Early prenatal treatment of ewes with testosterone completely masculinises external genitalia of female offspring but has no effects on early body weight changes

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Abstract. Treatment of pregnant ewes from day 20 of gestation with 100 mg implants of crystalline testosterone did not cause masculinisation of genitalia or affect growth rates of female lambs. Prenatal treatment from day 20 of gestation with testosterone propionate (1.2 g in divided doses for four weeks) or testosterone cypionate (600 mg in 3 doses over three weeks) completely masculinised the external genitalia of female lambs producing a ventral penis and scrotum with no external vaginal opening; ovaries and uteri were normal. Single male lambs were significantly heavier than female lambs during a 16 week measurement period, but masculinised female lambs were not different from controls. In the twin lamb groups, there were no differences between male and female or treated and control lambs. Body weights of castrated male lambs born as twins were not different from entire controls. It was concluded that testosterone treatment of developing lambs from 20 to 65 days of gestation produces complete masculinisation of external genitalia of female lambs but does not affect body weight during the first 16 weeks of age.

In the female rat it is well documented that exposure to androgens in the early postnatal period will produce the 'androgenisation syndrome' with vaginal cornification, sterility, and failure of normal mating behaviour even when primed with oestrogen and progesterone (Baraclough 1966; Gorski 1971; Harris 1964). Several investigators have shown that the androgenised female rat is significantly heavier than the normal female rat (Beatty et al 1970; Bell & Zucker 1971; Tarttelin et al. 1975). Tarttelin et al. (1976) reported that early postnatal treatment (up to day 3) was effective in increasing body weight (BWt) but that later treatment (day 4 or 5) was without effect, even with a large dose of testosterone, although classic androgenisation was still observed.

Little comparable work has been reported in larger domestic animals. There have been several unsuccessful attempts to reproduce the androgenisation syndrome in heifers, bitches and gilts (Zimbelman & Lauderdale 1973) and sheep (Przekop et al. 1974). Zimbelman & Lauderdale (1973) treated cows prenatally (83–133 days of gestation) with androgens and reported increases in clitoral size but no effects on BWt or fertility. Przekop et al. (1974) injected androgens directly into lamb foetuses at the 84th day of gestation but reported no masculinisation of external genitalia. However, Jost et al. (1973) reported that prenatal androgen treatment of pregnant cows from 40–60 days of gestation produced a fully differentiated penis in heifers but later treatment was ineffective in masculinising external genitalia. Several workers (Alifakiotis 1976; Clarke et al. 1976; Short 1974; Wilson & Tarttelin 1978a) have reported masculinisation of the external genitalia of female lambs providing androgen treatment was started early (from day 20 of gestation), but later treatment (after 70 days of gestation) was less effective, and treatment after 90 days was ineffective (Clarke et al. 1976). Hinz et al. (1974) reported masculinisation of external genitalia of
gilts following prenatal androgen treatment (42–77 days of gestation).


Steroids have been used for many years to increase live weight gains of large animals, particularly steers (Dinusson et al. 1950) and more recently comparative studies using oestrogens and commercially available anabolic agents such as Trenbolone (Friaplix; Heschst) and Zeranol (Ralgro; International Minerals Corporation) given to 12 to 15 month old steers have verified increased BWt gains (O’Lamhna & Roche 1984). Many studies using synthetic oestrogens, such as diethylstilboestrol, have demonstrated increased BWt gains in lambs (O’Mary et al. 1952) but this compound has deleterious effects on carcass finish and serious potential public health hazards (Herbst & Bern 1981).

A technique which could increase BWt gain in female lambs, such as has been described in the androgenised rat, applied during a limited period during gestation which would ensure no steroid residues in the offspring seemed worthy of investigation. Evidence of masculinisation of the female genitalia would be a useful marker of prenatal treatment. The present study describes 3 experiments conducted over a 2-year period when BWt during the first 16 weeks of age was measured in the offspring of ewes treated from the 20th day of gestation with, implants of testosterone, repeated injections of testosterone propionate, or repeated injections of testosterone cypionate.

