



## Review Article

## Seasonal Influences on Cooled-Shipped and Frozen-Thawed Stallion Semen



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## ABSTRACT

This review summarizes and discusses results from studies addressing the question if equine spermatozoal function is compromised outside the breeding season resulting in an inferior quality of processed semen obtained at certain times of the year. Differences among studies with regard to the time of the year best suited for cryopreservation may be caused by differences among methods applied to assess semen quality, stallions, and location. Nevertheless, researchers agree that differences among seasons are at best small and variation in the suitability of individual stallions for semen cryopreservation is obviously much more important. Less information is available with regard to cooled-stored semen quality among seasons. Because of the fact that early in the breeding season the number of artificial insemination (AI) doses needed per day is much lower than later in the season, the number of progressively motile spermatozoa per AI dose is usually high. This may counterbalance a decreased longevity of cooled-stored semen produced early in the year. Reduced gonadotropin release during winter is assumed to be the underlying cause of lower semen quality at this time of the year.

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## 1. Introduction

The horse is a seasonally breeding species with maximal reproductive activity occurring in late spring and summer. Neglecting this fact, many breeders aim for foals to be born as early as possible in the year because this provides an advantage for horses competing at an early age. This approach is not only followed in Thoroughbred horse racing but also in horses bred for European equestrian disciplines or Western riding. For this reason, the official breeding season for many horse registries starts well before the spring equinox and thus much earlier than the physiological breeding season of horses. In most breeds, horses born from December onward are already registered for the following birth year.

For practical reasons, many semen collection centers freeze semen early in the year. At that time, artificial insemination (AI) stallions usually have already passed all veterinary tests required for international semen shipment, but the number of mares for insemination is still low. In contrast, during the breeding season, semen collection centers focus on the production of cooled-stored semen and the production of cryopreserved semen is generally low. This leads to a peak production of frozen ejaculates in February and March also at the semen collection center of the Vetmeduni Vienna (Fig. 1).

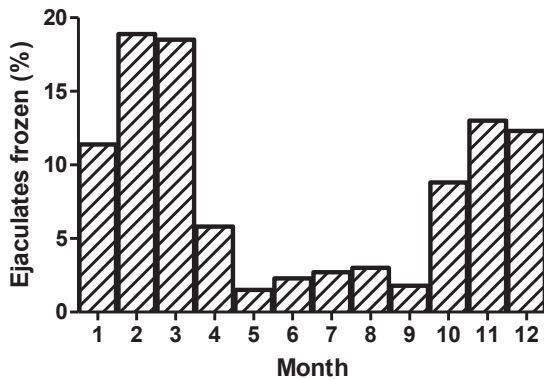
## 2. Seasonal Variation of Semen Quality in the Stallion

## 2.1. Seasonal Effects on the Quality of Cryopreserved Semen

In Europe, the French national studs were among the first to include frozen-thawed semen into their equine breeding program on a large scale [1]. Over the last 20 years, research groups from Europe and America have addressed the question whether the quality of

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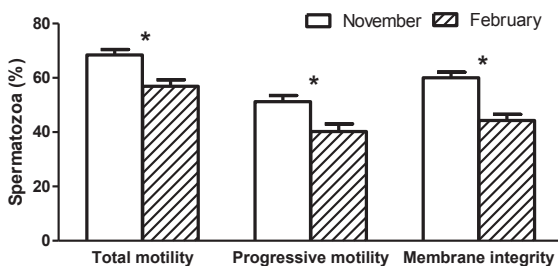
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**Fig. 1.** Ejaculates frozen in different months of the year (%;  $n = 1,021$  ejaculates) at the Vetmeduni Vienna semen collection center (1 = January).

cryopreserved semen changes with regard to the season when it is produced [2–8]. Semen quality was assessed by computer-assisted semen analysis and different tests for sperm plasma membrane integrity. Magistrini et al [2] from France analyzed frozen-thawed semen produced over the year and demonstrated that motility is highest in semen frozen during winter. The study included a low number of stallions (three ponies and three horses), and results are not easily comprehensible. The authors concluded that differences in the quality of cryopreserved semen produced in different seasons exist but are much smaller than the variability of semen quality among stallions. For many years, results of this study supported the approach to perform cryopreservation of stallion semen during the winter months, that is, before the start of the breeding season. However, more recent follow-up studies concluded that autumn may be a more favorable time of the year for freezing of stallion semen than early spring or late winter [4,5,7] because a setback in the quality of frozen-thawed semen was demonstrated in February/March, that is, approximately 10 weeks after the winter solstice (Fig. 2).

