

Physiological differences and implications to reproductive management of *Bos taurus* and *Bos indicus* cattle in a tropical environment

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In the current review the main fundamental biological differences in reproductive function between *Bos taurus* and *Bos indicus* cattle are discussed. Breed differences regarding puberty, estrous cycle patterns, estrous behavior, acquisition of ovulatory capacity, ovarian structures and reproductive hormones are presented. The main physiological differences that *Bos indicus* cattle present relative to *Bos taurus* cattle include: delayed age at puberty; higher circulating concentrations of hormones such as estradiol, progesterone, insulin and IGF-I, despite having smaller ovulatory follicle size and corpora lutea; greater population of small follicles and smaller size of the dominant follicle at deviation; and greater sensitivity of follicles to gonadotropins. Knowledge of the differences between *Bos indicus* and *Bos taurus* breeds help explain different management procedures and responses to hormonal treatments associated with artificial insemination, ovarian superstimulation, and in vivo and in vitro embryo production.

Introduction

Over the years, cattle have been raised and selected especially for meat and dairy production and the majority of breeds are either *Bos taurus* or *Bos indicus* (Zebu). Some of the *Bos taurus* breeds, such as Holstein and Jersey, have been selected for high milk production, and others for beef production. Due to intensive selection, *Bos taurus* breeds are very efficient for food production as compared to other breeds; however, they are not well adapted to tropical environments and cannot maximize their potential for production in the tropics. The main problem with *Bos taurus* cattle in the tropics is the very low tolerance to heat stress. As compared to *Bos taurus*, *Bos indicus* (Zebu) breeds experience a less severe reduction in feed intake, growth rate, milk yield and reproductive function in response to heat stress (Adeyemo *et al.* 1979; Bennett *et al.* 1985; Pegorer *et al.* 2007).

Although *Bos taurus* breeds are more precocious, Zebu cattle are extensively used in the tropics, especially in Brazil, due to their thermo tolerance and resistance to parasites. In fact, Brazil alone has nearly 200 million cattle of which 80% are Zebu or crossbred Zebu animals. The extensive systems of management which predominate in South America have continually exposed these cattle to a tropical climate and nutritional environment resulting in increased tolerance to that environment through selection pressure. This environmental tolerance means that Zebu cattle can thrive where *Bos taurus* cattle may not.

To overcome or minimize problems related to decreased production of purebred *Bos taurus* cattle in tropical regions, one strategy has been to produce *Bos taurus* X *Bos indicus* cross-breeds. Zebu cattle are known to be less fertile and have lower levels of milk production than *Bos taurus* breeds, but are better adapted to the environmental conditions which makes them more likely to reproduce successfully in the tropics. Crossbreeds incorporate the environmental adaptation of Zebu cattle and the higher production of *Bos taurus* cattle (Negussie et al. 1999) as well as the benefit of hybrid vigor.

Because most of the reproductive physiology studies in cattle have been performed with *Bos taurus* breeds in countries with a temperate climate, strategies for reproductive management that have been used in Zebu cattle were adapted from those studies and may not be ideal for *Bos indicus*. Therefore, the increase in knowledge of the physiological differences between *Bos taurus* and *Bos indicus* breeds has been useful to develop specific protocols or strategies for reproductive management to maximize production in different breeds of cattle raised in a tropical environment.

Similarities and differences in reproductive physiology of *Bos taurus* and *Bos indicus* cows and heifers

Puberty

Puberty is defined by the animal's ability to reproduce as a result of the development of hypothalamic neurons that secrete GnRH at the appropriate frequency and in sufficient quantities to release gonadotropins and promote gametogenesis. Although the first ovulatory estrus is the main marker of puberty (Rawlings et al. 2003), heifers usually only reach maximum fertility on their third estrus (Byerley et al. 1987). Increased LH pulse frequency in the circulation, which is the determining factor for sexual maturity of heifers, is due to decreased hypothalamic sensitivity to the negative feedback of estradiol (Day et al. 1987) which is influenced by genetic and environmental factors (Martin et al. 1992; Nogueira 2004).

Most heifers reared in the tropics are subjected to low quality forages and suffer from protein, energy and/or mineral deficiencies. Therefore, most of these animals cannot reach puberty until 2 years of age, while those in a temperate climate reach puberty around 9 to 12 months of age (Fajersson et al. 1991). According to Nogueira (2004), independent of nutrition, there also seems to be a genetic factor associated with puberty in Zebu cattle, since 30% of Nelore heifers (12 of 37) became pregnant at 16 months of age but there was no difference in age or weight from the group mean at first ovulation.

There are also clear differences in age at puberty between *Bos taurus* and *Bos indicus* cattle. Generally, Zebu heifers reach sexual maturity and puberty later than *Bos taurus* heifers. This was demonstrated in the study by Rodrigues et al. (2002), where the cessation of inhibition of LH pulses by estradiol occurred simultaneously with the onset of puberty in heifers of both genotypes, but it happened earlier in *Bos taurus* heifers, especially during the initial period of weight gain (16 to 18 months of age). The estimated age at puberty for Zebu in the tropics

and subtropics is between 16 and 40 months, with a mean of 25 months, i.e., 6 to 12 months later than *Bos taurus* (Abeygunawardena & Dematawewa 2004; Nogueira 2004). Moreover, smaller breeds, such as the Zebu breeds, normally reach puberty at an older age and heavier body weight than *Bos taurus* heifers (Martin *et al.* 1992; Bagley 1993). Finally, regardless of size and nutrition level, Zebu cattle are less precocious in regards to puberty because the selection pressure for puberty has been much less intensive than in *Bos taurus* cattle (Eler *et al.* 2002), even though there is a high correlation between scrotal circumference in *Bos indicus* bulls and younger age of puberty in their daughters (Forni & Albuquerque 2005; Eler *et al.* 2006). Moreover, age at first conception was highly heritable ($h^2 = 0.44$ to 0.67 ; Pereira *et al.* 2007) as was heifer pregnancy ($h^2 = 0.58$ to 0.66 ; Eler *et al.* 2006; Pereira *et al.* 2007). There was also a high genetic correlation between age at first calving and interval from the onset of breeding season to calving in the subsequent year (Forni & Albuquerque 2005). Therefore, the potential to improve age of puberty and shorten postpartum anestrus interval through selection in *Bos indicus* cattle does exist.

