



CRYOPRESERVATION OF EQUINE SPERMATOZOA

Puget Sound Equine Reproduction Center

HISTORY

Successful cryopreservation of semen (frozen semen) was first performed in the early 20th century with bull semen and is extensively used in the cattle industry today. The first pregnancy resulting from frozen stallion semen occurred in 1957 in the US when spermatozoa were taken from a stallion's epididymis, frozen, thawed and inseminated into a mare. Interest and necessity have rapidly grown to transform equine semen cryopreservation throughout the world into a successful industry.

Equine semen is far less tolerant of the freezing and thawing process than bull semen. Furthermore, not all stallion semen freezes alike. No single technique or magic formula exists to freeze all equine semen. Semen from some stallions fairs better than others with certain freezing media. Some stallion's semen does not freeze well and may require extensive testing and laboratory procedures in attempting to preserve the spermatozoa. Furthermore, at different times of the year and even at different collections, tolerance of the freezing and thawing process may show some variation in an individual stallion.

ADVANTAGES

The greatest advantage to the use of frozen semen probably belongs to the stallion owner. However, both mare and stallion owners have reason to benefit from its availability.

The reasons include:

1. Frozen semen is insurance against injury or death of the stallion.
2. Frozen semen allows international shipment of semen, thereby enhancing the equine gene pool in all countries. This requires semen to be processed at a USDA approved facility.
3. Horses from different hemispheres can be bred even during the stallion's "off " season. This may represent an additional source of income to the stallion owner.
4. The stallion's show schedule need not be interrupted for semen collection.
5. Overuse of the stallion is minimized since most freezing is performed after the breeding season. Furthermore, the use of frozen semen prevents low fertility due to heat stress in the summer months since semen can be preserved at a different time of the year.
6. Frozen semen may allow stallions with behavioral problems to be gelded.
7. Frozen semen allows more precise timing of insemination because semen may be shipped many days prior to ovulation, and so is available for insemination at the optimal time. This eliminates emergency collections and ensuing anxiety that semen may not arrive in time to cover the ovulation.
8. Delays in customs for international shipment or due to airline difficulties do not affect the viability of the frozen semen.
9. There is often a reduced cost for shipment of semen necessary for a single heat cycle. With fresh/cooled semen, collection and shipment fees can run as high as \$275.00 or greater per shipment. Sometimes it may require more than one shipment of fresh/cooled semen in one heat cycle.

Finally, frozen semen offers the same advantages of fresh-cooled transported semen in that it:

1. Allows the ability to breed to any stallion regardless of distance.
2. Eliminates the transportation and board fees to the mare owner at the stud farm.
3. Reduces the possibilities of injuries to both the mare and the stallion.
4. Reduces the management of the “difficult breeder”, i.e. a mare that does not show heat or stand for a stallion.
5. Reduces the chances of intrauterine infection since gross contamination is removed at the time of collection and antibiotics are added to semen freezing media.
6. Reduces the chance of infection in the stallion.

DISADVANTAGES

There are some disadvantages to frozen semen as well:

1. There is remarkable variability in the ability of the stallion spermatozoa to withstand the freezing and thawing process. Some stallion's semen may not be viable or fertile post-thaw.
2. There is considerable initial expense involved for the stallion owner. However, frozen semen can be quite cost effective when compared over a breeding season to other methods of breeding.
3. In some stallions conception rates are lower with frozen semen compared to fresh cooled semen. With education, familiarity, and subsequent improvement of post thaw technique this appears to be improving.
4. Veterinary involvement is more labor intensive, and thus more costly for the mare owner.
5. The number of capable inseminators continues to grow; however, it can be a source of frustration to both the mare and stallion owners in finding an individual who is both willing and trained to use frozen semen.

INSEMINATION

Frozen semen, after rapid thawing, should be inseminated into mares as close to ovulation as possible. Current research suggests that a mare should be inseminated within six hours of ovulation for best pregnancy rates. It is often recommended that insemination take place on either side of ovulation and at the time of ovulation. Therefore precise timing of ovulation is critical to success and requires intensive monitoring of the mare's reproductive tract by transrectal ultrasound.

