Effect of transient early hyperthyroidism on onset of puberty in Suffolk ram lambs

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The thyroid function and sexual development of eight 6-week-old Suffolk ram lambs were studied. The lambs were divided into either control or treatment groups and housed indoors. From 6 to 12 weeks of age, four lambs in the treatment group received 15 mg kg⁻¹ body weight per day of 6-propyl 2-thiouracil orally to suppress normal thyroid function. During the same period, thyroxine and tri-iodothyronine were injected s.c. at the rate of 8 and 16 µg kg⁻¹ body weight per day, respectively, to induce a hyperthyroid state. Four control lambs received sham injection and oral excipient. Concentrations of thyroxine, tri-iodothyronine, FSH, testosterone and insulin-like growth factor I were determined in blood collected by indwelling jugular catheters once a week, every 20 min from 09:00 to 15:20 h. Scrotal circumference was recorded each week. Semen collection was attempted by electro-ejaculation between weeks 17 and 36. Lambs were castrated at week 36 and testicular histology was examined. During the treatment period only, the concentration of thyroid hormones was

higher in treated lambs than in controls ($P < 0.05$). From week 6 to week 9 only, concentrations of FSH in treated lambs were lower than in controls ($P < 0.05$). Insulin-like growth factor I concentrations were lower in treated lambs than in controls from week 10 to week 13 ($P < 0.05$). Frequency of testosterone pulses was higher ($P < 0.01$) in the treated lambs but concentrations were similar in the control and treated lambs throughout the experiment. Scrotal circumference was greater in treated lambs from week 26 to week 36 ($P < 0.05$). Treated lambs produced viable spermatozoa earlier than did control lambs. At week 36, sperm concentration in treated lambs was higher than in controls ($P < 0.01$) but semen volumes were similar ($P > 0.1$). Diameter of the seminiferous tubules in treated lambs was larger than in controls ($P < 0.05$). In conclusion, transient neonatal hyperthyroidism decreased FSH and insulin-like growth factor I concentrations temporarily, increased testosterone pulses and sperm production and advanced puberty in Suffolk ram lambs.

Introduction

Thyroid hormones can affect testis functions directly and indirectly (Palmero *et al.*, 1988), but these effects are highly dependent upon the age of the animal (Meisami *et al.*, 1994). Testes of adult animals have been considered unresponsive to thyroid hormones (Oppenheimer *et al.*, 1974). However, recent evidence supports the concept of a critical role for thyroid hormones in testicular development during the perinatal period. High tri-iodothyronine (T₃) concentrations in immature rats reduced the postnatal period of Sertoli cell proliferation and the final testicular size (van Haaster *et al.*, 1993). Conversely, 6-propyl 2-thiouracil (PTU), a potent reversible goitrogen, induced transient neonatal hypothyroidism and resulted in increased adult testis size and sperm production in rats (Cooke and

Meisami, 1991), hamsters (Kirby *et al.*, 1993) and mice (Joyce *et al.*, 1993). PTU treatment in rats decreased thyroxine (T₄) concentration throughout the treatment period but suppressed T₃ to a lesser degree (Kirby *et al.*, 1992). Serum FSH and LH concentrations were reduced significantly in hypothyroid rats without affecting testosterone concentration.

Sertoli cells are the major determinant of the magnitude of sperm production (Orth *et al.*, 1988). Neonatal Sertoli cell proliferation was extended up to 15 days in PTU-treated rats (van Haaster *et al.*, 1992) and mice (Joyce *et al.*, 1993), leading to large increases in their numbers in adults (Hess *et al.*, 1993). However, transient neonatal hyperthyroidism decreased the proliferation rate but enhanced the differentiation of Sertoli cells (van Haaster *et al.*, 1993). Similarly, Palermo *et al.* (1992) found that excess T₃ inhibited DNA synthesis and presumably proliferation in the rat prepubertal Sertoli cell culture. Immediately after cessation of PTU treatment in hypothyroid rats, the testes appeared to be immature and the lumen formation was delayed or absent. Conversely, T₃ treatment accelerated the formation

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of a tubular lumen (van Haaster *et al.*, 1993), which reflected the fluid secretion by the differentiated Sertoli cells (Russell *et al.*, 1989). Cooke *et al.* (1994a) stated that the early suppression of Sertoli cell proliferation, the reduced number of these cells in adults, and the enhanced onset of Sertoli cell secretory activity in hyperthyroid young rats were all due to direct actions of thyroid hormones on the Sertoli cells.

