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ESTRUS SYNCHRONIZATION METHODS FOR EMBRYO TRANSFER IN *BOS INDICUS* CATTLE

C. R. Looney, J. S. Nelson, E. H. Riano and D.W. Forest
Ovagenix, Bryan, TX and
Department of Animal Science, Texas A&M University, College Station, TX

Introduction

Reproduction is the main limiting factor in production efficiency of beef cattle. The largest loss of the potential calf crop occurs because cows fail to become pregnant due to anestrus and postpartum infertility (Short et al., 1990). Estrus synchronization is a method that has been studied for 40 years to control reproductive efficiency of beef cattle. Its purpose is to manipulate the estrous cycle of a herd to allow for timed artificial insemination and/or superovulation with subsequent embryo transfer into recipient cows or heifers at a predetermined time (Odde, 1990). Estrus synchronization allows for increased production efficiency of a herd by achieving shorter breeding and calving seasons, along with possible control of anestrous cows (Odde, 1990). In turn, the shorter seasons allow for lower labor requirements throughout the year and increase the percentage of the herd that calves early in the calving season. Calves that are born earlier have heavier weaning weights and allow for a longer postpartum period before re-breeding (Burke and Macmillan, 1996). Estrous synchronization also allows for disease control among herds and genetic improvements through the use of AI/ET.

Estrous Synchronization Methods

Estrous synchronization, as the name implies, is the manipulation of the estrous cycle in order to bring a group of females, at random stages of the estrous cycle, into estrus at a precise time. The following section will discuss means to manipulate estrous cycles and synchronization in Bos Indicus influenced cattle.

Progesterone is the dominant ovarian hormone present in the circulation during the estrous cycle and is secreted from the corpus luteum (CL). This period of the estrous cycle is also referred to as the luteal phase and lasts from the time of ovulation until regression or luteolysis of the CL near the end of the cycle. Progestins suppress estrus in cattle and have been used extensively to alter the estrous cycle. Studies during the 1940s revealed that estrus could be delayed and therefore synchronized by administration of exogenous progesterone to cattle or sheep. This led to many studies in which progestins were administered by injection, released by an intravaginal sponge, or fed for a period of up to and exceeding the length of the estrus cycle to synchronize estrus following the cessation of administration. It was determined that an increased duration of progestin administration resulted in an increased rate of estrus synchronization. However, fertility was compromised following administration of progestins for 14 d or longer and pregnancy rates were unacceptable (Odde, 1990).

One of the first methods used to synchronize estrus in cattle was the long-term feeding of melengestrol acetate (MGA; Zimbelman and Smith, 1966). MGA is a synthetic progestin that suppresses estrus when fed at the rate of 0.5 mg/hd/d. MGA is still utilized extensively today by feedlots to suppress estrus in beef heifers that are being fed for harvest and used for estrous synchronization of heifers with a 14 d feeding program followed by a single injection of PGF 17 d after withdrawl of MGA feeding. It is well established that administration of exogenous progesterone can hasten the attainment of puberty in heifers and cause postpartum anestrous cows to become estrous cycling. The ability of exogenous progestins to induce estrus in anestrous cattle has been attributed to, in part, increased LH secretion both during and after treatment. It has been reported that progestin treatment increased LH secretion in postpartum beef (Garcia-Winder et al., 1986) as well as seasonal dairy cows (Rhodes et al., 2002). In addition, LH secretion following weaning was increased in cows with prior exposure to progestin (Bruel et al., 1993). This induced increase in LH is important because it mimics the proestrus increase in LH leading to the preovulatory LH surge (Day, 2004).

Prostaglandins are lipids consisting of 20-carbon unsaturated hydroxy fatty acids derived from arachidonic acid. Prostaglandin $F_2\alpha$ (PGF) is produced by the uterine endometrium and is responsible for luteolysis, or degradation of the CL, in cattle. The bovine estrous cycle can be divided into two phases, the follicular phase and the luteal phase. The follicular phase is characterized by follicle growth culminating in selection of a dominant follicle and subsequent ovulation. The luteal phase is the longest phase of the cycle (approximately d 6 to d 16 of the estrous cycle). The luteal phase is characterized by the functioning CL secreting progesterone. During the late luteal phase (d 16 – 18 of the cycle) PGF is released from the uterus and binds to the CL causing luteal regression. During the 1970s, it was discovered that PGF was luteolytic in cattle and could be used to synchronize estrus (Lauderdale et al., 1974). It was later determined that PGF had limited utility in synchronizing estrus because it was only effective in cattle that were cycling and had a CL (d 5 to 17 of the cycle). Therefore, prepubertal heifers, anestrous females, females on d 0 to 4 of the estrous cycle, and females in the final days of the estrous cycle subsequent to luteolysis were not responsive. It was later determined that the interval from treatment with PGF to estrus was dependent upon the stage of the follicular wave at treatment (Lucy et al., 1992). Larger, more mature follicles ovulated sooner than their smaller, less mature counterparts.

