Boar semen is collected using the hand glove technique and the gel fraction is removed using sterile gauze. The semen is diluted 1:1 (v:v) with Androhep Plus or Androstar (37 °C; Minitube, Verona, WI), cooled to 23 °C over 1 hour on the laboratory bench and then to 15 °C over 1.5 hours. The samples are then shipped to the repository at 15 °C for cryopreservation.

Upon receipt, the samples are centrifuged for 10 minutes at 800 x g at 15 °C. The supernatant is removed and the sperm pellets are consolidated by boar. The sperm concentration and motility are determined using spectrophotometry and a Hamilton Thorne motility analyzer (Beverly, MA), respectively (at least 5 fields of analysis and 500 sperm).

The samples are first diluted to 600 x 10^6 sperm/mL with 15 °C BF5 cooling extender (CE; see recipe section) and then cooled to 5 °C over 2 to 2.5 hours. The second dilution is performed drop-wise using 5 °C freezing extender (FE; see recipe section) over 5 minutes so that the samples are diluted to a final concentration of 400 x 10^6 sperm/mL. The samples are loaded into 0.5 mL CBS or wick and powder (aka French) straws and frozen using the Cryo Bio System Mini Digitcool UJ400 (IMV Corporation, Minneapolis, MN) with the following curve: 5 °C to -8 °C at -20 °C per minute, -8 °C to -120 °C at -69 °C per minute, -120 °C to -140 °C at -20 °C per minute. The samples are then plunged in liquid nitrogen for storage.

Samples are thawed for 20 seconds in a 50°C water bath and motility analysis is performed as described previously.


Beltsville Freezing Extender 5 (BF5)

**Cooling extender (CE)**
- 52mM TES
- 16.5mM Tris
- 178mM D-glucose
- 20% Egg yolk (v:v)

The CE should be centrifuged at 10000 x g for 25 minutes to remove egg yolk particles

**Freezing extender (FE)**
- 6% Glycerol (v:v)
- 2.5% Equex paste (v:v)
- 91.5% CE (v:v)
Artificial insemination:

Standard intracervical insemination (2 inseminations per sow or gilt) is performed using $1 \times 10^9$ motile sperm, per insemination, diluted to a final volume of 80 mL in Beltsville Thawing Solution (Pursel and Johnson, 1975) and inseminated.

Deep intrauterine insemination of swine is performed according to Roca et al. (2003). The semen straws ($1 \times 10^9$ motile sperm) are thawed, as described previously, and pooled. The sample is loaded into a syringe and inseminated as described by Martinez et al. (2001). After deposition of the sample into the uterine horns, an additional 2 mL of BTS is used to flush the insemination catheter and remove the remaining sperm sample.

References:

