ANALYSIS OF RECORDS OF EMBRYO PRODUCTION IN RED BRAHMAN COWS

A Thesis

by

EDGAR HERNANDO RIANO ROCHA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2005

Major Subject: Physiology of Reproduction

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Approved by:

Co-Chairs of Committee,	David Forrest
	Andy Herring
Committee Member,	Louise Abbott
Head of Department,	Gary Acuff

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ABSTRACT

Analysis of Records of Embryo Production in
Red Brahman Cows. (August 2005)
Edgar Hernando Riano Rocha, DVM., La Salle University
Co-Chairs of Advisory Committee: Dr. David Forrest
Dr. Andy Herring

Records of embryo production in Red Brahman donor cows (n=50) and F1 recipients (n=531) were evaluated from the collection day to the birth of the embryo produced. The effects of the sire of the donor and the embryo, protocol, season-protocol, and body condition of the donor on the total number of good, degenerated, unfertilized, and total embryos were evaluated. The number of donors collected for protocols 1, 2, and 3 were 50, 39, and 46 respectively. The production of good transferable embryos, and embryos/collection for protocols 1, 2, and 3 were 171 (4.6), 152 (4.6), and 208 (6.3) respectively. The final status of each recipient was recorded as non-pregnant, resorption, abortion, and live calf. The model used to analyze pregnancy state was: protocol, embryo stage, embryo quality, corpora lutea size, and season. The effects of sire of the embryo, season-protocol, protocol, embryo stage, embryo quality, body condition score, and corpora lutea size on gestation length and birth weight were analyzed.

Season-protocol affected ($P<0.05$) the number of degenerated embryos. Mean number of degenerated embryos were higher ($P<0.05$) during winter for protocols 2 and 3 than during other seasons. The ratio for good embryos differed ($P<0.01$) by sire of donor.

The final status of recipients was affected ($P<0.01$) by protocol. The maximum percentage of live calves and the minimum percentage of non-pregnant recipients were achieved for protocol 3.

Gestation length differed ($P<0.01$) by sire of the embryo, season-protocol, protocol, and body condition score. Spring-protocol 3 resulted in the shortest while Fall-protocol 2 resulted in the longest mean gestation length. Calf birth weight differed ($P<0.05$) by season-protocol and by embryo quality. The lightest birth weights resulted from embryo quality grade 2 and from spring-protocol 3.

These results indicate that using protocols that combine 17β-estradiol, FSH and GnRH (protocol 3) during the spring in conjunction with selection for sire of donor can increase embryo production by Red Brahman cows. Use of protocol 3 with donors in the spring, selection of embryo sire for short gestation length and transfer of quality grade 2 embryos can be used to minimize the incidence of dystocia in recipients.

DEDICATION

I would like to dedicate this thesis to my wonderful wife, Adriana, for all her love, support, sacrifice and patience with me during this period of my life to accomplish the goals I had for my professional life.

ACKNOWLEDGMENTS

I would like to gratefully thank Dr. David Forrest for the guidance and support he has given me in the physiology of reproduction area and during the preparation of this thesis.

I would also like to thank Dr. Andy Herring for orienting me in the area of beef cattle production and its situation in the southern part of the United States, for all his advice on the statistical evaluation and interpretation of my research.

I must also thank Dr. Louise Abbott, for what I learned during my class of mammalian embryology, for her objectivity in the evaluation of this research and her good disposition to help and support me.

I also need to thank Josephine and Alfredo Muskus, the owners of Santa Elena Ranch for facilitating all the needed information and making possible the accomplishment of this study.

I couldn't forget to thank God, my Father and Savior, for the opportunity to pursue this degree at Texas A&M University and for giving me the strength and perseverance to accomplish this goal in my life.

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INTRODUCTION

For most embryo transfer (ET) programs in cattle, a major concern is the ability to synchronize estrus with hormones in donors and recipients to ensure that they are at the correct stage of the estrus cycle to recover multiple embryos of good quality from a donor and transfer either a fresh or frozen embryo on a specific day into a recipient. Numerous factors affect the response of these donors and recipients to the hormonal treatments used by the industry. Those factors may include weather patterns, nutrition, age, body condition, number of days after calving, nursing calf aside and breed composition/genetics. The use of ET has been increasing within the cattle industry due to improvements achieved with the ET technique during the last 40 years. The technique has had many changes in animal preparation (donors and recipients), embryo collection, and transfer, freezing, and thawing processes. Bovine embryo transfer is the most available alternative to improve and produce large numbers of offspring from a donor-female with the highest genetic merit and performance record, in a shorter time than any other reproductive technology [1]. Currently, the embryo transfer technique is widely used around the world, with more than 500,000 embryos being transferred each year [2]. Embryo transfer (ET) has had a significant impact on the beef industry. Registrations of Angus cattle over the past 15 years give indication of the increased use of ET. In 1987, 3.6% (5,105) of all calves registered were a result of embryo transfer. In 2002, 25,093 calves resulting from ET were registered. This was 8.9% of all calves registered [3].

Over half the cattle in the world are found in the tropics (from the Tropic of Cancer to the Tropic of Capricorn). The subtropics of the United States include the Gulf Coast region of the southern states and all of Florida. Nearly 30% of

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This research follows the style and form of Theriogenology.

the U.S. beef herd is maintained in this zone [4]. Compatibility between beef cattle type (e.g., breed) and the environment is critical in harsh environmental zones such as the subtropics. In contrast to the abundance of beef cattle genotypes in the United States that are adapted to temperate climates, germ plasm resources with adaptation to warm climates, including the subtropics, are generally limited to the Zebu breeds *(Bos indicus)* and, within these, primarily to the American Brahman [4].

Unfortunately, there is not much information available about ET in *Bos indicus* cattle since most of the studies and improvements in embryo transfer have been done in *Bos taurus*. The majority of the embryo transfer protocols have been designed and modified in *Bos taurus*, and applied to *Bos indicus* with results very different than expected. Brahman, a *Bos indicus* breed is the most popular of all the zebu breeds, has been used widely in commercial beef herds throughout the warm regions of the United States because of the good performance in extreme heat stress not cold weather conditions, utilization of low quality of feed, and high resistance to parasites. Twenty five percent of the cattle in the United States are estimated to have some percentage of Brahman breeding [4]. The major niche for Brahman cattle has been in crossbreeding programs that combine the tropical adaptation of Brahman with the more desirable reproductive efficiency and carcass characteristics of temperate-adapted *Bos taurus* breeds [4]. A cross between *Bos indicus* and either an English or Continental breed produces animals with the very high fertility and production that can be used in a wide variety of environments. Moreover, one of the most cited negative factors of the Brahman cow is the sub-standard fertility, when compared to the English breeds of beef cattle [5, 6]. Differences in reproductive physiology between *Bos indicus* and *Bos taurus*, specifically with Brahman cattle are found in the reduced duration of estrus and a shorter period from onset of estrus to the luteinizing hormone (LH) surge as well as from the LH surge to ovulation [7]. In addition, *Bos indicus* females have a lower preovulatory LH surge than *Bos taurus* females [7]. *Bos indicus* also have higher numbers of ovarian follicles, smaller corpus

luteum (CL), lower serum progesterone concentration [7], and higher serum concentration of insulin growth factor I [8]. Researchers have recently found differences in timing of ovulation, fertilization or events leading up to cleavage of early embryos in Brahman compared to Holstein cattle [9]. Another difference found in Brahman cattle compared with British or Continental breeds is in the response to the super-ovulation protocols. Brahman cattle are generally thought to require smaller dosages of follicle stimulating hormone (FSH) than any other breed. Frozen Brahman embryos obtained with the traditional protocols produce lower pregnancy rates when compared with English or Continental breeds [10]. The theoretical reason for the decreased pregnancy rate with the frozen Brahman embryo is caused by the intracellular lipids. However, this has not yet been demonstrated [11]. It has been found that the different forms of estradiol stimulate variable responses in the donors. For example using 17ß-estradiol on Brahman donor cows will result in fewer unfertilized eggs and fewer degenerated embryos than using estradiol benzoate.

Even though the process of super-ovulation has demonstrated large differences in response [11], the use of lower dosages/frequencies of FSH in Brahman donor cows other than in *Bos taurus* or Continental breeds may produce more viable embryos per collection than the use of FSH suggested in standard protocols. The use of gonadotropin releasing hormone (GnRH) to induce ovulation or luteinization of the largest follicle present at the time of treatment is common [12]. One protocol for synchronization of ovulation involves administration of GnRH on the first day and a second GnRH injection is repeated 32 hours after the injection of prostaglandin (PGF 2α) for fixed-time AI in beef and dairy cattle [13,14,15]. It has been suggested to use a single injection of (GnRH) in Brahman donors in estrus 6 hours before AI to synchronize ovulation of the new dominant follicle because the FSH will produce ovulation in the following 24 hours after treatment which may increase the conception rate. The objectives of this study were to analyze records of a commercial ET program to determine if the use of 17ß-estradiol, the individualized dosage of FSH, and the

application of GnRH 6 hours before AI in Brahman donors would result in an increase in the number and quality of embryos per collection, which can be reflected with a higher conception rate in recipients, compared to the conventional existing protocols to produce Brahman embryos.

Research objectives

The overall aim of this study was to quantify the relationship of traits associated with Brahman donors and with recipient females based on the efficiency of embryo transfer technology.

The specific objectives were to:

1. Quantify the effects of superstimulation protocol, sire of donor, sire of embryo, season within protocol and donor body condition on embryo production (good, degenerate, unfertilized, and total embryos).
2. Determine the relationships of superstimulation protocol, embryo stage, embryo quality, corpus luteum size and season of collection with the outcomes of transfer of embryos into recipients (live calf, embryo resorption, abortion, and non-pregnant recipient).
3. Quantify the effects of sire of embryo, season within protocol, embryo stage, embryo quality, recipient body condition and corpus luteum size on gestation traits (gestation length and birth weight) in recipients of embryo transfer.

These results should provide information to accelerate the use of embryo transfer technology in Brahman females.

LITERATURE REVIEW

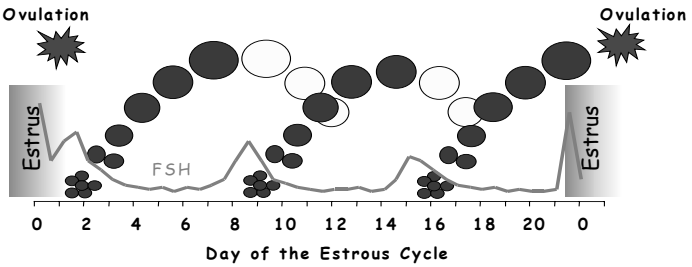
Hormonal regulation of follicles and the corpus luteum during the bovine estrus cycle

It is important to review the bovine estrus cycle and the role of the four main hormones involved in this process that produce changes in the pre-ovulatory follicle and the corpus luteum. Bovine estrus has been described as a short period (approximately 15 to 18 hours) of sexual receptivity that is manifested every 18 to 24 days, with ovulation occurring 10 to 14 hours after the cessation of behavioral signs of estrus [16,17]. 17ß-estradiol from the preovulatory follicle causes the cow to manifest estrus behavior and have a LH surge [18]. The LH surge causes ovulation of the preovulatory follicle about 28 hours later. The cells that remain from the preovulatory follicle develop into the corpus luteum. The corpus luteum grows in size during the first part of the estrus cycle and then reaches a plateau phase in which it maintains a large size (20-25 mm diameter) [19]. The major hormone coming from the corpus luteum is progesterone and the increase in size of the corpus luteum is reflected in increased concentrations of progesterone in the blood. If the cow becomes pregnant the corpus luteum is maintained, and progesterone concentration remains elevated. The high progesterone concentration prevents the cow from coming into estrus or having a subsequent ovulation. If the cow does not become pregnant, then the corpus luteum will degenerate at about day 17-20 of the estrus cycle (day of estrus = 0). The reason the corpus luteum regresses is because of secretion of prostaglandin (PGF 2α) from the non-pregnant uterus. After exposure to PGF 2α there is a decrease in circulating progesterone concentrations as well as a subsequent decrease in size of the corpus luteum [20]. The temporal profiles circulating concentrations of FSH, LH and estrogen during the estrus cycle are depicted in Figure 1. The changes in circulating progesterone concentration

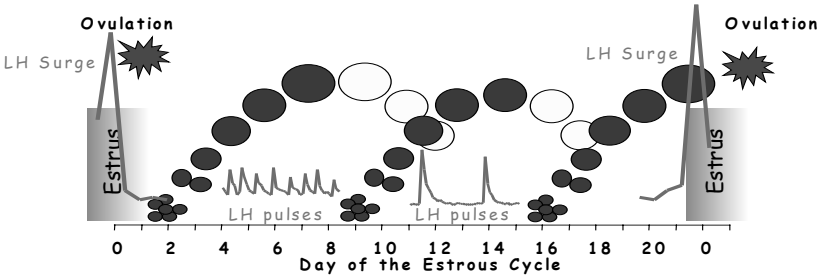
during the estrus cycle and associated with initiation of pregnancy are depicted in Figure 2.

