Modern Reproductive Technologies to Improve Cattle Production

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Abstract. Cattle contribute significantly to the global supply of animal-derived proteins which are an important part of a well-balanced human diet. With the human population increasing by 1 billion people every 13 years, there will be an estimated 9.6 billion people on planet Earth by the year 2050. To attain global food security the amount of available food will need to double between now and then. Enhancing reproductive efficiency is a prerequisite for boosting production of meat and milk from cattle, and, fortunately, an arsenal of modern reproductive technologies is available to assist with that effort. The objective of this manuscript is to provide an overview of reproductive biotechnologies that can bolster the efficient production of meat and milk from cattle. Protocols for synchronization of estrus and synchronization of ovulation facilitate more efficient artificial insemination using conventional or sex-sorted semen. *In vivo* and *in vitro* production of preimplantation embryos from genetically superior females enable creation of multiple offspring with high production potential. Biochemical and ultrasonographic methods for pregnancy testing identify non-pregnant females that can be re-mated or sold to prevent wastage of valuable feed resources. Somatic cell nuclear transfer is used to create copies of highly productive animals, and genome editing of zygotes provides a novel opportunity to selectively enhance the genetic makeup of cattle for the benefit of animal and human health. Wise use of these reproductive technologies will increase food production from cattle and will help alleviate world hunger.

Key words: Embryo transfer, *in vitro* fertilization, pregnancy testing, sperm sexing, synchronization of estrus.

Tecnologías Reproductivas Modernas para Mejorar la Producción Pecuaria

Resumen. El ganado contribuye significativamente al suministro global de proteínas de origen animal que son una parte importante de una dieta humana bien balanceada. Con el incremento de la población a un ritmo de mil millones de personas cada 13 años, habrá aproximadamente 9.6 mil millones de personas en el año 2050. Para alcanzar una seguridad alimentaria global, la cantidad de comida disponible deberá ser el doble entre la actual y la de esta fecha futura. Mejorar la eficiencia reproductiva es un prerrequisito para aumentar la producción de carne y leche, y afortunadamente, un arsenal de tecnologías reproductivas modernas está disponibles para asistir con este esfuerzo. El objetivo de este artículo es proveer un resumen de las biotecnologías reproductivas que pueden mejorar la eficiencia de producción de carne y leche. Con los protocolos para la sincronización del celo y de la ovulación se logra una inseminación artificial más eficiente usando semen convencional o sexado. La producción *in vitro* e *in vivo* de embriones a partir de hembras genéticamente superiores ayuda a la creación de crías múltiples con un alto potencial de producción. Los métodos bioquímicos y ultrasonográficos identifican de hembras vacías que pueden volver a ser servidas o vendidas para prevenir el desperdicio de recursos valiosos de alimento. La transferencia nuclear de células somáticas es usada para crear copias de animales altamente productivos, y la edición del genoma de cigotos provee una oportunidad nueva para mejorar selectivamente la genética del ganado para el beneficio de la salud animal y humana. El uso adecuado de estas tecnologías reproductivas aumentará la producción de alimento a partir del ganado y ayudará a aliviar el hambre mundial.

Palabras clave: Fertilización *in vitro*, prueba de preñez, sexado de semen, sincronización del celo, transferencia embrionaria.

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Introduction

Cattle are a major contributor to the world supply of milk and meat. In calendar year 2013, there were more than 1.42 billion head of cattle across the globe; those cows produced more than 635.5 million tons of whole fresh milk and more than 63.6 million tons of indigenous meat (FAOSTAT3 2014). The number of cattle lags behind only that of chickens (20.96 billion head) and sheep and goats (2.08 billion head).

Despite the enormous contribution of cattle-derived products to the human food supply, further increases in total production, as well as in the efficiency of production, are needed in the near future to meet the rapidly growing demand for food. By the year 2050, the world population will reach an estimated 9.6 billion people (Searchinger *et al.* 2014), and total availability of food must double by that time in order to avoid pervasive human hunger.

