



Novel bovine embryo transfer technologies in the United States

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Abstract

In the United States, the continued promotion of quality genetics in cattle necessitates the emergence of novel technologies. One such promising technology is the utilization of genetic testing to aid in improving herd selection for a variety of traits included but not limited to milk production, fertility and disease prone animals. Additionally, these genetic markers have identified many chromosome regions containing important genes that code for financially viable traits. Therefore the ability to test animals prior to investing would financially benefit both the industry and consumer. To further promote these genetics, a relatively new procedure for superovulation using a slow release formula (SRF; hyaluronan-based solution) for Follicle Stimulating Hormone (FSH) injections has shown to be as effective in comparison to traditional methods. The SRF protocol allows for 75% less handling thus lowering stress levels for both practitioner and donor animal. The application and benefits of recombinant technology in the use of superovulatory regimes, embryo production, semen processing in addition to vitrification will play an instrumental role in the future of cattle embryo transfer.

Keywords: cattle, embryos, hyaluronan, recombinant DNA, vitrification.

Introduction

In 2007, Angus Genetics Inc® (AGI), a subsidiary of the American Angus Association, began providing services to the beef industry that would assist in the genetic evaluation of traits of economic importance. In 2008, the United States Department of Agriculture (USDA) released its first unofficial genomic evaluations and by 2009 it became official for Jerseys, Holsteins and Brown Swiss cattle. The genomic age for the advancement of cattle genetics has arrived.

The repetitious handling of animals for superovulation has long been a procedure worth modifying. The traditional method of twice daily injections of FSH is more effective than a single dose unless a vehicle can be used to slow down the release of the hormone (Tribulo *et al.*, 2011). Hyaluronan is a biological polymer that, when injected as a diluent, can facilitate a slow-release of its carrier. When hyaluronan is added to culture medium, embryo blastocyst rates were

improved; and when submitted to cryopreservation, post-thaw re-expansion and hatching rates increased (Furnus *et al.*, 1998; Stojkovic *et al.*, 2002; Lane *et al.*, 2003).

The development of recombinant DNA technology has created a plethora of uses which include but are not limited to: proteins for the treatment or diagnosis of diseases, human growth hormones, genetically modified foods and somatotropins (Hasler *et al.*, 2003; Chandramohan *et al.*, 2012; Czarnecka *et al.*, 2012; Renehan *et al.*, 2012). Researchers have established that treatment of recombinant bovine somatotropin (rBST) increases the number of antral follicles and superovulatory response in cattle (Gong *et al.*, 1993).

Cryoprotectants and cooling-warming rates are the two main factors in the ability to decrease cryoinjuries (Vajta and Nagy, 2006). Vitrification (an instant solidification of a solution brought about by an extreme elevation in viscosity during cooling without ice crystal formation) is one such method in the reduction of cryoinjuries. This new technique of cryopreservation achieves rapid freezing without the use of a freezing machine, decreases the potential for injury associated with ice crystal formation and has much to offer in the way of cryoprotecting oocytes, IVP embryos and other tissues and cells difficult to freeze (Sommerfeld and Niemann, 1999).

Cattle genomics and genetic testing

For cattle ranchers to be successful at promoting quality progeny through embryo transfer (ET), management, nutrition and genetics must all be considered. For the sake of discussion, the authors will focus on genetics. Genomics is the branch of molecular biology concerned with the structure, function, evolution and mapping of genomes (i.e. the entirety of an organism's hereditary information). In cattle, the ability to have the entire genome sequence would be instrumental in the utilization of genome wide association studies (GWAS) utilizing sequence-based technologies which include: RNA interference, single-nucleotide polymorphism (SNP) genotyping assays, methylation and gene-expression profiling, and quantitative trait loci (QTL) of economic traits through genome mapping (Pareek *et al.*, 2002; Sellner *et al.*, 2007; Veerkamp and Beerda, 2007; Jiang *et al.*, 2010; Williamson *et al.*, 2010; Vanselow and Fürbass, 2011; Yamanaka *et al.*, 2011).

