IN VITRO FERTILIZATION (IVF) VERSUS MULTIPLE OVULATION EMBRYO TRANSFER (MOET): MAKING THE DECISION TO USE ONE OR BOTH

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Introduction

The utilization of embryo transfer offers a great opportunity for genetic improvement in beef cattle operations. Through embryo transfer, a single, genetically superior female is able to generate a greater number of offspring than through a conventional system (natural service or AI), and when coupled with spermatozoa from a genetically outstanding sire, embryos of exceptional genetic quality can be produced. In addition, recipient females of poor or average genetic merit have the opportunity to serve as surrogates and receive an embryo with high genetic value; therefore, these recipients can give birth to calves with greater genetics than they would otherwise. Through embryo transfer, genetic progress can be hastened, which is particularly useful in cattle due to their relatively long generation interval when compared to other livestock species. Another great advantage of this technology is the ability to transport embryos to areas where the production of beef needs to be advanced, instead of the live animals themselves.

Since 1951, when the first calf was produced by embryo transfer, biotechnologies have evolved to allow for embryo transfer to take place in a commercial setting (Willett et al., 1951). Initially, embryos were transferred using a surgical procedure where an embryo would be transferred into the lumen of the uterine horn by a technician, while the horn was exteriorized by a surgeon through the flank of the recipient cow. However, over time, improvements were made to allow for successful transcervical transfer of bovine embryos (Betteridge, 2003). Numerous short- and long-term factors contribute to donor's production of embryos and whether or not the recipient cows will maintain a transferred embryo, deliver a calf without assistance, and successfully raise that calf until weaning. These factors include, but are not restricted to: donor and recipient genetics, nutritional status, parity, and proper estrous cycle control. Taken together, these factors play a major role on the establishment of a successful embryo transfer program. The present proceedings provides an overview of our current understanding of the factors that impact the fertility of herds that utilize biotechnologies such as *in vitro* fertilization (IVF) and ET.

Available Options For Commercial Embryo Production

Multiple Ovulation Embryo Transfer

Currently, there are two methods utilized commercially for embryo production in cattle ET programs: multiple ovulation ET (MOET) and IVF followed by ET. In current MOET protocols, donor females are superovulated through treatment with follicle stimulating hormone (FSH) in order to stimulate the release of multiple oocytes at the time of ovulation. These superovulated females are AI, and if successful, a number of embryos will be recovered at the time of uterine flushing. Following embryo collection through the uterine flushing procedure, embryo viability is determined, and viable embryos are either transferred fresh to recipient females, or are frozen for future use. On average 6.9 viable embryos are recovered per flush in beef females (AETA, 2018); however, this number fluctuates depending on cow breed, age, and within breed variation. The transfer of fresh embryos typically yields 10 to 15% greater pregnancy rates than the use of frozen-

thawed embryos (Leibo, 1986; Sreenan and Diskin, 1987). Another important contributor to MOET success is the semen utilized to AI donors, as it has been well documented that donors AI with sex-sorted semen produce fewer embryos than donors AI with conventional semen.

According to the annual statistical survey of the American Embryo Transfer Association, the number of fresh and frozen bovine embryos transferred has increased exponentially from 200,000 in 2008, to just over 400,000 in 2017 (AETA, 2018). Of these embryos, 52% were transferred in dairy cattle, and 48% in beef cattle. Furthermore, the number of embryo transfers performed globally increased from 361,000 in 1997 to 506,000 in 2012 (Figure 1; Lamb et al., 2016). Overall, the success of embryo transfer depends on a variety of factors associated with the embryo, the recipient, the embryo transfer technician, or an interaction among these factors (Lamb and Mercadante, 2014). Suitability of recipients is dependent on several management, nutritional, and estrous cycle control factors to ensure the presence of a functional corpus luteum (CL) at the time of embryo transfer (Lamb and Mercadante, 2014). It is necessary to control the stage of the estrous cycle in order to achieve acceptable synchrony between donors and recipients. Development of estrus synchronization protocols has allowed this synchrony to be established, has reduced the amount of recipients required, and has increased the ease of incorporation of embryo transfer into cattle operations. Additionally, estrus synchronization has enabled the use of fixed-time embryo transfer (FTET), which eliminates the need for estrus detection in recipient cows. Pregnancy establishment is most successful when embryos are transferred into estrus synchronized cows 6 to 8 day after detected estrus or GnRH injection (Bó et al., 2002).

