



Endocrine and metabolic differences between *Bos taurus* and *Bos indicus* cows and implications for reproductive management

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Abstract

Based on the considerable differences in ovarian morphology and function, as well as circulating hormones and metabolites between *Bos indicus* (*B. indicus*) and *Bos taurus* (*B. taurus*), researchers are using this acquired knowledge to optimize protocols for fixed-time artificial insemination (FTAI), and production of *in vivo* derived embryos by multiple ovulation or by *in vitro* embryo production (IVP). In *B. indicus*, at the time of follicle deviation, the dominant follicle is smaller and acquires ovulatory capacity at a smaller diameter than *B. taurus*. Moreover, despite ovulating smaller follicles and having smaller corpora lutea (CL), circulating concentrations of estradiol (E2) and progesterone (P4) are greater in *B. indicus* than *B. taurus*. These physiological differences may be related to greater circulating cholesterol, insulin and IGF1 in *B. indicus* than in *B. taurus*. For both genetic groups there is a negative relationship between circulating P4 and ovulatory response to the first GnRH treatment of a fixed-time AI (FTAI) protocol. Moreover, despite lower clearance rates of steroid hormones in *B. indicus* than *B. taurus*, the dose of 2 mg estradiol benzoate seems to be the most effective either for Nelore (*B. indicus* beef), Angus (*B. taurus* beef), or Holstein (*B. taurus* dairy) cows at the initiation of an E2/P4-based FTAI protocol to optimize synchronization and pregnancy per AI (P/AI). Several studies have shown that only one recommended dose of PGF2 α at a FTAI protocol may be insufficient for adequate luteolysis in *B. indicus* and *B. taurus*. When submitted to multiple ovulation and embryo transfer, *B. indicus* cows and heifers need less FSH than *B. taurus* to achieve superovulation. Moreover, IVP has been more successful in *B. indicus* than *B. taurus* due to greater antral follicle count and anti-mullerian hormone, and better oocyte quality.

Keywords: artificial insemination, embryo transfer, Holstein, hormone, metabolism, Nelore.

Introduction

Recent studies have gathered a great deal of knowledge on the reproductive physiology of *Bos indicus* (*B. indicus*) heifers and cows, especially by performing direct comparisons with *Bos taurus* (*B. taurus*) under similar environmental, nutritional, and management conditions. Those studies have identified considerable differences in ovarian morphology and

function, as well as circulating hormones and metabolites between these two genetic groups.

Currently, researchers are using this acquired knowledge to optimize protocols for fixed-time artificial insemination (FTAI), and production of *in vivo* derived (IVD) embryos by multiple ovulation or by *in vitro* embryo production (IVP). Accordingly, this manuscript is divided into sections that describe: 1) Differences in reproductive physiology between *B. indicus* and *B. taurus*, 2) Practical implications of the physiological differences between *B. indicus* and *B. taurus* for FTAI protocols, and 3) Practical implications of the physiological differences between *B. indicus* and *B. taurus* for embryo production.

Because Nelore (*B. indicus*) and Holstein (*B. taurus*) are among the principal beef and dairy cattle breeds used in Brazil, respectively, they are the most representative of the studies discussed in this article.

Differences in reproductive physiology between *B. indicus* and *B. taurus*

During the last decade, there was a substantial increase in studies that compared reproductive physiology variables between *B. indicus* and *B. taurus*. Although below we present a summary of those results, and a compilation of data represented in Fig. 1 and Table 1, the aim of this manuscript is not to extensively describe those data. Therefore, for detailed information, we suggest that the reader consult the publications cited in this review article.

The average ovarian antral follicle count (AFC) in *B. indicus* is twice the number observed for *B. taurus* (Fig. 1; Table 1). For example, at wave emergence, the number of 2 to 5 mm follicles present in the ovaries was 42.7 (ranging from 25 to 100) in Nelore and 19.7 (ranging from 5 to 40) in Holstein cows (Bastos *et al.*, 2010). Greater AFC has been associated with greater circulating anti-mullerian hormone (AMH) in *B. indicus* as compared to *B. taurus* (Baldrihi *et al.*, 2014; Batista *et al.*, 2014). Another observed difference between *B. taurus* and *B. indicus* was the size of largest follicle at deviation. In Holstein cattle, the diameter of the future ovulatory follicle at the time of deviation was between 8.3 and 9.8 mm (Ginther *et al.*, 1996; Sartori *et al.*, 2004; Bastos *et al.*, 2010). In Nelore heifers, deviation happened when the largest follicle reached 5.4 to 6.2 mm (Sartorelli *et al.*, 2005; Gimenes *et al.*, 2008), and in nonlactating Nelore cows between 7.0 and 7.4 mm of

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diameter (Bastos *et al.*, 2010; Sartori *et al.*, 2016). Nevertheless, the time after ovulation or after wave emergence for follicle deviation was similar between *B. taurus* and *B. indicus* (Sartori *et al.*, 2010a, 2016; Fig. 1)

due to a slower growth rate of the follicle in Nelore (0.8 to 1.2 mm/d; Gimenes *et al.*, 2008; Sartori and Barros, 2011) than Holstein cattle (1.2 to 1.6 mm/d; Sartori *et al.*, 2001).

