

## FREEZING CANINE SEMEN

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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 breeding methods 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. This provides for the stud dog to sire puppies when he is no longer able to do so. Freezing semen also 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 breedings on the same day and in different places.

The most obvious benefit of frozen semen is the ability to store it indefinitely. Once semen is frozen it does not degenerate, and there have even been pregnancies reported from bull semen that is over 40 years old! So frozen semen can well and truly outlive the normal lifespan of the donor.

### 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 has 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 (two to five years old). 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 in the 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-what is involved?

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 then 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. The ingredients include egg yolk, antibiotics, and chemicals to protect the sperm during freezing (cryoprotectants).



Fig 1 Canine sperm viewed under the microscope

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 at 10 and 30 minutes after incubation at 37°C.

## How much semen should I freeze?

There are many different semen freezing techniques. There is no difference in pregnancy rate between semen frozen in pellets or straws. More important factors affecting the quality and fertility of frozen-thawed semen is 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 Glenbred, we use the "Uppsala" canine freezing technique and extenders which has been developed and published by Dr Catharina Linde-Forsberg from The Swedish University Veterinary School. 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 "intra-uterine artificial insemination (AI)" or per "dose" or "mating" is 100 million. 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 20 straws can be collected. An average collection will produce from 5 to 10 straws.

Once the semen is frozen, it is stored at  $-196^{\circ}\text{C}$  in a large tank containing liquid nitrogen. Once frozen the semen can be stored indefinitely in liquid nitrogen.



Fig 2: Photograph of large liquid nitrogen storage tanks holding thousands of straws and vials of frozen canine semen.

## 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.



Fig 3: This is a picture of a “dry shipper” container used to transport frozen semen all over the world.

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 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 MAF website: [www.maf.govt.nz](http://www.maf.govt.nz). Or AQIS website: <http://www.daffa.gov.au/>

### 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 400-2000 million sperm in a natural mating! For these reasons deposition of frozen-thawed semen directly into the uterus is recommended to maximise the chances of pregnancy and increased litter size.

*There are two intrauterine AI methods:*

- a) Transcervical Insemination (TCI): This is a technique developed in New Zealand by Dr. Marion Wilson in the 1980s. It involves placing a

rigid scope, with a camera attached, into the vagina. A special catheter is then guided through the cervix and into the uterus which deposits the semen directly into the uterus (see Fig 3). Most bitches in heat will tolerate this procedure without sedation. There have been some recent developments with the TCI scope. The newer scope ('renouretroscope') is longer and slimmer which facilitates the insemination of large breed bitches and maiden bitches which can have reduced space in the 'birth canal'. (see Fig 4)



Fig 4: Transcervical insemination procedure and visualisation of catheterisation of the cervix, and semen being injected into the uterus (photo courtesy of Dr Marion Wilson, 2003).

- b) 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. (See Fig 5)

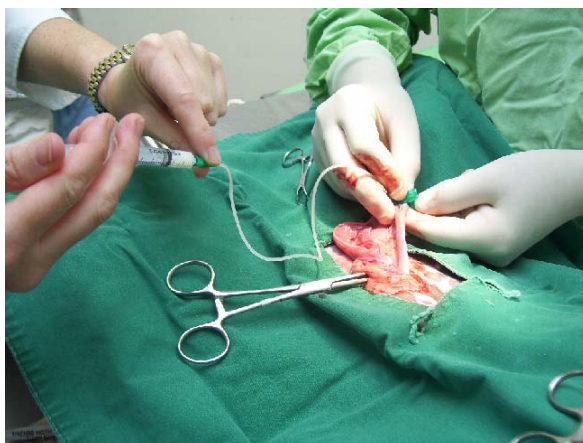


Fig 5: Photo of " Surgical AI". The bitch's uterine horns exteriorised through a surgical incision in the abdomen to allow for catheterisation and injection of the frozen-thawed semen.

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*".