Differential Staining of IVM/IVF and Cloned Sheep Embryo

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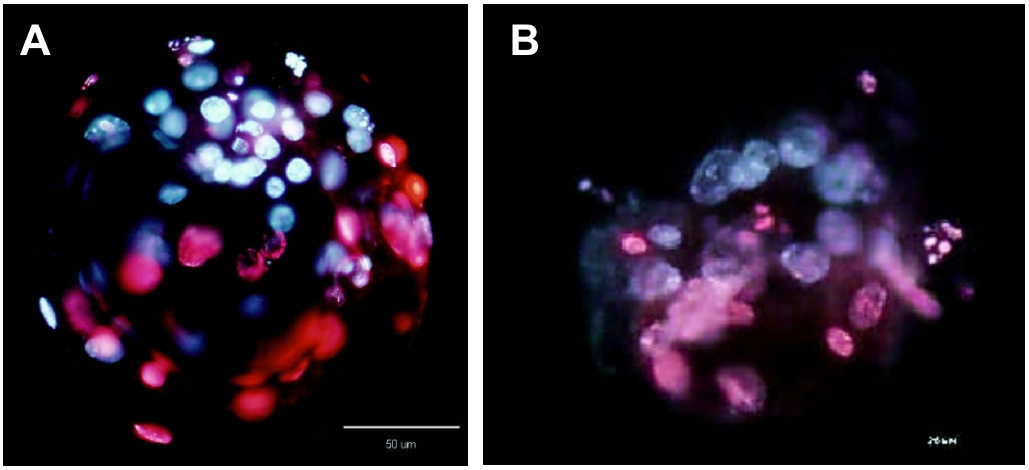

IVM/IVF Sheep embryo *Cloned sheep embryo*

Differential staining of the trophectoderm (TE) and ICM was performed according to the method described previously (1). The zona-intact blastocysts were first incubated in 500µl of 100µg/ml propidium iodide (PI; Sigma) with 1% Triton X-100 in serum free T6 medium for up to 45s or until trophectoderm visibly changed color to red and shrank slightly under a dissecting microscope. The blastocysts were then immediately transferred into 500µl of 25µg/ml bisbenzamide (Hoechst 33258; Sigma) in 100% ethanol at 4 ◦C overnight. Fixed and stained blastocysts were subsequently mounted in glycerol and observed under a microscope with an ultraviolet lamp (Nikon) and excitation filters (460 nm for blue and red fluorescence, and 560 nm for red only).

Reference:

1. Thouas GA, Korfiatis NA, French AJ, Jones GM, Trounson AO. Simplified technique for differential staining of inner cell mass and trophectoderm cells of mouse and bovine blastocyst. Reprod. Biomed. Online 2001; 3: 25–29