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Methods of Bovine Reproduction

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Introduction to beef and dairy cattle industries

In the United States, the cattle industry is integral to feeding the population and sustaining the economy. The beef and dairy industries are pressured to increase the output of product while using less land and minimizing environmental impact. Scientists have published numerous research studies to help farmers increase the efficiency of their cattle management programs. Many of these reports describe ways to improve bovine reproduction, one of the most essential aspects for the success of dairy and beef cattle farms.

The beef industry aims to produce large quantities of good quality protein per animal. Breeding selection is based on muscle mass and ideal marbling of the meat, which produces the best flavor. The beef industry represents the largest segment in US agriculture (Lowe and Gereffi, 2009) and had an estimated retail equivalent value of $79 billion in 2011. The largest cattle boom occurred between the 1920s and the 1970s and was approximately proportional to the increase in population during that time. The number of beef cattle peaked in 1978, and has decreased slowly until current day. There were 90.8 million beef cattle on farms in 2012 (United States Department of Agriculture, 2012a). The decline since 1978 was not because of decline in the industry, but actually due to greater meat production per animal (McCurry-Schmidt, 2011; Figure 1). The United States is the largest producer of beef in the world. The country exports over $5 billion of product, predominantly sending beef to Japan, Mexico, South Korea, and Canada (United States Department of Agriculture, 2012a).

The dairy industry is also significant and is valued at more than $35 billion (Lowe and Gereffi, 2009). The predominant milk-producing breed used in the United States is Holstein. This breed of cattle was shipped to America from Europe in the late 1880s and has proven to be the most productive dairy breed available to farmers (Holstein Association USA, 2013). Milk

production per cow has increased dramatically with reproductive technologies accelerating the selective breeding process (Sheldon et al., 2006). A great illustration of this is the world records for milk production over the decades. In 1971, the cow holding the record for greatest milk production in one year produced 44,019 lbs of milk. By 2010, the record was 72,170 lbs of milk (Holstein Association USA, 2013). Annual milk production in the United States increases every year, even though there has been a decrease of 4 million cows over the past 40 years, leaving a total of 9 million dairy cows in the United States in 2007 (United States Department of Agriculture, 2012a). The continuous increase in overall production is due to a significant increase in milk production per cow (Figure 2).

It is clear that both dairy and beef farms rely on effective and efficient breeding practices. Dairy and beef cattle need to regularly birth calves to produce enough product to meet the demand. Genetic selection is the most useful tool that exists for producing superior generations of cattle because it allows farmers to remove poor animals from their herd, and perpetuate the characteristics of the best animals by breeding them for better herd production. Current breeding practices aim to accelerate the selection process. However, there has been a 1% decrease in conception rate per year in cattle (Sheldon et al., 2006). Farmers must combat this and still manage to remain productive and profitable.

Overview of endocrine control of reproduction in cows

All mature female cows have an estrous cycle involving hormonal control of follicular development, estrus (heat), ovulation and the development of a corpus luteum. The primordial follicles on the ovary are established in embryonic development and all have the potential to become mature oocytes (Hansel and Convey, 1983). Most of these follicles will never ovulate

but will become atretic and die. Other follicles will be recruited to grow and will ovulate at the end of estrus (Ireland et al., 1979). In addition, estrus is the period of the estrous cycle where the cow is likely to become pregnant if she is inseminated. In cattle, it normally lasts 12 to 24 hours. After ovulation, the ovaries switch from primarily estrogen production to progesterone production, and the estrous cycle begins again.

At the start of the cycle, a group of follicles are recruited to grow from the pool of primordial follicles. These primary follicles are stimulated by follicle stimulating hormone (FSH), which is produced by the anterior pituitary gland. Oocytes are surrounded by two types of steroidogenic cell, theca interna cells and granulosa cells (Hansel and Convey, 1983). The theca interna cells synthesize testosterone, which diffuses to the granulosa cells. The granulosa cells, in turn, convert testosterone to estrogen when stimulated by FSH binding to their receptors (Ireland et al., 1979). A dominant follicle will emerge among the growing follicles and will continue to grow while the others will then decrease in size. Insulin-like growth factor 1 (IGF-1) binds to receptors on the dominant follicle and increases its potential for estrogen production (Velazquez et al., 2009). As estrogen production increases, the concentration of estrogen in the blood will increase feedback on the anterior pituitary gland, and will inhibit FSH production.

At this point, the follicle acquires the receptors necessary to prepare it to receive signals for ovulation. The theca interna cells already have luteinizing hormone (LH) receptors (Hansel and Convey, 1983). The granulosa cells will lose FSH receptors and acquire LH receptors. In the first wave of dominant follicle development, the blood concentration of LH is decreased, so a spike in LH will not occur. This spike is required for final growth and ovulation of the follicle, so instead of ovulation, the follicle will undergo functional atresia (Ireland et al., 1979). The LH spike does not occur because the ovary has just ovulated and has a corpus luteum that is actively

producing progesterone. The progesterone from the luteal cells of the corpus luteum reduces the LH concentration via a negative feedback on LH production, which works by blocking estrogen-induced activation of the LH surge (Hansel and Convey, 1983). The majority of cows will not ovulate until the second or third wave (Figure 3).

