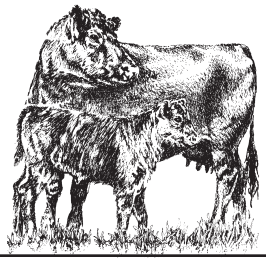
Western Beef Resource Committee

Fourth Edition



Cattle Producer's Handbook

Reproduction Section 460

Advances in Reproductive Biotechnology

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Recent advances in reproductive biotechnologies provide beef cattle producers with diverse opportunities to attain a high level of reproductive efficiency in their herd. The adoption and refinement of these advances will equip producers with the tools to feed a growing global population that is estimated to reach 9 billion by 2050 (Food and Agriculture Organization 2009).

An increase in beef production is important because beef provides the growing population with a nutrient dense food that contains minerals, vitamins, and protein in the form of essential and non-essential amino acids (Klurfeld 2015). The purpose of this fact sheet is to provide beef cattle producers with an overview of advances in reproductive biotechnologies that promote efficient beef production.

Artificial Insemination and Estrus Synchronization

The commercial use of artificial insemination (A.I.) and estrus synchronization in the beef industry has a long and rich history dating back more than 50 years. The process uses exogenous hormones to synchronize estrus and ovulation, which allows cattle producers to improve herd genetics by inseminating the females in their herd with semen from superior sires.

Approximately 14 percent of the cow-calf operations across the western United States use A.I. in their herds to promote an economically viable operation (NAHMS 2009). As of 2016, the Beef Reproductive Task Force—a multi-state extension activity made up of extension specialists—highlighted 19 different synchronization protocols that producers can use to A.I. beef cows and heifers (see fact sheet 405). Within the protocols, cow-calf producers will find that there are three primary groups of hormones commonly used to synchronize estrus in beef cattle—progestins, gonadotropin releasing hormone (GnRH), and prostaglandins.

Progestins are used in estrus synchronization to reduce follicular maturation and increase the likelihood that females are in a similar stage of the estrous cycle. They can be used in combination with GnRH to facilitate follicular development and/or prostaglandin, which induces ovulation for A.I.

Recent advancements in A.I. and estrus synchronization have established beef cow and heifer protocols in three different categories: heat detection, heat detection and timed A.I. (TAI), and fixed-TAI. These protocols are highlighted to minimize costs and the number of times cattle are handled while concurrently increasing pregnancy rates.

The heat detection protocol category is straightforward. It emphasizes A.I. between 6 and 12 hours after the first observation of standing heat, which can occur up to a week after a prostaglandin injection. Detecting heat is required for these protocols to be effective.

Another protocol category that adds a timed insemination after heat detection is TAI. Beef cows or heifers should be inseminated 6 to 12 hours after the onset of standing heat. If they are not observed in standing heat after a prostaglandins injection, they are injected with gonadotropin releasing hormone (GnRH) and inseminated as part of the TAI protocol.

Finally, fixed-time A.I. protocols are a slight modification of TAI. This protocol predetermines an insemination time and ignores standing heat.

The benefits of establishing an estrus synchronization and A.I. program go beyond improving the genetic potential of the beef cowherd and can be observed at weaning and during the calving season. In an economic

*Original authors of this fact sheet, written in 2003, were Dean Hawkins and Kim Kane, New Mexico State University.

evaluation of estrous synchronization and fixed-TAI in suckled beef cows, it was demonstrated that 84 percent of the cows exposed to the intended protocol weaned a calf in contrast to 78 percent of naturally serviced cows (Rodgers et al. 2012).

In the same study, cows exposed to fixed-TAI calved sooner compared to cows that were serviced naturally. These findings demonstrate that advances in A.I. technologies may provide beef producers the flexibility to produce older calves at weaning, which could correspond to heavier calves.

In Vitro Fertilization and Intracytoplasmic Sperm Injection (ICSI)

In vitro fertilization (IVF) is an additional valuable technology that has advanced beef cattle genetics. It involves the fertilization of an oocyte (egg) by sperm in a laboratory culture dish. Historically, the technique was used as a human infertility treatment before it successfully transferred to the beef cattle industry.

The IVF procedure simulates complex processes that normally occur within the beef cow or heifer. Oocytes retrieved from live cows without hormone treatments are undesirable because they are often immature. To overcome this problem, donor females undergo an exogenous hormone protocol to control the estrous cycle and synchronize follicular development allowing for ovum pickup at a time when the oocyte is mature.