Estrous cycle and follicular dynamics

During the estrous cycles of heifers and cows, there is growth and regression of follicles in a wave-like pattern (Pierson & Ginther 1984; Savio *et al.* 1988). Studies in *Bos taurus* cattle have described the occurrence of two to four follicular waves during the estrous cycle, with a predominance of two waves, and very rarely four waves (Sirois & Fortune 1988; Townson *et al.* 2002; Sartori *et al.* 2004). In a study with *Bos indicus* cattle in Brazil, Figueiredo *et al.* (1997) observed two- and three-wave cycles in Nelore cattle, with the majority of cows having two (83.3%) and heifers having three (64.7%) follicular waves. Other studies in Nelore heifers (Mollo *et al.* 2007), Gir cows (Gambini *et al.* 1998, Viana *et al.* 2000), and Brahman cows (Zeitoun *et al.* 1996) have detected a predominance of three waves, but also observed animals with two, four, and even a few with five waves during a cycle.

Although, several studies evaluated estrous cycle patterns in *Bos taurus* and *Bos indicus* cattle, very few have directly and simultaneously compared these genetic groups under the same environmental and management conditions. An experiment performed in Florida compared multiparous lactating Angus (temperate *Bos taurus*; $n = 12$), Brahman (tropical *Bos indicus*; $n = 12$), and Senepol (tropical *Bos taurus*; $n = 12$) cows throughout an estrous cycle during the summer (Alvarez *et al.* 2000). In this study, the majority of Angus and Brahman cows had two follicular waves during the estrous cycle (72.7% and 55.6%, respectively) and 70% of the cycles of Senepol cows had three waves.

A study in our laboratory compared estrous cycle patterns between *Bos indicus* and *Bos taurus* cows (Bastos *et al.* 2010). From January to April 2010, 5 to 10 year old multiparous nonlactating cows ($n = 12$ Nelore and 12 Holstein cows) were managed in individual stalls and fed a total mixed maintenance ration (TMR) according to the NRC (2000). The TMR consisted of 42.8% sugar cane bagasse, 45.7% corn, 5.7% molasses, 3.2% soybean hulls, 1.2% urea, and 1.4% mineral salt. Nelore and Holstein cows had a BCS (scale from 1 to 5) of 3.1 ± 0.1 and 2.8 ± 0.2 , and a body weight of 508 ± 17 and 575 ± 20 kg, respectively. After a 14 to 21-d period of adaptation, estrus was synchronized and cows were monitored daily by ovarian ultrasonography throughout an entire estrous cycle. Moreover, during the first follicular wave, ultrasound scanning was performed twice a day. Daily blood samples from the jugular vein were also collected for hormone assays during the entire period of study, except in two occasions in which blood was collected immediately before and 4 hours after feeding during the follicular and luteal phases. In this study, 80% of Nelore cows and 60% of Holstein cows

presented three waves of follicular development during the estrous cycle. All the remaining cows had two waves. Moreover, average estrous cycle length was 23 d (range of 21 to 26 d) and was similar between Nelore and Holstein cows ($P > 0.05$).

From other data, the average interestrus interval was 21 d, for both *Bos taurus* and *Bos indicus* cattle (discussed by Bó et al. 2003 and Sartori et al. 2004), and two-wave cycles were shorter than three-wave cycles (Savio et al. 1988, 1990; Sirois & Fortune 1988; Alvarez et al. 2000; Townson et al. 2002; Sartori et al. 2004). There were, however, exceptions, in which high-producing lactating Holstein cows tended to have a longer estrous cycle (23 d), due to a prolonged time between luteolysis and ovulation (discussed by Sartori et al. 2004).

Although there are similarities in estrous cycle length and follicular wave patterns between *Bos taurus* and *Bos indicus* cattle, differences in antral follicle population between genetic groups are well documented and are illustrated in Figure 1. At the onset of each follicular wave, approximately 24 small (2 to 5 mm) viable antral follicles were detected in *Bos taurus* cattle (Ginther et al. 1996), however, in *Bos indicus* cattle, there were greater numbers of small follicles during wave emergence. Buratini Jr. et al. (2000) described the occurrence of approximately 50 small follicles in the ovaries of Nelore heifers. High numbers of small follicles were also observed in the ovaries of Sindhi (*Bos indicus*) heifers (49.0 ± 6.4 , $n = 14$) and lactating cows (64.0 ± 5.1 , $n = 34$; MCC Mattos, unpublished observations). By directly comparing number of small follicles (3 to 5 mm) in the ovaries between *Bos taurus* and *Bos indicus* cattle, Alvarez et al. (2000) observed a greater number of follicles at wave emergence in Brahman (39 ± 4) compared to Senepol (33 ± 4), or Angus (21 ± 4) multiparous lactating cows. Recent studies performed with both genetic groups in contemporary environmental and nutritional conditions showed that *Bos indicus* had a greater number of follicles at wave emergence compared to *Bos taurus* cattle (Carvalho et al. 2008; Gimenes et al. 2009). Carvalho et al. (2008) synchronized ovulation in Nelore and Angus heifers and Gir and Holstein heifers, using a progesterone intravaginal device and 2 mg of estradiol benzoate with or without PGF $_{2\alpha}$ at the beginning of the treatment. There was no interaction of breed, therefore, genetic group was combined. In this trial, *Bos indicus* cattle recruited 33.4 ± 3.2 follicles, while *Bos taurus* heifers recruited 25.4 ± 2.5 follicles ($P = 0.09$). Gimenes et al. (2009), synchronized Nelore and Holstein heifers using a norgestomet auricular implant and 2 mg of estradiol benzoate with or without a 50 mg injection of progesterone. Nelore heifers had 29.7 ± 3.1 follicles at wave emergence; whereas, Holstein heifers had 15.0 ± 2.8 follicles ($P < 0.01$). Similarly, Bastos et al. (2010) detected significant differences in number of antral follicles between Nelore and Holstein cows. At wave emergence, the number of 2 to 5 mm follicles present on the ovaries was 42.7 ± 5.9 for Nelore (range of 25 to 100) and 19.7 ± 3.2 for Holstein (range of 5 to 40) cows ($P < 0.05$). There was little variation in number of small follicles throughout the entire estrous cycle in both breeds as reported by Alvarez et al. (2000) and Buratini Jr. et al. (2000). Significant differences in ovarian follicle population between *Bos indicus* and *Bos taurus* breeds may be associated with higher circulating concentrations of insulin and IGF-I in *Bos indicus* cattle, as described by Alvarez et al. (2000) and Bastos et al. (2010).

