Protocol

Freezing of Dog Semen

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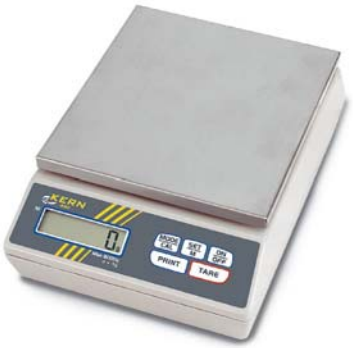
The following protocol is specifically designed for the freezing of dog semen in 0.25 ml or 0.5 ml straws:

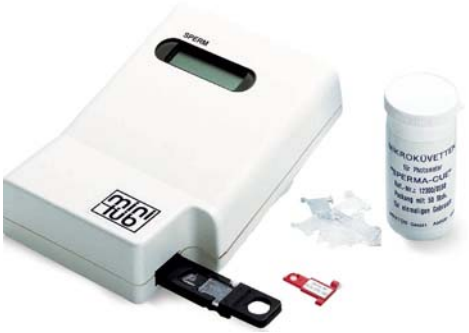
- Step 1: Semen Collection
- Step 2: Measure Concentration and Motility
- Step 3: Calculate TSE and Dilute
- Step 4: Centrifuge and Re-suspend
- Step 5: Determine the End-dilution
- Step 6: Fill the Straws
- Step 7: Freeze Semen Straws
- Step 8: Semen Thawing and AI Dose Preparation
- Step 9: Insemination Techniques

Step 1: Semen Collection

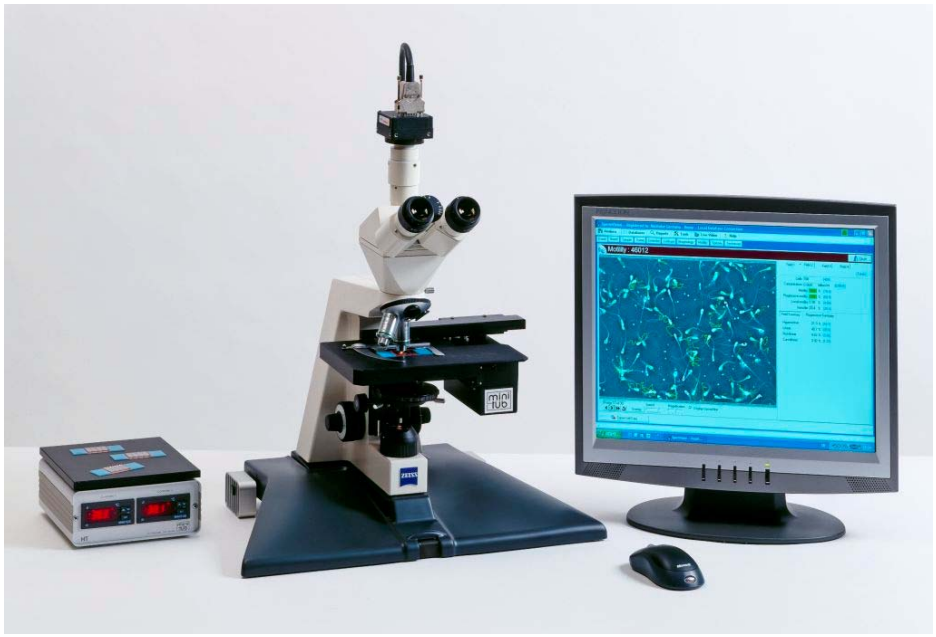
- Collect the sperm rich fraction of the dog semen using manual stimulation and funnels for collection. Measure the volume, preferably with a laboratory balance.



Step 2: Measure Concentration and Motility



- Determine the motility with a microscope and the sperm concentration with a photometer SpermaCue or, ideally, use the Sperm Vision™ CASA system for measuring both parameters.



