Epididymal sperm harvest and freezing

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Introduction

Artificial insemination of captive cervids is becoming more popular. As with raising traditional domestic species, deer farmers strive to improve the genetics of their herd in order to increase the value of their animals. Using advanced reproductive technologies is often a more economical way of accomplishing this without incurring the tremendous expense of purchasing a high quality breeding buck. Having the ability to harvest and preserve epididymal sperm from a deceased valuable breeding buck is of great value to a deer farmer. Moreover, being able to preserve sperm from a hunter-killed trophy buck can prove to be an economical method of adding quality genetics to a deer herd. It has been shown that successful pregnancies have been achieved by using epididymal sperm for artificial insemination.[1]

This manuscript describes methods of shipping testicles, harvesting epididymal sperm, sperm analysis, and sperm processing.

Keywords: Deer, sperm, epididymal, artificial insemination

Shipping and handling

Harvesting sperm immediately after death of the animal would be ideal. However, because veterinarians, technicians, and lab equipment are not usually present at the time of death, the specimens must be shipped to the appropriate location in a short period of time. Studies involving Iberian red deer have shown that cooling sperm maintained within the epididymis to 5°C, as soon as possible, keeps sperm viable up to four days.[1-3] Maintaining this temperature minimizes the deleterious pH and osmolality changes associated with decay.[1] Investigators have also found that the percentage of high quality sperm decreases by half every 24 hours that sperm are stored in refrigeration.[1] Therefore, processing the semen as soon as possible increases the chance for success.

An important consideration when harvesting epididymes is the time of year. Most cervid species exhibit strict seasonal reproductive patterns based on photoperiod.[4] It has been discovered that males display drastic changes in testicular size and function based on photoperiod changes.[4] This change in testicular size produces alternating periods of fertility and infertility. Additionally, aspermatogenesis is often found during the spring or summer antler regeneration period.[4] In short, sperm will be found from antler hardening to antler shedding.

Additionally, if the sperm will undergo cryopreservation, epididymes should not be harvested from the animal longer than two hours post-mortem, but in many cases this is not possible.[3] In such situations, it is still better to harvest the epididymides rather than not to. The entire scrotum containing the testes and their attached epididymides should be removed from the animal, placed in a closeable plastic bag, and put on ice or ice packs in a cooler. Including as much of the vas deferens as possible may yield additional mature sperm. This package should be overnight shipped to the laboratory. The scrotum helps to insulate the testes and epididymides from direct contact with the ice and allows gradual cooling. An alternative method is to remove the testes with attached epididymides from the scrotum, place them into a plastic bag, and use a towel (paper or cloth) as insulation from the ice.

Methods of sperm collection

The three most commonly described methods for obtaining sperm from the cauda epididymis include the flotation method, the cut method, and the flush method. The flotation method is performed by slicing or mincing the cauda in a buffered medium. The minced pieces are left in the medium for several minutes to allow the spermatozoa to swim into the medium.[5] This medium is then filtered to recover the spermatozoa.

The cut method involves making several cuts with a blade or multiple punctures with a needle into the cauda epididymis and gently squeezing the spermatic fluid out of the cut tubules. The recovered sample is then diluted with extender.[5] Flushing the cuts with extender may help with sperm recovery.

This author's preferred method is the flush method. The epididymis and extender are allowed to warm to room temperature. Once cleaned, the epididymis is carefully dissected away from the testicle and transected near the junction of the corpus epididymis and the proximal cauda. With experience, one will adjust the exact location of the transection based on the amount of sperm recovered. A plastic, latex-free conical tube is placed on a stable surface in an upright position. The cauda epididymis is placed into the opening of the plastic conical tube and the cut end of the vas deferens is catheterized. Depending on the lumen size of the vas deferens, one can use a blunted 25 gauge x 5/8 inch needle up to a 21 gauge x 1 inch needle. A tom cat catheter or a teflon IV catheter may also be used. The catheter that is used must fit tightly to prevent any back-flushing. A 3-6 cc, latex-free syringe containing freezing extender (Triladyl®, Minitube, Verona, WI) is used to retrograde flush the spermatic fluid out of the cauda epididymis into the collection tube. More extender may be used if necessary to complete the flushing process. Additional cuts also may be needed in the cauda epididymis to facilitate flushing. An immediate benefit to this method is that the sample is already diluted with extender.