Starting around the time of ovulation, a group of small follicles (cohort) begins to grow on the ovaries. This growth is known as the follicular wave. From this group of follicles a single dominant follicle is selected to continue growth, whereas, other follicles of the follicular wave undergo atresia or regression [21]. In cattle, follicular waves occur throughout the cycle, but under natural conditions only the dominant follicle of the wave present at the time of luteal regression subsequently ovulates [22, 23]. Due to the presence of a functional corpus luteum and high progesterone concentrations, this first dominant follicle does not cause estrus behavior and does not continue to ovulation. The first dominant follicle will become non- functional and a second follicular wave begins at about mid-cycle. A dominant follicle is again selected from this second follicular wave, and this follicle continues to ovulation because its growth corresponds to the time of regression of the corpus luteum [24]. Some cows also show three waves of follicular growth such that the second dominant follicle regresses, a third follicular wave is initiated, and the third dominant follicle becomes the ovulatory follicle. Both the first and the second follicular waves are preceded by an increase in FSH concentrations [21]. These increases in FSH are essential for the initiation of a follicular wave. The subsequent decrease in FSH is essential for selecting a single dominant follicle. There is also a FSH surge in association with the LH surge that causes ovulation. This FSH surge occurs near the onset of estrus and is of shorter duration than the subsequent FSH surge [25]. Near the time of estrus there are two surges in FSH that are difficult to discriminate because they are temporally adjacent. The first surge corresponds to the GnRH/LH surge that induces ovulation and a second occurs near the time of ovulation and is associated with emergence of the first follicular wave [25]. Emergence of the follicular wave has generally been retrospectively determined

(FSH)



(LH)



(Estrogen)

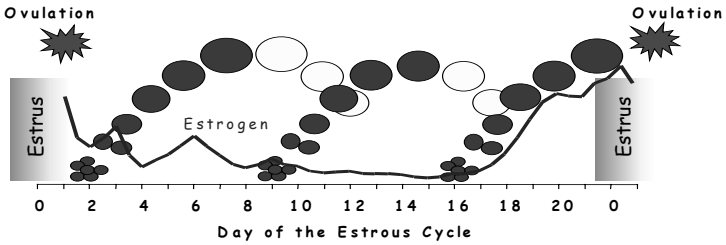
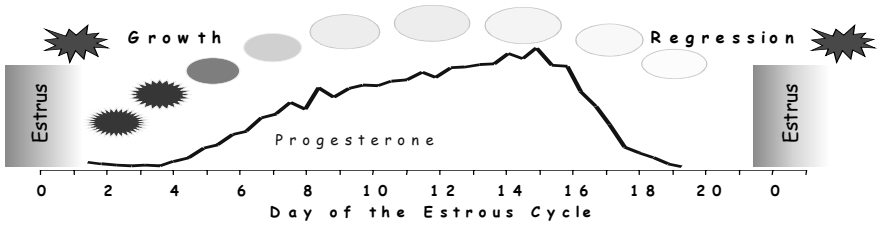
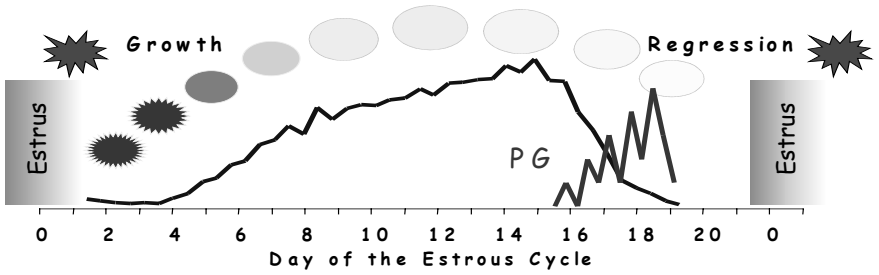


Fig. 1. Example shown for cattle having three follicular waves during a 21-day estrus cycle. Phillips G [26]. The smallest dark ovals represent the follicle recruitment. The middle size dark ovals represent the follicle selection. The large size dark oval represents the dominant follicle. The clear ovals represent the follicle regression.

Corpus Luteum (CL)
Progesterone



Corpus Luteum (CL) Regression
Prostaglandin $F_{2\alpha}$ (PG)



Corpus luteum (CL) Maintenance
When cow becomes pregnant

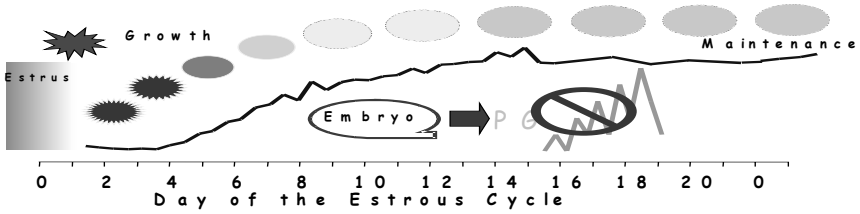


Fig. 2. The dark irregular ovals represent corpus luteums in development from the ovulated follicle. They take approximately 10 days to reach mature size. Corpus luteum produces progesterone. - Late in the estrus cycle, uterus produces PG, which causes regression of corpus luteum. - Presence of embryo blocks uterus to produce PG late in the estrus cycle which causes maintenance of corpus luteum and production of progesterone for maintenance of pregnancy. Phillips G [26].

as the time when the first follicles of the follicular wave reached ≥ 4 mm [25]. Following emergence, follicles continue growth and circulating FSH begins to decline up until the time of follicular deviation. Follicular deviation has been defined as the beginning of the greatest difference in growth rates between the largest follicle and the second largest follicle at or before the examination when the second largest follicle reached its maximum diameter [24].

Hormonal changes involved close to the time of estrus and ovulation

Standing estrus and the luteinizing hormone (LH) surge, which is requisite to induce ovulation are two events initiated by the high circulating estradiol concentration. The elevated estradiol is due to growth of a large preovulatory follicle on the ovary [25]. After regression of the corpus luteum the dominant follicle grows and produces increasing amounts of estradiol. The cow becomes sexually active prior to the onset of standing estrus due to the increasing amounts of estradiol in the absence of circulating progesterone [25]. Progesterone is low due to regression of the corpus luteum. If the corpus luteum does not regress and progesterone remains elevated some or all of the subsequent events such as the LH surge, estrus, and ovulation do not occur even when estradiol is elevated. After estradiol attains a threshold concentration for a certain time period, there is a change in the brain that causes the cow to begin to stand solidly during mounting or more exactly during the onset of estrus [25]. There is also secretion of gonadotropin-releasing hormone (GnRH) in large amounts from a region of the brain called hypothalamus. The secretion of GnRH causes the LH surge. Induction of a LH surge and ovulation can be accelerated with a GnRH injection prior to the normal time of ovulation [27]. The onset of estrus is due to the high circulating estradiol concentration. Estrus behavior ends prior to ovulation in cattle. The end of estrus may be due to a decrease in circulating estradiol because estradiol production in the follicle is dramatically

reduced following the LH surge. The time from the onset of estrus until ovulation is between 25 and 34 hours [28].

Use of CIDRs to synchronize estrus in donor cows

EAZI-BREED™ CIDR® (Controlled intravaginal progesterone-releasing device) was approved for use in the United States in 2002. The approved and manufacturer recommended protocol indicates that the CIDR should be administered intra-vaginally, one per animal, and left in place for 7 days. The CIDRs sold in the U.S. contain 1.38 grams of progesterone. Mean plasma progesterone concentration increases from less than 0.1 ng/mL to greater than 3 ng/ml within 12 minutes after insertion of CIDR [29]. The maximal concentration (6 ng/ml) occurs within the first 18 hours after insertion [30,31]. Plasma concentrations of progesterone decreased slowly during the 7-day insertion period, but remained greater than 2.5 ng/mL. Upon removal of the CIDR the mean plasma progesterone concentration decreases from less than 3 ng/mL to less than 0.5 ng/ml within 1 h [29].

Like other progestins, the CIDR suppresses the expression of estrus and ovulation by elevating the blood progesterone concentration throughout its duration. A significant problem with using synthetic progestins in synchronization programs is manifested in the compromised fertility of the synchronized estrus after either short-term (greater than 8 days) [32], or long-term treatments [33]. The reduction in fertility is a result of one of two mechanisms. The first is the maintenance of a dominant follicle on the ovary under the influence of long-term progestins like MGA [34,35] and CIDR [36] in the absence of a functional corpus luteum resulting in the ovulation of sub-fertile oocytes [37]. The second mechanism is the ovulation of aged oocytes [38], which would be more prevalent during short-term progestin treatments. To prevent development of either a persistent follicle or ovulation of an aged oocyte after a progestin treatment, estrogens can be administered to regress follicles [39,40], or

gonadotropin-releasing hormone (GnRH) can be administered to ovulate the dominant follicle [41,42] at the initiation of a progestin treatment, resulting in emergence of a new cohort of follicles 2 to 5 days later [39,40,43]. Estrus synchronization programs using CIDRs, are combined with prostaglandin PGF 2α one day before removing the CIDR or the same day of the insert removal. The timing of estrus following recommended administration of the CIDR and PGF 2α has been very synchronous [31]. More than 60% of the animals consistently exhibit estrus within a 24-hour period. Pregnancy rates of cattle bred 12 h after estrus detection have been satisfactory [31]. CIDRs are also combined with estradiol and gonadotropin releasing hormone (GnRH) at the time of the insertion to more effectively control follicular growth and to induce a timed ovulation following CIDR removal [44,45]. The timed ovulation programs have been useful to eliminate estrus detection of embryo transfer recipients [46]. Transfer of embryos to cows with a palpable corpus luteum 7 days after administration of GnRH or 8 days after administration of estradiol to induce estrus has been successful. Several basic CIDR synchronization protocols are shown in Fig. 3. All programs consist of a CIDR treatment for 7 days, with PGF 2α administered at CIDR removal. Either estradiol benzoate or GnRH can be administered at CIDR insertion as a means of initiating follicle turnover. Following CIDR removal, multiple AI programs can be used [47]. In option 1, cattle can be inseminated by observed estrus for approximately 5 days after CIDR removal. In option 2 and 3, estradiol benzoate can be injected 24 hours after CIDR removal and cattle can be inseminated either by observed estrus (option 2) or timed AI approximately 24 to 36 hours after estradiol benzoate (option 3). In option 4, cattle can be timed AI and injected with GnRH 48 hours after CIDR removal. With option 3 and 4, 48-hour calf removal can also be implemented in the timed AI programs [47].

Treatment of postpartum beef cows with a 7-day CIDR results in an increased percentage of cattle detected in estrus and conceiving early in the breeding

season in both cycling and anestrus postpartum beef cows. The use of estradiol benzoate or GnRH in combination with CIDRs can enhance pregnancy rates [47].

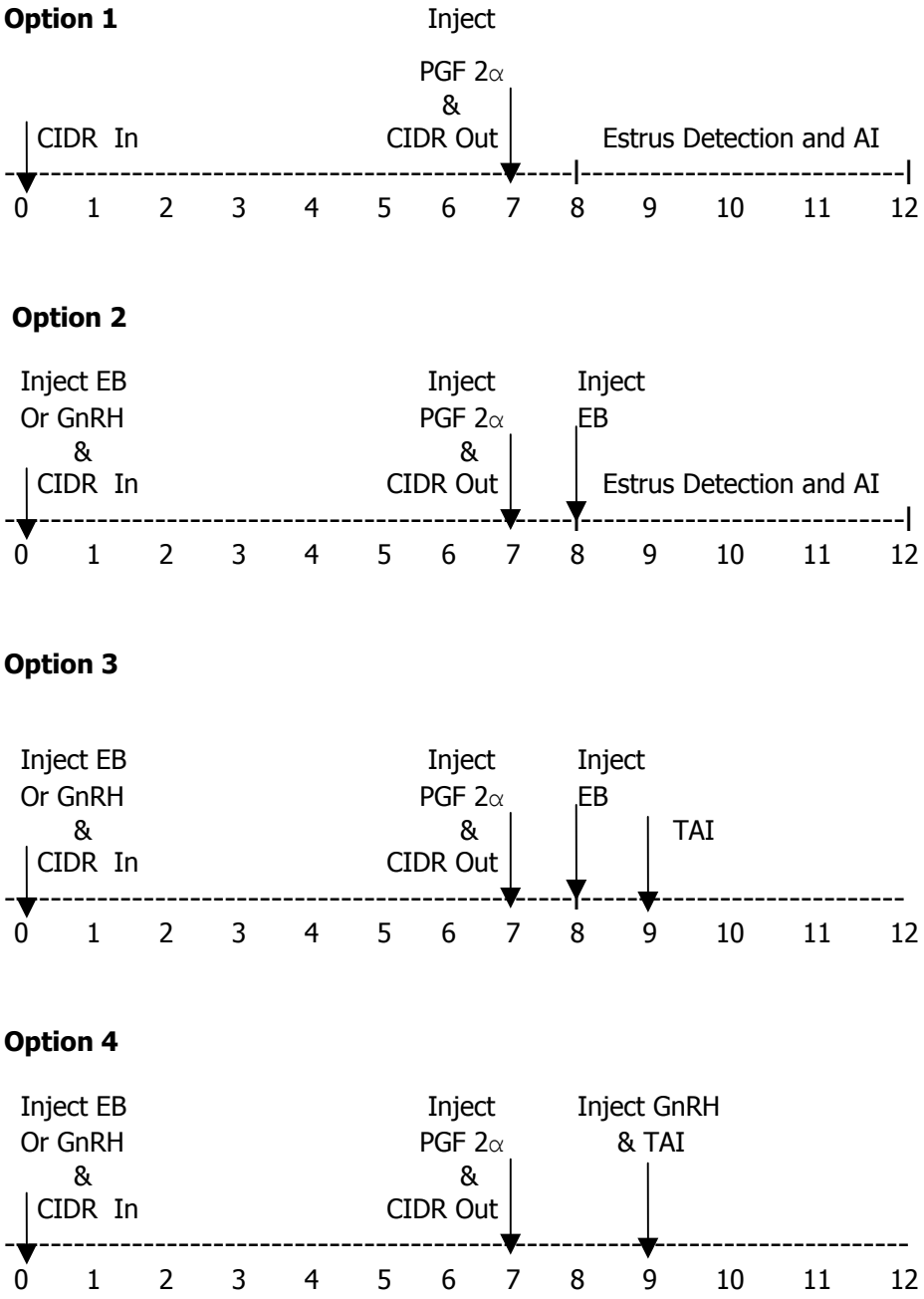


Fig. 3. Four different options used to synchronize estrus and/or ovulation in beef cows treated with intravaginal progesterone-releasing device (CIDR) in combination with prostaglandin F2α (PGF 2α) and with or without estradiol benzoate (EB) or gonadotropin-releasing hormone (GnRH).

