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Prenatal Androgen Exposure and Growth and Secretion of Growth Hormone and Prolactin in Ewes Postweaning[1,2] (42535)

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Abstract. Growth and secretion of growth hormone (GH) and prolactin (PRL) in ewe lambs exposed to androgen during fetal development were investigated. Testosterone cypionate was administered to the pregnant dams from approximately Days 28 to 84 of gestation. Ewe lambs from dams that received androgen exhibited masculinized external genitalia and some masculine behavioral characteristics. Intact androgenized ewe lambs grew faster ($P < 0.05$) and were more efficient in conversion of food to body gain ($P < 0.05$) than ewe lambs born to untreated dams over the period from 70 to 224 days of age. One-half of the ewe lambs in each group was ovariectomized at 58 days of age. Ovariectomy had no effect on subsequent growth or efficiency of growth in the control ewe lambs. However, ovariectomy of androgenized ewe lambs abolished the observed stimulated rate of growth and decreased the improvement in efficiency of food conversion. Blood samples were collected from the lambs at 85 and 136 days of age at 15-min intervals for 8 hr to determine parameters of GH and PRL secretion. Prenatal androgen exposure had no effect on any parameter of GH or PRL secretion. These data indicate that prenatal androgen exposure altered differentiation of growth potential in ewe lambs, but the growth response was not mediated through dramatic changes in secretion of adenohypophysial somatotropic hormones, GH and PRL.

In many species, rate and pattern of growth are sexually dimorphic. In domestic ruminant species males are heavier at birth and, whether intact or castrate, exhibit faster rates of growth which are characterized by relatively more protein and less fat accretion than that in females. The superior rate and pattern of growth demonstrated by males are the consequence of sexual differentiation which begins early in fetal development (see (1), for review). Testosterone production by embryonic testes is a major component of the sexual differentiation process. Testosterone and/or its metabolites are responsible for masculinization of the external and internal genitalia, the hypothalamic–hypophysial axis, and many behavioral characteristics. Neonatal administration of testosterone to female rats enhanced body weight gain and long bone growth when measured at 105 days of age (2). The profile of growth hormone (GH) secretion in those androgen-treated, ovariectomized female rats was more masculine in character (high maximal values with low minimal values) than feminine (lower maximal values with higher minimal values). Patterns of growth hormone secretion differ between sexes (higher in males than females), in sheep (3), cattle (4), and swine (5). Growth hormone response to administered growth hormone-releasing hormone is also greater in male than in female cattle (6). Prolactin (PRL) has many of the biological actions of GH and has been implicated in the regulation of rate and pattern of growth (7, 8). The objective of the present study is to determine the effect of altering the processes of sexual differentiation during fetal development on growth and secretion of the somatotropic hormones, GH and PRL, in ewe lambs and to determine whether expression of the effect required the ovaries.

Materials and Methods. Estrus in crossbred multiparous ewes ($\frac{1}{2}$ Finnish Landrace, $\frac{1}{4}$ Dorset, $\frac{1}{4}$ Rambouillet) was synchronized through use of intravaginal progestin pessaries

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(Synchro-Mate, G. D. Searle Co., Skokie, IL). Ewes were mated to Suffolk rams fitted with marking harnesses. One-half of the dams received one im injection of 200 mg testosterone cypionate (Depo-Testosterone, Upjohn Co.; 200 mg/ml cottonseed oil) during each of the following periods, 28–35, 35–42, 49–56, 63–70, and 77–84 days postmating. Lambs used in the study were born on Julian date 91.1 ± 1.9 (mean ± SD). Fourteen ewe lambs from untreated control dams (control lambs) and 14 ewe lambs from androgen-treated dams (androgenized lambs) were weaned at an average age of 44 days and were moved to individual pens in a controlled environment at 51 days of age. The pens were 1.1 m^2. Light: dark cycle was 14:10 and temperature was 18–24°C. At 58 days of age one-half of the lambs from each group was ovariectomized via midventral laparotomy under local anesthesia. Lambs left intact were subjected to sham surgeries. The growth performance trial was initiated when the lambs were 70 days of age. Each lamb was individually fed with orts weighed back weekly. Lamb weights were obtained at fortnightly intervals through 196 days of age. At 84 and 135 days of age the lambs were fitted with indwelling jugular cannulae and the next-day blood samples were collected at 15-min intervals for an 8-hr period (9). One lamb in each of the control–intact and androgenized–ovariectomized groups died before the end of the feeding trial and were excluded from all data analyzed.

Plasma concentrations of GH and PRL were quantified by radioimmunossay (8). Temporal concentrations for each animal were subjected to the PULSAR algorithmic procedure which defined the secretory patterns in terms of mean and baseline concentrations, and number and amplitude above baseline of secretory peaks or pulses (8, 10). The equation to predict the standard deviation (SD) for the concentration of GH ([GH]) was $SD = 0.535 + 0.041 * [GH]$ where [GH] was measured in nanograms per milliliter. The equation to predict SD for concentration of PRL ([PRL]) was $SD = 1.12 - 0.019 * [PRL] + 0.0004 * [PRL]^2$ where [PRL] was measured in nanograms per milliliter. The G_1 to G_5 values used were 3.80, 2.60, 1.90, 1.50, and 1.20, respectively, for both GH and PRL.

