Assisted Reproductive Technologies for Conservation Breeding of Equines

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Conservation of rare or endangered horse or donkey breeds depends on successful reproduction to propagate the animals and to conserve the genetic health of the populations. Despite the continued genetic health of some breeds, in general, rare breed foal registrations are down by 50%. The time to start conserving these genetic resources is now. Recent advances in assisted reproductive technologies (ART) are now available to support equine conservation efforts. Cryopreservation of semen, embryos and somatic cells can extend the reproductive lives of both mares and stallions.

1) **Semen collection for cryopreservation**—Semen can be collected from a stallion as he mounts a mare, or a phantom ("dummy") mare, and ejaculates into an artificial vagina. When a suitable mount is unavailable, e.g. for ponies, semen can often be collected by placing an artificial vagina on the penis while the stallion is standing. A teaser mare may be required. In the laboratory, the collected ejaculate is centrifuged to remove the seminal fluid, then the sperm is diluted in a cryoprotective extender and frozen at about 100 million sperm per straw. A single breeding dose is typically 200-300 million progressively motile sperm. The number of straws needed depends on the quality of the sperm after thawing; typically about 1 billion sperm total is used per dose. Once the sperm are frozen in liquid nitrogen, they can be stored indefinitely, with an estimated half-life of ~50,000 years. The average stallion produces 4-8 doses per collection. Semen is typically collected every other day. Very young stallions and older stallions may have fewer sperm or lower-quality sperm, but 80-90% of adult stallions produce freezable semen. Use of cryopreserved sperm is often more efficient than is shipping a stallion or a mare and makes possible the national or international shipment of semen. One series of collections yields multiple doses that can be used over many years, far extending the natural breeding life of the stallion. Frozen semen can be preserved long-term in a semen bank for an unforeseen future. The frozen semen (as well as other genetic material such as embryos) must be stored in a dedicated semen-storage facility to avoid the possibility of liquid nitrogen failure and thus loss of the samples. Dividing the sample among locations can further safeguard the collection. One estimate of the cost to house such samples is $50/quarter for 250 straws, but some facilities may offer lower prices. Collection and freezing of semen can cost about $500 -$750 per ejaculate; typically with board and multiple collections to provide a reasonable supply of frozen semen for future use, total cost is $2500-$3500.
2) **Chemical ejaculation**—In situations where standard semen collection is not possible, chemical ejaculation can be used to collect sperm for cryopreservation. The stallion does not have to travel to be collected, but can instead be collected at home under the supervision of a trained veterinarian. A medication is administered to reduce the threshold for ejaculation, which sedates the horse. In many cases, sedation will cause ejaculation and the concentrated fresh sperm can be harvested and shipped directly to a cryopreservation facility. Cost is variable for the sedation procedure (your veterinarian); cryopreservation of shipped semen can cost about $500-$750 per ejaculate.

3) **Epididymal sperm collection**—In the event of injury or death, or at gelding (if the testes are mature enough), testes and their associated tissues (epididymides) can be harvested and shipped cool to a freezing facility where sperm can be recovered from the epididymides and frozen. It is possible to retrieve many (30-50) breeding doses of frozen sperm from epididymal tissue of a normally-fertile stallion. For best results, freezing of semen should be completed within 48 hours of tissue harvesting. Cost of harvesting the epididymal sperm is $500-$750. Cryopreservation of the recovered sperm can cost about $750-$1000.

4) **Oocyte harvesting from live mares**—Mares may not be able to carry a pregnancy to full term due to a number of causes. In such cases, oocytes (unfertilized eggs) can be collected from the mare's ovaries and shipped to a laboratory for fertilization. The resulting embryos can be carried to term in a recipient mare or frozen for later embryo transfer. The half-life for frozen embryos is estimated to be ~50,000 years. Cost is ~$1000 for the oocyte harvest. Costs for the fertilization procedure (ICSI) are given below.