Control of follicular recruitment

It is known that 8 to 12 days after estrus (equivalent to days 7 to 11 after ovulation) would be the approximate time of emergence of the second follicular wave in two or three wave cycles) [48] and a cohort of growing follicles would be present around that time. Emergence of each follicular wave in the bovine estrus cycle is preceded by a transient increase in endogenous FSH [49], all 2 to 3 mm follicles that emerge in a wave have FSH receptors and for the first 2 days after emergence follicular development is supported by FSH. As the small follicles develop they secrete inhibin and estradiol, both of which inhibit FSH secretion [50]. By the second or third day after follicular wave emergence FSH reaches basal levels and continued follicular growth is dependent on LH secretion. A dominant follicle is selected approximately 2.5 days after emergence because it develops LH receptors while the subordinate follicles do not [24]. After selection of the dominant follicle, LH secretion is able to support the development of the single dominant follicle, but the subordinate follicles become atretic and regress.

The necessity of waiting until mid-cycle to initiate super-stimulatory treatments implies monitoring estrus and an obligatory delay. To obviate these problems, an alternative approach is to initiate super-stimulation treatments subsequent to the exogenous control of follicular wave emergence [51]. There are several possible ways to control follicular development in cattle. Follicular wave emergence has been altered experimentally by mechanical follicle ablation or by hormone treatments. Ultrasound-guided follicle aspiration (used for oocyte retrieval in IVF programs) has been used as a method of follicle ablation [52,53], the ablation of ovarian follicles (≥ 5mm), altering the endogenous release of LH and FSH or administration of exogenous steroids or gonadotropins can cause regression of a dominant follicle and emergence of a new follicular wave [54]. Estradiol-progesterone treatments (to hormonally suppress follicular development) have been extensively investigated [39,55,56]. All of these treatments have resulted in

comparable super-ovulatory response in synchronization of follicular wave emergence [57].

Predicting the functional activity of a dominant follicle and corpus luteum might be important before starting a super-ovulation regimen or a synchronization program [58]. Successful super-ovulation depends upon the ability to control the development of follicles with exogenous FSH. The timing of FSH administration to optimize super-ovulation must begin at a time when a new wave of follicles has emerged, but before a dominant follicle has been selected. After emergence, but before dominant follicle selection, all follicles have FSH receptors and injections of FSH can act to rescue follicles otherwise destined to become atretic [59]. The number of follicles that respond to FSH treatment and develop into ovulatory follicles is dependent on the number of small, antral follicles that emerge in the follicular wave. Control of the process whereby primordial follicles develop into antral follicles is poorly understood, however, the development of primordial follicles eligible to emerge in a follicular wave is not controlled by FSH and LH [60]. Instead, development of primordial follicles is dependent on several growth factors produced locally and systemically [59]. Major factors that influence pre-antral follicular growth include: growth-differentiating factor 9 (GDF-9), bone morphogenic proteins (BMPs) activins, inhibins, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), insulin and insulin-like growth factors (IGF-I and IGF-II) [59].

Variability in response continues to be one of the most frustrating problems associated with estrus synchronization and super-ovulation programs in cattle. The techniques designed to control follicular wave dynamics reduce the variability caused by treating cows at different stages of the estrus cycle [51]. Protocols involving synchronization of follicular wave emergence do offer the convenience of being able to initiate super-stimulatory treatments quickly and at a self-appointed time, without the necessity of estrus detection and without sacrificing results [51].

The use of estradiol in estrus synchronization

Estrogens have been shown to induce follicle regression, followed by synchronous follicular wave emergence, if administered in the face of high levels of progesterone, and induce LH release and ovulation of a dominant follicle if administered in the face of low levels of progesterone. It was found that estradiol valerate treatment, in some cases, caused prolonged suppression of the pre-wave FSH surge and delayed emergence of the next follicular wave [61]. This was attributed to the prolonged action of the valerate ester. Therefore, a series of experiments were designed to investigate the effects of a shorter acting estrogen, 17β-estradiol on follicle wave dynamics. When 17β-estradiol was injected in the presence of progesterone, FSH suppression occurred and a FSH surge preceded the new follicle wave 4 to 5 days later [61]. Therefore, it is recommended that if donors could not be injected with progesterone, they should be implanted with progesterone before treatment with estrogen. Estradiol benzoate (2 mg) and progesterone (50 mg) given at the time of device insertion resulted in more synchronous follicular wave emergence
than treatment with 2 mg estradiol benzoate alone [62].

Estradiol and progesterone treatments have been increasingly used over the past years in estrus synchronization programs in beef and dairy cattle [63]. Nowadays, treatments consist of insertion of a progesterone (CIDR) device and the administration of estradiol with progesterone on day 0 (to synchronize follicular wave emergence). Prostaglandin at the time of device removal on days 7 or 8 (to ensure luteolysis), and the subsequent application of a lower dose of estradiol 24 hours later or GnRH/LH 48 to 54 hours later to synchronize ovulation [63].

The use of GnRH in donor cows for embryo transfer programs

Administration of GnRH during the bovine estrus cycle causes regression or ovulation of the dominant follicle and initiates the emergence of a new wave of follicular growth an average of 2.5 days following treatment [27]. Atresia or ovulation of the dominant follicle depends on the status (growing, static or regressing) of the dominant follicle at the time of GnRH injection [64,65]. The scientific literature of the 1970s and 1980s demonstrated that injections of gonadotropin-releasing hormone (GnRH) could induce ovulation of ovarian follicles in milked [66] and suckled cows [67]. Injections of GnRH induced the release of luteinizing hormone (LH) [66] and follicle-stimulating hormone (FSH) [68] from the anterior pituitary gland. Resulting elevated blood concentrations of LH and FSH either caused follicular rupture (ovulation), if a follicle(s) was present, or may have induced some new follicular development.

Gonadotropin releasing hormone has been used widely since it first became available commercially in the 1970's as a treatment for follicular cysts [69]. The implementation of GnRH in donors has been used to select the ovulatory follicle, which should cause premature ovulation of that follicle. Using these concepts researchers have made tremendous strides in developing numerous systems to synchronize the estrus cycle for an artificial insemination and embryo transfer [30]. Martinez et al. [44] reported the efficacy of the GnRH products available in the United States. Cystorelin® induced a greater LH surge than Fertagyl® and Factrel®. Cystorelin® also induced a greater ovulation rate, but all products synchronized follicular wave emergence. GnRH is a decapeptide, a linear chain of ten amino acids. The base for Cystorelin®, Fertagyl® and Ovacyst® is diacetate, tetrahydrate. Therefore, these three products are chemically identical. Factrel® has a HCL base, which should not alter bioactivity. Even though the products are chemically similar, Martinez et al. [44] found differences among them, which can be related to the active compound in the product. The dose depended on the type of the ovaric cyst, the clinical claim for GnRH product.

Perhaps this explains the variability in response when doses less than the recommended dose are used. In a retrospective analysis between Cystorelin® and Factrel®, Stevenson et al. [70] did not detect an effect of GnRH product on AI pregnancy rates in cows treated with two different GnRH estrus synchronization protocols.

Twagiramungu et al. [71] proposed the use of a second GnRH treatment after prostaglandin treatment to ensure ovulation of the existent dominant follicle. They found that the second GnRH treatment improved the precision of ovulation and permitted fixed-time insemination without adversely affecting pregnancy rates. The method consists of an injection of GnRH followed by prostaglandin 7 days later, a second GnRH injection 48 hours after prostaglandin treatment, and fixed time insemination 24 hours later. The rationale for the treatment is that the first injection of GnRH is intended to induce an LH release and ovulation or luteinization of the dominant follicle present at the time, thus resulting in the emergence of a new follicular wave within 2 days. The administration of prostaglandin 7 days after treatment is intended to induce the regression of the original or induced CL, and the second GnRH injection is intended to induce synchronous ovulation of the new GnRH-induced dominant follicle. The importance of the second GnRH injection was demonstrated by a higher rate of ovulation than in cows that were not given a second injection (97% vs. 77% respectively) [55].

Uses of GnRH in timed AI protocols

One of the newest breeding programs in cattle is known as Ovsynch, which has been developed for dairy cows (Fig. 4). This protocol uses the initial GnRH injection of 100 µg to induce ovulation of a dominant follicle that develops into a new corpus luteum in the cycling dairy cow. In cycling dairy cows, about 60 percent of the cows given the initial GnRH injection ovulate a follicle in response to the LH released by the GnRH injection, depending on the stage of their estrus

cycle [72]. Following this induced ovulation, a new wave of follicles emerges from both ovaries within 48 hours, from which a new dominant follicle develops. Seven days after the initial GnRH injection, PGF 2α is injected to lyse or regress the original corpus luteum (if one was present at the time of the initial GnRH injection) and the new corpus luteum induced by the GnRH. During the next 48 hours the new dominant follicle rapidly matures, and at 48 hours after PGF 2α a second GnRH injection (100 ug) is administered, and the cow is inseminated in the next 8 to 24 hours. The highest pregnancy rate occurs when the timed AI occurred at 16 hours [73]. In practice, on dairy farms, cows are inseminated any time they are detected in estrus during this protocol and further hormonal injections are discontinued.

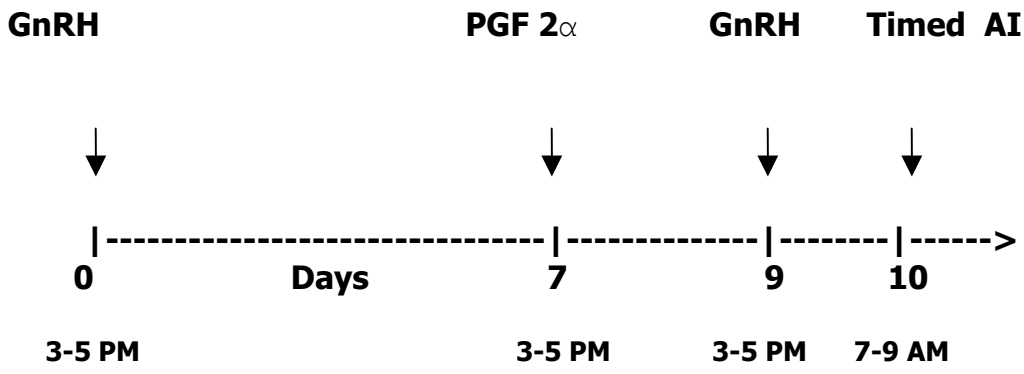


Fig. 4. Ovsynch Protocol.

Based on the distributions of estrus after the select Synch protocol and the early success with Ovsynch in beef cows, the Cosynch protocol was developed and tested [74,75]. This protocol was designed to reduce the number of trips through the working facility to three (Fig.5). Comparisons of Cosynch to Ovsynch were made and pregnancy rates were identical at 48% [74]. A further study, Geary et al. [76] also incorporated 48-hour calf removal (between the injections

of PGF 2α and the second GnRH injection) after both the Ovsynch and Cosynch protocols. Calf removal produced pregnancy rates that were 9 percentage points greater than rates after each protocol without calf removal.

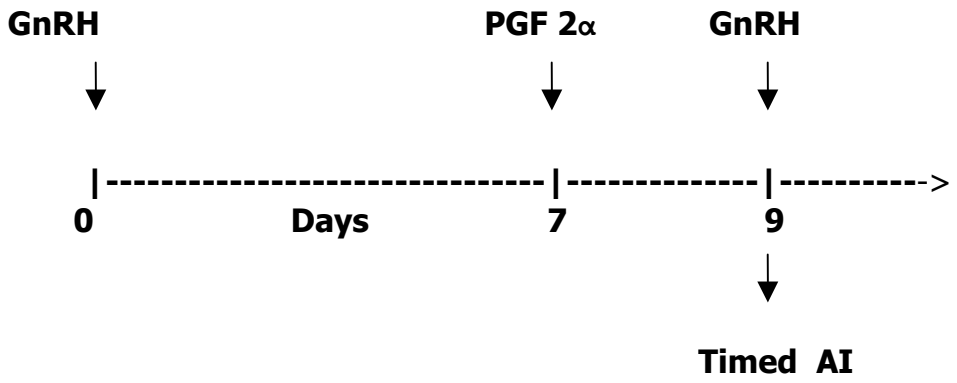


Fig.5. Cosynch protocol.

The combination of Cosynch with a progestin (CIDR inserted from day -7 to day 0) (Fig. 6) indicates that addition of a CIDR for progesterone supplementation improved pregnancy rates after a fixed-time AI [77]. But progesterone did not seem to improve pregnancy rates in suckled beef cows cycling at the initiation of treatments. Progesterone seemed to be more effective in a enhancing pregnancy rates in cows that were cycling but in the latter stages of the estrus cycle at first injection of GnRH and subsequently had no luteal structure at the PGF 2α injection or in non-cycling cows. Along with parity, days post-partum, calf removal, and cow condition, previous reports [77] also indicate that location variables (which could include differences in pasture and diet, breed composition, body condition, post-partum interval, and geographic location) may affect the success of fixed-time AI protocols. Therefore, a sound strategy for utilizing a GnRH protocol, in the absence of progesterone, may be to select cows that calved earlier in the calving season that tended to be in a good body condition. A high percentage of these cows should be cycling and result in

acceptable fertility rates [77]. To achieve optimal pregnancy rates after embryo transfer with a GnRH and CIDR synchronization protocol, cows should be in a good body condition (BCS=5) and treatments should be initiated only when cows are at least 50 days postpartum [78].

