New Technologies in Cattle Reproduction and the Correlated Acceleration of Genetic Gain

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Introduction

Over the last 50 years, the cattle industries, both beef and dairy, have dramatically increased the rate of genetic change for economically important traits by harnessing the power of quantitative population genetics theory coupled with the development and implementation of advanced reproductive technologies (ART). These technologies have increased the impact of genetically superior individuals in cattle populations for both sexes. Breakthroughs in the ART tool box continue to decrease generation interval, while at the same time, becoming more efficient in the production of animals. Such breakthroughs include *in vitro* fertilization (IVF) embryo production that equals or is greater than *in vivo* embryo production. Current technology advancements continue to accelerate genetic gain, not just in elite germplasm, the power of these technologies is beginning to be harnessed in the commercial production sectors as well. This discussion will review the current status of ART and the impact it has on genetic improvement in all sectors of the cattle industry.

Artificial Insemination

Without a doubt, the largest impact of a single technology affecting bovine genetic improvement in cattle over the last 50 years was the commercialization of artificial insemination, followed with the development of cryopreserved semen. These advancements greatly enhanced the widespread use of artificial insemination (Pickett and Berndtson, 1978; Foote, 2002). The ability to produce large numbers of progeny from a single sire across many geographical regions and multiple management practices was the cornerstone in making estimation of additive genetic merit in individual animals possible. Artificial insemination resulted in and continues to enable widespread dissemination of superior sires to all sizes of producers. Additionally, artificial insemination facilitates progeny testing, and is often a key component in cross breeding programs. This technology has accelerated the distribution of superior genetics worldwide through exportation, and is essential in the success of estrus synchronization programs when done on large groups of cattle.

Estimations of the use of artificial insemination in the beef and dairy industries, and its impact of genetic progress can be assessed from the current 2018 National Association of Animal Breeders (NAAB) reports of units semen sold domestically and exported in the United States, when compared with historic reports from 1980 (www.naab-css.org). In 1980, 13.3 million units were sold in the dairy industry, domestic, compared to 24.6 million units of semen sold in 2018. Beef domestic sales of semen increased 1980, with 1.0 million units semen sold compared with 4 million units of semen sold in 2018. Differences in the percent of domestic sales in beef and dairy can be attributed to the changes in management in the dairy industry observed over the last 50 years. This change resulted from the consolidation of dairy operations and a continued decreased use of natural service. Development of artificial insemination in beef has been considerably slower in the United States, when compared to dairy and its implantation in beef production in other countries. Range conditions create added hurdles to overcome for heat detection and insemination. However, the industry has seen steady growth over the last ten years (www.naab-css.org). One should note a portion of the increase in beef units sold over the last ten years is the use of beef semen to create beef dairy cross calves in the dairy industry. Presently, artificial insemination is a worldwide business, with an estimated greater than 100 million cattle inseminated annually (Verma et al., 2012).

Genetic change, resulting from AI, has been well documented over the last sixty years (Van Vleck, 1986; Wiggins 1991; Johnson and Jones 2008). During this same time, commercialization of artificial insemination has resulted in the growth of numerous companies on a global scale participating in the leasing/ownership of sires, collection, processing, distribution of semen, and insemination of females.

The acceptance and adoption of artificial insemination was the cornerstone making the development of other advanced reproductive technologies, such as sexing of sperm, estrus regulation, embryo harvesting, freezing, culture, transfer, and cloning possible. Without this game changing development, the rate of genetic improvement in beef and dairy would be a fraction of the present success.

Embryo Transfer

In vivo embryo transfer (ET) is the process of changing the normal ovulation of the bovine female from ovulating one ovum every 21-day estrus cycle, to having many (multiple) ovulations allowing for the production of multiple embryos produced that can be transferred to surrogate females, often referred to as recipient dams, to gestate, and if needed, raise the calf. Donor females can begin a superovulation program between 8-13 days of the estrus cycle if a corpus luetum (CL) is present. Donors are given scheduled injections of follicle stimulating hormone (FSH) over three to four days, every twelve hours. Upon the final does of FSH, an injection of prostaglandin f_2 alpha is administered to initiate ovulation. Donors are bred by natural service or

artificial insemination; embryos are allowed to develop for 7 days in utero, at which time they are non-surgically flushed from the uterus. At this time; they are either transferred to a recipient female that was synchronized to ovulate on the same schedule as the superovulated donor, or frozen for later transfer to recipient females.