Food availability can be increased through two primary avenues: 1) decreased pre- and post-harvest food losses, and 2) increased efficiency of food production. A prerequisite to the efficient production of cattle-derived foods is successful reproduction because cattle that do not become pregnant are unable not only to produce milk but also to produce calves for subsequent meat production. Thus, reproductive efficiency is of paramount importance to bolstering the production of human foods from cattle.

The objective of this manuscript is to provide an overview of modern reproductive biotechnologies that can enhance the efficient production of meat and milk from cattle.

Reproductive Biotechnologies for Cattle

For centuries, farmers have endeavored to breed better and more productive livestock. Robert Bakewell, an 18th century British agriculturalist, is often acknowledged as one of the first to implement systematic selective breeding of livestock. Although Bakewell's "breed the best to the best" philosophy was effective in improving livestock productivity, it was not until the pioneering efforts of animal geneticists such as Jay L. Lush (Chapman 1991) that the scientific basis of livestock genetic improvement became well understood.

Greater knowledge of genetic improvement principles was acquired concomitantly with pioneering studies on livestock reproductive physiology. Investigations on the estrous cycle, the postpartum period, and techniques for the regulation of the estrous cycle (reviewed in Lauderdale 2009) were crucial to advancing knowledge of how to improve livestock reproductive efficiency. It was the tandem development of reproductive biotechnologies and enhanced methods to identify genetically superior animals that revolutionized cattle breeding.

Artificial insemination (AI). One of the most impactful and widely utilized reproductive biotechnologies for cattle is artificial insemination (Foote 2002). Artificial insemination (AI) involves the collection of semen from genetically superior bulls, processing of that semen into several dozen doses, and placement of the processed semen into the reproductive tracts of cows or heifers. One ejaculate processed for AI can impregnate dozens of females, thus allowing the highest genetic merit bulls to produce a large number of offspring.

The first scientifically documented AI was performed in dogs in 1780 by Abbé Lazzaro Spallanzani (Heape 1897), but it was not until nearly two centuries later - when it was discovered that the chemical compound glycerol could protect spermatozoa against damage caused by cold temperatures (Polge *et al.* 1949) - that the bovine AI industry began to develop in earnest. The ability to freeze (cryopreserve) spermatozoa from bulls opened the door to shipment of semen from genetically superior bulls to any part of the world.

One needs only to look at the US dairy cattle industry to grasp the significant contribution AI has made to increased cattle productivity. In 1965 (prior to widespread use of AI by US dairy cattle farmers), there were 15 million dairy cows that produced a national supply of 56,445 kg of milk (3,775 kg of milk per cow). By the year 2000, the US dairy herd had dropped to 9.2 million head (a 39% decrease), the national milk supply had risen to 76,342 kg (a 35% increase), and productivity per cow had risen to 8,274 kg of milk (a 119% increase). Today, AI is an integral and essential part of cattle production. In 2015, more than 23.6 million units of cryopreserved dairy cattle semen and more than 2.6 million units of cryopreserved beef cattle semen were sold by members of the National Association of Animal Breeders based in Columbia,

Missouri, USA (NAAB 2016).

Sperm sexing. There has long been interest in pre-determining the genetic sex of offspring of cattle. Dairy cattle farmers usually prefer the production of heifer calves (because bulls do not lactate), whereas beef cattle producers typically prefer to produce bull calves (because male calves grow faster and are more feed efficient than heifer calves).

There are three major approaches to determining the genetic sex of an offspring prior to its birth – fetal sexing (Curran *et al.* 1989), embryo sexing (Bredbacka 1995), and sperm sexing (Garner 2006). Ultrasonographic sex determination is an accurate and commercially available technique (Youngs and Evans 2012). The location of the fetal genital tubercle between days 56-75 of gestation is highly predictive of fetal genetic sex, but farmers must either accept the genetic sex of the offspring or abort the pregnancy if the fetus is of the undesired genetic sex (the latter of which seems counterproductive, particularly for dairy cattle). Embryo sexing requires the collection of preimplantation embryos from mated females, so this technique is not practical for large numbers of cows within a herd. Although the embryo sexing technique is highly accurate, it is relatively expensive and farmers must decide the fate of embryos that are not of the desired genetic sex (discard, sell, or transfer into a recipient female knowing a calf of the undesired sex will be produced).