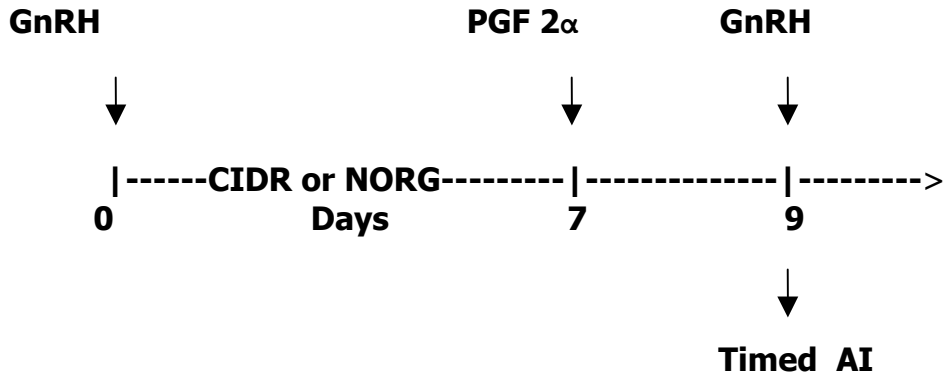


Fig. 6. Cosynch protocol with or without an exogenous source of progesterone administered during 7 days.

The GnRH/CIDR/ PGF 2α protocols utilize the advantage of both the GnRH injection to induce the formation of a new follicular wave and to prevent females from exhibiting estrus prior to the injection of PGF 2α. Conventional wisdom indicated that these protocols might be the most consistent protocol to ensure that the maximal number of recipients exhibit estrus within the ideal interval [78].

Cryopreservation

The development of successful techniques for the freezing and thawing of cattle embryos had a profound impact on the field of embryo transfer, which in turn, has had a significant influence on the cattle industry. An increasing demand for freezing embryos during the 1990s culminated with over 90 % of the

embryos having been frozen in 1999 [79]. Although not all cattle breed associations keep records of the animals produced by embryo transfer, the records of some associations indicate that embryo transfer is being widely used by breeders of genetically superior cattle. This is evidenced by the fact that 86 % of the top 100 Holstein bulls (as evaluated by Holstein Type-production Index in 2000) were produced by embryo transfer methodology [79].

The first published report of success in freezing and thawing mammalian embryos for the production of live-born young involved the use of dimethyl sulfoxide (DMSO) or glycerol as cryoprotectants for laboratory mouse embryos [80]. Shortly thereafter, cattle [81] embryos were successfully frozen and thawed with production of offspring. Embryos should be recovered between 6.5 and 7.5 days (normally range from late morula to mid-blastocysts) for optimum viability after freezing and thawing. The embryos to be frozen should be graded 1 (excellent or good) or 2 (fair) according to the International Embryo Transfer Society [82]. Improved protocols utilized 10% (1.4 M) glycerol as the cryoprotectant. A general freezing protocol cited by Hastler [79], utilizes glycerol as follows:

1. Equilibrate embryos in 10% glycerol for 10 to 20 minutes.
2. Load embryos in 0.25 cc straws; seal and label straws; (embryos can be loaded while they are equilibrating).
3. Place straws in freezer that is maintaining a holding temperature of -6°C
4. Seed straws and maintain at -6ºC for 15 minutes.
5. Decrease temperature at 0.5ºC per minute to -32ºC.
6. Plunge straws into liquid nitrogen.

Another method for freezing embryos is through the use of ethylene glycol, which is another cryoprotectant. Voelkel and Hu [83], reported acceptable pregnancy rates following the transfer of bovine embryos in ethylene glycol and transferred after thawing directly from the straws in which the embryos had

been frozen. This technique is now widely used on an international basis [83]. A comparison of reported pregnancy rates resulting from the frozen embryo transfer in glycerol versus those frozen in ethylene glycol is shown in Table 1. Ethylene glycol is a more suitable cryoprotectant than glycerol for direct transfer because it is a smaller molecule and penetrates the cell membranes of embryo cells faster [79]. This property is more important for the thawing stage than for the freezing stage of the process. Embryos can be equilibrated in ethylene glycol in the same manner as in glycerol, i.e.; they can be placed directly in the final ethylene glycol concentration of 1.5M at room temperature and loaded in straws during the equilibration period. Equilibration involves attaining equal concentrations of ethylene glycol outside and inside the cells and is probably completed within 5 minutes. Equilibration can be continued for as long as 20 minutes to allow for loading and sealing of a number of straws. It is very possible, however, that some of the reported problems with ethylene glycol may result from equilibration in-farm embryo transfer programs with much higher summer environmental temperatures. Ethylene glycerol may well be more toxic to embryos during extended exposure at higher temperature [79]. The use of ethylene glycol, having a higher permeability through the plasma membrane than glycerol would also improve pregnancy rates from blastocysts [84]. In a study by Martinez et al. [85] when different combinations of ethylene glycol or propylene glycol and sucrose were tested, it was observed that ethylene glycol and sucrose resulted in higher pregnancy rates, which were similar to those obtained from either fresh morula or blastocyst. In general terms, addition of sucrose to the freezing medium was beneficial when ethylene glycol was used but it was detrimental in combination with propylene glycol.

The technology with greatest variability when comparing the effects of environment and breed is embryo cryopreservation. Brahman and Zebu embryos when collected and frozen using industry standard protocol procedures exhibit lower and unacceptable pregnancy rates when compared to English or Continental breeds [86]. Pregnancy rates are within acceptable ranges for fresh

embryos, but are lower than expected when frozen and thawed. Brahman embryos frozen in glycerol have a lower pregnancy rate (30%) compared with 48% for direct transfer. The theoretical reasoning for the decreased pregnancy rate in frozen Brahman embryo is intracellular lipids [11].

Table 1
Comparison of pregnancy rates achieved after transfer of bovine embryos that were frozen in glycerol or in ethylene glycol. (X^2= P>0.05)

Glycerol		Ethylene Glycol		
No.	% Preg.	No.	% Preg.	References
92	48.9	189	58.2	[87]
185	56.8	780	59.6	[88]
56	69.6	56	50.0	[89]
423	45.9	97	47.4	[90]
838	55.3	228	51.8	*
1609	58	11,376	59.2	[91]
225	53.8	218	51.8	**
Total				
3428	55.4	12,944	58.8	

* Holland Genetics, Unpublished data
** Em Tram, Inc., Unpublished data

Factors affecting the embryonic survival in the cow

Embryonic mortality in cattle is the main source of economic loss for livestock producers. [92]. In beef herds, pregnancy losses represent an even more important economic factor because the number of calves sold determines most of the income.

In order to properly standardize bovine reproductive terms, the Committee on Bovine Reproductive Nomenclature [93], established that the embryonic period of gestation extends from conception to the end of the differentiation stage, at approximately 42 days of gestation, and that the fetal period extends from gestation day 42 to the delivery of the calf. Secretion of progesterone by the

corpus luteum is essential throughout gestation to achieve 100% success in the maintenance of uterine quiescence and survival of the embryo/fetus, and for normal parturition, in the cow [94]. Prior to and immediately after estrus, progesterone regulates the establishment and timing of mechanisms necessary for luteal regression in the non-pregnant cow and for maternal recognition of pregnancy. Utilizing this knowledge, researchers and breeders can transfer embryos into ovariectomized cows and pregnancy can be completed successfully by providing exogenous progesterone on a continuous daily basis [95].

Much of the pregnancy loss in cattle is due to early embryonic death [96] after either natural or artificial insemination or embryo transfer. According to the review by Thatcher et al. [42], approximately 30 % of repeat breeder cows experience embryonic loss by day 7 of pregnancy. Additional embryonic losses occur gradually from days 8 to 17 (approximately 40 % of total losses) and between days 17 and 24 (approximately 24 % of total losses). Losses between days 17 and 24 were estimated at 6 to 12 % of pregnancies in two studies in which dairy heifers were bred at synchronized estrus after two treatments with PGF 2α 11 days apart [97]. Published estimates of late embryonic death rate (days 27 to 42) average 10 to 12% [42,98,99]. These factors must be considered in conjunction with estimates of fertilization failure (12%) [100], and fetal losses that may range as high as 8% of animals pregnant at 42 days [99]. Thus, wastage occurs throughout pregnancy, but is concentrated in the embryonic period, or the first 40 days after breeding.

Conception postpartum

During the postpartum period, the cow undergoes a transition from an anovulatory, anestrus, infertile animal into a fertile animal with normal ovulatory estrus cycle and a higher conception rate. Holness et al [101] reported that only 12% of first postpartum ovulations were fertile and particularly low in the early

postpartum period in *Bos indicus* cattle. Ovulation failure was a significant factor limiting pregnancy rate in these early postpartum cows.

Early embryonic death associated with short duration of the luteal phase

A short luteal phase may occur following first ovulation, or first estrus [102,103]. Short-lived corpus luteum occurred in the pubertal heifer and the postpartum cow returning to ovulatory activity [104,105]. Obviously, a corpus luteum that had regressed before day 14 could not support pregnancy; maternal recognition of pregnancy in the cow occurs on days 14 to 17. Variables such as follicular development, pre-ovulatory and post-ovulatory concentrations of gonadotropins, and luteal receptors for LH were shown to be responsible for some variations in level of luteal function [106], but not for luteal duration. It has been observed that pretreatment with progesterone usually resulted in formation of a corpus luteum with a normal functional lifespan, in response to weaning or injection of gonadotropins [107,108]. Secretion of PGF 2α rose during treatment of anestrus cows with progesterone, in the same manner as during a short luteal phase after injection of hCG in control cows [109]. Thus, if the uterus had not been exposed previously to progesterone, secretion of PGF 2α increased prematurely when the first corpus luteum began to secrete progesterone.

Several studies have reported that beef cows, from which calves were weaned at about 35 days postpartum, would consistently exhibit estrus in 4 to 5 days and form a corpus luteum [110,111,112]. The studies provide evidence that ovulation and fertilization occurred at the expected time after an estrus preceding a short luteal phase in early weaned cows. Logically, fertility should be improved by pretreatment of the postpartum cow with progesterone because of the prevention of the shortened luteal phase [113]. Progesterone therapy was tested by providing a daily supplement of melengestrol acetate (MGA) in feed

beginning on day 4 after breeding [114]. No pregnancies were maintained in cows with short luteal phases. In contrast, 41% of all norgestomet pre-treated cows and 50% of those cows that had normal luteal phases maintained pregnancy regardless of whether or not they received MGA. Supplemental injections of 200 mg progesterone daily gave the same results. No pregnancies occurred in cows with short-lived corpus luteum. Surprisingly, 12 of the 13 control cows that were deleted from the experiment for having a short luteal phase before breeding conceived at the post-weaning estrus, even though they were bred at an average of only 33 days postpartum. Combining all of the above results, it was concluded that about half of the difference in ability to maintain pregnancy between cows with short and normal luteal phases (when supplemental progesterone was provided) could be attributed to effects on the oocyte or embryo before day 7 after estrus and the other half to a hostile uterine environment on or after day 7. The apparent timing of embryo loss was strikingly similar to the timing of increased uterine secretion of PGF 2α on days 4 through 9 after estrus in cows with a short luteal phase [109]. Moreover concentrations of PGF 2α in flushing from the uterine lumen of cows with short luteal phases were more than double the concentration from cows with normal luteal phases [115]. Embryo quality tended to be correlated negatively with concentrations of PGF 2α in flushing from the uterine lumen. Because embryo quality was lower on day 6 [115] than on day 3 [114]. It was proposed that the specific problem in short luteal phase cows was likely to have occurred after the embryo entered the uterus. A direct embryo-toxic effect of PGF 2α seemed possible because that had been suggested for mouse [116], and shown for rabbit [117] and rat [118] embryos. Effects of PGF 2α on embryo survival have been examined in several studies in cows in which daily supplemental progesterone was provided to replace the regressed corpus luteum. It was shown that PGF 2α was detrimental to embryos when given to normally cycling beef cows during days 4 to 7 after estrus and insemination, an interval similar to that during which high embryo

mortality had been observed in cows with short luteal phases. The majority of embryonic mortality in sub-fertile dairy cows occurred 6 to 7 days after estrus [119], when the morula was developing into the blastocyst. Maurer and Chenault [120] observed that 67% of embryonic mortality had occurred or was occurring by day 8 of gestation in beef cows. Seals et al. [121] showed that premature luteal regression by PGF 2α on days 5 through 8 caused embryonic death in cows supplemented with progesterone (confirming the results of Buford et al. [122), but treatment on either days 10 through 13 or 15 through 18 of pregnancy was not effective. Many products of luteolysis or partial luteolysis could play a role in embryonic loss [122]. Involvement of luteal PGF 2α is worthy of further evaluation, because it was observed that the short-lived corpus luteum produced more PGF 2α than did a corpus luteum with a normal life span.

Other factors that increase secretion of PGF 2α, such as heat stress [123], uterine infection [124], or mastitis [125,126] could cause embryonic death through this mechanism.

Other factors associated with pregnancy losses

In cattle sub-luteal concentrations of progesterone during the estrus cycle preceding insemination induce increased frequency of LH pulses resulting in a persistent dominant follicle [127,128]. In beef cows, larger pre-ovulatory follicles, that maintained dominance for an extended period before the LH surge, reduced conception rate compared to smaller pre-ovulatory follicles (36% versus 91%) [114]. Exposure of the oocyte to high peak frequency of LH induces the premature resumption of meiosis [37,129], and the biochemical and morphological changes in the oocyte in persistent follicles reduce fertility in cattle [127,130] due to embryo mortality before the 16-cell stage [131]. Therefore, extending the period of follicle dominance either by exogenous progestins [131], or when cows have cycles with two waves of follicle growth instead of three-

follicle growth waves [132], compromises fertility. Fortunately, the presence of a persistent follicle does not alter the developmental potential of oocytes from smaller follicles; that is, if the persistent follicle regresses, normal fertility is resumed [99].

