

## **A Big Thaw in the Use of Frozen Stallion Semen**

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There have been tremendous changes in the acceptance and use of frozen stallion semen for breeding programs over the past several years. Many more breed registries are now allowing its use. Some now even allow its use after the stallion has died. These are all good signs that the horse industry in general is becoming more progressive. But as many of you know, various Warmblood registries have embraced this technology for years.

So what's the big thaw about? Advances in the technique of freezing (cryopreservation) of stallion semen have been slowly progressing since the first foal conceived to a frozen semen insemination was born in 1957. These have primarily involved changes in recipes for semen extenders, concentrations of the cryoprotectants used (such as glycerol and dimethyl sulfoxide), variation in packaging (0.25 ml to 5.0 ml straws), and changes in how fast or how slow the semen is either frozen or thawed, or both. In spite of the many attempts at finding the right combination, the 'magic bullet' formula has not been discovered. What is known is that there is a lot of variation between stallions themselves! What may work for one stallion might not be so good for another. The hunt continues. Furthermore, a stallion that may have a really good conception rate when breeding by natural service (in hand), but may have very dismal results when trying to get conceptions from his frozen-thawed semen. This has not only discouraged stallion owners, but to a greater extent mare owners too have become disillusioned. They have gotten generally poor conception results after multiple attempts to get their mares pregnant. Take heart there may be a sunny day on the horizon.

Fertility or the components that go into a successful breeding between mare and stallion boils down to two things: timing and numbers. Both of these have been BIG problems when it comes to the use of frozen semen. Not only is it a challenge for the veterinarian and mare owner to get the mare ready for breeding, but then they must determine her exact ovulation time (or nearly so). Why? This is because frozen stallion sperm usually have a very short life once they are warmed back to normal body temperature. Some stallion sperm when deposited by natural (live) cover at breeding have been known to survive for 5 to 7 days. Sperm deposited by artificial insemination (AI) when collected fresh from a stallion on the farm typically are expected to survive for 48 to 72 hours. But frozen sperm may only survive for 1-6 hours after thawing and AI. The mare's egg is typically viable and readily fertilized for only an 8-hour window of opportunity after ovulation occurs. Sperm from a live cover, fresh AI, or even transported AI can be ready and waiting well in advance of the egg's arrival. Not so with sperm that were frozen, they have to hit the same jump as the egg at almost the same time, with little room for wriggle. Timing is critical, and will remain so.

But how do the numbers count? It has been accepted practice for decades to inseminate between 100 and 500 million live sperm per mare per breeding for optimum fertility. The more the merrier, right? Not necessarily so, too many sperm are an inflammatory challenge to the mare's uterus. She must get rid of the extra sperm, and the inflammatory products associated with their insemination within the next 5 days. The embryo descends from the oviduct to the womb by this time and needs a clean house to develop in. Too many sperm inseminated also wastes stallion resources. Too few sperm, especially too few living sperm are a really big problem also. Which brings up the point of the question, what about the numbers? How many living sperm are minimally needed to get the job done successfully? In truth, only one, but that is not the practical answer. While researching an entirely different problem, it was discovered that as few as 5 million live sperm could result in an acceptable conception rate. That's a big drop from 500 million! But what's the catch? The site of depositing the sperm is the key. Typical insemination of the mare occurs in the uterine body, or in one uterine horn. Low-dose insemination, as it is referred to, deposits semen right at the utero-tubal junction (UTJ), the communication between uterus and oviduct. This places sperm directly at the door, or as close to it as we can conveniently get. The rest is up to them.

The big deal in this technique applies severally to stallions and to mare owners alike. It was previously a challenge for those that are freezing semen to 'package' a sufficient number of live sperm cells into a convenient insemination dose (100 to 500 million live sperm). Stallion owners were disappointed that each semen collection for freezing may only result in 5 to 10 'good' breeding doses. (The lucky few got more). The game now suddenly has changed! Fewer sperm needed for each insemination means more breeding doses per stallion collected. Stallion owners may pay less to get more! Mare owners can appreciate this too. Some may have had direct experience with inseminating a 5cc (ml) dose of frozen semen, which amounts to a soda straw full! Others may have had the fun of thawing and inseminating 8 small straws (0.25ml) at a time for each insemination. Now it may only require one! And a small one at that!

There's got to be a catch, what is it? There is, but it's not a dramatically difficult one. The success of this new technique relies upon deep intrauterine insemination near the UTJ. The best way to get there is by using a flexible endoscope (hysteroscopy) inserted into the mare's uterus and up the horn on the side where the mare is ovulating a large follicle. Many veterinarians already use these instruments for respiratory examination of the throat and upper airway. Appropriately cleaned and disinfected between uses, these same instruments can now serve double duty. Of course the more advanced practitioner will choose to have separate instruments for each. Use of a videoendoscope, as opposed to the standard fiberoptic variety with a simple eye-piece, makes the whole thing much more viewer friendly. There will be an increased cost to the

mare owner to have this newer type of insemination performed, but the potential benefits may outweigh its cost.

Progressive breed registry rules, more breeding doses per stallion, smaller insemination doses per mare, and a simple solution to a problem that has been perplexing the industry for the past 10-20 years in my opinion combines to make a really big thaw. Further benefits to this new method means mares are exposed to less intrauterine sperm challenge. They will have a healthier uterine environment and be better able to nurture the embryo when they conceive to the low dose insemination. It also opens the door, or maybe even Pandora's Box, to the use of sex-sorted stallion semen. What gender do you want your next foal to be, a filly by design or a colt of course? That can be a topic for another sunny day.

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