Another method of estrous synchronization includes the use of gonadotropin releasing hormone (GnRH) or GnRH agonists in combination with and injection of PGF. This method is available to consumers as the Ovsynch program (Pursley et al., 1996). The protocol includes an injection (im) of GnRH (100 ug) and an injection of PGF (25 mg, im) 7 days later. Through day 9 of the protocol, 80% of treated cows and heifers were detected in estrus and fertility rates were as high as 85%. GnRH eliminates the large follicles by ovulation or atresia and induces emergence of a new follicular wave within 3 to 4 days after treatment during any stage of the estrous cycle (Twagiramungu et al., 1995). By the addition of CIDR with this GnRh program (Co-Synch) the additive effects of progesterone along with the ability to initiate a new follicle wave with the GnRh.

Bos Indicus vs. Bos Taurus Cattle

Brahman cattle are significantly different in several reproductive aspects than European and Continental cattle. Brahman cattle have longer gestations (292 vs. 285 days) shorter and less intense estrus and puberty occurs at an older age. In addition the twinning rate is less in *Bos Indicus* than in *Bos taurus* (Rutledge, 1975). In addition, secretory patterns of hormone production in *Bos indicus* have been shown to be different than *Bos taurus* and may change due to photoperiod or season. However, the single most cited negative factor of the Brahman cow is the sub-standard fertility when compared to the English breeds of beef cattle (Warnick 1956, Reynolds 1963).

There are other differences in reproductive physiology between *Bos Indicus* and *Bos Taurus,* with Brahman cattle having reduced duration of estrus and a shorter period from onset of estrus to the LH surge as well as from the LH surge to ovulation (Randel, 1984). In addition, *Bos Indicus* females have lower preovulatory LH surges than *Bos Taurus females* and their luteal cells are less responsive to LH in vitro especially in the winter (Randel, 1984). *Bos Indicus* also have higher number of follicles and higher serum concentrations of insulin growth factor I. Researchers have recently found differences in timing of ovulation, fertilization or events leading up to cleavage of early embryos in Brahman cattle compared to Holsteins (Krininger et.al. 2003). Brahman and Brahman influenced females are at times more difficult pass catheters through their crooked and large cervixes and their disposition make it more difficult to handle them in pens and corrals.

Donors

Traditionally, bovine superovulation programs have utilized detected estrus followed by FSH treatments beginning between days 9 through 13 of the estrous cycle. These programs require a large amount of time commitment and are inefficient at controlling follicular waves in cattle. The objective of this study was to compare traditional superovulation regimes with those implementing the use of an intravaginal progesterone releasing device (CIDR) with injections of estradiol benzoate (2.5 mg, im) and progesterone (50 mg, im) at CIDR insertion.

Meyer et al. (2000) detected estrus and performed 103 embryo collections (Table 1). Cows that showed estrus days 1-7 of the estrous cycle, days 8-16, and days 17-24 prior to CIDR, P4, EB treatment began FSH injections four days after CIDR insertion. Superovulation was achieved with varied doses of FSH (140-400 mg, im) given bid for 4 d followed by PGF on day 3 (Brahman influenced breeds) or day 4 (Continental and English breeds) of CIDR removal depending on breed, on the third or fourth day of superovulation. Estrus was detected and artificial inseminations performed at onset of estrus, 12 h and 24 h post-estrus. Embryos were collected 7 to 7.5 d from onset of estrus. Here were no differences in any parameter based on timing of CIDR insertion. From the results the authors concluded that donors could successfully be superovulated without regard to estrous cycle using the CIDR+P4+EB program in a commercial setting. Figures 1 and 2 compare collection results from initiation of FSH at different stages of the estrous cycle. No significant differences were detected, however it appears trends may be present and difference may not be seen due to low experimental numbers.

