THE 'ART' OF DELIVERING GENOMICS TO THE BEEF HERD

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Introduction

Genomic selection enables animal breeders to select animals of greater genetic merit with higher accuracy and at a younger age as compared to traditional breeding strategies, therefore achieving increased selection precision and decreased generation intervals. By combining genomic selection with assisted reproductive technologies (ART), it is possible to further reduce the generation interval by selecting animals while they are embryos or by the breeding of prepubertal animals. Furthermore, ART allow the maximization of selection intensity, allowing for the use of fewer animals contributing their desirable genetic traits to the next generation. Therefore, the combination of genomic selection and ART offers great potential to accelerate genetic progress. Here we present how different ART can be combined with genomic selection.

Fixed Timed Artificial Insemination

While cattle artificial insemination (AI) has been possible for more than 60 years, the widespread adoption of this technology has been slow. Given the increasing global demand for meat production and the advancement of genomic trait selection via DNA markers, there is an increase for the need and use of AI. Genomic selection allows the use of younger "genomic bulls", diminishing the male selection timeline by approximately one year of age as compared to a minimum of 4.5 years when using progeny-tested bulls (Schefers and Weigel, 2012). Female genotyping can also be used to identify elite heifers and increase selection pressure. The potential of genomic selection allows the improvement of bovine breeding programs and builds toward the shortening of the generation interval and a faster genetic gain.

AI programs require estrus detection and often utilize estrous synchronization strategies that make them labor intensive. Prostaglandin F2 alpha (PGF2α) is the most commonly used treatment for synchronization of estrus in cattle, but estrus is distributed over a six day period and is influenced by the response of the corpus luteum and the developmental stage of the dominant follicle at the time of PGF2α administration (Macmillan and Henderson, 1984). The strong variability in estrus response and pregnancy rate after PGF2α treatment led to the development of treatments that control follicular and luteal development. New estrous synchronization protocols were designed to control follicular wave dynamics, regress the corpus luteum, and induce ovulation of a dominant follicle with the objective to synchronize ovulation among a group of animals at various stages of the estrus cycle at the beginning of the program to allow fixed time artificial insemination (FTAI) (reviewed by Bo et al., 2003). Performing FTAI reduces the time and labor required for the detection of estrus and allows animals to be managed in groups rather than individually. Several hormonal treatments are available for performing FTAI. One of the most popular is the Ovsynch protocol that was developed to synchronize ovulation in dairy cattle and consists of a gonadotropin releasing hormone (GnRH) to synchronize follicular waves, a dose of PGF2α given seven days later to induce luteolysis, and a dose of GnRH given 36 to 48 hours after the PGF2α administration to induce ovulation at a predetermined time. AI is performed 16 to 24 hours after the second GnRH administration (Pursley et al., 1997). Since it was observed that estradiol treatment suppresses antral follicular growth, estradiol and progesterone treatments have been increasingly used during the past years

for FTAI programs in cattle. Currently, the use of intravaginal progesterone releasing devices and estradiol benzoate (EB) is one of the most popular treatments around the world for FTAI in beef and dairy cattle (Bo et al., 2003). However, estradiol is not an approved drug for estrus synchronization in the United States, so several GnRH and progesterone-based protocols were designed (e.g. Co-Synch and Co-Synch+CIDR).

The beef industry has also benefitted from the combination of using timed AI programs with sexed semen (Humblot et al., 2010). Despite the lower conception rates and higher costs, sexed semen is worthwhile when the production is gender specific. In this way, it is possible to produce specialized and genetically superior replacement heifers and then use the rest of the cows to produce male calves with sexed semen from bulls with superior genetic merit for growth, feed efficiency and carcass merit.

