Veterinary Reproduction Consultancy Pty Ltd.

Canine Semen Freezing

Canine semen* freezing was first described in 1954. Since this time, improvements in freezing and insemination techniques have proven semen freezing to be an invaluable tool in canine breeding and reproduction.

Advantages of Semen Freezing?

1. **Preservation** of bloodlines and valuable genetics **forever**
2. **Insurance** against loss, death or infertility secondary to age/disease
3. Enables **transport** of your bloodlines worldwide

What are the optimal circumstances for freezing canine semen?

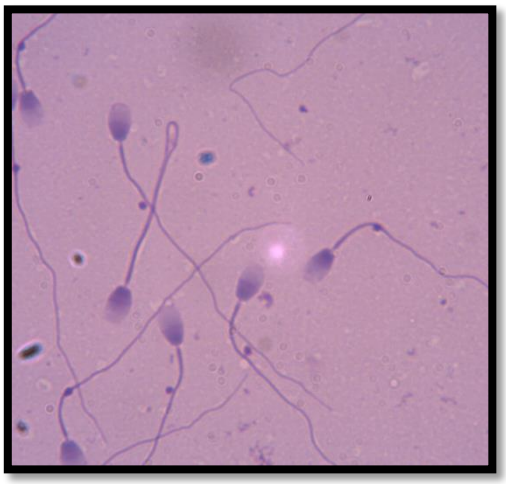
- It is best to freeze semen when your stud is YOUNG
  - In general, the optimal **age** for semen freezing is **2 years old** (and in some smaller breeds even earlier)
  - Semen quality and fertility significantly deteriorate in dogs **greater than 6 years old** secondary to prostate and testicular degeneration/disease
- There is **no** requirement to 'clean out' your dog prior to your consultation – in fact this can reduce the number of sperm available for freezing
- **A teaser bitch* is required to be present at any collections requiring freezing**. The presence of a teaser
  - Increases the **likelihood** of collection (particularly important in boys that have not been collected before or are used for natural matings)
  - Improves the **quality** of the ejaculate
  - Increases the **quantity** of the ejaculate, therefore supplying **more breeding units**

What is involved with canine semen collection?

At VRC Dr Hyatt uses the 'open hand' technique to collect an ejaculate. This technique is chosen as there is no involvement of plastic sheaths and cones that some studs can become adverse to. The entire collection process takes just a few minutes.

The ejaculate is divided into three parts, or 'fractions', that are released consecutively:

1. **Pre-sperm fraction**: This is clear fluid that does not contain sperm. We do not collect this fraction but its purpose in a natural mating is to lubricate the reproductive tract.
2. **Sperm- rich fraction**: When the ejaculate starts to become opaque the second fraction is being released. This fraction contains all of the sperm* cells, therefore being the important fraction for collection.
3. **Prostatic fluid**: This is the clear fluid released after the sperm- rich fraction is completed. It does not contain sperm cells. In a natural mating, this fluid is released during the tie to help push the semen through the vagina and up to 100 mls can be released. We collect a small portion of this fluid to assess prostatic and testicular health.



Following collection the sperm- rich fraction is assessed under the microscope and is visible to you via the microscope viewing screen.

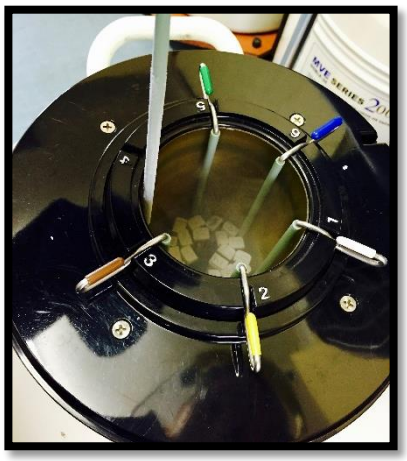
The freezing process

VRC uses the internationally renowned 'Uppsala' freezing technique and extenders developed by Professor Catharina Linde-Forseberg from Sweden. Semen is frozen in 0.5ml straws with 2 straws being the usual breeding unit if quality is sufficient.
The freezing process is a lengthy technique requiring approximately 7 hours to complete. A small amount of the sample is frozen in a 'test straw' which is then thawed to assess how the sample has withstood the freezing and thawing process.

It is important to note that there are many types of semen extenders and methods of semen freezing utilised worldwide. However the most crucial factor that determines the post-thaw quality of frozen semen is the skills and knowledge of the person doing the collection and freezing.

Storage of frozen semen

Frozen semen is stored in specialised tanks (image below) containing liquid nitrogen at a temperature of -196˚C. Once frozen, semen can be stored indefinitely.



How successful are inseminations with frozen semen?

The freeze- thawing process is rigorous and greatly impacts on sperm viability. When using frozen semen we anticipate it to last only **12 – 24 hours**, significantly less than the 5-7 days of fresh ejaculated semen. Some frozen semen will last longer than this however we never assume this will be the case.

The international minimum dose of frozen-thawed canine semen is **100 million motile sperm**. This is also significantly less than fresh, ejaculated semen which contains anywhere from 200 million to 3 billion sperm dependent on breed and size.

As you can appreciate from these facts, success of frozen semen insemination is not a given. It is dependent on a number of factors:

1. **Accurate timing**: With the anticipated survival time of frozen semen so low, accurate timing of insemination when the bitch has ovulated and her eggs are ready for fertilisation is of utmost importance
2. **Site of sperm deposition**: At VRC we use non- invasive transcervical insemination (TCI) to deposit semen right into the uterus where the eggs are waiting for fertilisation.
3. **Selection of dam**: A young (<5yo) and fertile (proven with fresh semen) bitch presents the ideal scenario for a successful insemination
4. **Selection of semen**: Utilising high quality (as determined by a thawing assessment) semen from a young, proven male is recommended.

Reported pregnancy rates with frozen canine semen vary from 0-100% with the international average pregnancy rate after AI 75%.

*Definitions:

Semen	Male reproductive fluid in its entirety. Semen contains sperm, fluid from the prostate and testicles, and may contain other cells.
Sperm	Abbreviation of 'spermatozoa', sperm are the cells resembling tadpoles that contain male DNA and fertilise the egg of the female.
Teaser Bitch	A bitch in standing heat (usually day 10-15 of heat), presence of which greatly improves the quality and quantity of a semen collection from the male.
Sperm morphology	Microscopic assessment of the form/shape of the sperm cells in the ejaculate. This enables us to determine the likelihood of fertilising ability of these cells. Morphology does NOT alter the phenotype of the offspring.
Sperm motility	Microscopic assessment of the movement of the sperm cells in the ejaculate. This is usually provided as a % of sperm motile in the sample.
Thawing	'Defrosting' of the frozen semen sample to regain fertilising ability.