CONSIDERATIONS FOR UTILIZING REPRODUCTIVE TECHNOLOGIES IN *BOS INDICUS*-INFLUENCED CATTLE

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INTRODUCTION

Bos indicus cattle, sometimes referred to as zebu, are derived from wild aurochs domesticated in the Indus valley region of modern-day Pakistan approximately 7,000 years ago. Domestication of *Bos indicus* cattle was a separate event from domestication of *Bos taurus* cattle, which is thought to have occurred approximately 10,500 years ago around the Taurus mountains and upper Euphrates River region of the Fertile Crescent, near modern-day Turkey (Loftus et al., 1994). Genomic analyses continue to shed light on the complicated relationships and ancestry of cattle breeds, and indications of both indicine and taurine ancestry are present in many breeds (Decker et al., 2014). Nevertheless, due in part to the distinct domestication events, indicine and taurine cattle are referred to as two separate subspecies: *Bos taurus taurus* and *Bos taurus indicus*. At one time, indicine and taurine cattle were taxonomically divided not as subspecies but as separate species; thus, the terms *Bos taurus* and *Bos indicus* are still commonly used. While the difference between species and subspecies classification is somewhat trivial, the fact that a difference in classification is needed at all highlights the fact that *Bos taurus* and *Bos indicus* cattle differ in meaningful ways.

Due in part to the location in which they were domesticated, *Bos indicus* cattle are well-adapted to tropical and subtropical conditions, exhibiting a high tolerance to parasites, solar exposure, high ambient temperature and relative humidity, and even low-quality forage diets. Due to this adaptation, use of *Bos indicus* breeds of cattle dwarfs use of *Bos taurus* breeds from a global perspective, as well over half of the world's cattle inventory is managed in subtropical or tropical environments (Cundiff et al., 2012). Conversely, in the United States, use of *Bos taurus* cattle predominates in both the beef and dairy industries, and it is uncommon for commercial producers to use purebred *Bos indicus* females in the United States. However, a large proportion of the beef industry in the southern United States lies in areas of high thermal stress, such as the humid Gulf Coast states and the arid Southwest. In these areas, beef producers frequently incorporate *Bos indicus* genetics in crossbreeding programs to confer thermal tolerance. In the United States, the most commonly used *Bos indicus* breed is the Brahman breed, developed in the United States as a composite from several different breeds of *Bos indicus* cattle imported from India in the mid to late 1800s and early 1900s. Today, most *Bos indicus*-influenced cattle in the southern United States are generated through crossbreeding programs with Brahman animals or Brahman cross animals (e.g. Brangus, Braford, Simbrah, Santa Gertrudis etc.). Due to growth, performance, and carcass advantages of *Bos taurus* cattle (Frisch and Vercoe, 1977; Thrift et al., 2010), some producers seek to incorporate only the minimum percentage *Bos indicus* genetics necessary to mitigate environmental effects. Other producers seek to generate F1 *Bos indicus* x *Bos taurus* animals to take advantage of maximum heterosis. Hence, there is considerable variation among herds in the southern United States relative to percentage *Bos indicus* influence.

Bos indicus females have been reported to differ from *Bos taurus* in several aspects of reproductive physiology, and other authors have written extensive reviews on this topic (Randel, 2005; Sartori et al., 2010). The present review therefore will be narrower in scope and will focus specifically on differences that pertain to response of *Bos indicus*-influenced females following estrus synchronization programs commonly used in the United States. More specifically, this review will pose the question, "Are *Bos indicus*-influenced females really all *that* different? And if so, what can we do differently?"

DISTINCT PHYSIOLOGY OF *BOS INDICUS*-INFLUENCED FEMALES

Attainment of Puberty. In the low forage quality environments to which *Bos indicus* females are adapted, growth may be limited by protein, energy, and/or mineral availability. Purebred *Bos indicus* heifers in the tropics and subtropics typically reach puberty between 16 and 40 months of age (Abeygunawardena & Dematawewa 2004; Nogueira 2004). In contrast, *Bos taurus* heifers raised in temperate environments generally reach puberty between 10 and 18 months of age (Dow et al., 1982). Due to genetics for later puberty attainment and limited nutrition, managing purebred *Bos indicus* heifers to calve at two years of age is challenging in tropical and subtropical systems. Certainly, crossbred *Bos indicus* x *Bos taurus* heifers and lower percentage *Bos indicus*-influenced heifers do reach puberty at later ages than *Bos taurus* heifers; however, researchers in the southern United States clearly demonstrated in the 1950s and 1960s that age at puberty attainment is still sufficiently early for these heifers to calve at two years of age with proper management (Warnick et al., 1956; Temple et al., 1961; Reynolds et al., 1963; Reynolds, 1967; Plasse et al., 1968a). Other authors in these proceedings will provide more exhaustive reviews of the literature relative to development of *Bos indicus*-influenced heifers. Here, we will simply highlight two key opportunities for producers to assess the effectiveness of their heifer development practices: target weight and reproductive tract score.

