

Recovery, storage, and preparation of epididymal and vas deferens sperm for insemination, cryopreservation, and shipping

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Abstract

The current technology allows preservation of sperm from testes and the distal reproductive tract if harvested after death determined largely by environmental and handling conditions (e.g. how long sperm remain viable within their nurturing structures postmortem) after death. Pregnancies are established using non-ejaculated and even immotile sperm with appropriate preparation. There are very simple techniques available that can be employed by any practitioner to recover and prepare sperm from testes and the distal reproductive tract. This paper focuses on techniques for recovery, storage, and preparation of epididymal and vas deferens sperm, and techniques for shipping and cryopreservation.

Keywords: Epididymis, vas deferens, postmortem, preparation, sperm, storage

Introduction

One of the more rewarding reproductive services that practitioners can offer their clients at the difficult time of euthanasia is sperm recovery from a valuable stud for cryopreservation. Under certain circumstances, this can also be successful after an untimely death. For example, goat sperm were still viable 3 days postmortem.¹ General practitioners do not need any specialized equipment, extender, or supplies in order to provide this service. Intact epididymides and vas deferens can be surgically removed easily and shipped to other facilities set up for cryopreservation or insemination. In many cases, sperm can be extended and cooled or held within these structures without processing for insemination a week later without cryopreservation.

Excellent reviews on epididymal sperm preservation and fertility are available.^{2,3} Pregnancies have been obtained using cryopreserved epididymal sperm in bulls and stallions, and embryos from cryopreserved epididymal boar sperm.⁴⁻⁶ In European mouflon rams, sperm obtained from the cauda epididymis resulted in higher pregnancy rates and embryo survival compared with ejaculated sperm.⁷

Sperm can also be obtained from testes; however, they are immotile. Sperm within the caput epididymis of most mammals are also immotile. Intracytoplasmic sperm injection (ICSI) is a technique wherein a single sperm is directly injected into the oocyte cytoplasm. For ICSI, sperm need not be motile to achieve pregnancies.⁸ In humans, testicular sperm obtained via biopsy are used to establish pregnancies following ICSI.⁹ Human sperm recovered via percutaneous epididymal aspiration has also resulted in pregnancies after ICSI.¹⁰ In veterinary practice, unejaculated sperm can be obtained after removal of the entire testis or from the accessory glands, particularly, the ampullae of stallions or ruminants.

Epididymal sperm maturation

Sperm mature as they are transported through the epididymis (transit takes ~ 10 days for most species). During epididymal transit, sperm become motile, shed cytoplasmic droplets, undergo acrosomal membrane change, and other osmotic and chemical changes.^{11,12} Although the capability of sperm to fertilize an oocyte increases as they enter the cauda epididymis, the author does not discriminate between specific regions of the epididymis during sperm recovery and combines all epididymal sperm (cauda, corpus, and caput).

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Sperm recovery

The method selected for sperm recovery depends upon: 1. intended disposition of the sperm (e.g. preservation, immediate insemination); 2. client's desire for obtaining the most insemination doses possible (e.g. including immotile sperm from testes for ICSI); and 3. the amount of sperm present within the reproductive tract.

The procedure to recover sperm from the reproductive tract begins with a modified castration procedure. Special care is necessary to remove the entire epididymis attached to each testis and dissect up into the inguinal canal to retrieve as much of the vas deferens as possible in one piece. The distal cut end of the vas deferens should be ligated to prevent sperm loss. For large animals, the proximal end of the caput epididymis should also be ligated and the testis separated. For small animals, testes can remain attached provided they will fit in the transport container. If there is no attempt to preserve testicular sperm for ICSI, testes are not shipped.

