OOCYTE TRANSFER:
UNDERSTANDING ITS APPLICATION, PROCEDURES AND SUCCESS RATES

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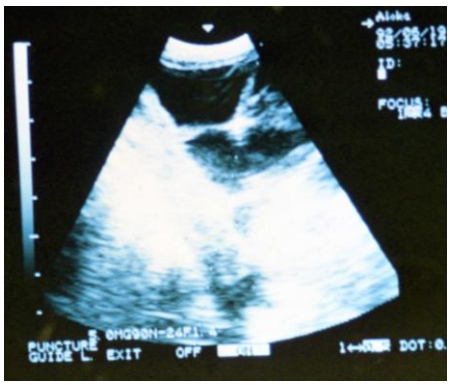
For years, advances in reproductive technology have directly resulted in obtaining foals from horses that otherwise could not provide offspring. One of these techniques, oocyte transfer, has been a topic of interest for over 20 years with the hope of assisting those mares with a uterine environment too compromised to yield viable embryos and/or pregnancies. Other possible issues that justify its use include cervical tears, oviductal occlusions, and ovulation failure. By definition, oocyte transfer is a method by which an unfertilized egg (oocyte) is harvested directly from a follicle within the ovary and transferred into the oviduct of another mare with a healthier reproductive tract. This in essence bypasses the compromised portion of the reproductive tract of the donor mare.

Figure 1 (Left). Aspiration of a preovulatory follicle utilizing TVA. Figure 2 (Below). Needle puncturing follicle to aspirate fluid and potential oocyte.

For oocytes to be harvested from the follicle of a live animal one must successfully puncture the follicle with a needle and aspirate the follicular contents. There have been three techniques used experimentally to collect oocytes: 1) flank laparotomy (surgical incision exteriorizing the ovary for puncture); 2) flank puncture (penetrating the flank with a small trocar and blindly puncturing the follicle via rectally-guided manipulation of the ovary); and 3) transvaginal aspiration (ultrasound-guided aspiration through the vaginal wall). Of the few reproductive facilities or universities that offer oocyte recovery procedures most utilize the transvaginal route of aspiration (TVA).

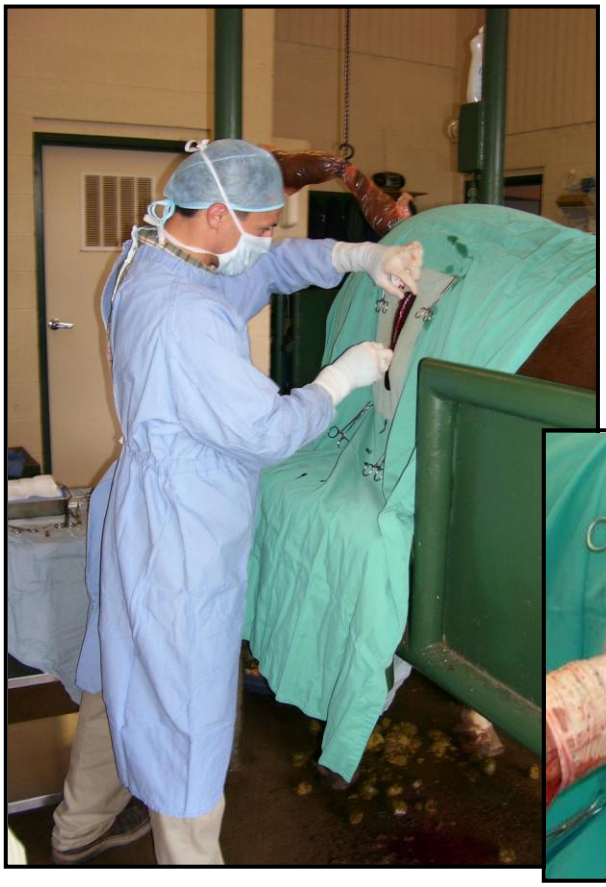
In preparation for TVA procedures, the mare is brought into a stanchion (stocks) and adequately tranquilized. This is a standing procedure so minimizing movement is essential. Once her perineal region is thoroughly washed, a transvaginal probe is guided into the vaginal cavity and placed adjacent to the cervix. The probe used at our facility is made up of a 5.0 MHz, curvilinear transducer and a long, specially-molded handle with a needle guide. Similar to rectal ultrasonographic exams, ultrasonic waves are emitted from the end of the probe and used to visualize the structure(s) of interest on the screen. Once the end of the probe is in place then the practitioner takes his/her arm out of the vagina and places it into the rectal cavity. The goal is to retract the ovary, via rectal palpation, to the abdominal side of the vaginal wall while holding the probe in place on the vaginal side. As the designated follicle(s) is seen on the screen, a



double lumen needle is passed inside the probe handle, through the vaginal wall and into the follicle of choice. The needle used is typically connected to two lines. One line goes to a pump that helps aspirate the follicular fluid and potential oocyte while the other is attached to a syringe containing specialized media used to flush the follicle after aspiration. The oocyte is very sensitive to changes in temperature, osmolality, pH and physical damage therefore it is important to pay close attention to good technique and media preparation.

Aspiration can be done to any follicle that can be visualized and effectively punctured however oocyte recovery rates tend to be lower when aspirating small- to medium-sized follicles (<30 mm in diameter, 10-40%) in comparison to large preovulatory follicles (≥35 mm in diameter, 60-85%). The premise is that the immature oocytes are more closely adhered to the follicular wall and thus harder to dislodge. Depending on the maturity of the oocyte, it either needs to be incubated for several hours (12-30 hrs) prior to transfer or immediately transferred into a recipient. With preovulatory follicles, it is common to use hormonal therapy (hCG or Deslorelin acetate) prior to aspiration in order to mature the oocyte in vivo (in the body). This minimizes the time needed for in vitro (outside the body) maturation and hastens the transfer process.

