USDA ARS National Animal Germplasm Program

Cattle Oocyte Collection, Transportation, Maturation and Vitrification Protocol

Collection and maturation:

Aspirate follicles between 2 and 8 mm using an 18-gauge needle and a vacuum pump (50 mmHg; 28 mL/min) or hypodermic needle.

Grade oocytes and only retain those classified as grade I (homogeneous cytoplasm and at least 3 layers of cumulus cells) or grade II (homogeneous cytoplasm and 1 or 2 layers of cumulus cells) for vitrification.

Mature oocytes in *in vitro* maturation medium (De La Torre-Sanchez et al., 2006; please see recipe listed below) in a 38.5 °C atmosphere of air containing 5% CO_2 for 22 hours.

Oocytes can be collected, graded and diluted in maturation medium, as previously described, and then transported using a portable incubator set at 38.5 °C so that the oocytes can be vitrified in a laboratory setting.

Oocyte vitrification:

Following maturation, wash groups of 5 oocytes through three 100 µL drops of Vitrification Medium 1 for a total of 3 min.

Wash a group of 5 oocytes through a single 100 µL drop of Vitrification Medium 2 for 45 to 60 s.

Load individual oocytes onto a vitrification device (e.g. Cryotop from Kitazato Corp, Tokyo, Japan or Vitringa from INGÁMED, Maringá, PR, Brazil) and plunge each directly into liquid nitrogen. Cap the vitrification device per the manufacturer's instructions. Ensure that no residual vitrification solution is present on the vitrification device when plunging the oocytes.

Oocyte thawing:

Warm thawing media to 37 °C.

Thaw oocytes by placing the frozen devices containing the vitrified oocytes into Thaw Medium 1 for 1 min.

Transfer the devices/oocytes to Thaw Medium 2 for 3 min.

Remove the oocytes from the devices and wash them 2 times in Basal Medium.

Culture for IVF.

Recipes:

Maturation medium recipe
HEPES-buffered TCM-199 supplemented with 1.0 mM glutamine, 0.2 mM Na-pyruvate, 0.1 mM cysteamine, 15 ng/mL ovine follicle stimulating hormone [NIDDK-oFSH-20], 1 µg/mL bovine luteinizing hormone [NIH-LH-S1], 1 µg/mL oestradiol 17β, 50 ng/mL human recombinant epidermal growth factor, and 0.5% fatty acid-free bovine serum albumin.

Basal Medium
80% TCM-199 and 20% FBS

Vitrification Medium 1
7.5% ethylene glycol, 7.5% dimethyl sulfoxide, and 85% Basal Medium

Vitrification Medium 2
15% ethylene glycol, 15% dimethyl sulfoxide, 0.5 M sucrose and Basal Medium

Thaw Medium 1
1 M sucrose in Basal Medium

Thaw Medium 2
0.5 M sucrose in Basal Medium

Reference:
De La Torre-Sanchez, J.F., Preis, K., Seidel Jr., G.E. 2006. Metabolic regulation of in-vitro-produced bovine embryos. I. Effects of metabolic regulators at different glucose concentrations with embryos produced by semen from different bulls. Reprod. Fert. Dev. 18:585-596.

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