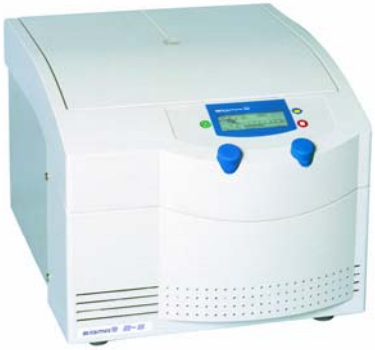
Step 3: Calculate TSE and Dilute

Minimum requirements for freezing dog semen:
Number of sperm cells per ejaculate: 700 x 10^6
Percentage of progressive motile sperm ≥ 60%
Percentage of normal sperm ≥ 70%

- Calculate **T**otal **S**perm cells count in **E**jaculate (TSE) by multiplying the volume of the ejaculate by the number of sperm cells per ml.
- Dilute the ejaculate with CaniPro™ Freeze Part A without egg yolk. The medium must have the same temperature as the ejaculate. Dilution rate is 1 + 1.

Step 4: Centrifuge and Re-suspend

- Centrifuge the semen with 700-800 g for 10 to 15 minutes. Centrifugation of the ejaculate is especially important if there is urine or blood in the ejaculate. Remove the supernatant and re-suspend the pellet with CaniPro™ Part A with egg yolk at ambient temperature to the initial volume or further if semen is highly concentrated. Place semen into a fridge at +5°C for a minimum of 2 hours.



If centrifugation is not necessary, the sperm rich fraction is diluted 1+1 with CaniPro™ Part A with egg yolk at semen temperature, and placed into a fridge at +5°C for a minimum of 2 hours.

Step 5: Determine the end-dilution

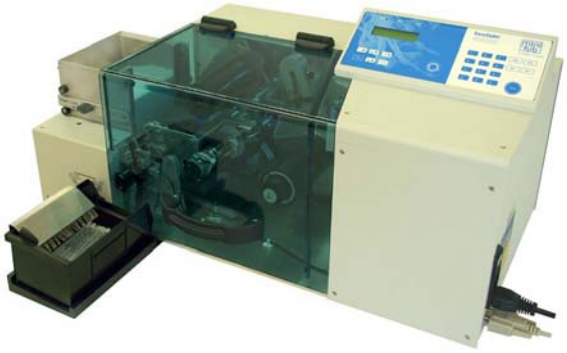
- Determine the number of the sperm cells in the re-suspended pellet with a counting chamber.

Total number of spermatozoa per breeding unit: 150-200 million. In most systems the final concentration is 50-100 million spermatozoa per 0.5 ml straws and 2-4 straws are used per AI.

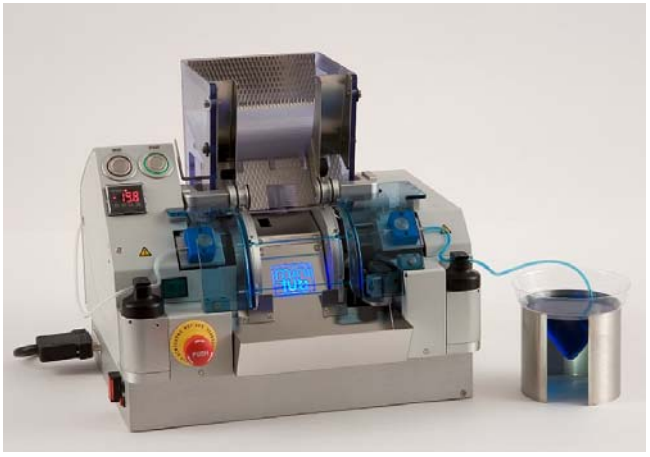
- Perform final dilution with CaniPro™ Part B with egg yolk at +5°C.
- Equilibrate at 5°C for 20 to 60 minutes while filling into straws.

Step 6: Fill the Straws

- Fill the final diluted semen in pre-printed 0.5 ml straws. The printing is preferably done using the EasyCoder straw printer. Cool printed straws to +5°C.