Follicular deviation and selection of the dominant follicle

After follicular wave emergence in cattle, there is a mechanism of selection in which only one follicle becomes dominant, inhibiting the growth of other follicles on the ovary (subordinate follicles; Ginther et al. 1996). Follicular deviation has been used to refer to the time at which differences in the growth rate between the future dominant and the future subordinate follicles become apparent (Ginther et al. 1996). In *Bos taurus* breeds, such as Holstein, follicular devia-

tion occurs when the largest developing follicle reaches 8.5 to 9.0 mm in diameter (Ginther *et al.* 1996; Sartori *et al.* 2001), whereas, in Zebu cattle, such as Nelore, deviation occurs when the largest growing follicle reaches 5 to 7 mm (Castilho *et al.* 2007; Figueiredo *et al.* 1997; Sartorelli *et al.* 2005; Ereno 2008; Gimenes *et al.* 2008). There are exceptions, however, in both genetic groups of animals. When comparing high-producing lactating Holstein cows to nulliparous Holstein heifers, Sartori *et al.* (2004) observed that deviation occurred when the largest growing follicle reached 9.8 mm and 8.3 mm in lactating cows and heifers, respectively ($P < 0.05$). Likewise, Bastos *et al.* (2010) detected that follicular deviation occurred when the largest developing follicle reached 7.0 ± 0.2 and 8.9 ± 0.4 mm in nonlactating Nelore and Holstein cows, respectively ($P < 0.05$).

Although follicle size at deviation differs substantially between breeds, the time of deviation during the first follicular wave in *Bos indicus* relative to the preceding ovulation (2.3 to 2.8 d; Sartorelli *et al.* 2005; Ereno 2008; Gimenes *et al.* 2008) was similar to that reported in *Bos taurus* cattle (Ginther *et al.* 1996; Sartori *et al.* 2001). In fact, when comparing Nelore to Holstein cows, Bastos *et al.* (2010) observed that deviation occurred, on average, 2.3 d after ovulation, independent of breed.

The mechanisms that control selection of the dominant follicle are complex and not fully understood. Ovarian theca cells express LH receptors (LHR) from preantral to preovulatory sizes (Xu *et al.* 1995). Although it is clear that LHR expression occurs in granulosa cells of dominant follicles (Xu *et al.* 1995; Bao *et al.* 1997), there is some controversy about the time when these cells start to express functional LHR and whether it is required to support dominant follicle growth at the time of deviation when FSH levels are declining or low. LHR mRNA abundance measured by real-time PCR was higher in granulosa cells from the largest follicle of the wave compared with the second largest follicle before morphological deviation, suggesting that acquisition of LH responsiveness by granulosa cells is part of the deviation process (Beg *et al.* 2001). Other studies utilizing *in situ* hybridization, failed to detect LHR mRNA in granulosa cells around deviation (Evans & Fortune 1997; Fortune *et al.* 2001; Garverick *et al.* 2002), which is in agreement with previous reports showing binding of LH to granulosa cells of only selected dominant follicles (Ireland & Roche 1982; Webb & England 1982).

The LHR gene in sheep and cattle follicles is subject to alternative splicing, and variants with deletion of exon 10 and/or partial deletion of exon 11 were reported (Bacich *et al.* 1994; Abdennebi *et al.* 2002; Robert *et al.* 2003). Although functional properties of LHR splice variants have not been fully elucidated, the variant with a partial deletion of exon 11 has been shown to be translated into a protein that is not translocated to the cell membrane (Bacich *et al.* 1999; Kawate 2004), and deletion of exon 10 resulted in impaired activation by LH in comparison with hCG in humans (Gromoll *et al.* 2000; Muller *et al.* 2003). Therefore, PCR strategies amplifying exon fragments present in all transcripts should permit quantification of LH receptor expression without identifying each of the variant forms. In a recent study assessing expression of LHR alternative transcripts by PCR in granulosa cells from crossbred Nelore cows, all isoforms were only detected in follicles at 7 mm in diameter or larger, and mRNA abundance increased with follicle size (Nogueira *et al.* 2007a). In Nelore cattle, follicle deviation occurs when the dominant follicle reaches 5 to 7 mm in diameter; therefore, these results suggest that expression of functional LHR only occurs in granulosa cells after deviation. In a follow up study using the same PCR strategy, Barros *et al.* (2009) assessed LHR expression in granulosa cells from the two largest follicles in the ovary of Nelore heifers taken before (Day 2 of the wave), during (Day 2.5), and after (Day 3) the expected time of follicular deviation. LHR mRNA expression was detected only in two out of seven largest follicles obtained on Day 2.5 (both in 7 mm follicles), but not on Day 2. Moreover, the majority of the largest follicles

obtained on Day 3 expressed LHR (64% positive follicles from 8 to 14 mm). Therefore, data obtained in Nelore cattle suggest that expression of functional LHR occurs after follicular deviation and is a consequence, rather a means of selection (Part of the review above was presented at the Annual Meeting of the IETS/2010 by Barros *et al.* 2010).

Acquisition of ovulatory capacity

Although follicle deviation occurs in *Bos indicus* when the dominant follicle reaches 5 to 7 mm in diameter (Figueiredo *et al.* 1997; Sartorelli *et al.* 2005; Castilho *et al.* 2007; Gimenes *et al.* 2008; Bastos *et al.* 2010) compared to 8 to 9 mm in *Bos taurus* (Ginther *et al.* 1996; Sartori *et al.* 2001; Bastos *et al.* 2010), it is possible that additional growth is necessary for the dominant follicle to acquire ovulatory capacity in both breeds.

Sartori *et al.* (2001) observed that Holstein cows with follicles 7 or 8.5 mm in diameter did not ovulate, even after administration of high doses of pLH (40 mg). However, 80% of the cows with follicles ≥ 10 mm ovulated after pLH administration. Conversely, Gimenes *et al.* (2008) reported that administration of 25 mg pLH in *Bos indicus* heifers induced ovulation in 33.3, 80.0 and 90.0% of animals with follicles that were 7.0 to 8.4, 8.5 to 10 and > 10 mm in diameter, respectively. These results were not unexpected, and corroborate the data showing differences in size of the growing follicle at follicular deviation between *Bos indicus* and *Bos taurus* cattle.