Table 1. Least Squares Means of superovulatory responses with or without CIDR-P4-EB.

	Total ova		Viable embryos		Degenerate		Unfertilized	
	Brahman	Cont.	Brahman	Cont.	Brahman	Cont.	Brahman	Cont.
Control	11.5[a]	12.4[a]	6.5[a]	5.9[a]	2.4[a]	2.5[a]	2.6[a]	3.9[a]
	± 0.86	± 0.86	± 0.57	± 0.58	± 0.31	± 0.31	± 0.50	± 0.50
CIDR	9.7[b]	9.4[b]	5.8[a]	5.1[a]	1.8[a]	2.1[a]	2.0[a]	2.2[b]
	± 0.97	± 0.70	± 0.65	± 0.47	± 0.34	± 0.25	± 0.56	± 0.40

[a-b]Data in columns with different superscripts are different ($P < 0.05$, ± SEM, Student's t-test).
Cont = is Continental and English breeds.

Figure 1. Embryo recovery in Bos Taurus-influenced females by stage of cycle at intitiation of the CIDR protocol.

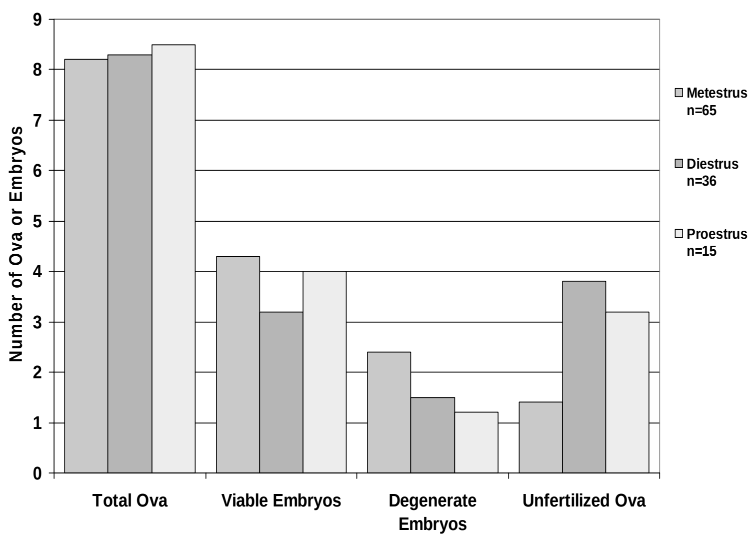
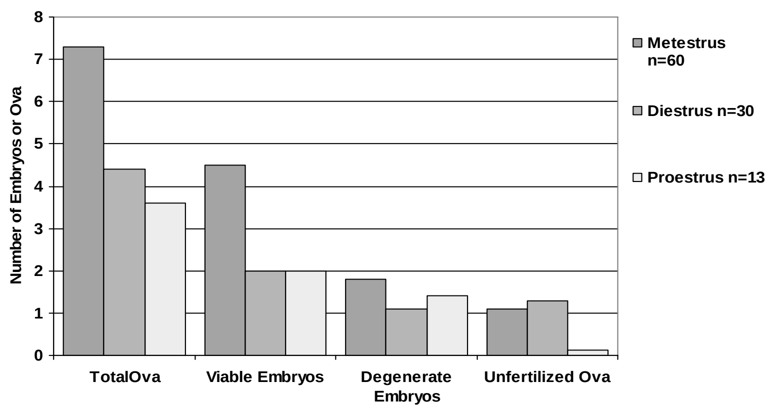


Figure 2. Embryo recovery in Bos Indicus-influenced females by stage of cycle at initiation of the CIDR protocol.



Meyer 2002

Recipients

Although much progress has been made in estrus synchronization in cattle in recent years, it remains to have the most limiting factors in widespread implementation of AI and ET technology. Most of the early protocols were developed by lengthening or shortening the luteal phase with either progesterone or prostaglandin. As researchers began to understand that estrus synchrony entailed ovulation control did programs develop to control the stage of follicular development at the beginning of treatment. Steroid hormone treatments can also be used to alter follicular growth. Both progesterone and estradiol influence the onset of subsequent follicular wave emergence and the combined effects of these steroids have demonstrated a controlled suppression on growth of the dominant follicle and is the most effective and consistent new wave emergence and subsequent ovulation control developed. Synchronization protocols allowing that enable tighter synchrony have been developed and higher overall pregnancy rates achieved (Table 2).