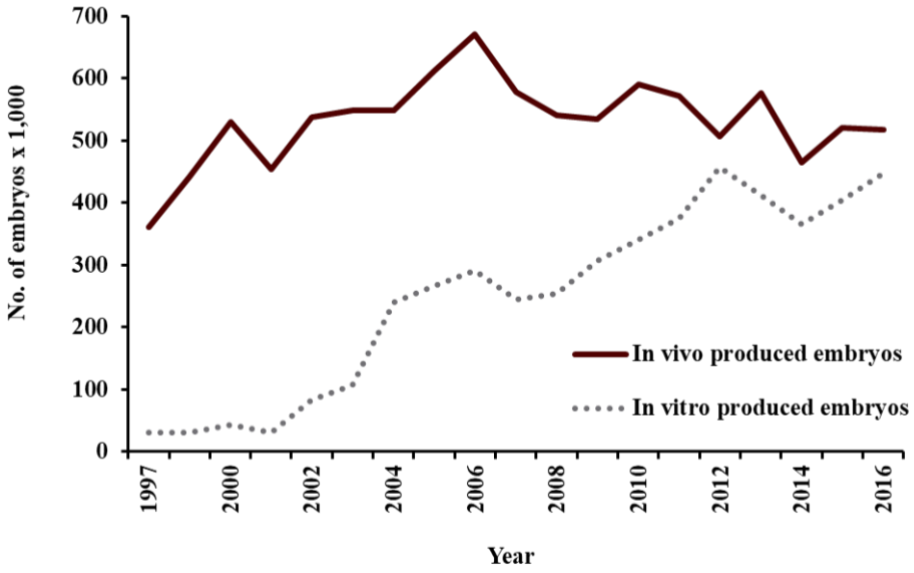

Figure 1. The number of embryos produced per year by *in vivo* and *in vitro* techniques (IETS, 2017).

Superovulation protocols increase the number of follicles that are ovulated per cycle, allow multiple oocytes to be fertilized at a time, and ultimately lead to the production of several embryos at once. Currently, the recommended superstimulatory protocol for *Bos taurus* donors involves inserting a CIDR on day 0, followed by a 100-µg injection of GnRH 2 days later. Beginning on day 4, donors receive injections of FSH every 12 hours. The amount of FSH per injection decreases each day until day 7, resulting in a total of 8 injections and 400 mg of FSH. On day 7, donors receive 2 injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 12 hours apart (AM/PM). At the time of the second $PGF_{2\alpha}$ injection, the CIDR insert is removed, and heat detection begins 24 hours after CIDR removal and continues until day 11. Donors that are detected in heat during this period are AI at both 12 and 24 hours after onset of estrus. Embryos are flushed 7 days after AI (Lamb et al., 2016a); Figure 2). A major limitation to the production of embryos from MOET has been the lack of reliability at successfully inducing superovulation in donor females (Betteridge, 2006). However, research into the development of superovulation protocols and techniques to predict which donor females may respond well to superovulation is ongoing.

