

REAL-TIME RT-PCR ANALYSIS OF THE ONTOGENY AND NUTRITIONAL REGULATION OF MYOSTATIN mRNA EXPRESSION IN SKELETAL MUSCLE OF FETAL SHEEP.

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Muscle fibre characteristics are not solely genetic in origin, but are influenced before birth with the pre-natal environment contributing significantly to the potential growth of muscle. The main variable affecting the pre-natal environment is that of nutrition, via placental restriction, as mediated by maternal nutrition and/ or litter size.

Myostatin is a negative regulator of myogenesis. ‘Myostatin knockout’ mice display greater muscle mass (McPherron *et al.* 1997), while double-muscled cattle, which have a mutation in the myostatin protein (Kambadur *et al.* 1997), have double the number of muscle fibres of other breeds (Wegner *et al.* 2000). The aims of the present experiment were to examine a) the developmental expression of functional myostatin in fetal sheep, and b) the influence of maternal nutrition on myostatin expression.

Mature age Merino ewes (n=119) were stratified on live weight and randomly allocated to 2 nutritional treatments at 1.8 and 0.6 times maintenance requirements (High (H) n=60, and Low (L) n=59, respectively) and fed a pelleted diet prior to, and throughout, pregnancy. Pregnant ewes, from each treatment group, were stratified on litter size and live weight and randomly allocated to one of three sample points (day 50, 92 and 133 of gestation). Ewe live weight and condition score were significantly different at each sample point (p<0.01). Ewes were euthanased and the fetuses recovered, weighed and dissected and muscle samples taken. Fetal weights tended to be less in fetuses from low nutrition ewes, although this was only significant for day 133 fetuses (P<0.01).

Relative abundance of myostatin mRNA was determined from a standard curve and normalised to 18S rRNA, by real-time RT-PCR. Our preliminary analysis on a subset of muscle samples (n=30), indicates for the first time the differential expression of myostatin mRNA during gestation (P<0.01) and some evidence for nutritional regulation (P<0.1) during fetal sheep development (Table 1).

Table 1. The effect of maternal nutrition on the abundance of myostatin mRNA, normalised to 18S rRNA and expressed as a percentage of mean myostatin abundance of day 50 Low (100%), in muscle of fetal sheep at different stages of gestation.

Stage of Pregnancy (days)	Level of Nutrition	
	Low (n=15)	High (n=15)
50	100 ^a	152 ^a
92	106 ^a	148 ^a
133	19 ^b	28 ^b

^{a, b} Values with different superscripts are significantly different (P<0.05).

The ontogeny of myostatin expression in fetal sheep is in concurrence with other reported declines in late gestation cattle and pig fetuses. The possible interaction between myostatin and nutrition warrants further investigation. We are currently analysing more samples to examine expression levels in different muscle types and the effect of litter size. The nutritional and developmental influences on myostatin expression at the protein level and the relationship with muscle cell development will also be examined.

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