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BRIEF COMMUNICATION

Development of a technique to evaluate the role of elevated maternal thyroid hormone concentrations in the birthweight response to mid-pregnancy shearing.

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INTRODUCTION

Several studies have shown that lambs born to mid-pregnancy shorn ewes are heavier at birth than those born to unshorn ewes (Morris *et al*. 2000, Kenyon *et al*. 2002a,b) but, the mechanism responsible has not been identified. Morris *et al*. (2000) observed elevated maternal triiodothyronine (T3) concentrations over a 20-day period after shearing at mid-pregnancy in both single- and twin-bearing ewes and subsequently found an increased birthweight in the singletons. Symonds *et al*. (1989) also reported maternal T3 and T4 concentrations to be higher in pregnancy-shorn housed ewes although, an altered birthweight was not reported.

Study one aims: Develop techniques to (i) maintain maternal T3 and T4 concentrations in shorn ewes at a concentration equivalent to that in unshorn ewes and (ii) elevate in a controlled fashion, maternal T3 and T4 concentrations in mid-pregnancy.

METHOD

Eight Romney ewes were thyroidectomised (to eliminate endogenous thyroid hormones) under anaesthesia induced with Ketamine (Parnell Laboratories, New Zealand) and Diazepam (Pamlin, Parnell Laboratories, New Zealand) and maintained with Halothane (Vet Companies of Australia Pty Ltd) in oxygen. These eight ewes were randomly allocated into one of four groups (n=2) and implanted with either a 20, 30, or 70 mg T4 implant or two 25mg implants (Glaxo, UK). Blood samples (10ml) were taken via jugular venepuncture (Lithium Heparin vacutainer, Becton Dickinson Vacutainer Systems, USA) from those ewes and three control (thyroid intact) ewes at weekly intervals for a minimum of five weeks (Kenyon 2002). At the end of this trial period, after concentrations of T3 and T4 had returned to basal concentrations the eight thyroidectomised ewes were injected either daily or every second day with either 0.5 or 0.75mg of T4 (Sigma Chemical Co, USA) in 1 ml of saline (0.9% NaCl) and 1ml of sesame oil (Sigma Chemical Co, USA) over a five-day period (n=2 in each group). Blood samples were collected daily for five days and then every second day for a further five days. Plasma was analysed for T3 and T4 concentrations by radioimmunoassay using diagnostic kits (Coat-A-Count, Diagnostic Products Corporation, CA, USA). The intra and inter coefficients of variation for T3 and T4 were 8.9, 3.8, 10.0 and 8.0% respectively.

RESULTS/DISCUSSION

T3 and T4 concentrations of control ewes ranged from 110 – 170 μg/dL and 4 to 6 μg/dL respectively over a twelve-week period. T3 and T4 concentrations of ewes implanted with a 70mg implant continually rose (to levels equal to and above that recorded in control ewes) during the first six-week measurement period before falling rapidly. This indicated that these implants were not suitable at maintaining a constant concentration of both T3 and T4, as required. Ewes implanted with either a 20 or 30mg implant displayed similar (although slightly higher levels in those with a 30mg implant), fairly constant, T3 and T4 concentrations (at levels approximately 50% lower than that of controls) during the measurement period. T3 and T4 concentrations of those implanted with two 25mg implants were very low, suggesting that these implants may have been defective. Daily injections with 0.75 mg of T4 for five days, raised T3 concentrations by least 40-50μg/dL above those recorded pre-injection regime. This is similar to the increase seen with shearing in an earlier study (Morris *et al*. 2000), however the elevation persisted for less than five days after the last injection. In addition T4 concentrations were raised by up to 7 μg/dL. All other T4 injection regimes failed to raise either T3 or T4 concentrations appreciably above basal levels. Therefore these were unsuitable for use in mimicking the rise in T3 concentrations reported post mid-pregnancy shearing.

Study two aim: Development a method that will separate effects due to elevated thyroid hormone concentrations from other effects associated with mid-pregnancy shearing on lamb birthweight.