Many cattle producers have opted to pre-determine the genetic sex of their calves by utilizing sperm sexing. Sperm sexing technology (refined and patented by the US Department of Agriculture) takes advantage of the fact that the X chromosome is larger and possesses more DNA than the Y chromosome. Sperm cells are incubated in a DNA-specific fluorescent dye and then are passed individually though a flow cytometer equipped with an ultraviolet laser. Bull sperm cells with an X chromosome possess approximately 3.8% more total DNA than those with a Y chromosome, and because they have more DNA they fluoresce more brightly. The flow cytometer segregates the brightly fluorescing sperm cells from others, yielding a population of spermatozoa that is approximately 94% X chromosome-bearing (and 6% Y chromosome-bearing). Insemination of heifers and cows with this enriched population of X chromosome-bearing spermatozoa will lead to the production of heifer calves in nearly all cases.

The first live offspring produced after separation of X chromosome- from Y chromosome-bearing spermatozoa was in the rabbit (Johnson *et al.* 1989), but this technology was quickly adapted for use in the cattle industry (Cran *et al.* 1993) - firstly via use *of in vitro* fertilization technologies, secondly via use of deep intrauterine AI, and lastly via use of traditional AI. Today, approximately 2 million units of bovine semen containing sexed sperm cells are sold annually (Seidel 2014).

One problem that has been observed routinely following AI with sex sorted spermatozoa is lower fertility. Pregnancy rates after AI with sex-sorted spermatozoa are typically 80% of those obtained after AI with traditional (non-sex sorted) semen from the same bull (Seidel 2014). For example, if pregnancy rate after AI with traditional semen is 60%, insemination with sex-sorted spermatozoa is expected to yield a 48% pregnancy rate (60% normal pregnancy rate X 80% fertility with sex-sorted semen = 48% pregnancy rate).

Recently, however, there has been significant improvement in sperm sexing technologies. Vishwanath (2014) reported that fertility after insemination of cows and heifers with frozen-thawed SexedULTRA™ sex-sorted semen containing 4 million cells (65.0% 56-day non-return rate [NRR]; 1,182 inseminations) was comparable to that obtained with conventional semen that contained 15 million cells (64.5% NRR; 50,143 inseminations). A patent is pending for the SexedULTRA™ method (Vishwanath *et al.* 2016), and refinements of the previously developed technology appear to relate to extending the sperm sample with a buffered holding medium (to regulate pH), adjusting the concentration of the extended sperm sample to a target concentration range, and catching sorted sperm cells in a medium that is supplemented with an antioxidant (to prevent cell damage due to oxygen free radicals).

Synchronization of estrus. Without a doubt, widespread and successful utilization of AI in the beef and dairy cattle industries was facilitated by the technology to control the timing of expression of estrus in cows and heifers. This technology is commonly known as synchronization of estrus. Cattle typically exhibit estrus (or heat) every 21 days. For more than 70 years, AI has been performed following the a.m./p.m. breeding rule (Trimberger 1944). This method involves inseminating females that are

observed in estrus in the morning (a.m.) in the evening of the same day and inseminating females that are observed in estrus in the evening (p.m.) in the morning of the following day. For farmers who are trained to perform AI, this breeding method not only is convenient but also is quite successful. However, for those farmers who have not been trained to perform AI, it is impractical and cost prohibitive to hire AI technicians to visit the farm every morning and evening for 21 days to AI cows as they come into heat. Thus, techniques were developed to regulate when females exhibit estrus (reviewed in Lauderdale 2009).

