When Nature Meets Science: Breeding With Frozen Semen

What you need to know and expect when using this breeding method

NATALIE DEFEE-MENDIK

Artificial insemination (AI) allows Mother Nature and science to work hand in hand, providing broodmare owners access to choice stallions worldwide. It also offers a safer breeding method than live cover for the mare and stallion, both from a physical and pathogenic standpoint.

Nowadays, all breed registries but The Jockey Club allow AI using fresh, cooled, or frozen semen. While all three techniques involve collecting semen from a stallion to inseminate a mare—immediately with fresh semen, within 48 hours with cooled, and indefinitely with frozen—only the latter offers the utmost convenience.

Breeding with frozen (cryopreserved) semen is extremely accommodating for both the mare and stallion manager or owner, allowing them the flexibility to fit both horses’ schedules. But, like anything, breeding with thawed frozen semen has its pros and cons. Here, two sources well-versed in the process will fill us in on realistic expectations for success and key points to keep in mind.
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A Head Start (for Her)

Before you start dreaming about foal names, have your veterinarian perform a breeding soundness exam on your mare to estimate her chances for successfully conceiving and carrying a healthy foal to term.

During this evaluation the veterinarian takes into account the mare's history and overall health, as well as any gynecological issues. In addition to conducting a whole-horse exam, ensuring she's physically able to carry a foal, your veterinarian will check the mare's external genitalia and perineal region, vagina, uterus, ovaries, and cervix by way of a speculum exam, palpation, ultrasound, low-volume lavage, biopsy, culture, and cytology.

While this is standard operating procedure for any broodmare each breeding season, it's particularly important for older and/or maiden (never been bred) mares.

Frozen semen is viable inside the mare for about 12 hours

A Head Start (for Him)

Stallion owners interested in freezing semen should start out with the standard stallion breeding soundness exam, in which a veterinarian ensures he's in good shape for breeding and his sperm is pathogen-free (no viruses, etc.) and within the normal ranges for motility, morphology, and count. The next step is performing a test freeze to see how his sperm handles the cryopreservation process, as not all stallions produce semen that freezes well.

During the test process on the stallion's sperm, the more intervention that is involved, typically the more difficult it is to keep the cells healthy," says Paul Loomis, MS, founder and CEO of Select Breeders Services Inc., an international provider of frozen semen services. He says that some stallions' semen might not tolerate being cooled, even if it's acceptably fertile with five cover. Further, some stallions' semen might be fertile after having been cooled, but not tolerate cryopreservation.

The test-freeze procedure involves dividing the ejaculate into samples and trying multiple freezing treatments, extenders, and cooling curves (measurements of the semen's changes in temperature as it cools) for that stallion to determine which, if any, of these procedures produce commercially acceptable semen samples. While there is no industry standard for stallion sperm quality in North America at the moment, Loomis says a standard dose of semen should have at least 30% progressive motility after thawing and contain at the very minimum 200 million progressively motile sperm.

Insemination Basics

Choosing when and how to inseminate a mare with frozen semen all comes down to how many doses the mare owner buys and the breeder supplies. "This changes the way I get a mare ready," says David Scofield, DVM, MS, Dipl. ACT, of SRS Veterinary Services, in Chesapeake City, Maryland. "The one difference we have to think about with frozen semen is that cryopreservation and subsequent thawing may decrease the longevity of the sperm. A dose of fresh or cooled semen is generally viable inside a mare 24 to 36 hours, whereas with frozen semen, we look at a time frame where it's viable inside the mare for about 12 hours."

This means there's a much smaller target window of time for insemination than with other methods. "Research has shown that after six hours from the time of ovulation, a mare's chance of conceiving is lower," says Scofield. "If I have two doses of semen, I try to make sure I inseminate the mare within 12 hours before ovulation and then again within six hours following ovulation, so you're covering ovulation on the front and back side with two separate inseminations."

"If I only have one dose of semen, I breed the mare within six hours of ovulation," he continues, a process which he kicks off with hormonal treatments. "I time her to ovulate in the middle of the day, when I can easily monitor her (confirming ovulation with palpation and ultrasound) to make sure that I am breeding her within that six-hour window. If I have multiple doses of semen, I set the mare up to ovulate overnight, so I breed her last thing before I go home, and then again first thing in the morning after she's ovulated. If you time the mare to ovulate at the right time, using either IGCS (human chorionic gonadotropin) or deslorelin acetate, you can breed the mare with minimal extra exams if you've done your due diligence. It doesn't have to be more intensive than with cooled semen, if you are setting the mare up properly."

He adds that, in some respects, it's easier to breed with frozen semen. Breeders don't have to worry about stallion collection days (if, for instance, a mare owner orders semen on a day the stallion is competing), shipping, and so on. "It takes the stallion out of the timing," says Scofield.

