	9 and 0^{b}	p<0.01
Cycles length (days)	20.8±0.4^{a}	20.9±0.5^{a}	21.1±0.5^{a}	20.6±0.5^{a}	p=0.85
Intact heifers body weight (kg)	583.6±8.8^{b}	629.3±13.7^{ab}	655.2±24.9^{a}	629.9±14.8^{ab}	p<0.01
Intact heifers body weight change (%)	0.6±0.3^{a}	3.4±0.8^{a}	0.1±1.9^{a}	-0.6±1.7^{a}	p=0.19
Ovariectomized heifers body weight (kg, n=5)	579.2±19.5^{a}	624±22.1^{a}	703.2±64.8^{a}	683.2±25.6^{a}	p=0.12
Ovariectomized heifers body weight change (%, n=5)	0.8±0.4^{a}	2.6±0.3^{a}	0.9±3.5^{a}	-2.4±0.3^{a}	p=0.28

Means followed by different letters on the line are different ($p < 0.05$) by Tukey test
1 Means followed by different letters on the line, are different ($p = 0.06$) by Tukey test
2 Means followed by different letters on the line are different ($p < 0.01$) by Fisher's test

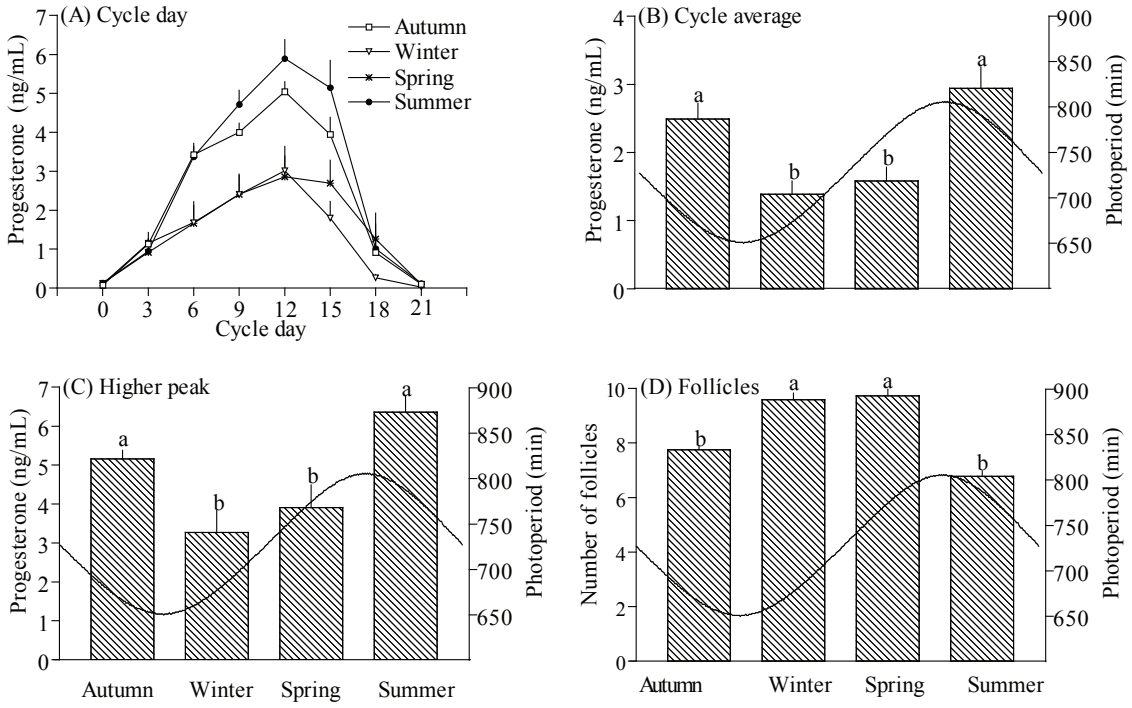

Figure 1 – (A) Plasma progesterone concentration from samples collected every 3 days; (B) average progesterone concentration, (C) Maximum progesterone concentration and (D) number of follicles ≥ 3 mm from daily observations (average±SEM) during estrous cycles evaluated at different seasons of the year (Autumn-n=11; Winter-n=8; Spring-n=9; Summer-n=9) in 8 Nellore heifers, depicted related to photoperiod variation. Bars with different superscript letters are different (p<0,01) according Tukey – Araçatuba-SP - 2009