MATERIALS AND METHODS

Criteria to select the records of Red Brahman donor cows and the recipients

Records of Red Brahman donor cows (n=50) were selected from 200 potential cow records available to develop this study. The selection was based on previous reproductive records and performance, and only the cows with a minimum of two births were included. The donor cows were cycling normally at the time of the reproductive evaluation by transrectal palpation with normal conformation of the uterus, including cervix, and ovaries. None of the cows had evidence of reproductive disease. Other additional selection criteria included, body condition (only the cows with a score between 5 to 7 on a scale 1 to 9), and cows had to be at least two months post partum. Some of the donors were collected two or three times per year, depending on their prior records, and some of them could not be collected at some point during the study because they were pregnant.

Records of recipients F1 Brahman–Holstein cows comprised 87% of the recipients for this study (n=531). During this study approximately 30% of the recipients were rejected before transferring them with an embryo. The selection criteria for recipients included a complete reproductive exam by transrectal palpation, analysis of performance records lactating cows with more than two births, a body condition score between 5 to 6 on the scale 1 to 9, good milking ability, feminine phenotype, and docile temperament.

Embryo transfer procedure

The donors and the recipients involved in this study belong to the Santa Elena Ranch, a pure Red Brahman cattle, located in Madisonville, Texas. The Brahman embryos were collected and transferred under the direction of Ultimate Genetics-

Embryo transfer division. The same technician collected, evaluated, and transferred the embryos produced by the three protocols from 2001-2004.

The process to produce bovine embryos involved the preparation of two groups of cows, the embryo donor cows and the embryo recipients. Both groups were synchronized with the use of hormones. Donors had been selected by genetics, production and reproductive performance. The synchronization started with CIDR's (1.38 mg of Progesterone intra-vaginal device) 2.5 mg of 17β-estradiol + 50 mg of progesterone on day 0. To improve the efficiency of the embryo production, it was important to increase the number of released oocytes at the time of ovulation and maximize the collected number of high quality transferable embryos using FSH from day 5 to 8. The total dosage of FSH depended on several factors such as age, weight, and previous records. The dosage used for GnRH was 0.0042 mg/mL (5mL im as a total dosage) to the donors, 6 hours before the AI (artificial insemination). Eight days after breeding the donor, the embryos were collected using non-surgical procedures. The embryos were classified according to the stage and quality by IETS [82], and were transferred to the recipients previously prepared with the same protocol without the application of FSH, and GnRH. The recipients that did not conceive to embryo transfer, after a new physical and reproductive evaluation, were synchronized again from 60 days after the last embryo collection for the next embryo transfer protocol.

Protocols for donors

Results from the new protocol used for the donors in 2004 were compared with results of the three previous years. The production, quality, quantity, and stage of the embryos, pregnancies, resorptions, abortions, non-pregnant cows and births of the calves through the conventional embryo transfer technique were evaluated. The quality of the embryo and the stage of development of the embryo are described according to the IETS. Quality is a number based on

morphological integrity of the embryo which ranges from "1" to "4" as follows: Code 1: Excellent or Good. Symmetrical and spherical embryo mass with individual blastomeres (cells) that are uniform in size, color, and density. This embryo is consistent with its expected stage of development. This judgement should be based on the percentage of embryonic cells represented by the extruded material in the perivitelline space. The zona pellucida should be smooth and have no concave or flat surfaces that might cause the embryo to adhere to a petri dish or a straw. Code 2: Fair. Moderate irregularities in overall shape of the embryonic mass or in size, color and density of individual cells. At least 50% of the cellular material should be an intact, viable embryonic mass. Code 3: Poor. Major irregularities in shape of the embryonic mass or in size, color and density of individual cells. At least 25% of the cellular material should be an intact, viable embryonic mass. Code 4: Dead or degenerating. Degenerating embryos, oocytes or 1-cell embryos: non-viable [82]. Only embryos with quality code 1 and 2 were transferred and evaluated. The code for stage of development is also numeric, ranging from "1", an unfertilized oocyte or a 1-cell embryo to "9", expanding hatched blastocyst [82]. Only embryos number 4 (Morula, day 6), number 5 (Early blastocyst, day 7), and number 6 (Blastocyst, day 7-8), were transferred and evaluated.

Pregnancies (the act of carrying a developing embryo than fetus within the uterus) [133], resorptions (the organic process in which the substance of some differentiated structure that has been produced by the body undergoes lysis and assimilation) [133], abortions (expulsion of the products of conception before the embryo or fetus is viable) [133], non-pregnant, and live calves were also evaluated.

The new protocol was based on the previous response of the donors to the FSH for the super-ovulation process, the substitution of estradiol benzoate for 17β-estradiol, and the addition of a single dose of GnRH 6 hours before the artificial insemination of the donor. The schedule for this protocol was:

Day 0: Application of CDIR (Intravaginal 1.38 mg progesterone), 50 mg progesterone and 2.5 mg of 17β-estradiol (2mL im) in the morning.

Day 5 to 8: Application of FSH am/pm. The dosage depended on the individual response of each donor to the treatment in previous flushes. The age and weight was also considered. Based on the above criteria, the total dosage ranged from 11mL to 14mL of FSH (Folltropin).

Day 7: Application of 25 mg of prostaglandin F2-α (Lutalyse, 5mL, im) in the morning and the CIDR removal in the afternoon,

Day 8-9: 25 hours after the CIDR removal, 0.0042 mg /mL (5mL im) of GnRH were injected. 6 hours later, the donors were bred through artificial insemination for the first time and then two more inseminations with a 10-hr interval, for a total of three inseminations.

Protocols for recipients

The new protocol for the recipients involved the elimination of the application of estradiol benzoate on day 0, and the addition of an injection of 50 mg progesterone and 2.5 mg of 17β-estradiol (2mL im), at the moment of the application of the CIDR.

The protocol used in this study was:

Day 0: a.m. Application of CIDR plus a mixture of 50 mg of progesterone and 2.5 mg of 17β- estradiol (2mL im)

Day 8: a.m. Application of 25 mg of prostaglandin F2-α (Lutalyse, 5mL im)

Day 9: a.m. CIDR removal

Day 10: Begin detection of estrus.

Estrus is typically observed in 87% of the recipients synchronized [11]. Before the embryo transfer all donors and recipients were again evaluated through transrectal palpation. Donors were evaluated to check the potential embryos than can be collected on day 8 after the insemination based upon the number of corpora lutea. Recipients were evaluated prior to embryo transfer to check the response and CL stage. Only recipients with a CL greater than 10 mm in diameter received a fresh embryo. The corpora lutea were graded from 1 to 3, with 1= excellent, 2= good (close to 10 mm), and 3 = small corpora lutea that are not good to receive an embryo. The embryo flush was non-surgical, using the standard media and protocols for this technology. The uterine horns of the donors were flushed to collect the embryos. Pregnancy in the recipients was determined by transrectal palpation three times at 39-49 days, at 60 days, and at 90-120 days after the embryo transfer to confirm their pregnancies.

Data analysis

Independent variables that were evaluated for donor cow traits included body condition on the day of the synchronization for the embryo transfer process, the type of protocols used, the season of the year, the sire used to fertilize the mature oocytes, and the sire of the donor collected. At embryo collection, the total number of embryos collected per donor was recorded, as well as the number of good embryos to be transferred to the recipients, degenerate ova, and unfertilized embryos.

For the recipient traits, the independent variables included body condition, CL size, and stage and quality code score of the embryo that was transferred. Dependent variables studied included, palpation results (positive pregnancy, non-pregnant, resorption, abortions), and characteristic traits of the calves born (births, birth weight).

Statistical analysis

Analysis of Variance (ANOVA) was used to determine the effects of the independent variables on the response in donor and recipient females. This model included the effects of the new protocol for Red Brahman donor cows, used in 2004, compared to the results obtained from the protocols used from 2001 to 2003 to produce Brahman embryos. The General Linear Model Procedure of the SAS (2002-2003) statistical package was used to perform the analysis of the response data for donors and recipients.

For donor traits the model included sire of the donor, sire of the embryo, type of protocol, season within protocol, and body condition. Least squares means of significant effects were separated by two tailed t-tests. Response traits analyzed included total, good, degenerated, and unfertilized embryos collected. Ratios of good, degenerated, and unfertilized embryos, based on the total embryos collected were also evaluated with the same statistical model.

For recipient traits, the three protocols used in this study, the embryo stage, embryo quality, corpora lutea size, and season within protocol were analyzed by the final status of the transferred embryos (non-pregnant, resorptions, abortions, and live calves) using Chi-square analysis Freq- Procedure in SAS (2002-2003) statistical package. The General Linear Model (GLM) procedure of SAS was also used to analyze birth weight and gestation length. The model included the sire of the embryo, season-protocol, protocol, embryo stage, embryo quality, body condition, and size of the corpora lutea. Least square means of significant effects were separated by two tailed t-tests. Differences were considered significant at ($P<0.05$).

RESULTS

A total of 135 collections were carried out on 50 Red Brahman donor cows during 2001 to 2004, using three different protocols. The number of good transferable embryos collected with protocol 1 (2001-2002) was 171, with protocol 2 (2003) 152 good transferable embryos were collected, and with protocol 3 (2004) 208 good transferable embryos were collected. The total number of embryos produced during this time was 531 good transferable embryos.

Donors

The traits analyzed in the donors (number of good, degenerated, unfertilized, and total embryos) were not affected by sire of donor, sire of embryo, protocol, and body condition score. The number of degenerated embryos differed by season-protocol ($P<0.0157$). Fig. 7 shows the least squares means for winter from protocol 2 and winter from protocol 3 as the two most affected seasons in the number of degenerated embryos. These two seasons are different from the other seasons involved in this study = ($P <0.05$), between 2001-2004. The traits of good, degenerated, unfertilized, and total embryos were not affected by sire of the donors ($P>0.05$), but there appear to be some underlying biological differences in the least squares means (±SEM) between the 13 sires of donors presented in this study (Table 2). The sires of the donors with the seemingly most consistent and highest embryo production were 70/7, 4/95, 3/42, 56/3, and 24/8 with mean of 7.3 good embryos per collection. The sires of the donors that were in the middle were 4/115, 80/3, and "x" with a mean of 5.0 good embryos per collection. Sires of donors with the lowest embryo production were 3/180, 2/40, 0/170, and 72/7 with a mean of 3.3 good embryos per collection.

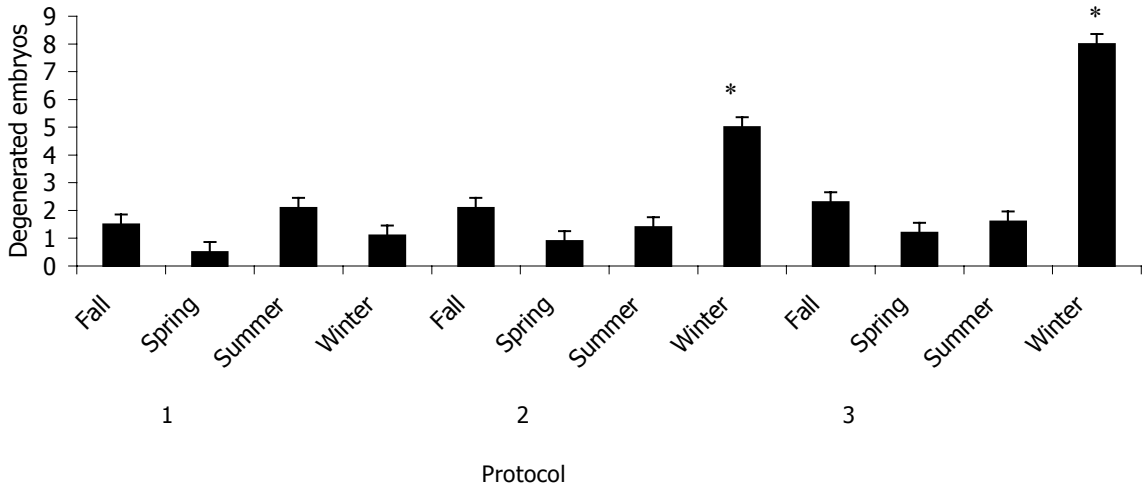


Fig. 7. Least squares means (+/- pooled SEM) of degenerated embryos by season of embryo collection for protocols 1,2 and 3. Winters (*) from protocol 2 and 3 are the highest in number for degenerated embryos ($P < 0.05$).

Table 2 also shows the sire of the donors organized from high to low based on the means of total and good embryo production, and from the low to high means of degenerated, and unfertilized embryos for the majority of this sires of the donors.

The traits evaluated (good, degenerated, unfertilized, and total embryos) did not differ statistically by the three types of protocol in the donors ($P>0.05$). However, Table 3 shows in protocol 3 a higher rank for mean production of good embryos, and a slight higher mean of degenerated embryos compared with protocol 1 and protocol 2, but protocol 3 had the lowest mean of unfertilized embryos of all protocols.

Table 2
Least squares means for number of good, degenerated, unfertilized, and total embryos by sire of donors (P>0.05).

Sire of Donor	N	Good	Degenerated	UFO	Total Embryo
70/7	3	11.6	4.7	0.0	16.2
4/95	11	6.7	2.2	1.0	9.8
3/42	5	6.6	3.5	0.3	10.4
56/3	20	6.5	1.4	1.9	10.0
4/115	5	6.0	5.3	2.3	13.7
24/8	9	5.0	1.4	-0.4	6.0
80/3	50	4.7	2.6	3.5	10.8
"x"	4	4.5	2.6	0.4	7.4
2/40	3	4.3	1.5	3.7	9.6
3/180	3	4.2	1.6	1.2	7.0
2/28	5	4.1	-1.7	0.2	2.6
0/170	13	2.7	1.8	4.0	8.5
72/7	4	0.1	2.7	5.5	8.3
	Pooled SEM	0.47	0.41	0.42	0.58

"x" Represents all the sires of donors that were collected only once.

The embryos were collected using three different protocols and in different years. Embryos collected by protocol 1 included 2001-2002, embryos collected by protocol 2 included 2003, and embryos collected by protocol 3 included 2004.

Table 3
Least squares means for number of good, degenerated, unfertilized, and total embryos by protocols (P>0.05).