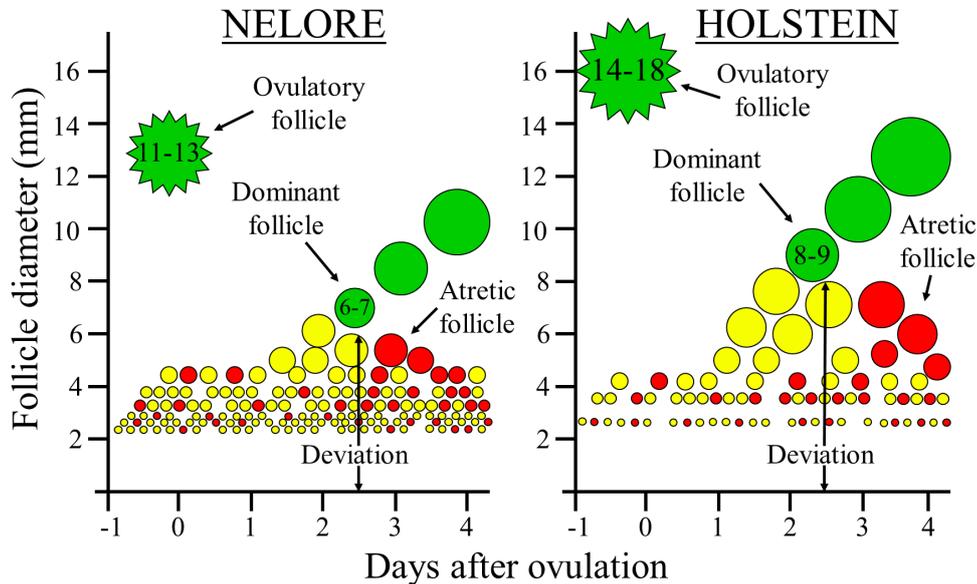


Figure 1. Schematic representation of follicle development in Nelore (*B. indicus*) and Holstein (*B. taurus*) cows based on data from the literature and personal data. Nelore have a greater population of small (2 to 5 mm) follicles in the ovaries throughout the entire estrous cycle than Holstein cattle. Moreover, although both breeds have 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 Holstein than Nelore cows (From Sartori *et al.*, 2010a).

Although follicle deviation occurs in *B. indicus* when the dominant follicle reaches 5 to 7.5 mm in diameter compared to 8 to 10 mm in *B. taurus*, 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 *B. 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.

Diameter of the largest ovulatory follicle and of the corpus luteum (CL) also differs between *B. taurus* and *B. indicus*. *B. taurus* have larger diameter of ovulatory follicle (Fig. 1) and greater CL volume than *B. indicus*. However, circulating concentration of steroid hormones in *B. taurus* are lower than in *B. indicus* (Sartori and Barros, 2011; Sartori *et al.*, 2016). In studies with Nelore cattle, the diameter of ovulatory follicle was between 11 and 14 mm (Figueiredo *et al.*, 1997; Sartorelli *et al.*, 2005; Sartori *et al.*, 2016), and the maximum diameter of the ovulatory follicle in Holstein cattle was between 13 and 19 mm (Ginther *et al.*, 1989; Sartori *et al.*, 2002a, 2004). Similarly, the CL diameter of *B. indicus* ranged from 17 to 21 mm or ~ 2.5 to 5.0 cm³ (Segerson *et al.*, 1984; Rhodes *et al.*, 1995; Figueiredo *et al.*, 1997; Bó *et al.*, 2003; Sartori *et al.*,

2016), whereas the CL diameter of *B. taurus* ranged from 20 and 30 mm or ~ 4.0 to 14.0 cm³ (Ginther *et al.*, 1989; Bó *et al.*, 2003; Sartori *et al.*, 2004, 2016).

Despite having larger follicles and CL, *B. taurus* have lower circulating steroid hormone concentrations. For example, we have shown that non-lactating Holstein cows had larger ovulatory follicle diameter (14.2 vs. 12.9 mm) and CL volume (5.2 vs. 3.9 cm³) than non-lactating Nelore cows (Sartori *et al.*, 2016); however, Holstein cows had a lower preovulatory peak of circulating estradiol (E₂; 12.5 vs. 16.2 pg/ml) and circulating progesterone (P₄) concentrations (1.9 vs. 2.7 ng/ml) on day 7 of the estrous cycle compared with Nelore cows.

This inverse relationship between size of ovarian structures and circulating hormone steroids may be related to the reported differences in circulating insulin, IGF1, and cholesterol between breeds. Most of the studies have described greater circulating insulin and IGF1 in *B. indicus* than in *B. taurus* (Alvarez *et al.*, 2000; Sartori *et al.*, 2010b, 2013). Moreover, it has also been reported that, under similar nutrition management conditions, Nelore heifers have $\sim 60\%$ greater circulating cholesterol (precursor for the biosynthesis of steroid hormones) than Holstein heifers (196.8 vs. 123.5 mg/dl; Gandra *et al.*, 2011). The potential effects of metabolic hormones or other substances on either different clearance rates of steroid hormones or E₂ and P₄ production by ovarian structures are discussed by Sartori *et al.* (2016).

Table 1. Comparison of reproductive variables between *B. indicus* and *B. taurus*.

	<i>B. indicus</i> vs. <i>B. taurus</i>
Estrous cycle length	=
Day of luteolysis	≤
Number of waves per cycle	>
Ovarian antral follicle count	>
Circulating AMH	>
Size of dominant follicle at deviation	<
Day of deviation after ovulation	=
Growth rate of dominant/ovulatory follicle	<
Maximum size of dominant/ovulatory follicle	<
Estradiol peak preceding ovulation	>
Duration of estrus	≤
Luteal tissue volume	<
Circulating progesterone	>
Steroid hormones clearance rate	<
Circulating insulin	>
Circulating IGF1	>
Circulating FSH	≤
Circulating cholesterol	>

*From Segerson *et al.* (1984); Alvarez *et al.* (2000); Bó *et al.* (2003); Carvalho *et al.* (2008); Gimenes *et al.* (2008); Sartori *et al.* (2010, 2013, 2016); Sartori and Barros (2011); Baldryghi *et al.* (2014); Batista *et al.* (2014).

Practical implications of the physiological differences between *Bos indicus* and *Bos taurus* for FTAI protocols

Artificial insemination is an important tool for genetic improvement. However, suboptimal estrus detection rates in cycling cows (Pinheiro *et al.*, 1998; Lopez *et al.*, 2004; Sartori and Barros, 2011; Fricke *et al.*, 2014) and a substantial percentage of postpartum cows that are not cycling (Wiltbank *et al.*, 2002; Meneghetti *et al.*, 2009; Santos *et al.*, 2009), produce the problem of low service rates (SR) in *B. indicus* and *B. taurus*. Thus, AI programs based on estrus detection may have lower reproductive efficiency and less cost-benefit than FTAI programs (Ribeiro *et al.*, 2012).

The FTAI program allows for a large number of cows to be inseminated on the same day, with high reproductive efficiency and relatively low cost. Nevertheless, the success of the FTAI program depends on several factors, such as synchronization of follicular waves to optimize the period of follicular dominance in order to not ovulate too old (Cerri *et al.*, 2009) or too young (Vasconcelos *et al.*, 2001) of a follicle; synchronization of corpus luteum (CL) function and circulating P4; complete luteolysis at the end of the protocol; and synchronization of final ovulation with optimally scheduled FTAI.