After atresia, FSH is produced again and there is another recruitment of primordial follicles to the growing phase. As before, a dominant follicle is eventually selected and will produce estrogen. Estrogen prepares the reproductive tract for conception, which includes growth of the uterus and oviducts and causes the cervix to secrete more mucus (Garverick et al, 1971). The estrogen produced from the granulosa cells of the follicle circulates in the bloodstream of the animal. This hormone reaches the brain, specifically the hypothalamus, which is responsible for regulating the activity of the anterior pituitary gland. When the concentration of estrogen in the blood reaches a certain threshold, which can be detected by concentrations of estradiol in the blood greater than 5 pg/mL (Maquivar et al., 2007), the gonadotropin-releasing hormone (GnRH) surge center of the hypothalamus is activated (Zolman et al., 1974). The GnRH released from the hypothalamus travels to the anterior pituitary gland via the hypothalamo-hypophyseal portal blood system (Harris and Jacobsohn, 1952). The GnRH binds to gonadotropic cells in the anterior pituitary gland, producing a surge of LH that signals the follicle to ovulate (Hansel and Convey, 1983).

After ovulation, the follicular cells undergo a change in function due to the effects of LH. The LH binds to the granulosa and theca interna cells that have been surrounding the follicle until ovulation. It activates adenylate cyclase, which produces cyclic AMP. These events trigger a cascade that will cause a change the function of the cells (Hansel and Convey, 1983). The granulosa cells and theca interna cells become large and small luteal cells, respectively, and the

tissue becomes a structure known as the corpus luteum. These cells produce increased amounts of progesterone. This causes uterine endometrial growth and inhibits GnRH release, which is the reason that the first wave of dominant follicles does not ovulate. If a cow does not receive any signals that she is pregnant, the uterus produces prostaglandin $F_2\alpha$ ($PGF_2\alpha$), which induces luteolysis by removing the blood supply of the corpus luteum (Hansel and Convey, 1983). This is when the cycle begins again with new primordial follicles. If the cow becomes pregnant, the CL will remain functional for a longer period of time to prevent subsequent ovulations by producing progesterone (Britt et al., 1981).

For cows that are bred or inseminated in the key 24-hour period of estrus, pregnancy is likely. Cows are monotocous, which means they usually produce one calf at birth. They have a gestation period of about nine months. Calf management varies depending on if it is a beef or a dairy calf. Beef cattle calves usually stay with their mother until they are six to nine months old (McCurry-Schmidt, 2011). Dairy cattle calves, on the other hand, are allowed to consume the colostrum from their mother, and then are removed shortly after and fed milk replacer so that the milk can be collected for human consumption (Holstein Association USA, 2013).

Development of our understanding

The collective understanding of bovine reproduction by the scientific community has been closely tied to technological advancements. As the interactions of the endocrine and reproductive systems were discovered, the effectiveness of methods to manipulate cow estrous cycles improved. Other practices, like artificial insemination (AI) could be timed more closely to increase efficiency. The last half of the twentieth century saw dramatic improvement in the

reproductive efficiency of the cattle industry and the technologies available to scientists and farmers.

In the 1960s, it became possible to accurately measure steroid and peptide hormones using radioimmunoassays and competitive protein binding techniques, which could be used to study cattle estrous cycles (Hansel and Convey, 1983). In the 1970s and into the 1980s, the connections between brain endocrinology and the reproductive system were being solidified. Using the hormone assays, the cycles of estrogen, progesterone, and LH became well understood. However, follicular development was still a mystery (Britt et al., 1981). Around this time new factors were being discovered. For example, $PGF_2\alpha$ was found to be the major luteolytic factor in bovine corpus luteum (Lauderdale et al., 1974).

The major developments in the understanding of follicular development came with accessibility to ultrasonography. This allowed scientists to actually observe the growth of follicles on the ovary of a live cow over the entire estrous cycle in a minimally invasive way (Lauderdale, 2009). This gave the first insight to the several follicular waves before ovulation in cattle and helped tie the hormonal changes in the animal to physical changes in the size and activity of the ovary, and the follicles or corpus luteum present on it (Savio et al., 1988). During these advancements in knowledge, new experiments with cows were conducted to find methods to manipulate the estrous cycle to improve conception rate. Lauderdale (1974) used $PGF_2\alpha$ to regress the corpus luteum, but this method still required estrus detection by farmers. Several years later, GnRH was found to either stimulate the ovulation of large or dominant follicles or continue follicles on their path towards atresia. This advancement would lead to the approval of Cystorelin, a synthetic GnRH hormone developed by Merial, for use in cattle to treat follicular cysts by the FDA in 1986 (Lauderdale, 2009).

Artificial Insemination

Well before there was a solid understanding of bovine reproductive physiology, the practice of AI was being evaluated and implemented. By the time the practice had spread to the United States, the basics technique had been described and published in journals in Europe. Milovanov (1938) had developed semen extender for stallions and described techniques in sheep and cattle breeding. He also developed artificial vaginas to collect semen from chosen sires instead of using sponges in the vagina of a mounted animal. Walton (1933) wrote a book describing AI in livestock species and studied preservation of ram semen. Sørensen (1940) found that storing semen in straws was an excellent way to freeze it and extend sperm vitality. It was known to be more efficient to deposit semen into the cervix or uterine body as opposed to the vagina to minimize the waste of sperm. However, there were still numerous obstacles to overcome in terms of long term storage of sperm and in determining and manipulating the ideal time to inseminate a cow (Foote, 2001). These challenges would have to be addressed before AI could become a commercially widespread practice.