The most commonly utilized methods for synchronization of estrus in cattle involve administration of exogenous hormones, and the exogenous hormones used in synchronization of estrus protocols (prostaglandin F2α [PGF], progesterone, and gonadotropin releasing hormone) are the same hormones produced naturally by the cow herself.

Exogenous PGF (or its analogues such as cloprostenol) may be given to lyse the *corpus luteum* (CL) present on the ovaries of the cow. When the CL is lysed, production of the hormone progesterone (P4) by the CL is stopped. As the liver continues to metabolize P4, blood concentrations of P4 are reduced, negative feedback of P4 on the hypothalamus and anterior pituitary is reduced, ovarian follicles grow larger and produce greater amounts of estradiol -17β (E2), and the cow comes into heat when the E2/P4 ratio is sufficiently high. This sequence of events is the same that occurs naturally in randomly cycling cows. Although PGF may be administered as a single injection, most cattle producers administer two injections of PGF 11 days apart in an attempt to have a greater percentage of cows possessing a CL at the time of the second injection. This will result in more cows synchronously exhibiting estrus.

Estrus and ovulation will not occur when blood concentrations of P4 are high, so at first glance it seems counterintuitive to administer exogenous P4 in an attempt to synchronize estrus. However, when one thinks about a cow very late in her estrous cycle – one whose CL is dying from exposure to endogenous PGF – it is easy to understand that something must be administered to that cow to prevent her from coming into heat. The most logical product to administer is P4 (or its analogues such as melengestrol acetate [MGA]) because it will block estrus and ovulation until the exogenous P4 is removed or metabolized.

The challenge when synchronizing estrus with exogenous P4 is to administer P4 for a sufficient number of days to allow all females to reach the stage of the estrous cycle where they no longer have a viable CL but not to administer P4 for too many days which leads to a reduction in fertility at the synchronized estrus. The P4 analogue MGA is typically fed for 14 days, and this length of treatment provides a good balance between estrus response rates and fertility. Intravaginal P4-containing devices such as the CIDR (controlled internal drug releasing device) or DIB (dispositivo intravaginal bovino), and subcutaneous P4 analogue-containing implants such as Crestar®, may also be used to deliver P4 or P4 analogues.

Due to the risk of reduced fertility at the synchronized estrus that may be observed with P4/P4 analogue treatment, protocols have been developed that utilize a combination of PGF and P4. By incorporation of PGF into P4 protocols, the number of days of treatment with P4 can be reduced (and the reduction in fertility typically associated with long-term exposure to P4 can be minimized/eliminated). These protocols have the advantage of being of shorter duration and requiring less “lead time” to implement.

A third exogenous hormone that can be incorporated into synchronization of estrus protocols is gonadotropin releasing hormone (GnRH). There are two reasons for including GnRH in synchronization of estrus protocols: 1) to force ovulation of a large antral follicle - which leads to the emergence of a new wave of ovarian follicular growth and development, and 2) to force ovulation of a large antral follicle that will release the oocyte to be fertilized by sperm shortly after AI. It should be noted that ovulation in response to exogenous GnRH is mediated through a surge release of the hormone LH (luteinizing hormone).

Researchers continue to develop new protocols for synchronization of estrus each year, and the constant barrage of new protocols can be confusing for farmers. The appropriateness of certain protocols differs between dairy and beef cattle, as well as between cows and heifers. The Beef Reproduction Task Force (2016) has developed a list of approved protocols for synchronization of estrus in cows and in heifers. Readers are referred to their web site (http://beefrepro.unl.edu/resources.html) for a detailed description of the 16 different approved protocols. Three of the protocols (Select Synch, Select Synch + CIDR®, and PG 6-day CIDR®) are true synchronization of estrus protocols for cows, and another three

protocols (1 shot PG, 7-day CIDR®-PG, and MGA®-PG) are true synchronization of estrus protocols for heifers. The remaining protocols involve timed artificial insemination (TAI) which will be discussed below.