Synchronization of emergence of follicle wave

There are two main methods for follicle wave emergence synchronization used in dairy and beef cattle: GnRH-based protocols, in which in the presence of a dominant follicle, GnRH induces ovulation and a new follicle wave starts (Pursley *et al.*, 1995), or E2/P4-based protocols, that induces regression of all follicles present in the ovaries and a new wave starts within the following days, depending on the E2 ester used (Bó *et al.*, 2003). In GnRH-based programs in dairy cattle, about 50 and 70% of the cows have a new wave

properly synchronized by the Ovsynch and Double-Ovsynch programs, respectively (Giordano *et al.*, 2012a). In E2/P4-based program in dairy cows about 70% of the cows have a new wave properly synchronized (Monteiro Jr *et al.*, 2015a).

Effectiveness of GnRH-based protocols for follicle wave emergence in *B. taurus* and *B. indicus*

There are several factors that influence the proportion of cows ovulating to a GnRH/LH surge at the initiation of a FTAI protocol, such as day of the estrous cycle (Vasconcelos *et al.*, 1999) or follicle diameter (Sartori *et al.*, 2001; Gimenes *et al.*, 2008). Although GnRH-based protocols have been successfully used in *B. taurus* cows (Pursley *et al.*, 1995, 1997; Bridges *et al.*, 2008), but not necessarily in *B. taurus* heifers (Pursley *et al.*, 1995, 1997), there are conflicting data regarding their effectiveness in *B. indicus* cows (Fernandes *et al.*, 2001; Bó *et al.*, 2003; Baruselli *et al.*, 2004; Ferraz Jr *et al.*, 2016). Below, we describe results of experiments that may explain why GnRH-based protocols may have had suboptimal outcomes in the studies cited above.

Because it has been shown that *B. taurus* did not have consistent ovulation to a GnRH treatment even in the presence of a dominant follicle >10 mm in diameter (Martinez *et al.*, 1999; Perry and Perry, 2009), factors other than follicle size, such as circulating concentration of P4, may affect ovulation to GnRH. For example, a study (Biehl *et al.*, 2013) compared ovulatory response to the first GnRH (100 µg gonadorelin) treatment of the 5 days CO-Synch+CIDR program in Nelore heifers. The treatments were: HiP4 (n = 62; GnRH in heifers with a CL present at P4 device insertion); LoP4 (n = 35; GnRH in heifers with no CL present at P4 device insertion); and PGF-LoP4 (n = 65; GnRH in heifers that had a CL but were treated with 25 mg of dinoprost [PGF] 2 days before GnRH treatment and P4 device insertion). Ovulation to GnRH was



greater for LoP4 (85.7%) and PGF-LoP4 (95.4%) compared with the HiP4 (25.8%). Melo *et al.* (2016) have also shown a negative correlation between circulating P4 and ovulatory response to GnRH. Lack of ovulation to GnRH is likely related to the negative relationship between circulating concentration of P4 and magnitude of the GnRH-induced LH surge, which has been demonstrated in *B. taurus* heifers or cows (Colazo *et al.*, 2008; Perry and Perry, 2009; Giordano *et al.*, 2012a). For example, treatment with 100 µg of GnRH in the presence of high vs. low P4 resulted in a much lower peak of the LH surge (3.3 vs. 15.7 ng/ml). Nevertheless, greater doses of GnRH, even in the presence of elevated P4, can result in a greater magnitude of the LH surge and greater ovulation (Giordano *et al.*, 2013). Moreover, it is possible that different analogs of GnRH may produce different outcomes, because it has been reported, for example, that gonadorelin induced a lower LH surge compared with other analogs of GnRH, such as lecorelin, fertirelin, or busorelin (Chenault *et al.*, 1990; Picard-Hagen *et al.*, 2015). Although the magnitude of a GnRH-induced LH surge in the presence of low vs. high P4 has not yet been tested in *B. indicus*, it seems likely that this problem may underlie some of the observed lack of ovulation to GnRH treatment in these cattle.

Thus, GnRH-based protocols are likely to have lower synchronization of the first follicular wave when they are initiated in the presence of a CL, primarily due to lack of ovulation of a dominant follicle at the initiation of the protocol. This is clearly related to the greatly reduced magnitude of the LH surge induced by GnRH in the presence of elevated P4. In addition, due to natural growth and regression of follicular waves during the luteal phase there are many times when an LH-responsive dominant follicle is not present, and therefore ovulation would not occur even with an optimized LH surge. Further, the presence of elevated P4 may reduce LH receptor expression in the dominant follicle and this could reduce responsiveness to an LH surge in dominant follicles (Dias *et al.*, 2014). In spite of reduced ovulation to GnRH in the presence of elevated P4, and therefore reduced follicular wave synchronization, fertility is generally relatively high in cows that are given the first GnRH of an Ovsynch protocol in the presence of elevated P4.

In contrast, in a low P4 environment, GnRH induces ovulation in a high proportion of lactating dairy cows (Gumen *et al.*, 2003). A low P4 environment would be expected in anovular cows or cows in the proestrous phase of the estrous cycle. In these cows, ovulation to the GnRH treatment is essential for optimal fertility, as cows with low P4 at the initiation of the protocol that did not have ovulation to GnRH had greatly reduced fertility (Giordano *et al.*, 2012b). Comparisons of the use of EB (2 mg) vs. GnRH at the beginning of a FTAI program have reported variable results. For example, Pereira *et al.* (2013a) compared the 5-days Cosynch protocol, a GnRH-based protocol, to an E2/P4 protocol in Holstein cattle (n = 1,190) during the summer. They reported better synchronization rate for the GnRH compared to the E2 protocol (78.2 vs. 70.7%; P = 0.02). However, using only synchronized cows, percentage pregnant/AI (P/AI) was not different at the 32 days pregnancy diagnosis but

was lower for GnRH than E2 at the 60 days pregnancy diagnosis (17.7 vs. 25.6%; P = 0.03) due to greater pregnancy loss for GnRH than E2-treated cows (21.7 vs. 6.7%; P = 0.01). In contrast, in high-producing dairy cows (n = 1,035), Melo *et al.* (2016) reported that, even with a low ovulation rate to GnRH (gonadorelin; ~ 35%) at the beginning of the protocol, cows that received GnRH tended to have greater P/AI than those receiving EB at the start of the protocol (38.2 vs. 33.7; P = 0.07).

