

Cryopreservation of canine semen

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Introduction

Freezing the valuable stud dog's semen preserves his genetics virtually indefinitely, well beyond the expected age-related decline in his fertility. In Australia, strict biosecurity protocols favour the import of frozen rather than fresh, chilled semen.

Selecting the semen freezing candidate

Semen freezing is best performed when a dog is at peak fertility. Prior to accepting a dog for semen freezing, a breeding soundness examination should be performed, including a thorough clinical examination with attention to potentially heritable defects. Identification of the dog should be verified through a microchip number, correlated to the pedigree certificate, at every semen collection. A thorough semen evaluation, including semen motility and morphology, should be performed. The freezing process is known to be detrimental to semen quality;¹ thus, only dogs with good to excellent semen quality should be selected for semen freezing. Ideally, this will be dogs with a progressive sperm motility above 70%² and a similar minimum proportion of morphologically normal sperm.

Semen extenders

A number of semen extenders are commercially available. Several semen extender recipes have also been published.³ Although details regarding the exact composition of a commercial extender and expected success rates with its use may be withheld by a supplier, commercial extenders are often practical and are expected to have undergone adequate quality control measures. The choice of extender may depend on whether a one-step or two-step freezing process will be followed, the availability of an extender, and the reliability and reputation of the company supplying a commercial extender.

The semen freezing process

Canine semen freezing involves the addition of a cryoprotectant, usually glycerol, in a one- or two-step process. The steps denote the number of sequentially increasing glycerol concentrations. The author is currently using a commercially available one-step protocol, with reasonably good post-thaw motility rates. Significant individual differences exist in response to semen freezing.⁴ It is therefore possible that a particular dog's semen that performs poorly during freezing with one particular protocol or extender, may benefit from attempting semen freezing using alternative extenders or testing minor variants of the semen freezing protocol.

Straws versus pellets

A common query when considering dog semen freezing is whether to freeze in straws or pellets. The general consensus among reproduction specialists seems to be a preference for semen straws, and it is generally assumed that little difference in semen quality exists between the two freezing methods. Personal experience has found semen frozen in pellets to be reasonably easy to handle and thaw, apart from the need to loosen the cap of a vial containing pellets soon after removal from liquid nitrogen to avoid

explosion of the vial and dispersal of its contents. Nevertheless, major reservations regarding the use of pellets arise from: (i) a perceived greater risk for contamination by infectious agents and foreign spermatozoa and (ii) risks regarding the identification of pellets, which can be easily transferred between vials.

Straw/vial identification and accompanying documentation

Just as identification of the dog is extremely important during the semen freezing process, so too is clear identification of the frozen semen produced. Semen straws are easily identified using an automated straw labelling machine or a permanent marker suitable for frozen semen straws. The following information should be included on each straw:

Clinic name • Registered name of dog • ID number (microchip) • Species • Breed • Date • Operator initials

Paperwork detailing the verified identification of the dog, identification of the semen straws (including straw and plug colour if relevant), the final concentration of semen frozen, the extender used, and the progressive motility of a thawed test straw should be held for all semen in storage and should accompany the semen once shipped. The recommended number of straws per insemination dose and the recommended thawing procedure should be included.

Thawing of frozen semen

Thawing instructions should always be provided in the paperwork accompanying a shipment of semen. For 0.5 mL straws, thawing at either 37°C for 30 seconds or 70°C for 8 seconds is usually recommended. Research suggests that, in general, thawing at 70°C results in better post-thaw semen quality than thawing at 37°C.⁴⁻⁶ Some practitioners prefer to thaw at 37°C in the belief that this is less dangerous, however in the author's experience thawing at 70°C for strictly 8 seconds can be done safely. Many specialists also advise the use of a thaw medium; the rationale being that glycerol is toxic to thawed spermatozoa and therefore the high glycerol content of freezing media should be diluted immediately post thawing. As with many factors in dog semen freezing and reproduction in general, the influence of a thaw medium on semen fertility is difficult to test. Dilution at a ratio of one part semen to two or four parts thaw medium was found to maintain sperm motility better than that of undiluted semen after 8 hours of incubation at 38°C.⁷ The extent to which this study resembles *in vivo* conditions is open to debate. The author currently avoids the use of thaw media unless provided with the semen or specifically requested in the thawing instructions, primarily because a thaw medium adds one more variable with potentially detrimental effects on semen quality. Also, semen is thawed only once access to the uterus has been achieved and is inseminated immediately, which could conceivably limit the length of time motile spermatozoa are in contact with glycerol.

References

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