Cattle are an excellent species for the use of AI for of several reasons. The collection of the semen of choice bulls is relatively easy. Cow anatomy makes it easier for technicians to navigate the reproductive tract with precision. One hand is inserted into the rectum of the cow while the insemination gun is guided through the vagina. The cervix is the major obstacle that the gun must pass through to the uterine body, where semen is deposited (Foote, 2001). The cervix can be palpated and grasped through the rectal wall, which enables the technician to guide the gun through the cervix (DeJarnette and Nebel, 2011). The semen is ideally expelled in the uterine body so that equal amounts can travel up either uterine horn, maximizing the likelihood of fertilization (Moore and Thatcher, 2006). This procedure has been very successful, but the two

areas that needed the most improvement were determining an ideal method to store sperm for long periods of time and avoiding the tedious process of estrus detection, especially in a large herd.

Semen collection, evaluation, and preservation improved greatly over the twentieth century. It was determined that a cow could be fertilized with a semen sample containing 4 $x10^6$ sperm as opposed to 100 $x10^6$ sperm (Foote, 2001). Also the optimal frequency of sperm collection was found to be six times per week, resulting in 200,000 doses for insemination per year from each bull (Salisbury et al., 1978). In the 1940s, a yolk-phosphate semen extender with a sodium citrate buffer was discovered to allow sperm to survive for three days at 5℃, and in the 1950s, antibiotics were added to semen to eliminate venereal diseases from the cattle population (Foote, 2001). Before being used for AI, semen samples were now evaluated for normal, straight swimming sperm. By the end of the 1950s, liquid nitrogen was the substance of choice to freeze the straws at -196℃ (Foote, 2001). This allowed for sperm survival for long periods of time and therefore, the ability to ship semen from bulls across the country became possible.

With the AI technique perfected, and semen storage becoming a very efficient practice, the next hurdle to improve reproductive efficiency was to improve estrus detection. Since other female cows will mount a cow that is in estrus, heat indicator patches were introduced. These patches were glued to the rump of a cow and change color when pressure is applied to them, therefore showing that she will stand to be mounted, which often indicates that she is in estrus (Sheldon et al., 2006). As soon as estrus was detected, AI would be performed that evening or the following morning. This may sound simple, but can be extremely labor intensive. The individual detection and treatment of each cow was impractical for large cattle farms, especially those with several hundred females. Many farms do not have a full time AI technician working

for them, so scheduling a technician to inseminate the cows is also a struggle (Moore and Thatcher, 2006). If there could be a way to eliminate the need for estrus detection, it would make AI much more efficient.

Ovsynch: Protocol, Deviations, and Efficacy

As knowledge of the hormonal control of the bovine estrous cycle became better understood, scientists experimented with hormone treatments of cows to time estrus. The goal was to render estrus detection unnecessary and provide a method to synchronize a herd of cattle so all could be inseminated on the same day. Prostaglandin $F_2\alpha$, the hormone responsible for causing luteolysis, was one of the first hormones used. Lauderdale et al. (1974) found that pregnancy rates of cows that were inseminated at 72 and 90 hours after injection with $PGF_2\alpha$ were comparable with those inseminated after normal estrus detection. This discovery would lead to the development of Ovsynch, a series of hormone injections making timed-AI possible and estrus detection unnecessary.

The typical procedure of Ovsynch combines $PGF_2\alpha$ injections with GnRH injections and was first reported by Pursley et al. (1995). An intramuscular injection of GnRH at any point in the cycle of a cow caused the ovulation of the dominant follicle, but had no effect on a new follicular wave. Seven days later, $PGF_2\alpha$ is injected to regress any corpus luteum. Forty-eight hours later (day 9 of procedure), GnRH is injected to stimulate the ovulation of the new dominant follicle, which should by then have sufficiently grown. Twenty-four hours after that (day 10 of procedure), the cow is bred by AI (Moore and Thatcher, 2006). This eliminates estrus detection completely, which can save farmers labor, and allow them to inseminate all of their cows at the same time. Many studies have evaluated the efficacy of Ovsynch compared with

estrus detection with AI and with natural breeding. Overall, few significant differences in the pregnancy rates were observed. Ovsynch produced the same pregnancy rates as natural breeding of cows (Rabiee et al., 2005).

As Ovsynch use became more widespread, researchers continued to evaluate methods to adjust or add to the protocol to enhance results. To prevent the production and ovulation of aged follicles that yield less fertile oocytes, a Presynch treatment was created. It was determined that the Ovsynch protocol was most effective when a cow was in the early luteal phase so that the initial Ovsynch injection of GnRH does cause the ovulation of a follicle (Gordon et al., 2009). The Presynch method comprised of two injections of $PGF_2\alpha$, 14 days apart, with the second injection occurring 12 to 14 days before starting Ovsynch. There are several studies with differing results on the effectiveness of Presynch. Moreira et al. (2001) found use of Presynch protocols to increase pregnancy rates by 18% when compared with Ovsynch alone. Gordon et al. (2009) found no significant difference in pregnancy rates among the treatments, even including an experiment with an additional injection of GnRH after insemination intended to ensure ovulation and stimulate the formation of a functional CL. Bello et al. (2006) reported greater success with synchronization using a $PGF_2\alpha$ injection first, GnRH two days later, and beginning Ovsynch 6 days after that. The studies have varied results for many reasons. Some experiments only used lactating cows for the experiments (Bello et al., 2006), while others included heifers in their protocol (Gordon et al., 2009). The timing of the Presynch injections differed slightly between the experiments, and the stage of lactation when AI was attempted varied as well. All of these factors introduce a multitude of influences on the fertility of a cow, making it difficult to objectively compare the Ovsynch and Presynch treatments. Today, the use of Presynch is mostly

a personal preference decision among farmers, as available data are not conclusive on the most effective Presynch protocol.