Development to Target Weight. Nutrition is permissive rather than causative of attainment of puberty (see review by Hall and Glaze, 2016). If genetics and other factors allow for early attainment of puberty, inadequate nutrition will delay attainment of puberty. Conversely, adequate nutrition results in early attainment of puberty only when genetics and other factors also allow for early puberty. Certainly, genetic variation for age at puberty exists among *Bos taurus* as well as *Bos indicus* females; for example, larger frame, higher growth *Bos taurus* breeds from continental Europe have traditionally been considered later maturing than smaller frame British breeds. In order to ensure adequate nutritional development among the majority of heifers, researchers proposed developing heifers to a target weight (Lamond, 1970). Expressing this target weight as a percentage of the weight of a mature cow, the long-standing recommendation for *Bos taurus* heifers has been development of heifers to target weight of 65 percent of mature body weight (Patterson et al., 1992). Development of *Bos taurus* heifers to a 55 percent target weight has been found to be sufficient in some studies (Funston, 2004; Funston and Deutscher, 2004; Roberts et al., 2009; Larson et al., 2009), while results from other studies have noted decreased reproductive performance of *Bos taurus* heifers developed to this lower target weight (Patterson et al., 1992;

Eborn et al., 2013). Thus, some debate exists as to whether development to a target weight lower than 65 percent may be an effective development strategy for some *Bos taurus* producers, particularly with lower input systems and/or herds with strong genetics for early attainment of puberty. However, when development to target weights of either 55 or 65 percent were compared among *Bos indicus*-influenced heifers, development to a 65 percent target weight resulted in earlier attainment of puberty and earlier pregnancy success during the breeding season (Patterson et al., 1989; 1991). This difference was much more pronounced among *Bos indicus*-influenced compared to *Bos taurus* heifers, suggesting that later maturing *Bos indicus*-influenced heifers are particularly sensitive to limited nutrition in the development phase. Based on these data, producers of *Bos indicus*-influenced heifers should consider the following steps to ensure nutritional development of heifers is not limiting potential puberty attainment: (1) accurately estimate mature cow weight by weighing cows (or a representative subset) or by correcting sale weights of cull cows for body condition score; (2) calculate a target weight of 65 percent of mature cow weight; (3) feed a diet with adequate protein and sufficient energy to develop heifers to the target weight; and (4) weigh heifers (or a representative subset) periodically during the development phase to verify average daily gain, adjusting diet or feeding as necessary.

Table 1. Characteristics associated with the reproductive tract scoring system.*

Pubertal Group	RTS	Cycling status	Uterine horns	Ovaries
Prepubertal	1	Infantile	no tone	No palpable follicles
	2	Non-cycling > 30 d to puberty	no tone	8 mm follicles
Peripubertal	3	Non-cycling < 30 d to puberty	slight tone	8-10 mm follicles
Pubertal	4	Estrous cycling; Follicular phase	coiled	> 10 mm follicles
	5	Estrous cycling; Luteal phase	distended	>10 mm follicles, corpus luteum present

*Anderson et al., 1991.

Reproductive Tract Scoring. The reproductive tract score (RTS) system (Table 1) was developed to assist beef producers in selecting replacement heifers that are good candidates for estrus synchronization (Anderson et al., 1991). In order for results to be informative, scoring should be performed close to the start of the breeding season, no more than two weeks before the start of estrus synchronization. Scores are determined by transrectal palpation of the reproductive tract to assess uterine and ovarian size as well as structures present on the ovaries (follicles and CL). Scores range from 1 to 5 as follows: 1 = prepubertal, with an infantile tract; 2 = prepubertal, likely over 30 days from attainment of puberty; 3 = peripubertal, likely within 30 days of attainment of puberty; 4 = pubertal, in the follicular phase of the estrous cycle; 5 = pubertal, in the luteal phase of the estrous cycle. By evaluating the actual reproductive tract directly, RTS offer an opportunity to directly assess pubertal status of individual females rather than relying only on weight as an indirect indicator. A common recommendation is that at least 50 percent of heifers are identified as pubertal (RTS 4 or 5) prior to estrus synchronization (Smith et al., 2011). Among *Bos taurus* heifers, reproductive tract scoring is repeatable and accurate for determining pubertal status (Rosenkrans and Hardin, 2003), and RTS is also an effective predictor of heifer pregnancy rates to fixed-time artificial insemination (FTAI), pregnancy rates by day 50 of the breeding season, and weaning weight of calves (Pence and BreDahl, 1998; Pence et al., 2007; Holm et al., 2009). We have recently reported results from two separate large scale experiments in which pregnancy rates of *Bos indicus*-influenced heifers were strongly associated with RTS in both natural service and timed AI systems (Thomas et al., 2017; Locke et al., 2018). In fact, when comparing results among *Bos indicus*-influenced heifers to field data collected among *Bos taurus* heifers in the Missouri Show-Me-Select™ replacement heifer program, results were quite similar between heifers within respective RTS categories (Table 2; adapted from Thomas et al., 2017). These results, discussed in more detail below in "Protocols for *Bos indicus*-Influenced Heifers," underscore the potential utility of RTS in assessing development of *Bos indicus*-influenced heifers.