PREGNANCY

With most stallions, conception rates are lower with frozen semen compared to fresh/cooled semen. It is generally agreed that frozen semen does not maintain fertility after thawing as long as cooled semen. Furthermore, a great deal of variation probably exists between stallions. With optimal timing and normal fertility (of the mare and stallion), an expected per cycle conception rate is approximately 40–45%.

With continuing improvement in the freezing techniques, especially with freeze media, as well as the familiarity of the inseminator with the procedures, overall conception rates should continue to improve.

PROCEDURES

Semen collected into an artificial vagina is rapidly processed in special cryopreservation media and cryopreserved in liquid nitrogen vapor at -196°C . It may be packaged in several different forms of straws. The straw sizes range from .5 ml to 5 ml straws and ampules, etc.

A test straw from each frozen batch is thawed, cultured for potential pathogenic bacteria, and examined for motility and velocity.

The straws that are processed at PSERC are available for commercial and/or private use. Each insemination dose is estimated to contain a minimum of 800 million progressively motile spermatozoa with no less than 30% post-thaw progressively motile sperm. The number of straws per insemination dose varies on the size of the straw used. Straws that do not meet these requirements are available to the stallion owners for use in their own mares, or they are deemed unusable and are destroyed.

PREPARATION OF THE STALLION

Dead spermatozoa accumulate in the reproductive track of the stallion when he is not being bred or collected every few days. Therefore, these dead sperm must be “flushed out” during the week before freezing to ensure optimal quality of the semen to be frozen. Also, at this time several cultures of the reproductive track are taken and examined for potential pathogenic growth and to determine appropriate antibiotics for the freezing media. Basic semen evaluations are performed during this preparatory week in order to evaluate sperm numbers and motility. Also the semen is tested in different media. There are many combinations to try if the most common extenders don't result in acceptable post-thaw motility. Lastly, this week allows the stallion to become familiar with semen collection methods, there by reducing the semen variability when collecting for a freeze.

Stallions must be tested for Equine Viral Arteritis (EVA) within a month of freezing or owners must furnish proof of vaccination following a positive titer that was performed within the last year.

Vaccinations for other diseases (Influenza, Rhinopneumonitis, and Tetanus) are required before a stallion stays at PSERC.

There is no concrete evidence as to the length of time frozen semen can be stored in liquid nitrogen without losing viability upon thawing. Foals conceived with semen stored for 20 years have been born in the past few years.

Again, there is tremendous variability in pregnancy rates due not only to stallion variability, mare fertility, and the level of expertise of the inseminator, but also due to many other factors that are beyond our control. There is no guarantee made concerning the fertilizing capacity of frozen semen, even with post-thaw motility that meets the current industry standard.

BREED REGISTRY

It is the responsibility of the stallion and mare owners to determine the policies regarding the transportation and the use of frozen semen that have been set by the individual breed registries.

STORAGE

Semen is stored in liquid nitrogen containers. This service is available at PSERC or the semen can be stored with the stallion owner if a tank is available. These containers need to be “topped off” with liquid nitrogen at regular intervals to avoid the risk of “running the tanks dry”, thus causing the sperm to thaw and die. There are also several commercial services across the country that store animal semen and ship it as well.

INTERNATIONAL SHIPMENT

Frozen semen intended for international shipment must be collected and stored at a USDA approved facility capable of stringent quarantine procedures. We can help you locate an approved facility if you anticipate an international market for your stallions semen.

Frozen semen intended for international shipment must meet the requirements of the country to which it is being imported. Often, a quarantine period and specific protocol are required. It is the responsibility of the stallion owner to obtain a permit to import as well as ensure that all criteria are met for exportation of the frozen semen. Plenty of time prior to freezing should be allowed for these procedures. The USDA/Federal veterinarian can be quite helpful in determining the protocols of various countries.

DOMESTIC SHIPMENT

Shipping your stallion's frozen semen around the US requires the use of a specialized "dry shipper" container designed specifically for transportation of frozen semen. This service is available through PSERC.