In-Vitro Fertilization in Cattle

Josh Vowell
Mississippi State University
College of Veterinary Medicine
Class of 2021
Clinicopathologic Conference
September 4, 2020

Advisors: Kevin Walters DVM, DACT; Cari Chisholm DVM

Introduction

In-vitro fertilization was first successful with humans in 1978 and then in cattle that exhibited infertility in 1981. The development of in-vitro fertilization in cattle was focused on improving genetic selection and decreasing the generation interval. By recovering ovaries from slaughterhouses and using frozen semen, a powerful research model was established. These resources provided 40 years of clinical application and research on the process for humans as well as cattle[7]. In-vitro fertilization was first successful in humans in 1978 by obtaining oocytes using a laparoscopic procedure. This was the initial effort in obtaining mature bovine oocytes. The first IVF calf was produced in 1981 by using a ventral midline laparotomy on a donor female to retrieve the oocyte and an additional laparotomy on the recipient female to deposit the embryo into the oviduct. After four years of research and clinical trials, a laparoscopic procedure was developed for the use of IVF in cattle. The oocytes were recovered laparoscopically and incubated in a rabbits' oviduct for seven days. The embryos were then surgically transferred to the bovine oviduct. Laparoscopy remained the preferred method for harvesting oocytes in cattle for ten years until it was replaced by ultrasound around 1990[7].

What is IVF?

In-vitro fertilization is the process of producing embryos from oocytes that are fertilized outside the animal in a petri dish in a bio-secure, climate-controlled environment. The oocytes are recovered by ultrasound-guided aspiration of the follicle on the ovary. The ultrasound is inserted into a specially designed probe that allows maximum visualization of the ovary during aspiration. The ovary is located via rectal palpation of the reproductive tract. The probe is inserted vaginally and is advanced to where the transducer contacts the ovary through the vaginal wall. Along the dorsal border of the probe, a guide is attached to a needle. Using the ultrasound as a visual aid, the needle will advance through the vaginal wall into the ovary and is inserted into the fluid filled follicles that contain oocytes. The content of the follicles is aspirated with a vacuum pump that maintains a negative pressure. The pump is

connected to the aspirating needle with medical grade teflon tubing, and with negative pressure, follicular fluid is aspirated into a sterile filtered container. The filter will separate the oocytes from the unwanted fluid to allow for sorting and grading of the oocytes. The container is taken to the lab where it is rinsed multiple times with a holding media that is supportive to the oocyte, and the fluid is then searched and oocytes are sorted and graded with a dissecting microscope. Once the oocytes are sorted, they are counted and given a grade. The sufficient oocytes are moved to a petri dish with a media that is designed to promote maturation. After 18-24 hours of incubation, the oocytes are fertilized, and the embryos are placed back into the incubator for an additional seven days. The incubator and media are designed to be a compatible environment with the bovine oviduct. Once the seven-day maturation cycle is complete, the viable embryos are evaluated and graded. The grade one, two, and three embryos may be transferred freshly into a prepared recipient, however only the grade one and two embryos will be frozen for transfer at a later date[1,4].

What is a donor?

When using advanced reproductive protocols, it requires a donor and a recipient. The donor female is one that is selected due to her superior genetics, and her lineage is desired to maintain. Desirable genetics may include high milking percentages, increased weaning weights, and desired calving ease in association with more heritable traits such as structure, longevity, and carcass quality. Additional selection of donor females includes females that no longer respond to conventional ways of reproduction due to age or other factors[2,4].

What is a recip?

A recipient female, or recip, is an animal that will carry a donor female's embryo. A recip female should be docile, have appropriate udder conformation, produce adequate milk, and possess excellent structure as these are traits that are vital to the viability of the embryo's survival and maturation[2,4].

Quantifying quality – EPD's

For analysis and accurate assessment of a mating, a select number of desired heritable characteristics have been quantified into what is called an expected progeny difference, EPD. An expected progeny difference is a predicted, computed number that validates an animal's merit based off its prior offspring's performance. The accuracy of an EPD is positively correlated with the number of offspring produced by a sire or dam. To make adequate breeding decisions, it is necessary for producers to have this information and provide it to breed registries. Misleading information or unreported information may result in poor mating and a decrease in productivity[3]. This can happen in young animals or animals that are sold and offspring information is not reported.

History and Presentation

23Y1 is a 4-year-old Brangus female that was strategically selected to be a donor female. The owner of MacMahon Ranch has a highly progressive Brangus ranch of approximately 100 head of producing cows. He uses Brangus bulls on a portion of his Brangus cows to maintain a registered group for extension of their genetics. Simmental and Angus bulls are used on the remainder of the herd as terminal crosses for meat production and to develop recipient cows for embryo transfer. He selected 23Y1 as a donor female for her good structure qualities, docility, and carcass merit. These traits will increase her calves' marketability as they are most valuable to feedlot and calf developing operations.