For dairy heifers, the Dairy Cattle Reproduction Council (2016b) has approved three protocols (2xPGF, CIDR®-PGF7, CIDR®-PGF6) for synchronization of estrus (http://www.dcrcouncil.org/protocols.aspx). There are no approved synchronization of estrus protocols for lactating dairy cows due to their low efficacy; however, some re-synchronization protocols (used in in mated and potentially pregnant females) do rely upon visual observation of estrus.

Synchronization of ovulation. One of the biological events that occurs approximately 24-27 hours after the onset of estrus is ovulation (release of the oocyte from the ovarian follicle). When farmers are synchronizing estrus in their cows and heifers, they are also synchronizing ovulation (although farmers cannot see ovulation like they can see estrus). However, what happens when a cow does not exhibit estrus? Does lack of estrus mean that she does not ovulate? Or what happens if a cow exhibits heat, but the farmer does not observe that heat?

There are various management schemes, facility designs, and times in the production cycle of cows which hinder a farmer's ability to detect estrus effectively. If a farmer does not detect a cow in heat, AI will not be performed and pregnancy will not be established. High-producing dairy cows can be particularly difficult to observe in estrus.

To circumvent problems associated with the detection of estrus and/or with anovulation, protocols were developed to control the time of ovulation through use of exogenous hormones. These protocols allow AI of cows and heifers at a fixed time without regard to the expression of estrus. Two acronyms are typically used to describe these timed inseminations: fixed time AI (FTAI) or timed AI (TAI).

The same three exogenous hormones that were used for synchronization of estrus protocols are also used for synchronization of ovulation protocols (GnRH, PGF, P4). In general, various combinations of exogenous hormones are given to induce the emergence of a new ovarian follicular wave, and females are subsequently treated with exogenous GnRH to induce a surge release of endogenous LH that will lead to ovulation of the dominant follicle of the new follicular wave.

One of the first TAI protocols for cattle was named OvSynch (Pursley *et al.* 1995). In the OvSynch protocol, which has been refined since its initial development, dairy cows are treated with GnRH after their voluntary waiting period. Seven days later cows receive PGF, and this is followed two days later with a second treatment with GnRH. Cows undergo TAI 16 hours after the second GnRH treatment. In the OvSynch protocol cows are handled a total of four times.

Beef cattle producers were intrigued with the idea of eliminating the need for detection of estrus by using a synchronization of ovulation protocol; however, because handling cows four times was not acceptable, a modified OvSynch protocol named CO Synch was developed (Geary and Whittier 1998). The CO Synch protocol is identical to the OvSynch protocol except that TAI is performed at the time of the second GnRH injection (eliminating one time of handling cows).

The Beef Reproduction Task Force (2016) has developed a list of approved protocols for synchronization of ovulation in cows and in heifers. Three of the protocols for cows (Select Synch & TAI, Select Synch + CIDR® & TAI, and PG 6-day CIDR® & TAI) and three of the protocols for heifers (Select Synch + CIDR® & TAI, MGA®-PG & TAI, and 14-day CIDR®-PG & TAI) also involve detection of estrus. There are two true synchronization of ovulation protocols approved for both cows and heifers (7-day CO-Synch + CIDR®, 5-day CO-Synch + CIDR®), one protocol that is approved for *Bos indicus* cows (PG 5-day CO-Synch + CIDR®), and two protocols for heifers that involve pre-treatment with P4 or MGA (14-day CIDR®-PG &TAI, MGA®-PG & TAI) to prepare the hypothalamus for cyclicity.

For dairy heifers, the Dairy Cattle Reproduction Council (2016b) has approved two protocols (5-day CIDR®_CO-Synch72-PGF2, 5-day CIDR®_CO-Synch72-PGF1) for synchronization of ovulation. For lactating dairy cows, the Dairy Cattle Reproduction Council (2016a) has approved four synchronization of ovulation protocols (OvSynch56, OvSynch48, 5-day CO-Synch-72, Co-Synch-72) that may be used with or without one of three different pre-synchronization methods (2X-PGF, GnRH-PGF-GnRH 72, PGF-GnRH-48).