Similar to oocyte recovery attempts, the transfer procedure is performed while the mare is standing and under sedation. Oocyte transfer is a surgical technique by which a 4 to 6 inch incision is made in the flank region of a recipient mare. The goal is to enter into the abdominal cavity in close proximity of the ovary. By separating the skin, muscle and peritoneal layers as an arm is being passed, a pathway is created to the abdomen. Once the ovary is identified, gentle but firm traction is used to exteriorize it through the incision. At that time, the ovary is positioned so that the infundibulum (end of the oviduct closest to the ovary) can be visualized. Following exposure of the entryway into the oviduct, the oocyte is passed as far as possible (typically ½ to 1 inch) utilizing a specialized catheter or pipette and then deposited. The ovary is then placed back into the abdominal cavity and efforts are made to suture the muscle and skin layers of the incision site.



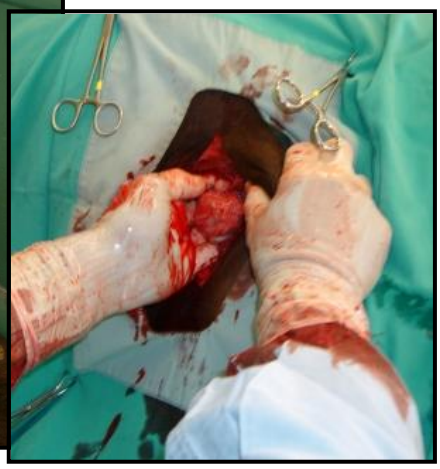


Figure 3 (Left). Creating an incision line during a standing surgical procedure. Figure 4 (Right). Exteriorizing the ovary in preparation for oocyte transfer.

Although the oocyte has been recovered and subsequently transferred, the job is not done. Remember your end goal is to get a pregnancy therefore the recipient needs to be bred during this time. Researchers have obtained pregnancies by transferring both the sperm and oocyte into the oviduct together (GIFT or gamete intrafallopian transfer) however most practitioners tend to inseminate the mares through the traditional

vaginal route. Timing the insemination(s) relative to the oocyte transfer is very important. Pregnancies have been achieved when mares were bred anywhere from 16 hrs before to 16 hrs after transfer but success rates tend to be higher when the mare is inseminated prior to the transfer.

For oocyte transfer to be successful, it is important that the donor and recipient mares be at the same stage of the cycle together. This can be challenging since both mares will be developing preovulatory follicles at the same time. If the recipient is allowed to ovulate her own follicle while you are transferring an oocyte into her, then upon insemination one wouldn't know if any subsequent pregnancy is genetically linked to the recipient mare or the mare from which the oocyte was transferred. Similarly, if she happens to get pregnant with twins a practitioner wouldn't know which one to manually reduce. There are two ways to avoid this complication. The first option is to perform transvaginal aspiration on the recipient mare's follicle as well. If you are able to recover her oocyte prior to transferring the other mare's oocyte then any resulting pregnancy has to be genetically linked to the donor mare. In recent years, efforts have been made to utilize non-cycling mares as recipients. With exogenous hormonal therapy, the oviductal and uterine environments can mimic that of a cycling mare but without the concern of a preovulatory follicle being involved.

Pregnancy rates after oocyte transfer have historically been anywhere from 31 to 92%. Much of the variability in success has to do with whether or not the oocytes are coming from older, subfertile mares or young, healthy mares. In addition, the type of semen used (fresh, cooled or frozen) to breed the mare has a dramatic affect on success rates. And lastly, one should expect higher success when in-vivo matured oocytes are harvested rather than oocytes that require several hours of in-vitro maturation prior to transfer. Given that most oocyte transfer services are dealing with aged mares with some reproductive compromise, one should expect pregnancy rates to be in the 35 – 40% range.

In the last 20 years, oocyte transfer services have helped many mares continue too produce offspring that otherwise would not be able to. Nevertheless, recent efforts have been made to expand on its application. One such endeavor is the ability to harvest oocytes from mares that are being euthanized. In 2003, Carnevale and coworkers achieved pregnancies after collecting oocytes from either slaughterhouse ovaries or ovaries from euthanized client animals. Pregnancy rates were low (15 to 18%) but to be expected given that it took 8 to 24 hrs for the ovaries to arrive at the laboratory and another 24 to 30 hrs to incubate the immature oocytes prior to transfer. Interest in the last decade has also been with the use of intracytoplasmic sperm injection (ICSI). Instead of transferring the unfertilized oocyte and then breeding the recipient, a technique has been adopted from human and cattle reproductive research that allows one to directly inject a single sperm into the oocyte thus forcing fertilization. Although the first ICSI foal was created in 1996 at Colorado State University, efforts have been made by several facilities in the last decade to promote this as a commercial service. Current research efforts are trying to

Figure 5 (Above). First ICSI foal was born in 1996 at Colorado State University.

come up with a successful method of shipping oocytes from one facility to another so ICSI procedures can be more readily used.

In 2008, a 27-year-old Arabian mare was entered into the embryo transfer program at our facility however shortly after arriving we discovered that she had an occluded (blocked) cervix. There was no access to her uterus so oocyte transfer was the only foreseeable option to obtain a foal. This mare had some difficulty cycling regularly but of the two cycles that developed preovulatory follicles, we were able to recover one oocyte via transvaginal aspiration. This oocyte was then shipped to Colorado State University in an attempt to perform the ICSI procedure. Unfortunately, no cellular division occurred after the sperm injection. Further attempts could not be performed during the 2009 breeding season because the mare failed to cycle again. As a final thought, we must remember that advanced reproductive techniques have the ability of overcoming some physiological barriers but often times Mother Nature has the last say.