Incidentally, several superovulation protocols have been developed to allow the harvest of several oocytes during one ovum pickup section. At that time, an ultrasound transducer is placed in the vagina and an oocyte pickup needle is used to perforate the vagina. A technician is able to view the follicles on an ultrasound monitor, which allows the technician to guide the needle into a follicle. Once in the follicle, a low vacuum pressure is applied to retrieve the oocyte. It is then transferred to a culture dish where it is induced through a second maturation step using a chemical shock treatment—a process that takes 20 to 26 hours. Likewise, sperm are treated to mature before being placed with an oocyte in the culture dish. After successful fertilization, the resulting embryo develops for about a week before it is either transferred into a recipient cow or frozen for transfer to a recipient cow at a later time.

Intracytoplasmic sperm injection (ICSI) is another valuable tool available in the beef cattle industry—especially when there is a limited supply of semen from an elite bull. The process consists of fertilizing an artificially activated egg by directly injecting a sperm cell (Fig. 1). After a week in culture, the resulting embryo is either transferred into a recipient or frozen for a later transfer. One of the advantages of this technique is the possibility of bypassing fertilization issues related to sperm quality.

Embryo Transfer

Embryo transfer (ET) is a rapid way beef producers can improve herd genetics. In simplest terms, ET is the production of multiple embryos by a donor cow and bull that have desired genetics. Those individual embryos are then transferred into recipient cows that will carry the embryo until birth. Beef cows or heifers with either average or poor genetic potential can serve as surrogates to calves of exceptional genetic merit.

The ET procedure is an extensive process that continues to evolve. It starts when the donor cow receives a series of exogenous hormone injections that will induce her to ovulate multiple ova. The donor cow is then artificially inseminated, usually twice.

After fertilization, the developing embryos are flushed from the donor cow and transferred to recipient cows the same day for gestation. Those embryos that are not transferred are typically frozen for future use. This allows for multiple offspring from the outstanding female and male, rather than the single offspring per year that they would be able to produce on their own.

Despite many advantages, the process associated with ET is expensive and is more common among seedstock producers compared to commercial cow-calf operators. The costs, inconsistent market for embryo transfer progeny, and inability to select outstanding female (donors) accurately contribute to the limited use of ET. Embryo transfer and related biotechnologies result in 40,000 to 50,000 beef calves per year in North America, which represents only 1 per 700 to 800 calves born annually in North America (Seidel 1995). Interestingly, the number of *in vitro*-derived embryos transferred internationally increased four-fold between 2000-2011 (Hasler 2014).

The process of freezing embryos—known as cryopreservation—has increased the flexibility of incorporating embryo procedures into production scenarios. Before

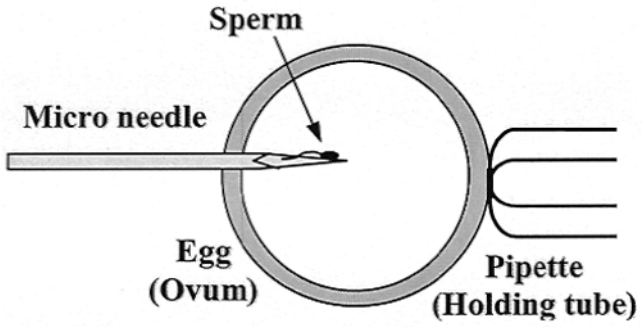

Fig. 1. Intracytoplasmic sperm injection (ICSI) is used to overcome defects in either the sperm or ovum. The ovum, in a culture dish, is held by a holding tube (micro pipette) by vacuum pressure. An individual sperm cell is loaded into a micro needle that penetrates the ovum. Once the micro needle enters the ovum, the sperm is injected directly into the ovum.

successful cryopreservation, recipient cows had to be available when embryos were collected. Variability in number of embryos collected is high and, therefore, the number of recipient cows prepared to receive embryos seldom matched the number of embryos collected. Cryopreservation provides the opportunity to store excess embryos until a later date, thus reducing the cost of maintaining excessive numbers of recipient cows that may or may not be used.

Female beef cattle have thousands of oocytes that never ovulate or yield offspring. Methods to harvest these ova from a cow's ovary and then subject them to *in vitro* fertilization and subsequent cryopreservation continue to be developed. Likewise, procedures are being refined to recover valuable genetics upon the death of a superior female. Removal of the ovaries immediately upon death and subsequent aspiration of follicles allows the recovery of eggs from valuable animals. After retrieval, the harvested eggs can be fertilized *in vitro* and the resulting embryos can be frozen or placed in recipient females. In bulls, collected semen can be frozen or used for A.I., ET, and/or IVF.

Somatic Cell Nuclear Transfer (Cloning)

Cloning animals using non-reproductive (somatic) cells from an adult gained worldwide attention in the 1990s after Dolly the sheep was successfully cloned in Scotland. Today, nearly 20 different animal species have been cloned using somatic cell nuclear cloning (Oback 2008), which transforms a somatic cell into a newborn animal (Rodriguez-Osorio et al. 2012). Specifically, the procedure involves the fusion between a somatic cell nucleus with a second cell that had the nucleus extracted. It is followed by an activation procedure that allows cells that compose the embryo to divide. That embryo remains managed until it is transferred into a recipient cow. The procedure is referred to as somatic cell nuclear transfer or cloning.