with three waves[16,26]. Despite the seasonality variation found in the number of follicular waves, some reports found no difference in these variables Nelore[14,27] or in Brahman[4]. The estrous cycle length with two follicular waves (20.53±0.26 days) were shorter (p=0.02) compared to estrous cycles with three follicular waves (21.61±0.35 days).

In estrous cycles with two follicular waves, there was no difference (p= 0.32) between the maximum diameter of the dominant follicle from the first wave (12.1 ± 0.3 mm) and pre-ovulatory follicle diameter (12.5 ± 0,3 mm). But in cycles with three follicular waves, the dominant follicle diameter from the second wave was smaller (9.7 ± 0.4 mm, p <0.01) than the dominant follicle from first wave (11.3 ± 0.4 mm) and the pre-ovulatory follicles (12.1 ± 0.2 mm). This difference between the dominant follicle diameter in cows presenting three follicular waves is consistent with the literature for both *Bos taurus*[17]and for *Bos indicus*[14] cattle and can be explained by the different P4 production from CL, that reaches a maximum between 5 and 11 days of the estrous cycle[29]. The higher P4 levels decreases LH levels[19] and decreases LH pulses frequency[20], leading to a smaller dominant follicle diameter, as consequence of a shorter growth period of the dominant follicle in the second follicular wave[16]. In contrast, low P4 concentrations results in a larger dominant follicle diameter, and a longer dominance phase, delaying the emergence of the next follicular wave [19,22].