A study was performed to compare the cut method and the flush method.[5] It was discovered that a greater percentage of the flushed samples were free of red blood cells and less contaminated than the cut samples. Furthermore, the pre-freezing and post-thawing analysis revealed better motility with the flushed samples than with the cut samples.

Evaluation of the sperm

Once the sperm sample has been obtained and diluted with extender, a drop is placed on a warm slide and examined for motility and morphology under 200x magnification. Although the sperm stored in the tubules of the cauda epididymis are capable of fertilizing an oocyte, the motility and morphology of the sperm will appear considerably worse than an ejaculated sample. Without undergoing normal ejaculation, the majority of the spermatozoa do not display progressive motility. If the sperm show any movement (wiggling), it is considered acceptable, but the more motility the better. Total motility improves as the sperm sample warms. However, one study in Iberian red deer indicated that harvested epididymal sperm refrigerated for 24 hours had 75% progressive motility.

Morphology will not resemble that of a normally ejaculated sample. It is not uncommon for the spermatozoa to retain their cytoplasmic droplets. Bent mid-piece is another fairly common abnormality. One study discovered that the percentage of spermatozoa with bent mid-piece increased drastically after three days of storage in the epididymis at 5^{o}C.[2] This phenomenon is thought to be a result of thermotropic phase transitions in the plasma membrane lipids due to prolonged exposure to low temperatures.[2] It is imperative that owners and breeders are aware of these differences from ejaculated sperm. This author recommends providing a sperm data sheet which describes the motility and morphology findings of the respective sample. This may help whomever buys or uses the semen realize these differences.

Freezing

Once motility and morphology data have been collected, concentration of the diluted sample is determined by using a hemocytometer. This is accomplished by placing drop of the diluted sample at the designated well on the hemocytometer and allowing the sample to spread across the gridded area. It is important to wait about 10 minutes before counting to allow all of the sperm to settle onto the hemocytometer. The count from the hemocytometer indicates the concentration of sperm (x 10^6) per milliliter of sample. The desired concentration of harvested epididymal sperm intended for cryopreservation is 75-100 x 10^6 sperm/half-milliliter straw. Therefore, this equates to 150-200 x 10^6 sperm/milliliter of total sample. It is usually required to quantitatively adjust the sample to reach the desired concentration by adding more extender. Once the desired concentration has been achieved, the entire sample is placed into the refrigerator for three to four hours to cool to 5^{o}C. The cooled sample is then loaded into pre-labelled straws, which have also been cooled to 5°C. Filling of the straws should be done under refrigeration. All straws should be pre-labeled with the buck's name, species, owner/farm name, date, and name of the person or lab that processed the semen. It is also a good idea to have straws available with a pre-printed code (i.e., WTD 1234) that can be used if testicles arrive unexpectedly. This code can then be designated to that particular buck, owner, and date.

Filled and sealed straws are then frozen in nitrogen vapor by placing them onto a rack 5 cm above the level of the liquid nitrogen for 15 minutes. It is common practice at the author's institution to plunge the vapor-frozen straws into the liquid nitrogen immediately prior to loading them into their respective storage canes. The loaded canes, which should be accurately labeled with the buck's name, owner's name and date, are then placed into a liquid nitrogen tank for long-term storage.

Summary

Epididymal sperm can be used to preserve genetic material from deceased males. Although proper handling of the epididymides and sperm is important, acceptable pregnancy rates can be achieved using harvested epididymal sperm.

Acknowledgements

I thank Clifford Shipley, DVM, DACT and Kim Kensell, BS, CVT for support in this manuscript preparation.

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