Embryo transfer. Embryo transfer is a reproductive

technology that involves production of embryos from genetically valuable donor females and subsequent transfer of harvested preimplantation embryos into the reproductive tract of lower genetic quality (but reproductively sound) recipient females (Youngs 2007). Embryo transfer (ET) is the female equivalent of AI and allows females of high genetic merit to produce more offspring in one year than would be possible through natural service.

More than 125 years ago the first successful ET in mammals occurred (Heape 1891). This pioneering research was conducted with rabbits, but more than 70 years passed before the birth of the first ET calf (Willett *et al.* 1951). Today, more than 825,000 bovine preimplantation embryos (includes *in vivo* derived and *in vitro* produced embryos) are transferred each year throughout the world (Perry 2015).

During the early years of the commercial bovine ET industry, many people referred to ET as MOET (multiple ovulation & embryo transfer). The reason for this name is because donor cows are treated with exogenous follicle stimulating hormone (FSH; the same hormone the cow herself naturally produces) to induce multiple ovulations and to make the overall process of ET more efficient. These superovulated donor females undergo AI two times at 12-hour intervals, and embryos are allowed to develop inside the cow for 6-7 days before they are flushed from the donor's uterus with a sterile saline solution. Embryos produced in this manner are called *in vivo* derived (IVD) embryos. Harvested IVD embryos are evaluated (Jahnke *et al*. 2015), and those deemed to be viable (an average of nearly seven IVD embryos per donor) are transferred individually into the uterus of a synchronized recipient female. The degree of synchrony of estrus between donor and recipient females plays an important role in the establishment of an ET pregnancy, and the aforementioned methods for synchronization of estrus and ovulation are routinely used in the ET industry. Pregnancy rates following transfer of fresh IVD embryos to synchronous recipient females is typically near 60-70%.

Analogous to the importance of frozen semen to the bovine AI industry, cryopreservation of embryos is also vitally important to the ET industry. Although more than 20 years passed between the birth of the first ET calf in 1950 and the birth of the first calf produced after transfer of a frozen-thawed embryo (Wilmut and Rowson 1973), transfer of frozen-thawed embryos is now an integral part of the bovine ET industry. More than 56% of bovine IVD embryos transferred globally to recipient females in 2014 had been previously frozen (Perry 2015).

Methods for embryo cryopreservation have been reviewed recently (Youngs *et al.* 2010; Youngs 2011a; Youngs 2011b). Historically, embryos had been frozen using glycerol as the cryoprotective agent to dehydrate the cells of the embryo prior to freezing. Although successful, it was necessary to remove glycerol from the embryo after thawing and prior to transfer to recipients. Voelkel and Hu (1992) developed a method known as "direct transfer" which allows embryos frozen in ethylene glycol to be thawed and directly transferred to recipient females without the necessity of removing the ethylene glycol first. Today, nearly 100% of bovine embryos frozen in the US are cryopreserved in ethylene glycol for use in direct transfer (Wehrman 2015). Pregnancy rates following transfer of frozen-thawed IVD embryos to synchronous recipient females is typically near 55-65%.

***In vitro* embryo production.** For many years, the bovine ET industry was based solely on the production, collection, and transfer of IVD embryos. However, the lack of consistency in the response of donor cows to superovulation, coupled with failure to obtain transferrable quality embryos from 20-25% of superovulated donors, led to development of alternate methods for embryo production.

One such alternative is the laboratory production of embryos using the test-tube procedure known as *in vitro* fertilization (IVF). This name (IVF) is actually a misnomer because the creation of *in vitro* produced (IVP) embryos involves more than IVF; it involves collection and *in vitro* maturation (IVM) of oocytes, *in vitro* capacitation of spermatozoa, IVF, and *in vitro* culture (IVC) of zygotes. An overview of bovine *in vitro* embryo production was recently published (Hasler and Barfield 2015), as was a review of methods for *in vitro* culture of embryos (Godke *et al.* 2014).

