Frozen semen unlimited shelf life

In most cases fresh-cooled semen is used for impregnating mares. However, frozen semen is also an option, Doeke Hoekstra explains. ‘The impregnation results are almost just as good, it does however, require more intensive supervision of the mare.’

TEXT: ALICE BOOIJ | **PHOTOS:** ALICE BOOIJ EN PETRA ZEELEN

Frozen semen is stored in liquid nitrogen at a temperature of -196 degrees.

It’s scarcely used for Friesian horses in the Netherlands but all the more so abroad: frozen semen. Doeke Hoekstra is employed at VDL Stud in Beers, Friesland, and has years of experience with the quality of stallion semen, also frozen semen. ‘Thanks to frozen semen we still see the birth of a foal by Nimmerdor every year’, he says by way of example. ‘Nimmerdor is

a KWPN stallion who was born in 1972; so nearly 50 years ago.'

Friesian stallions outside the season

The VDL Stud, the stallion station owned by the Van de Lageweg Family, houses mainly KWPN (showjumping) stallions whose precious semen is collected for distribution as fresh semen during the stud season and as frozen semen for exporting abroad. With about 1000 horses and 3500 stud services at the peak of the season it is one of the largest stallion stations in Europe. 'We don't have Friesian stallions here at VDL, that is not open for discussion after Tsjerk 328' , Doeke says with a laugh (see background). 'But outside the stud season we do get Friesian stallions here to collect their semen for freezing so that it can be exported overseas.' Friesian stallions that came to his station are for example Tsjalle 454, Jehannes 484, Nane 492, Ulbe 506, and also Teun 505. 'Stallions with very good semen quality', Doeke points out. 'The quality of semen from Friesian stallions keeps getting better', he states. 'On average, their semen is of a slightly lesser quality compared to warmblood horses, which is partly due to their high kinship and the closed Studbook.' The semen of the VDL showjumping stallions has a very high-quality average, Doeke has noticed. 'But this is an open studbook and the stallions have so many different origins. Still, there are Friesian stallions with very good semen and there are warmbloods with low-quality semen.' For the purpose of semen collection the Friesian stallions have to stay at the stud station for at least eight weeks. 'That's because of quarantine regulations. We have to make sure the semen complies with the highest health requirements for export.'

Seven days a week

At the peak of the season the stallions at VDL Stud do their stud services seven days a week. Every jump yields enough semen for around five to ten inseminations. 'For the production of frozen semen the stallions jump only three times a week', Doeke explains. 'That safeguards optimal production and quality for freezing. Ultimately, it is that one sperm cell that does the job of impregnating the mare. Everything involved in semen collection, treatment and processing evolves around as many active sperm cells as possible, because those are necessary to get that one sperm cell in the correct place where it has to go. Doeke has made a thorough study of it and throughout the year works in his laboratory to improve the quality and technique even further. 'When inseminating with freshly-cooled semen a billion sperm cells are injected into the uterus. Thousands enter the Fallopian tube with a few hundred reaching the egg cell.' In the time when they swim from the uterus to the egg cell the sperm cells mature, Doeke explains. 'Part of them temporarily attach to the oviduct wall and are released again over time so that after a minimum of 48 hours following the insemination there still are live sperm cells to fertilise ripened egg cells .'

Under the microscope

After the stallion has performed the stud service it is standard practice to check the semen for quality. The amount, the concentration, shape and motility are all investigated

Foreign countries: Payment per pregnancy

For the sale of frozen semen Doeke prefers a system of payment per pregnancy. 'Breeders in foreign countries pay a compensation of roughly € 100 per insemination dose plus expenses. When the mare's pregnancy is confirmed we send the invoice for the stud service of the stallion in question and complete the registration. Moreover, in partnership with a few stallion keepers in the Netherlands and The Friesian Connection (De Boer Family) in the USA we also offer something like a 'Live Foal Guarantee' for North America. When the mare does not become pregnant with frozen semen she can be inseminated with freshly-cooled semen from stallions stationed at The Friesian Connection, at the same price.'