The aim of the present study was to apply transient early hyperthyroidism in sheep to further understanding of the role of thyroid hormones and testicular development in peripubertal rams. Major differences in the rate of Sertoli cell proliferation of neonatal rats and sheep (Gondos and Berndson, 1993) make it of interest to document the effects of early hyperthyroidism on the ovine testis. In contrast to the case in rats (Dubois and Dussault, 1977), the sheep hypothalamic–pituitary–thyroid axis is well developed at the time of birth (Brzezinska-Slebodzinska and Krysin, 1990). Therefore, the potential differences between rodents and sheep in the application of transient early hyperthyroidism require investigation.

Materials and Methods

Animals, housing and environment

Eight Suffolk ram lambs were selected from a large flock after spring weaning (at 40 ± 2 days of age and weighing 14.4 ± 0.6 kg; mean $\pm$ SEM). Lambs were assigned randomly to either a control ($n = 4$) or PTU+H ($n = 4$) group. Each group was housed in a 180 cm $\times$ 300 cm pen on a concrete floor covered by wood shavings. Room light duration was adjusted each week to correspond to the natural photoperiod. Lambs had free access to fresh water and were fed *ad libitum* a ration of chopped hay and barley-based concentrate formulated to meet 1985 National Research Council requirements for fast growing lambs. They were maintained in accordance with the Canadian Council on Animal Care Guidelines (1993).

Treatments

PTU (Sigma-Aldrich Canada Ltd, Oakville, Ontario) was administered to the PTU+H lambs at a rate of 15 mg kg⁻¹ body weight per day from 6 to 12 weeks of age to suppress normal thyroid function. The daily dose of 0.1% (w/v) PTU was administered by drench in two equal parts at 08:00 and 16:00 h. PTU also suppresses hepatic and peripheral T₃ production. Therefore, PTU+H lambs were also given daily s.c. injections of 8 µg T₃ and 16 µg T₄ kg⁻¹ body weight (Sigma Chemical Co., St Louis, MO) to induce a hyperthyroid state during the treatment period. T₃ and T₄ solutions were prepared by dissolving both hormones in alkalized saline as described by Lynch *et al.* (1983) with some modifications as follows: 2500 µg T₄ and 1250 µg T₃ were dissolved in a mixture of 5 µl of 1 mol NaOH l⁻¹ and 495 µl of 95% ethanol, and vortexed. This solution was mixed with a solution comprising 2.5 ml propylene glycol

and 7 ml normal saline. The final solution had approximately 250 µg T₄ and 125 µg T₃ ml⁻¹. The control lambs were given placebo and injected s.c. with normal saline.

Blood sampling

Blood was collected from lambs each week between 6 and 36 weeks of age, every 20 min from 09:00 to 15:20 h, by indwelling jugular catheters put in place according to the procedure of Sakurai *et al.* (1993).

Haematocrits were determined every 2 h during the sampling day. Blood samples were stored for up to 16 h at 4°C. Serum was collected after centrifugation for 20 min at 2000 g and stored at -20°C until assayed for concentrations of T₄, T₃, FSH, testosterone and insulin-like growth factor I (IGF-I).

Growth measurements

Scrotal circumference of the lambs was measured each week from 6 to 36 weeks of age with a cloth measuring tape while the lambs were restrained in a sitting position. Scrotal wool was clipped off and the testes were pulled fully into the scrotum before measurement. Body weight, average daily gain, relative growth (increase in body weight as a percentage of the total body weight) and average daily feed intake were measured and calculated each week until 36 weeks of age.

Semen collection and evaluation

Attempts to collect semen were made twice a week from week 17 to week 36 by electro-ejaculation and semen samples were evaluated immediately. The sperm concentration was determined from a 20 µl sample of the ejaculate fixed in acetic acid. The number of sperm cells was counted using a haemocytometer (Reichert Scientific Instruments, Buffalo, NY). The volume of the semen was recorded and the percentage of non-motile spermatozoa was calculated.