Table 2. Pregnancy rates to fixed-time artificial insemination (FTAI) following the 14-d CIDR-PG protocol[1]: Comparing results based on reproductive tract score (RTS) in *Bos indicus*-influenced heifers with field results from *Bos taurus* heifers in the Missouri Show-Me-Select™ Replacement Heifer Program[3]

	RTS 2		RTS 3		RTS 4		RTS 5	
Treatment	Proportion	%	Proportion	%	Proportion	%	Proportion	%
Bos indicus-influenced (Thomas et al., 2017)	39/111	35	37/95	39	17/33	52	21/38	55
Bos taurus Show-Me-Select	182/572	32	2219/4773	46	2778/5604	50	3249/6266	52

[1]See Figure 2 for protocol diagram.
[2]See Table 1 for description of reproductive tract scores.
[3]Adapted from Locke et al., 2016.

Both of the above metrics (weights relative to target weight and reproductive tract scores) allow producers to identify heifers that are poor candidates for use of reproductive technologies due to age, performance, and/or genetic predisposition towards later attainment of puberty. Whether these heifers are culled or simply managed differently will likely depend on economic factors specific to an individual operation; however, producers should consider the long term genetic progress that can be made through retaining only early maturing females. Unfortunately, selection pressure for age at puberty has been less intense among *Bos indicus* compared to *Bos taurus* breeds (Eler et al., 2002). Heritability is relatively high for both age at first conception (h^2 = 0.44 to 0.67) and heifer pregnancy (h^2 = 0.58 to 0.66), however, suggesting these traits can be improved significantly if appropriate selection pressure is imposed (Eler et al., 2006; Pereira et al., 2007). Lastly, it should be emphasized that reproductive performance of heifers directly impacts lifetime reproductive performance of cows. Heifers that become pregnant earlier in the breeding season go on to remain in the herd longer and wean more pounds of calf over their productive lifespan (Lesmeister et al., 1973).

Anestrus. In mature cows, postpartum anestrus is defined as the interval from calving to the first estrus post-calving. Among both *Bos indicus* and *Bos taurus* cattle, length of the anestrous period is affected by negative feedback from calf suckling as well as nutritional status (see review by Jolly et al., 1995; Montiel and Ahuja, 2005). Historically, postpartum *Bos indicus* cows have been thought to have a longer period of anestrus following calving, perhaps due to increased sensitivity to the aforementioned stimuli. However, it is unclear whether this is true for *Bos indicus* broadly or more specifically in the context of harsh tropical environments, as some data published from U.S. trials suggest similar lengths of anestrus are observed among *Bos taurus* and *Bos indicus* cows (Stahringer et al., 1999; Webb et al., 2001; Strauch et al., 2003). It is possible that differences in length of the anestrous period were one more pronounced, but that selection pressure has decreased the length of postpartum anestrous among *Bos indicus*. However, it does appear that cyclicity and fertility of *Bos indicus* cows is more likely to be impacted by environmental conditions (Dale et al., 1959; Tomar, 1966; Luktuke and Subramanian, 1961; Plasse et al., 1970) and even influenced by seasonality through effects of day length (Anderson, 1944; Tomar, 1966; Jochle, 1972; Randel, 1984). Data also suggest that gestation length is longer among *Bos indicus* (292 days; Plasse et al., 1968b) compared to *Bos taurus* cows (282 days; Lush et al., 1945), although it should be noted that average length of gestation has decreased in many breeds due to selection for calving ease. In summary, when considering the net impact of all of these potential differences, it is certainly conceivable that higher rates of anestrus may be observed at the start of the breeding season in *Bos indicus*-influenced cow herds. Therefore, producers selecting reproductive management programs for *Bos indicus*-influenced females should carefully evaluate the reported efficacy among anestrous as well as cyclic cows.