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Fokkerij | Frozen semen

With improving the process and inseminating technique the results with frozen semen are simular to fresh semen. 'Frozen semen is practically every bit as good', Doeke states.

under the microscope. 'Sperm cells come in many variations: double-tailed sperm cells, crooked mid-sections and deviating acrosomes. But that is not always indicative of fertilising capacity', Doeke points out.
'They look different, but some do the job perfectly', says Doeke, who calls it advanced insight. Yet another check is done to determine the viability of the sperm cells. 'We have a special machine that measures viability/ sustainability. This check is carried out on fresh semen but again after freezing of the semen.'
After centrifuging and the addition of an extender the fresh semen is left to cool down gradually – in 14 ml tubes – from room temperature to 5 degrees Celsius so that it can be transported in a cool box. 'That will keep the semen ready for use for a minimum of 24 hours, which leaves enough time for transportation to locations within Europe.'
At VDL Stud the inseminators get on the road by mid-afternoon so that 80% of semen can be inseminated within 12 hours after collection has taken place.

‘The quality of semen from Friesian stallions keeps getting better’

Extra attention frozen semen

After the quality check, frozen semen gets a different extension than we do with fresh semen, Doeke clarifies procedures. ‘We have to avoid damaging the sperm cells during the process of freezing or defrosting so we add another agent to protect them during freezing.’ The agent added to the semen can vary between stallions, Doeke points out. ‘Not all semen reacts in the same way. We adjust it to guarantee the best quality. And yes, sometimes there are stallions whose semen cannot be frozen.’ After the semen has been inserted into the straws – of 0.5 ml – the meticulous process of freezing begins, which results in semen samples that are stored in liquid nitrogen at a temperature of -196 degrees. ‘That gives them unlimited shelf life.’

A dose of frozen semen contains about 300 million well-shaped, motile and viable sperm cells. ‘Research has shown that this is sufficient to impregnate a mare’, says Doeke, who has consulted a pile of scientific papers and constantly keeps on learning. ‘The technique for equine frozen semen is still quite new. We stay in close contact with people in foreign countries to help each other improve the process and consequently, improve results too.’

Improve insemination process

Successful insemination with frozen semen requires more precision at the moment of insemination. ‘We inseminate closer to the mare’s ovulation’, says Doeke, ‘preferably immediately before or after the ovulation. We also test out new techniques and insemination protocols by using ‘frozen-defrosted-cooled’ semen and less rigorous monitoring by a veterinarian.’ The advice for the best result: use the most fertile mares. Doeke sums up: healthy mares, good condition, not older than fifteen, no problem mares and do make sure the mares are clean. ‘If the mare is difficult to impregnate with fresh semen it won’t be any easier with frozen semen. So best practice is to take mares that have been pregnant before’, Doeke explains while pointing out that egg cells in Friesian horses take longer to ripen. ‘They usually develop bigger follicles before ovulating, ranging from 45 to 60 mm in Friesian mares. Your own vet knows the size of your mare’s follicles.’ Inseminating with frozen semen also differs from fresh semen: preferably deeper into the uterus, closer to the opening of the Fallopian tube. Particularly the intensive supervision of a veterinarian is what makes inseminating with frozen semen a bit more expensive.