In mammals, SNPs are the most common type

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of genetic variation with totals averaging more than 3.2 million for Black Angus and 3.7 million for the Holstein genome (Stothard *et al.*, 2011). Genes associated with disease can be located by using SNPs which act as biological markers. In addition, SNPs can be used to track inheritance of disease genes within families. A study utilizing SNP technology was performed in 2011 to estimate breed composition for individual breeds of cattle with the aim of providing a channel to transfer research results to the beef cattle industry (Kuehn *et al.*, 2011). Researchers collected DNA samples from 2,235 bulls (United States Meat Animal Research Center – USMARC 2000 bull project) representing 16 breeds that were genotyped using the Illumina Bovine SNP array (Illumina Inc., San Diego, CA, USA). Genotyping was successful for 52,156 markers with accuracy of marker-based breed composition between 88-89%. Capitalizing on this new technology, it is now possible to some extent to quantitate certain heritable traits such as low reproductive efficiency. Using the SNP marker assay, researchers discovered the presence of a Y-associated material in cattle with low reproductive tendencies. Confirmation was conducted using the Y chromosome PCR test used to sex embryos (Park *et al.*, 2001; McDanel *et al.*, 2012). With multiple herds across the United States exhibiting this abnormality, producers could reduce the incidence of reproductive failures with the aid of these markers. In addition, SNPs and QTLs were successfully identified in association with blastocyst and fertilization rates in bovine *in vitro* produced embryos and linked to milk production and non-return rates in heifers (Huang *et al.*, 2010; Olsen *et al.*, 2011; Pimentel *et al.*, 2011; Peñagaricano and Khatib, 2012).

The most important factor in increasing the rate of genetic improvement is shortening the generation interval. From 1980 to 2000 pregnancy rates declined 6% in dairy cattle which is equal to a 24 days increase from calving to conception (Shook, 2006). In addition, genetics is associated with approximately one third of the decline in pregnancy rates. With hormone usage becoming less acceptable, identification of genetic markers to aid in fertility will be instrumental in allowing for new management strategies. Researchers from the University of Wisconsin utilized a genome-wide search for ovulation rate QTL in two related sire families which lead to approximately 60 additional informative microsatellites to each family. With this added information along with pooled typing, a recognized ovulation rate QTL was located on the BTA14 chromosome (Arias and Kirkpatrick, 2004; Gonda *et al.*, 2004). Within the US as well as internationally, the utilization of QTL mapping to identify fertility markers have been performed on other species including plants (Buske *et al.*, 2006; Laissue *et al.*, 2009; Dou *et al.*, 2010; Pandit *et al.*, 2010; Coyral-Castel *et al.*, 2011).

Over time, bovine oocyte quantity declines with age, with reserves at calving ranging from 10,000 to 350,00 and by 12 months, an oocyte reduction of approximately 80% (Erickson, 1966; Kuhn *et al.*, 2006). Blood serum concentrations of anti-Müllerian hormone (AMH - endocrine marker of follicular population) can now be performed to help determine ovulation reserve potential in relation to fertility (Bonilla Musoles *et al.*, 2012; McDade *et al.*, 2012). With this valuable tool, young cattle with low circulating AMH could be detected prior to expensive superovulation attempts. Minitube of America (Verona, WI, USA) offers this test.

Breed composition is possibly one of the strongest genetic indicators of performance for feedlots and when combined with allele frequencies by breed can be helpful in tracking disease associated studies (Homer *et al.*, 2008). Bovine Spongiform Encephalopathy (BSE) is commonly known as mad-cow disease which is a fatal neurodegenerative disease in cattle that can infect humans if contaminated tissue is consumed. Researchers have found that BSE could be transmitted genetically thus validating the importance of genetic testing (Nicholson *et al.*, 2008). With BSE being detected in the USA and others abroad, measures using genetic profiling have been and are continuing to be developed (Heaton *et al.*, 2008; Nicholson *et al.*, 2008; Zhao *et al.*, 2009; Murdoch *et al.*, 2010, 2011). Other maladies, parasitic resistance and genetic disorders in cattle are being subjected to genetic testing however, much of this testing is based on research with little in the way of commercialization (Greenstein, 2003; Nilsen *et al.*, 2009; Allison *et al.*, 2010; Koets *et al.*, 2010; Porto Neto *et al.*, 2011; Seichter *et al.*, 2011; Hou *et al.*, 2012).