Ovarian Follicular Development. Several studies have raised questions as to general differences between *Bos indicus* and *Bos taurus* females with respect to estrous cycle length and/or number of ovarian follicular waves in a cycle. Some studies have found the most common estrous cycle among *Bos indicus* females consists of three ovarian follicular waves (Zeitoun et al., 1996; Gambini et al., 1998; Viana et al., 2000; Mollo et al., 2007; Bastos et al. 2010), while other researchers (Alvarez et al., 2000) have observed two-wave cycles as more common among *Bos indicus* females, as is the case among *Bos taurus* (Sirois and Fortune, 1988). In one study, mature *Bos indicus* cows most commonly had two-wave estrous cycles and *Bos indicus* heifers most commonly had three-wave estrous cycles (Figueiredo et al., 1997). Thus, it is possible that there is considerable variability observed among *Bos indicus* females in number of ovarian follicular waves per cycle, perhaps more so than is typically observed among *Bos taurus* females. Likewise, published ovarian follicular data from *Bos indicus* females indicate smaller diameter dominant follicles are typically observed among *Bos indicus* females (Figueiredo et al., 1997; Alvarez et al., 2000; Sartorelli et al., 2005; Mollo et al., 2007; Bastos et al., 2010) compared to *Bos taurus* females (Ginther et al., 1989). The functional consequences of these differences in the context of estrus synchronization are not fully understood. However, data generated among *Bos taurus* females indicates that response to exogenous GnRH administration is variable based on stage of follicular development and physiological maturity of the follicle (Vasconcelos et al., 1999; Geary et al., 2000; Moreira et al., 2000). Some (Fernandes et al., 2001) but not all (Portillo et al., 2008) studies have observed low rates of ovulatory response among *Bos indicus* females administered GnRH at a random stage of the estrous cycle. Thus, it is often suggested that the ovulatory response to exogenous GnRH administration is reduced or that the time window of responsiveness is narrower among *Bos indicus*-influenced females. Decreased ovulatory response to exogenous GnRH may also be influenced by differences in the magnitude of LH released by *Bos indicus* females. Multiple authors have reported reduced LH release by *Bos indicus* females in response to exogenous GnRH (Griffin and Randel, 1978) or exogenous estradiol (Rhodes et al., 1978), with some indication that the magnitude of LH released in crossbred females is inversely proportional to the percentage of *Bos indicus* influence in the female's breed composition (Portillo et al., 2008).

Due to the challenge presented by *Bos indicus*-influenced females relative to ovarian follicular development, some researchers feel that estrus synchronization protocols need to be modified to optimize results among *Bos indicus*-influenced females. Recently, a number of publications have suggested that circulating progesterone concentrations need to be taken into greater consideration when designing protocols for *Bos indicus*-influenced females, as progesterone is a key indirect regulator of ovarian follicular development. High circulating concentrations of progesterone decrease LH pulsatility, thereby limiting follicular development and reducing ovulatory capacity. *Bos indicus* females appear to be more sensitive to these effects of progesterone than are *Bos taurus* females (Randel, 1984; Carvalho et al., 2008). As a result, several research efforts have centered around reducing endogenous progesterone production or limiting exogenous progesterone administration during estrus synchronization (Carvalho et al., 2008; Peres et al., 2009; Claro et al., 2010; Mantovani et al., 2010; Cipriano et al., 2011; Williams et al., 2012).

Susceptibility to Stress. *Bos indicus*-influenced cattle are more susceptible to stressors, and the stress induced by animal handling and sorting has the potential to decrease success rates when using reproductive technologies (Galina et al., 1996; Dobson and Smith, 2000; Cooke, 2014). Cortisol, a dominant hormone associated with the stress response, is reported to be higher in temperamental cattle compared to cattle that are calm (Cooke et al., 2009a;2009b; Cooke et al., 2012a; Francisco et al., 2012a). Elevated concentrations of cortisol have a suppressive effect on LH pulsatility by decreasing responsiveness of the anterior pituitary to GnRH (Breen and Karsch, 2003). Potential effects of stress on ovarian follicular development should be carefully considered among *Bos indicus*-influenced females, especially given the challenges already discussed relative to ovarian follicular development in this biological type. In addition to minimizing stress placed upon animals, producers of *Bos indicus*-influenced cattle should consider opportunities to improve temperament through management that acclimates animals to human contact. In one study, efforts to acclimate heifers to human handling improved temperament, reduced cortisol, and hastened puberty and pregnancy attainment (Cooke et al., 2009a). Additionally, heritably of temperament is reported to be moderately to highly heritable ($h^2 = 0.4$), suggesting significant progress can be made through selection and culling decisions (O'Bleness et al., 1960).