Other protocols were developed to make estrus synchronization and timed-AI less time consuming. The Ovsynch protocol was modified to have time-AI performed concurrently with the second GnRH injection. This eliminated one day of treatment, making it more convenient for farmers to put into practice. This method is referred to as Co-Synch (Geary and Whittier, 1998). Minimizing individual treatment per cow is important, especially on larger farms. By reducing a synchronization treatment by just one injection, a cattle farm can save labor.

For effective estrus synchronization, it is important that a cow does not undergo estrus prematurely. To prevent this from happening, an intravaginal progesterone insert, called a CIDR (controlled internal drug release) was implemented (Figure 4). This device is inserted into the vagina of a cow on the first day of an Ovsynch or Co-Synch protocol, on the same day of the first injection of GnRH. It is removed seven days later, at the time of the $PGF_2\alpha$ injection (Pfizer Animal Health, 2007). While the device is inside of the cow, it consistently releases a small amount of progesterone. After insertion, plasma progesterone concentrations increase to near luteal levels (5 to 7 ng/mL) by 24 hours and then decrease to concentrations of 2 to 3 ng/mL after 2 to 3 days, where they remain until CIDR removal on day 7 (Mapletoft et al., 2003). This inhibits estrus by blocking the estradiol-induced LH release so that ovulation cannot occur (Martinez et al., 2007). Lamb et al. (2001) found that incorporating a CIDR in the Co-Synch synchronization regimen increased timed-AI conception rate by 25% in beef cattle.

Protocols using CIDR are popular because not only do they enhance synchronization among the cattle, but also they help postpartum cows to resume their estrous cycles. Small increases in progesterone occur during the natural resumption of ovulatory cycles postpartum,

and a CIDR imitates this process (Lamb et al., 2001). Saldarriaga et al. (2006) observed improved outcomes with CoSynch plus CIDR due to pretreatment with exogenous progesterone to help stimulate ovulation in a greater proportion of anestrous cows, and to synchronize follicular waves. It also can be used to accelerate the first pubertal estrus in heifers. Numerous studies have been published to refine the CIDR and Co-Synch protocols. For example, Dobbins et al. (2009) found that the second GnRH injection and timed-AI must occur at least 56 hours after the CIDR removal to maximize pregnancy rates. Lamb et al. (2001) found that a second injection of GnRH 60 hours after CIDR removal was most effective in postpartum beef cows.

Other researchers focused on using CIDR with protocols other than Co-Synch. Bartolome et al. (2009) examined dairy cow pregnancy under the Presynch/Ovsynch method with a CIDR. Interestingly, they did not find any significant differences in pregnancy rate per AI; however, they did report a decrease in pregnancy loss compared with the same treatment without a CIDR. Martinez et al. (2007) used a CIDR and an injection of 1mg of estradiol benzoate to synchronize beef cattle without any other hormone treatment. The CIDR was inserted and removed seven days later. It was determined that the shortest time possible for ovulation, that would be caused by an injection of estradiol benzoate, was 12 hours after CIDR removal. This could possibly cause a decrease in fertility by ovulating immature oocytes, so it would be safest, and most convenient for farmers, if the injection were given 24 hours after CIDR after removal. Artificial insemination should be performed at the time of the estradiol injection. In this case, estradiol has the same effect as GnRH from the Ovsynch protocol. Estradiol will activate GnRH release, and cause the LH surge for ovulation. If more research with this method shows promising results, this could be an easy, less labor-intensive method of synchronization.

Current and Future Improvements to Bovine Reproduction

There are many reproductive technologies that are not in widespread use today, but are currently being investigated and evaluated. Like AI and synchronization techniques, these practices will only become common in the commercial cattle industry after they have been refined and developed to be extremely effective and financially feasible for farmers. Other procedures will remain exclusive to research studies if practical commercial application is not appropriate. The theme continues with scientists searching for methods to decrease production costs and labor, increase the frequency of the most desirable traits in cattle herds, and learning more about the biology of bovine reproduction. The technologies that will be further discussed are superovulation and embryo transfer (ET), in vitro fertilization, sperm sexing, transgenics, cloning, and genomic selection.

It is possible to perpetuate the genes of a superior bull to many different offspring in just one year using AI. Since cows only carry one calf per pregnancy, perpetuating the desirable traits of a superior cow through her offspring is a more tedious process. To overcome this predicament, superovulation and ET are performed. Superovulation is when the ovaries are stimulated to develop many dominant follicles at once and ovulate several oocytes during estrus (Betteridge, 1977). Embryo transfer is when an embryo from one cow in inserted into the uterus of another cow for gestation. The first calf born from ET was in 1951 (Hasler, 2003). Until the early 1970s, embryo collection and ET were only possible by surgery. This was impractical because surgical facilities were needed, the recipient and the donor cow had to be synchronized in their estrous cycles, and the procedure was difficult because the udder is in the way of the reproductive tract (Hasler, 2003).

For it to be practical to perform the surgery, more than one embryo had to be collected per operation. Superovulation had been studied in other species, and it was found that pregnant mare's serum gonadotropin (PMSG) could induce superovulation in cattle. Later FSH was used to stimulate superovulation because, unlike PMSG, lactating cows were also responsive to it (Betteridge, 1977). The selected cow was artificially inseminated and her embryos were collected for ET. The surrogate mother had to be synchronized to the cycle of the donor cow and the embryos transferred. When invasive surgery was required for ET, it was mostly performed for research or for the interests of exotic cattle breeders (Hasler, 2003).