In 2009, the American Angus Association (AAA) entered into an exclusive agreement with Igenity® (Merial, a Sanofi company, www.merial.com) providing Angus breeders and their customers access to GE- EPDs powered by the high-density (HD-50K) 50 K platform. This new approach to combining EPDs with the industry's first and only 54,000-marker panel identifies 18 economically important traits. For production these traits include: calving ease, birth and weaning weight, yearling weight and height, mature weight/height, dry matter intake, residual feed intake, scrotal circumference and docility. In addition, maternal traits consisting of calving ease and milking ability are calculated with carcass traits of weight, fat thickness, ribeye area, marbling score and tenderness completing the list. Further tests are also available for: Arthrogryposis Multiplex (AM or "curly calf syndrome"), Neuropathic Hydrocephalus (NH or "water head") and coat color. Tail hair, blood or semen samples are collected and shipped for analysis. GeneMax™ (marbling and gain) and Pfizer Animal Health (GenSTAR®) are other companies working with AAA that offer deoxyribonucleic acid (DNA) trait testing. Results take 3-4 weeks and if



requested by the customer, are posted on the AAA's website (www.angus.org) which is updated weekly.

The first use of bovine embryo biopsies to determine sex was established and commercialized but was limited to gender selection only (Ellis *et al.*, 1988; Bondioli *et al.*, 1989; Shea, 1999). With recent advancements in multiple genotype analysis, small amounts of tissue (i.e., 1-5 embryo blastomeres) can be used to detect traits prior to implantation (Chrenek *et al.*, 2001; Le Bourhis *et al.*, 2011; Moghaddaszadeh-Ahrabi *et al.*, 2012). This information could be invaluable to the ET practitioner when animals of particular breeds are suspected carriers of undesirable traits.

Slow release formula (SRF) for follicle stimulating hormone (FSH)

Since the inception of superovulation, ET practitioners have sought ways to decrease the incidence of stress and possible injury to both humans and cattle when handling animals for superovulation injections. The process, however, of stimulating ovaries to produce multiple follicles requires a series of twice daily FSH injections for 4-5 days due to the half life of the hormone (Elsden *et al.*, 1976; Demoustier *et al.*, 1988). As early as 1981, researchers successfully superovulated donors by injecting 5 mg FSH once per day for 5 days using a 3.2% gelatin protein as a vehicle for FSH thus cutting animal handling in half (Looney *et al.*, 1981). However, utilizing this same protocol with only a one 50 mg injection gave similar results for the number of CL/donor while the number of viable embryos/collection was substantially less (Hill *et al.*, 1985). To determine if dosage and site of injection would alter the effect of FSH absorption, researchers concluded that a single bolus of 400 mg of NIH-FSH-P1 of Folltropin-V (Bioniche Animal Health Inc., Belleville, Ontario, Canada) given subcutaneously behind the shoulder to multiparous beef cross-bred cows with good body condition scores could yield similar results as compared to a twice-daily intramuscular (IM) treatment for 4 days (Hockley *et al.*, 1992; Bo *et al.*, 1994). Ideally, the combination of FSH with a slow-release carrier that could induce a superovulatory response over several days could have a profound impact on the ET industry. Al-gel (aluminum hydroxide gel) is beneficial as a carrier for both vaccines and hormones (Kamaraj *et al.*, 2009). One study using Al-gel in combination with 30 mg of pFSH was administered by a single injection in the muscle of Japanese black cattle and successfully induced superovulation (Kimura *et al.*, 2007). Other studies have recorded superovulatory responses in cattle, monkey, rabbits and sheep using a single administration of FSH combined with either polyvinylpyrrolidone (PVP) or hyaluronan, all of which would release its carrier slowly over a period of several days (Sugano and Shinogi,

1999; D'Alessandro *et al.*, 2001; Hashimoto *et al.*, 2007; Yang *et al.*, 2007; Tribulo *et al.*, 2011).

Hyaluronan (HA) is a biodegradable polymer of disaccharides (i.e., glycosaminoglycan) found in the mammalian uterus, connective, epithelial and neural tissues and plays an important role in cell function (Lee and Ax, 1984; Fraser, 1997; Bergqvist *et al.*, 2005). It was first discovered in the vitreous humor of bovine eyes in 1934 (Meyer and Palmer, 1934). It is a stable compound that does not cause tissue reaction when injected either into humans or animals (Neustadt *et al.*, 2005; Kaplan *et al.*, 2009). In one bovine study, a single-split protocol was designed in that the second injection of FSH would correspond with the administration of prostaglandin thus reducing animal handling (Tribulo *et al.*, 2012). Two concentrations (5 and 10 mg/ml) of HA (MAP-5, Bioniche, Inc.) were combined with 300 mg of Folltropin-V (Bioniche, Inc.) and administered in a split-single IM injection on days 4 and 6 post follicular wave emergence. No differences were noted between follicular growth profiles and transferable embryos when compared to traditional twice-daily IM injections of the equivalent concentration of FSH.