The commercialization of embryo transfer in cattle had a huge impact in the ability of the producer to leverage the contribution of genetics from the superior female. The ability to produce upwards of 20-40 progeny from a female in a given year -compared to 6 or 7 in a lifetime- resulted in much of the cattle seedstock industry adopting ET technology into genetic selection programs over the past 3 decades. Embryo transfer allows the producer to mate superior parents to produce multiple full-sibs per procedure, resulting in increased rates of genetic gain. By enhancing selection intensity in the female population and harnessing the power of sampling many more individuals from a single mating. This enhances the probability of identifying superior progeny for the next generation (Land and Hill, 1975). This has resulted in genetic gain by improving reproductive rates in bovine females, increased selection intensity, shorter generation intervals (Church et al., 1977), and with the incorporation of genomics in genetic evaluation, enhanced accuracy of selection in younger animals.

The first reported successful bovine embryo transfer was reported by Willet et al. in 1951. Another key event includes the first commercial embryo company, Alberta Livestock Transplants, Ltd., in 1971. This was followed by key scientific advancements in this field: 1) the production of offspring from frozen embryos (Wilmut and Rowson, 1973); and 2) the development of non-surgical transfer (Greve and Lehn-Jensen, 1979). The ability to cryopreserve embryos like semen created a vehicle to make on farm embryo transfer extremely manageable. Additionally, the producer has an additional genetic product to generate revenue and facilitate greater numbers of progeny from superior parents. Estrus synchronization programs evolved and developed in conjunction with the in vivo embryo transfer, which added to the success and adaption of ET worldwide.

In vivo embryo transfer technology became common throughout the world in the 1980's and 90's. About 17,000 bovine pregnancies were produced by superovulation and embryo transfer in North America in 1979 (Seidel, 1981). It has been estimated that greater than 500,000 ET embryos are produced world-wide annually from super ovulated cows (Mapletoft and Hasler, 2005).

The impact of ET has been dramatic in enhancing the rate of genetic gain in cattle world- wide. The theoretical modeling of genetic change estimated that twice the rate of improvement would be achievable for moderately heritable traits when harnessing the power of ET, when compared to a traditional conventional performance testing program (Land and Hill, 1975). Additionally, it was estimated that the impact on generation interval would also enhance genetic improvement

even though the accuracy of selection in unproven females is less than adult females (Nicolas and Smith, 1983). Since these early publications, the impact of ET on genetic advancements has become a reality. For dairy and beef seedstock sectors, ET has become an integral part of the world's industry leading companies.

In vitro Embryo Production

In vitro embryo production (IVP) is the process of creating embryos from oocytes (unfertilized female gametes) by fertilization and early development outside of the uterus in a laboratory setting. Oocytes are either collected (aspirated) from slaughter house ovaries, surgical collection or the through the use of ultrasound-guided transvaginal follicular aspiration on the donor female. Oocytes go through a maturation period and are fertilized the following maturation with conventional or sexed-sorted semen. After fertilization, they are allowed to develop in an incubator for seven additional days, and the resulting viable embryos are transferred into recipient dams or frozen for future transfers.

This evolution of in vitro embryo production technology has been under development for three decades. Early key pieces of IVP science that allowed scientists to begin to take oocyte maturation, fertilization and embryo development to the blastocyst stage in the lab for bovine occurred in the 1980's (Freis and Ruvinsky, 1999). A very important development for IVP adoption that occurred in the early 1990's was a procedure/technique that was less invasive than earlier surgical procedures, allowing oocyte retrieval from live cows at a much more efficient rate (Merton et al., 2009). Transvaginal, ultrasound-guided oocyte recovery, often referred to as Ovum Pick Up (OPU) (Kruip et al., 1991)- is used in a commercial setting to recover oocytes from antral follicles that will be matured, fertilized and cultured to the blastocyst stage using *in vitro* procedures (Hasler et al., 1995). The procedure, which is minimally invasive, can be used with superovulation every two weeks or done without superovulation twice a week on a single donor (Kruip et al., 1994; Hasler et al., 1995).

The benefits of IVP, like ET, allow for the increased number of progeny from valuable cows, production of progeny from females no longer able to produce naturally or through in vivo embryo transfer, ability to produce embryos from pregnant donors from days 40-100 of pregnancy, and with the advent of sorted semen, the ability to produce large numbers of calves of a desired sex (Hasler et al., 1995). The ability to produce embryos weekly, or every other week, from a donor female allows for a greater number of progeny to be produced in a shorter period of time when contrasted to ET. These gains in efficiency of IVP in time will lead to the development of the technology being used beyond the nucleus seedstock sector to the commercial production portion of the industry. First, it will likely impact the commercial dairy female replacement programs for both purebred and cross breeding programs. Such programs allow producers to further capture greater portion of the gains made in genetic improvement

programs. Additionally, the value of heterosis through the crossing complementary breeds to create f1 progeny maternally designed to match production environment will likely evolve in the dairy and beef commercial industries.

