Steroid Levels After Intramuscular Injection of Testosterone Propionate in the Caprine

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ABSTRACT

Nine sexually mature intact grade does were injected intramuscularly with testosterone propionate and subsequent plasma steroid concentrations determined and male-like behavior recorded. The does received either 100 mg testosterone propionate every three days for six treatments, total dose 600 mg (N = 5); 50 mg testosterone propionate daily for eighteen days, total dose 900 mg (N = 2) or 10 mg testosterone propionate daily for eighteen days, total dose 180 mg (N = 2). The treatments induced male-like sex behavior, the intensity of which was related to the dose of exogenous testosterone used, the regimen of administration, and the plasma levels of testosterone. Exogenous testosterone treatment had minimal effect on the subsequent reproductive activity of the does.

RÉSUMÉ

Cette expérience impliquait neuf chèvres adultes, croisées et intactes; elle consistait à leur administrer du propionate de testostérone, par la voie intramusculaire, et à déterminer ensuite la teneur de leur plasma en stéroïdes, ainsi qu'à enregistrer tout comportement d'allure mâle. Cinq d'entre elles reçurent 600 mg de cette hormone, à raison de 100 mg à tous les trois jours, à six reprises; deux autres en reçurent 900 mg, à raison de 50 mg par jour, pendant 18 jours; les deux dernières en reçurent 180 mg, à raison de 10 mg par jour, pendant 18 jours.

Ces interventions provoquèrent un comportement sexuel mâle dont l'intensité se révéla proportionnelle à la quantité d'hormone injectée, à la façon d'en étaler les injections et à la concentration plasmatique ultérieure en testostérone. L'expérience n'exerça qu'une influence mitigée sur l'activité reproductrice ultérieure de ces chèvres.

INTRODUCTION

Accurate detection of estrus in goats is important for maximum fertility, especially where artificial insemination or controlled natural mating is used. Estrus detection includes observations for the signs of estrus such as continuous bleating and flagging of the tail (7). More recently teaser bucks have been used to assist in the detection of estrus. Intact bucks or intact bucks surgically altered by deviating the penis from the midline to a position 45° from the midline (2) have been used for this purpose. However, the maintenance of a buck for detection of estrus is not profitable where only a few animals are kept. Furthermore, the keeping of intact bucks in dairy herds is undesirable because of the odor and the fear of tainting the milk. In addition, teaser bucks and occasionally surgically altered bucks have been known to achieve intromission and thus the possibility of spreading venereal disease exists.

More recently cows (8, 9, 10) and ewes (12) treated with testosterone have been used to detect estrus in cattle and sheep. No such studies have been carried out in goats. In our earlier report, we have described the effects of various treatments with testosterone propionate treatments on the induction of male-like behavior in female goats (3). This report details the plasma steroid patterns in female goats treated with testosterone propionate, and the subsequent reproductive events following the termination of treatment.

MATERIALS AND METHODS

EXPERIMENT I

Six sexually mature grade does which had kidded during the previous kidding season, March to May 1977 were used. The does were paired into three groups and each pair confined to a boxstall. The does received the same kind of managment, care and feed during the breeding season which commenced in September, 1977. They were observed and teased daily for signs of estrus for a period of one month prior to the commencement of the testosterone injections. Three dosage levels of testosterone propionate[1] were used. One pair (does 7 and 16) recieved intramuscular injections of 10 mg testosterone propionate each daily for 18

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[1]Sterivet Laboratories, Bolton, Ontario.

days (180 mg); in the second pair (does 14 and 15) each doe received 50 mg intramuscularly daily for 18 days; (900 mg) and in the third pair (does 10 and 11) each doe received 100 mg intramuscularly every third day for a total of six injections (600 mg).

Two adult ovariectomized does were treated, one with ECP[2] 4 mg, and the other with DES[3] 10 mg every third day for the purpose of estrus induction and were used as stimulus animals.

Daily observations were made on sexual behavior of the testosterone treated does by parading one of the ovariectomized estrogen induced estrous does in front of their pens or by restraining an estrous doe in a small stanchion and presenting a testosterone treated doe to this stimulus subject. In addition the treated does were exposed to an intact buck for the purpose of determining estrous behavior.