The world's first calf produced via *in vitro* methods was born nearly 35 years ago (Brackett *et al.* 1982); however, commercial application of this technology took many years to develop. The production of IVP embryos was led by the Brazilians, but the once-popular production of IVP embryos is Asia has declined. Nonetheless, global production of IVP embryos is quite common today, as evidenced by the transfer of nearly 300,000 fresh and 68,000 cryopreserved IVP embryos in 2014 (Perry 2015).

In the beginning stages of commercial *in vitro* embryo production, genetically elite donor cows were brought into a highly specialized clinic for ultrasound-guided aspiration of oocytes directly from ovarian follicles of living donor cows. This procedure is known as ovum pick up (OPU; Pieterse *et al.* 1988). As equipment (e.g., aspiration needles, vacuum pumps, portable incubators) and protocols for OPU and IVM improved, OPU evolved to enable oocytes to be collected from a high-volume of donors brought to a central collection facility that is not highly specialized. Theoretically, OPU can be performed on individual farms, although it is not as economical as OPU done at a central collection point. During 2014, an average of 19.2 oocytes and 5.2 transferrable quality embryos were produced per OPU session (Wehrman 2015) by members of the American Embryo Transfer Association.

Pregnancy rates obtained after transfer of fresh Day 7 IVP embryos into Day 7 or Day 8 recipients typically ranges between 50-55% (Hasler and Barfield 2015). However, pregnancy rates after transfer of frozen-thawed IVP embryos is typically only 40-50% (Youngs 2011a). Substantial research in underway on the cryopreservation of bovine IVP embryos using the ultra-rapid cooling method known as vitrification, and in the near future it is likely that pregnancy rates after transfer of vitrified/warmed embryos will approach those obtained with fresh IVP embryos.

The *in vitro* production of embryos also provides at least four unique opportunities not available via conventional MOET approaches. Oocytes can be collected from: 1) pregnant females who are no more than 100 days pregnant (Meintjes *et al.* 1995), 2) females in the early post-partum period (Perez *et al.* 2000), recently deceased females (if ovaries are obtained no later than 6-8 hours after death), and 4) prepubertal females (Brogliatti and Adams 1996).

Pregnancy testing. One very practical, but often underutilized, reproductive tool that can aid in better reproductive management of cattle operations is pregnancy testing. There is tremendous economic incentive to identify mated females who are not pregnant so that those females may be either re-mated or culled. In the US, it costs more than $600 USD to maintain one cow for one year (USDA, 2015), and a non-pregnant cow that does not produce a calf hurts the economic viability of the farm. Each day that a dairy cow is NOT pregnant past the end of the voluntary waiting period (e.g., Day 60) can cost a dairy farmer as much as $3 USD (Groenendaal *et al.* 2004). Methods for determination of pregnancy in cattle has recently been reviewed (Youngs and Klemesrud 2015).

Although the most accurate methods for pregnancy testing typically require farmers to hire a trained technician, there is a protocol known as FastBack™ that farmers can use themselves to help identify non-pregnant cows. Cows mated by natural service or AI can be treated for seven days with P4 (e.g., CIDR, DIB, MGA) 14 days after AI, and at the end of the treatment period cows are monitored for signs of heat. Non-pregnant cows will exhibit estrus and can be re-mated, and cows that do not return to estrus are presumed pregnant (but pregnancy should be confirmed at a later date via an alternate testing method).

Transrectal palpation is one of the oldest and most commonly utilized techniques for pregnancy testing, and it may be used during days 32-90 for accurate determination of pregnancy (Christiansen 2015). However, palpation is a technique that is subject to error. One study revealed that 51.9% of "non-pregnant" dairy cows purchased from a sale barn after transrectal palpation were actually pregnant (Howard *et al.* 2007). In the past, questions had been raised about the impact of transrectal palpation on fetal loss; however, there was no difference observed between palpated and non-palpated dairy cows on pregnancy loss (12.2% and 13.2%, respectively) between days 29 and 90 of gestation (Romano and Fahning 2013).

