



VET CHAT

Canine Frozen Semen

The why(s), when(s) and what(s)

by *Dr Jasmin Hyatt BVSc (Hons) DipACT MANZCVS (Reproduction)*

Registered Specialist in Veterinary Reproduction. Veterinary Reproduction Consultancy Pty Ltd (02 6301 9550)

Most of our dog breeders are, at the very least, aware of frozen semen. Many of you have had semen frozen from your studs, used frozen semen for breedings and imported/exported frozen semen doses internationally. So WHY do we rely on it so heavily, WHEN should we be collecting and using it, and WHAT actually is 'frozen' semen?

The WHY(s)?

There are significant advantages of frozen semen:

- Preservation and storage of the gene pool indefinitely
- Insurance from a young age against unexpected death or infertility
- Transport of genetic material both nationally and internationally
- Availability of the stud dog for multiple breedings on the same day internationally.

The WHEN(s)?

1. WHEN to freeze the MALE

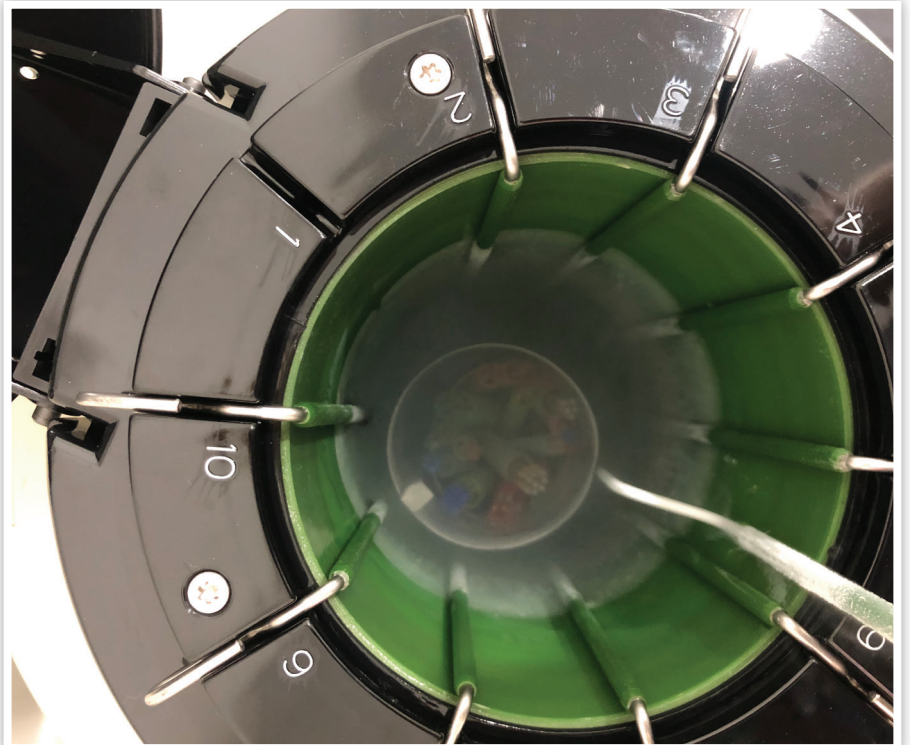
The ideal age for collecting semen from stud dogs for freezing is **between 18 months and 3 years of age**. At this age the stud is most likely to provide the best quality and quantity of semen, ensuring the collection consultation is as productive and cost efficient as possible.

From **6 years of age onwards**, the incidence of **prostate disease** increases, which has an adverse effect on semen quality. We also know that semen frozen from dogs over 6 years of age has an inherent lower fertility when compared with semen frozen from dogs under 6 years old.

Service dog breeding organisations **freeze all stud dogs by the age of 2 years**, regardless of expected potential. This ensures a high-quality sample is frozen and their future genetic potential is insured, much before we have decided on the working ability of the individual stud. This is an approach that breeders with valuable lines would be remiss to sidestep.

2. WHEN to inseminate the FEMALE

It is ideal to use frozen semen in **proven** bitches under 5 years of age. Of course, we perform many inseminations where this is not the case, however bitches in this category provide us with the highest chance of conception when using valuable frozen semen doses.





VET CHAT

WHY?

