DEVELOPMENT AND USE OF OOCYTE TRANSFER IN THE MARE

Elaine M. Carnevale, DVM, MS, PhD

Oocyte transfer involves the transfer of a donor's oocyte into the oviduct of a recipient mare. The recipient is inseminated, and fertilization and embryo development occur within the recipient.

The first oocyte transfer was recorded in a mare in 1988 (McKinnon et al.). The procedure involved the collection of oocytes from preovulatory follicles and transfer into recipients' oviducts. The recipients were inseminated; and approximately 2 days later, recipients' oviducts were removed. From 15 oocytes transferred, 3 oviductal embryos were collected. The embryos were transferred into the oviducts of second recipients. One of the 3 recipients carried a foal to term. By the mid 1990's, further research resulted in the collection and transfer of oocytes with high success rates.

Procedures for oocyte transfer

Equine oocytes have been collected from estrus, diestrus and pregnant mares. Historically, methods used to collect oocytes included: laparotomies, colpotomies, flank transcutaneous punctures, and transvaginal, ultrasound-guided aspirations. Because of the firm attachment of the cumulus complex to the follicular wall in the equine follicle, collection of oocytes from immature follicles is difficult. Collection rates vary among aspirators, but collection rates from immature follicles are typically less than 50%. For experienced aspirators, collection rates from preovulatory follicles are often greater than 70%.

Oocytes have been successfully collected and transferred between 20 and 36 hours after hCG administration. Oocytes collected between 20 and 24 hours after hCG are probably in Metaphase I and are cultured for approximately 12 hours before transfer. In one study (Carnevale et al., 2000), oocytes were collected at 24 hours after hCG and transferred immediately into recipients' oviducts. Recipients were inseminated at the approximate time of oocyte maturation (12 hours after transfer). Pregnancies resulted, suggesting that the completion of oocyte maturation occurred within oviducts.

Collection of oocytes from preovulatory follicles simplifies requirements for oocyte culture. A typical culture system for oocytes collected approximately 24 hours after administration of human chorionic gonadotropin to the donor, would include: base medium (e.g., TCM-199) with additions of a protein source (e.g., 10% fetal calf serum), antibiotic (e.g., gentamicin or penicillin/streptomycin), and pyruvate. Oocytes are cultured at 38.5 to 39ºC in an atmosphere of 5 or 6% CO_2 and air. If the oocyte is transferred, time of transfer is determined to be approximately 36 hours from administration of hCG to the donor.

Oocytes are transferred through standing flank laparotomy. The surgical incision allows for exposure of the ovary and oviduct. Oocytes are placed into a buffered medium (e.g., Dulbecco's PBS; SOF or TCM-199 with HEPES or MOPS), and pulled into a fire-polished glass pipette for transfer. The pipette is advanced approximately 3 cm through the infundibulum, and the oocytes are expelled.

Recipients have been inseminated before, after or before and after oocyte transfer with success (Carnevale et al., 2001a). Use of a fertile stallion is essential to optimize pregnancy rates.

Recipients used for oocyte transfer can be cyclic or noncyclic. In cyclic recipients, the oocyte from the recipient's preovulatory follicle must be removed before transfer to assure that the recipient's oocyte is not fertilized. Noncyclic recipients are treated with estrogen for 3 to 6 days before transfer and with progesterone after transfer. In our lab, 3 mg of estrogen is administered daily before transfer; and 150 to 200 mg of progesterone is administered after transfer. If a pregnancy is detected at 12 to 14 days after transfer, altrenogest is used to maintain the pregnancy until 150 days of gestation.

Use of oocyte transfer in a commercial program

Embryo transfer has provided the equine breeder with a method to obtain foals from mares that could not carry a foal to term. For embryo transfer to be successful, fertilization and the first week of embryo development must occur within the donor. In some donors, reproductive pathologies (e.g., ovulatory failure, blocked oviducts, uterine or cervical pathology) prevent the successful collection of embryos. In recent years, oocyte transfer has been used to obtain offspring from mares with various reproductive pathologies that were unsuccessful embryo donors (Carnevale et al., 2001b).

Colorado State University began a commercial program for oocyte transfer in 1998. Most donors in the program are older mares, approximately 20 years of age, with histories of reproductive failure in embryo transfer programs. Donors are managed similar to donors in an embryo transfer program. Ovarian activity is monitored during estrus until a follicle, that will respond to hCG, is observed. Criteria for most cycles are: a follicle >35 mm in diameter, uterine edema, and relaxed uterine and cervical tone. However, criteria for individual mares vary. Donors are administered hCG and/or deslorelin acetate (2.1 mg implant, Ovuplant™, Fort Dodge, Fort Dodge IA) to induce follicular maturation. Because response to hCG is not consistent with some old mares, the author uses a combination of hCG and deslorelin acetate.

In the commercial program at CSU, pregnancy rates per transfer are approximately 35%. In the program, the majority of mares are $\geq$ 20 years of age; and semen was obtained from various stallions. Semen is primarily cooled and transported. In 2002, the commercial pregnancy rate at 16 days after transfer was 33%. Experimental transfers were done using the same culture system and procedures. However, oocytes were collected from young donors, and semen was collected and cooled from stallions with good fertility. Embryo development rates after experimental transfers were greater than 80%. The disparity between pregnancy rates for commercial and experimental programs was probably caused by two primary factors -- mare age and semen quality.

