Removal of Zona Pellucida with Pronase in Cattle

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Materials

Phosphate buffered saline (PBS) - one can use either Dulbecco’s PBS for ease of preparation, 10 mM KPO₄, pH 7.4 containing 0.154 M NaCl or the same buffer with NaPO₄.

8% (w/v) paraformaldehyde stock solution: Dissolve 8 g of powdered paraformaldehyde in 100 ml water. Heat and stir (55-60°C – do not go higher). Add a few drops of 2 N sodium hydroxide until the solution clears. Make fresh each day. Alternatively, 8% paraformaldehyde can be purchased from Electron Microscopy Sciences as a custom formulation in 4 ml aliquots (cat. no. 15710-SP). Throw away whatever is not used in one day.

4% (v/v) paraformaldehyde: 1:1 solution of 8% paraformaldehyde stock solution and PBS. Make up on the day of use.

Protease (0.1%, w/v) - weigh 0.1 g protease, add to a volumetric flask and add 100 ml of PBS (0.1 M). Aliquot in 1 ml tubes and store at -20°C until use.

Protocol

1) Remove the embryos from the culture drop and wash them at least 3 times in PBS containing 1 mg/ml polyvinylpyrrolidone (PBS-PVP).

2) Put the embryos (as few as possible at each time) in a 50 µl drop of 0.1% protease and immediately watching them using a high magnification microscope that allows you to see the zona clearly. Leave the embryos in the drop until the zona is removed (up to 1 – 5 minutes). BE CAREFUL!!! Otherwise the protease will digest the embryo too!!!!!! Differences between stages of development from oocyte to expanded blastocyst may affect the time necessary for protease action.

3) Wash embryos very quickly 3 times in PBS-PVP and place them in a 50 µl drop of paraformaldehyde solution [4% (w/v) in PBS]. The digestion seems to stop only when the embryos are in the paraformaldehyde drop (so be fast!!!!).

4) Keep embryos in the paraformaldehyde drop for 1 h at room temperature for fixation.

5) Wash the embryos 3 times in a 50 µl drop PBS/ PVP by transferring the embryos from drop to drop (2 min for each wash).

6) Store the embryos at 4 °C in 4-well plates until further use in other procedures.

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