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Suppression of secondary wool follicle development by administration of placental lactogen to ovine foetuses

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ABSTRACT

The effects of ovine placental lactogen (oPL) on wool follicle development were examined in chronically catheterised singleton Dorset foetuses. From days 122 to 136 of gestation, five foetuses were infused intra-arterially with purified oPL (1.2 mg/day) in a carrier medium consisting of sterile physiological saline plus 15% plasma from non-pregnant ewes while another five were infused with carrier medium alone. Foetal arterial plasma concentrations of oPL were increased six-fold by the infusion compared with those in controls. Foetuses were euthanased on day 136 of gestation and skin samples excised from the midsides for histological examination. Treatment of foetuses with oPL did not influence primary follicle density but reduced the ratio of immature secondary follicles to primary follicles (Control, 1.22 vs oPL-treated 1.01, Pooled SE = 0.06, P = 0.06). These data provide the first evidence that follicle development in the lamb may be regulated by ovine placental lactogen.

Keywords Foetus, ovine placental lactogen, wool, follicle development, Dorset sheep.

INTRODUCTION

It has been known for many years that events associated with wool follicle development during prenatal and early postnatal life are important determinants of the type of wool produced during the life of a sheep (Wickham 1963). In particular, changes in the branching of secondary follicles may markedly alter follicle populations and hence the diameter of wool which is one of the major factors influencing price.

Although follicle development is obviously under strong genetic control (as evidenced by marked differences between breeds in follicle densities), little is known about the hormonal factors which may be involved in this process. A few studies have shown that treatment of foetal or newborn lambs with hormones or growth factors such as thyroxine (Williams, 1984), growth hormone (GH) (Labban, 1957) or epidermal growth factor (Thorburn, et al., 1981) can alter follicle development. However, there has never been a concerted effort to identify the factors regulating follicle development.

In recent years there has been increasing interest in the developmental role of the placental lactogens (chorionic somatomammotropins). These proteins, which are produced in the placenta, have structural similarity to GH and prolactin and may be involved in the regulation of foetal growth (Hill, et al., 1986). As a result, studies conducted at Cornell University to examine the impact of ovine placental lactogen (oPL) on foetal growth (Schoknecht 1991), have been extended to examine the effects of this hormone on follicle development. We present here preliminary evidence that high levels of oPL in the foetus suppress secondary follicle development and hence the ratio of secondary to primary follicles.

MATERIALS AND METHODS

Animals and treatment

Ten Dorset ewes mated to Dorset rams and carrying singleton lambs (as determined by ultrasonic scanning) were housed in metabolism crates from day 100 of gestation. On day 115 of gestation, foetuses were surgically prepared by implantation of catheters in the abdominal aorta and vena cava via vessels in an exteriorised hind leg. Infusions commenced on day 122 of gestation and continued until day 136. Five foetuses (2 male, 3 female) were infused intra-arterially with 1.2 mg/day purified oPL in a carrier medium consisting of sterile physiological saline plus 15% plasma from non-pregnant ewes while the other 5 (1 male, 4 female) were infused with the carrier medium alone. Blood samples for determination of oPL concentrations (Bell, et al., 1989) were obtained via the arterial catheter on the day before infusion commenced and then on days 1,3,5,8,11 and 14 of infusion. The infused oPL was purified from extracts of ovine placentomes by anion and cation exchange chromatography, chromatofocusing and high resolution molecular filtration. The preparation was homogeneous as assessed by SDS-polyacrylamide gel electrophoresis followed by staining with Coomassie Blue. On the basis of relative binding activity in radioreceptor assays and more sensitive silver staining of gels, the preparations of oPL were judged to be consistently greater than 95% pure (Bell, et al., 1989).

Determination of follicle populations

Foetuses were euthanased at the completion of treatment on day 136 of gestation and immediately weighed. Skin samples were removed from the left and right midsides of each foetus (over the last rib) using a trephine of 1 cm diameter and fixed in Bouin’s fluid. Longitudinal and cross-sections were then prepared for histological examination as described by Maddocks and Jackson (1988). Follicle populations were determined by counting 10 fields of 1 mm² area per midside sample (ie 20 fields per foetus) at the level of the sebaceous gland and classifying follicles as primaries, secondaries with fibre present and immature secondaries (no fibre present). Mean follicle depths were measured on 20 follicles containing fibres per sample (40 follicles per foetus) in the longitudinal sections.

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Statistical methods

All data were subjected to one-way analyses of variance comparing control and oPL-treated foetuses. In preliminary analyses, follicle population data were adjusted by covariance analysis for foetal weight and sex. However, since neither of these variables was significantly related to follicle population characteristics, unadjusted data have been presented.

