Embryo transfer: managing recipients and donors

G. Cliff Lamb, Ph.D., Professor and Head, Department of Animal Science, Texas A&M University;
Vitor R. G. Mercadante, Ph.D., Assistant Professor, Virginia Polytechnic Institute and State University;
Pedro L. P. Fontes, D.V.M., Graduate Research Assistant, North Florida Research and Education Center, University of Florida.

Abstract

For an embryo transfer program to be effective, numerous factors need to be put in place to ensure success. Nutrition, estrous cycle control, donor and recipient management are all factors responsible for the success or failure in fertility for a given herd. Utilization of body condition score is a practical method to determine nutritional status of both recipients and donors. Prepartum nutrition is critical to ensure that recipient cows calve in adequate body condition to reinitiate postpartum estrous cycles early enough to respond to synchronization protocols. Estrus synchronization for embryo transfer after detected estrus or for fixed-time embryo transfer are effective methods to increase the number of calves produced by embryo transfer. In addition, resynchronization of nonpregnant females effectively ensures that a high percentage of recipients will return to estrus and are eligible for subsequent embryo transfers. Numerous additional factors need to be assessed to ensure that the herd achieves its reproductive potential. These factors include assessing the merits of nulliparous, primiparous, or multiparous cows, ensuring that facilities allow for minimal stress, and that the herd health program is well-defined and followed. Numerous short- and long-term factors contribute to donor's production of embryos and whether or not the recipients will maintain a transferred embryo, deliver a calf without assistance, or raise and wean a healthy calf.

Introduction

The primary use of embryo transfer in cattle has been to amplify reproductive rates of valuable females. Embryo transfer is especially useful with cattle because of their relatively low reproductive rate and long generation interval (Seidel, 1991) when compared to other livestock species.

The success of embryo transfer depends on factors associated with the embryo, the recipient, or an interaction between both. Once transferable embryos are collected from a donor cow, a decision is made as to which of the available recipients should receive embryos to achieve the greatest number of pregnancies (Wright, 1981). Suitability of recipients is dependent on numerous management, nutrition, and estrous cycle control factors that affect whether or not the recipient will have a functional CL at the time of embryo transfer. Although studies have focused on these factors, differences in techniques, sample sizes and other elements have limited the applicability of the results of these studies.

In many ways, management of the donor and recipient is critical to ensure the success of an embryo transfer program since donors are expected to produce good quality embryos and the recipients must be able to conceive to the transferred embryo, maintain the pregnancy until full term, calve without assistance, and raise a calf of high genetic merit. Therefore, the available

management strategies that allow for optimization of embryo transfer programs will be discussed in this article.

Managing Donors

Nutrition Management of Donors

Many factors may influence how donors respond to superstimulation and generate a high number of fertilized good to excellent quality embryos. Outside of genetics, nutrition likely is the single greatest factor that influences the response of donor cows to superstimulation. It is important to ensure that cows are maintained on a positive plane of nutrition and are fed a diet that meets maintenance requirements.

Throughout the embryo transfer industry, the current dogma exists that feeding an organic source of mineral prior to superovulation of donors will enhance the total number and quality of transferable embryos. One previous unpublished study has demonstrated that donors receiving organic mineral may yield a greater quantity of embryos, but this report failed to demonstrate that organic mineral enhanced the quality or quantity of embryos. Therefore, we conducted a study to determine whether trace mineral supplementation prior to embryo collection affected embryo production and quality. In this study (Lamb et al., 2008), among all heifers, the total number of recovered embryos was similar among treatments. The number of unfertilized embryos was greater for Inorganic than Organic heifers, whereas Control heifers were intermediate. In addition, Control heifers had a greater number of degenerate embryos than Organic or Inorganic heifers. Organic heifers produced a greater number of transferable embryos than Inorganic and Control heifers remained intermediate (Table 1). Although the appearance occurs that Organic heifers produced more transferable embryos than Inorganic heifers, there is not an explanation for not having differences in the Control heifers. Therefore, we concluded that mineral source probably does not influence embryo quality or number.

In another study that cows received a blend of bioactive peptides and oligosaccharides to support immune function has shown some promising results. In this study (Marquezini et al., 2010) we determined that supplementation of Nutrition Horizons Grade One™ (Brookville, OH) may alter quality of embryos after superovulation. Donors received either: 1) 6 Grade One™ capsules (13 g/capsule) containing a blend of bioactive peptides and oligosaccharides (NHG1; n = 35); or 2) donors received 6 placebo capsules (13 g/capsule; Control; n = 37). After superovulation the embryos were evaluated and classified by stage and quality. The percentage of grade 1 embryos collected compared to recovered transferable embryos was greater ($P = 0.062$) for NHG1 than Control. In addition, the percentage of grade 2 embryos collected compared to recovered transferable embryos was greater ($P < 0.05$) for Control (76.6%) than NHG1 (59.9%). We concluded that the number of transferable embryos collected per flush did not differ between treatments; however, the quality of transferable embryos was improved after embryo donor cows received NHG1 prior to embryo collection.

Table 1. Embryo production in heifers receiving inorganic, organic or no mineral after superovulation with follicle stimulating hormone (Lamb et al., 2008).

