Case File: Epididymal Semen
An emergency procedure to salvage valuable genetic material

Signalment and History

- An 18-year-old Quarter Horse stallion suffered a severe musculoskeletal injury to a front leg.
- The owner elected to euthanize the stallion and requested that the CSU Equine Reproduction Laboratory harvest and cryopreserve the stallion’s spermatozoa for later use.

Salvaging Genetic Material

Spermatozoa can be recovered from a stallion that sustains a catastrophic injury, colic, or other life-threatening medical condition. The epididymis contains billions of spermatozoa that can be harvested and cryopreserved for future use. In most instances, spermatozoa will survive and maintain capacity for fertilization for up to 24 hours after the stallion’s death. However, it is recommended that sperm be harvested and cryopreserved as soon as possible following death, euthanasia, or elective castration of a stallion.

The Epididymis

Spermatozoa are produced within seminiferous tubules of the testes by a process known as spermatogenesis. During spermatogenesis, primary sperm cells of the male undergo meiosis and eventually form spermatozoa containing a haploid number of chromosomes. Spermatogenesis takes approximately 57 days in the stallion. Spermatozoa produced in each testis pass into the respective epididymis and are subsequently transported from the caput (head) through the corpus (body) and into the cauda (tail) of the epididymis over a period of 10-11 days. The epididymides are the primary sperm storage sites, containing an average of 8 billion to 10 billion spermatozoa (or more) between the two combined epididymides. Millions of additional spermatozoa are contained within the ductus deferens (vas deferens), the tubular tract that connects the tail of the epididymis with the pelvic urethra (Fig. 1).

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Fig. 1: Stallion testis (T), head (H), body (B), and tail (T) of the epididymis, along with the ductus deferens (D).
Smooth muscle surrounding the epididymal tail and ductus deferens contracts during normal ejaculation, propelling concentrated spermatozoa distally into the urethra where it mixes with fluids from the accessory sex glands and the semen eventually exits the penis.

**Epididymal Sperm**

Sperm mature as they transit the length of the highly coiled epididymis and gain the capacity for fertilization and become motile. In general, sperm in the head of the epididymis have limited capacity for fertilization and movement, while sperm in the tail of the epididymis can fertilize an oocyte and have the ability to swim. However, motility is limited while sperm are stored in the epididymis. Motility is dramatically enhanced once sperm are ejaculated and come in contact with other secretory fluids of the accessory sex glands (i.e., seminal plasma).

**Case Management**

Immediately following euthanasia, a modified open castration was performed to collect each of the paired testes, epididymides, and ductus deferens from the stallion. Each ductus deferens was identified and ligated with surgical suture to prevent loss of spermatozoa during collection and transport. The ligation was placed as far proximal on the ductus deferens as possible to maximize recovery of spermatozoa. Each testes, epididymis, and associated ductus deferens were removed as an intact unit. No attempt was made to separate the epididymis from the testis.

The testes, epididymides, and ductus deferens were rinsed with sterile saline and placed in a sterile bag. The bag was placed into an Equitainer® containing two frozen coolant cans. The container was transported to the Equine Reproduction Laboratory located on the Foothills Campus of CSU.

**Harvesting Epididymal Sperm**

The tissue was removed from the container and each epididymal tail and associated ductus deferens was separated from each testis. The epididymal tail was placed into a sterile glass flask and the ductus deferens was elevated and cannulated with a glass pipette. The ductus deferens and epididymis were flushed with seminal plasma previously harvested from other stallions followed by a second flush with semen extender (Fig. 2). This process was repeated for the second epididymis.

The fluid containing the spermatozoa flushed from each side was subsequently combined. A total of 12.3 billion sperm were harvested. Assessment of motion characteristics revealed that the total motility of the harvested sperm was 50 percent and the progressive motility was 40 percent (Fig. 3).

The spermatozoa were divided into three aliquots and added to either lactose EDTA, MFR-5, or Botu-Crio freezing extenders to a final concentration of 200 million sperm per ml of extender. The extended semen was transferred into 0.5 ml semen straws each containing 100 million spermatozoa. The semen was subsequently frozen according to protocols appropriate for each extender. Straws of frozen semen were transferred into a tank of liquid nitrogen for storage (Fig. 4). Ultimately, 92 straws of epididymal semen were frozen.

**Fig. 2:** Flushing seminal plasma through the ductus deferens (white arrow) and out the epididymal tail (grey arrow) to collect spermatozoa into a glass flask.

**Fig. 3:** Epididymal spermatozoa
A straw from each type of extender was thawed and evaluated the next day. Semen frozen in Lactose EDTA and Botu-Crio extenders had the highest post-thaw motility (Table 1) and was considered of adequate quality for use with traditional artificial insemination techniques. Semen frozen in MFR-5 had lower post-thaw motility, and advanced assisted reproduction techniques may be required to generate pregnancies using that cryopreserved semen.

**Discussion Point**

Approximately 10 to 15 billion sperm can be harvested from the paired epididymides of most stallions for cryopreservation. A total of 12 to 20 breeding doses (eight straws per dose, with each straw containing 100 million sperm) are usually obtained. Mares may be inseminated with one or two doses of frozen semen per estrous cycle.

Pregnancy rates for mares bred with frozen epididymal semen are often lower than that of mares bred with frozen ejaculated semen from the same stallion. However, pregnancy rates can be increased by optimizing mare management, timing insemination relative to ovulation, and incorporation of deep horn insemination techniques. Pregnancy rates with frozen epididymal semen have ranged from 0 percent to 66 percent. The wide range in pregnancy rates is due to factors such as health status of the stallion prior to castration or euthanasia, interval from castration to harvest of epididymal spermatozoa, post-thaw motility, and reproductive status of mares that are bred. Stallions that have a sustained an acute life-threatening injury, such as a musculoskeletal injury, typically have better quality epididymal semen than stallions with chronic disease processes.

Intracytoplasmic sperm injection (ICSI) may also be used with epididymal semen to conserve spermatozoa, as the procedure requires injection of only one spermatozoon into an oocyte. Frozen epididymal semen yields an equivalent pregnancy rate to frozen ejaculated semen when using ICSI.

**Table 1:** Results of post-thaw motility evaluation for the epididymal semen collected and frozen from the injured stallion.

<table>
<thead>
<tr>
<th>Extender</th>
<th>Total and Progressive Motility (10 min Post Thaw)</th>
<th>Number of Straws Frozen</th>
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<tbody>
<tr>
<td>Lactose EDTA</td>
<td>30 / 17% (Total / Progressive Motility)</td>
<td>35</td>
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<tr>
<td>MFR-5</td>
<td>14 / 7%</td>
<td>35</td>
</tr>
<tr>
<td>Botu-Crio</td>
<td>39 / 27%</td>
<td>22</td>
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**Take Home Message**

It is possible to preserve genetic material from stallions that sustain a catastrophic injury by harvesting and freezing epididymal spermatozoa. The frozen epididymal semen may be used in a traditional artificial insemination program or by advanced reproductive procedures such as intracytoplasmic sperm injection. Please contact the Equine Reproduction Laboratory at Colorado State University for more information.

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