Estrous Response. Accurate detection of estrus is more difficult among *Bos indicus*-influenced breeds of beef cattle compared to *Bos taurus* for several reasons (Rae et al., 1999). *Bos indicus* females have a shorter period of standing estrus (Anderson, 1936; De Alba et al., 1961; Plasse et al., 1970; Mizuta, 2003). Purebred *Bos indicus* and crossbred *Bos indicus* x *Bos taurus* females induced to express estrus through use of exogenous estradiol also expressed estrus for a shorter period of time than did purebred *Bos taurus* females, indicating potential subspecies differences in physiological responsiveness to estradiol (Rhodes and Randel, 1978). Differences in timing of estrus onset may also result in more difficulty detecting estrus among *Bos indicus*-influenced females, as the majority of *Bos indicus* cows exhibit estrus in the late evening or at night (Pineheiro et al., 1998). As a result, AI programs that rely on accurate estrus detection may be less efficient among *Bos indicus*-influenced females, especially when considering the susceptibility of *Bos indicus*-influenced females to handling stress and the potential stress induced by separating off estrous females for AI. Hence, there is significant interest in use of timed AI programs that eliminate the potential error and stress associated with estrus detection. It should be noted, however, that estrous response still affects fertility in timed AI programs, as pregnancy rates of females that express estrus prior to timed AI are 27% higher than those of females that fail to express estrus (meta-analysis by Richardson et al., 2016). Therefore, estrous response prior to timed AI is carefully considered when designing such protocols.

ESTRUS SYNCHRONIZATION FOR *BOS INDICUS*-INFLUENCED FEMALES

Other authors in these proceedings will provide a detailed overview of estrus synchronization protocols developed for *Bos taurus* females. Unfortunately, producers of *Bos indicus*-influenced cattle may find their options for estrus synchronization are limited. While numerous protocols have been developed for effective synchronization of estrus among *Bos indicus* cattle, the majority of the research in this area has come out of South America, Australia, and other areas outside of the United States. In these countries, protocols often rely heavily on use of estradiol products, such as estradiol benzoate and estradiol cypionate. Currently, estradiol products are not available or legal for estrus synchronization in the United States. This has not always been the case. For example, the Syncro-Mate-B system used in the 1980s and early 1990s involved administration of estradiol valerate. Likewise, an estradiol cypionate product was marketed by Pfizer Animal Health until it was voluntarily removed from the market. No estradiol products are currently available, however, as none have been approved by the United States Food and Drug Administration's Center for Veterinary Medicine for use in estrus synchronization. Moreover, FDA regards use of estradiol in food producing animals as illegal and has repeatedly warned veterinarians and pharmacies against compounding estradiol products for reproductive purposes. Lastly, it appears unlikely that a pharmaceutical manufacturer will seek label approval in the U.S. for a new estradiol product, given the current regulatory attitude toward use of steroid hormones, the high cost of animal clearance trials, and the limited market for such products (Lauderdale, 2016). Therefore, the following discussion will focus on approaches that are available to U.S. beef producers.

Protocols for Bos indicus-Influenced Beef Cows. One of the first estrus synchronization protocols evaluated extensively among *Bos indicus*-influenced females in the United States was the Syncro-Mate-B protocol previously discussed, which is no longer available on the U.S. market. Although average pregnancy rates with this protocol were somewhat acceptable, results varied considerably among herds and were often discouraging (see review by Williams et al., 2005). Since that time, applied research in *Bos indicus*-influenced mature cows has generally focused on adaptation of protocols originally developed for *Bos taurus* females, as early results were discouraging using the standard version of these synchronization systems among *Bos indicus*-influenced females (Hiers et al., 2003; Saldarriaga et al., 2007). For an extensive overview of the data relating to assessment of protocols among mature *Bos indicus* cows, see review by Yelich and Bridges (2012).

Currently, only one protocol for *Bos indicus*-influenced cows is widely publicized by the Beef Reproduction Task Force: the PG 5-d CO-Synch + CIDR (Figure 1). This protocol, more commonly referred to as "Bee Synch" due to its development at the Texas A&M AgriLife Research Station in Beeville, Texas, has been adopted by a number of producers in the southern United States due to encouraging results observed in *Bos indicus*-influenced cow herds. The PG 5-d CO-Synch + CIDR ("Bee Synch") protocol is similar to the 5-d CO-Synch + CIDR protocol for mature beef cows, with two modifications. First, PG is administered at the start of the protocol concurrent with GnRH administration and CIDR insertion. This induces luteolysis among the majority of cyclic cows and thereby reduces circulating progesterone concentrations during treatment. This enhances follicular development among *Bos indicus*-influenced females, as previously discussed. Second, FTAI is performed at 66 h following CIDR removal in the PG 5-d CO-Synch + CIDR ("Bee Synch") protocol, compared to 72 h as in the standard 5-d CO-Synch + CIDR protocol. This is to accommodate for earlier onset of estrus in the 5-d CO-Synch + CIDR ("Bee Synch") protocol, since the administration of PG at the start of the protocol ultimately results in altered timing of estrous response compared to the standard 5-d CO-Synch + CIDR protocol.