Item	Treatments[a]			P-value
	Control	Inorganic	Organic	
	----------n ± SE----------			
All treated heifers[b]:				
No. of heifers	49	51	51	
Total embryos recovered	4.24 ± 0.60	3.64 ± 0.60	3.29 ± 0.58	0.5219
Degenerate/cleaved	0.93 ± 0.24^{x}	0.26 ± 0.23^{y}	0.25 ± 0.23^{y}	0.0632
Unfertilized	1.31 ± 0.37^{x}	2.32 ± 0.36^{y}	0.82 ± 0.36^{x}	0.0135
Transferable	2.01 ± 0.39^{xy}	1.07 ± 0.38^{y}	2.18 ± 0.38^{x}	0.0900
Grade 1	1.43 ± 0.33	0.82 ± 0.32	1.43 ± 0.32	0.2955
Grade 2	0.56 ± 0.14^{xy}	0.23 ± 0.13^{x}	0.68 ± 0.13^{y}	0.0494
Grade 3	0.00 ± 0.03	0.02 ± 0.03	0.06 ± 0.03	0.1948

[a] Heifers received either 0.11 kg of organic mineral, 0.11 kg inorganic mineral, or no mineral for the 23 days prior to embryo collection.
[b] All heifers receiving FSH.
[x,y] Uncommon means within a row differ ($P < 0.05$).

Superovulation of Donors

Significant progress has been made in the understanding of cattle reproductive physiology. This knowledge has been used for the development of applied technologies that allow us to control reproductive events in the cow with the use of exogenous hormones. Within those technologies, the objective of superovulation protocols is to increase the number of follicles ovulated per cycle, allowing the fertilization of multiple oocytes and consequently, the production of several embryos at once.

One relevant strategy is the use of hormonal treatments to synchronize the follicular wave in such a way that the beginning of a new follicular wave coincides with the beginning of FSH administration. Bo et al. (1991, 1996) tested the association of estrogen (estradiol 17-13 or estradiol valerate) with a progestin (progesterone, CIDR-B, or norgestomet, Synchromate-B) to induce follicle turnover and synchronize a new wave. The progestin was present for 6 to 7 days and the estrogen (2.5 to 5.0 mg of estradiol benzoate) is administered one day after progestin administration, or alternatively it is injected simultaneously with the progesterone (i.m. 50 to 100 mg) at the time that donors receive the intravaginal device (CIDR) or the ear implant (norgestomet). The authors observed that estrogen when associated with progestin induced the synchronized growth of a new follicular wave, approximately 4 to 5 days after its administration. Therefore, the superovulatory treatment (single s.c. injection or 8 injections every 12 h, total

dose 400 mg NIH-FSH-PI Folltropin-V) is initiated 4 to 5 d after estrogen injection, coinciding with the beginning of a new follicular wave.

This strategy has the convenience of initiating the superovulation protocol in a self-appointed time, regardless of the stage of the estrus cycle of the donor. The commercial impact of synchronizing the new follicular wave is significant for the embryo transfer industry, since it allows embryologists to initiate superovulation of multiple donors at the same time. However, estradiol is not FDA approved for commercial use in the United States; therefore, an alternative to the use of estrogen, as a technique to synchronize a new follicular wave, is the association of a progestin with an exogenous gonadotropin-releasing hormone (GnRH) analogue, which will cause the ovulation of a dominant follicle followed by the emergence of a new follicular wave (Pursley et al., 1995). The downside of this protocol is the fact that administration of GnRH at a random stage of the estrus cycle causes ovulation in less than 60% of the animals, thereby decreasing the synchronization rate (Martinez et al., 1999; Colazo et al., 2009). For that reason, the use of GnRH at the beginning of superovulation protocols has a lower superovulatory response when compared with estradiol.

A luteolytic agent (PGF2α) should be administered towards the completion of the superovulatory treatment and the progestin source removed 12 h after PGF2α administration. Artificial insemination is performed 12 and 24 h after the beginning of estrus, around 60 and 72 h after the PGF2α injection (Figure 1). These treatments resulted in a number of transferable embryos similar or superior to those found on conventional superovulatory treatments performed between the day 8 and 12 of the estrous cycle (Bo et al., 1991, 1996).

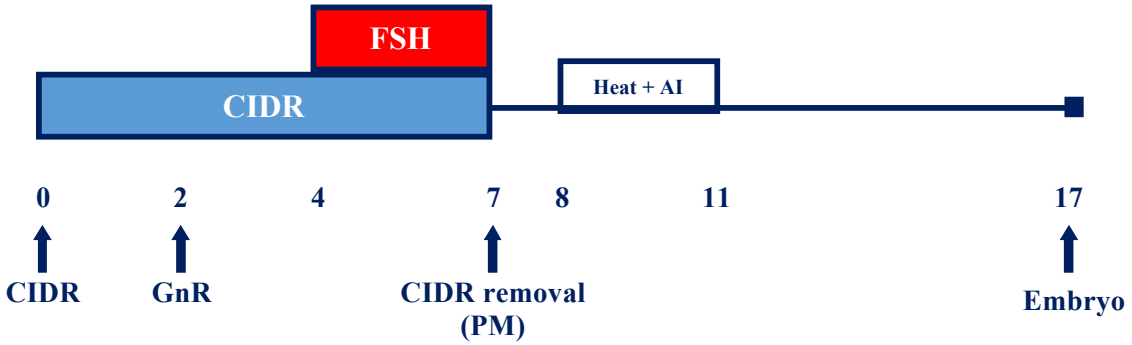

Figure 1. Current superstimulation protocol for *Bos taurus* donors. CIDR is inserted on d 0, followed by a 100-µg injection of GnRH 2 days later. The superovulatory treatment (8 injections every 12 h, total dose 400 mg NIH-FSH-PI Folltropin-V) is initiated at d 4. Donors receive 2 injection of prostaglandin F2α (PGF2α) on d 7, 12 h a part (AM/PM). At the time of the second PGF2α, CIDR insert is removed. Heat detection starts 24 h after CIDR removal and goes until d 11. Donors detected in heat are artificial inseminated (AI) 12 and 24 h after onset of estrus. Embryos are flushed 7 days after AI.