RESULTS

Foetal arterial concentrations of oPL were substantially increased by oPL infusion. In control animals they averaged 28.6 ± 1.0 ng/ml (mean ± SE) immediately prior to commencement of the infusion and 30.2 ± 1.2 ng/ml during the 14-day infusion period (mean of values for blood samples taken during the treatment period). Foetuses treated with oPL had significantly higher circulating oPL levels than controls on the day prior to infusion commencing (48.5 ± 6.8 ng/ml, P < 0.05), a chance effect reflecting the randomisation of small numbers of foetuses into the two groups. These levels were further increased to 136.3 ± 28.1 ng/ml during the infusion period, a six-fold elevation compared with control foetuses during the same period (P<0.05).

Follicle densities, ratios and depths are presented in Table 1. Compared with control foetuses, those treated with oPL had a higher density of primary follicles (by 4%) and a lower density of immature secondary follicles (by 13%). The net effect of these changes was a 17% lower ratio of immature secondary to primary follicles in oPL-treated foetuses (P = 0.06). Treatment of foetuses with oPL did not appreciably alter the density of secondary follicles with fibres or the ratio of these follicles to primaries. Foetal weight was not influenced by treatment.

TABLE 1 Characteristics of follicle populations in control foetuses and those treated with 1.2 mg/day ovine placental lactogen (oPL) during days 122 to 136 of gestation. Foetal weight is also shown.

<table>
<thead>
<tr>
<th>Control</th>
<th>oPL-treated</th>
<th>Pooled</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary (P)</td>
<td>22.9</td>
<td>23.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Secondary-Immature (SI)</td>
<td>27.7</td>
<td>24.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Secondary-with fibres (SF)</td>
<td>56.9</td>
<td>56.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Total</td>
<td>107.4</td>
<td>104.7</td>
<td>3.1</td>
</tr>
<tr>
<td>Follicle ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SI/P</td>
<td>1.22</td>
<td>1.01</td>
<td>0.06</td>
</tr>
<tr>
<td>SI/P</td>
<td>2.49</td>
<td>2.37</td>
<td>0.05</td>
</tr>
<tr>
<td>(SI + SF)/P</td>
<td>3.1</td>
<td>3.38</td>
<td>0.13</td>
</tr>
<tr>
<td>Follicle depth (µm)</td>
<td>926</td>
<td>962</td>
<td>26.9</td>
</tr>
<tr>
<td>Foetal weight (kg)</td>
<td>4.31</td>
<td>4.45</td>
<td>0.15</td>
</tr>
</tbody>
</table>

DISCUSSION

Although the results of this study require further verification, they suggest that ovine placental lactogen may have a role in regulating the development of secondary wool follicle populations. The decline in secondary follicle density observed in oPL-treated foetuses could not have been simply a reflection of oPL effects on foetal size and hence skin stretching because there was no difference between the groups in foetal weight. The lack of difference in the ratio of mature secondaries to primaries indicates that the ability of initiated follicles to produce fibres was not affected. The 17% reduction in immature secondary/primary follicle ratio therefore appears to have been due to the infused oPL suppressing the initiation of new secondary follicles. It seems unlikely that the effects of oPL were related to the previously-reported effects of epidermal growth factor (EGF) and thyroxine on follicle populations. Treatment of ovine foetuses with EGF, which substantially reduces secondary to primary ratios, does not influence foetal plasma concentrations of oPL (Thorburn et al., 1981). Moreover, infusion of oPL into these and other foetuses did not alter their circulating thyroxine levels despite the fact that it suppressed foetal thyroid weights (Schoknecht 1991).

A direct effect of oPL on follicle development would require the presence of receptors for placental lactogen in the skin. While such receptors have been reported in foetal hepatocytes, fibroblasts and myoblasts (Adams et al., 1983; Hill et al., 1985; Freemark and Handwerger, 1986) they do not appear to have been examined in skin. Alternatively, oPL could act via other growth factors such as insulin-like growth factor-1 (IGF-1) since the stimulatory effects of placental lactogen on DNA synthesis in isolated human foetal fibroblasts and myoblasts appear to be mediated partly through increased local production of IGF-1 (Hill et al., 1986). Conversely, Labban (1957) showed that newborn sheep treated with putitutary-derived bovine GH (and which would be expected to have elevated circulating IGF-1 concentrations) had increased secondary/primary ratios (ie the opposite effect to that of oPL in our foetal lambs) although we found no such effect in neonatal lambs treated with recombinantly-derived bovine somatotropin (Y.X. Sun, A. et al., 1992).

Finally, it should be noted that the two-week infusion period used in this study represents less than 30% of the time period during which secondary follicles accumulate and less than half the time needed for a newly initiated follicle to attain maturity (Hardy and Lyne, 1956). Treated foetuses did have higher plasma oPL concentrations than control foetuses prior to infusion but this difference was small compared with that due to the infusion. More marked effects of oPL on secondary to primary ratio may therefore become apparent with longer periods of treatment.

ACKNOWLEDGEMENTS

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