### Materials and Methods

The subjects in experiment 1 and 2 were 2–3 year old cross-bred (Romney × Border Leicester) ewes kept at pasture: each ewe was identified with a numbered plastic neck label. Oestrous cycles were synchronised during the second month of the breeding season with polyurethane tampons impregnated with 40 mg methyl-acetox-propodeosterone removed after 14 days, mating started 2 weeks later. Experiment 3 used 3–5 year old unsynchronised Romney ewes. Two rams wearing marker crayons were put with the ewes 2 weeks after removal of tampons and successful mating was verified by twice daily examination of the ewes: day 1 of

| Table 1. Birth weight (means (SEM)) and number of lambs analysed (N) according to treatment group. |
| ----------------------------------------------- | ----------------------------------------------- | ----------------------------------------------- |
| Experiment | Treatment | Male | | Female | |
| | | N | Birth weight | | N | Birth weight | |
| 1 | Twins | T | 5 | 4.5 (0.1) | | 8 | 3.8 (0.2) | |
| | Control | | 6 | 4.1 (0.3) | | 7 | 4.2 (0.2) | |
| | Castrate | | 8 | 3.6 (0.2) | | | | |
| 2 | Singles | TP | 3 | 5.1 (0.1) | | 9 | 4.1 (0.2) | |
| | Control | | 6 | 5.7 (0.2) | | 5 | 5.2 (0.2) | |
| 3 | Twins | TC | 3 | 3.9 (0.2) | | 9 | 3.9 (0.4) | |
| | Control | | 5 | 4.3 (0.3) | | 7 | 4.0 (0.5) | |
| | Castrate | | 8 | 3.8 (0.4) | | | | |
| | Singles | TC | 10 | 4.7 (0.3) | | 11 | 4.4 (0.2) | |
| | Control | | 6 | 4.9 (0.3) | | 3 | 3.3 (0.1) | |

T: testosterone implant (100 mg).
TP: testosterone propionate injection (total dose 1.2 g).
TC: testosterone cypionate injection (total dose 600 mg).
Scatterplot showing highly significant correlation between mean body weight (n = 11) and SEM (filled circles) and loss of this correlation when body weight is transformed to logarithms (base 10) (open circles).

Treatment

All treatments started on day 20 of gestation.

1) 25 ewes were implanted with a 100 mg pellet of testosterone: 15 controls received a cholesterol implant. Prior to lambing all sites of implantation were examined and all implants were fully absorbed.

2) 25 ewes were injected with 100 mg testosterone propionate in 2 ml ethyl oleate, 3 times weekly for 4 weeks: 15 control ewes received 2 ml of vehicle.

3) 40 ewes were injected with 3 doses of 200 mg testosterone cypionate (Depo-testosterone, Upjohn Ltd.) given on day 20, 27 and 40 of gestation: 20 untreated ewes acted as controls.

Data analysis

Arithmetic means of BWt data collected over several weeks are highly correlated with variance which may invalidate statistical tests such as those used in a regression analysis; in analysis of variance (ANOVA) homogeneity of variance amongst groups of samples is assumed. Fig. 1 shows the significantly high correlation between BWt, expressed as the mean of a typical group measured over a 16 weeks period, plotted against the standard error of the mean (r = 0.98; P < 0.001). A simple transformation to logarithms (base 10) removes this correlation (r = 0.28; NS), therefore all data were analysed as logarithms. However, to simplify interpretation of graphs, results are graphed in the 'raw' state. Analysis was by oneway ANOVA at each week; weeks in which a significant 'F' value was obtained were analysed further. Most analyses involved four groups: treated males and females, and control males and females. The sum of squares amongst treatments yielded a set of three orthogonal single degree of freedom comparisons: 1) all females vs all males, 2) treated females vs control females and 3) treated males vs control males.