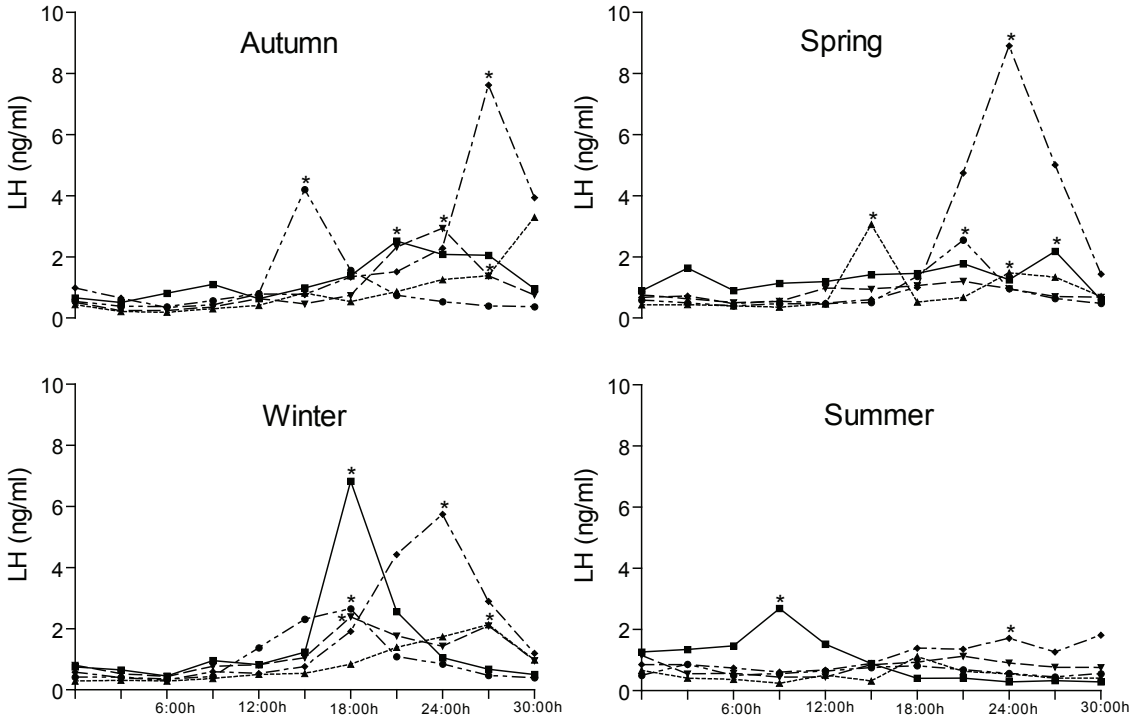

Figure 2 – LH plasmatic concentration from 5 ovariectomized Nellore heifers every 3 hours after 17β-estradiol (2μg/kg) injection, in different seasons of the year. * = identified LH peaks – Araçatuba-SP - 2009

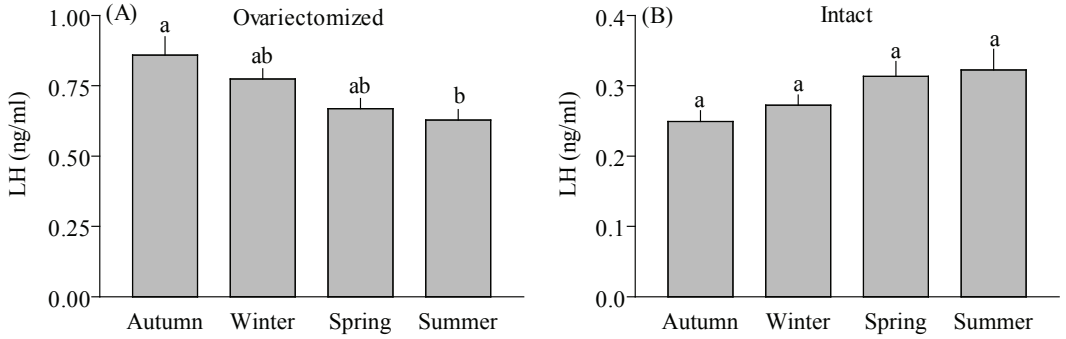

Figure 3 - (A) Average LH concentration in each season of the year, blood samples were collected every 4 days, from 5 ovariectomized Nellore heifers (excluding 17β-estradiol injection day) (average±SEM). (B) Average LH concentration, blood samples collected every 4 days (ovulatory LH peaks were excluded) from 6 intact Nellore heifers during different seasons of the year. Bars with different superscripts are different (p<0,05) according Tukey– Araçatuba-SP - 2009

During the autumn and summer (i.e. decreasing photoperiod) there was a higher plasma P4 concentration (average over the entire cycle) in intact heifers, compared winter and spring (i.e. increasing photoperiod) (Figure 1B). The maximum P4 concentration in each cycle was higher at seasons with decreasing pho-

toperiod (p <0.01, Figure 1C). Higher concentrations of P4 in cows under decreasing photoperiod were also found by other authors[3,4]. One possible explanation would be a higher sensitivity of CL to LH in animals exposed to decreasing photoperiod, with greater P4 production, as previously observed in cultured luteal cells from *Bos taurus indicus*[6,7].

Knowing that the energy balance influences P4 concentration[30], heifers were weighed monthly. Intact heifers average weight during the experiment was 622.5±8.9 kg, while the weight of ovariectomized heifers was 647.4± 20.7 kg. The average weight of intact heifers in the autumn was lower (p = 0.01) than in the spring, but ovariectomized heifers showed no weight difference (p = 0.12) between seasons (Table 2). The average weight percentile change of intact (p= 0.21) and ovariectomized (p= 0.28) heifers showed no seasonal variation (Table 2). There was no correlation between the maximum P4 values found in each estrous cycle with the weight percentile change (Δ%) in the respective month of the cycle (p= 0.66, r= -0.08). Also the periods in which supplementation was more intense (i.e. autumn and winter) were not associated with lower P4 concentrations periods (i.e. winter and spring), suggesting that P4 variation was not influenced by diet. Since the daily photoperiod percentage change (Δ%) were highly negatively correlated with maximum P4 value (p <0.01, r =- 0.45), a possible seasonal effect on luteal function of Zebu heifer can be considered. It should be noted that the heifers were maintained on pasture and feeding with sugar cane bagasse and supplemented with up to 2 kg of concentrate per animal/day. Considering the intact heifers average weight of 622.5 kg, they received no more than 0.3% live weight of high energy food/day.