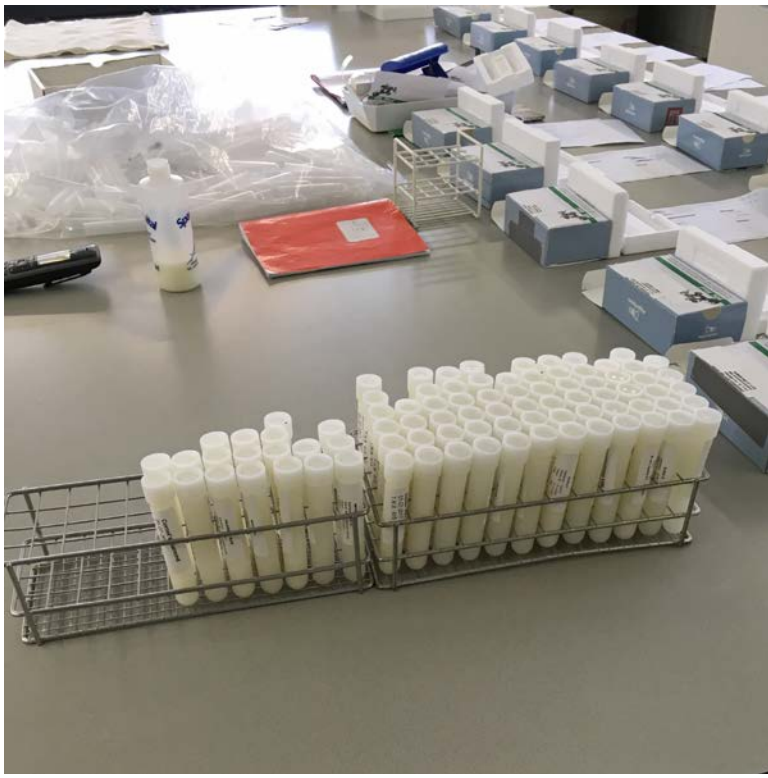
TNB

The quality of semen used for the insemination is closely monitored, Doeke says. ‘Various research has shown that by injecting a minimum of 300 million normal-shaped, motile sperm cells (TNB) into a mare, the number of sperm cells is not the limiting factor in terms of the likelihood of a mare becoming pregnant’, he explains. ‘Injecting more sperm cells therefore does not increase the chances of pregnancy. And for some stallions 100 to 200 million TNB already suffices.’

If freshly-cooled semen is dispatched, especially if it involves overnight travel, we often send a double dose of TNB. ‘This is done to ensure that if part of it becomes damaged during transport

From Tsjerk 328 to stallion keeper and VDL Stud

In his young years foundation sire Tsjerk 328 was stationed at VDL Stud. He was however, not exactly one of the fastest stallions, we learn from Doeke. ‘For Tsjerk it was absolutely normal to take 30 to 45 minutes before he was ready to mount the phantom mare. And sometimes even much longer. If I tell you that in the high season as many as twenty stallions have to perform their stud services before 9 am, then it’s not difficult to figure out that this did not combine too well with the busy VDL schedule.’ Around that time Doeke and his girlfriend Petra Zeelen started their own stallion station (Groot Archem), and they could welcome Tsjerk 328 at their station. Over the next few years and with a bit of patience, Tsjerk could easily service about 200 mares per year. They discontinued their stallion station ten years ago. Meanwhile Doeke has been working in the laboratory at VDL Stud for five years already, he is the owner of various Friesian horses and is a member of the KFPS Member Council.



‘Frozen semen is more vulnerable, so you have to be closer to the moment of ovulation’

there still are a minimum of 300 million TNB left to inject into the mare.’ The same rule applies to frozen semen: one insemination dose contains at least 300 million TNB. The number of straws per dose is determined by the quality and concentration of the sperm cells in the straw. ‘From every ejaculate meant for freezing a few straws are examined for this quality. Usually, one straw contains between 40-100 million TNB’, Doeke states. ‘Over the past few years other methods for insemination have been developed, which means we can use fewer TNB per insemination without diminishing the chances of a pregnancy.’
With normal inseminations the semen is injected into the ‘body’ of the uterus. With Deep Horn insemination the pipette is inserted deeper into the uterus, right to the opening of the Fallopian tube, which is why less semen is needed. ‘We usually use 1/3 of a normal dose.’
A next development is ICSI. ‘With this technique one sperm cell is injected into the follicle. A complicated and expensive procedure, which can however be effective when dealing with problem mares or when very little frozen semen is available.’

Fresh semen ready for transport. At the right fresh semen, in 14 ml tubes, with a minium of 600 TNB.

Closer to the ovulation

Doeke explains that inseminating with frozen semen requires a higher degree of supervision. ‘The semen is more vulnerable so you have to be closer to the moment of ovulation, preferably six hours before or after the ovulation.’
When the follicle is ready hormone injections are used to stimulate the ovulation. In order to determine the exact moment of insemination the mare is checked frequently. Every six hours, so that insemination can be carried our shortly after the ovulation. ‘If you have more than one dose at your disposal then it is possible to use an insemination schedule’, Doeke continues. ‘That gives you the room to inject two doses at fixed intervals after the hormone injection. In 90% of all cases ovulation then takes place within six hours before or after insemination. That means more semen is needed, but it requires less supervision by a veterinarian.’ With this method it is very well possible to achieve the same results as with fresh semen. ‘50-60% pregnancies’, Doeke states. ‘This percentage is only slightly higher for fresh semen, so frozen semen is practically every bit as good.’