Procedure

On the morning of April 29, 2020, 23Y1 presented at Pine Belt Veterinary Clinic in Hattiesburg, Mississippi for follicular aspiration and ovum pick-up. She was placed into a Flying W hydraulic squeeze chute where she was restrained for the procedure. Using betadine, followed by an alcohol swab, her tail head was cleaned, and a caudal epidural was performed using 6 mls of 2% lidocaine. The entire perineal area was gently scrubbed using Ivory soap, rinsed, and dried to remove all debris. The preferred method of ovum pick-up is now via ultrasound guided aspiration through the vaginal wall. A 20-gauge, 2.5-inch

needle was attached to a needle guide and was inserted into the transvaginal probe. Medical grade teflon tubing was attached to the opposing end of the needle guide and was connected to a sterile collecting cylinder containing a filter and 2 mls of oocyte culture media. The collecting cylinder was additionally connected to a vacuum pump that facilitates follicular aspiration by maintaining negative pressure (60-75 mmHg) until the procedure is complete. Negative pressure was checked by inserting the aspirating needle into an additional sterile cylinder containing sterile oocyte culture media. A sterile covering was then placed over the wand which houses the transvaginal probe of the ultrasound and needle guide. Sterile lube was applied to the transducer to decrease friction and increase contact. With rectal palpation, the ovary was isolated. The labia were then separated, and the transvaginal probe was inserted at a 45-degree angle and then raised until parallel with the reproductive tract. The probe was advanced until a clear image of the ovary was made through the vaginal wall. Once the follicles were visualized via ultrasound, using the needle guide, the needle was advanced through the sterile probe covering, through the vaginal wall, and into the follicle, aspirating the fluid containing the oocyte. Once the aspiration process was completed on the right ovary, the process was repeated on the left ovary. After the aspiration process achieved a satisfactory status, the sterile collecting cylinder was carried to the lab for searching and sorting of viable oocytes. The fluid in the collecting cylinder was transported to a sterile beaker with a filter containing ABT holding media which supports cellular metabolism. The collecting cylinder was thoroughly rinsed to ensure all oocytes were retrieved. The filter in the sterile beaker was then removed and rinsed, using ABT holding media, into a petri dish. The petri dish was then placed under a dissecting microscope, and the oocytes were sorted, graded and then collected and transported to sterile cylinders that were labeled with a grade 1,2, or 3. The graded oocytes were placed in their corresponding sterile cylinders containing ABT holding media. The sterile cylinders containing the graded oocytes were packaged into a thermos that maintains a consistent temperature compatible

to the bovine oviduct and were shipped the same day as follicle aspiration. Forty total oocytes were aspirated from 23Y1, and 39 oocytes were of quality to ship to Transova for fertilization.

Physiology

Oocyte Maturation

Oocytes are initially graded by the degree of competent cumulus cells that are surrounding the oocyte. Oocytes with a thicker and healthier cumulus have increased chances of survival and fertilization. Cumulus cells are a sub-population of granulosa cells with a clear purpose of providing nutrients to the oocyte during maturation, aid in forming the zona, and synthesize the protein and hyaluronic acid matrix after the LH surge which is important for oviductal transport for fertilization. Cumulus size and integrity vary regarding the size and health of the follicle. When using in-vitro fertilization, exogenous ovarian stimulation leading to super-ovulation is not required although it can be utilized. Follicle stimulating hormone is an exogenous hormone product that facilitates super-ovulation in the ovary, increases follicle size, and assists in maturation of the oocyte in-vivo. Research shows that a larger follicle contains an oocyte that has a higher potential of becoming an embryo. Despite the larger follicles leading to increased production of embryos, the development rate per oocyte retrieved was not improved by follicle stimulating hormone administration[6].

Breeding with conventional semen vs. sexed semen

Reverse-sorted semen is defined as sorting or sexing semen after it has been frozen and then thawed. When comparing the success rate between conventional semen and reverse-sorted sexed semen, the results are similar. However, success rates are decreased when using semen that was sorted or sexed prior to freezing. Sexed semen is desired when a heifer calf crop will deem most productive as such in a dairy setting[4,5]. Studies show that an average pregnancy rate using reverse-sorted semen and in-vitro fertilization is 41%. Similar statistics were reported using semen that is sexed prior to freezing,

which was a 39% pregnancy using in-vitro fertilization. Other studies have shown a 3-4% difference in pregnancy rates when using reverse sorted semen and sexed semen prior to freezing[5].

IVF vs. Conventional Flush

When utilizing in-vitro fertilization there is no hormonal stimulation required as ovulation is not a priority to be achieved. Oocytes may be recovered in two-week intervals as long as the donor female is deemed healthy. With each aspiration having similar results with mild variation, 15-18 oocytes are generally the average. This number is subject to change regarding the donor female's health status, age, and nutrition since reproduction is neglected when the body is immunocompromised. Of the oocytes that are aspirated, on average, 30 percent will develop into viable embryos.