METHOD

Based on the results of study one, it was decided to implant all thyroidectomised ewes with both a 20mg and a 30mg T4 implant (as the results of study one indicated the combined effect of these would mimic that observed in the unshorn ewe) to maintain thyroid hormone concentrations at a constant level (as seen in unshorn ewes) and for Groups 3 and 4 to use an injection regimen of 0.75 mg of T4 daily for 10 consecutive days (as daily injections for 5 days in study one failed to elevate both T3 and T4 concentrations for more than ten days) to try to achieve a 20-day elevation in T3 (as previously observed in mid-pregnancy shorn ewes).

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Forty-two, twin-bearing Romney ewes which conceived over a two-day mating period post-progesterone-synchronization (CIDR, type G, Livestock Improvement Corporation, Hamilton, New Zealand) were randomly allocated into one of the following four treatment groups:

Group 1, Unshorn control (n=9). Unshorn ewes were sham-operated (between P48 and P52). During the immediate surgery recovery period, all ewes (regardless of group) were given long-acting antibiotic (Terramycin/LA, Pfizer Animal Health, New Zealand) and analgesic (Ketoprofen, Merial New Zealand Ltd). Ewes were injected daily during P71-80 with 1ml of saline and 1ml of sesame oil.

Group 2, Shorn-controlled T4 (n=10). Thyroid gland removed and two T4 implants (one 20 and one 30 mg) inserted subcutaneously under the right front leg. Ewes were shorn at P70 and injected daily during P71-80 with 1ml of saline and 1ml of sesame oil.

Group 3, Unshorn-high T4 (n=12). Thyroid gland removed and two T4 implants (one 20 and one 30 mg) inserted subcutaneously under the right front leg. During the period P71-P80, ewes were injected daily with 0.75 mg of T4 in 1 ml of saline and 1ml of sesame oil.

Group 4, Shorn-high T4 (n=11). Thyroid gland removed and two T4 implants (one 20 and one 30 mg) inserted subcutaneously under the right front leg. Ewes were shorn at P70 and injected daily with 0.75 mg of T4 in 1 ml of saline and 1ml of sesame oil between P71 and 80.

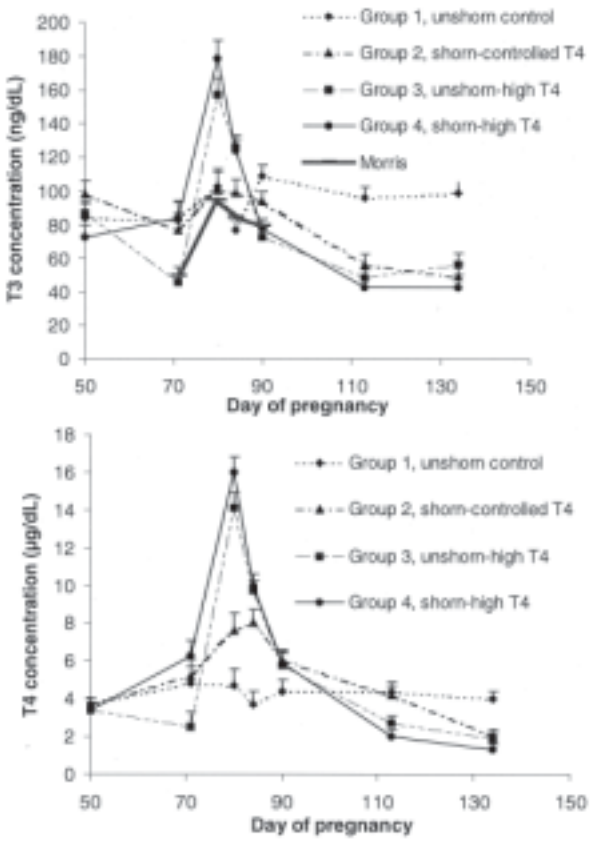
DATA ANALYSIS

All data were analysed by one-way analysis of variance using the Generalised Linear Model procedure of the statistical package 'Minitab' (Minitab 1998).