Three experiments were conducted using SRF with FSH in a single IM injection to determine the efficacy of the site of the injection, concentration of the SRF diluent and superovulatory response as compared to the traditional twice-daily injections in cattle (Tribulo *et al.*, 2011). In experiment one, FSH (400 mg NIH-FSH-P1)/SRF (10 ml at 20 mg/ml) injections were either given in the neck (IM) or subcutaneously (SC) at the base of the ear on day 4 post estradiol-17 β , progesterone and intravaginal device treatment (day 0). After artificial insemination (AI) and uterine flushing, control treatment was significantly higher in the total number of transferable embryos, fertilized ova and ova/embryo as compared to the SC group with no differences noted in the IM group as compared to the other treatment groups. Two concentrations of SRF (22 mg/ml vs. 11 mg/ml) in a single IM dose were tested with greater numbers in embryo production coming from the higher dose. To confirm the above results, a single IM injection of FSH/SRF (22 mg/ml) was compared with traditional twice-daily treatments with results showing no differences between previously discussed traits and affirming its effectiveness across superovulation and resulting variables.

A recent study was conducted on *Bos taurus* using Folltropin-V (Bioniche) reconstituted with a 0.5% hyaluronan-based SRF (Map 5, Bioniche) diluent (Hasler and Hockley, 2012). Six commercial ET programs utilizing four synchronization protocols were conducted from 2010-2011. Authors compared responses to the traditional eight twice-daily descending doses of FSH totaling 260 to 400 mg protocol (control group; n = 109) with responses to FSH in SRF totaling 67% of the total amount of FSH given to control donors on day 0 with the remaining 33% of SRF FSH given on



day 3 (treatment group; n = 103). On days 3 and 4 both groups received two injections of prostaglandin and CIDRs were removed on day 4. Donors were inseminated 12 to 24 h post-estrus and non-surgically collected 7 days later. These researchers found no differences between control and SRF treatment groups for the number of ova (14.0 ± 1.0 vs. 12.2 ± 1.0), transferrable (7.9 ± 0.8 vs. 6.8 ± 0.8), degenerate embryos (2.7 ± 0.4 vs. 2.4 ± 0.4) or unfertilized ova (3.4 ± 0.5 vs. 3.1 ± 0.5), respectively.

Another use for hyaluronan is in embryo culture (Furnus *et al.*, 1998). Hyaluronan has the ability to elevate the viscosity of media and regulate water distribution and water binding functions of the cell (Stojkovic *et al.*, 1999). Concentrations of HA are found in cumulus cells of developing oocytes and are correlated with normal embryo development thus facilitating its use in culture (Ball *et al.*, 1982; Tirone *et al.*, 1997). When used as a replacement for protein in culture media for bovine *in vitro* produced embryos, blastocyst development, total trophoctoderm and cell counts were significantly higher than for embryos cultured in media containing bovine serum albumin (BSA; (Furnus *et al.*, 1998; Stojkovic *et al.*, 2002). Additionally, these embryos when cultured in hyaluronan prior to freezing showed lower cell damage post-thawing as compared to those cultured in BSA. Interestingly, when HA was combined with recombinant albumin and citrate during culture of bovine *in vitro* fertilized (IVF) embryos, post-thawing hatching rates were significantly higher than those cultured in BSA or polyvinyl alcohol, respectively (60, 36.8, 5%; Lane *et al.*, 2003). Other uses are medically related for the treatment of osteoarthritis, ophthalmic surgical procedures, medical device coverings (in accordance to ASTM F2347 Medical Device Standards and Implant Standards; www.astm.org) and veterinary medicine (Dick *et al.*, 1999; Dougados, 2000; Cribb *et al.*, 2009).

Recombinant technology

Recombinant DNA (rDNA) refers to the process whereby DNA from one organism is placed into the genome of a different organism where the resulting DNA copied is isolated and then analyzed for the intended construct. Scientists are now able to isolate genes, determine nucleotide sequence, evaluate transcripts, transform and then reinsert the changed sequence into a living organism. Essentially every area of biological research has been affected by the use of rDNA technology.

Superovulatory responses in cattle are known to be highly variable. In order to optimize conditions to obtain a maximal response, a gonadotropic preparation with consistent biological activity must be available. Commercial preparations of pituitary-derived gonadotropins exhibit tremendous lot-to-lot variation. This may be the result of seasonal changes in donor

animal pituitary gonadotropic content and/or biological activity, as well as variations in purification procedures. Recombinant hormones could reduce such instability in product variation as studied by this author previously (Looney and Bondioli, 1988).

