In the new millennium, research focused on methods of inseminating and, from the early 1980s’ practice of using a full dose of semen that was thawed and deposited into the body of the uterus, researchers advanced to deep intra-uterine insemination, using extended catheters which deposit sperm at the junction of the uterus and fallopian tube. This gives a 65% success rate and uses doses as low as one tenth of a semen straw, rather than a full dose which can be anything from one to 15 straws.

Why use frozen semen?
The convenience of having semen available at any time, even from stallions on the other side of the world is a huge bonus to mare owners. Frozen semen can be ordered months before the breeding season, can be stored indefinitely and mares can be inseminated without waiting for shipments of chilled semen that may not arrive in time. The advantage for stallion owners is that the stallion can continue with his competitive career without taking time out for the breeding season, semen can be exported to almost anywhere in the world and, should the stallion become ill or die, his semen is still available.

Disadvantages
With frozen semen the timing of insemination is critical and the procedure requires more intensive mare management and more attention to detail than insemination with fresh or chilled semen. Not all stallions produce semen that will freeze successfully and there are few vets who are experienced in the process or have the facilities and equipment necessary to inseminate using frozen-thawed semen. Conception rates with frozen semen are often very low at between 33-65% per cycle compared with 70% per cycle for natural cover or insemination using fresh or chilled semen.

How is it collected and frozen?
Semen is collected from the stallion using an artificial vagina, exactly the same as for chilled transported semen or for semen analysis. Once collected, the semen is evaluated to establish sperm concentration and motility then the sperm are separated from the seminal plasma by spinning the ejaculate in a centrifuge. The resulting pellet of sperm is re-suspended in an extender containing antibiotics, nutrients and substances to protect the sperm during the freezing process. The sperm and extender mixture is then loaded into the straws in which they will be frozen, in doses ranging from 0.5 ml to 5 ml, depending on the laboratory (a single dose of 500 million sperm may be stored in from one to fifteen straws). The straws are frozen in liquid nitrogen vapour for a period of time and, once frozen, they are maintained at -196°C in liquid nitrogen in storage containers.

Not all stallions are suitable
Not all stallions have sperm that will survive the freezing and thawing process, even when it is frozen, stored and thawed correctly. It is estimated that between a quarter and a third of all stallions have semen that, when frozen, thawed and inseminated into a suitably fertile...
mare, will not result in pregnancy. A great deal of effort goes into determining the best way to freeze the semen of each stallion, however, sometimes it just doesn’t work and many stallions with excellent fertility when used with natural service have semen which performs poorly or not at all when frozen and then thawed. Unfortunately the only way to tell whether a stallion’s sperm will survive the freezing process is to actually freeze and then thaw it.

**CHECK FERTILITY OF CHOSEN STALLIONS AND FROZEN SEMEN**

A fertile stallion will produce on average five billion progressively motile sperm (that is, sperm with one head and one tail, moving in a forward direction) in a single ejaculate. It is accepted that 500 million progressively motile sperm are needed for each frozen insemination dose, so one ejaculate can be divided into up to ten insemination doses.

It is important to know the first-cycle pregnancy rate for your chosen stallion’s frozen-thawed semen and how the mares were bred to achieve this pregnancy rate. Were the mares bred before ovulation, after ovulation, or before and after ovulation with a full dose of semen or a dose that was split in half to allow breeding before and after ovulation? Was the semen deposited in the uterine body or was deep-uterine insemination used?

To obtain the greatest chance of a pregnancy, it is important to use exactly the same technique with your mare. Ask to be referred to breeders whose mares are in foal to your chosen stallion or have had foals by him. They will tell you how easy, or how difficult and expensive, it was to get their mares in foal. If you are still not sure, surf the web and check out forums devoted to frozen stallion semen.

**IS YOUR MARE SUITABLE FOR BREEDING WITH FROZEN SEMEN?**

There are a number of important things to take into account before considering breeding your mare with frozen semen. Working with frozen semen requires the mare to be handled a lot as, close to ovulation, she needs to be palpated every couple of hours to determine precisely when she will ovulate and thus when she will be inseminated. She should therefore be well handled and not object to the procedures that will be carried out on her at regular intervals. Flighty or touchy mares are probably not good candidates for frozen semen as stress can adversely affect them going in foal.

