EPIDERMAL GROWTH FACTOR (EGF) INDUCES CESSATION OF FIBRE GROWTH IN CULTURED WOOL FOLLICLES

J.J. BOND[A], *P.C. WYNN*[A] *and G.P.M. MOORE*[B]

[A]University of Sydney, Department of Animal Science, Camden, N.S.W. 2570
[B]CSIRO Division of Animal Production, Locked Bag 1 Delivery Centre, Blacktown, N.S.W. 2148

We have established procedures for the isolation and culture of individual wool follicles from Merino sheep skin (Bond *et al.* 1994). This technique provides a model with which to study the direct effects of growth regulating molecules on fibre quality independently of systemic influences on the animals metabolism. Here we report the influence of epidermal growth factor (EGF) on follicle morphology. fibre growth and wool keratin synthesis in cultured follicles.

Follicles were microdissected from midside skin (22 μm) samples of 2-year-old Haddon Rig wethers and transferred individually, to 24 well tissue culture plates containing supplemented serum-free Williams' E medium (1 mL). After 24 hour in culture, culture wells containing follicles damaged by dissection were removed from culture and EGF (1 or 10 ng/mL) was added. Due to the variation in depilatory response to EGF at low doses between individual sheep observed *in vivo* (Hollis *et al.* 1983) follicles were dissected from the 6 sheep. Each day 1 complete replication of each treatment (n = 8) per culture plate on each sheep was completed. The protocol was repeated 4 times for each sheep. Control follicles (n = 85) grew for 6 days at a rate of 67 ($\pm$ 7) μm per day. Examination of sectioned follicles revealed that normal morphology of an actively growing follicle was maintained during this period. Using antibodies that specifically recognize wool keratin (αK6 French and Hewish 1986) we found that controls also continued to produce wool hard keratin during this period.

EGF was added to the culture medium 24 hours after dissection. Follicles grown in the presence of EGF (10 ng/mL) ceased to produce a fibre after 4 days in culture. Although follicles continued to elongate this was not associated with fibre growth. Fibres formed with tapered ends which resembled those found in response to the administration of a depilatory dose of EGF *in vivo* (Hollis *et al.* 1983). However, unlike the effects observed *in vivo,* cessation of fibre growth was not accompanied by the characteristics of follicle shutdown where the follicle bulb and dermal papilla regress towards the skin surface. In addition, EGF induced a different pattern of keratin synthesis in the cells remaining in the lower bulb region of the follicle. Hard keratin synthesis was no longer detected in these cells after 4 davs in culture but, cytokeratins associated with outer root sheath (ORS) cells were detected. The inhibition of fibre growth in response to 1 ng/mL of EGF was more variable between individual sheep. Some follicles in this treatment group did not completely cease producing a fibre, in agreement with studies *in vivo* directed towards achieving a partial 'break' in the fleece (Hollis *et al.* 1983).

Our results confirm that EGF causes cessation of wool fibre growth in cultured follicles and suggest EGF or a related molecule plays an important role in the functions controlling fibre strength and the maintenance of cell specific functions associated with fibre growth. However, the differences observed in follicle morphology *in vitro* and *in vivo,* imply that EGF does not act to produce a wool break in isolation from other growth factors and hormones *in vivo.*

This work was supported by the Australian Wool Research and Development Coporation.

BOND, J.J., WYNN, P.C. and MOORE, G.P.M. (1994). *In vitro* (in press).

HOLLIS, D.E., CHAPMAN, R.E., PANARETTO, B.A. and MOORE, G.P.M. (1983). *Aust. J. Biol. Sci. 36:* 419-34.

FRENCH, P.W. and HEWISH, D.R. (1986). *J. Cell. Biol.* 102: 1412-18.