Within the ovary, high concentrations of IGF1 (Insulin-like Growth Factor-1) can be found in the follicle (Spicer and Chamberlain, 2000). The synthesis of IGF1 is stimulated by somatotropin in both humans and animals thus increasing the potential for superovulation (Homburg *et al.*, 1990; Gong *et al.*, 1996a). Recombinant bovine somatotropin (rBST) when given in conjunction with pFSH in Holstein heifers twice-daily over a period of 4 days gave similar results to control groups not receiving rBST for total number of embryos and transferable embryos collected, respectively (8.3 ± 5.3 and 7.2 ± 6.6 ; 5.3 ± 4.0 and 5.2 ± 4.5 ; Rieger *et al.*, 1991). The superovulatory response was improved by the co-treatment of rBST with a significant increase of progesterone activity as compared to controls. Further studies in mature beef heifers determined that the effects of rBST could be detected within 3 days of the initial injection with an increase in population of small follicles in all stages of the estrus cycle without inhibiting the effect of the dominant follicle on its secondary follicles (Gong *et al.*, 1993). Increased levels of peripheral IGF1 and insulin were detected 48 h post-treatment correlating to the increase in the number of small follicles which occurred 24 h later verifying a link between rBST and increased follicular population.

Researchers looked at the effect of pre-treating heifers with a single SC dose of rBST (320 mg in a sustained release formula) 7 days post-estrus (Gong *et al.*, 1996b). Five days later, FSH was administered over 4 days at twice daily injections. Following non-surgical collections, number of ovulations, total number of ova/embryos recovered and number of transferable embryos were all significantly increased. Additionally, poor ovulatory responses and the occurrence of follicular cysts were reduced in animals receiving rBST.

With concerns emanating from the use of animal byproducts, recombinant technology is now playing an important role in the production of embryo/semen media. Embryos collected for export can now be treated with recombinant trypsin (Trypzean™, Sigma-Aldrich Corp., St Louis, MO, USA) which has shown to be as effective as the animal derived 0.25% trypsin for inactivating bovine herpes virus-1 (Seidel *et al.*, 2006). TrypLE™ Select (Invitrogen, Carlsbad, CA, USA) another recombinant trypsin source was also used on *in vivo* produced embryos and proved effective in removing BHV-1 (Marley *et al.*, 2005). Trypsin was originally designed as a disinfectant to eliminate pathogens and bacteria from embryos (Stringfellow, 1998). In a preliminary IVF study, semen was washed through a density gradient product (BoviPure™, Nidacon, Sweden) treated with recombinant trypsin



(untested for virus inactivation for this study) resulting in no differences between controls for cleavage rates and embryo development (Aaltonen *et al.*, 2008). Further experiments utilizing BoviPure™ in combination with recombinant trypsin on freshly collected *Bos indicus* semen resulted in significantly higher fertilization rates and transferable embryos post-AI and collection as compared to controls, respectively (88.4 vs. 63.1% and 70.3 vs. 51.8%; Blevins *et al.*, 2008). When human semen was exposed to a similar soy-based trypsin/gradient treatment, the infection potential of the pathogen was reduced to below levels of clinical relevance (Loskutoff *et al.*, 2005). In addition, the same human study used a side-by-side comparison to test fertilization capabilities in *Bos taurus* semen for IVF and found a significant increase in development rates as compared to controls, respectively (41.8 vs. 16.7%). Theoretically, frozen/thawed semen of low concentration may potentially have better fertilization rates when submitted to recombinant trypsin.

The identification of heparin-binding proteins (HBP) can be used as a diagnostic indicator for bull fertility (McCaughey *et al.*, 2001). Elevated HBP found in seminal plasma act to enhance fertility in bulls (Bellin *et al.*, 1996). One study examined the use of recombinant HBP (i.e., fertility-associated antigen (FAA) and Type-2 tissue inhibitor of metalloproteinase (TIMP-2) on sexed sorted semen for IVF. Significant differences were noted between control and treatment group for cleavage rates (61.4 and 83.7%) with development rates not affected. Thus the addition of HBP to sexed sorted semen could increase fertility competence.