Daily blood samples were collected *via* jugular venipuncture (immediately prior to all injections) from the hormone treated does starting four days before and continued for the period of testosterone treatment (18 days). The plasma was separated and stored at -20°C until needed for assay. At the conclusion of the behavioral studies the testosterone treated does were bred.

In order to provide information regarding the plasma levels of testosterone in the intact buck during the breeding season and for comparison to levels achieved following testosterone injection into does, the following study was carried out. Two adult bucks which had been used successfully for breeding during the previous season were used. Blood samples were obtained at hourly intervals from these bucks between 0800 and 1800 on three separate occasions during the breeding season (September, October and November).

ASSAYS

Progesterone was measured using a specific antiserum to 11 α -hydroxyprogesterone hemiscuccinate-BSA raised in sheep. The behavior of this antiserum in a rapid radioimmunoassay method and its crossreaction with the major steroids have been previously described (4,14). Progesterone was extracted from duplicate aliquots of 200 microliters of the plasma samples for the assay. To establish whether or not plasma would yield blank values, plasma samples from ovariectomized goats were extracted and analysed with the method. The plasma blank was less than 50 pg which was less than assay sensitivity. The mean percent recovery of known amounts of unlabelled progesterone in 200 microliters of ovariectomized goat plasma was 95 ± 0.7% (N=11). The interassay coefficient of variation was 12.2% (N=12).

Plasma concentrations of testosterone were determined by a radioimmunoassay method (1). The antiserum was supplied by Dr. Lars-Eric Edqvist, Royal Veterinary High School, Uppsala, Sweden. The crossreaction of the antiserum with the major steroids have been described (1). Testosterone was extracted from duplicate aliquots of 400 microliters of the plasma samples for the assay. A blank of 60 pg was found in ovariectomized goat plasma at this extraction volume, and the results were corrected for this plasma blank. Percent recovery of known amounts of unlabelled testosterone added to 400 microliters of ovariectomized goat plasma was 93.4 ± 2.8% (SD) for 21 assays. The within and between assay coefficients of variation were found to be 14 and 18% respectively.

Total immunoreactive estrogens expressed in the results as estrogens were determined in one assay using a radioimmunoassay method (6). The crossreaction of the antiserum used have been described (11). Its crossreaction with diethylstilbestrol was not determined, but is highly unlikely.

EXPERIMENT II

Since there was no information regarding the reproductive activity and fertility of female goats treated with testosterone, and because of the occurrence of one case of cystic ovarian degeneration in one doe and the failure of conception in another doe in experiment I, it was decided to determine the effect of the treatment on ovarian function in the doe. Accordingly, three mature does, #77, 78, 79 were treated with testosterone during the next breeding season, in November, 1978. The does received intramuscular injections of PG-F_{2a}[4] on October 24; and on November 2, the testosterone injections were started. Intramuscular injections of 100 mg testosterone propionate were administered to each doe every third day for a total of six treatments (600 mg). The observations on the sexual behavior of these does were as described in experiment I. The does were bred on the first estrus following the testosterone treatment. Daily blood samples were collected from these does starting at five days before the first testosterone injections and continued for thirty-six days. The plasma was separated and stored until assayed for testosterone and progesterone.

RESULTS

Observations on sexual behavior including emergence and duration of virilistic behavior, and the stimulus values of treated does as teaser animals have been reported elsewhere (3). In brief, male-like behavior was induced in all the does treated with testosterone and the virilistic behavior was highest in does treated with 50 mg testosterone daily and least in the does treated with 10 mg testosterone daily.

The results of breeding following the testosterone treatments are summarized in Table I. One doe (#14) manifested persistent estrus

[2]ECP, Estradiolcypionate, Upjohn Company, Don Mills, Ontario.
[3]DES, Diethylstilbestrol, M.T.C. Pharmaceuticals, Hamilton, Ontario.
[4]Lutalyse injection, Tuco Products, Orangeville, Ontario.