This relationship between follicular diameter, acquisition of ovulatory capacity and gene expression of LHR isoforms, was recently investigated (Simões, 2009). In the first experiment, the minimum pLH dose (Lutropin-V, Bioniche Animal Health Inc, Belleville, ON, Canada) necessary to induce ovulation in Nelore cows that had a first wave dominant follicle of 10 to 11 mm in diameter was found to be 3.125 mg. In the second experiment, 6.25 mg of pLH was utilized to induce ovulation of first wave dominant follicles of 7 to 8.0, 8.1 to 9.0 and 9.1 to 10 mm in diameter. In a third experiment, theca and granulosa cells, obtained from abattoir-derived ovarian follicles, were separated for total RNA extraction, and gene expression of LHR isoforms was measured by semiquantitative RT-PCR using GAPDH as the internal control. Simões (2009) observed that with the increase in follicle diameter (7.0 to 8.0, 8.1 to 9.0 and 9.1 to 10.0 mm) in Nelore or crossbred Nelore females, there was a corresponding increase in ovulation rates (9.0, 36.0 and 90.0%, respectively) and expression of LHR isoforms in granulosa cells (16.5, 21.0 and 37.6; mRNA LHR/mRNA GAPDH), but not in theca cells. It was concluded that in Zebu cattle ovulatory capacity is related to an increase in follicle diameter and expression of LH receptors in granulosa cells.

Maximum size of dominant/ovulatory follicle and CL and circulating hormones

Ginther *et al.* (1989), working with Holstein females, observed that heifers with two follicular waves had dominant follicles with maximum diameters of 17.1 and 16.5 mm for the first and second wave, respectively. In contrast, in *Bos indicus* females, the diameters reported were 11.3 and 12.3 mm, respectively (Figueiredo *et al.* 1997; Sartorelli *et al.* 2005; Figure 1). Similarly, in Zebu cattle, the size of the CL ranged from 17 to 21 mm in diameter (Segerson *et al.* 1984, Rhodes *et al.* 1995, Figueiredo *et al.* 1997); whereas, in *Bos taurus* cattle diameters of 20 to 30 mm were detected (Ginther *et al.* 1989). Although there seem to be clear differences in size of ovarian structures between *Bos taurus* and *Bos indicus*, follicle size, and consequently CL size may be determined by factors other than breed.

Studies have reported that dairy cows develop larger ovulatory follicles, but have lower serum estradiol concentrations than heifers (Sartori *et al.* 2004; Wolfenson *et al.* 2004) or dry cows (De La Sota *et al.* 1993), suggesting an increased steroid metabolism in dairy cows, as reported by Sangsritavong *et al.* 2002). In relation to serum concentration of estradiol, there are few data comparing *Bos indicus* to *Bos taurus*. In the study by Alvarez *et al.* (2000), there were no differences in maximum circulating estradiol among Brahman, Angus, and Senepol cows (8.9 ± 1.6 , 9.1 ± 1.4 , and 8.7 ± 1.4 pg/mL, respectively). This seems to be the only study in which the maximum diameter of the ovulatory follicle was greater in *Bos indicus* (15.6 ± 0.5 mm) than in *Bos taurus* cattle (12.8 ± 0.4 and 13.6 ± 0.4 mm for Angus and Senepol, respectively). Conversely, Segerson *et al.* (1984) compared Angus to Brahman cattle and observed higher serum concentrations of estradiol-17 β and progesterone in *Bos taurus* cows.

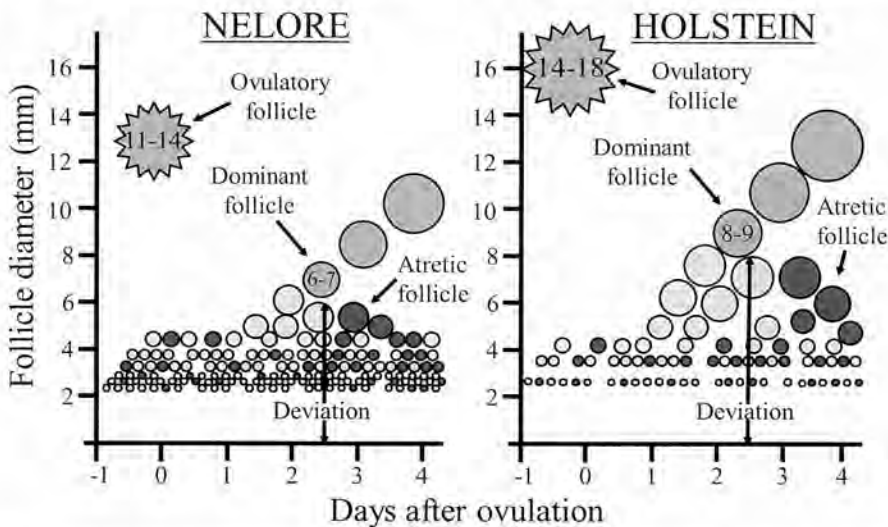


Fig. 1. Schematic representation of follicle development in Nelore (*Bos indicus*) and Holstein (*Bos taurus*) cows based on data from the literature and personal data. *Bos indicus* (Nelore) have a greater population of small (2 to 5 mm) follicles in the ovaries throughout the entire estrous cycle than *Bos taurus* (Holstein) cattle. Moreover, although both breeds present follicle deviation between Day 2 and 3 after ovulation, the diameter of the future dominant follicle at deviation is greater in Holstein cattle. The diameter of the ovulatory follicle is also greater in Holsteins than in Nelore cows.

According to Randel (1976) *Bos indicus* and *Bos indicus* crossbred females had lower progesterone concentration per gram of luteal tissue than in *Bos taurus* females. However, Segerson *et al.* (1984) did not detect differences in progesterone concentration in the CL between Brahman and Angus cows (75.8 ± 11.3 and 65.9 ± 5.3 $\mu\text{g/g}$ of CL, respectively; $P > 0.10$). Conversely, studies performed in Brazil detected higher circulating progesterone in *Bos indicus* (Nelore or Gir) compared to *Bos taurus* (Angus or Holstein) cattle. Carvalho *et al.* (2008) compared *Bos indicus* (Nelore and Gir), *Bos taurus* (Angus and Holstein) and crossbreds (Angus x Nelore and Gir x Holstein) throughout the period of an estrus synchronization protocol. Two PGF2 α treatments, 12 days apart were performed 24 and 12 days before treatment with an intravaginal source of progesterone was initiated. On Day 0, each heifer received a progesterone intravaginal device plus estradiol benzoate. During that period (with a progesterone device), serum progesterone concentrations were higher in *Bos indicus* heifers. Similarly, Bastos *et al.*

(2010) reported that although the maximum diameter of the ovulatory follicle (15.7 ± 0.3 mm vs 13.4 ± 0.3 mm; Figure 1) and the maximum CL volume (7610.5 ± 512.0 mm³ vs 4916.6 ± 548.1 mm³) were greater in Holsteins, plasma peak estradiol (7.70 ± 0.67 vs 12.71 ± 0.98 pg/mL) and progesterone (4.06 ± 0.18 vs 4.64 ± 0.40 ng/mL) concentrations were higher in Nelore cows. This suggests that *Bos indicus* females may be slower to metabolize estradiol and progesterone.