Myth Busting (for Her)

One persistent misconceptions surrounding frozen semen is that it involves intensive management measures to be successful. "Dr. Scofield describes two management issues. One is very similar to cooled semen and basically involves one examination per day, maybe at the most two on the day of ovulation; the other involves checking a mare every six hours until she ovulates," Loomis says. "The difference between these two protocols is strictly dependent upon how much semen is available. When stallion owners sell semen by the dose and mare owners try to purchase a pregnancy at low cost by purchasing only one dose of frozen semen, it forces the veterinarian into intensive management of the mare.

Again, when a veterinarian has access to only one semen dose, the mare must be bred within six hours of ovulation. Inseminate too early and you risk waiting too long for the mare's follicle to erupt, decreasing the chance of conception.

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Myth Bustling (for Him)

You might be jumping the gun if you rule out a stallion whose semen loses fertility when cooled. "Some people think if a stallion's semen doesn't cool well, there's no way he can be a candidate for frozen semen, and that may or may not be true," says Loomis. If the stallion's sperm cells are very sensitive to temperature change, it's likely they'll neither cool nor freeze well. But if the stallion's semen doesn't cool well because of the type of ejaculate he produces (e.g., high-volume, low-concentration), freezing might result in a better outcome, says Loomis.

In natural mating, Loomis explains, the stallion ejaculates in five to seven jets with the first few jets containing most of the sperm, known as the sperm-rich fraction. The following jets consist primarily of accessory sex gland fluid. In live cover, the stallion ejects the sperm-rich fraction directly into the uterus, and the mare excites the excess fluid naturally. "It sounds counterintuitive, but seminal plasma, the ejaculate fluid the sperm cells are in, is a really bad medium for keeping cells alive," says Loomis. "When you take a collection sample in a collection vessel, you are essentially incubating the sperm cells in those fluids. However, the process of preserving sperm—freezing—actually minimizes the cells' exposure to seminal fluids.

"With cooled semen, one of the critical factors in retaining semen quality is adequate dilution (using extenders) of the seminal fluid," he continues. "The procedures for freezing semen, on the other hand, involve removing most of the seminal plasma via centrifugation. If you have a stallion that typically produces high-volume, low-concentration ejaculate, and the cooling dilution rate is not adequate to minimize the effects of the seminal plasma, then freezing that stallion's semen would do better than cooling. In freezing, you centrifuge the semen, taking away most of the seminal plasma and replacing it with a special extender that protects the cells.

Trends: Past, Present, Future

Technological advances in the past decade have propelled the use of frozen semen to mainstream breeding. "Frozen semen has become so much more popular in the last five to eight years than it was 20 years ago," says Loomis, who attributes this trend to developments such as multiple extenders with cryoprotectors and control-rate cell freezers that provide more uniform cooling curves. The ability to screen sperm samples provides further useful information regarding potential fertility. Loomis explains that veterinarians have always looked at sperm morphology and motility, but "we now have really good fluorescent probes that can look at other aspects of semen quality...such as sperm membrane proteins and mitochondrial function, that can be assessed, which is helpful when developing new cryopreservation techniques," says Loomis. In other words, these probes help veterinarians determine how freezing impacts certain cell components that are crucial for fertility.

"With more sophisticated techniques, we can better determine if that particular sample has a high or low potential for fertility," he continues. "We have a lot more tools in the tool chest now and can therefore freeze (sperm of) a higher percentage of stallions in the overall population than we used to. We do a lot of customization of freezing protocols in order to get semen from stallions that, without that customized approach, wouldn't be producing semen that would pass commercial quality standard."

On the female side of the equation, the latest in ultrasonic technology, coupled with greater consistency in ovulation-induction medications, has proven a boon to mare management, making frozen semen a much more viable option.

"With advancements in imaging quality, it's much easier for us to predict what a follicle will come close to ovulating; we can actually see subtle changes in follicular shape, wall thickness, and consistency of intrafollicular fluid—all these things that help me predict when a mare's going to ovulate," explains Scofield. "In addition, we now have better quality control for ovulation induction medication; these products are now manufactured by major pharmaceutical companies—historically they were compounded products, so there was a degree of variability."

More widespread knowledge among breeding professionals has also contributed to breeding protocol consistency.

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Over the years, a full battery of people who are very knowledgeable about breeding with frozen semen have been delivering information to veterinary schools and the symposia we all attend," says Scofield. "So what lies ahead? In one research project that's underway, scientists are looking at identifying markers on the equine genome related to sperm freezing ability. It appears that (viable-ness of semen is a heritable trait—it certainly is in other species," says Loomis. "It's pretty exciting that you might be able to do DNA typing on a stallion to determine what level of success you might have with his semen freezing."