Testicular histology

Rams were castrated surgically at 36 weeks of age after their scrotal circumference had reached a plateau and at a time corresponding to the late breeding season. Testes were weighed and histological sections prepared by the haematoxylin and eosin staining method to examine the number, diameter and number of layers of cells in the seminiferous tubules. Diameter of seminiferous tubules was determined with the aid of a calibrated microscopic slide. Number of layers of cells was determined by counting the average number of cells standing in a column from the periphery to the lumen of seminiferous tubules in four different directions. Owing to the large diameter of the testes, it was not possible to count the total number of seminiferous tubules in the cross-section of the testis and therefore the number of seminiferous tubules per view field of the microscope was counted at a magnification of $\times 100$.

At least ten fields of view were observed to obtain the average number and diameter of seminiferous tubules. Longitudinal sections were avoided as much as possible.

Hormone concentrations and pulse calculations

Mean hormone values for each sheep were calculated from the 19 samples taken at 20 min intervals on sampling days for testosterone and from samples taken hourly for T_4 , T_3 , FSH and IGF-I. Pulses of testosterone were calculated according to the method described by Veldhuis *et al.* (1984), which is based on the measurements of the hormone that exceed the preceding local minimum by a value that is fourfold greater than the intra-assay coefficient of variation (CV) of the radioimmunoassay. This method allows elimination of the noises produced by the error of the assay and produces fewer false positive pulses compared with other methods. The ratio of $T_4:T_3$ during and after treatment period was calculated.

Testosterone radioimmunoassay

Concentrations of testosterone were determined in duplicate by radioimmunoassay using the procedure described by Sanford *et al.* (1974). The mean intra-assay CVs for high, medium and low reference pools of serum were calculated according to the procedure of Wilson and Lapwood (1979) and were 13.1, 12.2 and 12.9%, respectively, whereas the mean interassay CV was 14.2%. The concentrations of testosterone were expressed as ng ml⁻¹.

Thyroxine and tri-iodothyronine radioimmunoassays

Total T_4 and T_3 assays were performed by radioimmunoassay using Coat-A-Count® kits (total T_4 1081 and total T_3 501 for T_4 and T_3 , respectively; Diagnostic Products Corp. Los Angeles, CA). Similar procedures have been used by others (Milter and Albyl, 1985; Wrutniak *et al.*, 1985) in sheep. Mean T_4 and T_3 intra-assay CVs were 3.9 and 6.3%, respectively, whereas inter-assay CVs were 7.3% for T_4 and 14.95% for T_3 . The values of T_4 and T_3 are expressed as µg and ng dl⁻¹, respectively. Sensitivity of the assay was 0.25 µg dl⁻¹ for T_4 and 7 ng dl⁻¹ for T_3 .

ELISA for insulin-like growth factor I

IGF-I concentrations were determined using IGF-I ELISA kits (Diagnostic Systems Laboratories, Inc., Webster, TX). This method involves acid ethanol extraction, which has been confirmed to be the most reliable method of extraction (Breier *et al.*, 1986; Daughaday *et al.*, 1989). Similarity of purified ovine IGF-I to human IGF-I in structure and biological activity has been demonstrated (Breier *et al.*, 1986; Francis *et al.*, 1989). The amino acid sequence of ovine IGF-I is identical to that of human IGF-I except for amino acid number 66 (Francis *et al.*, 1989).

The mean intra-assay CV for IGF-I was 3.2% and the inter-assay CV was 5.6%. The values of IGF-I are expressed as ng ml⁻¹. The sensitivity of the assay was 0.03 ng ml⁻¹.

FSH radioimmunoassay

Serum samples were assayed for FSH according to the method described by Evans *et al.* (1992). The average intra- and inter-assay CVs were 3.1% and 7.8%, respectively. Sensitivity of the assay was 0.1 ng ml⁻¹.

Statistical analysis

The design of the experiment was a completely randomized design (CRD) with repeated measures over time for hormone values. All the data are shown as mean ± SEM unless stated otherwise. Statistical analysis used ANOVA and Student's *t* test, where appropriate. For measurements that were taken once a week, the analysis was a CRD. For measurements taken repeatedly within a week, the analysis was a CRD with repeated measures. For the repeated measures procedure, animal within treatment was used as the error term to test treatment, whereas time and treatment × time effects were tested using animal within treatment × time as the error term. If significant treatment or treatment interaction effects were found, they were further investigated using the LSD test (Steel and Torrie, 1960), holding experimental error at 0.05. Haematocrit measurements from 09:00 and 15:20 h were analysed using a paired *t* test assuming equal variances. Correlation coefficients (*r* values) were calculated and if the absolute value was more than 0.5, it was considered a significant correlation. Owing to changes in the concentration of thyroid hormones during the treatment period, correlation coefficients have been calculated from the end of treatment to the end of the experiment.