It was not until the late 1970s that non-surgical flushing for embryo collection was developed (Hasler, 2003), and in the 1980s embryos were being successfully frozen, eliminating the need to synchronize the recipient cow to the donor. The non-surgical flushing method was much more practical than surgery and produced embryos of good fertility. In a study by Elsden et al. (1974), embryos were successfully collected in 92% of cows with an average of 6.9 eggs per recovery. This demonstrates the effectiveness of superovulation. Elsden et al. (1974) noted that considerable care is necessary in searching through the flushing medium, which contains more mucus and cellular debris than medium collected from the surgical method. Finally it was possible to use the genetics from the best available bull and the best available cow to add genetically superior calves to the herd.

An extension of superovulation and ET is in vitro fertilization (IVF). Instead of artificially inseminating a cow and collecting embryos, the unfertilized oocytes are collected and inseminated in a petri dish in the laboratory. Oocytes can be collected after ovulation using the same flushing method as with embryos (Betteridge, 1977) or immature oocytes can be aspirated with a ultrasound guided needle from the ovaries themselves in a process called ovum pick-up

(OPU; Moore and Thatcher, 2006). Collection is more efficient because cows can undergo OPU twice a week, resulting in a greater number of collected oocytes. It is also different from embryo collection because oocytes can be collected from a larger population of cows, including pre-pubescent heifers and pregnant cows (Moore and Thatcher, 2006). This can increase the lifetime productivity of a cow that exhibits desirable genetic traits. Efficiency is also improved slightly because less sperm is necessary to fertilize an oocyte in vitro as opposed to AI. In vitro fertilization allows scientists to analyze the embryo before it is implanted, including determination of sex (Betteridge, 1977). This can be useful to increase the amount of female births.

Ovum pick-up and IVF has drawbacks. Betteridge (1977) reported that a 2 to 8 cell in vivo produced embryo persists much better after ET than an IVF embryo. Embryos produced from IVF have more complications ranging from increased abortions, large calf size at birth, and dystocia (Moore and Thatcher, 2006). It is still not well understood why these difficulties occur, but clearly, the medium and environment in which the oocyte is fertilized and allowed to develop interferes with normal development in some way. Pregnancies attempted by IVF have widely been reported to be successful less than 50% of the time, which is also attributed to the fact that embryos produced by IVF do not freeze nearly as well as embryos produced in vivo (Moore and Thatcher, 2006). In vitro fertilization may have important uses, but it can end up being more costly than it is worth.

Unfortunately embryo transfer by IVF or by AI is a static industry today. There have not been any significant improvements in decades (Hasler, 2003; Hesser et al., 2011). This is largely due to the fact that it is still an expensive, labor-intensive process. Also, the method of superovulation has not progressed enough to make it an economical endeavor (Table 1).

Government trading restrictions inhibit transportation of embryos, which can impede research as well (Hasler, 2003). For further advancement in this field, superovulation needs to be improved and the complications with IVF need to be researched and corrected. More recent research endeavors, like that of Monniaux et al. (2010), have shifted the focus from increasing the average number of embryos collected from donor cows to instead, selecting those cows that are able to produce much more than the average number of embryos when induced to superovulate.

It would be a great advantage to be able to choose the sex of offspring in herd management. The dairy cattle industry could benefit greatly if cows had mostly heifer calves. But, sexing embryos in vitro presents the same difficulties as IVF in general. A promising technology that has the potential to increase efficiency of the bovine reproduction industry is sexed semen. Johnson et al. (1989) developed the first machine capable of effectively sorting sperm based on their X or Y chromosome. It works on the basis that a single spermatozoa that has an X chromosome contains 3.8% more DNA than one containing a Y chromosome (Seidel, 2003). Using a DNA-binding fluorescent dye, sperm are stained and sorted by a laser in a process called flow cytometry (Moore and Thatcher, 2006). The original 'standard speed' machine was capable of sorting 350,000 sperm per hour (Johnson, 2000). This was very slow considering most straws of unsexed semen used for AI contain about 4 X 10^6 sperm (Foote, 2001). The process of sorting also decreased the fertility of the sperm, and freezing the sample after sorting decreased fertility even more. The reduced pregnancy rates made it uneconomical to use sorted sperm because with traditional AI, the same amount of female calves would be produced because the fertility rate was so much greater (Moore and Thatcher, 2006).

Despite the difficulties, Johnson (2000) is optimistic about the developing technology. He reported that the newer 'high speed' flow cytometry machine that has been developed can sort 6

million X sperm and 6 million Y sperm per hour. Dead sperm from the semen sample are discarded. If only X sperm are sorted out, sorting speeds can reach 11 million X sperm per hour at 85 to 90% purity (Johnson, 2000). The machine also causes less damage to sperm during sorting to boost fertility. During the process of sorting, each sperm is surrounded by a small amount of 0.1% bovine serum albumin liquid, which dilutes the sample when sorted, but centrifugation is performed to increase the concentration to near the original sample. This increases the chances of successful insemination, even with a reduced number of sperm (2×10^5 per insemination). Johnson (2000) reported that AI with sorted sperm results in pregnancy with the desired sex, 83% of the time.