Other important factors that influence donor response to superovulation is the donor's breed. *Bos indicus* cows respond differently to *Bos taurus* in terms of the quantity of hormone administrated or the timing of the hormonal treatment. Zebu cattle have a greater variability in the ovarian follicular response to gonadotropin when multiple ovulation protocols are used (Adams, 1994), even though considerable research was done in this area (Hyttel et al., 1991; Stock et al., 1996).

After the characterization of follicular dynamics in *Bos indicus* (Castillo et al., 2000) it was possible to develop hormonal treatments to control the follicular growth and the time of ovulation, in order to allow fixed time artificial insemination. Similarly, the follicular development and ovulation can be pharmacologically manipulated to improve multiple ovulation embryo transfer (MOET) programs in Zebu cattle (Barros et al., 2000). However, the physiological differences must be considered when superovulating *Bos indicus* donors. Studies indicate that Zebu donors may require less FSH than *Bos taurus* cows to achieve optimal superovulatory response. Therefore, lower doses of FSH are recommended for the production of *Bos indicus* embryos through MOET.

In summary, even though, progress has been made in manipulating the bovine follicular development in order to facilitate donor management, the high variability in the ovarian follicular response to gonadotropin stimulation continues to be major problem in embryo transfer programs and warrants further research.

Managing Recipients

Nutrition Management Using Body Conditional Score

Insufficient intake of energy, protein, vitamins, and micro- and macrominerals has been associated with suboptimal reproductive performance. Of these nutritional effects on reproduction, energy balance is probably the single most important nutritional factor related to poor reproductive function in cattle. The metabolic use of available energy in ruminants for each physiological state is ranked in order of importance, as follows: 1) basal metabolism; 2) activity; 3) growth; 4) energy reserves; 5) pregnancy; 6) lactation; 7) additional energy reserves; 8) estrous cycles and initiation of pregnancy; and 9) excess energy reserves (3). Based on this list of metabolic priorities, reproductive function is compromised because available energy is directed towards meeting minimum energy reserves and milk production. Generally, beef cows do not experience a period of negative energy balance because they fail to produce the quantity of milk that dairy cows produce; however, beef cows need to be in sufficient body condition to overcome postpartum anestrus and become pregnant every year.

Body condition scoring (BCS) is a reliable method to assess the nutritional status of recipients. A visual BCS system developed for beef cattle uses a scale from 1 to 9, with 1 representing emaciated and 9 obese cattle (Whitman, 1975). A linear relationship exists between body weight change and BCS, where an approximate 40 kg weight change is associated with each unit change in BCS. Managers of recipients should understand when cows can be maintained on a decreasing plane of nutrition, when they should be maintained on an increasing plane of nutrition, or when they can be kept on a maintenance diet. Understanding the production cycle of the cow and how to manipulate the diet will improve the ability of the recipients to conceive to the transferred embryo (Mapletoft et al., 1986; Beal, 1999).

Pre and Postpartum Nutritional Effects on Reproduction

The general belief is that cows maintained on an increasing plane of nutrition prior to parturition usually have a shorter interval to their first ovulation than cows on a decreasing plane of nutrition. Energy restriction during the prepartum period results in a low BCS at calving, prolonged postpartum anestrus, and a decrease in the percentage of cows exhibiting estrus during the breeding season (Perry et al., 1991). Pregnancy rates and intervals from parturition to pregnancy are also affected by level of prepartum energy (Perry et al., 1991). Conversely, when prepartum nutrient restriction was followed by increased postpartum nutrient intake, the negative effect of prepartum nutrient restriction was partially overcome; however, the effectiveness of elevated postpartum nutrient intake depended on the severity of prepartum nutrient restriction (Lalman et al., 1997; Perry et al., 1991). The effect of BCS prior to calving also has implications for calf birth and weaning weights. When cows were fed to achieve a BCS of either 4 or 6 prior to calving, body weights were greater and calf birth and weaning weights (with similar genetics) also were greater for those cows in a BCS of 6 (Spitzer et al., 1995). Despite the greater birth weights, there was no difference in calving difficulty, demonstrating the added advantage for recipients to wean calves with greater weaning weights. In addition, there tended to be an increased number of cows calving with a medium BCS that were cycling at the beginning of breeding season and after a 60 day breeding season than cows in poor condition, resulting in a greater proportion of cycling cows at various stages of the breeding season (Spitzer et al., 1995).

Numerous studies document that increasing nutritional levels following parturition increase conception and pregnancy rates in beef cows (Wiltbank et al., 1962; Whitman,1975). Increasing the postpartum dietary energy density increased body weight and BCS and decreased the interval to first estrus (Lalman et al., 1997). However, suckled beef cows in relatively poor body condition gaining in excess of 1 kg/d while consuming an 85% concentrate diet did not resume cyclic ovarian activity before 70 days postpartum (Lalman et al.,1997). Therefore, although an enhanced plane of nutrition after calving may partially overcome the negative effects of poor prepartum nutrition, the added stress and negative impact of suckling and lactation also must be considered. Strategic feeding to obtain ideal BCS can be achieved by understanding the production cycle of the cow. The period of greatest nutritional need occurs shortly after calving; a cow is required to produce milk for a growing calf, regain weight lost shortly before and after parturition, and repair her reproductive tract to become pregnant within 3 months after calving. During this stage, a cow usually is consuming as much feed as she can and adjusting BCS at this time often is futile. Cows usually are grazing and tend to consume their full protein, vitamin and mineral requirements; however, the grass is often lush with a high percentage of moisture, which occasionally can cause a deficiency in energy (NRC, 1996).

Development of Estrus Synchronization Protocols.