Results

Body and testicular growth

Average daily feed intake kg⁻¹ body weight and average daily gain were similar between groups and within the acceptable range for rapidly growing ram lambs (National Research Council, 1985). Average rectal temperatures of the lambs in both groups were similar throughout the experiment. Hematocrit measurements were similar in the two groups on each sampling day, indicating that there were no deteriorating effects due to frequent sampling.

The initial mean scrotal circumference of the control and PTU+H groups were identical (12.5 ± 0.3 cm) and remained similar until 21 weeks of age. From week 21 to week 26, testes of PTU+H lambs tended to grow faster than those of control lambs but the difference was not significant until week 26 through to the end of experiment, when scrotal circumference of PTU+H lambs was greater than that of controls (*P* < 0.05; Fig. 1). At the end of the experiment, average scrotal circumference was 37.5 ± 2.7 and 42.4 ± 1.9 cm for control and PTU+H groups, respectively. At 31 weeks of age, scrotal circumference reached a plateau in both groups. The average final testicular mass of the two groups were different at

Fig. 1. Scrotal circumference of lambs treated with 6-propyl 2-thiouracil orally and thyroxine and tri-iodothyronine s.c. (PTU+H; ■) and of control lambs (□) from 22 to 36 weeks of age. Asterisks indicate significant differences between the two groups ($P < 0.05$). Treatment started at week 6 and ended at week 12 of age. The SEM for each individual data point has been omitted for clarity. The range of SEMs was 1.00–1.82 and the overall SD of the means was ± 4.0 .

513.3 $\pm$ 40.1 and 674.5 $\pm$ 33.7 g for control and PTU+H groups, respectively ($P < 0.05$). There was no within group difference between right and left testicular mass.

Semen quality

Semen was collected between 17 and 36 weeks of age. In the final ejaculation, semen volume was similar in the two groups (1.05 $\pm$ 0.09 ml for control versus 1.2 $\pm$ 0.09 ml for PTU+H lambs; $P > 0.1$) whereas sperm cell concentration was different (2.07 $\times 10^9 \pm 0.06$ spermatozoa ml⁻¹ for control versus 2.54 $\times 10^9 \pm 0.09$ spermatozoa ml⁻¹ for PTU+H lambs; $P < 0.05$). In the control group, viable sperm cells were first found at 20 weeks of age in one lamb and at 24 weeks of age in the second lamb. The third and fourth control lambs showed motile spermatozoa at 25 and 30 weeks of age, respectively. In the PTU+H group, the first and second lambs had viable spermatozoa at 18 weeks of age and the third and fourth lambs had viable spermatozoa at 19 weeks of age.

Histology of the testis

At 36 weeks of age, testicular development was greater in the PTU+H lambs and therefore more sperm cells were present in the lumen of seminiferous tubules. Although there were fewer seminiferous tubules in PTU+H (20 $\pm$ 0.5) than in control lambs (25 $\pm$ 0.6; $P < 0.05$), the diameter of the seminiferous tubules was greater in PTU+H (250–350 μ m) than in control lambs (200–300 μ m; $P < 0.05$). The diameter of the lumen was similar in both groups (150 $\pm$ 7.9). The number of layers of cells in the seminiferous tubules of the two groups was variable (6 $\pm$ 0.9 layers of cells).

Fig. 2. Testosterone pulse frequencies per 6 h period during the experiment in lambs treated with 6-propyl 2-thiouracil orally and thyroxine and tri-iodothyronine s.c. (PTU+H; ■) and in control lambs (□). The average pulse frequency was higher in the PTU+H than in the control lambs. Asterisks indicate significant differences between the two groups ($P < 0.01$). Treatment started at week 6 and ended at week 12 of age. The SEM for each individual data point has been omitted for clarity. The range of SEMs was 0.13–0.38 and the overall SD of the means was ± 0.80 .