Sexed sperm is different from IVF because it does not result in any differences in the offspring that are born. Characteristics like birth weight, mortality, abnormalities at birth, and gestation length are no different from controls (Seidel, 2003). Although using sexed sperm decreases fertility rates to 70 to 80% of rates using unsexed sperm, it has no negative effect on the mother or offspring if pregnancy does occur (Seidel, 2003). This is an extremely positive feature of sexed sperm. More research needs to be conducted for use of sexed sperm in populations of cattle other than heifers. Data would especially be useful for lactating cows and superovulated cows. As the sorting and cryopreservation technology improves, and sexed sperm becomes less expensive, it will likely become of widespread use in the cattle industry.

As comprehension of reproduction and embryonic development in mammals advanced, researchers challenged their understanding with endeavors like cloning and transgenics. Cloning in cattle has been successfully performed, but is very difficult and has a less than one percent success rate (Moore and Thatcher, 2006). The procedure involves collecting a somatic cell from the animal that is to be cloned and an oocyte from a donor cow (Figure 5). The nuclear DNA

from the oocyte is removed and is replaced with the somatic cell. They are fused and the adult cell is reprogrammed to be embryonic-like by chemical or electrical impulse. The embryo is cultured for 6 to 9 days and then transferred to a synchronized recipient. The embryo is then carried to term (Gurdon and Colman, 1999). Cloning is extremely inefficient and expensive. Complications include increased abortion rates, and since it is similar to the procedure of IVF, large offspring syndrome is prevalent (Hasler, 2003). This results in dystocia and often makes cesearan sections imperative (Moore and Thatcher, 2006).

This technology probably will not result in the production of herds of identical cattle on farms because it is financially impractical and could reduce genetic diversity. It may have specific uses for perpetuating the genomes of excellent specimens of cattle. Old, injured, or recently diseased animals that cannot contribute their gametes through traditional breeding practices or more common reproductive technologies could be cloned instead (Moore and Thatcher, 2006). Cloning also provide a means for transgenic alterations and improvements that could produce better offspring. The possibilites include improved efficiency, modified milk composition, and improved disease resistance (Hasler, 2003). Scientists may be able to inactivate the genes that cause prion disease like bovine spongiform encephalopathy, which has threatened the modern cattle industry (Gurdon and Colman, 1999). All forms of in vitro production give scientists the opportunity to genetically screen the embryo before implantation for any abnormalities or defects. A single cell can be removed from a blastocyst and be analyzed using polymerase chain reaction, karyotyping, and fluorescence in situ hybridization (Moore and Thatcher, 2006).

Transgenic technologies provide the opportunity to indroduce traits that are not found in normal breeding. Genes from other species can be incorporated into the nuclear genome of the

cow. For example, it was shown that bacterial genes that are created to be expressed in the mammary glands of cattle could make the animal resistant to mastitis (Moore and Thatcher, 2006). Some pharmaceutical companies are investigating the use of transgenic cattle to produce drugs through their milk. After the milk is collected, the drug can be purified out of solution and put on the market. However, this is a very expensive process and there is some public objection, based on perceived moral or ethical issues (Gurdon and Colman, 1999).

Because of the many complications and expenses associated with in vitro production of genetically ideal calves, it seems that genomic selection through breeding in vivo will continue to be the forefront of progress in the cattle industry. Currently, the reproductive value of bulls is assessed through the tedious process of progengy testing. A select bull must reach sexual maturity and father about 100 daughter calves. After a nine month gestation, the calves are born, but then must grow to reach sexual maturity, become pregnant and calve before they start producing milk. Finally their milk production can be measured and traced back to their father and contribute to the value of his semen (Hunt et al., 1972). Obviously this process is long, expensive, and requires an abundance of record keeping to be effective.

A reliable method of genomic selection would be a valuable addition to the cattle industry. Genomic selection involves the use of DNA markers to improve the rate of genetic gain, which is difficult because production and health is affected by many different loci of genes (Hayes et al., 2009). The entire bovine genome has been sequenced, but the function and interaction of the individual genes is not entirely known. As the genome becomes better understood, it is possible to make very accurate selection decisions based on assigned breeding values.

A scoring system using genomic breeding values continues to be developed and implemented in the United States today. Genomic breeding values are determined from a reference population of bulls that are identified as elite sires (Hayes et al. 2009). This allows the siring potential of a bull to be assessed when it is only a calf. This method has the potential to eliminate progeny testing. It reduces the generation interval in determining the value of the genetics of a bull, and is cheaper than progeny testing (Lillehammer et al., 2010). Inbreeding could be more easily managed with genomic selection. The United States and Canada lead the world in implementing the practice of genomic selection in the dairy cattle breeding programs (Table 2). Over 13,000 bulls are genotyped per year in the US, giving the country the best reference population for cattle in the dairy industry throughout the world (Smaragdov, 2013).

Conclusion

The beef and dairy cattle industries are of immense economic value in the United States and have made incredible advancements over the past century. Their success depends strongly upon the reproductive efficiency of their breeding programs. Today cattle are producing record amounts of beef and milk per animal (McCurry-Schmidt, 2011; Holstein Association USA, 2013). This is due to the advent of numerous reproductive technologies that have improved the genetics of production cattle much faster than traditional breeding ever could.

The technology that has by far had the strongest impact on bovine reproduction is AI. It reduces the amount of semen necessary to fertilize a cow, and enables one superior bull to be used to sire thousands of calves. With the great improvements in cryopreservation, the semen of a bull can be stored and shipped over long distances, helping to improve the genetic value and diversity in herds thousands of miles away (Foote, 2001). When performed correctly by a trained AI technician, AI can produce the same fertilization rates as traditional breeding (Salisbury et al.,

1978). It also eliminates the need for bulls to be present on farms, which removes management concerns of keeping a bull. Since the widespread incorporation of AI, the rate of genetic gain of American cattle has increased dramatically.