Table 2. Comparing ET service and pregnancy rate of PGF single injection synchronization method to CIDR+P4/E2+PGF+E2.

Synch Method	N	Synchrony (%)	Interval to Estrus (d)	Service (%)	FSCR (%)	FSPR (%)
25 mg PGF	1390	51[a]	3.6	93	63	29[a]
7d CIDR+ P4/E2+PGF+E2	753	94[b]	2.1	83	53	41[b]

[a b] Differing subscripts significant at P<0.05.
Data from Ovagenix's In-Clinic Programs

The following tables contain data from synchronization studies performed at a large Registered Brangus ranch in Central Texas. Information gained from these trials were instrumental in gaining the initial approval issues of the CIDR insert in beef cattel Post-partum Interval (PPI) has a significant effect on first service conception rate and first service pregnancy rate to embryo transfer and final pregnancy rate to natural service (Table 3). Early post-partum females had a lower ($P < 0.01$) first service conception rate and first service pregnancy rate to embryo transfer when compared to medium and late post-partum females. In addition, early post-partum females had a lower ($P < 0.01$) final pregnancy rate to natural service when compared to medium and late post-partum females. PPI did not have an effect ($P = 0.7$) on synchrony rate, interval to onset of estrus from time of CIDR removal, and service rate to embryo transfer.

Table 3. Synchrony rate, interval to estrus, first service conception rate (FSCR) and first service pregnancy rate (FSPR) to embryo transfer after CIDR administration and final pregnancy rate (Final PR) by post-partum interval (PPI) in females.

PPI	n	Synchrony (%)	Interval to Estrus (d)	Service (%)	FSCR (%)	FSPR (%)	Final PR (%)
Early (≤ 45 d)	120	99.2	1.9	79.0	46.9 [b]	31.6 [b]	84.2 [b]
Medium (46 – 75 d)	207	99.5	2.0	89.6	60.3 [a]	53.4 [a]	96.1 [a]
Late (≥ 76 d)	106	99.1	1.9	83.9	57.5 [a]	51.8 [a]	96.2 [a]

Columns with different superscripts are different; $P < 0.01$.
Meyer 2002.

Lactational state had a significant effect on synchrony rate and interval to onset of estrus from time of CIDR removal (Table 4). Non-lactating females had a higher ($P < 0.01$) synchrony rate and a significantly longer ($P < 0.01$) interval to onset of estrus from time of CIDR removal than lactating females. Lactational state had no effect ($P = 0.6$) on service rate, first service conception rate, and first service pregnancy rate to embryo transfer and final pregnancy rate to natural service.

Table 4. Synchrony rate, interval to estrus, service rate, first service conception rate (FSCR) and first service pregnancy rate (FSPR) to embryo transfer after CIDR administration and final pregnancy rate (Final PR) by lactational state of females.

Lactational State	n	Synchrony (%)	Interval to Estrus (d)	Service (%)	FSCR (%)	FSPR (%)	Final PR (%)
Non-lactating	253	95.6 [a]	2.5 [a]	90.1	50.8	43.3	87.7
Lactating	1165	86.3 [b]	2.0 [b]	89.3	55.3	43.4	83.8

Columns with different superscripts are different; P < 0.01.
Meyer 2002.

Duration of CIDR treatment (7 or 8-d) had a significant effect on synchrony rate (Table 5). Females that were administered a 7-d CIDR had a lower (P < 0.01) synchrony rate than did those females that received an 8-d CIDR. There was no difference (P = 0.8) in interval to onset of estrus from time of CIDR removal, service rate, first service conception rate or first service pregnancy rate to embryo transfer and final pregnancy rate to natural service between females that received either a 7 or 8-d CIDR.

Table 5. Synchrony rate, interval to estrus, service rate, first service conception rate (FSCR) and first service pregnancy rate (FSPR) to embryo transfer after CIDR administration and final pregnancy rate (Final PR) by CIDR administration for a duration of either 7 or 8 days.

Days of CIDR Insertion	n	Synchrony (%)	Interval to Estrus (d)	Service (%)	FSCR (%)	FSPR (%)	Final PR (%)
7	712	82.9 [b]	1.8	90.2	54.6	41.9	78.2
8	457	92.2 [a]	1.7	87.9	56.5	46.6	86.7

Columns with different superscripts are different; P < 0.01.
Meyer 2002.