A reproductively healthy mare should always be used when attempting a pregnancy with frozen semen. Fertility in the mare declines significantly from the age of about 14 and, while she may not be infertile, conception can be more frustrating, challenging and less successful. Any mare that has previously failed to become pregnant through using frozen semen should not be used again and older maiden mares are not good candidates because, in many of them as they age, the cervix is not able to relax properly. This is important as insemination with frozen semen initiates an inflammatory response in the uterus and this often produces fluid which, if it cannot drain through the cervix, increases the inflammation and reduces the likelihood of her becoming pregnant.
PROCEDURE FOR INSEMINATING WITH FROZEN SEMEN

Any time you manipulate semen, the per-cycle pregnancy rate decreases. Generally, the conception rate is highest for natural service. It is lower for chilled shipped semen and lower still for frozen-thawed semen. Normally a mare will cycle and ovulate every 21 days. Follicles containing eggs develop on the ovaries toward the end of each cycle and matures usually ovulate when these follicles reach 5 cm in diameter. After the egg is released from the follicle it enters the top of the fallopian tube and travels towards the uterus, entering the uterus only if it is fertilised while it is in the fallopian tube.

Sperm released into the uterine body swim into both horns of the uterus, eventually reaching and entering the fallopian tubes. By scanning the mare's ovaries, the vet knows which one is producing a mature follicle and can deposit the thawed sperm directly at the junction of the uterus and fallopian tube on that side, a procedure which has a significantly higher success rate than dumping the sperm in the uterine body. After the sperm have swum into the fallopian tube and fertilised a waiting egg, the fertilised embryo descends into the uterus and fizzes around for five or six days before attaching to the uterine wall. The pregnancy should be detectable by an ultrasound scanner 12-14 days after ovulation.

When inseminating with frozen semen, in the build-up to ovulation the mare's ovaries will be scanned at least once a day and as she gets closer to ovulating she will be scanned every two to four hours until ovulation occurs. For some reason, freezing sperm changes its makeup, enabling it to instantly fertilise the egg when they meet. By trial and error, researchers have found that the closer to ovulation the sperm is inseminated, the better the chances of success. Inseminating within three hours of ovulation gives a much better conception rate than inseminating before ovulation or three to six hours after.

There are three means of artificial insemination using frozen semen. The easiest and cheapest is to inseminate the full dose of semen into the mare’s uterine body. However, the conception rate for this procedure is very low at only 37-40% per cycle. Full dose insemination would not be recommended if you are being charged per dose of semen used or if the stallion you have selected has low fertility. On average, it takes three attempts (over three oestrus cycles) using full dose insemination to get each mare pregnant and this has consequent increases in costs for agistment and three lots of scanning and inseminating.

In deep-uterine insemination, a special catheter is inserted through the cervix and deep into the uterine horn on the side on which the ovulation occurred. This allows the semen to be deposited closer to the junction of the fallopian tube and uterine horn than by inseminating into the body of the uterus. This technique allows efficient use of the semen it is deposited where it is needed. However, it can sometimes be difficult to get the catheter all the way up the uterine horn, in which case deep-uterine insemination may be no more efficient than inseminating into the body of the uterus. The conception rate per cycle using deep-uterine insemination is 50%.

Hysteroscopic Insemination (HI) uses a flexible endoscope with a video camera and bright light on the tip. As it is passed through the cervix the uterus is inflated with filtered air, enabling the scope to be steered easily into the uterine horn to the utero-tubal junction. When the tip of the scope reaches the point where the fallopian tube enters the uterus, a highly specialised narrow catheter is threaded through the scope to the end of the fallopian tube. The semen (usually only a single straw is needed) is deposited precisely in the right spot by an operator who can see exactly what he is doing. By depositing the semen so close to the site of fertilisation, the sperm arrives fresh and in adequate numbers, with minimal wastage. Conception rates using HI are 65-68%, close to conception rates achieved with natural service.

CHECK THE VET, HIS FACILITIES AND THE COST

Do you have access to a vet who is experienced with the challenges of frozen semen and who has top-of-the-line ultrasound scanning and laboratory facilities and a good track record of getting mares in foal using frozen semen? Can you see the facilities for yourself? Is he prepared to show you previous years’ results using frozen semen? Are you happy for your mare to be hospitalised there before and during the insemination process and are you comfortable with the people who will be handling her while she is there? Are they prepared to work around the clock to ensure your mare becomes pregnant? Do you understand the extra costs involved with using this technique?

Breedling contracts vary enormously but most frozen semen sold in Australia is on the basis of a cost per dose, payable in advance and offering no guarantee and no money back if your mare doesn’t conceive. There are no guarantees for semen from stallions with low fertility so it is wise to ensure you breed to a stallion whose fertility is as high as possible. Read the fine print in any contract before you sign.

HOW LONG DOES FROZEN SEMEN LAST?

If frozen and stored correctly, it is believed that frozen semen will remain viable indefinitely. Frozen bull semen stored in the 1950s is still highly fertile and until recently the longest that horse semen had been frozen and stored before being thawed and used successfully anywhere in the world was 10 to 12 years. However, in March this year, Dennis O’Halloran of Kilkivan in Queensland arranged for 23-year-old semen from his deceased stallion, Hollywood Return* to be tested. Much to Dennis’s delight, the semen is still viable and he plans to use it to breed two mares this season.