During autumn and summer there was a lower daily total number of follicles (p <0.01) compared with winter and spring (Table 2 and Figure 1B). Among the categories, there were fewer follicles ≤3 mm ≤5 mm (p= 0.02) in summer compared to winter and spring (Table 2). Comparing the estrous cycles, fewer follicles ≥3mm were observed in seasons with a higher P4 concentration (Figures 1C and 1D). Different from the lower number of ≥4mm follicles observed in Holstein heifers[22] treated with lower P4 than physiological concentration, probably consequence of the extended dominant follicular phase, delaying the FSH surge and the next wave growth phase. Thus, in Nelore heifers there was a greater number of follicles emerging on estrous cycles presenting a lower concentration of P4. Despite the difference between seasons on P4 concentration, there was no difference on LH secretion patterns. Specifically, both the total area (p = 0.32), the area under the peak (p = 0.22), peak amplitude (interval between the first and the last point of the peak; p = 0.54), the LH maximum concentration (p = 0.30), baseline plasma LH during the cycle (p = 0.35) and circannual plasma LH concentrations (season average of samples collected every four days; p = 0.32; Figure 3C) were similar among seasons.

From the five ovariectomized heifers stimulated with 17β estradiol, three showed no LH peak in summer (Figure 2), as a result, the LH secretion after estradiol stimulation was lower (p <0.01) in summer (0.8±0.2 ng/mL) than in autumn (1.2±0.4 ng/mL), winter (1.3±0.4 ng/mL) or spring (1.3±0.5 ng/mL). Also in ovariectomized heifers the circannual LH plasma concentration (samples taken every four days) was lower in summer than in autumn (p =0.02, Figure 3A) similar to described previously[24].

However, alike the intact animals, in ovariectomized heifers showed no seasonal variation in LH secretion pattern, considering total secretion area (p =0.20), area under the peak (p = 0.05), peak amplitude (p =0.69) and maximum LH concentration (p = 0.18). Interestingly, in the summer, only three out of five ovariectomized heifers showed a peak LH in response to estradiol (Figure 2). A decrease in LH plasma concentration in ovariectomized Brahman cows in the summer was observed previously[8]. Also in

ovariectomized *Bos taurus taurus* cows there were an increased LH secretion in response to estradiol[9,10]in the spring equinox and during the winter[31].

Throughout the estrous cycle, LH is secreted in pulses every 1-2 h when there is lower P4 concentration after ovulation, decreasing to one every 3-4 h, in the middle of estrous cycle when P4 is maximum. LH pulse frequency increases again after luteolysis and stimulates estradiol production, culminating in the preovulatory peak of GnRH[20,29]. As there was variation in P4 concentration between seasons (Figure 1A), LH concentration was quantified in estrous cycle samples from days D6, D9, D12 and D15, aiming to evaluate the LH influence on P4 concentration and of P4concentration on LH. However, there was no seasonal variation in LH plasma concentrations from these samples ($p = 0.47$), which reinforces the hypothesis that between seasons, there is some variation in the luteal cells sensitivity to LH, as described in Zebu by Randel[6] and Rhodes, Randel and Long[7].

Although it has a suppressive effect on the LH secretion[20,29], previous P4 exposure is required for the hypothalamic sensitization by estradiol that is needed to stimulate the GnRH/LH peak[21]. This happens because P4 increases the concentration of estradiol receptors in the mediobasal hypothalamus, increasing the sensitivity to estradiol that will lead to the release of the GnRH (and, consequently, the LH) peak[32,33]. The reason for fewer follicles observed, in seasons with higher P4 concentrations during this experiment (Figure 1C and 1D) may be a consequence of estradiol sensitivity variation in the hypothalamus. The greater hypothalamic sensitivity to estradiol during the luteal phase (with higher concentrations of P4) allowed estradiol to suppress FSH secretion prematurely, anticipating the moment of follicular deviation.