As AI became more common, and the understanding of estrous cycle endocrinology became better understood, efforts to synchronize and easily detect estrus moved to the forefront of bovine reproduction research. Different combinations of hormone injections were investigated and Ovsynch was developed (Pursley et al., 1995). This allowed for timed-AI without the need for estrus detection. Presynch, which may improve the efficacy of Ovsynch, was also created. Additionally, many programs use CIDR devices to prevent premature estrus in their hormone synchronization programs, which inhibits estrus by blocking LH release with low continuous release of progesterone (Martinez et al., 2007). Different hormones and timing schemes of the injections are still being researched to increase the efficiency of the practice by reducing the number of injections necessary and improving the accuracy of estrus synchronization and timed-AI.

There are many technologies that are in development and may soon have widespread use in the beef and dairy cattle industries. Ovum pick-up, cloning, and transgenics are all in vitro methods of reproduction. These practices can allow several cows to carry the offspring derived from the oocyte of a different cow, so that a superior cow can more efficiently transer her genetics to the next generation (Betteridge, 1977). Cloning can help to prevent old or injured animals from being lost from the gene pool if they are unable to breed. Transgenics, though publicly controversial, may help prevent cattle diseases like mastitis and enable cows to produce drugs for pharmaceutical purposes (Moore and Thatcher, 2006). These technologies are very expensive and currently are not very effective. They result in complications like large offspring

syndrome and other abnormalities that are not well understood. However, they may become more useful with further improvements.

Sexed sperm is a technology with promise as it is becoming more affordable and can be very useful to farmers. An increase in female calves would be especially useful in the dairy cattle industry. The flow cytometry method has become much faster and can now sort up to 12 million sperm per hour with 90% purity (Johnson, 2000). Unlike IVF methods of reproduction, there are no differences in the offspring at birth compared to traditional breeding and AI (Seidel, 2003). The lack of side effects on the mother or fetus makes it a much easier technology to integrate into the current market. The major limiting factor on this technology is the commercial patent held by only one company in the country, which may limit production (Seidel, 2003).

Genomic selection and genomic breeding values are quickly becoming popular. It improves efficiency by minimizing the need for progeny testing for bulls, which is a tedious process with a large generation gap before a bull can be assessed. Genetic markers are becoming better understood everyday and can predict the production value of a calf fairly accurately. A standard scoring system will have to be put into use if this method is to become more widespread. The reference population should also be reassessed on a regular basis to ensure continued accuracy of the score (Hayes et al., 2009). Genomic selection can save money compared to progeny testing and further increase the rate of genetic gain in cattle herds throughout the US and the world (Lillehammer et al., 2010).

The scientific community has made significant progress in improving the reproductive efficiency and rate of genetic gain in the cattle industry. Artificial insemination and Ovsynch programs are extremely common throughout the modern world today. It will be interesting to see

how the developing technologies like sexed sperm, transgenics, and genomic selection change the field of bovine reproduction in the coming decades.

Figures and Tables

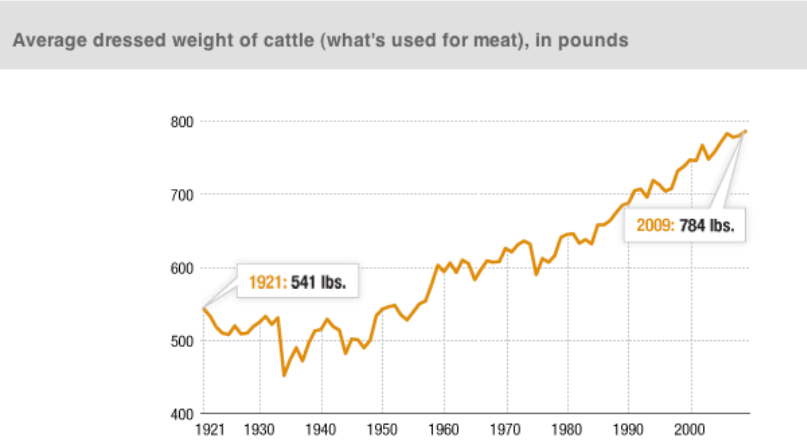

Figure 1. (Barclay, 2012)
The body weight per beef cow of usable meat after slaughter has increased significantly since 1921. This is due to improving genetics in cattle in the US.

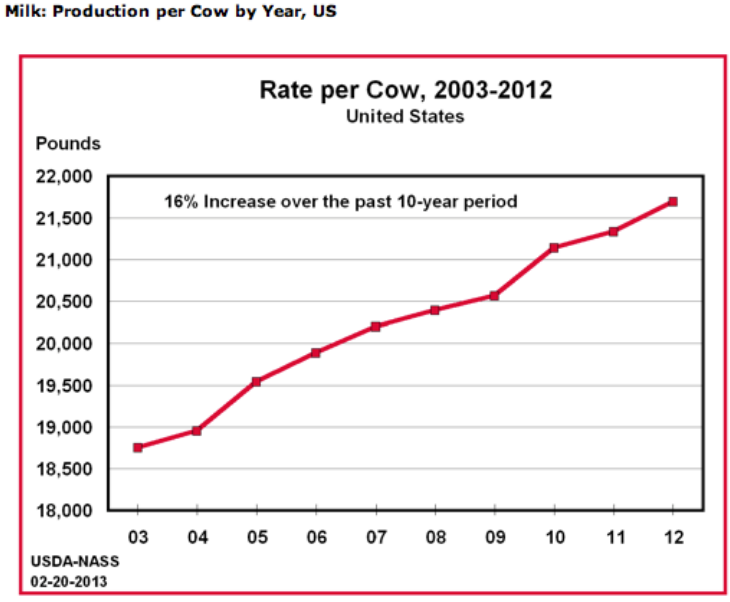

Figure 2. (United States Department of Agriculture, 2012b)
The amount of milk produced per dairy cow in the US continues to increase dramatically over the past decade.