Sperm recovery from vas deferens

Recovery of sperm from vas deferens can be accomplished using manual expression (Figures 1–3). After removing small blood vessels that run closely parallel to the vas deferens, the vas deferens is cut into ~ 5 cm segments in a petri dish. The sterile cover of a culture plate can be used if a petri dish is not available. Holding one end of the vas deferens segment closed, the contents from the vas deferens are stripped out of the other end into the dish. The vas deferens contents are thick (like batter). Semen extender should be used to rinse the vas deferens contents out of the dish and into a centrifuge tube. If sperm cryopreservation is planned, the vas deferens contents should be diluted directly with an extender containing a cryoprotectant. This approach would not stimulate motility prior to freezing. However, there is an experimental possibility of

stimulating sperm motility by adding heterologous seminal plasma as in other species (e.g. ram).¹³

Sperm recovery from epididymis

Sperm recovery from the epididymis is more challenging given its convoluted tubule construction. Manual expression can be accomplished but requires more patience and repeatedly stripping the epididymis from the proximal to distal direction for several minutes. This method leaves a substantial number of sperm in epididymis but the majority of sperm recovered will be from the cauda epididymis. After manual expression, the epididymis can be flushed by catheterizing a tubule in the caput epididymis using an appropriately sized needle and all-plastic syringe containing semen extender. Cauda epididymis should be held vertically with the tip of a mosquito forceps over a petri dish. After flushing, repeating manual expression of the epididymis might recover more sperm.

If more complete recovery is desired, the epididymis can then be minced in a petri dish. Epididymis should not be minced until the serous membrane covering the epididymis and any prominent blood vessels adjacent to the epididymis are removed. While blood itself is not spermicidal, breakdown products of blood (e.g. degraded hemoglobin) is spermicidal, so efforts should be made to minimize blood contamination. The epididymis is then minced into 2–3 mm segments. In author's experience, a stiff-backed office razor blade is more effective at mincing than a scalpel blade. Extender is then added to epididymal segments and the mixture is stirred for a couple of minutes. The mixture should be poured through a semen filter into a graduated cylinder to filter out as much of the non-sperm cellular components as possible. Additional extender should then be used to rinse the petri dish and rinse the filter to recover remaining sperm. Finally, the recovered sperm in extender should be centrifuged. It is important to note that the



Figure 1. Intact pair of canine testes and intact spermatic cords resected to the level of the abdominal wall from a deceased patient. Note the thin, pale vas deferens parallel to the rest of the spermatic cord, and the suture tying off its distal end to prevent sperm leakage.



Figure 2. Intact vas deferens (horizontal structure in foreground) as it is stripped away from the rest of the spermatic cord. Paper towel can be used to absorb any blood from the surface of the vas deferens upon removal. Intact spermatic cord with attached vas deferens clearly visible in the background awaits processing.

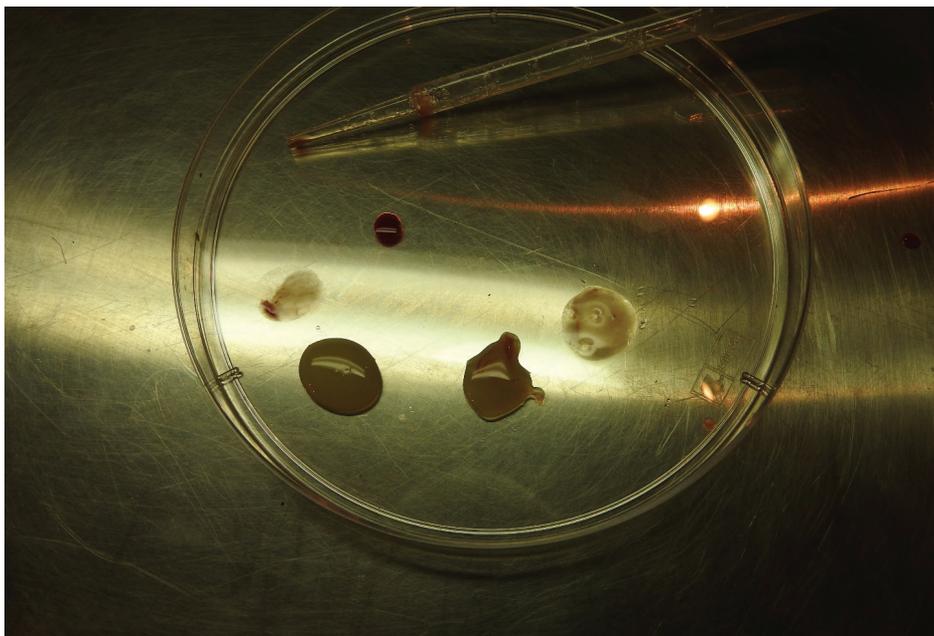


Figure 3. Drops of expressed vas deferens and epididymal sperm with and without blood contamination in a petri dish. An extender of choice is added to centrifuge away as much blood as possible (if necessary) and a glycerol-containing freeze medium is then used for cryopreservation.