It is important to evaluate differences on estradiol sensitivity as the hormonal manipulation of the estrous cycle allows a valuable increase in livestock production. Estrus synchronization protocols have been widely applied in different species, however animals with seasonal estrous cyclicity do not respond adequately to progestins and estrogens treatment during the unfavorable breeding season. This was observed in ovariectomized goats[34], demonstrating a possible change in estradiol/progesterone receptor expression across the seasons. In buffaloes, the follicular development decrease after estradiol valerate and norgestomet injection was less intense outside the breeding season[35]. Basal 17β-estradiol and LH concentrations in water-buffaloes with regular estrous cycles, did not vary throughout the year, however, concentrations were significantly higher close to estrus during the favorable breeding season[36]. In sheep there was increased estradiol receptors expression in the preoptic area during long days compared to short days[37]. In the current experiment, because of the seasonal variation on LH concentration after 17β-estradiol injection (Figure 3) it is possible to suppose that in Nelore heifers there was a lower expression hypothalamic estradiol receptors (responsible for negative feedback) during summer, the most favorable season for LH peak occurrence.

During zebu domestication, animals were selected against seasonal reproductive characteristic, today these subspecies shows reproductive capacity throughout the year[38] confirmed in this experiment, but some features still have a heritage from the evolutionary process.

Conclusion

Nelore heifers showed seasonal changes during the estrous cycle. Specific changes were in the P4 concentration, the number of follicles and number of follicular waves. However, there was no change in LH between seasons, despite the variation in LH release in response to 17β-estradiol injection in ovariectomized heifers throughout the seasons.We speculate that there are seasonal effects on luteal cells sensitivity to LH and also on the hypothalamic response to estradiol.