Protocol	N	Good	Degenerated	UFO	Total Embryo
1	50	4.6	1.3	2.0	7.9
2	39	4.6	2.3	2.1	9.0
3	46	6.3	3.3	1.4	10.9
	Pooled SEM	0.16	0.14	0.14	0.20

The analysis of the traits (good, degenerated, unfertilized, and total embryos) by season-protocol was only different for degenerated embryos (P<0.0157). The other types of embryo production (good, unfertilized, and total) did not differ by season-protocol combination in the donors evaluated, however, there are some

differences in the means of the embryos for the 3 protocols and the 12 seasons evaluated. Table 4 shows that the highest production of good embryos was in the protocol 3 for spring, winter, and summer. The highest number of degenerated embryos was in protocol 3 for winter and fall, and in protocol 2 for winter, the seasons that showed the least production of unfertilized embryos were summer and spring for protocol 3, and summer and spring for protocol 1.

Table 4
Least squares means for number of good, degenerated, unfertilized, and total embryos by season-protocol. Degenerated embryos differed by season-protocol (P<0.05). Good, unfertilized, and total embryos were not significant by season-protocol (P>0.05).

Season	Protocol	n	Good	Degenerated	UFO	Total Embryo
Spring	3	11	8.3	1.3	0.9	10.4
Winter	3	7	6.1	1.6	1.3	15.3
Summer	3	22	5.7	1.0	0.3	7.3
Winter	1	9	5.7	1.1	3.4	10.3
Fall	3	6	5.4	1.6	3.0	10.6
Winter	2	9	5.3	1.5	1.6	11.6
Summer	2	16	5.2	1.1	1.3	8.0
Fall	1	11	4.7	1.5	3.6	9.8
Summer	1	25	4.2	2.1	0.3	6.7
Spring	2	10	4.1	1.3	1.0	5.9
Spring	1	5	3.8	0.4	0.5	4.8
Fall	2	4	3.8	2.1	4.4	10.4
		Pooled SEM	0.41	0.36	0.37	0.53

The ratios for good, degenerated, and unfertilized embryos were evaluated, based on the total embryos produced by donors with the same model selected for the donors (Sire of the donor, sire of the embryo, type of protocol, season-protocol, and body condition).

The ratio for good embryos differed by sire of the donor (P<0.0089). Fig. 8 shows the 13 least squares means (±Pooled SEM) of good-ratio embryos at the collection time by the sire of the donors involved in this study. The least squares means for sire and respective ratio were: 2/28: 0.90, 24/8: 0.87, 3/180: 0.70,

4/95: 0.65, 70/7: 0.65, "x": 0.60, 56/3: 0.58, 3/42: 0.53, 2/40: 0.47, 80/3:0.41, for 4/115: 0.40, 0/170: 0.34, and 72/7: 0.10. The pooled SEM for the ratio of good embryos was 0.12. Eight sires of the donors accounted for over 50% of the ratio to produce good embryos. The ratio for good embryos in donors did not differ by sire of the embryo, type of protocol, season-protocol, or body condition score at the moment of the embryo collection (P >0.05).

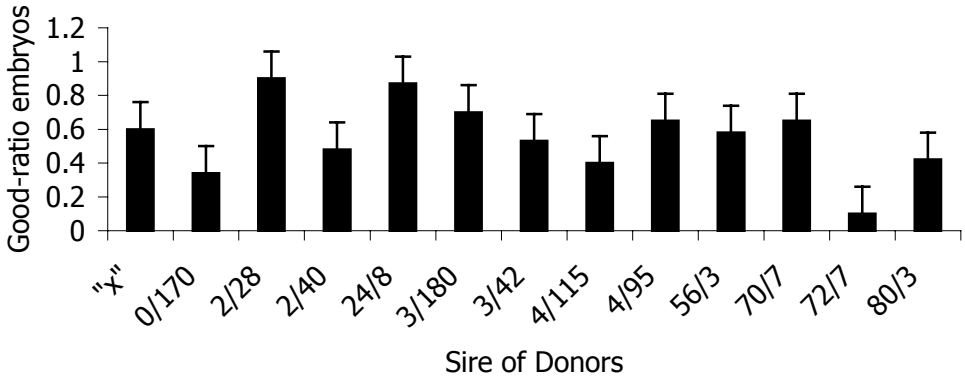


Fig. 8. Least squares means (+/- pooled SEM) of good embryo-ratio at the collection time for sire of donors (P<0.05).

The ratio for degenerated embryos differed by season-protocol (P<0.0018). Fig. 9 shows the least squares means (± Pooled SEM) of degenerated-ratio embryos at the collection time by season-protocol. They were: fall 1: 0.10, spring 1: 0.13, summer 1: 0.21, winter 1:0.18, fall 2: 0.10, spring 2: 0.10, summer 2: 0.14, winter 2: 0.48, fall 3: 0.18, spring 3: 0.11, summer 3: 0.18, and winter 3: 0.56. Winter for protocol 3 and winter for protocol 2 are the two seasons that showed different means from all the other seasons. Winter was the season that produced the most degenerated embryo-ratio.

The ratio for unfertilized embryos in donors did not differ by sire of the embryo, type of protocol, season-protocol, and body condition score at the moment of the embryo collection (P >0.05).

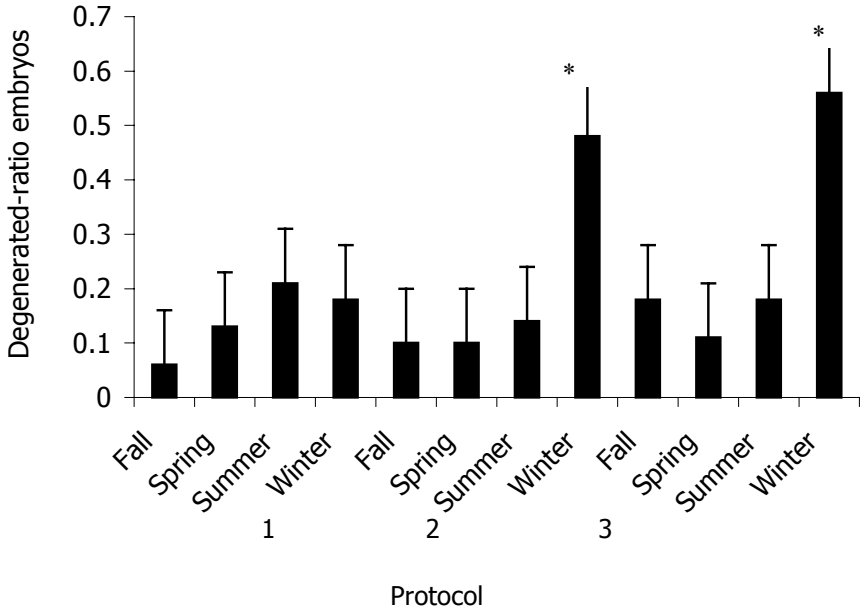


Fig. 9. Least squares means of degenerated embryo-ratio at the collection time for season-protocol (P<0.05). Winters (*) from protocol 2 and 3 are the highest to produce degenerated ratio embryos.

Recipients

The recipients were analyzed using two sets of criteria. The first set of criteria, included the three protocols, the three types of embryo stage, the two types of embryo quality, the three sizes of the corpora lutea of the recipient at the time of the embryo transfer, and the four seasons involved in the process. This model was evaluated by the final status of the embryo (Non-pregnant, resorptions, abortions, and live calves). The distribution of the final status of the embryos (Non- pregnant, resorptions, abortions, and live calves) at the end of the study

was different across protocols (P<0.0008). Fig.10 shows the percentages of the final status for the three protocols used.

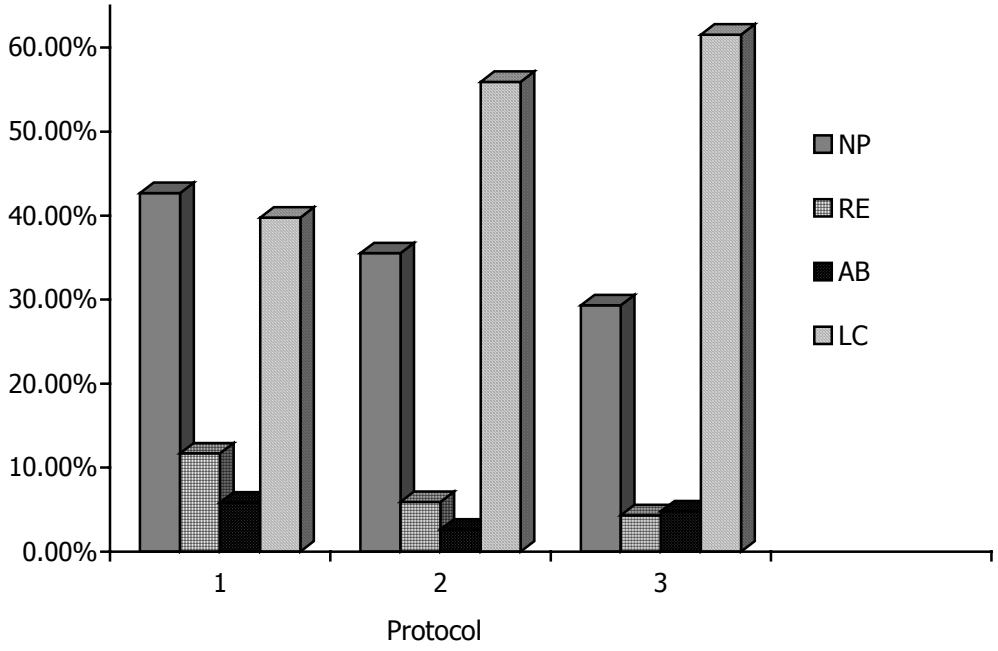


Fig. 10. Percentage of recipients of Brahman embryos that were non pregnant (NP), underwent resorption (RE), aborted (AB), or delivered a live calf (LC), by protocol (P<0.05).

Pregnancy rates were recorded three times after good embryos were transferred in the recipients. Transrectal palpations in the recipients were performed for the first time at 39-49 days, for the second time at 60 days, and for the third time at 90-120 days after the transfer. Fig. 11 shows the pregnancy rates of recipients with transferred embryos for the three protocols as follows: For protocol 1: 57.3% were pregnant at the first transrectal palpation, 45.6% were pregnant at the second transrectal palpation, and 40.90% were pregnant at the third transrectal palpation. For protocol 2: 64.50 % were pregnant at the

first transrectal palpation, 58.60% were pregnant at the second transrectal palpation, and 55.90% were pregnant at the third transrectal palpation. For protocol 3: 70.70% were pregnant at the first transrectal palpation. 66.30% were pregnant at the second transrectal palpation, and 62.50% were pregnant at the third transrectal palpation. These results show that with protocol 1 the percentage difference between the first palpation and the live calves is very large (17.5%) compared with the difference seen in protocols 2 (8.6%) and 3 (9.2%) where a significant decrease is noticeable.

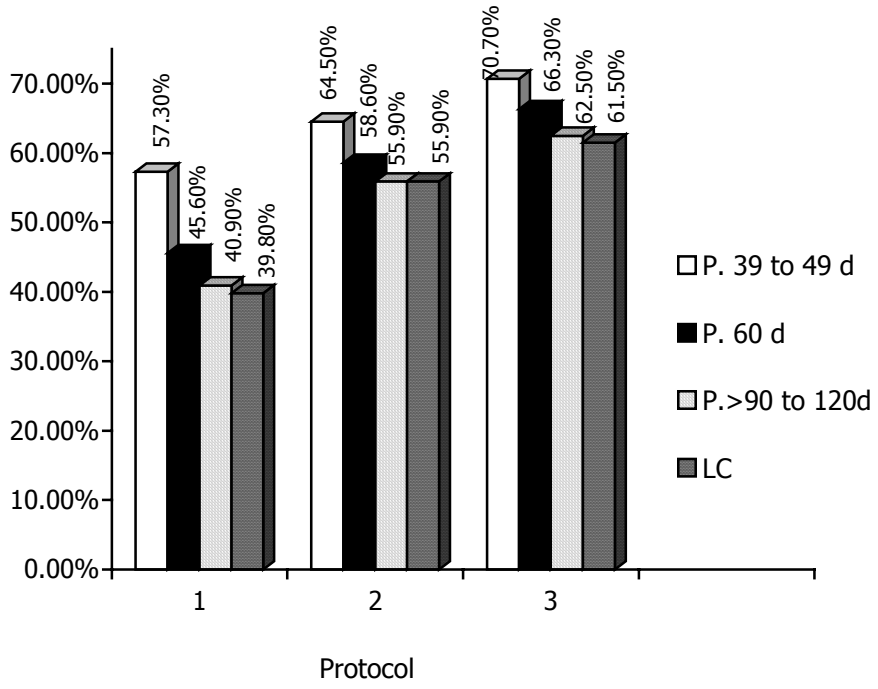


Fig. 11. Percentage of pregnancies in recipients of fresh Brahman embryos at the first palpation (39 - 49 days), second palpation at 60 days, third palpation (>90 - 120 days), and delivered live calves (LC), by protocol.

The distribution of the final status for other variables (embryo quality transferred, the size of the corpora lutea at the time of the embryo transfer, and the seasons) did not differ. However, Fig. 12 shows a trend (P<0.1332) in the percentages of live calves with more calves born from stage 6 than stage 5 or stage 4 embryos.