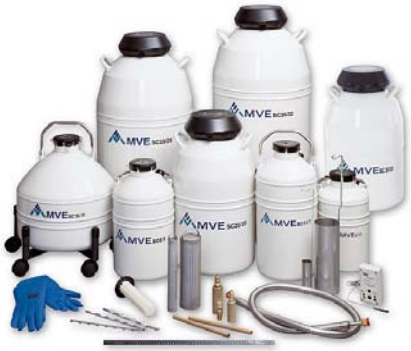


- For filling and sealing, ideally use the automatic Filling and Sealing machine MPP Uno or a manual method. Filling and sealing has to be performed at +5°C in a cooling cabinet or transportable cooler.

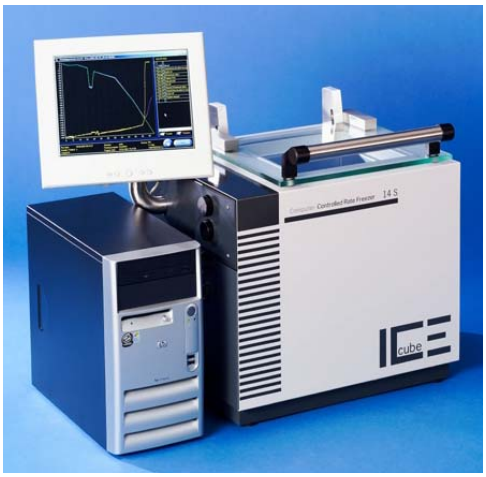


Step 7: Freeze Semen Straws

- For manual freezing, place the straws on a freezing rack and position the rack horizontally in nitrogen vapour at a distance of 4 cm above liquid nitrogen, in a Styrofoam box for 10 minutes. Afterwards, straws are plunged immediately into liquid nitrogen for storage.



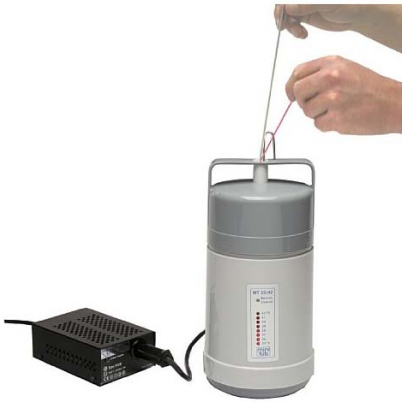
- Alternatively, a programmable Freezer (IceCube) can be used for freezing the straws. In general, freezing rates of -12 ºC/min or -28 ºC/min give good results.



- Store frozen straws in LN storage container within goblets and goblet canes.

Step 8: Semen Thawing and AI Dose Preparation

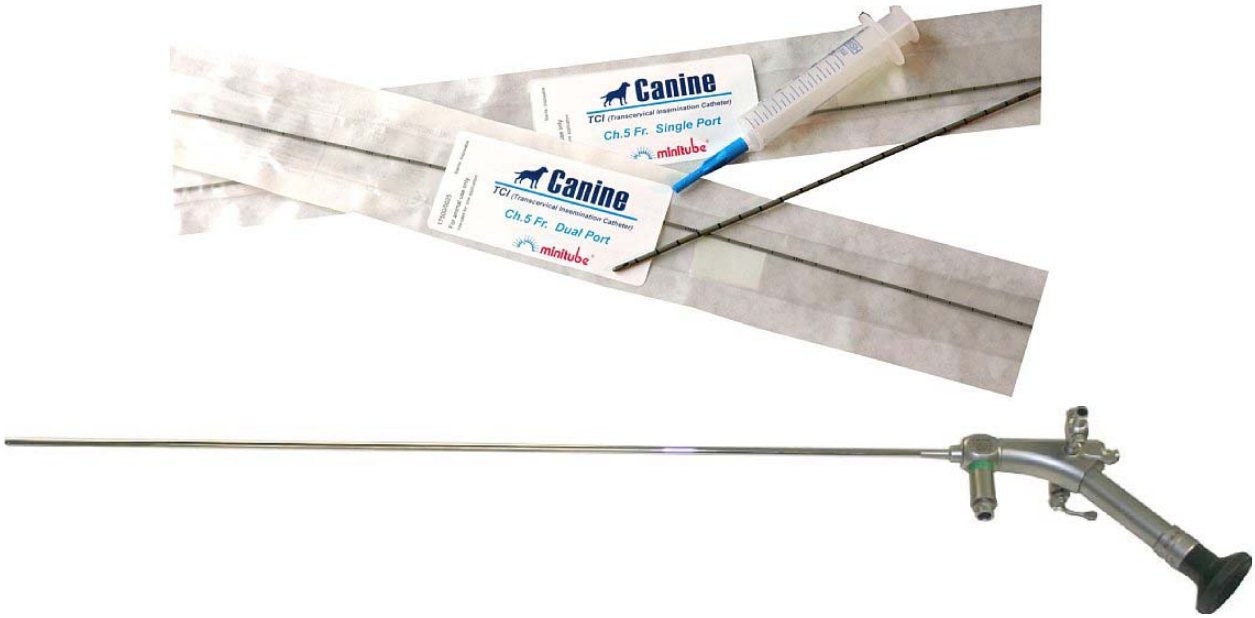
- Remove straws from the liquid nitrogen container and immediately transfer to the thawing unit at +37ºC. The number of straws may vary per insemination dose.