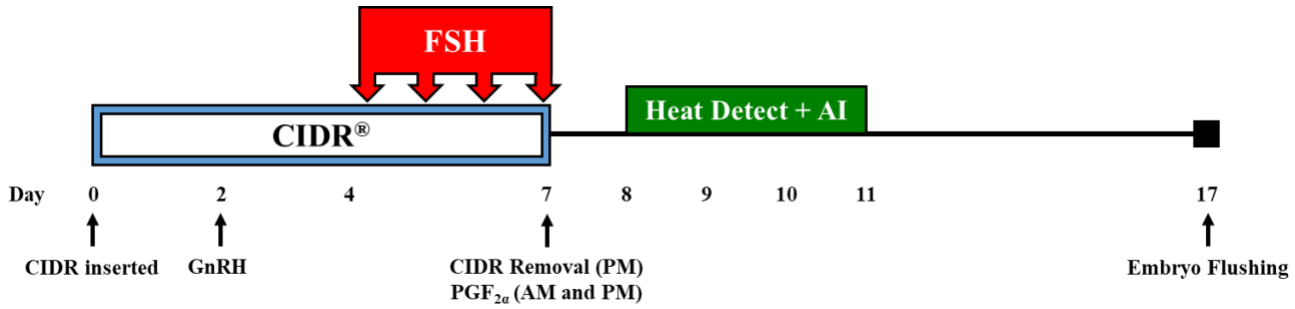

Figure 2. Current superstimulation protocol for *Bos taurus* donors. A CIDR is inserted on day 0, followed by a 100-µg injection of GnRH 2 days later. Superovulatory treatment is initiated on day 4, where donors receive an injection of FSH every 12 hours till day 7 (a total of 8 injections or 400 mg NIH-FSH-PI Folltropin-V). Donors receive 2 injections of $PGF_{2\alpha}$ on day 7, 12 hours apart (AM/PM). At the time of the second injection of $PGF_{2\alpha}$, the CIDR insert is removed. Heat detection starts 24 hours after CIDR removal and continues until day 11. Donors detected in heat are AI twice, first at 12 and then 24 hours after onset of estrus. Embryos are flushed 7 days after AI.

In Vitro Fertilization

An alternative to embryos derived from MOET is the production of embryos in a laboratory via *in vitro* maturation (IVM), IVF, and *in vitro* embryo culture (IVC), which are collectively referred to as IVF. Over the past 15 years the number of *in vitro*-produced embryos has increased by more than 300% (Figure 1; Lamb et al., 2016), and according to the AETA (2018), 58% of the embryo transfers performed in 2017 were with embryos generated through IVF. In vitro fertilization can generate pregnancies from a donor female that is already pregnant, and requires fewer units of semen when compared to MOET. In fact, one single semen straw can provide enough sperm to fertilize as much as 800-1000 oocytes (Tribulo et al., 2019). Furthermore, oocytes can be collected from the antral follicles of ovaries obtained from slaughter facilities, which greatly increases the number of embryos that can be produced, and can hypothetically eliminate the need for donor females. Finally, the potential disadvantage of a poor response to a superovulation protocol can be avoided by utilizing IVF.

During the IVF process, oocyte maturation needs to take place, where oocytes complete their first meiotic division (Leibfried-Rutledge et al., 1987). Similarly, spermatozoa used for IVF need to undergo capacitation before they are able to penetrate the zona pellucida of the oocyte (Parrish et al., 1986). Oocytes that mature spontaneously *in vitro* or *in vivo* are highly capable of sperm penetration. However, oocytes matured *in vitro* have shown reduced developmental capacities in comparison to those matured *in vivo* (Leibfried-Rutledge et al., 1987). In addition, the viability of IVF-derived embryos decreases with cryopreservation to a greater extent than *in vivo*-derived embryos; therefore, these embryos are more likely to be transferred fresh (Palasz and Mapletoft, 1996).

The predominant oocyte collection technique is known as aspiration or ovum pick up (OPU). Through OPU, unfertilized oocytes can be harvested directly from the ovaries of a donor cow or heifer using an ultrasound probe and an aspiration needle. This technique may be performed 2 to 3 times during a cow's estrous cycle for as long as 6 months (Greve and Madison, 1991), which is more frequent than what MOET can be performed. Therefore, a greater number of transferrable embryos per donor can be generated through IVF than through MOET. On the other hand, embryos that are produced by IVF are known to yield pregnancy rates approximately 10% lower compared to embryos produced by MOET. Furthermore, pregnancy losses throughout pregnancy are greater in IVF embryos compared to MOET (Ealy et al., 2019).

Management Of Donors

Superovulation

Significant progress has been made in the understanding of cattle reproductive physiology. This knowledge has been used to develop technologies that allow us to control reproductive events in the cow with the use of exogenous hormones. Within these technologies, superovulation protocols in MOET result in an increase in the number of follicles ovulated in small window of time, allowing the fertilization of multiple oocytes and consequently, the production of several embryos at once. This strategy also allows for the initiation of the superovulation protocol in a self-appointed time, regardless of the stage of the estrus cycle of the donor. The economic impact of the coupling of estrus synchronization and superovulation protocols a significant, since it allows embryologists to initiate superovulation of multiple donors at the same time.

The physiological principle of superovulation protocols are similar to estrus synchronization protocols (thoroughly described by other authors in these proceedings). However, superovulation protocols include the use of an exogenous FSH regimen. The rationale for the administration of FSH is to overcome the decrease in endogenous FSH that occurs as a result of the negative feedback that inhibin has on the endogenous secretion of FSH by anterior pituitary. Therefore, supplementation of FSH allows multiple selected follicles to continue to grow, reach pre-ovulatory size and ovulate in response to an LH surge (endogenous or induced via GnRH injection).