In research conducted in 1993 (Carnevale and Ginther, 1995), oocytes were collected from the follicles of old mares (6-10 years) and young mares (20-26 years). Oocytes were transferred into the oviducts of young, inseminated recipients. Embryo development rates for oocytes from old and young mares were 31% and 92%, respectively. Therefore, using oocyte transfer, the researchers demonstrated the effect of aging on quality of the mare's oocyte.

For commercial transfers, the author tries to obtain 1 to 2 x 10^9 motile sperm for insemination. Owners are encouraged to use young, highly fertile stallions with flexible or consistent collection schedules.

Gamete Intrafallopian Transfer

During GIFT, sperm are transferred into the oviduct with the oocyte. This technique has been used in human medicine for cases of male subfertility, because a limited number of sperm are required for oviductal transfers.

The first successful GIFT in the mare was reported in 2000. Since that time, further research has been done. Coutinho da Silva (2002) reported the highest success rates when 200,000 progressively motile sperm and oocytes were transferred into oviducts. Embryo development rates of 55% (12/22) were obtained after GIFT, and rates were similar to embryo development rates for intrauterine insemination (65%, 17/26).

For oviductal transfers, raw semen was collected from fertile stallions and spun through a percoll gradient. The use of GIFT for subfertile stallions is currently being researched.

In vitro maturation of oocytes

Maturation of oocytes in vitro is important for obtaining oocytes for research. In most protocols, the end point for success of oocyte maturation is nuclear maturation. Recently, oocytes were collected from the follicles of diestrus mares or from slaughterhouse ovaries and were matured in vitro. The oocytes were transferred into recipients, and embryo development rates were compared to oocytes collected from preovulatory follicles (Scott et al., 2001). Embryo development rates were 10%, 7% and 82% for oocytes collected from slaughterhouse ovaries, diestrus follicles and preovulatory follicles, respectively.

One potential commercial use of immature oocytes in a commercial program is after the death of a valuable mare. When a mare dies or is euthanized, her genetic potential is lost. However, oocytes can be salvaged from the ovaries, matured and transferred to try and produce additional offspring. In 2002 at Colorado State University, oocytes were collected from the ovaries of 5 mares that were euthanized for various medical reasons. In two cases, the ovaries were shipped from a distant location by airplane. From the five donors, four pregnancies resulted; however, three pregnancies were lost before 60 days of gestation. The final recipient gave birth to a healthy foal. Currently, additional research is being conducted to determine the optimal method for shipment of ovaries.

Oocyte cryopreservation

Methods to successfully cryopreserve equine oocytes have been investigated during recent years. In 2001, the first foals were born after the cryopreservation and transfer of equine oocytes by Maclellan et al. (2002). Oocytes were collected from mares 24 hours after hCG administration, vitrified in a cryoloop, and cultured for 12 hours after thawing. Oocytes, which appeared viable, were transferred into recipient mares. Of 26 oocytes assigned to the project, 3 developed into embryonic vesicles. Two vesicles developed in one recipient, and manual crushing was used to reduce one of the vesicles.

Future considerations

Use of oocyte transfer provides the researcher with a method to examine interactions among the equine oocyte, sperm and oviduct. For the clinician, oocyte transfer can be used to obtain foal from mares that are infertile using other methods, including embryo transfer. Further developments in oocyte transfer could result in use of GIFT for subfertile stallions, transport of ovaries from dead mares for oocyte collection and transfer, and oocyte cryopreservation to preserve the genetics of valuable mares.

References

Carnevale EM and Ginther OJ. Defective oocytes as a cause of subfertility in old mares. Biol Reprod Mono 1995;1:209-214.

Carnevale EM, Maclellan LJ, Coutinho da Silva MA, Scott TJ, and Scott EL. Comparison of culture and insemination techniques for equine oocyte transfer. Theriogenology 2000;54:981-987.

Carnevale EM, Maclellan LJ, Coutinho da Silva MA, Checura CM, Scoggin CF, and Squires EL. Equine sperm-oocyte interaction: results after intraoviductal and intrauterine inseminations of recipients for oocyte transfer. Animal Reprod Sci 2001a;68:305-314.

Carnevale EM, Squires EL, Maclellan LJ, Alvarenga MA, and Scott TJ. Use of oocyte transfer in a commercial breeding program for mares with reproductive abnormalities. JAVMA 2001b;218:87-91.

Coutinho da Silva MA, Carnevale EM, Maclellan LJ, Seidel GE Jr., and Squires EL. Effect of time of oocyte collection and site of insemination on oocyte transfer in mares. J Animal Science 2002;80:1275-1279.

Maclellan LJ, Carnevale EM, Coutinho da Silva MA, Scoggin CF, and Squires EL. Birth of foals after transfer of vitrified equine oocytes collected from superstimulated and non-stimulated mares. Theriogenology 2002;57:468.

Scott TJ, Carnevale EM, Maclellan LJ, Scoggin CF, and Squires EL. Embryo development rates after transfer of oocytes matured in vivo, in vitro, or within the oviducts of mares. Theriogenology 2001;55:705-715.