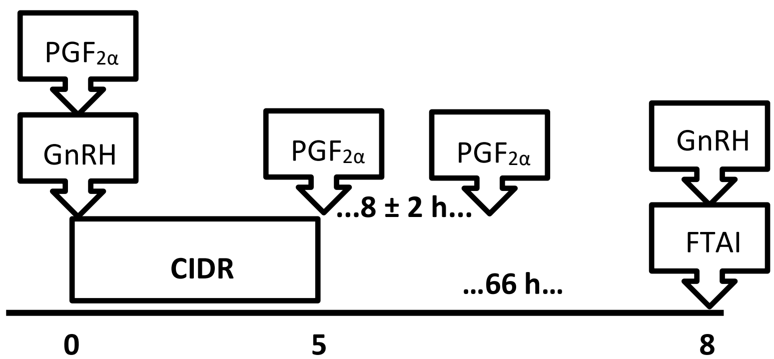

Figure 1. The PG 5-d CO-Synch + CIDR ("Bee Synch") protocol followed by FTAI

Ongoing efforts with the PG 5-d CO-Synch + CIDR ("Bee Synch") protocol are evaluating the potential to remove GnRH administration from the start of the protocol (Scarpa et al., 2017). Early results suggest that, specifically in *Bos indicus*-influenced cows, this administration of GnRH may not enhance synchrony of ovarian follicular waves, ovulatory follicle size, or any other variable associated with fertility. If final results in fact indicate this GnRH administration may be removed from the protocol, it may also allow producers to administer a single dose of PG at CIDR removal rather than two separate doses. This could be a substantial savings in both cost and labor. However, final results from this work are forthcoming, and producers should refer to the protocol sheets published each year by the Beef Reproductive Task Force for current recommendations.

Protocols for *Bos indicus*-Influenced Heifers. For estrus synchronization and FTAI of heifers, the Beef Reproduction Task Force currently recommends four protocols: two short-term protocols (5-d CO-Synch + CIDR and 7-d CO-Synch + CIDR) and two long-term protocols (MGA-PG and 14-d CIDR-PG). For a detailed discussion of the development of these protocols, see review by

Patterson et al. in these proceedings. Currently, no estrus synchronization protocol is recommended by the Beef Reproduction Task Force specifically for use in *Bos indicus*-influenced heifers, and published results for *Bos indicus*-influenced heifers are limited. However, several recent research efforts by our lab and others have sought to evaluate commonly used systems in this biological type.

Short-term protocols to synchronize estrus among *Bos indicus*-influenced heifers have been evaluated with mixed results. A recent report by Oosthuizen et al. (2018) reported use of the 5-d and 7-d CO-Synch + CIDR protocols in *Bos indicus*-influenced heifers, finding use of either protocol followed by FTAI increased the proportion of heifers becoming pregnant early in the breeding season compared to a non-synchronized, natural service control. In contrast, some datasets (data from Bischoff et al. reviewed by Yelich and Bridges, 2012; Williams et al., 2012) report these protocols, particularly the 5-d CO-Synch + CIDR, performed poorly among *Bos indicus*-influenced heifers. It is possible that outcomes following short-term protocols are particularly influenced by rates of cyclicity prior to estrus synchronization. In the data reported by Oosthuizen et al. (2018), a high proportion of heifers were pubertal, likely contributing to strong results. It is unclear at this time whether modifications to the short-term protocols can improve consistency of results among *Bos indicus*-influenced heifers. Naturally, there is interest in the effect of PG administration at the start of the protocols, similar to the approach used in the PG 5-d CO-Synch + CIDR ("Bee Synch") protocol for *Bos indicus*-influenced cows; however, data is limited at this time.