- Thaw 0.5 ml-straws for 45 to 60 seconds at the same time or one after the other.
- Dry the straws by wiping them with a clean paper towel.
- Shake the air bubble to the sealed end (opposite to the cotton plug) and cut the sealed end holding that end up.
- Re-dilute semen with CaniPro™ AI.

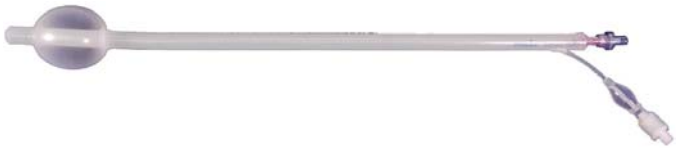
Step 9: Insemination techniques

Artificial insemination in bitches can be performed using vaginal deposition of semen or intrauterine deposition of semen. Intrauterine AI is performed by using a rigid endoscope (Minitüb TCI endoscope, Ref. 23700/1510) to visualize the cervix and a TCI catheter to transverse it.



For vaginal AI, the person should learn to locate the cervix of the bitch through abdominal palpation in order to be able to deposit the semen in the correct place.

For vaginal AI, we recommend to use the MiniMAVIC, MAVIC 250 or MAVIC 400 catheter.



Appendix: Equipment and Supplies

CaniPro™ Freeze A&B	(13700/0060)
CaniPro™ AI	(13700/0070)
Canine semen collection set	(17500/0200)
Semen collection funnels	(11521/0000)
Glass or plastic beakers	(15423/0500)
Lint-free paper towels	(15230/9001)
Balance	(14295/0444)
Water bath	(14078/0001)
Centrifuge	(14602/0515)
Pump for removing supernatant	(15520/0005)
SpermaCue	(12300/0500)
Microcuvettes, 50/bag	(12300/0550)
Fixed or variable pipette	(12425/4810)
Disposable pipette tip	(12427/0255)
Phase contrast microscope	(12004/0331)
Micro slides	(15400/2400)
Cover glasses	(15401/0990)
Capillary tube, disposable	(15404/1000)
Counting chamber „Thoma neu“	(15150/4771)
Straws 0.5 ml	(13408/0010)
Sealing balls	(13400/9900)
EasyCoder	(13038/0000)
Colour ribbon for EasyCoder	(13038/0010)
IceCube 14S	(16821/1000)
Accessories for IceCube	
Liquid nitrogen	
Aluminum Cane	(16965/6013)
Goblets, 13 mm	(16913/0133)
Tweezers	(17060/0025)
Cryo protective gloves	(23830/0520)
MVE Cryogenic container	(16520/2006)
Thawing device	(17043/3542)
Endoscope for insemination	(23700/1510)
TCI Catheter for endoscope	(24000/0115)
MAVIC™ mini, 120 mm	(17500/0052)
MAVIC, 400 mm	(17500/0070)
MAVIC, 250 mm	(17500/0075)

Sperm Vision® system and accessories: please ask your Minitüb contact.