

## STRESS APPLIED TO EWES AT DAY 2 AND 3 OF GESTATION INCREASES DAY 6 EMBRYO CELL COUNTS

***M. Butler<sup>2</sup>, G. Hinch<sup>2</sup>, J. R. Hill<sup>1</sup>***

<sup>1</sup>CSIRO Livestock Industries, Armidale, NSW, Australia; <sup>2</sup>Rural Science and Agriculture, University of New England, Armidale, NSW, Australia

The effect of stress during pregnancy on fetal development has been demonstrated in studies such as the administration of glucocorticoids at 4 weeks of gestation which then 'programs' arterial blood pressure to be elevated during subsequent adult life (1). In the current experiment we have explored the effect of an acute stressful event applied to pregnant ewes at Day 2 and 3 of gestation.

The morphology and cell counts of Day 2 and 3 embryos were evaluated following surgical collection from 15 superovulated ewes. Seven of these ewes were subjected on Days 2 and 3 after insemination to a 15 min period of shearing (partial), isolation and confinement adjacent to a working dog. These stresses were intended to produce a short-term cortisol peak at the time embryos were at the 4–8 cell stage.

Embryos recovered from the 7 treated and 8 control ewes were graded according to IETS guidelines using a Nikon TE 300 inverted microscope. Cell counts were performed following staining of embryos with Hoechst 33342. Only embryos with >8 nuclei were included in the analysis to exclude embryos that were either unfertilised or died prior to the stressful events.

Subjective grading showed no difference in grading embryos as transferable (stage of compact morula or later with quality grade of 1 or 2) between embryos derived from stressed and non-stressed ewes (45/63 v. 45/64). However cell counts were dramatically different as stressed ewes produced embryos with higher cell counts ( $74.7 \pm 32.0$  v.  $43.3 \pm 22.9$ ;  $P < 0.001$  using one-way ANOVA).

As it is presumed that cell counts are directly related to embryo quality, our results suggest that stress may be manipulated to increase embryo cell numbers and thus embryo quality. The apparently beneficial effect of stress is surprising as it is commonly stated that minimising stress during commercial animal embryo transfer programs results in improved results. We look forward to the opportunity to follow the *in vivo* development of embryos derived from ewes stressed at Day 2 and 3 of gestation.

(1) Dodic M. *et al.* (1998) An early prenatal exposure to excess glucocorticoid leads to hypertensive offspring in sheep. *Clin. Sci (Lond.)* **94**, 149–155.