TABLE I. Testosterone Treatment in Female Goats

	Goat #	Stage of Cycle	Treatment	Remarks
		Experiment I (1977)		
	10	Anestrus		Bred — twins
Group I			100 mg Test[b]/ 3 d/6x	
	11	12 d[a] postestrus		Bred — twins
	14	Anestrus		Cystic ovaries; ovariectomized
Group II			50 mg Test daily/18 d	
	15	4 d postestrus		Bred — NP[c]
	7	2 d postestrus		Bred — triplets
Group III			10 mg Test daily/18 d	
	16	4 d postestrus		Bred — twins
		Experiment II (1978)		
	77	6 d postestrus		Bred — twins
	78	5 d postestrus	100 mg Test/3 d/6	Bred — twins
	79	6 d postestrus		Bred — twins

[a]d = days
[b]Test = testosterone propionate
[c]NP = not pregnant

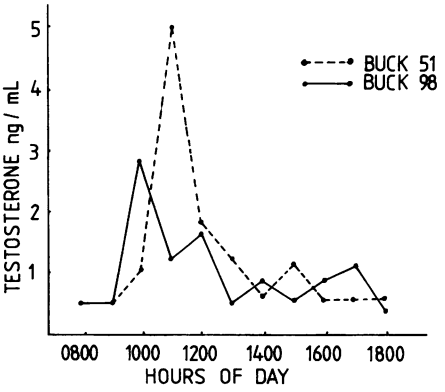

Fig. 2. Plasma testosterone levels in two bucks during an eight hour period on a day in the breeding season.

for three weeks following the last treatment; the ovaries were removed, and were found to contain three follicles, 0.6 to 1.2 cm in diameter (Fig. 1). Seven other does conceived and produced twins or triplets after the treatment.

The patterns of plasma testosterone in two bucks during an eight hour period of a day in October, are shown in Fig. 2. As can be seen the basal concentration of testosterone was 0.5 ng/mL or less in both bucks. One clearly defined peak occurred in each buck between 1000 and 1100 hr. The peak levels were 2.9 and 5.0 ng/mL. The overall testosterone profile was similar in the two bucks, except for the time of occurrence of peak concentration. The plasma testosterone patterns were qualitatively and quantitatively similar in the samples collected in September and November.

Treatment with estradiol cypionate or diethylstilbestrol induced estrus receptivity in the ovariectomized does. Estrous behavior was first observed 24 hours after the first injections, and was maintained throughout the experimental period. The intensity of estrous behavior waned gradually after the last injection, so that by seven to ten days after the last estrogen injection, the does refused to stand for a teaser buck. The plasma concentrations of estrogens (total immunoreactive estrogens) in the ovariectomized doe treated with

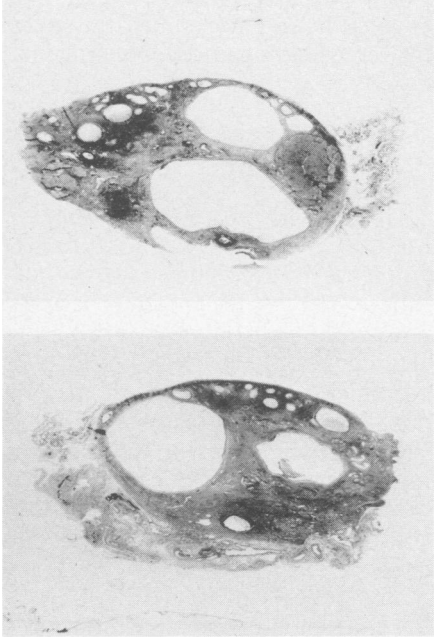

Fig. 1. Photomicrograph of ovaries removed from a doe (#14) with persistent estrus following treatment with testosterone propionate. The ovaries contained cystic structures and a corpus luteum.