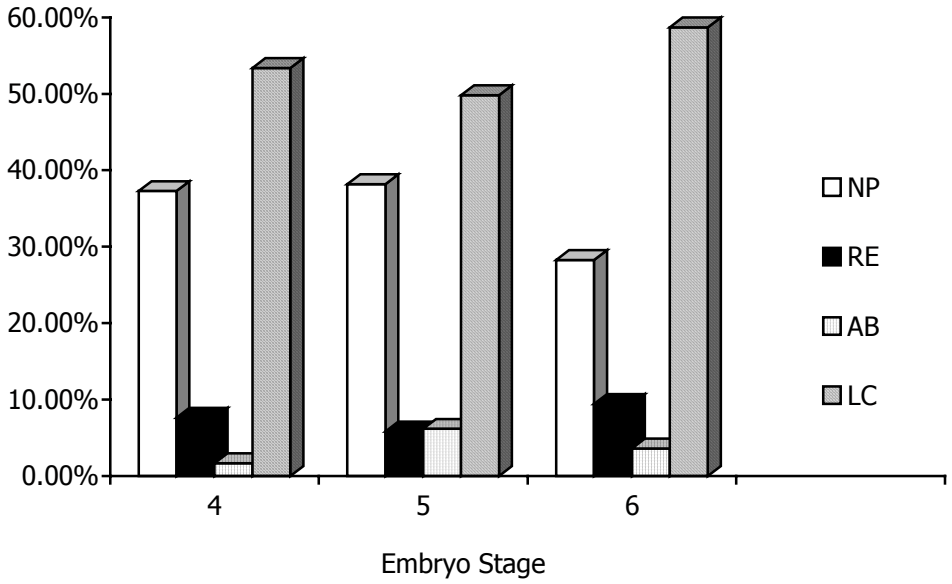


Fig. 12. Percentage of recipients of Brahman embryos that were non pregnant (NP), underwent resorption (RE), aborted (AB), or delivered a live calf (LC), by embryo stage.

Even though season did not affect the final status of the embryo (P<0.1026). Fig . 13 shows what may be biological differences in the percentages of the final status of the embryos. This study found that winter was the best season to get live calves from embryos transferred in recipients in the previous spring, and the seasons with less number of resorptions were summer and winter. The season with least number of abortions was spring.

The second aspect to the analysis of the recipient data was evaluation of gestation length and birth weight of the born calves, which were evaluated by sire of the embryo, season-protocol, protocol, embryo stage, embryo quality, body condition and corpora lutea size in the recipients at the time of the embryo transfer. Gestation length differed by sire of the embryo ($P<0.0007$), by season-protocol ($P<0.0001$), by protocol ($P<0.0067$), and by body condition score ($P<0.0042$). Table 5 shows the least squares means (±Pooled SEM) with the differences found in gestation length by the 14 sires of the embryos in this study.

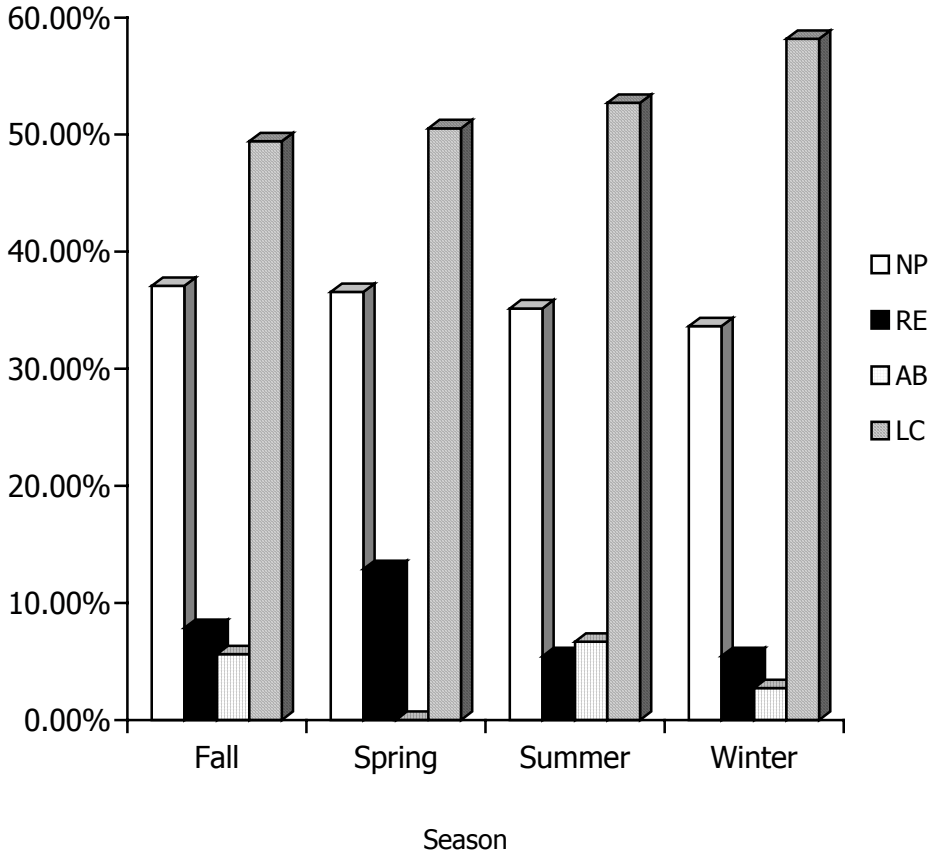


Fig. 13. Percentage of recipients of Brahman embryos that were non pregnant (NP), underwent resorption (RE), aborted (AB), or delivered a live calf (LC), by season.

The sires of the embryos with low least squares means (± Pooled SEM) for gestation length were: 2/400 with 279.9 days, and 56/3 and 2/28 with 280.2 days. The sires of the embryos that were in the middle for gestation length were: 3/180 with 283.5 days, 80/3 and 92/1 with 283.6, 4/95 with 284.0, 4/45 with 284.4, and 70/1 with 284.9, and the sires of the embryos that had the highest gestation length were: 6/125 with 285.5, 10/9 with 286.9, 4/115 with 288, 4/128 with 290.0, and 80/2 with 292.6.

Table 5
Least squares means (± pooled SEM) for gestation length by sire of the embryo (P<0.05).

Sire of embryo	n	Gestation length (d)
		LS Means
80/2	5	292.6
4/128	5	290.0
4/115	14	288.0
10/9	12	286.9
6/125	33	285.5
70/1	4	284.9
4/45	5	284.4
4/95	34	284.0
80/3	12	283.6
92/1	1	283.6
3/180	35	283.5
2/28	16	280.2
56/3	3	280.2
2/400	6	279.9
	Pooled SEM	0.38

Table 6
Least squares means (± pooled SEM) for gestation length by season-protocol (P<0.05).

Season	Protocol	N	LS means
Spring	3	16	276.4
Spring	2	14	283.8
Summer	1	30	283.9
Winter	2	28	284.2
Fall	1	15	285.3
Winter	1	20	286.5
Winter	3	16	287.4
Summer	2	37	288.2
Spring	1	3	288.9
Fall	2	6	289.0
		Pooled SEM	0.3

The gestation length in recipients differed by season-protocol (P<0.0001). In Table 6, spring-protocol 3 had the lowest least squares means (± Pooled SEM) for gestation length (276.4), summer-protocol 2, spring-protocol 1, and fall-protocol 2 had the highest least squares means (± Pooled SEM) for gestation length (288.2, 288.9, and 289.0), respectively.

The analysis for gestation length differed by protocol (P<0.0067) in the recipients. Table 7 shows protocol 3 as the least square mean (± Pooled SEM 281.89 (± 0.13) with the lowest gestation length of the 3 protocols. The least square means (± Pooled SEM) for protocol 1 and 2 were 286.14, and 286.31, respectively.

Table 7
Least squares means (± pooled SEM) for gestation length by protocol. (P<0.05).

Protocol	n		LS means
1	68		286.14
2	85		286.31
3	32		281.89
		Pooled SEM	0.13

The regression of gestation length on body condition score (on the scale of 1 to 9) of the recipients (P<0.0042) showed that gestation length was affected by 1.8 more days per each point more in the body condition of the recipient.

The last trait analyzed in this study was birth weight of the calves born of the embryo transfer program with the same model used for gestation length (sire of the embryo, season-protocol, protocol, embryo stage, embryo quality, body condition score, and corpora lutea size).

Birth weight differed by season-protocol combination (P<0.0109). Table 8 shows that the least squares means with the lowest birth weights were for spring-3 with 74.7, followed by fall-2 with 77.2, and summer-2 with 79.6. The birth weights found with middle least squares means were for summer-1 with

80.1, spring-2 with 80.6, fall-1 with 81.9, winter-2 with 84.0, and winter-1 with 84.7. The birth weights found with the highest least squares means were for spring 1-with 87.3, and winter-3 with 89.4.

Table 8
Least squares means for birth weight by season-protocol. (P<0.05).

Season	Protocol	N		LS means
Fall	1	15		81.9
Spring	1	3		87.3
Summer	1	30		80.1
Winter	1	20		84.7
Fall	2	6		77.2
Spring	2	14		80.6
Summer	2	37		79.6
Winter	2	28		84.0
Spring	3	16		74.7
Winter	3	16		89.4
			Pooled SEM	0.4

Birth weight of the calves born through the embryo transfer technology differed by embryo quality at the moment of the transfer (P<0.031). The highest weight was for calves from embryos classified as quality 1 at the moment of the embryo collection that were later transferred to the recipients (Table 9).

Table 9
Least squares means for birth weight by embryo quality (P<0.05).

Embryo Quality	N		LS means
1	75		84.0
2	110		80.2
		Pooled SEM	0.1

Birth weight of the calves born through the embryo transfer technology differed when regressed on corpora lutea size of the recipients (P<0.0018). The

calves born from recipients that had CL1 were 2.05 (kg) heavier than calves from recipients that had CL2 at the day of the embryo transfer.

Other models were evaluated in the traits for donors such as number of AI, sire of the donors within protocols, sire of the embryos within AI, age of the donor, weight of the donor, FSH mL, protocols within FSH mL, protocols within age, protocols within weight, and protocols within body condition score, but the results were not significant, and were excluded for the final analysis.

For recipients age was evaluated with the models used in SAS with no significant values. The breed of recipients was not evaluated because most of them (87%) were crossed F1 Brahman-Holstein, and the remaining 13% were represented by few recipients of many different crosses.

DISCUSSION

Donors

The number of degenerated embryos, as well as the ratio for the same trait evaluated in Red Brahman cows, was affected by season-protocol. The highest number of degenerated embryos was produced in winter protocol 3 and winter protocol 2. If we compare the degenerated embryos for winter for the three protocols, winter protocol 3 produced the highest number. For the ratio of degenerated embryos, winter protocol 3 and winter protocol 2 were the seasons that produced the most degenerated embryo-ratio. The highest embryo productions are compared in bovine embryo transfer technology, the production of gametes has been affected by seasons in both genders [134]. Indicating that embryo production is affected by the season of the year. *Bos indicus* cattle have reduced reproductive performance in winter [134]. In a study of reproduction of *Bos indicus* breeds and crosses by Bastidas and Randell [135], the number of transferable embryos produced per Brahman donor cow was lower in winter [135]. The analysis of good, unfertilized, and total embryos did not differ by season-protocol, even though there were differences on their least squares means for the embryo production. Spring, winter, and summer, for protocol 3, were the seasons with the highest number of good embryos produced, and also the seasons with the lowest number of unfertilized embryos found, not including winter for the same protocol 3. In Summary, the better combination to produce good embryos were the seasons that used protocol 3, not including winter. These results could be due to the improvements of protocol 3 using the FSH for superovulation under different criteria from the one used for protocol 1 and 2. Also the addition of only one dosage of GnRH to synchronize ovulation in the donors, 24 hours after the CIDR removal, or 36 hours after the application of PFG 2α (induction the regression of the corpora lutea) to allow the follicle to ovulate and the female to demonstrate behavioral estrus. GnRH has been used

with CIDRs and estradiol to control follicular growth, and a second dosage of either GnRH or estradiol to induce timed ovulation following CIDR removal [44].

Season could also have some effect on the production of embryos due to the variability of embryo production through the length of the 4 seasons and even more through the 12 periods evaluated from 2001-2004. A study on Brahman cows found that Brahman donors produced the greatest number of good embryos in the spring season [136]. A possible explanation for some of the seasonal influences found in *Bos indicus* cattle may be the function of the pituitary which is altered during the winter, suggesting that day length affects the estral cycle, especially in the Brahman breed. Brahman cows have a lower pre-ovulatory luteinizing hormone surge during the winter compared with the spring or summer periods [137].

The number of good, degenerated, unfertilized, and total embryos was not affected by sire of the donors. However the ratio for good embryos differed by sire of the donor. The differences in the least squares means for embryos produced by the 13 sires of the donors evaluated are shown in Table 2, and Fig. 8. The differences found could be related to genetic factors related to their fertility. A number of genetic based variations are known to have direct effects on fertility and reproductive outcome in cattle [138]. Currently, the cattle industry is requesting the use of genetic markers to predict fertility in males as well as in females. Genetic markers can be used to identify inheritance of specific genes of animals from their parents [139]. An example of fertility tests that identify genetic markers is Fertility Associated Antigen (FAA) to select bulls.

Differences in production of good, degenerated, unfertilized, and total embryos were not significant by the three types of protocols used in the donors studied. However, the three protocols showed slight differences using the least squares mean analysis of embryo production (Table 3). Protocol 3 had the highest least squares mean for good embryos compared with protocol 2 and 1. Protocol 3 also had the lowest least squares means for unfertilized embryos compared with protocol 2 and 1, but at the same time protocol 3 had the highest least squares

means for degenerated embryos compared with protocol 2 and 1. The better response in protocol 3 to produce more good embryos per collection can be related to the changes made to the super ovulation protocol. The dosage and criteria to use FSH in the donors was based per each individual, according to their previous records, age, and weight. However the dosage used in protocol 3 was lower than that used in protocols 2 and 1. Massive ovarian responses are often associated with a number of problems, and the final goal of super ovulation is to obtain good quality embryos. Large ovarian responses often result in the reduced embryo quality, massive ovarian responses may be associated with a reduction in the proportion of follicles that ovulate [140]. Follicular maturation is also strongly altered when super ovulation is induced [141]. The alterations are in the differentiation of granullosa/thecal cells induced by PMSG or FHS [142,143]. The other factor involved in the highest number of good embryos produced by protocol 3 over protocols 2 and 1 was the use of 17β-estradiol. 17β-estradiol intramuscularly induces regression of the dominant follicle and resets the development of follicles on the ovaries of a donor. This allows the use of FSH to be started 4.5 days later when a new wave of follicles is developed [144]. The number of good embryos produced by protocol 3 in this study could be compared with a study made in 58 cows superovulated following the application of 17β-estradiol to reset follicular development, producing a total of 394 embryos, with an average of 6.8 grade 1 or 2 embryos per donor [144]. This same author reported that when a control group of 56 donors not treated with 17β, resulted in 353 embryos and a rate of 6.3 grade 1 or 2 embryos per donor [144]. There are other aspects relevant to the embryo transfer industry, called variability between females and within each female. The first can be detected as soon as super ovulation is attempted, the second requires successive attempts at super ovulation in the same female [141]. Variation among donors is still one of the most frustrating aspects of embryo transfer.