Estrous behavior

Behavioral signs of estrus can be observed in cattle and used for estrus detection for the purposes of artificial insemination (AI). However, these characteristics are influenced by age, milk yield, environment and hierarchy (revised in Landaeta-Hernández *et al.* 2004). Additionally, differences can be noted among breeds (Rae *et al.* 1999), and even among genetic groups (*Bos indicus* vs *Bos taurus*).

Mizuta (2003) compared the reproductive behavior of Nelore cows with Angus cows using radiotelemetry (Heat-Watch). Nelore cows exhibited a shorter estrus compared to Angus cows (12.9 ± 2.9 vs 16.3 ± 4.8 hours, respectively). Additionally, more than 50% of Nelore cows showed estrus during the evening and night (between 6:00 PM and 6:00 AM; Pinheiro *et al.* 1998; Membrive 2000), and about 30% exhibited estrus only during darkness (Pinheiro *et al.* 1998). These factors decrease the efficacy of estrous detection for traditional AI. However, short estrus duration has been observed in high-producing Holstein cows. In a recent study evaluating the association between levels of milk production and estrous behavior, Lopez *et al.* (2004) observed a shorter duration (6.2 versus 10.9 hours) and intensity (6.3 versus 8.8 mounts) of estrus in cows with higher (> 39.5 kg/d) compared to lower (< 39.5 kg/d) milk production. These differences within the same breed may be related to lower circulating concentrations of estradiol in higher vs lower milk producing cows (Lopez *et al.* 2004) as well as in lactating cows compared to heifers (Sartori *et al.* 2002, 2004). Based on such results, Wiltbank *et al.* (2006) hypothesized that high producing cows (above 40 kg of milk/d) have low circulating estradiol due to a high metabolism of this steroid. The effect of higher metabolism on estrus behavior was also observed in beef cattle. Nelore heifers submitted to high dietary intake exhibited less intense and shorter duration of estrus as compared with Nelore heifers receiving low dietary intake (Mollo *et al.* 2007).

Although there are differences between *Bos taurus* and *Bos indicus* in duration and intensity of estrus, other factors such as feed intake, body size, and steroid metabolism may exert profound effects over this behavior.

Tolerance to heat stress

Heat stress is a particularly severe problem in cattle reproduction because of reduction in estrus expression, lower fertilization rates, increased embryonic loss and, consequently, lower fertility. Over 50% of the bovine population is located in the tropics and it is estimated that heat stress causes severe economic loss in approximately 60% of dairy farms around the world (Wolfenson *et al.* 2000). The magnitude of this effect on reproduction in dairy cattle is increasing, as augmented milk yield enhances susceptibility of cows to the deleterious effects of heat stress (Al-Katanani *et al.* 1999; Sartori *et al.* 2002; Lopez-Gatius 2003).

Bos indicus breeds experience a less severe reduction in reproductive function in response to heat stress than *Bos taurus* breeds (Adeyemo *et al.* 1979; Bennett *et al.* 1985; Rocha *et al.* 1998; Barros *et al.* 2006). Most of this adaptation to elevated temperature is due to superior

ability of thermo-tolerant breeds to regulate body temperature (Adeyemo *et al.* 1979; Gaughan *et al.* 1999) as well as intrinsic cellular resistance to elevated temperature (Malayer & Hansen 1990; Sartorelli *et al.* 2006). High environmental temperature and humidity resulted in a marked decline in oocyte quality from Holstein and crossbred Angus cows (Rocha *et al.* 1998). In contrast, a high percentage of oocytes retrieved from Brahman cows exhibited normal morphology and yielded a high proportion of blastocysts, regardless of season (Rocha *et al.* 1998). Although *Bos indicus* cattle are more resistant to heat stress than *Bos taurus* cattle, exposure of Gir cows to a 28-d period of heat stress exerted a delayed effect on reproductive function, manifested by an increased incidence of large follicles, more follicular codominance, and reductions in estrous cycle length, progesterone concentrations, and oocyte developmental capacity (Torres-Junior *et al.* 2008).

Infertility in the male caused by heat stress can be eliminated through the use of AI with semen collected and frozen from males in cool environments. In females, transfer of in vivo or in vitro produced (IVP) embryos have been utilized as an attempt to bypass effects of heat stress (Hammond *et al.* 1996; Al-Katanani *et al.* 2002; Sartori *et al.* 2006). For example, a retrospective study was performed in high-producing Holstein cows (average milk production 28.4 ± 2.3 kg/d) submitted to ET ($n = 2112$) or AI ($n = 7501$) during the period of 2000 to 2003 (Rodrigues *et al.* 2004). Estrus was detected in cows, which were subjected to AI 12 hours later or ET 7 d later. Pregnancy rates were higher in those receiving embryos than those undergoing AI during the summer months, but no differences were observed during the cooler months.