Neither the study by Janett et al [4] nor by Schmid-Lausigk and Aurich [7] included semen frozen in spring and summer. When this was done, highest post-thaw motility of cryopreserved semen was again seen in



**Fig. 2.** Total motility, progressive motility, and membrane integrity (%) of spermatozoa in frozen-thawed semen from Warmblood stallions ( $n = 10$ ). Semen was produced either in November ( $n = 30$  ejaculates) or February ( $n = 30$  ejaculates). Adapted from Schmid-Lausigk and Aurich 2014. Values are means  $\pm$  standard error of mean. \* $P < .001$  between months.

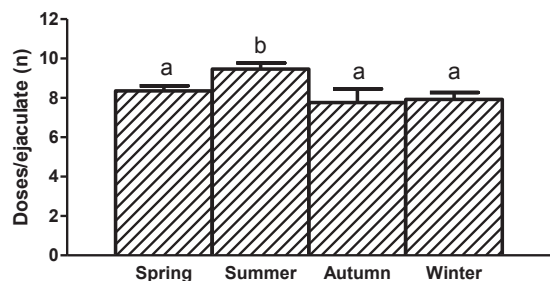
autumn (September–November) whereas the hypoosmotic swelling test revealed the lowest percentage of membrane-intact spermatozoa frozen in winter [5]. This study is therefore in agreement with the finding that autumn is the best time of the year for cryopreservation of stallion semen. However, under commercial conditions, the production of cryopreserved semen from stallions was most effective if performed in summer when the largest number of AI doses per ejaculate with acceptable quality could be produced (Aurich et al, unpublished observations, Fig. 3).

When evaluating the influence of season on cryopreserved stallion semen quality, Wrench et al [6] included the expression of the fertility-related protein SP22 into their analysis. A significant effect of season on spermatozoa staining positive for SP22 on the equatorial segment was demonstrated. The proportion of these sperm was also affected by stallion. In contrast to former studies, highest susceptibility of sperm to freezing damage was found in September when the percentage of sperm staining for SP22 was greatest [6]. A relationship between expression of SP22 and fertility of stallion semen has not been confirmed so far, but in rats, the protein is involved in sperm–egg interaction and its expression highly correlated with fertility [8].

Taken together, almost all studies demonstrated seasonal effects mainly on the motility of frozen-thawed stallion semen. Differences among studies with regard to the time of the year best suited for cryopreservation may be caused by differences among laboratory methods applied, breed, stallions, and location. However, numerically, differences among seasons are small, and variation in the suitability of individual stallions for cryopreservation of their spermatozoa is obviously much more important than seasonal variation. Optimization of season will not change a “bad” freezer into a “good” one [2]. It should be concluded that—proper management of the stallion presumed—the production of cryopreserved semen is possible all over the year.

## 2.2. Seasonal Effects on the Quality of Cooled-Stored Semen

The production of cooled-stored stallion semen obviously is restricted to the breeding season. In Europe, most



**Fig. 3.** Artificial insemination doses with acceptable quality (progressive motility  $\geq 35\%$ ,  $300 \times 10^6$  motile sperm/doses) per cryopreserved ejaculate ( $n$ ) produced in different seasons (spring: March–May, summer: June–August, autumn: September–November, winter: December–February) at the Vetmeduni Vienna semen collection center ( $n = 780$  ejaculates); values are means  $\pm$  standard error of mean. ab,  $P = .05$ .

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