While the primary use of cloning is to maintain and disseminate superior genetics throughout the beef herd, it does have setbacks. The process includes inefficiencies in the cloning process that make it costly and unlikely to offset costs. Additionally, nuclear cloned calves commonly have delayed parturition, which result in scheduled cesarean sections. Finally, this procedure has high neonatal mortality rates that has also preempted wide use of nuclear transfer in the beef cattle industry.

Transgenic Animals

Transgenic animals are unique in that they carry a foreign gene—one that is rearranged from the same species or from another species. The new (or foreign) genes are inserted into the chromosomes of developing embryos, allowing the animal to produce proteins that would not normally be produced. The most apparent use of transgenic animals is the inclusion of genes that produce compounds critical for humans suffering from a gene that is functioning improperly. The advantage to using farm animals is that the transgene may be expressed in the mammary glands, allowing large quantities of the desired gene product to be produced in and purified from the animal's milk.

Transgenic technology has the potential to produce benefits across the beef industry. For example, there are currently efforts whereby genes within double muscled cattle breeds can be modified to increase meat production without the negative effects of dystocia and decreased fertility (Tessanne et al. 2012). Additionally, there are efforts to modify genes to prevent disease and promote efficiency.

One researcher postulated that there might be an opportunity to incorporate select genes expressed on either the X- or Y-chromosomes (Seidel 2014). For example, males with low birth weights may receive traits inserted on the Y-chromosome and exhibit rapid growth or improved efficiency. Additionally, transgenic technologies can potentially be used to alter the sex ratio to produce a disproportionate number of calves of one sex over the other. Transgenic technologies continue to make advances that will one day be relevant to the commercial cow-calf producer.

Sex Selection

Sex selection, coupled with trait selection and embryo methodologies, represent the ultimate in animal selection and propagation. The sex of mammals is determined by the male sperm that contains either X- or Y-chromosomes. Oocytes from females contain only X-chromosomes. If an X-bearing sperm fertilizes an oocyte, a female (XX) results, and if a Y-bearing sperm fertilizes the oocyte, a male (XY) results.

Segregating X- or Y-bearing sperm has been attempted since the advent of frozen semen. Recently, flow cytometry methods have been used successfully for this purpose, and sex-specific calves have been produced as a result of sperm sorted by flow cytometry (Figs. 2 and 3). This methodology provides advantages for animal systems that receive economic benefits for production animals of one sex over the other. As a commercial cattle producer, expect to pay more for sex sorted semen and balance that out with the potential positive outcomes.

Cow-calf producers may carefully consider sex-sorted semen for several reasons. One may be the opportunity to mate genetically superior cows to produce replacement heifers while terminal sires breed the remaining cows. Alternatively, cows can be bred with sex-sorted semen to shift sex ratios for a marketing advantage. Altering calf production by increasing steer calf ratios creates a distinct market advantage because steers can weigh more at weaning compared to their heifer counterparts. Furthermore, they generally bring in a higher price per

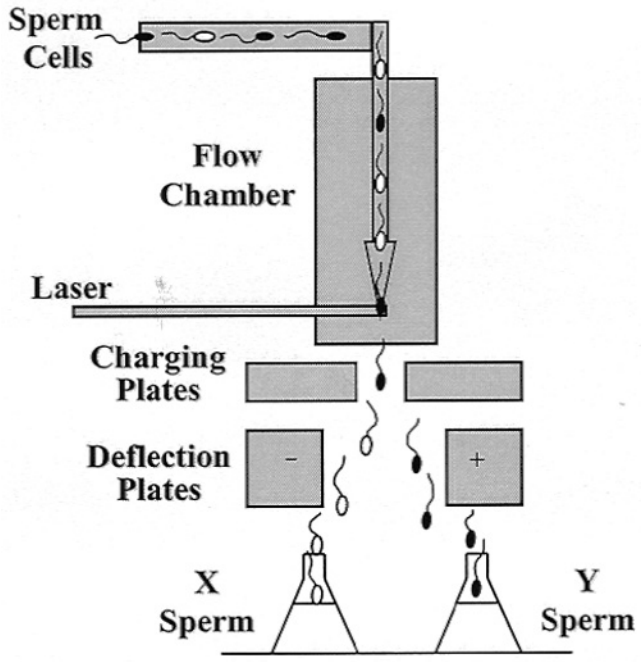

Fig. 2. Sperm sorting by flow cytometry. Sperm containing either an X- or Y-chromosome are individually sorted based on the content of DNA. Sperm containing an X-chromosome have slightly more DNA and take up more fluorescence stain than sperm with a Y-chromosome. The difference in amount of stain taken up by an individual sperm cell permits sorting into X- or Y-bearing fractions.

unit weight compared to heifers of a similar weight (USDA-AMS 2016).