Sexed Semen

The use of sexed semen in the dairy and beef industries has increased dramatically over the last 10 years. In 2008 very few, if any, commercially available AI beef bulls had gender sorted semen available for use in AI programs (Garner and Seidel, 2008). By 2011 greater than 70 commercially available AI beef sires had gender sorted semen available, which brought about a dramatic increase. In dairy, the number of units of commercially available semen increased dramatically from 2006 with 18,000 to 170,000 units of semen in the U.S. in 2008. The estimated number of females produced from sexed semen that entered the U. S. dairy herd in 2008, 2009, 2010, 2011, and 2012 is 8,000, 63,000, 156,0000, 258,000 and 237,000, respectively (De Vries, 2010). This trend has continued to increase dramatically, over the last ten years, in numbers of diary replacement female production. Currently, dairy genetic companies have begun to only sell X bearing semen on the highest genetic merit young genomic enhanced sires.

Simulation studies have shown the impact of sexed semen on the selection intensity, resulting in a future genetic impact on production traits when used in cows and heifers compared with conventional semen programs (Weigel, 2004). It should be noted that a negative effect on reproductive performance of dairy cows was found. Suggesting the appropriate use of sexed semen maybe in the dairy heifer to limit the negative impact of overall herd reproductive performance when used on lactating cows (Khalajzadeh et al., 2012). Sexed semen has the ability to greatly impact genetic gain in both the nucleus selection and commercial populations in both the beef and dairy industries.

Cloning

Since the first announcement of cloning Dolly the sheep (Wilmut et al., 1997), somatic nuclear transfer technology (SCNT) has led to a wide variety of mammals being cloned, including cattle. Over the last decade, the number of cattle created using SCNT technology has increased from a handful to thousands. Academic groups across the world have successfully cloned cattle and continue to study the biology and ways to improve efficiency (Wells et al., 2003). In the beef, dairy and bio-pharma industries commercialization of the SCNT cloning has become an additional tool in the ART toolbox across many regions of the globe.

The principal application of cloning in cattle can be separated into three main categories. First, some of the early models that were discussed were based on the principle of mass production of cloned animals that had more desirable genetic characteristics for traits well suited for commercial livestock production. The benefit for such a program would be the dissemination of

superior genetic material on a large scale with a great reduction in variation final product (Bousquet and Blondin, 2004). One example discussed would be a two-line cloning system. Terminal clones would be produced based on superior output qualities of a genetic donor(s). These clones would gestate in female clones that were derived from a superior maternal genetic donor(s) (Smith, 1989). This type of production, although attractive in theory, has some major shortcomings associated with risk, including proper identification of correct target traits and profit models, possible bottle necks in genetic variability (Van Vleck, 1999). In addition, the present costs of cloning and other ART technologies make the possibility of such systems production by cloning developing in the short term very unlikely. Second, is the use of cloning to produce genetic superior individuals. These are individuals that are identified as being superior for genotype. The producer may use cloning because of the need to propagate greater numbers of progeny from these individuals or use the technology as a type of insurance for elite genetics. Coupling cloning of superior females with other ART allows for increased selection intensity in a population from the contribution of the elite genetic donor. Additional examples include, superior cattle that become injured, reproductive inactivity due to age, and/or die unexpectedly. Cloning is a viable option to capture potential genetics that may be lost. Third is the use of cloning in the bio-pharma industry. Cloning technology has greatly impacted the ability to produce genetic engineered cattle to be used as medical models and production of pharma products through milk and blood in genetic engineered cattle.

Genetic Selection for Traits Associated with ART

Genetic selection for traits that enhance reproductive performance in cattle has been slow to almost non-existent in production populations, with very little research done on traits that impact ART. Church et al., 1977 discussed the impact that embryo transfer would have with the development of nonsurgical techniques and cryopreservation of bovine embryos. Interestingly, discussion in the article was extremely optimistic that the advancements covered in management and protocols would lead to the possibility of understanding genetic variation for traits impacting in vivo embryo transfer technology. Over the last 30 plus years, very little improvement was made in understanding the genetic contribution or implementing the selection of traits impacting superovulation. Like many traits related to reproduction in bovine, the progress and understanding has been slow. Much of this slow progress results from low heritability of reproductive traits and the multi-trait nature of so many of the measurable reproductive traits recorded (Cushman et al., 2007). Because environment is greatly influenced by management, the antagonistic relationships between the selection of traits have resulted in increased outputs of production. This has led to a negative genetic trend for traits related to reproduction in many selection programs. Genetic improvement for increased milk yield in Holstein cattle in the US has led to a dramatic reduction in fertility, as measured by open daughter pregnancy rate (VanRaden et al., 2004). One of the primary reasons for these negative trends is the low, narrow sense heritability of reproductive and correlated traits (Cushman et al.,

2007). Much of this reduction can also be attributed to the lack of inclusion of traits measuring fertility in selection models. For these reasons, genetic selection for animals that perform well in ART programs has been limited to none.