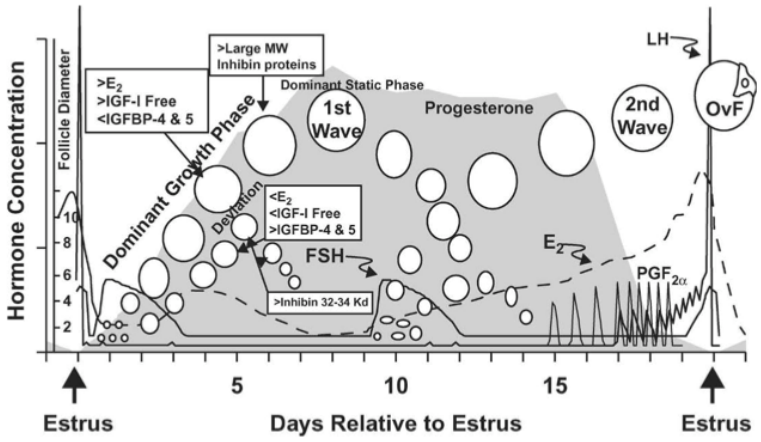

Figure 3. (Moore and Thatcher, 2006)
This picture illustrates the changing hormone concentrations over the estrous cycle of a cow. It also shows the development of follicles throughout the cycle.

Figure 4. (Pfizer Animal Health, 2007)
This is a photo of a CIDR, which is used to slowly release progesterone intravaginally in cattle. It is inserted using the blue applicator, so that it only assumes the T-shape after it has been inserted to keep the device in place. It is important to wear gloves when working with hormones because humans can be sensitive to the effects of them as well.

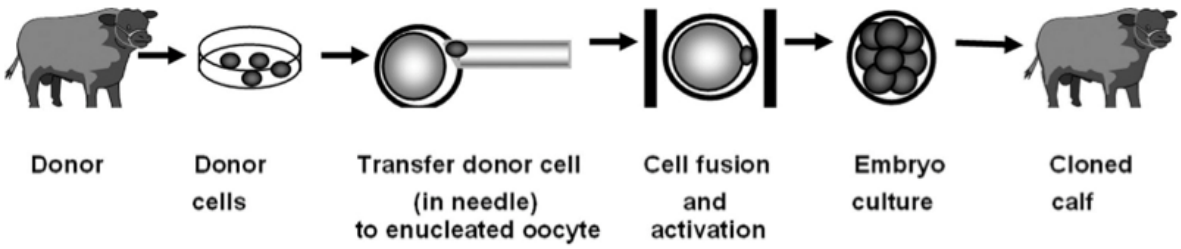

Figure 5. (Moore and Thatcher, 2006)
This is diagram illustrating the basic procedure of cloning. A somatic cell from the donor is fused with an enucleated oocyte. They are fused and activated, cultured, and implanted into a surrogate mother cow for gestation.

Year	No. of donors	% Beef	% Dairy	No. of Embryos	Mean no. of embryos/donor
1979	248	2.4	97.6	1,143	4.6
1999	1,485	69.1	30.9	7,135	4.8
2007 (worldwide)	122,000			764,000	6

Table 1. Embryos from Superovulated Donors from 1979 to 2007 (Hasler, 2003; Hesser et al., 2011)
This table shows how there has been little change at all in the average number of embryos collected from donor cows in superovulation over the nearly 30-year period. The data from 1979 and 1999 are samples from one company in the United States, while the data from 2007 is reported from the International Embryo Society Data Retrieval Committee.

Variables	Australia	Ireland	New Zealand	France	Germany	The Netherlands	Denmark, Sweden, Finland	United States, Canada
Year of GS beginning	2011	2009	2008	2009	2010	2010	2008	2008
Size of the reference population of bulls	2,247	4,500	3,600	19,377	19,377	19,377	19,377	12,152
Reliability (total merit index) %	43	54	55-60	65	65	60	55-60	62
Reliability (protein yield) %	50	61	55-60	65	72	66	63	71
Number of cows included in the reference population	10,000	No	16,000	No	0	0	0	11,473
Number of genotyped bulls per year	300	1,000	1,500	12,000-15,000	6,000	2,100	1,800	13,070
Number of bulls progeny tested	100	70	160	0	<500	140	175	2000
Age of used bulls, months	16	24	14	16	15	20	20	12
Cost relatively to traditionally valued bulls	The same	Lower	Higher	Lower	The same	The same	The same	The same
The number of bulls evaluated via GS among 20 elite bulls ranged for the country index	11	10	20	20	17	11	12	20
Percent of GS bulls in the market	No data	50	15-35	30	<30	25	45	43

Table 2. International Data on Prevalence of Genomic Selection (GS) in Dairy Cattle (Smaragdov, 2013)
This table shows data collected in 2012 and illustrates how widespread genotyping of cattle and genomic selection has become. The United States and Canada are the leaders in implementing this technology in the dairy cattle market, with the largest reference population in the world and the most genotyped bulls per year.

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