Unfortunately, fertility of sexed-sorted semen is lower compared to conventional semen. Factors contributing to the reduced fertility include fewer sperm within the inseminate combined with damage to the sperm from the sorting process. Commercial cow-calf producers that choose this option should expect a 10 percent decrease in fertility rates with sexed-sorted semen compared to rates with conventional semen (Seidel 2014).

Embryo transfer and related technologies should carefully consider sex-sorted semen. Within the ET paradigm, reproductive efficiency is reduced in cows that were superovulated with exogenous hormones and inseminated with sex-sorted semen because the number of embryos healthy enough to warrant transfer was reduced between 20 to 35 percent (Hall and Glaze 2014).

Embryo sexing is another methodology that is being used by producers for selection of a desired gender. Once embryos have been produced, as previously described in ET and cloning, a biopsy or cell sample is taken from the embryo and the sex is determined by presence or absence of Y-chromosome-specific DNA. If the embryo is the desired sex, it may be transferred to a recipient cow or frozen for future use.

Accuracy of embryo sexing is reported to be 85 percent. After transfer, pregnancy rates are about 60 percent for

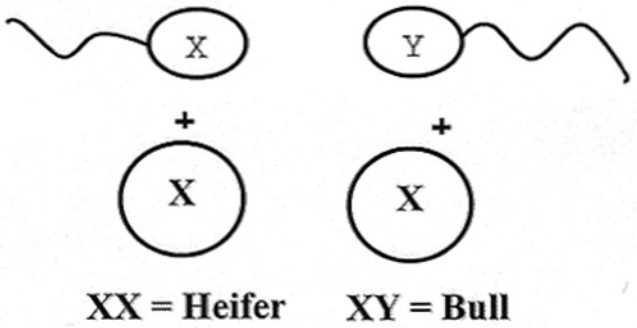

Fig. 3. Sperm sorted into X- or Y-chromosome can be used in artificial insemination, embryo transfer, or *in vitro* fertilization to preferentially produce bull or heifer calves. All oocytes contain X-chromosomes. If females are preferred, a producer would purchase X-bearing sperm (XX = female), and if males are preferred, Y-bearing sperm would be purchased (XY = male).

fresh embryos and 50 percent for frozen embryos. Currently, companies are offering services to sex embryos. However, the same limitations exist as noted for ET, with the added cost of sexing procedures, making this technology unprofitable for commercial cattle producers under current market conditions.

Stem Cell Technology

One of the newest reproductive advances in biotechnology is stem cell technology. Stem cells are unique in that they are able to differentiate into specific phenotypes. Stem cell technology is a powerful tool with several implications for the future of commercial cow-calf production. For example, this technology can potentially be used to take the genetics from a somatic stem cell of a genetically superior bull and insert them into the testes of other bulls that may not have optimal genetics.

Moreover, stem cells from genetically superior bulls whose sperm counts are tolerant to heat stress may be transplanted in the seminiferous tubules of less desirable bulls to simultaneously improve the semen and genetic quality. This technology may even reach a point where males may be able to produce sperm cells that carry only either the X- or Y-chromosome.

Marker-Assisted Selection and Genomic Selection

Current methods for sire selection are based on expected progeny differences (EPD) and/or performance testing, which consist of following bulls and/or their offspring for a defined period of time and measuring performance. These methods have provided advances in genetic selection for economically important traits. Yet, the process is less than perfect as the producer's ability to select bulls is often based on limited data and poor performing bulls exist in every performance test regardless of selection criteria.

Beef producers can use genetic markers to get an understanding of an animal's genetic composition before making a commitment to an investment. A host of factors that will not be present until later in life or after harvest, such as reproductive performance and carcass quality, can be assessed when the genomic information is incorporated into breeding programs.

Conclusion

Development of reproductive biotechnologies in animal agriculture is occurring at a phenomenal rate. However, producers must decide if the economic benefits outweigh the costs associated with many of these techniques. Additionally, caution should be advised that reproductive technologies may produce negative results that can impede genetic progress. While science can provide methods to create pregnancies and offspring, it is important to realize that although reproductive rates are low in heritability, reproduction is a heritable trait. Cattle that reproduce yearly in the environment in which they live should take priority over those unable to reproduce in similar conditions.

Reproductive advances have provided producers the ability to propagate superior animals beyond what was previously thought to be the normal reproductive lifespan of cattle. Animal agriculture will continue to reap the benefits of university, government, and private sectors actively advancing and commercializing reproductive biotechnology.

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Fourth edition; December 2016 Update