When a castrated group was included in the analysis then an additional comparison was made between castrated and entire males in the same treatment group. Further analysis was by regression using a technique of fitting individual regression lines to each lamb's BWt over the 16 week period similar to that described for the rat (Clark & Tartellin 1978) but using the combining techniques and ANOVA described by Sokal & Rohlf (1969). This technique allows combining data for a group of animals and has the advantage of separating within animal variation from between animal variation in the combined analysis. In the results section references to growth rates are derived from the regression analysis.
Results

Behaviour

Experiment 1. No abnormal behaviour was observed in the treated ewes. Experiments 2 and 3. Treated ewes showed mounting behaviour and were aggressive in their reaction to the sheepdogs and handlers with excessive foot stamping and head-down butting threats.

Lamb mortality

Experiment 1. There were 2 returns to service, and these ewes proved to be barren: there was no neonatal mortality.

Experiment 2. One ewe was barren and dystokia occurred in 2 ewes. Neonatal mortality was 17% and 22% in controls and treated ewes, respectively.

Experiment 3. Dystokia occurred in 3 ewes. Lamb mortality was 14% and 6% in controls and treated ewes, respectively.

Table 1 illustrates the numbers of lambs analysed in the various treatment groups. Groups of less than three were excluded from the study.

Appearance of external genitalia

Experiment 1. There were no obvious differences between female lambs from treated or control ewes.

Experiments 2 and 3. At birth all lambs in the treated group were sexed as male by the shep-

Fig. 2A and B.

A. Weekly body weight data of twin lambs in Experiment 1. Data are expressed as group arithmetic means (vertical error bars are given at week 16). Graph symbols are as follows: open circles: treated females; filled circles: control females; open triangles: treated males; filled triangles: control males.

B. Weekly body weight data of single lambs in Experiment 2. Data and key as in Fig. 2A.
herd, a decision based on the presence of a penis and scrotum. Six treated lambs which died neonatally were autopsied. In three lambs testes were absent, but normal uteri and ovaries were present. Normal Fallopian tubes, uterine horns, cervixes and vaginas were present, but there were no external vaginal openings. The penes were identical with those of the other three lambs (which were normal males, born to the treated ewes, but with normal testes in the scrota) and included a sigmoid flexure and a retractor penis muscle: the empty scrota remained at birth size. At 6 months of age, several lambs were slaughtered, and those with masculinised genitalia were found to have poorly developed penes with no processus urethrae, poorly developed retractor penis muscles and prepucial adhesions. An area of excoriation of the skin around the prepuce (urinary scald) was present in most of the masculinised female lambs. Final confirmation of sex identify was made by recovery of ovaries at slaughter in all treated female lambs.

**Body weight**

Birth weights are given in Table 1.

Experiment 1. Weekly BWt data are illustrated in Fig. 2A and show no differences between groups, from birth, in either absolute BWt or rates of gain. Eight male lambs were selected at random from 14 males and castrated, and ANOVA showed a significant difference in BWt from birth until 16 weeks ($P < 0.01$), with the entire lambs being heavier, but there was no difference in growth rate between the two groups (these data are not illustrated).

Experiment 2. Weekly BWt data are illustrated

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**Fig. 3A and B.**

A. Weekly body weight data of single lambs in Experiment 3. Data and key as in Fig. 2A.
B. Weekly body weight data of twin lambs in Experiment 3.
in Fig. 2B, and ANOVA proved consistent significant differences ($P < 0.01$) with males heavier than females from birth, but there were no significant differences between treated and control groups of either sex (except that control females were heavier than treated females, $P < 0.01$, from birth until 3 weeks of age). There were no significant differences between growth rates in any of the treatment groups.