Limited work has been done on the estimation of genetic parameter associated with ART in cattle. One of the earliest studies, looking at the repeatability and heritability of response the superovulation in Holsteins, (analyzed using Multiple Trait Derivative-Free Restricted Maximum Likehood (MTDFREML) repeatability animal model), found the repeatability of the number of transferable embryos to be low, with an extremely low heritability of 0.03. The conclusion by the authors was that little evidence existed in predicting future, superovulation responses based previous treatment(s) and that superovulation may not be a heritable trait that can be selected for (Tonhati et al., 1999). Historically, investigations in understanding the genetic parameters of traits associated with multiple ovulations have shown that it may be possible to enhance embryo transfer production through maternal selection of traits associated with superovulation in cattle. In Nellore cattle, heritability estimates for palpable corpora lutea (CL) ranged from .47 to .57 viable embryos from .20 to .65 (Peixoto et al., 2004). In Holstein, dairy heritability's was estimated to be .23 for number of flushed ova and .1 for transferable viable embryos. The number of flushed ova was also found to have a positive correlation with transferable embryos of .74. The authors concluded that selection for number of ova flushed would have an indirect positive increase of 22% transferable viable embryos, a key profit driver in embryo transfer programs (Konig, et al., 2007). Heritability estimates of .25 for number of oocytes collected and .16 for number of transferable embryos at day 7 were found in Holstein Friesian cattle. In that study, sires estimated breeding values for oocyte number and transferable embryos showed no correlation to the sires breeding index for female fertility in this population. Genetic parameter estimates for oocyte number and embryo production using in vitro embryo production systems support the possibility of introducing such traits into breeding programs to enhance the number of off-spring produced from a superior dam. And, as an important result, an improvement in cost per progeny produced in IVP (Merton, et al., 2009). The genetic components of direct and correlated traits for embryo production in the female give evidence that efficiency of ART programs can be improved through donor selection. From these studies, it would be useful to have genetic breeding value estimates for: 1) the traits number of ova produced; and 2) numbers of viable embryos. This will provide important data and enhance the efficiencies of in vivo embryo transfer and in vitro embryo production. The reality of such data making its way into genetic evaluation is hindered by the difficulty of collecting large numbers of phenotypic data. Because of these hurdles, the first impact of such data will likely be in those genetic evaluation programs that exist within breeding companies.

Incorporation of ART the future

Incorporation of ART will be extremely important as the world continues to see an increased need for high quality animal protein production. The manipulation of gametes and embryos in farm animals will become increasingly important. It will help meet the growing demand of agricultural products in emerging economies world-wide and impact in the biomedical field.

In the early years of ET, sources of variation in donor females and recipient dams were observed and discussion included such factors as genetics that may not respond to management practices (Church and Shea, 1977). This observation has been confirmed with the large amounts of documented individual female phenotypic variability both in vivo and in vitro embryo production resulting in the estimation of genetic components for traits associated ART (Merton et al., 2009; Konig, et al., 2007). No matter how excellent the management of donor females and the excellence execution of ART protocols, poor production of embryos in many cases cannot be overcome. In order to enhance efficiency of ART in the production of embryos selection programs, cattlemen will have to incorporate genetic selection for traits that are impacting embryo production. This will affect female selection first, but holds great potential to impact selection of males used in ART programs, as the world begins to understand the impact of male fertility in the successful formation of the bovine zygote. In the future, it will be important to take high genetic index females for traits and correlated traits that indicate females that will excel in production of embryos. One specialized phenotype that holds promise is the use of ultrasonography of the ovaries in assessing antral follicle count. This use of antral follicle count has been shown to be associated with a females' response to superovulation protocols and embryo production (Ireland et al., 2009; Mossa et al., 2012). With a heritability estimated of .44 (Snelling et al., 2012), antral follicle count would definitely respond to genetic selection, making it a good candidate for enhancing embryo production in MOET programs.

The desire to shorten generation interval has been greatly enhanced by the accuracy of genomic enhanced evaluations in young animals. The next logical step is selecting the next generation of parents by using embryo selection to increase selection intensity, resulting in another jump in genetic progress. It should be noted that mistakes will also be magnified and that continued phenotypic data in the genetic evaluations will be critical for the success of these types of programs.

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