addition of prostatic fluid to epididymal canine sperm prior to freezing can significantly increase pregnancy rates.¹⁴

Shipping tissues for delayed sperm recovery

If a practitioner is not able to perform sperm recovery, vas deferens, epididymides, and testes can be shipped to another facility for sperm recovery. As mentioned, the distal cut end of

vas deferens should be ligated to prevent sperm loss. For large animals, epididymis proximal end should also be ligated and the testis separated. Prior to shipping, isolated vas deferens and epididymides should be rinsed with sterile saline and packaged inside a moist whirl-pak or ziploc bag excluding excess air. Reproductive organs should be then packed in a semen shipping container or, if unavailable, a styrofoam vaccine shipping container with a frozen ice pack on both the top and bottom. Reproductive organs should be wrapped

in ~ 1 inch of bubble wrap or roll cotton to insulate tissues from direct contact with ice packs. If immediate shipping is not possible, then reproductive organs should be stored in a refrigerator and prepared for shipping (as described earlier). If the receiving organization has specific instructions for shipping the reproductive organ, those should be followed. Sperm recovery for cryopreservation and insemination can then be undertaken upon receipt.

Preparation for insemination without shipping or preservation

If sperm can be used for insemination within a week after recovery, isolated vas deferens and epididymides should be rinsed off with sterile saline after closing the ends with mosquito forceps. Use a litter bag with either saline or lactated Ringer's solution for placing the organs. Cut the top of the bag, expel some fluid to accommodate organs, and add twice the recommended dose (mg/kg) of antibiotic (either ceftiofur or penicillin/gentamicin), place the organs, roll down top and hold shut with mosquito forceps and stand upright in back of a refrigerator. The litter bag provides good insulation, allowing for a slow, steady cooling rate. Saline also prevents excessive desiccation of the tissues. Antibiotics inhibit bacterial growth and prevent premature tissue decomposition. Tissues should be placed in a refrigerator as soon as possible to start the cooling process. Because sperm are still within the reproductive tract, their very low metabolic state is not disturbed.

When needed for insemination, sperms are removed from the vas deferens and epididymides, diluted with an extender appropriate for the species and evaluated (for motility and concentration) to determine the volume needed for each insemination dose. It is important to note that the motility of the unextended caudal epididymal/vas deferens sperm is not progressive, but rather has a slight vibratory motion. Sperm will become motile after dilution with semen extenders or homologous seminal plasma. In author's experience, motility development might be almost instantaneous or may take several minutes. The author has used this method to produce foals from a stallion that died a week before mares were to be inseminated. It is possible that sperm may retain their fertilization ability for substantially longer using this in situ storage method.

Semen extenders

For flushing, extending, filtering, and centrifuging the collected sperm, any cryoprotectant-free medium (homemade or commercial) that is appropriate for the given species can be used. This is also true for the extender containing a cryoprotectant.¹⁵ There are individual biological differences in post-thaw motility among various extenders. For example, this author recently collected and processed sperm from a 25-year-old Arabian stallion that was euthanized because of a shattered hock. The stallion had small, firm testes at euthanasia and had not bred or been collected in more than 3 years. As described earlier, sperm were collected from vas deferens and epididymides and pooled together. Sperm sample was divided into equal aliquots and extended in two commonly used equine centrifuging media. Extended sperm were slow-cooled over 90 min and then gradually mixed with the freezing medium (the same freezing medium used for both aliquots). Aliquots were frozen simultaneously on the same rack. Post-thaw motility of the two aliquots differed significantly, with < 1% progressive motility (still presumably useful for ICSI) in one aliquot and 35% progressive

motility (also vigorous) in the other. The latter aliquot yielded more than 11 commercial quality doses of at least 300×10^6 postthaw motile sperm.

Conclusion

Postmortem preservation of sperm is not only feasible but also pregnancies are possible using available recovery and shipping techniques that any practitioner can perform. When confronted with an impending or unexpected loss of a valuable stud, practitioners should inform their clients that sperm collection and preservation are possible.

Conflict of interest

None to declare.

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