

# Advances in Stallion Semen Cryopreservation



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## KEYWORDS

- Stallion • Frozen semen • Sperm selection • Epididymal sperm • Centrifugation
- Cushion • Spermfilter

## KEY POINTS

- Recent advances in extender composition have allowed increased quality and fertility of frozen stallion semen from ejaculated and epididymal sperm.
- The use of new laboratory techniques to select and better protect sperm cells has allowed successful freezing of semen from stallions with sperm more susceptible to damage during the freezing-thawing process.
- Different quality control tests are available to better determine the quality of frozen semen. However, there is no reliable in vitro test to predict fertility.

## INTRODUCTION

The use of stallion frozen semen minimizes the spread of disease, eliminates geographic barriers, and preserves the genetic material of the animal for unlimited time. Significant progress in the process of frozen thawed stallion semen and consequently fertility has been achieved over the last decade. Progress has been associated with the use of (1) new AI techniques, such as deep uterine artificial insemination (AI), which permits the use of a small number of sperm cells; (2) other cryoprotectants than glycerol and new commercially available extenders that results in better sperm cryosurvival; and (3) sperm selection techniques to increase the quality of frozen semen. New laboratory approaches are also available to evaluate and overcome the deleterious effects of cryopreservation.

These improvements not only increased fertility rates but also allowed cryopreservation of semen from “poor freezer” stallions, inducing a positive impact in the interest of different horse breed associations and owners in the use of stallion frozen semen

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with thousands of mares being inseminated yearly worldwide. Approximately 20% of embryo donor mares have been bred in Brazil with frozen semen and 90% of foals from Standardbred mares are produced by AI in Australia<sup>1</sup> with frozen or cooled semen.

This article reviews traditional steps and new strategies for stallion semen handling and processing, which are performed to overcome the deleterious effects of semen preservation and consequently improve frozen semen quality and fertility.

## PROCESSING SEMEN FOR FREEZING

After collection, semen must be filtered to remove the gelatinous portion and debris from the ejaculate. Semen must then be diluted with skim milk base extender and evaluated for motility, viability, and sperm concentration to determine the number of straws. After this, centrifugation is performed to remove the seminal plasma and the sperm pellet is resuspended in extender containing cryoprotectant to the volume required to achieve the desired concentration. Finally, semen is loaded into straws. It is recommended after this time to perform a new analysis of motility, viability, and sperm concentration to ensure the quality and quantity of sperm placed in each straw. Next, semen is frozen using a predetermined freezing curve according to the medium to be used. The straws are stored in liquid nitrogen containers until thawing and used for insemination. It is recommended to perform another analysis of motility and sperm viability after thawing, to verify the quality of the semen to be inseminated (Fig. 1).

### *Standard Procedures for Semen Collection*

The penis should be washed, especially the urethral fossa, with warm water to eliminate smegma and microorganisms just before collection. It is important to avoid the use of bactericidal solutions that can disrupt the normal bacterial flora from the penis.

The two most traditional methods to collect semen from a stallion involve the use of a phantom or a mare in heat. The use of phantoms is safest for the animal and operator and stallions can easily be trained to perform a collection into an artificial vagina.

There are different models of artificial vagina available to collect semen from stallions. The Missouri and Colorado models in the United States, Hannover in Europe,



**Fig. 1.** Steps in the semen freezing process. First the semen is collected, filtered to remove gel, and diluted with milk-skimmed media extender. After seminal plasma is removed, the pellet of sperm is resuspended with freezing extender and finally frozen.

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