A Potpourri of Advances in Equine Reproduction

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Harvesting and handling of equine testicles for recovery and freezing of epididymal sperm

Sperm can be harvested from the caudal epididymis and ductus deferens following death, castration or euthanasia of a stallion and frozen for future use. Approximately 54 billion sperm are stored in the two caudal epididymides of a normal, sexually rested stallion so there is great potential to preserve the genetics of a stallion in the form of frozen semen.

As spermatozoa pass through the head and body of the epididymis they undergo maturation. Once the spermatozoa reach the tail of the epididymis, they gain motility as well as the ability to fertilize oocytes. Both the epididymal tail and ductus deferens contain fertile, motile spermatozoa.

Stallions to be euthanized should ideally be castrated prior to euthanasia because the effects of the euthanasia compounds on sperm viability are still not known. The testes and attached epididymides are removed using standard castration techniques. It is imperative that a large portion of the ductus deferens remain with the testicle as it contains billions of viable, motile sperm. The ductus deferens will require ligation before transection in order to avoid the loss of spermatozoa. Once the testes, epididymis and ductus deferens have been removed, the entire structure should be gently rinsed with saline or lactated ringers (body temperature) and placed in a rectal sleeve or plastic bag. Epididymal sperm recovery should begin immediately otherwise the testes, epididymis and ductus deferens should be placed in a passive cooling device designed for shipping semen and kept chilled (5 degrees C) until processed. The Equitainer is an excellent shipping container for testicles. Place the frozen coolant cans in the bottom of the container and the bag containing the testicles on top. Be sure to wrap the bag containing the testicles in a towel so the tissues are not in direct contact with the coolant cans. Referral centers performing equine reproduction should be able to harvest and freeze epididymal sperm. Contact the facility immediately and arrange to have the testicles shipped as soon as possible. It is best if the testicles arrive the same day but sperm remain viable within the epididymis and ductus deferens for 24 and even 48 hours. Blood should be drawn from the stallion and submitted for EVA testing.

Epididymal sperm can be frozen for standard artificial insemination as well as ICSI (intracytoplasmic sperm injection). The quality of frozen thawed epididymal sperm is best when originating from a normal, healthy, reproductively sound stallion. In general, frozen thawed epididymal sperm is of poorer quality than semen frozen following ejaculation. The quality of semen and the number of doses obtained is influenced by a variety of factors
including: the age of stallion, cause of death, size of the testicles, the inherent quality of the stallion’s semen and time and conditions from harvest to freezing. Pregnancy rates will be dependent on the quality of the frozen thawed semen. Studies conducted at New Bolton Center and elsewhere have shown a 60% pregnancy rate in mares bred with epididymal sperm that was stored for 24 hours at 5 degrees C before harvest and freezing. The same study at New Bolton Center showed a 40% pregnancy rate in mares bred with epididymal sperm stored for 48 hours at 5 degrees C before harvest and freezing. In all the above studies, the epididymal sperm originated from healthy stallions undergoing routine castration.

**Harvesting and handling of ovaries for oocyte recovery and ICSI**

In mares it is possible to establish pregnancies following death or euthanasia using oocytes recovered from the ovaries of the deceased mare. The oocytes are harvested from the ovaries by transecting each individual follicle with a scalpel blade and then meticulously scraping the inside of the follicle with a bone curette until the oocyte is recovered. The recovered oocytes are placed in maturation media and incubated for 24-30 hours. Those oocytes that mature are injected with a single sperm using a technique called ICSI or intracytoplasmic sperm injection. The fertilized oocytes are cultured in the laboratory for 7-10 days after which time any resulting blastocysts or embryos are transferred into synchronized recipient mares.

Ovaries should be harvested from the deceased or euthanized mare as quickly as possible. The mare’s ovaries can be removed following euthanasia with a barbiturate overdose or under general anesthesia. Once the ovaries are removed, they should be gently rinsed with warm saline (37 degrees C) and placed in a plastic bag or rectal sleeve, with or without a small amount of warm saline. DO NOT REFRIGERATE THE OVARIES. Ideally the oocytes should be removed from the ovaries within 6 hours of death or euthanasia therefore it is imperative that the ovaries arrive at a referral center, capable of oocyte recovery, as soon as possible. New Bolton Center is able to harvest oocytes from deceased mare ovaries 24 hours a day, 7 days a week. If the ovaries are within 2 hours of the referral center, they can be maintained at body temperature (32-37 degrees C). Place the ovaries in a styrofoam container with a 1 liter bag of saline warmed to 37 degrees C. If transport time is between 2-8 hours, then the ovaries should be cooled to room temperature or slightly lower. Place the ovaries in an Equitainer or well insulated Styrofoam container with coolant packs at room temperature. For transport times greater than 8 hours, the ovaries should be cooled to a temperature no lower than 12 degrees C. Ovaries can be shipped in an Equitainer with one frozen coolant can on the bottom, a room temperature coolant can in the middle and then the ovaries on top in the isothermalizer cup. A semen shipper or Styrofoam box can also be used. Place the frozen pack in the shipper or box with good insulation between the ovaries and the frozen pack. It is best to have a room temperature Ballast bag surrounding the ovaries. It is critical to contact the referral center harvesting the oocytes as soon as possible as well as the facility performing the ICSI. Semen, either fresh or frozen, will need to arrive at the facility performing the ICSI the day after the oocytes or ovaries arrive.
Typically 10-15 oocytes will be recovered from post mortem ovaries. If the mare is old or has been ill for an extended period of time, the number of oocytes recovered will be reduced. On average, 50% of oocytes will mature in culture and undergo ICSI. Approximately 20% of the fertilized oocytes will become blastocysts that can be transferred into synchronized recipient mares. Pregnancy rates are approximately 60% for transferred embryos and 25% of the transferred embryos will undergo early embryonic loss, a rate slightly higher than that seen in vivo transferred embryos. ICSI produced embryos can be vitrified however pregnancy rates following transfer are generally 25% lower than non-vitrified embryos. One can expect lower embryo development rates and higher rates of pregnancy loss for oocytes that remain within the ovary for extended periods of time (ie. >6 hours following death/euthanasia). The major facilities performing ICSI in the mare in the USA are: Equine Embryo Laboratory, Texas A & M University, College State, TX; Equine Medical Services, Columbia, MO; Equine Reproduction Laboratory, Colorado State University, Fort Collins, CO.