Many GnRH-based protocols in *B. taurus* now use presynchronization strategies to assure that cows are at a stage of the estrous cycle that optimizes ovulation to GnRH. For example, Double Ovsynch uses an Ovsynch protocol to presynchronize the cows so that almost all cows (~94%; Herlihy *et al.*, 2012) are cycling and at day 6 or 7 of the estrous cycle at the start of the breeding Ovsynch protocol. This produces a high ovulation rate to GnRH and high fertility during the protocol. These presynchronization strategies have not been adequately tested in *B. indicus* but are likely to be too complicated for most *B. indicus* management systems. Unfortunately, studies have not yet been performed that directly tested the ovarian responses and fertility of *B. taurus* vs. *B. indicus* in response to the same GnRH-based protocols.

Effectiveness of E2/P4-based protocols for follicle wave emergence in B. taurus and B. indicus

Early studies that established the physiological basis for these protocols utilized beef heifers and found that treatment with different esters of E2 simultaneously with P4 treatment led to suppression of the gonadotropins, regression of growing follicles, and emergence a new follicular wave about 4 days after E2 treatment (Bo *et al.*, 1993, 1994, 1995). Although E2/P4-based FTAI protocols are widely used in *B. taurus* and *B. indicus* (Martinez *et al.*, 2000; Ayres *et al.*, 2008; Meneghetti *et al.*, 2009; Souza *et al.*, 2009; Sá Filho *et al.*, 2011; Pereira *et al.*, 2013b; Monteiro Jr *et al.*, 2015b), the metabolism of E2 is different between *B. taurus* and *B. indicus* (Sartori *et al.*, 2016). For example, in a 3 x 2 Latin Square design study (Bastos *et al.*, 2011), the effect of dose of estradiol benzoate (EB; 1, 2 or 4 mg), given at the same time as a P4 intravaginal insert, on the synchronization of follicular wave emergence was evaluated in non-lactating Nelore (n = 13) and Holstein (n = 11) cows receiving a maintenance diet. The BCS and body weight were kept at 3.5 ± 0.1 and 3.0 ± 0.2 (scale of 1 to 5) and 535 ± 14 and 600 ± 23 kg for Nelore and Holstein cows, respectively. Two doses of prostaglandin F2α (PGF2α) were given 11 days apart, and simultaneously with the second PGF2α, cows were treated with EB and with an intravaginal P4 insert, which remained for 10 days. Ovarian dynamics were monitored daily by means of ultrasonography for 10 days after EB treatment. Only cows with follicular wave emergence synchronized by the protocol were included, i.e., when the emergence occurred between 1 and 6 days after treatment with EB + P4. In response to the treatments, 2.5% (1/39) of Nelore cows did not have a synchronized follicular wave emergence, with the only non-synchronized cow



being treated with 1 mg EB. As for Holstein cows, 15.1% (5/33) did not have a synchronized follicular wave emergence (three cows had received 1 mg and the other two cows, 2 or 4 mg EB). Regardless of breed, there was a dose-dependent effect of treatment with EB on the following variables: day of the follicular wave emergence, diameter of the dominant follicle 9 days after treatment with EB, circulating concentration of E2 24 h after treatment with EB, time that circulating FSH began to rise (beginning of FSH peak) after EB, and time of occurrence of the peak of circulating FSH (Table 2). The follicular wave emergence occurred 3.1 ± 0.3^a , 3.3 ± 0.1^{ab} and 3.9 ± 0.2^b days after treatment with 1, 2, or 4 mg of EB, respectively ($^{a,b}P \leq 0.05$), independent of breed, although circulating concentrations of E2 24 h after treatment with EB was much greater in Nelore than Holstein cows (Table 2). The diameter of the largest follicle 9 days after treatment was 12.2 ± 0.5^a , 11.5 ± 0.8^{ab} and 9.9 ± 0.7^b mm in cows that received 1, 2 or 4 mg of EB, respectively ($^{a,b}P \leq 0.05$), but there was no breed effect (Table 2). At follicle wave emergence, the number of 2 to 5 mm follicles present in the ovaries was greater in Nelore than in Holstein cows (30.8 ± 4.5 vs. 13.6 ± 1.1 AFC; Table 2). Furthermore, follicle deviation occurred, on average, 3.0 ± 0.2 days after wave emergence, independent of breed, when the diameter of the largest follicle reached 7.3 ± 0.4 and 9.0 ± 0.5 mm in Nelore and Holstein cows, respectively ($P < 0.05$). It was concluded that the timing of follicle wave emergence after treatment with EB + P4 was EB dose-dependent for both breeds.

The results presented above are, somewhat, contradictory, because despite a very different clearance rate of E2 (same doses, but very distinct concentrations of circulating E2 after EB treatment [>2 -fold difference] in cows with little difference in body weight - Holsteins were only 12% heavier than Nelore cows), the behavior of wave emergence after EB + P4 treatment was not different between breeds (Table 2). Therefore, based on differences in circulating E2 after EB treatment between breeds, one might expect that lower EB doses should be used for Nelore and higher EB doses should be used for Holstein cattle at the initiation of an E2/P4-based FTAI protocol in order to optimize synchronization of follicle wave emergence. In contrast, based on the similar responses of both breeds to EB treatments in terms of follicle wave emergence, a similar E2 dose might be optimal for Nelore and Holstein cows.

Currently, both in *B. indicus* and *B. taurus*, the dose of EB recommended for initiation of a FTAI protocol is 2.0 mg given at the same time as treatment with P4/progestin. However, hypothesizing that the dose of 2.0 mg EB would be insufficient to effectively synchronize follicular wave emergence in lactating dairy cows, Monteiro Jr *et al.* (2015a) compared 2.0 vs. 3.0 mg EB at the beginning of a FTAI protocol. All cows were treated with EB (2.0 or 3.0 mg) at the time of introduction of a P4 insert (day 0). On day 7, cows were given 25 mg of PGF 2α ; on day 8, the insert was removed and cows were given 1 mg of estradiol cypionate (ECP). All cows received FTAI on day 10. Daily transrectal ultrasound evaluations of the ovaries

were performed. There was no difference ($P > 0.10$) between treatments with 3.0 vs. 2.0 mg EB for proportion of cows with synchronized follicular wave emergence (71.4% [15/21] vs. 82.6% [19/23]), and time to wave emergence (3.6 ± 0.19 vs. 3.4 ± 0.17 d). However, treatment with 3.0 mg EB decreased the percentage of cows with a CL on day 7 of the FTAI protocol (19.8% [4/17] vs. 55.3% [13/21]; $P < 0.05$), indicating that the higher dose of EB caused increased CL regression during the protocol.