Frozen semen undergoes a rigorous process to enable indefinite storage in liquid nitrogen. For this reason, on thawing we anticipate good quality frozen semen to **survive 12-24 hours** in the bitch. This is in comparison to fresh semen which we anticipate to last 3 days (and actually can last up to 10!).

Additionally, the international minimum for an intrauterine frozen semen dose is 100 million motile live sperm. In comparison, a fresh or chilled ejaculate from a medium breed dog will contain on average **EIGHT times this amount**.

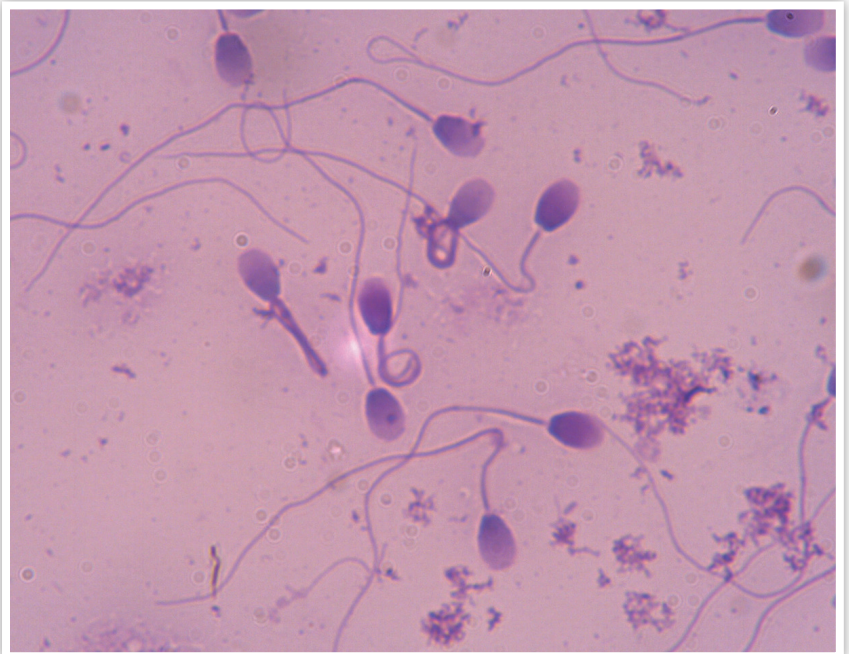
So it is only understandable that when performing inseminations with frozen semen we have to be selective and fastidious; it **doesn't survive long**, the sperm have been **stressed**, and the **numbers** are significantly **reduced**.

The WHAT(s)?

Collection: The process of semen freezing starts with separating the canine ejaculate into its' three fractions – the clear pre-sperm, white **sperm-rich fraction** (the important one!), and the final prostatic fraction.

Freezing: There are many different methods of processing to get this initial fraction ready for 'freezing' practiced worldwide. First we determine the **total number** of sperm in the ejaculate, enabling us to add the correct amount of "**extender**" to create the desired **concentration** of sperm cells per straw. The extender we use contains antibiotics, energy forms, and a protective agent against cold. We also assess the morphology (shape) of the sperm to count how many have abnormalities (*see image right*).

Image below: Abnormal sperm morphology: Note the curled up tails



This sample is then slow-cooled to 4° C over several hours before adding a second extender with an increased concentration of the protective agent. If the sperm are exposed to either reduced temperatures or the protective agent **too quickly** it is very detrimental – this slow method ensures they have time to **adapt** to their gradually changing environment and get through the rigorous process as happily as possible.

There is **NO** difference between sperm frozen in straws or those frozen in vials – what determines fertility is the skill of the facility personnel that freeze it and the extender freezing technique that was performed. At VRC we load our semen into 0.5ml straws prior to rapidly freezing in the -196° C in liquid nitrogen vapour.

Thawing: Once frozen, one "test straw" is immediately thawed in order to assess how well the semen survived the freeze-thawing process. The motility and forward progressive motion of the frozen-thawed semen is assessed using a powerful microscope immediately after thawing and at 10 minutes after incubation at 37° C. If a good quality post-thaw sample is achieved, the intrauterine insemination dose is 2 straws (total 200 million sperm).

Storage: Once the semen is frozen, it is stored at -196° C in a large tank containing liquid nitrogen. Frozen semen can be stored forever in these tanks.