The most useful alternative to increasing the number of animals receiving embryos is to utilize protocols that allow for embryo transfer without the need for estrus detection, usually called fixed-time embryo transfer (FTET) protocols. However, much of the research related to the systems currently used in embryo transfer programs were developed for fixed-time artificial insemination (TAI) rather than FTET. Transfer of embryos into estrus synchronized cows has been most effective when embryos were transferred 6 to 8 d after detected estrus or GnRH injection (Bó et al., 2002). Early estrous synchronization systems focused on altering the estrous cycle by inducing luteolysis with an injection of PGF2α followed by estrus detection. Once

systems involving a single PGF2α treatment became successful, researchers focused on multiple injections of PGF2α to further reduce days required for estrus detection (Lauderdale et al, 1974; Seguin et al., 1978). The next generation of estrous synchronization systems involved the use of exogenous progestins, such as an intravaginal progesterone release insert (CIDR) or melegestrol acetate (MGA), which were used to delay the time of estrus following natural or induced luteolysis and extend the length of the estrous cycle (Brown et al., 1988; Lucy et al., 2001).

Not until the discovery that growth of follicles in cattle occurs in distinct wave-like patterns (Fortune at al., 1988) were scientists able to embark on the third generation of estrous synchronization systems. Controlling follicular waves with a single injection of GnRH at random stages of the estrous cycle involves release of an LH surge, which causes synchronized ovulation or luteinization of dominant follicles (Garverick et al., 1980; Bao et al., 1998; Sartori et al., 2001). Consequently, a new follicular wave is initiated in most (> 60%) cows within 1 to 3 d of GnRH administration. Luteal tissue that forms after GnRH administration will undergo PGF2α - induced luteolysis 6 or 7 d later (Twagiramungu et al., 1995). A drawback to this method of estrus synchronization is that approximately 5 to 15% of cows are detected in estrus on, or before, the day of PGF2α treatment, reducing the proportion of females that are detected in estrus during the synchronized period (Kojima et al., 2000; Lamb et al., 2001; Martinez et al., 2001).

Advances in Protocols for Beef Cows.

Preliminary studies identified significant improvements in fertility among cows that received MGA prior to the administration of PGF2α compared with cows that received only PGF2α (Patterson et al., 2001). When cows received a CIDR for 7 d and an injection of PGF2α the day before CIDR removal, estrus synchrony and pregnancy rates were improved (Lucy et al., 2001). When GnRH was given 6 or 7 d prior to PGF2α, 70 to 83% of cows were in estrus within a 4 d period (Twagiramungu et al., 1995).

The use of GnRH to control follicular wave emergence, ovulation, and PGF2α to induce luteolysis led to the development of the Ovsynch protocol for dairy cows (Pursley et al., 1995). Combining the second injection of GnRH with TAI (CO-synch) proved to be more practical than estrus detection for beef producers because it had no negative effects on fertility (Geary et al., 2001). However, a disadvantage of this protocol is that approximately 5 to 15% of suckled beef cows exhibit estrus prior to, or immediately after the PGF2α treatment (Lamb et al., 2001). Unless these cows are detected in estrus and inseminated, they will fail to become pregnant to TAI. Therefore, we hypothesized that the addition of a CIDR to a GnRH-based protocol would prevent the premature occurrence of estrus and result in enhanced fertility following TAI. Overall pregnancy rates were enhanced by the addition of a CIDR to a GnRH-based TAI protocol (59 vs. 48%, respectively). The CIDR delayed the onset of ovulation, resulting in more synchronous ovulation, and induced cyclicity in noncycling cows (Lamb et al., 2001). However, the efficacy of these CIDR-based TAI protocols had not been evaluated concurrently with AI protocols requiring detection of estrus in suckled beef cows. Therefore, we implemented and coordinated a multi-state, multi-location experiment to discern whether a GnRH-based + CIDR protocol for TAI could yield pregnancy rates similar to protocols requiring detection of estrus (Larson et al., 2006). Results demonstrated that the TAI protocol yielded pregnancy rates that were similar to the estrus detection protocol, even though 35% of the cows were in postpartum

anestrous at the time of treatment. A detailed version of current estrus synchronization and TAI protocols was reviewed by the Beef Reproduction Task Force. Utilizing a similar protocol on recipients using FTET is practical and effective in yielding high pregnancy rates in recipients.

Advances in Protocols for Beef Heifers.

Early studies in beef heifers demonstrated that feeding MGA for 14 d followed by PGF2α 17 d later was an effective method of estrous cycle control in heifers (16; 30). However, when heifers were treated with PGF2α 19 d after the 14 d MGA feeding period, there was no difference in fertility but estrus was more synchronous (Lamb et al., 2000). Following the success of this protocol, researchers began to include GnRH in estrus synchronization protocols for TAI. However, addition of GnRH to the above protocol failed to increase pregnancy rates following TAI in heifers (Wood-Follis et al., 2004). Estrus synchronization using GnRH followed by PGF2α successfully synchronized heifers, but the above MGA-PGF2α protocol led to greater synchrony of estrus and, therefore, tended to be more effective (Lamb et al., 2000).

Development of a TAI protocol in beefs heifers has not been as straightforward as in cows, especially considering that at the time of estrus synchronization, a majority (greater than 85%) of heifers have attained puberty (Lamb et al., 2006). The primary reason for failure of TAI in heifers appears to be the inability to synchronize follicular waves with GnRH. After an injection of GnRH at random stages of the estrous cycle, 75 to 90% of postpartum beef cows ovulated (Thompson et al., 1999; El-Zarkouny et al., 2000), whereas only 48 to 60% of beef and dairy heifers ovulated in response to the same treatment (Macmillan et al 1991; Pursley et al 1995; Moreira et al., 2000). We have found no difference in synchrony of estrus or pregnancy rate in CIDR-treated heifers whether or not GnRH is administered at CIDR insertion, suggesting that response to GnRH in heifers at CIDR insertion may be of limited value (Lamb et al., 2006).