Administration of estradiol benzoate (EB) 24 h post-CIDR removal had a significant effect on synchrony rate, interval to onset of estrus from time of CIDR removal, and first service pregnancy rate to embryo transfer (Table 6). Females that received EB 24 h post-CIDR removal had a higher (P < 0.01) synchrony rate and first service pregnancy rate to embryo transfer than females that received No EB. Females that received No EB 24 h post-CIDR removal had a longer (P < 0.01) interval to onset of estrus from time of CIDR removal when compared to females that received EB. There was no difference (P = 0.5) in service rate, first service conception rate to embryo transfer, and final pregnancy rate to natural service between females that were treated with EB and those that were not.

Table 6. Synchrony rate, interval to estrus, service rate, first service conception rate (FSCR) and first service pregnancy rate (FSPR) to embryo transfer after CIDR administration and final pregnancy rate with estradiol benzoate (EB) or without estradiol benzoate (No EB) administration 24 h post-CIDR removal.

Treatment	N	Synchrony (%)	Interval to Estrus (d)	Service (%)	FSCR (%)	FSPR (%)	Final PR (%)
EB	1041	94.3 [a]	1.5 [b]	88.7	55.2	46.1 [a]	85.3
No EB	377	73.5 [b]	2.5 [a]	91.5	52.8	37.2 [b]	82.2

Columns with different superscripts are different; P < 0.01. Meyer 2002.

Parity had a significant effect on synchrony rate, interval to onset of estrus, service rate to embryo transfer and final pregnancy rate (Table 7). Primiparous females had a higher (P < 0.01) synchrony rate and final pregnancy rate than did multiparous females. Multiparous females had a longer (P < 0.01) interval to onset of estrus from time of CIDR removal than did primiparous females. Multiparous females also had a higher (P < 0.01) service rate than did primiparous females. There was no difference (P = 0.4) between first service conception rate and first service pregnancy rate to embryo transfer between primiparous and multiparous females.

Table 7. Synchrony rate, interval to estrus, service rate, first service conception rate (FSCR) and first service pregnancy rate (FSPR) to embryo transfer after CIDR administration and final pregnancy rate (Final PR) by parity in both lactating and non-lactating females.

Parity	n	Synchrony (%)	Interval to Estrus (d)	Service (%)	FSCR (%)	FSPR (%)	Final PR (%)
Primiparous	250	91.4 [a]	1.8 [b]	83.6 [b]	58.4	44.4	95.2 [a]
Multiparous	1168	87.2 [b]	2.1 [a]	90.7 [a]	53.8	43.1	82.2 [b]

Columns with different superscripts are different; P < 0.01. Meyer 2002.

There was a significant interaction between parity and EB administration on synchrony and service rate (Table 8). Primiparous and multiparous females that were administered EB 24 h post-CIDR removal had a higher (P < 0.01) synchrony rate than primiparous and multiparous females that were not administered EB. Multiparous females that received No EB had a higher (P < 0.01) service rate to embryo transfer than multiparous females that received EB.

Between treatments, primiparous females that were not administered EB had a higher (P < 0.01) synchrony rate than multiparous females that received No EB. However, multiparous females that were not administered EB had a higher (P < 0.01) service rate than did primiparous females that received No EB. There was no difference (P = 0.3) between synchrony rate and service rate to embryo transfer in both primiparous and multiparous females that received EB 24 h post-CIDR removal.

Table 8. Synchrony rate and service rate after CIDR administration by parity of female of female with estradiol benzoate (EB) or without estradiol benzoate (No EB).

Parity	Synchrony (%)				Service (%)			
	EB	(N)	No EB	(N)	EB	(N)	No EB	(N)
Primiparous	93.7 $^{a\,1}$	(191)	86.2 $^{b\,1}$	(87)	85.8 $^{a\,1}$	(191)	78.4 $^{a\,2}$	(87)
Multiparous	94.5 $^{a\,1}$	(923)	70.9 $^{b\,2}$	(412)	89.3 $^{b\,1}$	(923)	94.7 $^{a\,1}$	(412)

Columns with different numerical superscripts are different and rows with different alphabetical superscripts are different; P < 0.01. Meyer 2002.