RESULTS

At surgery (P48-52) none of the groups differed significantly in plasma concentrations of either T3 or T4 (Figure 1). Interestingly, by P71, Group 3 ewes had lower T4 concentrations ($P<0.05$) than Group 4 ewes, even though both groups had identical implants inserted. Additionally, T3 concentrations of Group 3 were lower ($P<0.05$) than those observed in both Groups 1 and 4 at P71. Daily injections of T4 (from P71 to P80) increased ($P<0.05$) T3 and T4 concentrations in both Groups 3 and 4 above those seen in either of Groups 1 or 2 for at least 10 days. By P84, T3 and T4 concentrations in Group 3 and 4 ewes were still significantly ($P<0.05$) greater than those observed in Group 1 but not in Group 2. T4 concentrations of Group 2 were significantly ($P<0.05$) greater than those of Group 1 at P84. At P90, Group 1 had significantly higher ($P<0.05$) T3 concentrations than both Groups 3 and 4 and by late pregnancy (P113 and P134) had significantly ($P<0.05$) higher T3 concentrations than all other groups. At P113, Group 4 ewes had significantly ($P<0.05$) lower T4 concentrations than both Group 1 and 2 ewes, and by P134, Group 1 ewes had significantly higher T4 concentrations than all other groups. In the late pregnancy period (P90-134) thyroidectomised ewe groups (Groups 2, 3 and 4) had similar T4 and T3 hormone profiles.

FIGURE 1. Effect of dam treatment on mean maternal plasma T3 (μg/dL) (upper graph) and T4 (μg/dL) (lower graph) concentrations during mid- to late-pregnancy. In the T3 graph 'Morris' indicates T3 concentrations reported by Morris *et al*. (2000). The vertical bars indicate standard errors of the means.



DISCUSSION

Morris *et al*. (2000) observed elevated T3 concentrations after shearing ewes in mid-pregnancy and subsequently observed an increase in birthweight of singletons. This, together with known effects of thyroid hormones on foetal growth and development (Spencer & Robinson 1993, Symonds 1995), suggests that elevated maternal thyroid hormone concentrations could be the mechanism responsible for increased lamb birthweights. The first study indicated it was possible to both maintain and elevate thyroid hormone levels in a thyroidectomised ewe. In the second study, T4 implants inserted in Group 2 ewes maintained maternal T3 and T4 concentrations at a level similar to that observed in the unshorn pregnant ewe (Group 1) during P50-113. Although at P84, T4 concentrations of Groups 1 and 2 did differ. This difference in T4 at one time point only does not indicate that the implants used are unsuitable. T3 is the active form of thyroid hormones within the target cell and concentrations of these did not differ between Groups 1 and 2 in the period P50 to P90. However, in late pregnancy, both the T3 and T4 concentrations of thyroidectomised ewes were lower than those found in Group 1. This indicates that implants need to be replaced approximately 7-8 weeks after the first insertion to maintain a constant level throughout pregnancy and to

avoid any possible negative effects of lower T3/T4 concentrations in late pregnancy. Basal T3 concentrations in all ewe groups (including Group 1) in the present study were above those reported by Morris *et al*. (2000). This difference could have been due to yearly variation, type of sheep or variation due to radioimmunoassay procedures.

Daily T4 injections from P71 to P81 in Groups 3 and 4 successfully elevated both T3 and T4 concentrations for at least 10 to 14 days above those observed in Groups 1 and 2, however, this was less than the intended 20-day elevation. This relatively quick T3/T4 clearance was also seen after the five-day injection regimen in study one. Therefore, to achieve a longer elevation, the injection regime needs to run for a period of at least 15 days. The peak T3 elevation reached by Group 3 and 4 ewes was greater than those reported by Morris *et al*. (2000), although, the relative elevation above levels observed in unshorn ewes in both studies was similar.

CONCLUSION

The design of the second study permits the separation of the effects of shearing from the effects of elevated thyroid hormone concentrations on lamb birth weight. However, although the magnitude of the T3 elevation in thyroidectomised ewes was similar to that previously reported in pregnancy-shorn ewes, the duration of the elevation was shorter and therefore a longer injection regime is required. In addition, the T4 implants used failed to maintain T3 and T4 concentrations at a level seen in unshorn ewes in late pregnancy, indicating that additional thyroid hormone implants would be required to maintain concentrations in late pregnancy. After these modifications the protocol developed here can be applied to future studies to determine if elevation of maternal thyroid hormones are responsible for the increase in birthweight resulting from mid-pregnancy shearing.

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