108 REVERSE-TRANSCRIPTASE PCR (RT-PCR) TO DETECT GENE EXPRESSION IN CULTURED CELLS FROM THE DEER ANTLER. Sue Francis, Dan Fitzgibbon, Mehri Sadighi and Jimmy Suttie. AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel.

Plasma IGF-I levels are elevated during the period of antler growth. Insulin-like growth factor I (IGF-I) has a mitogenic effect on antler cells *in vitro*. Previous work has shown that transforming growth factor-ß1 (TGF-ß1) is expressed in the pre-cartilagenous zone of the antler from which the cultured cells were derived. The objective of this work was to use the RT-PCR technique to detect the expression of TGF-ß1 and c-fos genes in cultured antler cells after stimulation with IGF-I. Cells were seeded in 12 well plates at a density of $2x10^5$ cells/well and cultured in BGJ$_b$/F-12 medium supplemented with 10% fetal bovine serum for 72 hours. After a further 24 hours incubation in serum-free medium, triplicate wells were treated with 0 or 10 nM IGF-I for 0, 15, 30 or 60 minutes and 2, 4, 8, 16, 24 or 48 hours. At the end of treatments, the medium was removed and total RNA was extracted from the cells using a guanidium isothiocyanate/phenol/chloroform method. 600 ng of total RNA was reversed transcribed and amplified by PCR using primers to generate a 200 bp TGF-ß1 or 222 bp c-fos fragment. Samples were analyzed in ethidium bromide stained 2% agarose gels.

TGF-ß1 was constitutively expressed and did not appear to be affected by IGF-I treatment in the first 24 hours. There was considerable variability in the band intensity between triplicates, despite the same amount of RNA being used in each reaction. At 48 hours, the intensity of the TGF-ß1 band in IGF-I treated cells appeared to be greater than in control cells. The c-fos gene was not detected in the 0 or 15 minute treatments. However, at 30 minutes there was a band in IGF-I treated but also in control cells albeit of lower intensity. At 60 minutes, lower intensity bands were detected in both control and IGF-I treated cells. It is apparent that the antler cells transiently produce c-fos in response to a stimulus. Further controls are needed to make this technique fully quantitative.