



Bull Semen Processing and Cryopreservation

Semen Processing

The sperm concentration and motility of the semen sample are determined and the samples are diluted to 120×10^6 sperm/mL in 37 °C Tris-Egg yolk A (TCA; see recipe below).

- If the samples will be transported overnight then they are placed in a shipping box for cooling to 5 °C and shipping per the Bull Semen Collection and Shipping Protocol.
- OR-
- If samples will not be held for an extended period of time (shipping) and will be frozen onsite, then they are placed in a 37 °C water jacket and cooled to 5 °C over 2 h.

After reaching 5 °C, or when the samples arrive at the laboratory for freezing, the samples are diluted 1:1 (volume to volume) with 5°C Tris-egg yolk B (TCB; see recipe below). This results in a final sperm concentration of 60×10^6 sperm/mL. The samples are then loaded into 0.5 mL semen straws.

Cryopreservation

Samples can be frozen one of two ways:

1) Box freezing: Samples are placed on a rack and frozen in liquid nitrogen vapor (4 cm above liquid nitrogen) for 10 min and plunged into the liquid nitrogen for storage.

2) Programmable freezer: The samples are frozen using the Cryo Bio System Mini Digitcool UJ400 (IMV Corporation, Minneapolis, MN) with the following curve: 5 °C to -10 °C at 5 °C/min; -10 °C to -110°C at 40 °C/min; -110°C to -140°C at 20°C/min and then plunged into liquid nitrogen for storage.

Cryopreserved samples are thawed for 30 seconds in a 37°C water bath.

Semen Cryopreservation Media Recipe from: Purdy and Graham, 2004.

Tris-Egg yolk A (TCA): 200 mM Tris, 65 mM citric acid monohydrate, 55 mM glucose

Tris-Egg yolk B (TCB): TCA with 14% glycerol by volume

Both of these solutions can be frozen in aliquots until the day of use when they can be thawed and used as described.

Reference: P. H. Purdy and J. K. Graham. 2004. Effect of cholesterol-loaded cyclodextrin on the cryosurvival of bull sperm. *Cryobiology* 48:36-45.

Originally created May 2015