5) **Post mortem oocyte recovery**—The death of a mare does not mean that her reproductive potential must be lost. Instead, ovaries can be harvested post mortem and shipped at room temperature to a laboratory. This must be done quickly; best results are obtained if the laboratory receives the ovaries within 6 hours of death. Unfertilized oocytes are not stable for freezing. Oocytes can be recovered from the fresh ovary and introduced surgically into an inseminated recipient mare for immediate fertilization, or the oocytes may be fertilized in the laboratory and the embryos frozen or transferred. Therefore, at the time of ovary recovery, steps should be taken to make suitable semen available to the laboratory. Cost is variable for ovary removal (your veterinarian); ~$500 for dissection of the ovary to recover oocytes. Costs for the surgical transfer to an inseminated mare are ~1500 per surgery; cost of the laboratory fertilization procedure (ICSI) are given below.

6) **ICSI**—intra-cytoplasmic sperm injection—Oocytes harvested from live mares or harvested post mortem can be fertilized by the injection of a single sperm into the cytoplasm of the oocyte. This technique is especially useful when numbers of oocytes or sperm are limited or sperm are of lower quality. The resulting embryo can be cryopreserved for later use or placed into a recipient mare to produce a pregnancy. The cost is about $500-$1000 for the oocyte maturation and ICSI procedures, then most laboratories charge an additional $500 to $1000 for each embryo produced as a result. The cost to transfer an embryo to a recipient mare and purchase the pregnant recipient is typically ~$2000-$5000.

7) **Somatic cell preservation and cloning**—Cloning can be used to produce a live foal having the same genetics as the donor animal. Cloning involves transferring the nucleus of a somatic cell (usually a skin cell) of a donor animal into a host oocyte whose own nuclear material has been removed. The oocyte is stimulated to divide and develop into an embryo, which can then be transferred to a recipient mare. A clone has the nuclear genetic material of the original skin cell donor. The purpose of cloning for conservation is not necessarily to produce a copy of a horse for performance, but instead to produce a clone that is a genetic twin of the somatic cell donor. This cloned animal can be used for breeding. This is especially valuable if the original animal was lost or was gelded before his or her genetic value was known. Although the cost of cloning an equine is high (currently about $70,000 commercially), prices continue to decrease. In any event, tissue harvesting (needs only a pea-sized bit of skin tissue from the donor animal) and freezing of somatic cells from important animals is both highly feasible and affordable. The decision to clone the animal can be made in the future. Cost for tissue collection is variable (your veterinarian); processing of the sample for cell culture and storage is ~$1000-$2000.

8) **Breed conservation using Assisted Reproductive Technologies.** These techniques can be used by the individual horse owner as they see fit. When these techniques are used by breed associations to preserve selected genetics for long term use or as a "backup," associations should actively participate in selecting animals for preservation. The optimal collection is a broad representation of the breed, including both major and minor bloodlines. At the very least, the sample should reflect the genetic ratios in place at the time it is created. More optimally, the sample would contain equal representation of all genotypes present in the breed, whether

currently desirable or undesirable. In addition, a protocol should be standardized to collect information on the origin of germplasm in the collection, for example pedigree of the donor, and phenotypic characteristics.

For breeds with populations in more than one country, it is worthwhile to investigate health testing requirements in all of the possible countries to which germplasm or tissue samples might be exported (including the U.S.A.) Following required procedures (such as quarantine and testing before collection and storage), including use of approved facilities, may add to the additional cost but this will preserve options for future transfer of critical genetics between countries.

Online Equine Assisted Reproductive Technology Resources

- Colorado State University: College of Veterinary Medicine
- Select Breeder Services
- Equine Medical Services
- Texas A&M University: College of Veterinary Medicine & Biomedical Sciences. Equine embryo laboratory http://vetmed.tamu.edu/equine-embryo-laboratory ; Theriogenology laboratory http://vethospital.tamu.edu/large-animal-hospital/equine-theriogenology/stallion-services.

The Endangered Equine Alliance is a project of The Livestock Conservancy. For more information on the program and endangered equine breed conservation visit the Conservancy's website or contact the office.

The Livestock Conservancy

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