One important factor that influences donor response to superovulation is the high variation from individual cows in response to these treatments. Another important factor is the genetics of the donors. *Bos indicus* females have a greater variability in the ovarian follicular response to gonadotropins when multiple ovulation protocols are used (Adams, 1994). 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. Tremendous progress has been made over the last few decades on the manipulation of follicular development and superovulation in cattle; however, individual variation in response to these treatments still represent a major challenge for *in vivo* production of embryos for MOET.

The stress experienced by embryos after being removed from the uterine environment, and then frozen and thawed in many cases, results in a decreased survival rate following transfer. Our results indicating a decrease in pregnancy rates from 83 to 69% with fresh (n = 122) and frozen-thawed embryos (n = 326), respectively, are similar to the 10 to 15% decrease in pregnancy rates previously reported (Leibo et al., 1986; Sreenan et al., 1987). Furthermore, our results are similar to the averages reported by the American Embryo Transfer Association and the International Embryo Transfer Association (Savoy, IL). It was previously reported that pregnancy rates among cows receiving a Grade 1 or Grade 2 fresh embryo (Hasler et al., 1987; Spell et al., 2001) did not differ. Moreover, a decrease in pregnancy rate has been reported with each corresponding decrease in quality score (Coleman et al., 1987; Hasler et al., 1987; Schneider et al., 1980; Wright 1981).

Nutritional Considerations

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.

An additional study where cows received a blend of bioactive peptides and oligosaccharides to support immune function, reported promising results. It was reported that supplementation of Nutrition Horizons Grade One™ (Brookville, OH) may alter quality of embryos after superovulation (Marquezini et al., 2010). 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).

	Treatments[a]			
Item	Control	Inorganic	Organic	*P*-value
	----------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	0.522
Degenerate/cleaved	0.93 ± 0.24^{x}	0.26 ± 0.23^{y}	0.25 ± 0.23^{y}	0.063
Unfertilized	1.31 ± 0.37^{x}	2.32 ± 0.36^{y}	0.82 ± 0.36^{x}	0.014
Transferable	2.01 ± 0.39^{xy}	1.07 ± 0.38^{y}	2.18 ± 0.38^{x}	0.090
Grade 1	1.43 ± 0.33	0.82 ± 0.32	1.43 ± 0.32	0.296
Grade 2	0.56 ± 0.14^{xy}	0.23 ± 0.13^{x}	0.68 ± 0.13^{y}	0.049
Grade 3	0.00 ± 0.03	0.02 ± 0.03	0.06 ± 0.03	0.195

[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 ($P < 0.05$).

Managing Recipient Females

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

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). Hence, estrus synchronization and superovulation protocols can be strategically utilized to optimize recipient and donor synchrony, respectively.

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 and 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 after 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 ET programs. Resynchronization strategies vary depending on the resources and capabilities of each operation. 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. Resynchronization with a progestin can increase synchronized return rates of nonpregnant females (Stevenson et al., 2003a; Colazo et al., 2006), facilitating the utilization of AI or ET in recipients that failed to become pregnant after the first round of ET. 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 AI and ET; therefore, increasing its chances of becoming pregnant and generating a genetic superior offspring.

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 as 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); be on a high plane of nutrition; have an adequately-sized, normally shaped, pelvic canal; and have no history of receiving growth implants.

When introducing recipient cows into an ET program, it is beneficial to know their reproductive history. Open cows with an unknown reproductive history need to be carefully examined prior to being included in a recipient herd or program, in order to potentially identify abnormalities that could impact reproductive success after ET (Stroud and Hasler, 2006). Recipients that carry an ET

calf to term, but do not raise a normal calf to weaning, should be re-evaluated as a recipient prospect. Recipient reproductive tracts needs to be thoroughly examined via rectal palpation or trans-rectal ultrasonography for potential pregnancies; uterine anomalies such as fluid and fetal remnants; signs of metritis or endometritis; and the ovaries should be examined for normal follicular and 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. Greatest ET success occurs in herds where facilities are designed to ensure that cattle are handled with minimal 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 days, 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 calving cows on 'fresh' pastures that haven't had cows-calf pairs in them, have been shown to reduce calf morbidity and mortality due to a reduction in exposure of calves to infectious pathogens (Smith et al., 2003).

Conclusions

Both MOET and IVF can be successfully incorporated into beef cattle operations to enhance genetic improvement; however, there are a number of considerations that need to be made to ensure success. Nutrition, estrous cycle control, donor, and recipient management contribute to the success or failure in any given ET program. Therefore, 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 ET program.

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