Testosterone concentration and pulse frequency

Average concentration of testosterone was similar in both groups throughout the experiment. There was a positive correlation between testosterone concentration and age of the lambs ($r = +0.74$ and $+0.87$ for control and PTU+H groups, respectively) indicating an effect of age on the testosterone concentration. The average number of testosterone pulses per 6 h sampling period was greater in PTU+H than in control lambs by 1 week from induction of hyperthyroidism through until 4 weeks after ending treatment, and then again from 25 to 31 weeks of age ($P < 0.01$; Fig. 2). There were positive correlations between serum testosterone concentrations and testosterone pulse frequencies ($r = +0.68$ and $+0.34$ in control and PTU+H lambs, respectively).

FSH concentration

FSH concentration in the two groups was different from 6 to 9 weeks of age only ($P < 0.05$; Fig. 3). The pattern of the FSH profile was the same in both groups and there was a positive correlation between FSH and age of the lambs in both groups ($r = +0.77$ and $+0.81$ for PTU+H and control lambs, respectively). Likewise, there was a positive correlation between FSH and testosterone concentration in both groups ($r = +0.55$ and $+0.47$ for control and PTU+H lambs, respectively). The correlation coefficients for FSH and scrotal circumference were $+0.74$ in the PTU+H and $+0.72$ in the control lambs.

Insulin-like growth factor I concentration

In PTU+H lambs, IGF-I concentrations were lower than

Fig. 3. Serum FSH concentrations in lambs treated with 6-propyl 2-thiouracil orally and thyroxine and tri-iodothyronine s.c. (PTU+H; ■) and in control lambs (□). FSH was lower at weeks 7, 8 and 9 in the PTU+H than in the control lambs. Asterisks indicate significant differences between the two groups ($P < 0.05$). Treatment started at week 6 and ended at week 12 of age. The SEM for each individual data point has been omitted for clarity. The range of SEMs for weeks 7–13 was 0.14–0.42 and, for weeks 15–31 was 0.10–0.82. Overall SD of the means was ± 0.70 and ± 1.31 for weeks 7–13 and 15–31, respectively.

those in controls at weeks 10, 11, 12 and 13 of age ($P < 0.05$) but were similar at other ages. The average IGF-I concentrations at week 11 were 412.5 ± 19 and 930 ± 55 ng ml⁻¹ for PTU+H and control lambs, respectively. There was an effect of age on the IGF-I concentrations in both groups ($P < 0.05$). In the control group, the increase in the IGF-I concentration was biphasic. IGF-I increased from 7 to 15 weeks of age and then decreased until week 27 and increased again at 27 to 34 weeks of age ($P < 0.05$). In the PTU+H lambs, an increase in IGF-I concentrations started at weeks 8–10 and then declined from week 10 to week 12. After week 13, the IGF-I pattern was similar between the groups (Fig. 4) and from week 13 to the end of the experiment, there was a positive correlation between IGF-I, T_3 and T_4 ($r = +0.48$ versus 0.79 for T_3 and $+0.79$ versus $+0.93$ for T_4 in the PTU+H and control groups, respectively).

Concentrations and ratio of T_4 and T_3

In the PTU+H group, serum T_4 and T_3 concentrations were higher during the treatment period ($P < 0.01$) whereas their concentrations were similar thereafter. At week 6 (before treatment), concentrations of T_4 and T_3 were similar between the two groups; T_4 concentrations were 5.8 ± 0.2 versus 5.8 ± 0.3 $\mu\text{g dl}^{-1}$ for control and PTU+H lambs, respectively, and T_3 concentrations were 126.6 ± 5.2 and 105.6 ± 5.5 ng dl⁻¹ for control and PTU+H lambs, respectively. At week 10, mean T_4 concentration reached a maximum value at 13.2 ± 0.4 $\mu\text{g dl}^{-1}$ in PTU+H lambs while, in controls, it was 5.14 ± 0.5 $\mu\text{g dl}^{-1}$ (Fig. 5). At week 9, in the PTU+H group, T_3 concentration reached a

Fig. 4. Serum concentrations of insulin-like growth factor I (IGF-I) in lambs treated with 6-propyl 2-thiouracil orally and thyroxine and tri-iodothyronine s.c. (PTU+H; ■) and in control lambs (□). Average IGF-I concentrations were lower in the PTU+H than in the control lambs at 10, 11, 12 and 13 weeks of age. Asterisks indicate significant differences between the two groups ($P < 0.05$). Treatment started at week 6 and ended at week 12 of age. The SEM for each individual data point has been omitted for clarity. The range of SEMs was 15.90–53.15 and the overall SD of the means was ± 75.98 .