In a large, multi-location study using GnRH, PGF2α, and CIDR, GnRH did not enhance pregnancy rates following estrus detection but the addition of a CIDR to a GnRH-based TAI protocol yielded similar pregnancy rates to those utilizing estrus detection (Lamb et al., 2006). Nevertheless, a bewildering fact remains that the average pregnancy rate for these protocols ranged from 53 and 58%, whereas pregnancy rates in MGA (with PGF2α administered 19 days after MGA removal) or a long-term CIDR (with PGF2α administered 16 days after MGA removal) protocols followed by PGF2α have been reported to range from 60 and 75% (Kojima et al., 2000; Lamb et al., 2000; Lamb et al., 2006; Patterson et al., 2003). A detailed version of current estrus synchronization and TAI protocols was reviewed by the Beef Reproduction Task Force. Utilizing a similar protocol on recipients using FTET would be practical and effective in yielding high pregnancy rates in heifer recipients.

Resynchronization of Estrus and Efficient Recipient Utilization

Effective management of a recipient herd requires getting the recipient ready to receive an embryo and identifying and preparing open cows to be resynchronized and re-used or inseminated. In any group of synchronized recipients, a small percentage will not be detected in estrus and not all detected in estrus will receive an embryo, either due to an asynchronous estrus or lack of a suitable CL at the time of transfer. If 80% of the synchronized recipients are detected

in estrus and 90% of those receive embryos and 60% become pregnant, then less than 45% of any group of recipients will become pregnant. Therefore, it is important to devise a strategy to resynchronize recipients as soon as possible.

Re-insemination of nonpregnant cows at the first eligible estrus can be facilitated by resynchronization of the estrous cycle (Van Cleeff et al., 1996), which has a wide application in intense embryo transfer programs. Resynchronization strategies vary depending on the resources and capabilities of the ranch. With the use of ultrasonography, non-pregnant recipients may be identified and resynchronized as early as 3 wk after embryo transfer (Jones et al., 1990). However, to most effectively condense the calving season, the second round of estrus synchronization should begin before the pregnancy status of the animals is known. Although resynchronization with a progestin increased synchronized return rates of nonpregnant females (Stevenson et al., 2003a; Colazo et al., 2006), resynchronization with CIDR devices and estradiol cypionate or estradiol benzoate decreased subsequent conception rates to AI (Stevenson et al., 2003a). In contrast, further studies did not note a decrease in fertility when estrogens were utilized for resynchronization with a CIDR (Cavalieri et al., 2007). Furthermore, insertion of a CIDR for 13 d on the day of embryo transfer, 7 d after estrus (Purcell et al., 2005) or from 5 d after TAI until day 21 (Larson et al., 2006), was effective in resychronizing estrus in non-pregnant cows. Hence, resynchronization of estrus is a strategy that increases the number of times a female can be exposed to biotechnologies such as artificial insemination and embryo transfer; therefore, increasing its chances of becoming pregnant and generating a genetic superior offspring.

Recipient Related Factors

Embryo Transfer Factors

The procedure of removing an embryo from its natural uterine environment and in many cases, freezing and thawing, increases the stress experienced by those embryos resulting in a decreased survival rate following transfer. Our findings of a decrease in pregnancy rate from 83% with fresh embryos (n = 122) to 69% with frozen-thawed embryos (n = 326) are similar to the 10 to 15% decrease in pregnancy rates reported previously (Leibo et al., 1986; Sreenan et al., 1987), which is similar to the difference in averages reported by the American Embryo Transfer Association and the International Embryo Transfer Association (Savoy, IL). Results from two studies reveal that pregnancy rates among cows receiving a Grade 1 or Grade 2 fresh embryo (Hasler et al., 1987; Spell et al., 2001) were not different. Previous reports (Coleman et al., 1987; Hasler et al., 1987; Schneider et al., 1980; Wright 1981) noted a decrease in pregnancy rate with each corresponding decrease in quality score.

Pregnancy rates have been shown to vary with the synchrony of the donor and recipient. Higher pregnancy rates were observed when recipients were in estrus coinciding with the donor or 12 h before the donor. Pregnancy rates decreased in recipients in estrus 12 h after the donor (Schneider et al., 1980), but not until 24 h in another reports (Sreenan et al., 1987; Hasler et al 2001; Spell et al., 2001).

The variability in progesterone concentrations in recipients reflects a combination of different rates of CL development and the fluctuation of progesterone secretion during the early luteal phase. It has been suggested that the optimum circulating concentration of progesterone to establish pregnancy was reported to range between 2.0 and 5.0 ng/mL (Niemann et al., 1985). However, a recent study has revealed that the minimum threshold progesterone concentration on the day of embryo transfer essential for the establishment and maintenance of pregnancy may be lower than previously reported; there were no differences in pregnancy rates when progesterone concentrations were as low as 0.58 ng/mL or exceeded 16.0 ng/mL (n = 448) (Spell et al., 2001). In another study, eight of 177 pregnant recipients had concentrations of progesterone <0.5 ng/mL on days 10, 11, and 12 of the transfer cycle (Hasler et al., 1980). In addition, the diameter and volume of the CL differed among recipients that received embryos from 6.5 to 8.5 d after estrus (Spell et al., 2001), increasing as days post-estrus increased. However, pregnancy rates did not differ among recipients receiving embryos 6.5 to 8.5 d after estrus.

General Recipient Considerations

Selection and identification of high-quality recipients is not simple. Many prefer the use of virgin heifers, whereas others choose cows with a known history of high fertility. When heifers are to be used for recipients, the selection criteria should be the same as for high quality replacement heifers. Heifers need to be cycling, which can be assessed indirectly by using reproductive tract scores (Patterson et al., 1999), on a high plane of nutrition, have an adequately-sized, normally shaped, pelvic canal and have no history of receiving growth implants.