Conventional embryo transfer is another advanced reproductive technique that involves in-vivo fertilization and collection. Donor females are administered a series of 8 injections, 12 hours apart, of exogenous follicle stimulating hormone, FSH, for follicular growth. An injection of prostaglandin F2 alpha is administered with the seventh and eighth injection of FSH to lyse any present corpus luteum. Estrus is expected to occur within 36 to 48 hours later. Approximately 12 hours following observation of heat detection, the female should be administered a single injection of GnRH to induce ovulation. Artificial insemination should immediately follow the GnRH injection. Theoretically, since FSH stimulates growth in multiple follicles, multiple follicles should ovulate. The embryos are matured in the donor's oviduct for 7 days following insemination. Embryos are collected on day 7 using a flush media that is a supportive transfer media. A sterile foley catheter is introduced into the vagina and passed through the cervix and into either of the uterine horns. The cuff is inflated to inhibit any fluid bypassing the foley catheter. The horn is then flushed with a media containing growth promoting enzymes for maturation of the embryos. The opposing horn is flushed in the same fashion. The embryos are then sorted and graded under a dissecting microscope in a lab. They are then either frozen for transfer at a later date or freshly transferred into a recipient female at that time. Conventional embryo collection can be

performed every 45-60 days which is generally enough time to allow the donor female to recover from stimulation and ovulation. The average transferable embryos per collection is 6-7 though it may range from 0 to 20 embryos depending upon the donor female[4]. The disadvantages of conventional transfer are the drug administration and the extensive interval between each flush. Lower input cost for the procedure and less equipment required are advantages of conventional embryo transfer. Another disadvantage that varies with the individual animal, is a lower average of the total amount of viable embryos produced. The advantages of follicular aspiration and in-vitro fertilization are having the ability to fertilize oocytes from one ovum pick-up to multiple bulls, no drug administration required, smaller intervals between collections, and increased average of total viable embryos produced. The main disadvantages of in-vitro fertilization are increased input cost, more equipment required, and the necessary need of an outside company to fertilize the oocytes.

Case Outcome

We aspirated 40 oocytes from 23Y1. Of those 40, there were 39 that were viable to ship to Transova for fertilization. Transova fertilized 20 oocytes with conventional semen to Boulder 10D7, a Simmental bull. The remaining 19 oocytes were fertilized using conventional semen to DMR Resource 535F30, a Brangus bull. Of the 39 oocytes that were fertilized, 7 embryos that were inseminated by Boulder were frozen, and 10 embryos by DMR Resource were frozen. The embryos were shipped back and stored in a liquid nitrogen tank to remain frozen for transfer at a later date. The remaining 22 oocytes were not viable for freezing due to poor maturation.

Conclusion

In-vitro fertilization plays a pivotal role in beef and dairy production systems as it provides the ability for superior genetic selection, crossbreeding, and the ability to produce numerous offspring from a dam in a given year. By utilizing in-vitro fertilization, the rate of selection for qualitative and quantitative traits are increased by improving accuracy, intensity of selection, and reducing the

generation interval. In addition to improving, expanding, and extending superior genetics within a herd, in-vitro fertilization can also be a method to improve subfertility in certain circumstances that would inhibit a cow from carrying or delivering a calf or from other life threatening medical emergencies[1]. With regards to the future of advanced reproductive technologies, additional research is needed for better understanding of fertilization timing, oocyte maturation time and medias, and recipient selection as well as other factors. Genomic evaluation in cattle is becoming more popular because it allows the producers to screen a bull calf's genetics for assessment without it having to sire offspring. In time this will decrease input cost by eliminating mismating, unwanted embryo transfer, service costs, recipients, and unwanted calves. Genomic evaluation of embryos will potentially decrease the generation interval[2,7]. In-vitro fertilization is of a class of advanced reproductive technologies that is utilized to increase production to meet the demands of superior genetics and offspring numbers.

References

1) Hansen, P.j. “Realizing the Promise of IVF in Cattle—an Overview.” Theriogenology, vol. 65, no. 1, 2006, pp. 119–125., doi:10.1016/j.theriogenology.2005.09.019

2) Hasler, John F. “Forty Years of Embryo Transfer in Cattle: A Review Focusing on the Journal Theriogenology, the Growth of the Industry in North America, and Personal Reminisces.” Theriogenology, vol. 81, no. 1, 2014, pp. 152–169., doi:10.1016/j.theriogenology.2013.09.010.

3) Golden, B. L., et al. “A Framework for the Next Generation of EPD's.” Economically Relevant Traits, 2000.

4) “In Vitro Fertilization.” Trans Ova Genetics, 9 July 2020, transova.com/.

5) Morotti, F., et al. “Pregnancy Rate and Birth Rate of Calves from a Large-Scale IVF Program Using Reverse-Sorted Semen in Bos Indicus, Bos Indicus-Taurus, and Bos Taurus Cattle.” Theriogenology, vol. 81, no. 5, 2014, pp. 696–701., doi:10.1016/j.theriogenology.2013.12.002.

6) Sirard, M.a., and P. Blondin. “Oocyte Maturation and IVF in Cattle.” Animal Reproduction Science, vol. 42, no. 1-4, 1996, pp. 417–426., doi:10.1016/0378-4320(96)01518-7.

7) Sirard, Marc-André. “40 Years of Bovine IVF in the New Genomic Selection Context.” Reproduction, vol. 156, no. 1, 2018, doi:10.1530/rep-18-0008.