Vitrification

Traditionally, embryos were cryopreserved in dimethylsulfoxide (DMSO) utilizing a step-wise procedure (Wilmot and Rowson, 1973). Glycerol was then introduced as a new cryoprotectant agent and was successfully implemented into bovine embryo transfer (Bilton and Moore, 1977). In order to transfer embryos cryopreserved in glycerol, the cryoprotectant must be removed in a step-wise manner. This is an effective way to cryopreserve embryos; however, it can prove to be more challenging in a field setting. Recently, the use of ethylene glycol as a cryoprotectant agent has been widely accepted. This method of cryopreservation allows for direct transfer of the embryo within the straw just minutes post-thaw (Voelkel and Hu, 1992).

Vitrification is an alternative route to traditionally freezing embryos (van Wagendonk-de Leeuw *et al.*, 1997). This form of freezing utilizes high levels of cryoprotectant (3.4 M or higher), multiple cryoprotectants, rapid cooling rates and small volumes (Campos-Chillón *et al.*, 2009). The most common cryoprotectants used in combination for vitrification are glycerol, 1,2 propanediol, polyethylene glycol and ethylene glycol. This process requires direct contact of

the cryoprotectant with a pre-cooled metal block, liquid nitrogen or liquid nitrogen slush. In combination, these techniques bypass the crystalline ice phase and produce a glass-like state within the embryo (Fahning and Garcia, 1992).

The utilization of vitrification in a field setting has been validated by researchers (Massip *et al.*, 1987; van Wagendonk-de Leeuw *et al.*, 1997). Research has reported no differences in pregnancy rates when bovine embryos were frozen by either a slow-cooling protocol or by vitrification (Vajta, 2000). In cattle, *in vitro* studies have been conducted comparing vitrification to slow-cool freezing protocols (van Wagendonk-de Leeuw *et al.*, 1997; Campos-Chillón *et al.*, 2006). In comparison, researchers have reported no differences between hatching rates when bovine embryos were subjected to either a slow freezing protocol (45.1%) or vitrification (44.5%; van Wagendonk-de Leeuw *et al.*, 1997). Pregnancy rates from frozen Brahman *in vivo* derived embryos can be improved by vitrification when compared with conventional direct transfer (Pryor *et al.*, 2007). Embryos were frozen/vitrified utilizing 0.25 ml straws for in straw direct transfer post thaw/warming. The ease of this technique decreased handling time post-thaw/warming, eliminated microscope use and expedited transfers.

The packaging method for embryo subjected to vitrification is an issue of interest. The use of cryotops, cryoloops, cryoleafs, open pulled straws and closed pulled straws are currently used as packaging methods (Vajta, 2000; Saragusty and Arav, 2011). The extremely high freezing curve of the packaging system requires that the surface area be smaller when compared with traditional freezing methods. One study suggests that the open pulled straw method of packaging when utilizing in conjunction with vitrification results in equivalent hatching rates to control freezing (Vajta *et al.*, 1998). A recent study comparing conventional freezing *versus* vitrified bovine IVF embryos yielded similar hatching results ($61.1 \pm 29.3\%$, $63.2 \pm 24.5\%$, respectively) with real-time PCR indicating a significant difference between 4 of 8 developmental gene transcripts thus indicating vitrification to be a more suitable method of cryopreservation (Stinshoff *et al.*, 2011). Vitrification with its potential to be equal to or better than conventional freezing, will have to be modified to better suit field condition status (i.e., make procedure less time consuming and more applicable for direct transfer) before its acceptance within the cattle industry.

Conclusions

The access and availability of all breed genomics will one day become a reality. Identifying genetic markers for disease prone carriers, embryo blastocyst and fertilization rates, milk production and nonreturn rates in heifers are just the beginning of what



this new approach to genetic management can do. Hyaluronan has shown to be effective in its ability to slow-release drugs when administered via injection. Other applications also indicate its usefulness as a protein replacement in embryo culture systems. The use of recombinant therapy for superovulation in cattle has yielded positive results in many variables studied. Its efficacy in inactivating viruses and enhancing fertilization rates continue to aid the ET industry in promoting a higher level of healthy genetics. New methods of cryopreservation that can provide an accurate fast and economical approach are being researched. The use of transgenic, nuclear transfer and IVF techniques are requiring a more protocol-specific approach to cryopreservation to yield similar if not higher results as compared to conventional methods. The utilization of vitrification may be the answer to this approach. The utilization of these novel approaches in combination with good management and nutritional practices in cattle will enable embryo transfer practitioners the ability to promote a more desirable product. Not covered in this review but undoubtedly an up and coming futuristic approach to further promote embryo production is the use of stem cell and RNA interference technology.

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