



Rooster Semen Cryopreservation Protocol

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Semen is collected from roosters by digital manipulation and the sample is observed to make sure it is free of feces and urine. Following collection the sample is diluted to approximately 200 μ l with Lakes Low Temperature non-glycerol medium (LLT; see recipe below) which was pre-cooled to 5°C. The samples are then placed in a rack in a styrofoam box containing ice and transported to the laboratory. Then the samples are diluted 1:2 (v:v; sample to cryopreservation medium) with LLT glycerol medium and loaded into 0.5ml straws. The samples are then frozen in liquid nitrogen vapor (2.5 in above liquid nitrogen) for 10min and plunged into the liquid nitrogen for storage.

Frozen samples are thawed in a 5°C water bath for 2 min and then warmed to room temperature for use or analysis.

Recipes:

Low Lakes Temperature (LLT) Medium

Non-glycerol

169.1mM Sodium glutamate-monosodium salt
33.3mM Fructose
4mM Magnesium Acetate (4H₂O)
62mM Sodium Acetate (Anhydrous)
3.7mM Potassium citrate
pH 7.5

Glycerol

Identical to the medium above except it includes 16% glycerol (by volume)

Reference:

Seigneurin, F., Blesbois, E. 1995. Effects of the freezing rate on viability and fertility of frozen-thawed fowl spermatozoa. *Theriogenology*. 43, 1351-1358.