The “Hose Technique” to treat retained fetal membranes in mares

Mares generally pass their fetal membranes within 1-2 hours after foaling. A placenta is considered retained in the mare if it has not passed within 3 hours of foaling. The cause of retained fetal membranes in the mare is believed to be a mechanical interference or hormonal imbalance that prevents the detachment of the chorioallantois from the endometrium, especially at the tip of the non-pregnant horn. Retained fetal membranes have an incidence of 2-10% in the mare and are more commonly seen following: dystocia, placentitis, prolonged gestation, hydrops, cesarean section and in certain horse breeds (ie. drafts and Freisians). Retention of the fetal membranes can lead to metritis, laminitis, septicemia and even death. Traditionally retained fetal membranes are managed with oxytocin delivered in a bolus injection or as an intravenous infusion. Uterine lavage is also used to try and loosen the attachment between the chorioallantois and the endometrium. Some veterinarians will attempt to manually remove the retained fetal membranes using traction combined with twisting the placenta and even placing a hand between the placenta and uterus to pry the two apart. This procedure can result is hemorrhage, delayed uterine involution, tearing of the placenta, damage to the endometrium and even uterine prolapse. The Burns technique is another procedure used to facilitate removal of retained fetal membranes. It involves infusing a large volume of fluid into the allantoic space thereby distending the chorionic microvilli sufficiently to bring about their release from the endometrium. Unfortunately it can be difficult to adequately seal the allantoic cavity for maximal fluid distension.

A technique that has been around for many years but has regained popularity is the umbilical vessel water infusion method or “hose technique” for removing intact fetal membranes. With this technique the placenta is filled with water which causes swelling and edema of the tissue. The microvilli are stretched which brings about their detachment from the endometrium. In order to perform this procedure, the mare is sedated and the perineal area of the mare is prepared for a sterile vaginal procedure (ie. tail wrapped and perineum washed and dried). A foal nasogastric tube or stallion catheter (maximum diameter of 9 mm) is attached to a water
hose using a hose connector and flow control valve. The umbilical artery or vein of the placenta is incised longitudinally using a scalpel blade. Alternatively, the catheter can be threaded into the umbilical vessels through the actual opening of the vessel. It is often easier to use the umbilical vein rather than the umbilical artery because it is less muscular and is more amenable to advancing the catheter. The catheter is slowly advanced into the vessel until several centimeters of catheter are within the umbilical vessel. The catheter is secured in the vessel using a zip tie or hand pressure; generally a zip tie is placed at the opening of the vessel and then approximately 10 cm cranial to the opening. The water to the hose is slowly turned on and allowed to enter into the placenta. The flow of water is adjusted according to the reaction of the mare. If the mare is uncomfortable, the flow of water is temporarily decreased or discontinued and then restarted once the mare settles. After 3-5 minutes, gentle traction can be applied to the fetal membranes. Often it is helpful to go into the mare vaginally during the procedure and place a hand between the uterus and the placenta to help encourage separation. Administration of 5-10 IU of oxytocin IV can also help with separation. Continue to fill the placenta with water and apply traction until the membranes are passed. It is often helpful to have an assistant support any expelled placenta to prevent the remainder of the placenta from tearing before it is released. Discontinue traction if the membranes tear or the mare becomes uncomfortable. According to one study, most mares (91%) will pass their fetal membranes within 5-10 minutes of umbilical vessel infusion. In a few instances, 5% of cases, it may take 15-30 minutes for the fetal membranes to be released from the uterus. About 5% of mares may show mild signs of abdominal discomfort during the procedure. If the fetal membranes have been retained for greater than 12 hours they may tear during the procedure which will prevent the technique from working. Fertility in mares following the procedure is reportedly good.

References


