Extender Preparation:

- Warm both bottles of CaniPRO ApX² Culture Medium for Freezing Canine Semen - Part A & Part B to room temperature.
- Add 20% of egg yolk to volume of CaniPRO ApX² Freeze Part A & Part B (i.e. add 4 ml of egg yolk to a 20 ml bottle of media; add 2 ml of egg yolk to a 10 ml bottle of media). Gently mix.
- Keep Part A bottle at room temperature to be used in Step 1.
- Cool and use Part B bottle at 4°C to be used in Step 2.
- Use only the sperm rich fraction of the ejaculate (2nd fraction) for freezing. Do not include the first fraction of the ejaculate (clear prostatic fraction before sperm rich fraction) or the third fraction (prostatic fraction post-sperm rich fraction) of the ejaculate in the sample to freeze because the quality of the semen will decrease.

Washing of semen: If the ejaculate collected is contaminated with prostatic fluid, wash the semen with the CaniPRO ApX² Freeze - Part A. In order to wash it, dilute 1 part of semen in 3 to 5 parts of CaniPRO ApX² Freeze - Part A (i.e. 1 ml of semen in 3 to 5 ml of CaniPRO ApX² Freeze - Part A). Centrifuge at 700g for 10-15 minutes. After centrifugation, discard the supernatant and re-suspend the sperm pellet to original ejaculate volume using CaniPRO ApX² Freeze - Part A.

Freezing Process:

- Step 1
  Slowly add Part A of the Freeze, using 1 part of semen per 1 part of Freeze - Part A (i.e. 2 ml of semen, then 2 ml of CaniPRO ApX² Freeze - Part A). Cool extended semen at 4°C for 2 hours minimum.

- Step 2
  After cooling 4°C for a minimum of 2 hours, slowly (1 to 3 minutes) add Freeze - Part B, which must be pre-cooled at 4°C. The addition of Freeze - Part B containing glycerol is absolutely required for the freezing process. The volume of - Part B to be added must be the same amount of the semen volume collected (i.e. if the sperm rich fraction collected was 2 ml, then add 2 ml of Part B).
  Fill the straws, which must be pre-cooled at 4°C, seal them and keep them at 4°C for 20 – 60 minutes.
  Put the straws on a rack above liquid nitrogen (4 - 5 cm above the LN2) for 20 minutes.
  Plunge the straws in liquid nitrogen.

Thawing:

- Thaw the straws at 38°C for 1 minute
- For best results dilute the frozen/thawed semen in CaniPRO ApX² AI (13574/0270), 1 part of thawed semen in minimum 1 part of CaniPRO ApX² AI (i.e. 0.5 ml of thawed semen in minimum 0.5 ml of CaniPRO ApX² AI) before performing the surgical or TCI insemination.

To perform insemination with thawed canine semen using CaniPRO ApX² AI (13574/0270):

- Warm extender to 38°C
- Thaw frozen semen at 38°C for 1 minute. Empty straws into a tube.

Continued on next side
• Slowly dilute the thawed semen in CaniPRO ApX² AI, 1 part of thawed semen in minimum 1 part of CaniPRO ApX² AI (i.e. 0.5 ml of thawed semen in minimum 0.5 ml of CaniPRO ApX² AI).

• Perform surgical or TCI insemination using 1-3 ml of extended semen depending on the size of the bitch.

Preparation & Addition of Egg Yolk for CaniPRO ApX² Extender

• Obtain fresh eggs.

• Wash, rinse, dry and store in a refrigerator until use.

• Place a metal egg separator over a 400 ml beaker.

• Crack the egg and separate the yolk using the above separator and beaker.

• When the yolk and white have separated sufficiently, place the yolk with membrane intact on a folded square of paper towel.

• Roll the yolk very gently to remove any excess white. The egg yolk can be aspirated from the membrane using a 5 ml syringe and a 16-18 gauge needle. Alternatively the yolk membrane can be lanced and the yolk drained into a beaker or measuring cylinder. It is very important that the yolk membrane not be used in the extender, as it may be harmful.

• Carefully add the exact amount of egg yolk required by the extender using a calibrated instrument (syringe, pipette, etc).