Multiple Ovulation and Embryo Transfer

Increasing the number of candidates submitted to genomic selection procedures to maximize the chances of obtaining interesting individuals that will be positively evaluated for a large number of traits is one of the main goals of genomic selection and could be achieved by the use of embryo-based biotechnologies (Humblot et al., 2010). Since the use of AI alone may not be enough to generate sufficient animals in a given period of time, the multiple ovulation and embryo transfer (MOET) strategy has become a more viable alternative. One of the main advantages of this is approach is its ability to increase both the selection intensity on the female side and the reproductive rates of valuable cows by tenfold on average or more in a year, and fivefold or more per lifetime as compared to AI. Although embryo transfer techniques are widely used around the world, with more than 500,000 bovine embryos being transferred each year, the variability in response to superovulatory treatment remains the main drawback of this technique (Bo et al., 2006). As seen with the FTAI programs, the recently acquired better understanding of ovarian function allowed for the development of protocols that control follicular dynamics and ovulation. The most common approaches consist of the synchronization of a follicular wave emergence and the administration of several doses of follicle stimulating hormone (FSH) to induce superovulation. Several studies showed that starting the superovulatory treatment without a dominant follicle increases the donor response. The use of embryo biotechnologies permits an intensive production with a reduced number of elite females, increasing the selection intensity on cows. On the other hand, MOET programs could increase the rate of inbreeding due to repeated embryo production sessions with a reduced pool of animals. However, the use of female genotyping also provides an important tool for the generation of genomic selection-assisted mating plans to avoid genetic defects from mating bull and cow carriers of deleterious recessive alleles and to control the level of inbreeding (reviewed by Ponsart et al., 2014).

Ovum Pick-Up and *In Vitro* Fertilization

In vitro embryo production (IVP) is an important tool for increasing the number of embryos produced over a determined period of time (Humblot et al., 2010). Ovum-pick up (OPU), also referred to as transvaginal oocyte retrieval or oocyte collection, consists of the removal of oocytes by ultrasonography and a vacuum aspiration system from donor females for IVP. Oocyte collection can be repeated once a week without risk to animal health or reproductive activity and can be performed throughout the female's reproductive life. This allows a great number of oocytes, and therefore embryos, to be collected from a single animal. Among the advantages of OPU/IVF is that the technique can be applied to a female in a variety of physiological states,

including prepubertal animals, pregnant animals, and castrated animals (i.e. ovaries removed at castration). In all these cases, MOET cannot be performed. OPU/IVF is often used in animals that do not respond well to superovulation treatments because of genital tract defects cannot produce embryos collectable in the uterus. Further, IVF permits generation of embryos from recently deceased animals, allowing preservation of valuable genetic material when unexpected circumstances arise.

After oocyte collection, *in vitro* maturation is performed and metaphase II oocytes can be fertilized. *In vitro* fertilization is then performed and consists of incubating metaphase II oocytes with sperm in a petri dish. Bulls of high genetic merit could be selected and their sperm used for IVF, increasing the efficiency of the genetic selection process. Because IVF occurs in a small volume of culture medium, the use of semen can be greatly optimized. Typically, 20 to 30 oocytes are fertilized in 50 microliters of medium with a sperm concentration of 1 million spermatozoa/mL; or the equivalent of 2,000 sperm/oocyte. Therefore, a frozen straw of sexed-semen, containing approximately 2 million sperm (of which about 1 million would be appropriate for IVF after thawing and purification) should be enough to inseminate approximately 500 oocytes. This would allow production of 150-200 transferable embryos and 50-100 calves (of a desired sex) from a single semen straw. This efficiency contrasts greatly with that of semen utilization during AI, at about 0.6 calves per straw, or that of MOET at 1-2 calves per straw, and justifies the use of top (expensive) bull genetics in most IVF programs. *In vitro* culture is the last step of the process and much research has been done to mimic the oviductal fluid environment. Synthetic oviductal fluid (SOF)-based culture systems are the most commonly used and the average development rate to the blastocyst stage ranges from 30 to 40% for most laboratories. The use of OPU-IVP leads to the production of approximately 70 calves per donor per year.

Even though OPU-IVP presents additional advantages compared with the MOET programs, still there is room for improvement. Genomic selection provides new tools for improving the efficiency of IVP program by selecting female donors and bulls with proven efficiency in *in vitro* production. SNP associated with number of viable oocytes, as well as fertilization, cleavage and development rates, have been determined by different research groups (reviewed by Ponsart et al., 2014). The development of all these technologies makes IVP more available and efficient for the beef cattle industry.