Fig. 5. Serum thyroxine (T_4) concentrations in lambs treated with 6-propyl 2-thiouracil orally and thyroxine and tri-iodothyronine s.c. (PTU+H; ■) and in control lambs (□). During weeks 7–11, T_4 concentrations were higher in the PTU+H than in the control lambs ($P < 0.01$), but were lower in the PTU+H lambs at week 12. Asterisks indicate significant differences between the two groups ($P < 0.01$). Treatment started at week 6 and ended at week 12 of age. The SEM for each individual data point has been omitted for clarity. The range of SEMs was 0.07–0.57 and the overall SD of the means was ± 0.97 .

maximum value at 544 ± 47.0 versus 157.3 ± 26 ng dl⁻¹ in the control group (Fig. 6). The ratio of $T_4:T_3$ during the treatment period was 38.9 ± 2.5 in control and 32.9 ± 6.6 in PTU+H lambs, whereas after treatment until the end of experiment it was 43.9 ± 2.4 versus 46.7 ± 2.6 for control and PTU+H lambs, respectively. There was a negative

Fig. 6. Serum tri-iodothyronine (T_3) concentrations in lambs treated with 6-propyl 2-thiouracil orally and thyroxine and tri-iodothyronine s.c. (PTU+H; ■) and in control lambs (□). During weeks 7–10, T_3 concentrations were higher in the PTU+H than in the control lambs ($P < 0.01$). At week 12, the concentration of T_3 decreased in the PTU+H lambs to nearly zero. Asterisks indicate significant differences between the two groups ($P < 0.01$). Treatment started at week 6 and ended at week 12 of age. The SEM for each individual data point has been omitted for clarity. The range of SEMs for weeks 6–13 was 2.75–39.66 and the overall SD was ± 38.94 .

correlation between the T_3 concentration and age of the lambs ($r = -0.58$ for PTU+H and -0.8 for control lambs). The Pearson product-moments for T_4 and age of the lambs were -0.65 and -0.78 for PTU+H and control lambs, respectively, indicating the effect of age on the concentration of thyroid hormones. After administration of the hormones ended at 12 weeks of age, the serum concentration of the hormones in the PTU+H group decreased markedly (to $0.32 \pm 0.15 \mu\text{g dl}^{-1}$ for T_4 and $38.1 \pm 4.9 \text{ ng dl}^{-1}$ for T_3). The concentrations of both T_4 and T_3 increased and were comparable to controls within the next 2 weeks.

Discussion

In other studies, administration of T_4 to adult sheep at the rate of $13.15 \mu\text{g kg}^{-1}$ body weight per day did not produce catabolic effects of thyrotoxicosis, although live weight was slightly reduced (Godden and Weekes, 1984). Moreover, T_3 injection into young lambs at the rate of $5 \mu\text{g kg}^{-1}$ body weight per day did not result in serum T_3 concentrations within the toxic range (Lynch *et al.*, 1983). Likewise, in the present study, administration of 8 and $16 \mu\text{g kg}^{-1}$ body weight per day of T_3 and T_4 , respectively, did not result in symptoms of thyrotoxicosis. Thyroid hormones in treated lambs returned to concentrations similar to those in controls within 2 weeks of stopping treatment. The reduced T_4 and T_3 concentrations in the first week after treatment were likely due to residual depressor effects of the treatment on thyroid function, TSH and TRH release (Hadley, 1992). The

ratio of $T_4:T_3$ was similar during and after treatment, implying that the amounts administered corresponded to the normal physiological ratio.

In spite of the initial lower relative growth, the transient hyperthyroidism did advance testicular function and onset of puberty in treated lambs. This finding contrasts with results from rats in which hyperthyroidism reduced final testicular size (van Haaster *et al.*, 1993), and hypothyroidism increased testicular size at puberty (van Haaster *et al.*, 1992). Hypothyroidism leading to increased testicular size at puberty was determined to be the result of hyperplasia and hypertrophy of Sertoli and Leydig cells (Cooke *et al.*, 1992). Cooke *et al.* (1994a) noted that T_3 directly inhibited mitogenesis of cultured neonatal rat Sertoli cells. Therefore, smaller testes with fewer more differentiated Sertoli cells might have been expected in PTU+H lambs compared with controls. However, the converse occurred.