Field data collected among *Bos taurus* heifers in the Missouri Show-Me-Select™ Replacement Heifer Program suggest that results may be more consistent across varying pubertal status groups when using long term protocols, potentially due to the fact that cyclicity is induced among prepubertal heifers earlier in the treatment schedule (Locke et al., 2016). Short-term and long-term protocols have not been directly compared among *Bos indicus*-influenced females. However, long-term progestin-based protocols appear to be very effective. In one recent publication using the 14-d CIDR-PG protocol, we reported similar pregnancy rates among *Bos indicus*-influenced heifers in a subtropical environment and *Bos taurus* heifers in a temperate environment, provided that results were analyzed relative to the reproductive tract score of the heifers (Table 2; Thomas et al., 2017). While pregnancy rates were lower among *Bos indicus*-influenced heifers overall due to lower average RTS, similar pregnancy rates were observed within respective RTS categories. We proposed this indicated similar efficacy of the protocol among *Bos indicus*-influenced and *Bos taurus* heifers from a physiological perspective, and increasing rates of puberty attainment prior to the breeding season would likely improve response.

In a subsequent, more comprehensive experiment, we evaluated use of long-term protocols among *Bos indicus*-influenced heifers (n = 1,456) prior to either FTAI or natural service (Locke et al., 2018). Weights and reproductive tract scores (RTS; Scale 1-5) were obtained for heifers prior to assignment of one of five treatments: Non-synchronized + natural service (NS); melengesterol acetate (MGA) + natural service; CIDR + natural service; MGA-PG + FTAI; and 14-d CIDR-PG + FTAI. Heifers in the three natural service treatments were exposed to fertile bulls for 65 days, beginning 10 days after progestin removal for CIDR + NS and MGA + NS groups. Heifers in FTAI treatments were administered PG 16 or 19 days following CIDR or MGA removal. Fixed-time AI was performed 66 and 72 h after PG for CIDR-PG and MGA-PG treatments respectively, and heifers in FTAI treatments were exposed to fertile bulls 12 days following FTAI. Pregnancy status was determined via ultrasound at the end of a 65-d breeding period. Estrous response after PG (52% versus 53%) and pregnancy rates after FTAI (40%) did not differ between MGA-PG and 14-d CIDR-PG treatments. Across all treatments, the proportion of heifers becoming pregnant in the first 21 days of the breeding period and total pregnancy rate at the end of the 65 day breeding period were compared relative to pubertal status (prepubertal RTS = 1 and 2; peripubertal RTS = 3; pubertal RTS = 4 and 5). Pregnancy rates by day 21 and by the end of the breeding season were influenced by pretreatment pubertal status ($P \leq 0.02$) and weight ($P \leq 0.05$), illustrating the importance of adequate heifer development. Pregnancy rates observed among *Bos indicus*-influenced heifers using the long-term protocols were decreased on the average in comparison to data published for *Bos taurus* heifers; however, pregnancy rates again appeared similar when accounting for differences in reproductive tract score.

It is unclear whether the long-term protocols commonly used among *Bos taurus* heifers need to be modified in order to accommodate differences in the reproductive physiology of *Bos indicus*-influenced heifers. One publication suggested results with the MGA-PG protocol could be improved through use of a "split" dose of PG 24 h apart (Bridges et al., 2005); however, this modification has seen little adoption by producers, and the additional animal handling required may be impractical. Moreover, since the pregnancy rates we reported among *Bos indicus*-influenced heifers were similar to published results among *Bos taurus* heifers when accounting for reproductive tract score (Thomas et al., 2017; Locke et al., 2018), we suggest the long-term protocols in their current forms (Figure 2) function adequately in both biological types. For producers seeking optimal FTAI results, we suggest use of a long-term progestin-based protocol (MGA-PG or 14-d CIDR-PG) whenever scheduling allows, simply due to the consistency observed among groups with mixed pubertal status. However, the importance of adequate heifer development cannot be overstated regardless of the protocol used.