Advanced Embryo-based Biotechnologies

OPU-IVP can be combined with other advanced reproductive technologies to reduce the generation interval by producing high genetic merit calves in a shorter period of time. Embryo genotyping constitutes one of the advanced reproductive tools for embryonic selection and is performed by embryo biopsy. An important aspect of this procedure is the balance between removing a small number of blastomeres in order to preserve viability while still sampling a sufficient quantity of DNA for further analysis. Embryo biopsies require highly skilled operators and micromanipulation equipment. Depending on the embryo stage, microblade, aspiration or needle biopsy techniques can be used (Cenariu et al., 2012). When a biopsy is performed, 5-15 cells are removed and used for DNA amplification and genotyping. Embryos can then be either cultured *in vitro* for another 3-48 hours and transferred into synchronized recipients, or frozen. Pregnancy rates range from 31% to 62.3% after direct transfer of frozen biopsied embryos.

Recently, more intensive methods combining OPU-IVF and cloning were used to accelerate genetic progress (Kasinathan et al., 2015). Transfer of 5-8 embryos to a single recipient allowed for the efficient production of early embryos (24 days of gestation), from which cell lines could be efficiently established. From these cultured cells, DNA was isolated and genotyped. The best cell line, selected based on the genomic performance index value, was selected to produce offspring. These cells were used for somatic cell nuclear transfer (SCNT) cloning, producing multiple calves (Kasinathan et al., 2015).

This report shows how genetic selection combined with advanced reproductive technologies is an approach that substantially reduces the generation interval while producing high genetic merit calves by SCNT with efficiency comparable to that of IVF embryos. Generation interval is reduced by approximately 7 months and the method offers the possibility to produce multiple animals simultaneously while pre-implantation stage embryo biopsy permits the production of only one animal. Even more intensive approaches could be envisioned in which combinations of IVP, derivation of embryonic stem cells (ESC), genomic selection of elite genotypes, and generation of gametes and embryos from top genomic ESCs could greatly reduce the generation interval by achieving a completely *in vitro* breeding scheme, which could be repeated until the genetic gain is maximized and animals are produced. However, such an approach cannot be readily applied as the generation of functional gametes from ESC has not been possible solely using *in vitro* procedures. That being said, genomic technologies combined with ART still offer great opportunities for beef herd genetic improvement and open the way for developing intensive schemes for rapid genetic progress.

References

Bo G.A., Barusseli P.S., Martinez M.F. 2003. Pattern of follicular development in Bos indicus cattle. Animal Reproduction Science;78:307-26.

Bo G.A., Baruselli P.S., Chesta P.M., Martins C.M. 2006. The timing of ovulation and insemination schedules in superstimulated cattle. Theriogenology;65:89-101.

Cassell, B. 2012. Impact of genomics on dairy cattle breeding. Proc. 2012 Virginia Tech Cow College.

Cenariu M., Pall E., Cernea C., Groza I. 2012. Evaluation of bovine embryo biopsy techniques according to their ability to preserve embryo viability. Journal Biomedicine Biotechnology. Article ID 541384. doi:10.1155/2012/541384

Humblot P., Le Bourhis D., Fritz S., Colleau J.J., Gonzalez C., Joly C.G., Malafosse A., Heyman Y., Amigues Y., Tissier M., Ponsart C. 2010. Reproductive Technologies and Genomic Selection in Cattle. SAGE-Hindawi Access to Research Veterinary Medicine International.

Kasinathan P., Wei H., Xiang T., Molina J.A., Metzger J., Broek D., Kasinathan S., Faber D.C., Allan M.F. 2015. Acceleration of genetic gain in cattle by reduction of generation interval. Scientific Reports;5:8674.

Macmillan K.L., Henderson H.V. 1984. Analysis of the variation in the interval from an injection of prostaglandin F2 to oestrus as a method of studying patterns of follicle development during dioestrus in dairy cows. Animal Reproduction Science;6:245-54.

Ponsart C., Le Bourhis D., Knijn H., Fritz S., Guyader-Joly C., Otter T., Lacaze S., Charreaux F., Schibler L., Dupassieux D., Mullaart E. 2014. Reproductive technologies and genomic selection in dairy cattle. Reproduction, Fertility and Development;26:12-21.

Pursley J.R., Kosorok M.R., Wiltbank M.C. 1997. Reproductive management of lactating dairy cows using synchronization of ovulation. Journal Dairy Science;80:301-6.

Schaeffer L. 206 Strategy for applying genome-wide selection in dairy cattle. J Anim Breed Genet;123:218-23.

Schefers JM, Weigel KA. 2012. Genomic selection in dairy cattle: Integration of DNA testing into breeding programs. Animal Frontiers;2:4-9.