Using similar reasoning as in the study of Bastos *et al.* (2011), a study was done in *B. taurus* and *B. indicus* beef cattle comparing 1.0 vs. 2.0 mg EB + P4 at the initiation of a FTAI protocol. Pessoa *et al.* (2015) compared the treatments above during a resynchronization protocol starting 22 day (day 22) after the first FTAI and evaluated P/AI, pregnancy loss and induction of new follicular wave emergence in suckled beef cows. Thus, on day 22 after first FTAI all cows received an intravaginal P4 insert and, regardless of pregnancy status, 1426 cows (768 *B. taurus* and 728 *B. indicus*) were treated with either 1 or 2 mg EB. After 8 days (day 30), the P4 insert was removed and pregnancy diagnosis was accomplished by ultrasound. Non-pregnant cows were then treated with cloprostenol. On the same day, *B. taurus* cows received 10 mg FSH and 1 mg EB, whereas *B. indicus* cows were treated with 300 IU eCG and 1 mg ECP. The FTAI was performed 44 or 48 h after P4 removal on *B. indicus* and *B. taurus* cows, respectively. Pregnancy diagnosis was conducted again at 62 days after first FTAI. The P/AI after the first FTAI was similar (~44.0%; $P = 0.85$) between treatments regardless of breed. However, P/AI after resynchronization was lower ($P = 0.0001$) in cows treated with 1 compared to 2 mg EB (36.1 vs. 47.3%). Pregnancy loss at first FTAI was similar ($P = 0.37$) between treatments (3.8 vs. 5.5% for 1 and 2 mg EB), but the cumulative pregnancy was greater ($P = 0.01$) in cows treated with 2 mg EB (68.2%) than those treated with 1 mg EB (62.8%). Moreover, *B. indicus* cows had ovarian dynamics evaluated by ultrasound to assess induction of a new follicular wave emergence after treatment with 1 mg ($n = 12$) vs. 2 mg ($n = 12$) EB. Despite a similar interval from EB treatment to new follicular wave emergence ($P = 0.13$), the emergence of a new wave was more dispersed ($P = 0.03$) in cows treated with 1 mg EB (1.8 ± 1.3 d) compared with cows treated with 2 mg EB (2.3 ± 0.6 d). Therefore, a dose of 2 mg EB produced a more uniform emergence of the follicular wave and greater P/AI after resynchronization, without compromising the pregnancy established at the first FTAI in suckled *B. taurus* and *B. indicus* beef cows. Preliminary results of a study (Prata and Sartori, 2016; ESALQ, USP, Piracicaba, SP, Brazil, unpublished) that is being performed with postpartum lactating Nelore cows followed a similar trend in P/AI for 1 vs. 2 mg EB treatment at the initiation of a FTAI protocol (40.9% [36/88] vs. 47.2% [42/89]; $P = 0.40$) but was not significantly different (day 0: EB + P4 insert; day 8: P4 insert removal, 0.6 mg ECP, 500 μ g cloprostenol, and 300 IU eCG; day 10: FTAI).

Table 2. Results (least squares means \pm SEM) of reproductive variables of non-lactating Nelore (*B. indicus*) and Holstein (*B. taurus*) cows treated with 1, 2, or 4 mg of estradiol benzoate (EB) and an intravaginal progesterone insert.

	Breed						P-value		
	Nelore			Holstein			Breed	Dose	BxD
	1 mg EB (n = 11)	2 mg EB (n = 12)	4 mg EB (n = 12)	1 mg EB (n = 11)	2 mg EB (n = 11)	4 mg EB (n = 11)			
Wave emergence after EB; day	3.1 \pm 0.3 ^b	3.4 \pm 0.2 ^{ab}	4.0 \pm 0.2 ^a	3.0 \pm 0.3 ^b	3.3 \pm 0.3 ^b	3.9 \pm 0.3 ^a	0.66	0.01	0.98
Antral follicle count at emergence; number	31.1 \pm 5.8	32.4 \pm 5.8	30.9 \pm 5.8	11.8 \pm 6.2	13.8 \pm 6.1	14.1 \pm 6.1	0.03	0.67	0.79
Diameter of largest follicle 9 days after EB; mm	11.5 \pm 0.9 ^a	11.5 \pm 0.9 ^{ab}	9.8 \pm 0.8 ^b	13.7 \pm 1.2 ^a	11.4 \pm 1.1 ^{ab}	10.4 \pm 1.2 ^b	0.34	0.05	0.47
Growth rate of dominant follicle; mm/day	1.3 \pm 0.1	1.3 \pm 0.1	1.1 \pm 0.1	1.6 \pm 0.1	1.6 \pm 0.1	1.4 \pm 0.2	0.03	0.22	0.95
Circulating estradiol 24 h after EB; pg/ml	57.0 \pm 9.1 ^c	85.4 \pm 9.5 ^b	148.1 \pm 9.1 ^a	25.8 \pm 12.0 ^c	42.3 \pm 11.2 ^b	71.0 \pm 11.9 ^a	< 0.01	< 0.01	0.06
Maximum circulating FSH after EB, ng/ml	1.06 \pm 0.06	1.07 \pm 0.06	1.06 \pm 0.06	1.30 \pm 0.08	1.27 \pm 0.08	1.10 \pm 0.09	0.04	0.26	0.30
Onset of FSH surge after EB, h	57.8 \pm 5.4 ^{ab}	53.3 \pm 3.5 ^b	64.0 \pm 3.2 ^a	46.0 \pm 4.7 ^b	56.0 \pm 4.4 ^{ab}	60.9 \pm 4.0 ^a	0.26	0.04	0.30
Time of FSH surge after EB, h	78.0 \pm 5.7 ^b	82.7 \pm 6.3 ^b	110.0 \pm 5.7 ^a	91.6 \pm 7.5 ^b	90.1 \pm 7.0 ^b	101.9 \pm 7.5 ^a	0.46	< 0.01	0.25

^{a,b,c}Effect of dose within breed (P < 0.05).

