USDA ARS National Animal Germplasm Program

Bull Semen Collection, Transportation, Processing and Cryopreservation Protocol

Semen collection and processing:

Collect semen from sexually mature bulls via an artificial vagina or electroejaculation.

Inspect sample to ensure it is free of urine and other contaminants.

Determine the sperm concentration and motility using spectrophotometry and a Hamilton Thorne motility analyzer (Beverly, MA), respectively (at least 5 fields of analysis and 500 sperm) or microscopy and a hemocytometer.

Add antibiotics per the National Association of Animal Breeders (NAAB) Certified Semen Services standards (https://www.naab-css.org/). The antibiotics are added to the neat semen and both cryopreservation media. Consult the NAAB website for the timely information regarding the appropriate antibiotics and dosages.

Dilute the samples in a 15 or 50 mL tube to 120 x 10^6 sperm/mL with 37 °C Tris-egg yolk A (TCA; see recipe below).

If the samples will be transported overnight then, after dilution with TCA, they are placed in a 37 °C water jacket and cooled to 5 °C over 2 hours in a refrigerator, packaged in an Impact Shipper (see Transportation section below) shipped to the National Animal Germplasm Program laboratory.

-OR-

If samples will not be held for an extended period (e.g. shipping) and will be frozen on sight, then, after dilution with TCA, they are placed in a 37 °C water jacket and cooled to 5 °C over 2 h.

When the samples reach 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 x 106 sperm/mL. The samples are then loaded into 0.5 mL CBS or wick and powder (aka French) semen straws.

Semen cryopreservation:

Samples can be frozen one of two ways:

Box freezing: Samples are placed on a rack and frozen in liquid nitrogen vapor (4.5 cm above liquid nitrogen) for 10 min and plunged into the liquid nitrogen for storage.

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.

Thawing:

Thaw samples for 30 seconds in a 37 ºC water bath and evaluate motility as described previously.

Artificial insemination:

Following estrous synchronization, single semen straws are used per insemination. Either single or double (2 inseminations per estrus) inseminations may be performed.

Transportation:

Please see the Impact Shipper Protocol on the Animal GRIN webpage noting the specific temperatures for each species and type of tissue:

https://www.ars.usda.gov/plains-area/fort-collins-co/center-for-agricultural-resources-research/paagrpru/docs/animal/animal-protocols/

Recipes:

Preferred 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 solutions can be frozen in aliquots and then thawed and used as described.

NOTE: The NAAB lists alternative Tris-Egg yolk, milk and other acceptable freezing diluents for use with bull semen. However, if holding/shipping the samples is necessary then the use of the milk and other diluents is discouraged because the post-thaw quality will be diminished.

References:

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

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