Lactating recipients have an advantage of a known reproductive history. Recipients that carry an embryo transfer calf to term but do not raise a normal calf to weaning should be re-evaluated as a recipient prospect. Similarly, open cows with an unknown reproductive history need to be carefully examined prior to being included in a recipient herd or program (Stroud and Hasler, 2006). The reproductive tract needs to be thoroughly examined via rectal palpation or trans-rectal ultrasonography for pregnancy, or uterine anomalies such as fluid or fetal remnants or evidence of metritis or endometritis and the ovaries examined for normal follicular or luteal structures. In addition, recipients should have good teeth and eyes, a good udder, be less than 8 years of age, and be structurally sound. Also, highest fertility occurs in herds where handling facilities are designed to ensure that cattle are handled with a minimum of stress.

Pregnancy Diagnosis

Knowing when cows conceive and when they will calve helps concentrate calving supervision. Ultrasonography can be used to accurately determine the presence of a conceptus as early as 28 d, but it is recommended to recheck all cows after 45 d to confirm pregnancy (Jones and Beal, 2003). Through the use of ultrasonography and breeding dates to determine the estimated date of calving, cows can be sorted into calving groups and managed to save on feed, labor, and veterinary expenses. Pre-calving vaccinations can also be timed to insure the most effective response. Also, avoiding over-crowding of calving pastures and/or calving cows on ‘fresh’ pastures that haven’t had cows with calves in it has been shown to reduce calf morbidity and mortality due to infectious calfhood scours (Smith et al., 2003).

Conclusion

For the embryo transfer technology to be effective, numerous factors need to be put in place to ensure success. Nutrition, estrous cycle control, donor, and recipient management are all responsible for the success or failure in a given program. Therefore, the producers, embryologists, veterinarians, and all members of the herd management team need to be aware of the short- and long-term factors that contribute to a successful embryo transfer program.

References

Adams, G.P. 1994. Control of ovarian follicular wave dynamics in cattle: implications for synchronization and superstimulation. Theriogenology 41:19-24

Bao B., Garverick H.A. 1998 Expression of steroidogenic enzyme and gonadotropin receptor genes in bovine follicles during ovarian follicular waves: A review. J Anim Sci 76:1903-1921.

Barros, C.M., M.F.G. Nogueira, and P.A.T. Andreussi. 2000. Superovulation and fixed time artificial insemination. Arquivos da Faculdade de Veterin8.ria UFRGS 28(Suppl. l):52-64.

Beal W.B. 1999. Streamlining embryo transfer. 18th Annual Convention AETA, Colorado Springs, CO, USA; 78-85.

Bó G.A., P.S. Baruselli, D. Moreno, L. Cutaia, M. Caccia, R. Tríbulo, H. Tríbulo, R.J. Mapletoft. 2002. The control of follicular wave development for self-appointed embryo transfer programs in cattle. Theriogenology 57:53-72.

Bo, G.A., G.P. Adams, R.A. Pierson, and R.J. Mapletoft.1996. Effects of progestogen plus estradiol-17β treatment on superovulatory response in beef cattle. Theriogenology 45:897-910

Bó, G.A., L.C. Peres, L.E. Cutaia, D. Pincinato, P.S. Baruselli, R.J. Mapletoft. 2011. Treatments for the synchronization of bovine recipients for fixed-time embryo transfer and improvement of pregnancy rates. Reproduction, Fertility and Development 24(1) 272-277.

Bo, G.A., R.A. Pierson, and R.J. Mapletoft. 1991. The effect of estradiol valerate on follicular dynamics and superovulatory response in cows with Synchro-Mate B implants. Theriogenology 36:169-183.

Brown L.N., K.G. Odde, D.G. LeFever, M.E. King, C.J. Neubauer. 1988. Comparison of MGA-PGF2α to Syncro-Mate B for estrous synchronization in beef heifers. Theriogenology 30:1-12.

Castillo, C., A.L.G. Gambini, P. Fernandes, L.A. Trinca, A.B. Teixeira, and C.M. Barros. 2000. Synchronization of ovulation in crossbred dairy heifers using gonadotropin-releasing hormone agonist, prostaglandin F2a and human chorionic gonadotropin or estradiol benzoate. Brazilian J. Medical and Biol. Res. 33:91-101

Cavalieri J, G. Hepworth, V.M. Smart, M. Ryan, and K.L.Macmillan. 2007. Reproductive performance of lactating dairy cows and heifers resynchronized for a second insemination with an intravaginal progesterone-releasing device for 7 or 8d with estradiol benzoate injected at the time of device insertion and 24h after removal. Theriogenology 67(4):824-834.

Colazo M., M. Gordon, R. Rajamahendran, R. Mapletoft, D. Ambrose. 2009. Pregnancy rates to timed artificial insemination in dairy cows treated with gonadotropin-releasing hormone or porcine luteinizing hormone. Theriogenology 72:262-270

Colazo M.G., J.P. Kastelic, R.C. Mainar-Jaime, Q.A. Gavaga, P.R. Whittaker, J.A. Small, M.F. Martinez, R.E. Wilde, D.M. Veira, R.J. Mapletoft. 2006. Resynchronization of previously timed-inseminated beef heifers with progestins. Theriogenology 65:557-572.

Coleman D.A., R.A. Dailey, R.E. Leffel, R.D. Baker. 1987. Estrous synchronization and establishment of pregnancy in bovine embryo transfer recipients. J Dairy Sci. 70:858-866.