There was a significant interaction between parity and EB administration 24 h post-CIDR removal on first service conception rate and first service pregnancy rate to embryo transfer (Table 9). Primiparous females that received No EB had a higher (P < 0.01) first service conception rate and first service pregnancy rate to embryo transfer than did multiparous females that received No EB. There was no difference (P = 0.6) in first service conception rate and first service pregnancy rate in both primiparous and multiparous females that were treated with or without EB administration 24 h post-CIDR removal.

Table 9. First service conception rate (FSCR) and first service pregnancy rate (FSPR) to embryo transfer after CIDR administration by parity of female with estradiol benzoate (EB) or without estradiol benzoate (No EB).

Parity	FSCR (%)				FSPR (%)			
	EB	(N)	No EB	(N)	EB	(N)	No EB	(N)
Primiparous	54.9 $^{a\,1}$	(191)	67.2 $^{a\,1}$	(87)	43.7 $^{a\,1}$	(191)	45.9 $^{a\,1}$	(87)
Multiparous	55.2 $^{a\,1}$	(923)	49.8 $^{a\,2}$	(412)	46.6 $^{a\,1}$	(923)	35.4 $^{b\,2}$	(412)

Columns with different numerical superscripts are different and rows with different alphabetical superscripts are different; P < 0.01. Meyer 2002.

Estradiol benzoate (EB) administration, (1 mg, im) at CIDR insertion had a significant effect on final pregnancy rate to natural service (Table 10). Females that were treated with EB at CIDR insertion had a higher ($P < 0.05$) final pregnancy rate than did those females that received No EB at CIDR insertion. EB administration at CIDR insertion had no effect ($P = 0.7$) on synchrony rate, interval to estrus from time of CIDR removal, service rate, first service conception rate and first service pregnancy rate to embryo transfer.

Table 10. Synchrony rate, interval to estrus, first service conception rate (FSCR) and first service pregnancy rate (FSPR) to embryo transfer after CIDR administration and final pregnancy rate (Final PR) with estradiol benzoate (EB) or without estradiol benzoate (No EB) at time of CIDR insertion.

Treatment CIDR In	n	Synchrony (%)	Interval to Estrus (d)	Service (%)	FSCR (%)	FSPR (%)	Final PR (%)
EB							
	85	98.8	2.0	82.8	64.6	54.1	98.8 [a]
No EB							
	348	99.4	1.9	84.9	55.1	47.5	91.4 [b]

Columns with different superscripts are different; $P < 0.05$. Meyer 2002.

Table 11. Use of CIDR and Estradiol Synchronization in Brahman-Influenced Recipients.

	Total	No. Trans. %	No. Preg. (conception %)
Estrus Observed	860(87%)	765(89%)	443(58%)
Estrus Not Observed	137(13%)	77(56%)	50(65%)
Total	997(100%)	842(85%)	493(58%)

Total preg (493) / Total Treated (997) x 100 = 49.3%
Data from V8 Ranch, Boling, Texas

Table 11 shows data from a single Brahman-influenced client owned recipient herd in South Texas. A total of 997 cows were synchronized using CIDR and 50 mg P4 and 2.5 mg E17β upon insertion and removal of the CIDR along with an im injection of PGF on day 7 or 8 followed by 1 mg E17β 24 h post CIDR removal. Calves were removed and estrus was detected for 2 d following the final estradiol injection.

Estrus was observed in 87% of the recipients synchronized. All recipients were ultrasounded prior to ET on day 7 and only recipients with CL>10mm in diameter received either a fresh of frozen embryo. Overall service rate was 85%. Conception rate was detected by ultrasound at 35-45 days of gestation was 58%. The overall pregnancy rate was 49.3%. On one replicate or day of work on 123 head of recipients we achieved 72.4% overall pregnancy rate. We have found no other estrus synchronization protocol work better.

Conclusions

The most significant improvement in the last 10 years of embryo transfer technology has been the ability to synchronize estrus and ovulation of cattle. These improvements have enabled AI and ET to be performed utilizing less total labor. The advances in timed embryo transfer and artificial insemination has somewhat eliminated the necessity to monitor estrus without sacrificing overall success rates. More research needs to be performed to better synchronize Bos Indicus influenced cattle. This is very important along the gulf coast as their ability to thrive in this region makes Brahman crossed cattle essential in many embryo transfer programs as recipients.

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