Using *Bos indicus* genotype embryos during summer months can also improve fertility. In vitro studies have shown that *Bos indicus* embryos submitted to heat shock at early stages of development are better able to survive as compared to *Bos taurus* embryos (Malayer *et al.* 1990; Kamwanja *et al.* 1994; Barros *et al.* 2006; Sartorelli *et al.* 2006). More recently, effects of heat stress on embryonic development in culture were evaluated in Nelore and crossbred (*Bos indicus* X *Bos taurus*) oocytes fertilized with Nelore or Angus (*Bos taurus*) spermatozoa. The decrease in blastocyst development rates caused by exposure to 41°C during 12 hours was more evident when the heat shock was applied at earlier stages of development, particularly for embryos that had a predominant *Bos taurus* genotype (Erberhardt *et al.* 2009). In the study reported by Sartorelli *et al.* (2006), embryos from Angus or Nelore cows produced using oocytes obtained by ovum pickup (OPU) procedures were exposed to a culture temperature of 41°C for 12 hours beginning 96 hours after fertilization. Thereafter, embryos were transferred at the blastocyst stage to crossbred recipient heifers. Pregnancy rates after transfer were: 29.4% (15/51) for non-stressed Nelore embryos, 29.0% (11/38) for stressed Nelore embryos, 21.4% (6/28) for non-stressed Angus embryos and 7.1% (1/14) for stressed Angus embryos. These results and previous reports (Malayer *et al.* 1990; Erberhardt *et al.* 2009) clearly indicate that Nelore embryos are better able to survive during heat stress at early stages of development and more capable of establishing pregnancies following heat stress than Angus embryos.

Although heat stress induces a deleterious effect on *Bos taurus* oocytes and embryos, this sensitivity differs between categories, i.e. heifers (H), high-producing cows in peak lactation (PL) and repeat-breeders (RB; Ferreira *et al.* 2010). In this study, previously cited categories of Holstein cattle were evaluated during summer heat stress. At OPU, heifers had greater number of follicles than PL cows ($H = 18.5 \pm 1.9^a$, $PL = 12.4 \pm 1.1^b$, $RB = 17.2 \pm 2.0^{ab}$; $P = 0.04$). Heifers also had greater number of total oocytes ($H = 9.6 \pm 1.6^a$, $PL = 5.0 \pm 0.9^b$, $RB = 8.8 \pm 1.3^{ab}$; $P = 0.03$) and viable oocytes ($H = 7.6 \pm 1.5^a$, $PL = 3.6 \pm 0.8^b$, $RB = 6.8 \pm 1.2^{ab}$; $P = 0.05$) recovered at OPU than PL cows. During IVP, embryos from heifers performed better than PL and RB embryos (cleavage at Day 3: $H = 47.8\%^a$, $PL = 31.1\%^b$, $RB = 35.4\%^b$, $P = 0.008$; blastocyst at Day 7: $H = 21.0\%^a$, $PL = 4.1\%^b$, $RB = 3.8\%^b$, $P < 0.0001$; and grade I embryos: $H = 1.3 \pm 0.4^a$, $PL = 0.3 \pm 0.2^b$, $RB = 0.5 \pm 0.2^b$, $P = 0.04$). The differences

observed among heifers and cows are probably related to their metabolism under heat stress, comprising oocyte number and quality. Also, although RB had similar results at OPU (number of oocytes) as heifers, they probably had compromised oocytes, because their *in vitro* production or performance was poorer.

Reproductive management

Use of Bos indicus genotype

The utilization of thermo tolerant breeds has been employed by the beef industry in countries with a hot climate. However, the short duration of estrus in Zebu cattle is one of the major factors that limit the widespread use of AI in these breeds. This problem can be overcome with the use of fixed-time artificial insemination (FTAI) protocols, developed or adapted specifically for these cattle. Additionally, *in vivo* and *in vitro* embryo production has been increasing in tropical countries like Brazil, particularly in the Nelore breed (Viana & Camargo 2007).

Fixed-time artificial insemination (FTAI)

Although AI is a good alternative to introduce *Bos taurus* genetics into a *Bos indicus* herd, its traditional use limits the widespread application and success of this breeding technology. Factors already mentioned, such as short duration of estrus and estrus expression during the night reduce the estrus detection rate and consequently decrease pregnancy success. Postpartum cyclicity is influenced primarily by suckling and poor nutrition which are difficult to overcome in *Bos indicus* cattle. Suckling results in inhibition of GnRH/LH pulse frequency and reduces follicular development (reviewed by Willians *et al.* 1996). Poor nutrition delays the development of large ovarian follicles in postpartum cows reducing the maximum diameter and persistence of dominant follicles (reviewed by Bó *et al.* 2003).

For these reasons, FTAI has become a tool for increasing the efficiency of AI, since it eliminates the need for estrus detection. Estradiol and progestogen/progesterone treatments have been used increasingly over the past years in estrus synchronization programs in cattle (Macmillan & Peterson 1993; Macmillan & Burke 1996). This association promotes regression of antral follicles and the emergence of a new follicular wave, on average, 4 d after the beginning of the protocol, depending on the estradiol source utilized (reviewed in Bó *et al.* 2003). However, the successful application of AI in *Bos indicus* cattle, not only has to overcome the problem of estrus detection but also the problem of nutritionally and suckling-induced anestrus. One alternative to increase pregnancy rates in FTAI programs in anestrus *Bos indicus* cattle may be the addition of 300 to 500 IU of equine chorionic gonadotropin (eCG) at the time of removal of the progesterone releasing devices (Macmillan & Peterson 1993; Macmillan & Burke 1996; Baruselli *et al.*, 2004).

Other differences between *Bos indicus* and *Bos taurus* cattle emphasize the need for different strategies to manipulate the estrous cycle. Carvalho *et al.* (2008) observed that *Bos indicus* heifers can maintain higher circulating progesterone concentrations than *Bos taurus* or cross-bred heifers. Therefore, these authors hypothesized that *Bos indicus* heifers are more sensitive to high concentrations of circulating progesterone, which may have a suppressive effect on LH pulsatility and, consequently, reduce follicular growth during a synchronization protocol. In this study, the administration of PGF2 α at the beginning of a synchronization protocol in the *Bos indicus* protocol decreased circulating progesterone concentrations during treatment and increased follicular growth rate, dominant follicle diameter and ovulation rate. Another alternative is the use of norgestomet implants in *Bos indicus* heifers instead of progesterone

devices, because of a reduced suppressive effect of norgestomet on LH pulsatility (Kojima *et al.* 1992). In a previous study of follicular dynamics using cyclic Nelore heifers treated with a norgestomet ear implant or progesterone-releasing intravaginal device (CIDR), follicular growth, maximum diameter of the dominant follicle, and ovulation rate were greater in norgestomet-treated heifers (Sá Filho *et al.* 2005).