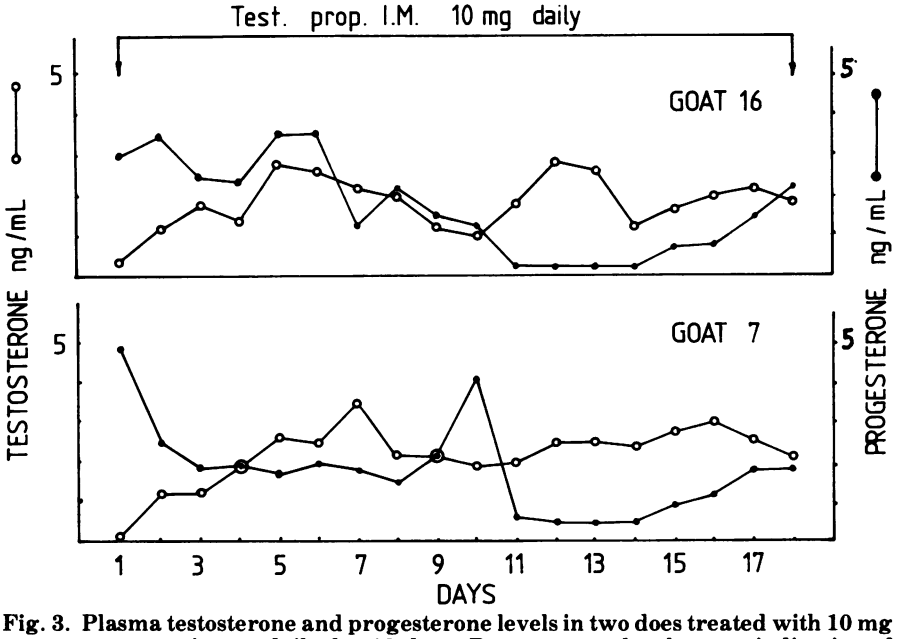

Fig. 3. Plasma testosterone and progesterone levels in two does treated with 10 mg testosterone propionate daily for 18 days. Progesterone levels were indicative of ovulation in both does during treatment.

estradiol cypionate increased from concentrations of 20 pg/mL or less, to concentrations between 120-750 pg/mL during treatment. On the other hand, the plasma estrogen levels in the ovariectomized doe treated with diethylstilbestrol, varied between 10 and 50 pg/mL during treatment.

Concentrations of plasma testosterone and progresterone in the testosterone treated does are reported in Figures 3 to 7. Levels of testosterone in the plasma of the two does treated with 10 mg testosterone propionate daily, increased to 1 ng/mL or more a day after the first injection, and thereafter the testosterone concentrations varied between 1.2 and 3.0 ng/mL during the experimental period (Figs. 3 and 6). In does treated with 50 mg testosterone daily, plasma testosterone levels increased gradually to attain concentrations of 8 ng/mL or more after the first injections. Thereafter, the plasma testosterone concentrations varied from 8 to 13 ng/mL during treatment (Figs. 4 and 6). The testosterone levels fluctuated widely in one doe, while in another doe there was very little daily variation.

The largest daily fluctuations in the plasma testosterone concentration were in the does treated with 100 mg testosterone propionate every three days (Figs. 5 and 6). The highest plasma testosterone concentration occurred a day after each injection, and the least concentration occurred on the third day after each injection. In three of the does where the plasma testosterone levels after the last treatments were studied, a gradual decrease to 0.5 ng/mL or less occurred within six to seven days (Fig. 6).

The plasma progesterone levels showed cyclical variations in the testosterone treated does (Figs. 3-5). In one case only, No. 14 (Fig. 4), the progesterone values were 0.5 ng/ML or less throughout the observation period.

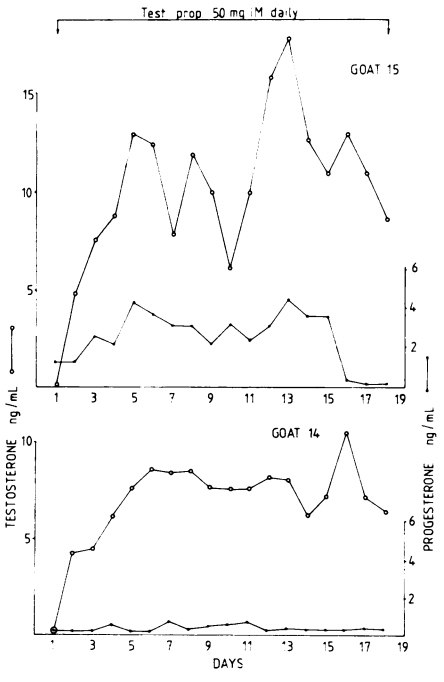

Fig. 4. Plasma testosterone and progesterone levels in two does treated with 50 mg testosterone propionate daily for 18 days. Note the low progesterone levels in doe #14.