El-Zarkouny, S. Z., J. A. Cartmill, B. A. Hensley, and J. S. Stevenson. 2000. Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone**.** J. Dairy Sci. 87:1024-1037.

Fortune J.E., J. Sirois, S.M. Quirk. 1988. The growth and differentiation of ovarian follicles during the bovine estrous cycle. Theriogenology 29:95-109.

Garverick H.A., R.G. Elmore, D.H. Vaillancourt, and A.J. Sharp. 1980 Ovarian response to gonadotropin-releasing hormone in postpartum dairy cows. Amer. J. Vet. Res. 41:1582-1585.

Geary T.W., J.C. Whittier, D.M. Hallford, and M.D. MacNeil. 2001. Calf removal improves conception rates to the Ovsynch and CO-Synch protocols. J. Anim. Sci. 79:1-4.

Hasler J.F. 2001. Factors affecting frozen and fresh embryo transfer pregnancy rates in cattle. Theriogenology 56:1401-1415.

Hasler J.F., A.D. McCauley, W.F. Lathrop, and R.H. Foote. 1987. Effect of donor-recipient interactions on pregnancy rate in a large - scale bovine embryo transfer program. Theriogenology 27:139-168.

Hyttel, P., H. Callesen, T. Greve, and M. Schmidt. 1991. Oocyte maturation and sperm transport in superovulated cattle. Theriogenology 35:91-108.

Jones A.L. and W.E. Beal. 2003. Reproductive applications of ultrasound in the cow. Bovine Practitioner 37:1-9.

Jones A.L., D.E. Marek, J.M. Wilson, and C.R. Looney. 1990. The use of ultrasonography to increase recipient efficiency through early pregnancy diagnosis. Theriogenology 33:259.

Kojima F.N., B.E. Salfen, W.A. Ricke, M.C. Lucy, M.F. Smith, D.J. Patterson. 2000. Development of an estrus synchronization protocol for beef cattle with short-term feeding of melengestrol acetate: 7-11 Synch. J. Anim. Sci. 78:2186-2191.

Lalman D.L., D.H. Keisler, J.E. Williams, E.J. Scholljegerdes, D.M. Mallet. 1997. Influence of postpartum weight and body condition change on duration of anestrus by undernourished suckled beef heifers. J. Anim. Sci. 75:2003-2008.

Lauderdale J.W., B.E. Seguin, J.N. Stellflung, J.R. Chenault, W.W. Thatcher, C.K. Vincent, and A.F. Loyancano. 1974. Fertility of cattle following $PGF_{2\alpha}$ injection. J. Anim. Sci. 1974;38:964-967.

Lamb, G.C., D.R. Brown, J.E. Larson, C.R. Dahlen, N. DiLorenzo, J.D. Arthington, and A. DiCostanzo. 2008. Effect of organic or inorganic trace mineral supplementation on follicular response, ovulation, and embryo production in superovulated Angus heifers. Anim. Reprod. Sci. 106:221-231.67.

Lamb, G.C., J.E. Larson, T.W. Geary, J.S. Stevenson, S.K. Johnson, M.L. Day, R.P. Ansotegui, D.J. Kesler, J.M. DeJarnette, D. Landblom. 2006. Synchronization of estrus and artificial insemination in replacement beef heifers using GnRH, $PGF_{2\alpha}$ and progesterone. J. Anim. Sci. 84:3000-3009

Lamb, G.C., D.W. Nix, J.S. Stevenson, and L.R. Corah. 2000. Prolonging the MGA-prostaglandin $F_{2\alpha}$ interval from 17 to 19 days in an estrus synchronization system for heifers. Theriogenology 53:691-698.

Lamb, G.C., J.S. Stevenson, D.J. Kesler, H.A. Garverick, D.R. Brown, and B.E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin $F_{2\alpha}$ for ovulation control in postpartum suckled beef cows. J. Anim. Sci. 79:2253-2259.

Larson, J.E., G.C. Lamb, J.S. Stevenson, S.K. Johnson, M.L. Day, T.W. Geary, D.J. Kesler, J.M. DeJarnette, F.N. Schrick, A. DiCostanzo, J.D. Arseneau. 2006. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin F2alpha, and progesterone. J. Anim. Sci. 84:332-342.

Leibo, S.P. 1986. Commercial production of pregnancies from one-step diluted frozen-thawed bovine embryos. Theriogenology 25:166 [Abstract].

Lucy, M.C., H.J. Billings, W.R. Butler, L.R. Ehnis, M.J. Fields, D.J. Kesler, J.E. Kinder, R.C. Mattos, R.E. Short, W.W. Thatcher, R.P. Wettemann, J.V. Yelich, and H.D. Hafs. 2001. Efficacy of an intravaginal progesterone insert and an injection of PGF2alpha for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers. J. Anim. Sci. 70:1904-1910.

Macmillan, K.L. and W.W. Thatcher. 1991. Effects of an agonist of gonadotropin-releasing

hormone on ovarian follicles in cattle. Biol. Reprod. 45:883-889.

Mapletoft, R. J. and Bo, G. A. 2015. Superovulation in cattle. In: Wiley Blackwell, editor. Bovine Reproduction. John Wiley $ Sons. Ames, IA. pp. 698-702.

Mapletoft, R.J., C.E. Lindsell, and V. Pawlyshyn. 1986. Effects of clenbuterol, body condition and non-surgical embryo transfer equipment on pregnancy rates in bovine recipients. Theriogenology 25:172 (abstract).

Marquezini, G.H.L., V.R.G. Mercadante, M.M. Ward, A.R. Spell, J.A. Carter, N.D. Paton, G.C. Lamb. 2010. Embryo Quality Characteristics from Superovulated Cows receiving a blend of bioactive peptides and oligosaccharides to support immune function (Grade One™). J. Anim. Sci. 88 (E-Suppl. 2):683. (Abstr.)