Treatments to induce multiple ovulations

Barros & Nogueira (2001) examined the efficacy of different superstimulatory protocols in which the expected time of ovulation was postponed by 6 to 12 hours and ovulation was induced by administration of LH or GnRH (Barros & Nogueira 2001; Nogueira *et al.* 2002). Although these protocols did not significantly increase the quantity of viable embryos compared to estrus detection protocols, it was possible to control the time of ovulation with hormonal treatments, allowing the use of FTAI. From these experiments, a new protocol was developed called the P-36 protocol (Barros & Nogueira 2005). The protocol included insertion of a CIDR for 36 hours after PGF2 α administration and induction of ovulation with exogenous LH, administered 12 hours after CIDR removal (48 hours after PGF2 α administration). FTAI was performed 12 and 24 hours later since ovulation occurs between 24 and 36 hours after LH administration (Nogueira & Barros, 2003). The effectiveness of the P-36 protocol has been confirmed (Barros & Nogueira 2001, 2005; Baruselli *et al.* 2006), and more recently, an average of 13.3 ± 0.75 total structures and 9.4 ± 0.63 viable embryos, with a viability rate of 71.0% (1279/1807) following 136 embryo collections in Nelore cows has been reported (Nogueira *et al.* 2007b). These results were comparable to those reported in studies in which Nelore cows were inseminated 12 and 24 hours after onset of behavioral estrus (Nogueira *et al.* 2002; Nogueira & Barros 2003).

A variation of the P-36 protocol in which the progesterone device is removed 24 hours after PGF2 α (protocol P-24) and LH is administered 24 hours later (48 hours after PGF2 α), has been utilized in Nelore females, apparently with comparable results to those obtained with P-36 protocol (Zanenga *et al.* 2003; Baruselli *et al.* 2006).

The use of the P-36 protocol in *Bos taurus* breeds has resulted in a decrease in the number of viable embryos in comparison with conventional protocols with estrus detection. In Holstein (Martins *et al.* 2005; Rodrigues *et al.* 2005; Baruselli *et al.* 2006) and Angus donors (Bó *et al.* 2006; Chesta *et al.* 2007), viable embryo production was increased with the P-36 protocol when the ovulation induction treatment (LH or GnRH) was administered at 60 hours (P-36/LH60), rather than 48 hours (P-36/LH48) after PGF2 α administration. Similarly, the results obtained by Barcelos *et al.* (2006), in Bonsmara donors (5/8 Afrikaner and 3/8 Hereford/Shorthorn) indicated that the P-36/LH60 protocol was more efficacious.

On the other hand, even though delaying ovulation for 12 hours in the P-36 protocol in *Bos taurus* breeds (P-36/LH60 protocol) had positive effects on embryo production, the opposite occurred when used in *Bos indicus* breeds. The P-36/LH60 protocol caused a decrease in embryo production when compared to P-36/LH48 protocol (Baruselli *et al.* 2006). Therefore, it can be inferred that ovulation in superstimulation protocols must be induced earlier in *Bos indicus* donors; whereas, in *Bos taurus* donors, it seems necessary to delay treatment with an ovulation inducer, thereby allowing an increase in follicle size, and acquisition of LH receptors.

Bos indicus breeds have a reduced capacity for LH secretion and a greater sensitivity to exogenous gonadotropins than *Bos taurus* cattle (Randel 1984). Superovulatory response was evaluated in Nelore cows submitted to three different doses of Follitropin-V (100, 133 or 200 mg) in a crossover design. There were no significant differences in any of the variables evaluated, indicating that it is possible to reduce the dose of FSH to 100 mg in Nelore cows

submitted to a FTAI superstimulatory protocol, without compromising superovulatory response and embryo production (Baruselli et al. 2006). Others studies in Nelore heifers successfully induced superovulation and embryo production using a smaller dose of FSH (70 mg; Sartori et al. 2009), which is very unlikely to be effective in *Bos taurus* cattle.

In vitro embryo production

Especially due to the greater antral follicle population in *Bos indicus* cattle in relation to *Bos taurus* cattle, *in vitro* embryo production is much more successful in *Bos indicus* cattle (Viana & Camargo, 2007). In a recent study, (Pontes et al. 2010) Gir, Holstein and crossbreds (1/4 Holstein x 3/4 Gir or 1/2 Holstein-Gir) were compared for total and viable (good and regular quality grades according to Seneda et al. 2001) oocyte yield, and *in vitro* embryo production. The number of total and viable oocytes, and embryos produced were greater in Gir than in Holstein cattle (17.1 vs 11.4; 12.1 vs 8.0; 3.2 vs 2.2, respectively). Moreover, embryo production (5.5 blastocysts) was even greater in Holstein-Gir crossbreds compared to the other breeds.

A recent study was conducted comparing *Bos indicus* and *Bos taurus* cattle in which Nelore and Holstein heifers were submitted to a synchronization protocol to induce the emergence of a new follicular wave. Follicles were aspirated 1, 3 or 5 d after expected wave emergence (Gimenes et al. 2010). There was no effect of time of OPU on the variables evaluated. However, more oocytes were recovered and more embryos were produced from Nelore than Holstein heifers, as demonstrated in Table 1.

Table 1. Effect of genetic group on oocyte recovery and quality, and developmental competence of *Bos indicus* (Nelore) and *Bos taurus* (Holstein) heifers.

	GENETIC GROUP	
	Nelore (n = 9)	Holstein (n = 9)
Number of Replicates	6	6
OOCYTE RECOVERY AND QUALITY		
Visualized follicles	41.0 ± 2.1 ^a	22.1 ± 1.3 ^b
Total oocytes	37.1 ± 2.6 ^a	15.4 ± 1.2 ^b
Recovery rate (%)	82.3 ± 2.5 ^a	66.8 ± 2.8 ^b
Oocytes submitted to IVC	25.6 ± 1.8 ^a	9.1 ± 0.9 ^b
DEVELOPMENTAL COMPETENCE		
Cleaved structures	21.1 ± 1.6 ^a	5.2 ± 0.5 ^b
Cleavage rate (%)	82.6 ± 1.6 ^a	59.9 ± 3.6 ^b
Blastocysts 7 d after IVF	7.3 ± 0.9 ^a	1.1 ± 0.2 ^b
Blastocyst rate (%)	28.3 ± 2.8 ^a	14.1 ± 2.9 ^b

^{a,b}P < 0.05.

Similar results were found in another recent study (Sales et al., 2010). Oocyte quality and quantity of nonlactating cattle was evaluated. In this study, 14 Gir and 14 Holstein cows were submitted to eight successive OPU sessions performed every 14 d. Gir cows had a greater number and better quality of oocytes recovered by OPU than Holstein donors (Table 2).