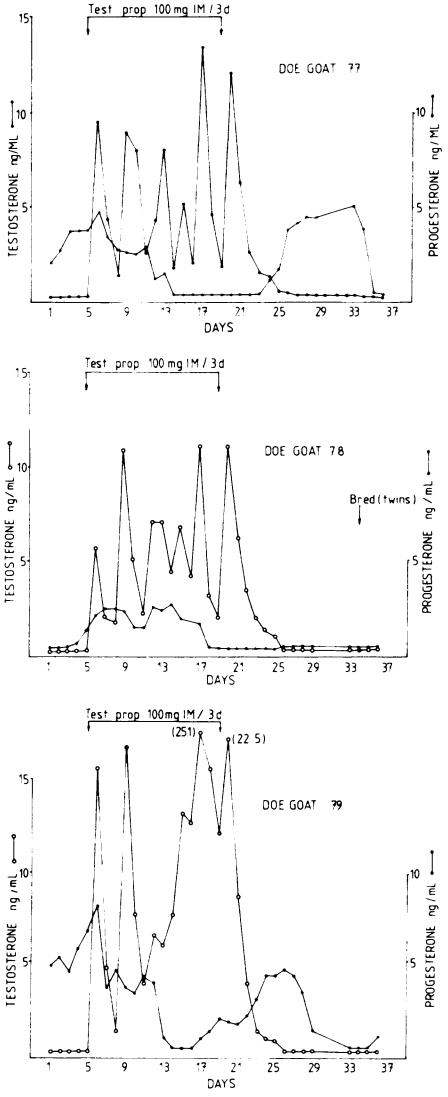

Fig. 5. Plasma testosterone and progesterone levels in three does treated with 100 mg testosterone every third day for six treatments.

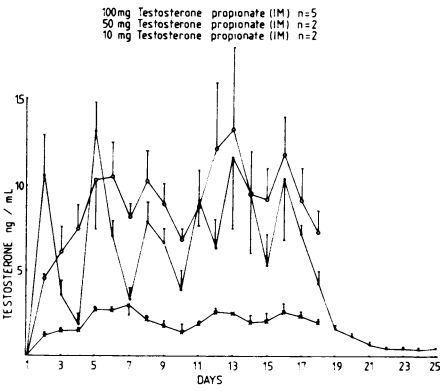

Fig. 6. Mean levels of plasma testosterone in does treated with testosterone propionate. Note the wide fluctuations in does treated with 100 mg testosterone propionate every three days.

DISCUSSION

Administration of testosterone propionate induced male sex behavior in intact female goats as previously reported for the ewe (12) and the cow (10). Testosterone treated does can, therefore, be used as teaser animals and would obviate the use of intact or surgically altered bucks for detection of estrus. The cost involved in the testosterone treatments was minimal, and on the basis of the behavioral responses induced in each group, it would appear that the administration of testosterone propionate at 50 mg/day would be most effective although impractical due to the daily administration. For practical purposes the administration of 100 mg testosterone propionate every three days is advocated. This study did not determine the regimen necessary to maintain virilistic behavior in does following induction. In a similar study conducted in ewes, male sex behavior was successfully induced by the administration of 50 mg of testosterone propionate every other day for twenty days and was maintained with similar injections every ten days thereafter (12). It would appear, therefore, that a similar treatment following the

period of induction would be effective in maintenance of male sex behavior in the female goat.

In the present study, seven out of nine does conceived and produced normal kids following the testosterone treatment. Thus the adverse affect of the testosterone treatment on fertility was minimal.

Neither the testosterone concentration nor the endogenous profile of this hormone appeared to have been hitherto reported in the buck when this study was started. While this manuscript was in preparation, Muduuli *et al* (13) reported on a detailed study of the secretory patterns, circadian and seasonal changes in testosterone in the male pygmy goat. The mean serum concentration of testosterone was 2.3 ± 0.5 ng/mL, which is similar to the levels found in this study. However, the episodic secretions of testosterone described by Muduuli *et al* (13), was more obvious than that found in the present study, and this may be due to the difference between intervals of blood sampling. In our study , samples were obtained at hourly intervals whilst Muduuli *et al* (13) collected samples at half-hourly intervals. The difference in levels from month to month in the breeding season was not observed in this study.

Examination of the plasma progesterone profiles in the animals treated with testosterone was indicative of normal ovarian function. Thus the testosterone treatment did not appear to affect the corpus luteum function. Some of the does ovulated during treatment while others ovulated shortly after completion of the treatments. The plasma progesterone levels in these does were similar to those described for the doe during the normal estrous cycle (14).

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