

FREEZING CANINE SEMEN

The ability to collect, freeze and store semen from dogs was first described in 1954. Since that time, improvements in freezing and insemination techniques, in conjunction with better methods to monitor the optimum time to inseminate the bitch during her cycle, have meant that these reproductive technologies are a commercial reality.

Why freeze canine semen?

There are many reasons for freezing canine semen. Perhaps the most important reason is to preserve and insure the breeding potential of a dog against loss, death or infertility. With the ability to be stored indefinitely, frozen semen does not degenerate, and we have had pregnancies as a result from semen that was over 30 years old!

Furthermore, freezing semen allows for the transport of genetic material both within and between countries. This obviously increases the marketing potential for stud dogs located in countries such as New Zealand where shipment of dogs to the northern hemisphere for mating is impractical, and likewise allows for the importation of genetic material into New Zealand from overseas, thereby increasing the gene pool.

The use of frozen semen, rather than natural mating, also allows a dog to be used for several breeding's on the same day and in different places.

When is the optimal time to freeze semen from my dog?

Ideally semen should be collected from males that are between 18 months and 4 years of age. From 5 years of age onwards, the incidence of prostatic disease increases, which can have an adverse effect on semen quality. While an older male can still be frozen, a better collection can be expected from a young, mature dog. Therefore, it is better to collect and store a dog at a young age based on potential, rather than wait until a male is in great demand but unfortunately much older.

Ensure the male has been fit and healthy both at the time of collection and for the previous few months. A sick, or stressed dog will not provide a quality collection. Furthermore, a high fever or other illness in the recent past can adversely affect semen quality for several months after the event.

If the male is over 6 years old, has a history of prostatic disease, or has questionable fertility, it may be advisable to have a semen evaluation and "test-freeze" performed before committing to storage of large amounts of semen.



The semen freezing process

Once the semen is collected a sample is removed and assessed under the microscope for a number of parameters including motility, concentration, morphology, and cytology. At this point, if semen quality is marginal, a decision can be made to delay freezing to another time, or to investigate potential causes for the poor quality semen. If the semen is of good quality and meets the minimum required standards to be frozen, the sperm rich fraction of the ejaculate is diluted in a special fluid called "semen extender" and cooled to 4°C over several hours before being loaded into 0.5 ml straws and rapidly frozen to -196°C in liquid nitrogen. The freezing extender contains a number of ingredients to protect the sperm through the cooling, freezing and thawing process, including egg yolk, antibiotics, and chemicals to protect the sperm during freezing (cryoprotectants).

Once frozen, one "test straw" is immediately thawed in order to assess how well the sperm survived the freeze-thawing process. The motility and forward progressive motion of the thawed semen is assessed using a powerful microscope immediately after thawing and then again at 10 minutes after incubation at 37°C.



How much semen should I freeze?

There are many different semen-freezing techniques. Semen can be frozen in either pellets or in straws, however, there is no difference in pregnancy rates between semen frozen in each of these forms.

More important factors affecting the quality and fertility of frozen-thawed semen is the operator (someone with experience and understanding in sperm cryobiology and semen freezing), age of the male at the time of collection, inherent fertility of the male, quality of semen at time of collection, semen freezing technique and freezing extenders.

At TCI Glenbred, we use the Minitube canine freezing technique and extenders. This involves freezing semen in 0.5 ml straws with a total of 100 million sperm per straw. The recommended minimum number of motile sperm required per “intrauterine artificial insemination (AI)” or per “dose” or “mating” is 100 million. However, at TCI GlenBred we aim for at least 150 million sperm per mating. Therefore, the number of straws required per mating with frozen semen depends on how well the semen freezes and at what concentration the semen is frozen at. On average, 2 straws per mating are required.

The number of straws frozen per semen collection varies between breeds and individuals. Anything from none to 25 straws can be collected. An average collection will produce approximately 5 to 10 straws.

Once the semen is frozen, it is stored at -196°C in a large tank containing liquid nitrogen. Once frozen the semen can be stored indefinitely in liquid nitrogen.

Shipment of frozen semen: National and international export and import Once frozen, semen can be shipped within and between countries in specially designed shipping containers.

There are different import requirements for different countries and unfortunately these can change without notice. Furthermore, there are health requirements that must be met at the time of collection of the ejaculate if intended for exportation. A large amount of paperwork is often involved with the exportation and importation of frozen semen. Further information can be obtained from the MPI website: www.mpi.govt.nz Or AQIS website: www.agriculture.gov.au/biosecurity

HOW THE PRICING WORKS

For a semen collection and assessment, the fee is \$285.00. It is then \$74.50 per straw, the number of straws we get depends on factors such as the breed and age of the dog. So for example 4 straws is \$298.00. Storage with TCI GlenBred per year is \$95.00. (Prices current at time of publication and subject to change)

Artificial insemination (AI) with frozen semen

The freeze-thawing process significantly reduces the sperm's lifespan in the bitches' reproductive tract compared to fresh or fresh-chilled semen. Furthermore, usually lower numbers of sperm are available per mating, i.e. 100 million motile frozen-thawed sperm compared to 400-3000 million sperm in a natural mating! For these reasons, deposition of frozen-thawed semen directly into the uterus is essential to maximise the chances of pregnancy and increased litter size.

There are two intrauterine AI methods:

Transcervical Insemination (TCI):

This is a technique developed in New Zealand by Dr. Marion Wilson in the 1980s. This is a non-surgical, non-invasive insemination technique whereby a catheter is passed through the cervix into the uterus using a specialised rigid endoscope. The semen is then flushed through the cervix and deposited directly into the uterus. It has the advantages of being anaesthetic and sedation free, non-surgical and relatively stress free. It also has the distinct advantage in comparison to surgical AI in that more than one insemination can be carried out during the bitch's heat.

Surgical insemination:

This involves the bitch undergoing a general anaesthetic so that an incision can be made into the abdomen to allow the exteriorisation and catheterisation of the uterus. Once exteriorised semen is injected into the uterine horns. At TCI GlenBred, surgical AI is reserved for cases where the cervix cannot be catheterised during TCI. This happens in around one in every 500 bitches.

Regardless of the type of insemination, the most critical variable is the timing of the procedure to match the bitches' fertile period. Accurate ovulation timing is essential because of the reduced lifespan of frozen/thawed sperm and is accomplished by a series of blood tests for progesterone levels and vaginal cytology assessment to identify the most fertile period. See article on “Artificial Insemination in Bitches”.