Ovarian superstimulation with gonadotropins has been reported to increase the number of oocytes retrieved by OPU as compared to nonstimulated *Bos taurus* cattle (Blondin *et al.*, 1997, 2002). However, this beneficial effect of superstimulation has not been confirmed in *Bos indicus* females (Seneda *et al.* 2004; Monteiro *et al.* 2009).

Evidence suggests that when *Bos taurus* cattle experience a "coasting" period (i.e. deprivation of FSH to induce follicle atresia) between hormonal stimulation and slaughterhouse ovary collection (Goodhand *et al.* 1999) and from ovary collection to oocyte aspiration (Blondin *et al.* 1997) in vitro developmental potential of cumulus-oocyte complex (COC) is effected. In both situations, follicles driven into phases of pseudodominance or early atresia provided COC with an ideal environment in which to acquire developmental competence (Blondin *et al.* 2002). A very high blastocyst rate (80%) was reported by Blondin *et al.* (2002) after superstimulating Holstein heifers with FSH and delaying COC retrieval by OPU using a coasting period of 48 hours. Additionally, LH was administered 6 hours before OPU in an attempt to render the COC more competent. Recently, the same protocol proposed by Blondin *et al.* (2002) was simultaneously compared to other protocols used for OPU and in vitro embryo production (IVP) in Nelore cattle (Monteiro *et al.* 2009). Their results indicate that ovarian superstimulation associated with deprivation of FSH and OPU did not increase in vitro embryo production in Nelore cattle. On the contrary, the highest rate of hatched blastocysts was observed in oocytes from nonstimulated cows.

Table 2. Effect of genetic group on oocyte recovery and quality of nonlactating *Bos indicus* (Gir) and *Bos taurus* (Holstein) cows.

	GENETIC GROUP	
	Gir (n = 14)	Holstein (n = 14)
Number of Replicates	8	8
Visualized follicles	25.5 ± 1.2	23.8 ± 1.1
Total oocytes	23.4 ± 1.6 ^a	14.9 ± 0.9 ^b
Recovery rate (%)	91.2 ^a (2604/2856)	61.1 ^b (1633/2673)
Oocyte quality		
Grade 1	5.3 ± 0.5 ^a	1.6 ± 0.2 ^b
Grade 2	9.8 ± 0.7 ^a	5.2 ± 0.4 ^b
Grade 3	4.8 ± 0.5	4.3 ± 0.4
Grade 4	0.9 ± 0.2	1.0 ± 0.2
Apoptosis by TUNEL (%)	16.6 ^b (21/117)	40.6 ^a (34/82)

^{a,b}P < 0.05.

Further experiments are necessary to understand why Nelore cattle did not respond as well as *Bos taurus* breeds to the protocol proposed by Blondin *et al.* (2002). It may be necessary to adjust FSH dosage and the "coasting" period to obtain better results in *Bos indicus* cattle. On the other hand, perhaps the fact that Nelore females usually have a larger number of follicles available for OPU precludes the use of superstimulatory protocols that might be useful for animals with lower number of follicles (*Bos taurus*).

Final considerations

Bos indicus and *Bos taurus* cattle have been mainly used for milk and beef production all over the world. Although, in general, *Bos taurus* cattle have been more intensely selected for production, a better adaptation to the tropical and sub-tropical environments makes *Bos indicus* cattle and crossbreds feasible options for production.

There are, however, significant differences in the reproductive physiology of those genetic groups, as seen in Table 3, that affect the application of adequate tools for reproductive management. For example, *Bos taurus* in general reach puberty sooner and have a shorter gestation length as compared to *Bos indicus* cattle (Paschal et al, 1991). Therefore, in order to have a 12 month calving interval, *Bos indicus* cows must conceive 10 d earlier than *Bos taurus* cattle.

Table 3. Main differences on reproductive physiology of *Bos taurus* and *Bos indicus* females.

Physiology	<i>Bos indicus</i>	References	<i>Bos taurus</i>	References
Age at puberty (months)	16 to 40	Abeygunawardena & Dematawewa (2004) Nogueira (2004)	9 to 12	Fajersson et al. (1991)
Duration of estrus (hours)	10,9 to 12,9	Pinheiro et al. (1998) Mizuta (2003)	16,3	Mizuta (2003)
Number of small follicles	30 to 60	Alvarez et al. (2000) Buratini Jr. et al. (2000) Carvalho et al. (2008) Gimenes et al. (2009) Bastos et al. (2010)	15 to 33	Ginther et al. (1996) Alvarez et al. (2000) Carvalho et al. (2008) Gimenes et al. (2009) Bastos et al. (2010)
Diameter of dominant follicle at deviation (mm)	5.4 to 7.0	Sartorelli et al. (2005) Castilho et al. (2007) Ereno et al. (2008) Gimenes et al. (2008) Bastos et al. (2010)	8.3 to 9.8	Ginther et al. (1996) Sartori et al. (2001) Sartori et al. (2004) Bastos et al. (2010)
Diameter of largest subordinate follicle at deviation (mm)	5.3 to 5.9	Sartorelli et al. (2005) Castilho et al. (2007) Gimenes et al. (2008)	7.2	Ginther et al. (1996)
Diameter related to acquisition of ovulatory capacity (mm)	7.0 to 8,5	Gimenes et al. (2008)	10.0	Sartori et al. (2001)
Diameter of ovulatory follicle (mm)	11.3 to 14.0	Figueiredo et al. (1997) Sartorelli et al. (2005) Mollo et al. (2007)	13.9 to 17.1	Ginther et al. (1989) Sartori et al. (2004)
Diameter of corpus luteum (mm)	17 to 21	Segerson et al. (1984) Rhodes et al. (1995) Figueiredo et al. (1997) Bastos et al. (2010)	20 to 30	Ginther et al. (1989) Kastelic et al. (1990) Bastos et al. (2010)

Differences in estrus behavior and ovarian function also make some adjustments of reproductive management necessary, such as the use of FTAI in postpartum cows. These strategies are also very useful for high milk-producing cows, due to their lower circulating estradiol. Moreover, the greater antral follicle population and sensitivity to gonadotropins in *Bos indicus* cattle, make the use of superovulation much more affordable than in *Bos taurus* cattle especially in vitro situations. Finally, greater sensitivity to the negative feedback of steroid hormones on the hypothalamus/pituitary makes a dose reduction necessary during hormone treatments in *Bos indicus* cattle.

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