Experiment 3. Weekly BWt data of single lambs are illustrated in Fig. 3A and the results of the analysis are as described in Experiment 2. above (except that treated females were heavier than control females, $P < 0.05$, from birth until week 4). Weekly BWt data of twin lambs are illustrated in Fig. 3B and show no significant differences between groups, from birth, either in absolute BWt or in growth rates. A group of eight lambs were castrated and showed no differences in absolute BWt or growth rates when compared to entire male lambs (these data are not illustrated).

**Discussion**

Testosterone treatment of ewes, either in the form of propionate (total dose of 1.2 g from 20–50 days of gestation) or cypionate (total dose of 600 mg from 20–40 days of gestation), caused full masculinisation of external genitalia of female lambs: a testosterone implant (100 mg) was ineffective. Calculations based on the data of Cowie & Flux (1954) would indicate that the dose and shape of the implant used would have given a daily release rate of 1 mg (roughly equivalent to a dose of 20 µg/kg BWt). Short (1974) and Clarke et al. (1976) used implants of 1 g testosterone and reported successful masculinisation of female lambs. Testosterone absorbed from propionate injections would not be expected to persist beyond day 50, but significant levels of testosterone, following cypionate injections, have been reported until 65 days of gestation (Wilson & Tarttelin 1978a). These findings confirm earlier reports that the form of testosterone treatment, which induces masculinisation of external genitalia, is not important if an adequate dosage is maintained during the period of maximum sensitivity (Alifakiotis 1976; Clarke et al. 1976; Short 1974) which would appear to coincide with the onset of testicular testosterone secreting activity in the normal lamb (Attal et al. 1969; Pomerantz & Nalbandov 1975).

The present study confirms a sexually dimorphic difference in BWt with male lambs being heavier than female lambs but only in groups of single lambs during the first 16 weeks. There was no evidence of increased BWt in female lambs treated during the period 20–65 days of gestation so it is clear that in the lamb there is no correlation between the timing of the masculinisation of genitalia and that of modifications to the growth control process. Schlenker & Hinz (1974) reported a similar conclusion in gilts. However, there is some evidence for hypothalamic change induced by androgen treatment during this early period. Clarke et al. (1976) reported abnormal ovarian cyclicity in their treated female lambs (which had masculinised external genitalia). Wilson & Tarttelin (1978a) reported significant depressions in spontaneous luteinising hormone and testosterone plasma levels in male lambs born to sheep treated prenatally with testosterone (20–40 days of gestation). Furthermore, Wilson & Tarttelin (1978b) provided evidence that prenatal androgenisation had affected the production and/or release of gonadotropin hormone releasing hormone at hypothalamic levels. So it appears that testosterone treatment from 20–65 days of gestation will masculinise external genitalia of female lambs and will affect control of the hypophyseal-gonadal axis, but not growth control processes. It is possible that treatment of ewes during later stages of gestation (70–140 days) might affect BWt in female lambs.

The penes and scrotum of the treated female lambs did not grow significantly from birth; these structures depend on a continual source of androgens for normal growth and development. The persistence of an infantile penis, with prepuce infections and absence of a processus urethrae, interfered with normal micturition and caused ‘urine scald’ which predisposed to ‘fly strike’ and local infections. Such a condition would severely limit the practicality of any commercial application of prenatal androgen treatment even if significant increases in BWt could be stimulated.

The present study failed to show a significant weight difference following castration of male lambs. Everitt & Jury (1966) reported depression in growth in castrated lambs, but only at the 10% level of probability. In Experiment 1 of the present study, control lambs were significantly heavier
than the castrated group from birth, even though the two groups were selected into their treatment groups at random. However, regression analysis proved that the growth rates were identical during the 16 week study period. The groups selected for castration in Experiments 1 and 3 were born as twins from treated ewes, which may have influenced BWt changes following castration. Also twin lambs have a lower birth weight than single lambs, so it is possible that BWt at birth is a factor affecting early growth rate. It was not possible to study castrated males born as singles as the group numbers were too small.

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References


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