Corpus luteum function, luteolysis, and growth and ovulation of the preovulatory follicle

Despite having smaller CL, *B. indicus* have greater circulating P4, probably due to greater production, but also, a reduced metabolic clearance rate for P4 than in *B. taurus* (Sartori et al., 2016). Moreover, among *B. taurus* breeds, lactating dairy cows have even lower circulating steroid hormones (Sartori et al., 2002a, 2004) due to greater metabolic clearance associated with greater dry matter intake (Sangsrivong et al., 2002; Vasconcelos et al., 2003).

One of the first studies (Carvalho et al., 2008) to suggest lower P4 clearance rates in *B. indicus* than *B. taurus* was done using 70 nulliparous heifers of different genetic groups [*B. indicus* (Nelore and Gir), *B. indicus* x *B. taurus* (Nelore x Angus and Gir x Holstein), *B. taurus* (Angus and Holstein)]. Two PGF2 α treatments were performed 24 and 12 days (day -24 and day -12) before a P4 implant insertion. On day 0, each heifer received the P4 implant, and 2 mg EB. In addition, half of the heifers from each genetic group received 25 mg of PGF2 α on day 0. A second PGF2 α treatment was performed on all heifers at the time of P4

implant withdrawal (day 8). On day 9, all heifers received 1 mg EB. There were no detectable differences in serum P4 concentrations among genetic groups on day 0, but P4 concentrations were greater on day 3 ($P < 0.05$) in *B. indicus* (6.8 ± 0.8 ng/ml) and *B. indicus* x *B. taurus* (5.7 ± 0.7 ng/ml) than in *B. taurus* (3.9 ± 0.4 ng/ml) heifers. Circulating P4 in *B. indicus* heifers on day 6 (5.7 ± 0.6^a vs. 4.0 ± 0.6^b vs. 3.2 ± 0.5^b ng/ml) and day 8 (5.3 ± 0.7^a vs. 3.3 ± 0.4^b vs. 3.0 ± 0.5^b ng/ml) exceeded ($^{a,b}P < 0.05$) those of *B. indicus* x *B. taurus* or *B. taurus* heifers, respectively. These results were corroborated by another study (Nascimento et al., 2012; Sartori et al., 2016) designed to compare circulating P4 profile in non-lactating Holstein ($n = 20$) and Nelore ($n = 20$) cows fed a maintenance diet after insertion of intravaginal P4 inserts. Cows did not have a functional CL at the time of implant insertion. There was an effect ($P < 0.05$) of breed (1.2 vs. 2.2 ng/ml, Holstein and Nelore, respectively), in which P4 concentrations were about 90% greater in Nelore than in Holstein cows, probably due the greater P4 metabolism in Holsteins (Fig. 2A). After removal of the P4 device, blood was sampled every 40 minutes (0.65 h) until 240 min, and a lower P4 concentration was observed in Holstein than in Nelore cows (Fig. 2B).

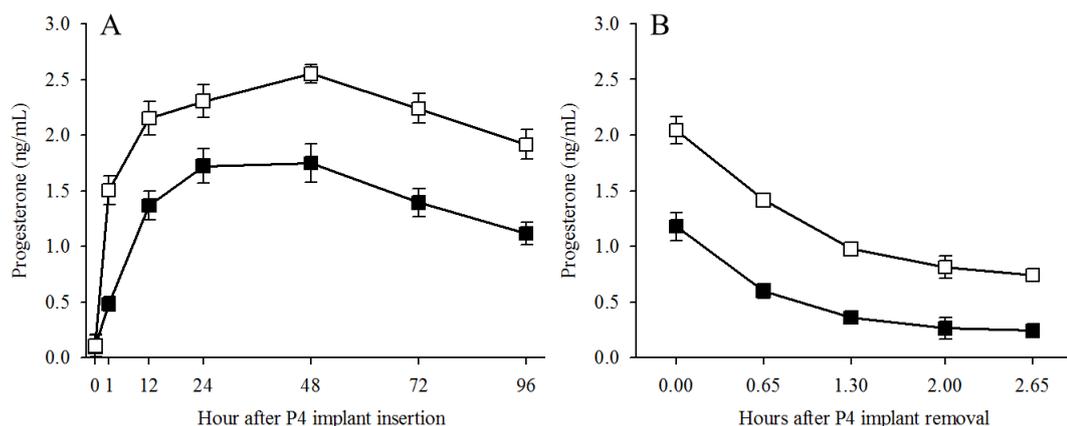


Figure 2. Circulating progesterone (P4) concentrations (least squares means \pm standard error) of non-lactating Holstein ($n = 20$; ■) and Nelore ($n = 20$; □) cows fed with a maintenance diet. (A) Blood was sampled before (0 h), and 1, 6, 12, 24, and 48 h after P4 implant insertion. There were effects of breed and day ($P \leq 0.05$). (B) Blood was sampled immediately before (0 h) and 0.65, 1.30, 2.00, and 2.65 h after P4 implant removal. There were effects of breed and day ($P \leq 0.05$; From Sartori et al., 2016).