Transrectal ultrasonography is a technology that permits experienced technicians to detect pregnancy as early as day 27 or 28 of gestation (Youngs and Evans 2012). If performed during days 56-75 of gestation, transrectal ultrasonography can be used to also determine fetal genetic sex (Curran *et al.* 1989). One additional benefit of ultrasonographic pregnancy testing is that pathologies of the reproductive tract and/or dead fetuses can be detected more easily than with palpation.

One additional method to determine pregnancy in cows is by measuring various pregnancy-specific biochemical compounds that are present only in the blood of pregnant cows. With proper facilities and minimal training, farmers can learn how to collect blood samples from their cows. The blood samples can then be sent to a laboratory that is capable of measuring pregnancy-associated glycoproteins such as pregnancy-specific protein B (PSPB; Sasser *et al.*

1986). The PSPB (BioPRYN™) ELISA is 93% accurate beginning at ~ Day 30 of gestation, but because of its long half-life it does not make a good pregnancy test in early postpartum cows (Cain and Christiansen 2015).

Somatic cell nuclear transfer. The world's most famous sheep is probably Dolly. Dolly was born in 1996, and she was created through a ground-breaking advancement in nuclear transfer technology (Wilmut *et al.* 1997). Even though cloning had been done before, Dolly was unique because she originated from a differentiated cell taken from the mammary gland of an adult animal. This technological breakthrough revealed that it is biologically possible to take a specialized cell and re-program it to behave as an undifferentiated cell.

Although a number of cattle have been produced using somatic cell nuclear transfer (SCNT) technology, its utility is limited to cattle producers who raise high-dollar animals (e.g., bulls that become AI sires, purebred cows that produce AI sires). In the United States, it costs approximately $15,000 - $20,000 to produce one calf via SCNT. To date, the most popular use of SCNT is to re-create a prize-winning steer as an intact bull that subsequently can be used for breeding. The SCNT technology has also been used to re-create a prominent AI bull, as well as a bull carrying a disease-resistance gene. As exciting as SCNT is, most people agree that is has little (if any) application in the commercial cattle industries.

Genome editing of zygotes. One of the more exciting scientific developments that has the potential to drastically alter cattle breeding is that of genome editing in recently fertilized eggs (zygotes). Although, technically speaking, genome editing is considered a genetic rather than reproductive technique, the application of genome editing is intertwined with embryos and ET technology so it is important to mention.

There are three basic approaches to making specific alteration of the DNA sequence of a zygote: 1) zinc finger nucleases (ZFNs), 2) transcription activator-like effector nucleases (TALENs), and 3) clustered regularly interspaced short palindromic repeats (CRISPR)/ CRISPR-associated endonuclease cas9 (Cas9; Carlson *et al.* 2014). These enzymes can be utilized to direct site-specific homologous recombination to knock out a gene, to repair a gene, or to introduce a novel mutation at a specific location.

More than 300 pigs, cattle, sheep, and goats have been generated using genome editors (Tan *et al.* 2016). These include modifications of the low-density lipoprotein receptor, myostatin gene, polled gene, and genes related to disease resistance. One simulation study indicated that the rate of livestock genetic improvement using genome editing could be 1-4 times higher than that possible via traditional breeding programs (Jenko *et al.* 2015). Clearly, an exciting future lies ahead!

Conclusions

Today's cattle producers have a variety of modern reproductive biotechnologies available to help them increase efficiency of meat and milk production. Synchronization of estrus and synchronization of ovulation protocols facilitate higher pregnancy rates from artificial insemination with traditional or sex-sorted semen. Use of genomically enhanced breeding values for selection of genetically meritorious donor females permit production of high genetic value *in vivo* derived and *in vitro* produced embryos. Pregnancy testing identifies non-pregnant females that can be re-mated or culled. Finally, in the very near future genome editing of zygotes will create novel genotypes that are more feed efficient, more disease resistant, and produce greater quantities of healthier animal-derived proteins to help feed the world in the year 2050 and beyond.

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