Acknowledgements : FAPESP and UNESP-Araçatuba

References

1. WHITE, F. J., WETTEMANN, R. P., LOOPER, M. L., PRADO, T. M., MORGAN G. L. Seazonal effects on estrous behavior and time of ovulation in nonlactating beefs cows. **Journal of Animal Science**, v. 80, n. 12, p. 3053-3059, 2002.
2. GAMA FILHO, R. V.; FONSECA, F. A.; UENO, V. G.; FONTES, R. S.; QUIRINO, C. R.; RAMOS J. L. G.; Sazonalidade na dinâmica folicular ovariana e produção embrionária em novilhas da raça Guzerá, **Brazilian Journal of VeterinarianResearch Animal Science**, v. 44, n. 6, p. 422-427, 2007.
3. LAMOTHE-ZAVALETA, C.; FREDRIKSSON, G.; MADEJ, A. Reproductive performance of zebu cattle in Mexico 2. Seasonal influence on the levels of progesterone, estradiol-17β, cortisol and LH during the estrous cycle.**Theriogenology**, v. 36, n. 6, p. 897-912, 1991.
4. ZEITOUN, M. M.; RODRIGUEZ, H. F.; RANDEL, R. D. Effect of season on ovarian follicular dynamics in Brahman cows. **Theriogenology**, v. 45, n. 8, p. 1577-1581, 1996.
5. BORGES, A. M.; TORRES, C. A. A.; RUAS, J. R. M.; ROCHA JUNIOR, V. R.; CARVALHO, G. R. Desenvolvimento luteal e concentrações plasmáticas de progesterona em vacas das raças Gir e Nelore. **RevistaBrasileira de Zootecnia**, v. 32, n. 2, p. 276-283, 2003.
6. RANDEL, R. D. Seasonal effects on female reproductive functions in the bovine (Indian breeds). **Theriogenology**, v. 21, n. 1, p. 170-185, 1984.
7. RHODES, R. C.; RANDEL, R. D.; LONG, C. R. Corpus luteum in the bovine: in vivo in vitro evidence for both a seasonal and breedtype effect. **Journal of Animal Science**, v. 55, n. 1, p. 159-167, 1982.
8. KINDER, J. E.; WHYTE, T. R.; CREED, A.; ASPDEN, W. J.; D'OCCHIO, M. J. Seasonal fluctuations in plasma concentrations of luteinizing hormone and progesterone in Brahman (*Bos indicus*) and Hereford-Shorthorn (*Bos taurus*) cows grazing pastures at two stocking rates in a subtropical environment. **Animal Reproduction Science**, v. 49, n. 2-3, p. 101-111, 1997.
9. STUMPF, T. T.; DAY, M. L.; WOLFE, P. L.; WLFE, M. W.; CLUTTER, A. C.; KITTOK, R. J.; KINDER, J. E. Feedback of 17β-estradiol on secretion of luteinizing hormone during different seasons of the year. **Journal of Animal Science**, v. 66, n. 2, p. 447-451, 1988.
10. DAY, M. L.; IMAKAWA, K.; PENNEL, P. L.; ZALESKY, D. D.; CLUTTER, A. C.; KITTOK, R. J.; KINDER, J. E. Influence of season and estradiol on secretion of luteinizing hormone in ovariectomized cows. **Biology of Reproduction**, v. 35, n. 3, p. 549-553, 1986.
11. MADAN, M. L.; DAS, S. K.; PALTA, P. Application of reproductive technology to buffaloes. **Animal Reproduction Science**, v. 42, n. 2, p. 299-306, 1996.
12. WIKIPÉDIA. **Clima de monções**. [2009]. Disponível em: <http://pt.wikipedia.org/wiki/Mon%C3%A7%C3%A3o>. Acesso em: 10 fev. 2009.
13. GINTHER, O. J.; KNOPF, L.; KASTELIC, J. P. Temporal associations among ovarian events in cattle during estrous cycles with two and three follicular waves. **Journal of Reproduction and Fertility**, v. 87, n. 1, p. 223-230, 1989.
14. FIGUEIREDO, R. D.; BARROS, C. M.; PINHEIRO, O. L.; SOLER, J. M. P. Ovarian follicular dynamics in Nelore breed (Bos indicus) cattle. **Theriogenology**, v. 47, n. 8, p. 1489-1505, 1997.
15. RHODES, F. M.; DE'ATH, G.; ENTWISTLE, K. W. Animal and temporal effects on ovarian follicular dynamics in Brahman heifers. **Animal Reproduction Science**, v. 38, n. 4, p. 265-277, 1995.
16. ADAMS, G. P.; JAISWAL, R.; SINGH, J.; MALHI, P. Progress in understanding ovarian follicular dynamics in cattle. **Theriogenology**, v. 69, n. 1, p. 72-80, 2008.
17. SIROIS, J.; FORTUNE, J. E. Ovarian follicular dynamics during the estrous cycles in heifers monitored by real-time ultrasonography. **Biology of Reproduction**, v. 39, n. 2, p. 308-317, 1988.
18. GINTHER, O. J.; WILTBANK, M. C.; FRICKE, P. M.; GIBBONS, J. R.; KOT K. Selection of the dominant follicle in cattle. **Biology of Reproduction**, v.55, n. 6, p. 1187-1194, 1996.
19. GINTHER, O. J.; BERGFELT, D. R.; BEG, M. A.; KOT, K. Follicle selection in cattle: Role of luteinizing hormone. **Biology of reproduction**, v. 64, n. 1, p. 197-205, 2001.
20. MIHM, M. Follicle wave growth in cattle. **Reproduction in Domestic Animals**, v. 37, n 4, p. 191-200, 2002.
21. GÜMEN, A.; WILTBANK, M. C. An alteration in the hypothalamic action of estradiol due to lack of progesterone exposure can cause follicular cysts in cattle. **Biology of Reproduction**, v. 66, n. 6, p. 1689-1695, 2002.
22. ADAMS, G. P.; MATTERI, R. L.; GINTHER, O. J. Effect of progesterone on ovarian follicles, emergence of follicular waves and circulating follicle-stimulating hormone in heifers. **Journals of Reproduction & Fertility**, v. 95, n. 2, p. 627-640, 1992.
23. BOLT, D. J.; SCOTT, V.; KIRACOFE, G. H. Plasma LH and FSH after estradiol. norgestomet and Gn-RH treatment in ovariectomized beef heifers.**Animal Reproduction Science**, v. 23, n. 4, p. 263-271, 1990.
24. NOGUEIRA, G. P.; OLIVEIRA, D. J. C.; CIPRIANO, R. S. Variação sazonal da secreção de LH em novilhas Nelore. **Acta ScientiaeVeterinariae**, v. 35, n. 3, p. 1018, 2007.
25. Efemérides de Araçatuba-SP. [2009]. Disponível em: <www.apolo11.com/efemerides.php>. Acesso em: 10 fev. 2009.
26. BO, G. A.; BARUSELLI, P. S.; MARTINEZ, M. F. Pattern and manipulation of follicular development in Bos indicus cattle. **Animal Reproduction Science**, v. 78, n. 3-4, p. 307-326, 2003.
27. BORGES, A. M.; TORRES, C. A. A.; ROCHA JUNIOR, V. R.; RUAS, J. R. M.; GIOSO, M. M.; FONSECA, J. F.; CARVALHO, G. R.; MALFFILI, V. V. Dinâmica folicular e momento da ovulação em vacas não lactantes das raças Gir e Nelore durante duas estações do ano. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 56, n. 3, p. 346-354, 2004.
28. FORTUNE, J. E. Ovarian follicular growth and development in mammals. **Biology of Reproduction**, v. 50, n. 2, p. 225-232, 1994.
29. MIHM, M.; DISKIN, M. G.; ROCHE, J. F. Regulation of follicular growth in cattle. **Reproduction in Domestic Animals**, v. 31, n. 3, p. 531-538, 1996.
30. BUTTLER, W. R. Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. **Journal of Dairy Science**, v. 81, n. 9, p. 2533-2539, 1998.
31. CRITZER, J. K.; MILLER, K. F.; GUNSETT, F. C.; GINTHER, O. J. Seasonal LH profile in ovariectomized cattle. **Theriogenology**, v. 19, n. 2, p. 181-191, 1983.
32. BLACHE, D.; FABRE-NYS, C. J.; VENIER, G. Ventromedial hypothalamus as a target for oestradiol action on proceptivity, receptivity and luteinizing hormone surge of the ewe. **Brain Research**, v. 546, n. 2, p. 241-249, 1991.