It has been shown that serum LH concentrations during the natural (Randel, 1976; Randel and Moseley, 1977), E2-induced (Rhodes et al., 1978), or GnRH-induced (Griffin and Randel, 1978) preovulatory LH-surge were lower in Brahman (*B. indicus*) than in British (*B. taurus*) breeds. Likewise, as discussed by Bó et al. (2003), gonadotrophin secretion patterns in the postpartum period also differ between *B. taurus* and *B. indicus*. Under the same conditions, *B. taurus* (Hereford x Shorthorn suckled cows) had higher plasma LH concentrations (0.7 ± 0.1 ng/ml) than suckled Brahman cows (0.6 ± 0.1 ng/ml) after 30 days postpartum and this difference appeared to increase over time (D'Occhio et al., 1990). In addition, a greater proportion of *B. taurus* cows had a higher pulsatile LH secretion than *B. indicus* cows (D'Occhio et al., 1990). In this study, LH concentrations were also affected by

body condition and postpartum management (D'Occhio et al., 1990) suggesting that nutrition is one of the major concerns when evaluating postpartum activity in *B. indicus* and *B. taurus*. Moreover, independent of breed, or genetic group, suckling cows have lower LH-pulse frequency than lactating cows not carrying calf (discussed by Stevenson and Lamb, 2016). Despite that, non-pregnant, lactating Holstein cows had greater LH-pulse frequency than non-pregnant non-lactating Holstein cows (Vasconcelos et al., 2003). It has been shown that LH pulses are essential for growth of the dominant follicle (Ginther et al., 1996). Circulating P4 concentrations are expected to influence LH-pulse frequency because P4 inhibited the dominant follicle development in a dose-dependent manner (Adams et al., 1992; Santos et al., 2016). Therefore, cattle with too high of circulating P4, nursing cows, or cows under



nutritional restrictions may have reduced development of the dominant/preovulatory follicle potentially leading to reduced likelihood of ovulation and reduced size of the ovulatory follicle, which may compromise fertility under these conditions. These problems may be even more likely in *B. indicus* due to several factors, such as described above.

The importance of circulating concentrations of P4 during development of the preovulatory follicle on ovulation and fertility in cattle has been discussed by several authors (Wiltbank *et al.*, 2006; Carvalho *et al.*, 2008; Santos *et al.*, 2016; Stevenson and Lamb, 2016). The general idea is that due to high LH-pulse frequency and physiologically low circulating P4 due to elevated steroid metabolism, lactating dairy cows ovulate larger follicles, and have high incidence of double ovulations, however oocyte and embryo quality may be hampered (Sartori *et al.*, 2002b; Wiltbank *et al.*, 2006, 2014; Santos *et al.*, 2016). In *B. taurus* beef cattle, Stevenson and Lamb (2016) reported little effect of P4 environment during development of the preovulatory follicle on pregnancy risk. Moreover, Holstein heifers that received PGF2 α at the initiation of a Cosynch + CIDR protocol, and therefore, had lower circulating P4 during the protocol, resulting in larger ovulatory follicles, had similar P/AI as heifers not receiving PGF2 α at the initiation of the protocol (Stevenson *et al.*, 2008). In contrast, studies in *B. indicus* heifers or non-lactating cows indicated that high circulating P4 concentrations during synchronization of ovulation protocols reduced the growth of the dominant follicle, which negatively affected ovulation rate and pregnancy success (Carvalho *et al.*, 2008; Dias *et al.*, 2009; Peres *et al.*, 2009).

The results presented above may explain the contrasting differences regarding the effects of equine chorionic gonadotrophin (eCG) at the end of a FTAI protocol on final follicle development and ovulation between *B. indicus* and *B. taurus*. Whereas in *B. indicus*, the majority of studies has shown a positive effect of eCG on percentage of heifers or cows that ovulate at the end of the protocol, greater circulating P4 during the subsequent diestrus, and improved P/AI (Baruselli *et al.*, 2004; Peres *et al.*, 2009; Sá Filho *et al.*, 2009, 2010a, b; Lemes *et al.*, 2011; Sales *et al.*, 2011), data in *B. taurus* are conflicting and, although some studies in beef cattle have shown improved ovulation rate and fertility by adding eCG to the FTAI protocol (Pessoa *et al.*, 2016), several studies have shown no or little benefit of eCG, especially in lactating Holstein cows (Small *et al.*, 2009; Souza *et al.*, 2009; Ferreira *et al.*, 2013; Pulley *et al.*, 2013).

Regarding induced luteolysis by exogenous treatments, the responsiveness of CL to PGF2 α seems to be very similar between *B. indicus* and *B. taurus*. To study the effect of the dose of a PGF2 α analog and day of the estrous cycle at the time of treatment, Ferraz Jr *et al.* (2016) performed an experiment with non-lactating Nelore cows, in a 3 x 2 factorial design, in which three different doses of dinoprost tromethamine (12.5, 25.0, and 50.0 mg) were used on days 5 or 7 of the estrous cycle. In this study luteolysis was dose-dependent.

Therefore, increasing the dose of PGF2 α increased the proportion of cows detected in estrus and augmented the percentage of cows with circulating P4 concentration <1.0 ng/ml at 72 h after PGF2 α treatment. Similar results were reported by Nascimento *et al.* (2014) who found that complete luteolysis did not occur in non-lactating Holstein cows on day 5 of the estrous cycle with either a full dose of PGF2 α (25 mg of dinoprost tromethamine), two full doses 8 h apart, or double dose (50.0 mg). Giordano *et al.* (2013) evaluated whether increasing the dose of PGF2 α (cloprostenol) during the Breeding-Ovsynch portion of the Double-Ovsynch protocol could improve the rates of ovulation and luteolysis and therefore increase P/AI. The authors observed a better luteolytic response to PGF2 α (% of cows with complete CL regression) when the dose was increased from 500 μ g to 750 μ g in lactating Holstein cows (84.3 vs. 90.8%; P = 0.03). The greater cloprostenol dose increased luteal regression primarily in multiparous cows and increased fertility (P = 0.05) only at the pregnancy diagnosis 39 days after FTAI.

In addition to the dose of PGF2 α , it is well known that the day of the estrous cycle impacts the proportion of cows with luteolysis after PGF2 α (dinoprost tromethamine) treatment. Ferraz Jr *et al.* (2016) observed that Nelore cows that had CL on day 5 of the estrous cycle were more refractory to PGF2 α than those on day 7 of the cycle. Moreover, in lactating Holstein cows, a second treatment of dinoprost tromethamine (+PGF2 α ; 24 h apart) increased the percentage of cows that had complete luteal regression (95.6%) compared with control cows (84.6%). Although one study found no detectable effect of the additional PGF2 α on P/AI (control = 41.5% vs. +PGF2 α = 44.7%; P = 0.34; Brusveen *et al.*, 2009), recent studies have found significant improvements in P/AI when a second PGF2 α treatment is given, particularly in multiparous cows (Carvalho *et al.*, 2015; Wiltbank *et al.*, 2015). Therefore, complete luteolysis is essential to optimize ovulation and fertility to a FTAI protocol. In fact, more lactating Holstein cows with P4 < 0.1 ng/ml, compared with P4 \geq 0.1 and < 0.22 ng/mL at the time of AI, ovulated to an E2/P4-based FTAI protocol (81.2 vs. 58.0%) and had increased P/AI (47.4 vs. 21.4%; Monteiro Jr *et al.*, 2015a). Similar results were observed by Pereira *et al.* (2013b) when lactating dairy cows were also synchronized with an E2/P4-based protocol and were either inseminated or served as embryo recipients. The P4 concentration at the time of AI or 7 days before embryo transfer (ET) altered fertility in FTAI cows, with higher P/AI in cows with P4 concentration <0.1 ng/ml compared with cows with P4 concentration \geq 0.1 ng/ml. In ET cows, higher P/ET was found in cows with P4 concentration <0.22 ng/ml compared with cows with P4 concentration \geq 0.22 ng/ml.