Transiently hyperthyroid lambs produced more viable sperm cells earlier than did euthyroid lambs, indicating that the Sertoli cells of the PTU+H lambs were at more advanced stages of maturation and able to support and nourish a greater number of sperm cells. The increase in testicular size of PTU+H lambs may be due to hypertrophy of Sertoli cells and hyperplasia of the germ cells. An eightfold increase in Sertoli cell protoplasm normally occurs during the peripubertal period in lambs (Monet-Kuntz *et al.*, 1988). Mature Sertoli cells produce androgen-binding protein (ABP), which binds to testosterone (Martin *et al.*, 1991), thereby increasing testosterone concentration in the seminiferous tubules. The maintenance of high intratesticular testosterone concentrations enhances spermatogenesis and the nourishment of the developing germ cells (Mita *et al.*, 1982). Therefore, it appears that excess thyroid hormones during early development enhance maturation and growth of the testes and advance the chronology of testicular growth in ram lambs. However, the mechanisms remain to be clarified.

The relationship between hyperthyroidism and testosterone concentration has not been defined. The higher testosterone pulse frequency associated with advancing puberty (Renaville *et al.*, 1993) in the PTU+H lambs was more likely due to increased pulsatility of GnRH and LH (Sanford *et al.*, 1978) than to the changes in the FSH concentration. Throughout the sampling periods, FSH concentration was not higher in the PTU+H lambs than it was in controls. Reductions in the concentration of FSH in the hyperthyroid lambs were transient, whereas reduction of FSH in hyperthyroid (van Haaster *et al.*, 1993) and hypothyroid rats (Kirby *et al.*, 1992) was permanent. In cultured neonatal rat Sertoli cells, T_3 alone or in combination with FSH stimulated production of inhibin- β_b mRNA (Cooke *et al.*, 1994a). It is conceivable that high concentrations of thyroid hormones in the PTU+H lambs increased inhibin production by the Sertoli cells, thereby causing a reduction in FSH concentration. There was no apparent deleterious effect on testes development or spermatogenesis.

The lower IGF-I concentration in the hyperthyroid lambs during treatment is consistent with the results of Elsasser *et al.* (1991) in steers. At physiological concentrations, thyroid hormones stimulate pituitary GH production which, in turn, stimulates IGF-I production in the liver and other tissues (McGuire *et al.*, 1992). Alteration of thyroid function can affect pituitary GH mRNA production (Samuels *et al.*, 1989) and hepatic IGF-I production. Although transiently lower peripheral IGF-I concentrations may reflect central suppression of GH, testicular concentrations of IGF-I may have been quite different. Thyroid hormones control production and secretion of IGF-1 in Sertoli cells (Smith *et al.*, 1987). Therefore, the transient increase in thyroid hormone concentrations may have promoted Sertoli cell maturation and spermatogenesis through increased IGF-I concentration in the microenvironment of the seminiferous tubules.

This is the first report of juvenile hyperthyroidism in sheep. The present results show that transient hyperthyroidism enhanced the sequence of events leading to the onset of puberty in hyperthyroid lambs, and enhanced testicular size and sperm output. These findings are in contrast with the situation in rats and may reflect developmental differences between the species. Maximum proliferation of rat Sertoli cells occurs perinatally coincident with the onset of neonatal thyroid function (Jannini *et al.*, 1990). In lambs, thyroid activity begins *in utero* and maximal Sertoli cell proliferation occurs early in post-natal life (Gordos and Berndston, 1993). However, even though the time window of effectiveness and rate of proliferation may differ between lambs and rats (Cook *et al.*, 1994b), it remains unclear why the response to thyroid hormones should be opposite. A number of questions remain unanswered. The reason for the higher testosterone pulse frequency, increased testicular size and function in the PTU+H lambs compared with controls remains to be determined. However, the present study provides some insight into the complexity of neonatal gonad development and the involvement of thyroid hormones in this process. Even short-term hyperthyroidism can have provocative influences on gonad development and function in ram lambs.

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