Martinez, M., G. Adams, D. Berfelt, J. Kastelic, and R. Mapletoft. 1999. Effect of LH or GnRH on the dominant follicle of the first follicular wave in heifers. Anim. Reprod. Sci. 57:23-33.

Martinez, M.F., J.P. Kastelic, G.P. Adams, and R.J. Mapletoft. 2001. The use of GnRH or estradiol to facilitate fixed-time insemination in an MGA-based synchronization regimen in beef cattle. Anim. Reprod. Sci. 67:221-229.

Moreira, F., R.L. de la Sota, T. Diaz, and W.W. Thatcher. 2000. Effects of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. J. Anim. Sci. 78:1568-1576.

Niemann, H., B. Sacher, and F. Elasaesser. 1985. Pregnancy rates relative to recipient plasma progesterone levels on the day of non - surgical transfer of frozen/thawed bovine embryos. Theriogenology 23:631-639.

NRC. Nutrient Requirements of Beef Cattle (7th Ed.). National Academy Press, Washington, DC. 1996;85-96.

Patterson, D.J., S.L. Wood, and R.F. Randle. 2003. Procedures that support reproductive management of replacement beef heifers. Proceedings of the Amer Scociety of Anim Sci. http://www.asas.org/JAS/symposia/proceedings/0902.pdf

Patterson, D.J., S.L. Wood, and R.F. Randle. 1999. Procedures that support reproductive management of replacement beef heifers. J. Anim. Sci. 77:1-15.

Perry, R.C., L.R. Corah, R.C. Cochran, W.E. Beal, J.S. Stevenson, J.E. Minton, D.D. Simms, and J.R. Brethour. 1991. Influence of dietary energy on follicular development, serum gonadotropins, and first postpartum ovulation in suckled beef cows. J. Anim. Sci. 69:3762-3773.

Purcell, S.H., W.E. Beal, and K.R. Gray. 2005. Effect of a CIDR insert and flunixin meglumine, administered at the time of embryo transfer, on pregnancy rate and resynchronization of estrus in beef cattle. Theriogenology. 64(4):867-78.

Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF2a and GnRH. Theriogenology 44:915.

Schneider, Jr., H.J., R.S. Castleberry, and J.L. Griffin. 1980. Commercial aspects of bovine embryo transfer. Theriogenology 13:73-85.

Seguin, B.E., B.K. Gustafson, J.P. Hurtgen, E.C. Mather, K.R. Refsal, R.A. Wescott, and H.L. Withmore. 1978. Use of the prostaglandin $F_{2\alpha}$ analog cloprostenal (ICI 80,996) in dairy cattle with unobserved estrus. Theriogenology 10:55-64.

Smith, D.R., D.M. Grotelueschen, T. Knott, and S. Ensley. 2003. Managing to alleviate calf scours: the sandhills calving system. Proc, The Range Beef Cow Symposium XVIII, Mitchell, NE 2003.

Spell, A.R., W.E. Beal, L.R. Corah, and G.C. Lamb. 2001. Evaluating recipient and embryo factors that affect pregnancy rates of embryo transfer in beef cattle. Theriogenology 56:287-298.

Spitzer, J.C., D.G. Morrison, R.P. Wetteman, and L.C. Faulkner. 1995. Reproductive responses and calf birth and weaning weights as affected by body condition at parturition and postpartum weight gain in primiparous beef cows. J. Anim. Sci. 73:1251-1257.

Sreenan, J.M., and M.G. Diskin. 1987. Factors affecting pregnancy rate following embryo transfer in the cow. Theriogenology 27:99-113.

Stevenson, J.S., S.K. Johnson, M.A. Medina-Britos, A.M. Richardson-Adams, and G.C. Lamb. 2003. Resynchrnonization of estrus in cattle of unknown pregnancy status using estrogen, progesterone, or both. J. Anim. Sci. 81:1681-1692.

Stock, A.E., J.E. Ellington, and J.E. Fortune. 1996. A dominant follicle does not affect follicular recruitment by superovulatory doses of FSH in cattle but can inhibit ovulation. Theriogenology 45:1091-1102.

Thompson, K. E., J. S. Stevenson, G. C. Lamb, D. M. Grieger, and C. A. Loest. 1999. Follicular, hormonal, and pregnancy responses of early postpartum suckled beef cows to GnRH, norgestomet, and prostaglandin$F_{2\alpha}$. J. Anim. Sci. 77:1823-1832.

Twagiramungu, H.L., A. Guilbault, J.J. Dufour. 1995. Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: A review. J. Anim. Sci. 73:3141-3151.

Van Cleeff, J., K. L. Macmillan, M. Drost, and W. W. Thatcher. 1996. Effects of administering progesterone at selected intervals after insemination of synchronized heifers on pregnancy rates and resynchronization of returns to estrus. Theriogenology 4:1117-1130.

Whitman, R.W. 1975. Weight change, body condition and beef-cow reproduction. Ph.D. Dissertation. Colorado State Univ., Fort Collins.

Wiltbank, J.N., W.W. Rowden, J.E. Ingalls, K.E. Gregory, and R.M. Koch. 1962. Effect of energy level on reproductive phenomena of mature Hereford cows. J. Anim. Sci. 21:219-225.

Wood-Follis, S.L., F.N. Kojima, M.C. Lucy, M.F. Smith, and D.J. Patterson. 2004. Estrus synchronization in beef heifers with progestin-based protocols. I. Differences in response based on pubertal status at the initiation of treatment. Theriogenology 62:1518-1528.

Wright, J.M. 1981. Non-surgical embryo transfer in cattle, embryo-recipient interaction. Theriogenology 15:43-56.