Practical implications of the physiological differences between *B. indicus* and *B. taurus* for embryo production

The physiological differences between *B. indicus* and *B. taurus* described above, need also to be



considered when managing females for *in vivo* derived (IVD) embryos by multiple ovulation and embryo transfer (MOET) or *in vitro* embryo production (IVP).

One of the protocols most used in *B. indicus* cows and heifers for MOET was developed by Barros and Nogueira (2005) and was called P-36. The protocol included insertion of an intravaginal P4 insert for 36 h after PGF2 α administration and induction of ovulation with exogenous LH, administered 12 h after P4 insert removal (48 h after PGF2 α administration). The FTAI was performed 12 and 24 h later since ovulation occurs between 24 and 36 h after LH administration. The effectiveness of the P-36 protocol has been confirmed (Baruselli *et al.*, 2006; Nogueira *et al.*, 2007). A variation of the P-36 protocol, in which the P4 device is removed 24 h after PGF2 α (protocol P-24) and LH is administered 24 h later (48 h after PGF2 α), has been utilized in Nelore cattle, apparently with comparable results to those obtained with P-36 protocol (Baruselli *et al.*, 2006).

The use of the P-36 protocol in *B. taurus* breeds, however, has resulted in decreased number of viable embryos in comparison with conventional protocols with estrus detection. In Holstein (Baruselli *et al.*, 2006) and Angus donors (Bó *et al.*, 2006), viable embryo production was increased with the P-36 protocol when the ovulation induction treatment (LH or GnRH) was administered at 60 h (P-36/LH60), rather than 48 h (P-36/LH48) after PGF2 α administration. On the other hand, even though delaying ovulation for 12 h in the P-36 protocol in *B. taurus* breeds (P-36/LH60 protocol) had positive effects on embryo production, the opposite occurred when used in *B. 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 *B. indicus* donors; whereas, in *B. 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 *B. taurus* (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 was 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). Other studies in Nelore heifers successfully induced superovulation and embryo production using a smaller dose of FSH (70 mg; Guardieiro *et al.*, 2014), which is very unlikely to be effective in *B. taurus*.

Especially due to the greater AFC and better oocyte quality in *B. indicus* in relation to *B. taurus*, IVP is much more successful in *B. indicus*. For example, Gir (*B. indicus*), Holstein and crossbreds (1/4 Holstein x 3/4 Gir or 1/2 Holstein-Gir) were compared for total and

viable oocyte yield, and IVP embryos (Pontes *et al.*, 2010). 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. Another study was conducted to compare IVP between Nelore and Holstein heifers (Gimenes *et al.*, 2015). More oocytes were recovered (37.1 vs. 15.4), more embryos were produced (7.3 vs. 1.1), and a greater blastocyst rate was obtained (28.3 vs. 14.1%) from Nelore than Holstein heifers. In another study (Sales *et al.*, 2015), Gir cows had a greater number of oocytes recovered by ovum pickup (OPU; 23.4 vs. 14.9), better quality of oocytes demonstrated by greater cleavage rates (73.6 vs. 40.8%), greater number of blastocysts (3.8 vs. 0.7) and better blastocyst rates (36.7 vs. 12.1%) than Holstein donors.

Overall, the main findings of the studies described above indicate that *B. taurus* yielded less oocytes, as well as produced less blastocysts per OPU than *B. indicus* donors. This pattern seems to occur even when other *B. taurus* breeds are studied. Sudano *et al.* (2014) conducted an experiment in which non-lactating Simmental (*B. taurus*) and Nelore cows were compared for *in vivo* embryo production and IVP. Although the total number of recovered ova/embryos per cow (5.5 vs. 3.7) and transferable IVD embryos per cow (3.8 vs. 2.3) were not different between Nelore and Simmental, respectively, when IVP was performed, Nelore produced more oocytes per OPU (14.9 vs. 8.1) and had greater blastocyst rates per OPU (41.5 vs. 23.4%) than Simmental cows. The potential impact of metabolic or steroid hormones, associated or not with different feed intake regimens on embryo production in *B. indicus* and *B. taurus* cattle, has been discussed in more details by Sartori *et al.* (2016).

Final considerations

Bos indicus and *B. taurus* have been mainly used for milk and beef production around the world. Although, in general, *B. taurus* have been more intensely selected for production, a better adaptation to the tropical and sub-tropical environments makes *B. indicus* and crossbreds feasible options for production. Moreover, there are significant differences in the reproductive physiology between those genetic groups that affect the application of adequate tools for reproductive management. For example, *B. taurus* in general reach puberty sooner (Sartori *et al.*, 2010a) and have a shorter gestation length as compared to *B. indicus* (Paschal *et al.*, 1991). Therefore, in order to have a 12-months calving interval, *B. indicus* cows must conceive 10 days earlier than *B. taurus*. Differences in estrus behavior and ovarian function also make some adjustments of reproductive management necessary. The greater sensitivity to the negative feedback of steroid hormones on the hypothalamus-pituitary axis makes a dose reduction sometimes necessary during hormone treatments in *B. indicus*. On the other hand, due to greater steroid hormone clearance, especially in



lactating *B. taurus* dairy cows, higher doses of hormones may be necessary to optimize reproductive management strategies. Moreover, the greater AFC, greater oocyte quality, and greater sensitivity to gonadotropins in *B. indicus*, make embryo production much more affordable than in *B. taurus*.

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