33. CARATY, A.; SKINNER, D. C.Progesterone priming is essential for the full expression of the positive feedback effect of estradiol in inducing the preovulatory gonadotropin-releasing hormone surge in the ewe. **Endocrinology**, v. 140, n. 1, p. 165-170, 1999.

34. BILLINGS, H. J.; KATZ, L. S. Progesterone facilitation and inhibition of estradiol-induced sexual behavior in the female goat. **Hormones and Behavior**, v. 31, n. 1, p. 47-53, 1997.

35. BARTOLOMEU, C. C. **Estudo da dinâmica folicular durante o tratamento com CIDR-B e Crestar visando, a inseminação artificial em tempo fixo em fêmeas bubalinas (Bubalusbubalis)**. 2003. 123 f. Tese (Doutorado em Reprodução Animal) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2003.

36. RAO, L. V.; PANDEY, R. S. Seasonal variations in oestradiol-17 deta and luteinizing hormone in the blood of buffalo cows (Bubalusbubalis). **Journal of Endocrinology**, v. 98, n. 2, p. 251-255, 1983.

37. SKINNER, D. C.; HERBISON, A. E. Effects of photoperiod on estrogen receptor, tyrosine hydroxylase, neuropeptide Y, and β-endorphin immunoreactivity in the ewe hypothalamus. **Endocrinology**, v. 138, n. 6, p. 2585-2595, 1997.

38. HAFEZ, E. S. E.; HAFEZ, B. **Reprodução animal**. 7. ed. Barueri: Manole, 2004. p. 160.