Recipients

The results showed significant differences in the final status (non-pregnant, resorptions, abortions, and live calves) for the three protocols used (Fig. 10). Transrectal palpations in the pregnant recipients revealed loss of early and late pregnancies through the full length of gestation (Fig. 11). Analysis of the results for recipients for protocol 1, showed resorptions of 11.7% between the first and the second transrectal palpation, abortions of 4.7% between the second and the third transrectal palpations, and more abortions of 1.1% between the third transrectal palpation and the birth time of the calves. Recipients with transferred embryos using protocol 2 showed resorptions of 5.9% between the first and the second transrectal palpation, abortions of 2.7% between the second and the third transrectal palpations, and 0% abortions between the third transrectal palpation and the birth time of the calves. Finally recipients with transferred embryos using protocol 3 showed resorptions of 4.4% between the first and the second transrectal palpation, abortions of 3.8% between the second and the third transrectal palpations, and 1% of abortions between the third transrectal palpation and the birth time of the calves. Most of the lost pregnancies occurred between the first and the second palpation, and the protocols that showed the least lost pregnancies were 2 and 3. Between protocol 1 and 3 there is a big difference in the percentages of total lost embryos. Losses of pregnancy are characterized by early embryonic death, which occurs prior to the period of corpus luteum maintenance in the cow at days 15-17 of the cycle, and late embryonic death, which occurs from corpus luteum maintenance to the end of the differentiation stage, at approximately 42 days of gestation [91, 92]. After 50 days of gestation, pregnancy losses are less frequent and characterize fetal death [91, 92].

It is interesting to compare the fetal loss rates in this study with the fetal loss rates reported by [145,146] under field conditions. This evaluation showed fetal losses of 5.8% for protocol 1, 2.7% for protocol 2, and 4.8% for protocol 3 while

fetal loss rates of 5.6% between the time of pregnancy diagnosis and parturition are thought to be common in cattle [145,146]. The fetal loss rates reported by Dunne and Baxter et al [145,146] include the losses by reproductive diseases, in this study Red Brahman cows donors and recipients were tested for reproductive diseases, even though the herds of origin of the donors and recipients were free of the main reproductive diseases. This also explains the lower fetal loss rates in embryo transfer with the three protocols used compared with the results reported by Dunne and Baxter et al [145,146]. However, there are other many factors involved in the pregnancy losses and fetal death such genetic, nutritional, and management aspect that are not discussed in this study.

The analysis of the recipients by the stage of the embryo on the day of transfer approached significance but was not significant of ($P<0.05$). The slight difference shown in the percentages of live calves between embryo stage 4 and embryo stage 6 may suppose that embryos have better chances of survival through the whole gestation when transferred as morula or blastocyst, but not with early blastocyst. Spell et al [147| determined effect of type and developmental stage of embryos on pregnancy rates after embryo transfer in Angus cows for both groups, donors and recipients. The embryos collected were assigned a developmental stage and quality grade according to standards set forth by the International Embryo Transfer Society. Developmental stage codes were: 3 for early morula, 4 for morula, 5 for early blastocyst, and 6 for blastocyst. Pregnancy diagnosis was performed 30 days after the transfer (37 total days of gestation). The pregnancy rates for embryos staged 3, 4, 5, and 6 were: 50%, 73.4%, 73.1%, and 63.2% respectively [147]. These results can not be compared because the cited study evaluated pregnancy rates only at 37 days of gestation, while in this study (Red Brahmans) the recipients were evaluated through the length of the whole pregnancy, until the birth of the calves. A major problem for the cattle breeding industry is the high rate of early embryo loss [148]. The evaluation of any kind of effect of an embryo at 37 days of pregnancy rate is considered too premature to make any kind of definitive statement.

The last part of this study was the gestation length and birth weight of the embryo calves born. Gestation length differed in the analysis by the sire of the embryos, season-protocol, and corpora lutea size in the recipient on the day of the transfer. The differences found among the 14 sires of the embryos for the least squares means for gestation length were: The sires of the embryos that had the lowest least squares means were: 2/400, 56/3, and 2/28. The sires of the embryos that had the highest least squares means were: 80/2, 4/128, 4/115, and 10/9. The rest of sires of the embryos are in between the lowest and the highest least squares means for gestation length. However the 14 Red Brahman bulls used to produce embryos showed that the gestation length for the recipients (dam of these embryos), was under normal parameters. Randel [134] reported the gestation length by other sources: *Bos taurus* with 282 days [134,149], Brangus 286 days [134,150], Brahman 293 days [134, 151], Nelore 291 days [134, 152]. In another study by Amen et al [153] that evaluated birth weight and gestation length (n =511), *Bos indicus- Bos taurus* reciprocal backcrossed embryo transfer calves were studied. The average for gestation length in males and females was 291.2 and 288.5, respectively [153]. Results for gestation length were affected by the breed composition of the embryo transfer calves themselves, as well as the cross that produced the calves when *Bos indicus* and *Bos taurus* combinations are involved [153].

The use of EPDs (Expected Progeny Difference) from the American Brahman Breeders Association [154], which is an estimate of an animal's genetic value as a potential parent as compared to another animal of the same breed [155]. The birth weights can be predicted by using the EPDs as an alternative to predict the gestation length that can be influenced by the sire of the donor. The birth weight EPDs for some of the lowest least squares means for the red Brahman bulls used in this study coincide with a shorter gestation length as shown in Table 10 for bulls 2/400, 56/3 and 2/28. In this case it was more accurate to predict short gestation length based on the EPDs of the sires because these sires had a high accuracy to predict low birth weights furthermore short gestation length.

Accuracy value, expressed on a 0 to 1 scale gives an indication of how much confidence one can place in the EPD, and is based on how thoroughly the animal has been evaluated genetically [155]. For the highest least squares means for gestation length, the bull: 80/2 was not possible to evaluate because its EPDs are not avaliable yet. The sires 4/128, 4/115 and 10/9 were the ones with the highest gestation length and high EPDs for birth weight, but the accuracy of those EPDs are not high compared to the sires with low EPDs. For that reason in this study we can not predict high gestation length for the sires of the donors. However, cows of different breeds have different gestation lengths. The breeds derived from India have gestation lengths about 10 days longer than *Bos taurus* breeds [134].

Table 10
Least squares means for gestation length and EPD's for birth weights by sire of the embryo.

Sire of embryo	N	Birth Weight		Gestation length (d)	Birth weight (lb)
		EPD	ACC	LS Means	LS Means
80/2	5	-	-	292.6	82.0
4/128	5	1.2	0.64	290.0	81.6
4/115	14	1.0	0.73	288.0	83.0
10/9	12	1.5	0.46	286.9	78.9
6/125	33	3.1	0.62	285.5	81.5
70/1	4	-0.1	0.65	284.9	81.5
4/45	5	2.9	0.63	284.4	81.6
4/95	34	2.6	0.70	284.0	85.4
80/3	12	5.4	0.88	283.6	82.4
92/1	1	1.6	0.65	283.6	81.0
3/180	35	3.9	0.71	283.5	84.3
2/28	16	-0.2	0.83	280.2	81.1
56/3	3	0.2	0.86	280.2	81.9
2/400	6	0.65	0.71	279.9	78.2

The gestation length in recipients differed by season-protocol. Spring-protocol 3 and 2 had the lowest least squares means for gestation length, in contrast Summer-protocol 2, Spring-protocol 1 and Fall-protocol 2 had the highest least squares means for gestation length. Contrary to this study Ritchie et al. [156] reported that calves born in the fall season are in general lighter in weight and

experience less dystocia that those born in the spring. Then this leads us to think that the protocol has an important influence on the gestation length. The analysis for the gestation length differs by protocol, and Protocol 3 was the one with lowest least squares means for gestation length.

For the analysis of gestation length by body condition, no literature was found to compare with the results obtained in this study. However there are many other factors that affect gestation length through the body condition of the recipient such as genetic and nutritional factors, age, breed of the recipient and breed of the embryo as well as EPDs of the sires of donors and embryos.

Birth weight differed based on embryo quality. This result could be used to match embryos quality grade 1 with recipients with big frame score and mature, embryos with quality grade 2 with recipients with small frame score and young. This would prevent potential dystocias at the birth time with some embryos from sires known as carriers of high birth EPDs.

The last trait is the corpora lutea. Birth weight is influenced by corpora lutea, which means that calves from recipients that had CL1 were 2.05 kg heavier than calves from recipients with CL2. Since recipients with CL1 produce heavier calves it would be desirable to transfer grade 2 embryos to them, and recipients with CL2 transferred with embryos grade 1.

CONCLUSIONS

Registered Red Brahman donor cows, and recipients F1 Brahman-Holstein, from Santa Elena Ranch, located in Madisonville, Texas were evaluated. The study involved the whole process of embryo transfer, through the analysis of records of fresh embryo production from the day of the collection to the birth of the embryos produced. Fifty donors were selected based on reproductive and production records. The study included records produced during years 2001 to 2004 with three different protocols. For 2001 and 2002 with protocol 1, 2003 with protocol 2, and 2004 with protocol 3. A total of 135 collections were made. For donors the traits evaluated were: sire of the donor, sire of the embryo, protocol, season-protocol, and body condition by the production of the donor at the collection time as follows: good, degenerated, unfertilized, and total embryos produced. The total number of good embryos collected was 531 categorized as follows: 171 embryos for protocol 1, 152 embryos for protocol 2, and 208 for protocol 3. The embryos were transferred immediately to 531 recipients previously selected by reproductive and production records. Records of transrectal palpation were recorded at 39 to 49 days, at 60 days, and between 90 to 120 days after the embryo transfer. These results were evaluated as non-pregnant, resorptions, abortions, and number of live calves born through this reproductive technology. The model used for these traits was: protocol, embryo stage, embryo quality, corpora lutea size, and season. The recipients were evaluated according to the gestation length and birth weight of the calves by the traits: sire of the embryo, season-protocol, protocol, embryo stage, embryo quality, body condition score, and corpora lutea size.

Spring and summer for protocol 3 were the best seasons to produce good numbers of embryos, and the least number of unfertilized embryos for the donors.

Protocol 3 with the use of 17ß-estradiol in combination with FSH in individual treatments, dosages, and criteria, plus the use of GnRH in Red Brahman donors

previously synchronized with (CIDRs), was the best alternative to produce quality, and quantity number of embryos per collection for the majority of the seasons except winter. Winter was identified as the season that produced the highest number of degenerated embryos. Further studies are needed to determine the cause resulting in produce unfertilized, and degenerated embryos at the collection time in order to maximize the production.

There are genetic variations present in the sires of the donors that affect the embryo production independently of the protocols used and season of the collection. The sires of the donors with the most regular production of embryos were: 70/7, 2/28, 4/95, 3/42, 56/3, and 24/8. The sires of the donor with average production of good embryos were: 4/115, 80/3, and "X". The sires of the embryos with the lowest embryo production were: 3/180, 2/40, 0/170, and 72/7.

There are differences between the protocols with respect to the final result of the transferred embryos. Protocol 3 had the highest percentage of live calves (61.54%) compared with protocol 2 and 1, respectively (55.92%, and 39.77%). Protocol 3 also had the lowest percentage of resorptions of the three protocols evaluated. Protocol 2 had the lowest percentage of abortions (2.63%) compared with protocol 3 (4.81%), and protocol 1 (5.85%). Based on the results on the donors collection and the embryo transfer to the recipients in this study, protocol 1 was found different from protocol 3.

The majority of the lost pregnancies for the embryos were between the first and the second palpation after the transfer. The protocol 3 showed the lowest number of lost pregnancies.

It is believed that embryos in stage 4 and in stage 6 have better chances of survival once they are transferred in the recipient. However, it requires further study with a larger numbers of embryos and recipients to validate this potential hypothesis.

The results showed that the sires of the embryos with the lowest gestation lengths were: 2/400, 56/3, and 2/28. The sires of embryos with the highest

gestations lengths were: 80/2, 4/128, 4/115, and 10/9. However the 14 Red Brahman bulls used in this study showed normal gestation lengths for the recipients.

The use of EPDs to predict birth weights in calves from the sire of the donor could be used to predict gestation length. According to the results of this study, the donor's sires: 2/400, 56/3, and 2/28 with high ACC should be selected for this trait.

The results showed that gestation length in recipients differed by season-protocol, with the protocol more influential on the gestation length. For further studies, it is important to analyze the effect of gestation length by body condition of the recipient on the day of the embryo transfer because this study found that it was significant. However there may be other factors involved that were not evaluated in this analysis.

Birth weight by embryo quality, and birth weight by corpora lutea size of the recipient can be used together as a parameter to choose the right embryo from the collection for the right recipient at the moment of the embryo transfer, to decrease the risk of loosing the pregnancy and reduce dystocias.

This study should be very beneficial to the Brahman breeders in that it is a model designed specially for the breed. It should help them to have a better understanding of the unique attributes of the breed with respect to reproductive and production performance. However, there are still many unanswered questions to solve in this field, and further research is needed relative to embryo production in Brahman cattle.

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