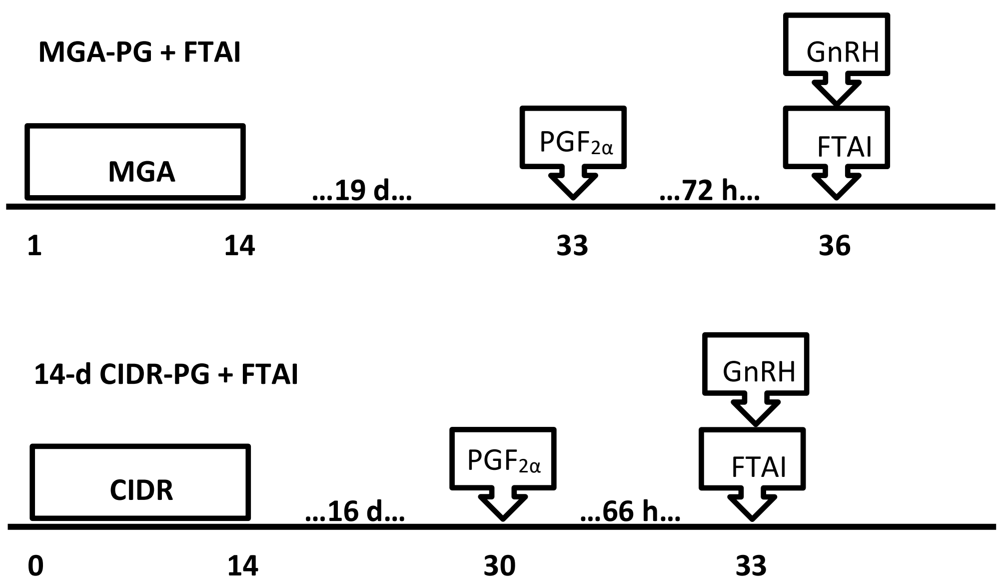

Figure 2. The MGA-PG and 14-d CIDR-PG protocols followed by fixed-time artificial insemination (FTAI).

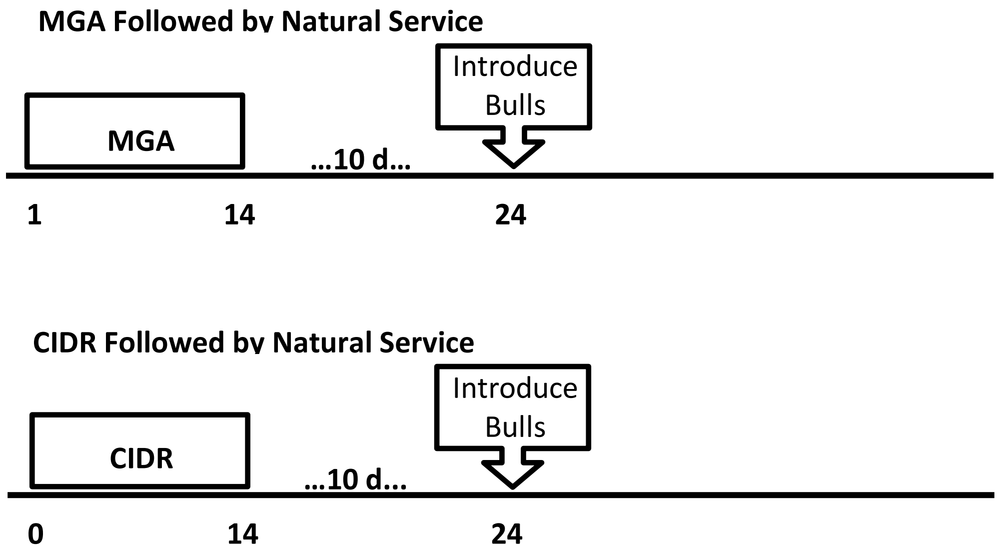

Figure 3. Estrus synchronization with either MGA or CIDR prior to natural service. Note that bulls are introduced 10 days after progestin treatment and PG is not administered.

One clear advantage of estrus synchronization is the induction of puberty among a large proportion of prepubertal and peripubertal heifers. Similar to the results discussed above with the long-term protocols (Locke et al., 2018), other efforts to induce puberty among *Bos indicus*-influenced heifers reported earlier attainment of pregnancy when heifers were treated with progestin prior to the start of a natural service breeding season (Moriel et al., 2017). Therefore, even producers who are unable or unwilling to implement an AI program should strongly consider use of estrus synchronization protocols prior to natural service. We suggest use of the long-term progestin treatments that form the basis of the MGA-PG and 14-d CIDR-PG protocols. An initial subfertile estrus will occur in the days following withdrawal of MGA from the feed or removal of CIDR inserts; therefore, producers should wait to introduce bulls until approximately 10 days after progestin withdrawal. Producers using these protocols with natural service are also advised not to administer PG but rather allow for a loosely synchronized return to estrus (Figure 3).

SUMMARY

Meaningful physiological differences exist between *Bos indicus*-influenced and *Bos taurus* females, and reproductive programs designed for *Bos indicus*-influenced females must take these differences into account. Research does suggest that, on the average, lower pregnancy rates are obtained among *Bos indicus*-influenced compared to *Bos taurus* females when using many reproductive technologies. Likewise, estrus synchronization options are admittedly more limited for producers of *Bos indicus*-influenced cattle. However, the systems discussed here are effective options, and more are likely to be developed in the coming years. As implementing an estrus synchronization and AI program has been demonstrated to result in continued improvement in reproductive performance and profitability over successive years (Lamb, 2015), producers of *Bos indicus*-influenced cattle should recognize these technologies as viable options with long-term potential impact.

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