

## ENHANCED DEVELOPMENT OF ONE DAY OLD CAPRINE EMBRYOS WHEN CO-CULTURED WITH OVIDUCTAL EPITHELIAL CELLS IN TCM-199

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In the past, a major hindrance to the development in vitro of domestic animal embryos to a stage where they could be stored or transferred was the provision of a suitable culture medium. Earlier attempts at in vitro culture usually failed to develop the embryos past the eight cell stage (Eyestone *et al.* 1987). Compact morulas (embryos containing 32 to 64 cells) are more suitable for transfer and freezing.

Enhanced development of 1 and 2 cell ovine and bovine embryos respectively will occur when co-cultured with oviductal epithelial cells (Gandolfi and Moore 1987) and (Eyestone and First 1989). These studies found that 97% of ovine and 46% of bovine embryos developed into morula.

In this study, embryos containing 1 to 4 cells were surgically collected from 5 2-year-old, Angora does 36 hours after mating. The embryos were pooled and 18 were randomly selected and cultured in Tissue Culture Medium, TCM- 199 (plus 10% foetal calf serum) and 20 were cultured in TCM-199 (plus 10% foetal calf serum) containing a lo-day-old monolayer of oviductal epithelial cells.

After culturing for 6 days, embryos from both groups were fixed in 1% formalin and stained with Hoechst 33342 DNA stain. This is a fluorescent stain which shows up the cell nucleus when examined in the dark with an ultra violet microscope.

None of the embryos cultured in TCM-199 developed past the 8 cell block stage, while 68% of the 20 embryos co-cultured in TCM-199 with oviductal epithelial cells developed past this stage, with 50% developing into morulas.

The number of cells in each fluorescent-stained embryo was easy to count up to 32 but became difficult after 64 cells. This difficulty was caused by a flat plane effect on the microscope. Because the embryo is spherical, movement of the focus through the embryo made it impossible to focus on all cells in the embryo at the same time. However the authors were confident that cell numbers observed were sufficient to classify the embryos as being between the morula and blastocyst stage ie 32 to 128.

The mean cell numbers in embryos cultured in the TCM- 199 only medium was  $4.06 \pm 0.41$ . While it was impossible to accurately count the number of cells in the co-cultured embryos, the mean cell count was well over 32.

The results indicate that it is possible to develop 1 to 4 cell caprine embryos past the 8 cell block by co-culture with oviductal epithelial cells. Fifty per cent of these embryos developed past the 32 cell stage and were classified as morulas. None of the embryos cultured in TCM- 199 only, developed past the 8 cell stage.

The annual value to Australia of goat meat for export is \$25 million (Elliott 1995). With the recognition of Australia's large feral goat population as a valuable resource when crossed with meat goats such as the Boer, there is as a result a large demand for Boer goats. Multiple ovulation and embryo transfer programmes, as well as *in vitro* fertilisation and culture of oocytes, are a way of satisfying this demand.

The results of this project suggest that goat embryos show similar development responses to cattle when co-cultured with oviductal epithelial cells and is one means of developing the embryos to a transferrable stage.

ELLIOTT, T.K. (1995). Master of Applied Science (Rural Management) Thesis, University of Queensland.

EYESTONE, W.H. and FIRST, N.L. (1989). *J. Reprod. Fertil.* 85: 715-20.

EYESTONE, W.H., VIGNIERI, J. and FIRST, N.L. (1987) *Theriogenology* 27: 228 (Abstract).

GANDOLFI, F. and MOORE, R.M. (1987) *J. Reprod. Fert.* **81: 23-8.**