The growth performance estimates analyzed for individual animals were average daily feed consumption, efficiency of gain, and average daily gain (ADG) which was estimated by regression analysis. Data for feed consumption and ADG were analyzed by one-way analysis of variance. Efficiency (conversion of feed to live body weight) was estimated by the allometric autoregressive model (11). The allometric autoregressive model was chosen because it presents the nonlinear term, change in weight relative to change in food intake, in a linear manner. The slope of the resulting equation, the rate of change in body weight as a function of rate of change in feed intake, was utilized as the estimate of efficiency. Treatments were compared by orthogonal contrasts. Parameters of GH and PRL secretory patterns were analyzed by split-plot analysis of variance. The whole plot consisted of treatment tested by animal within treatment. The subplot contained the effects of age and the interaction of age and treatment. The multiple regression analysis initially included treatment and the linear effects for baseline concentrations, number of peaks, and mean amplitude of peaks of GH and PRL. Mean concentrations were omitted since they are comprised of the other parameters. Using minimum error mean square criterium, terms were deleted sequentially following step-down procedures. This procedure was continued until a final model with minimum error mean square was obtained. The process was repeated at that point by including treatment and endocrine trait cross products for the remaining endocrine traits in the multiple regression model.

Results. Ewe lambs from dams which received exogenous androgen during early gestation had masculinized external genitalia, i.e., penis and empty scrotum. The internal genitalia, as observed at ovariectomy, was unaltered. Some androgenized ewe lambs demonstrated masculine posture during urination.

Growth performance data are presented in Table I. Intact lambs born to dams treated with testosterone cypionate during early gestation had rates and efficiencies of gain superior to those of the lambs from untreated dams. Ovariectomy had no significant influence on the growth performance of control ewe lambs. Rate of gain of the androgenized ovariectomized ewes was similar to that of control ewe lambs and efficiency was reduced to a level intermediate between intact androgenized and control ewe lambs.

TABLE I. LEAST-SQUARES MEANS AND STANDARD ERRORS FOR GROWTH PERFORMANCE OF EWE LAMBS

Treatment group	*n*	Average daily gain (kg/d)	Feed consumption (kg/d)	Efficiency[a]
Control–intact	6	0.23 ± 0.01^{b}	0.96 ± 0.04^{b}	0.31 ± 0.01^{b}
Control–ovx	7	0.25 ± 0.01^{b}	$1.02 \pm 0.03^{b,c}$	0.32 ± 0.01^{b}
Androgenized–intact	7	0.30 ± 0.01^{c}	1.10 ± 0.03^{c}	0.36 ± 0.01^{c}
Androgenized–ovx	6	0.24 ± 0.01^{b}	0.97 ± 0.04^{b}	$0.34 \pm 0.01^{b,c}$

[a] Efficiency = $\frac{\ln \text{Body wt} - \ln \text{Intercept}}{\ln \text{Cumulative Feed Intake}}$ (Roux, Ref. (11)).

[b,c] Values within a column with common superscripts are not different ($P < 0.05$).

Estimates of parameters of GH and PRL secretory patterns are presented in Tables II and III. Neither treatment nor interaction of treatment and age had a significant effect on parameters of GH or PRL secretion. Mean and baseline PRL concentrations and number and amplitude of GH peaks decreased ($P < 0.05$) with age whereas number of PRL peaks increased ($P < 0.05$). Effect of age approached significance ($P = 0.01$) for mean GH concentration.

Discussion. Intact ewe lambs exposed to exogenously administered androgen during fetal development had greater rates of gain and were more efficient in conversion of feed to body tissue. This agrees with results from rats (2, 12, 13) and cattle (14). In a preliminary report, DeHaan *et al.* (15) observed similar results in ewe lambs from dams implanted with testosterone.

In the present study, ovariectomy had no effect on growth rate or efficiency of feed conversion in the control ewes. This is contrary to previous reports in which growth rate was depressed after ovariectomy (16, 17). Surprisingly, in androgenized ewes castration abolished the response in growth rate and reduced the response in efficiency of feed utilization for growth. It is apparent that mediation of the growth response of ewes to prenatal androgen treatment requires ovarian factors.

The manner of GH secretion in female cattle (14) and ovariectomized rats (2) was altered by androgen administration during early development. Jansson *et al.* (2) suggest that alteration of GH secretion in female rats to a more masculine manner of secretion is the mechanism by which the growth response to androgen administration during early development is mediated (2). However, Millard *et al.* (18) reported that the GH secretory patterns in adult female rats treated neonatally with testosterone propionate were not different from those in untreated female rats. The rats in that study were intact, whereas those used by Jansson *et al.* (2) were castrated neonatally. Also, Millard *et al.* (18) examined the secretory pattern by quantification of temporal concentrations of GH in samples collected in a manner similar to that in the present study. Jansson *et al.* (2) utilized multiple daily samples collected at different times on each day. The pre-

TABLE II. LEAST-SQUARES MEANS AND STANDARD ERRORS FOR GROWTH HORMONE SECRETORY PARAMETERS

Treatment group	Age (d)	Overall mean (ng/ml)	Baseline mean (ng/ml)	Peaks (No./8 hr)	Amplitude (ng/ml)
Control–intact	85	9.80 ± 0.92	7.40 ± 0.65	2.00 ± 0.54	17.40 ± 3.15
	136	8.10 ± 0.92	7.30 ± 0.65	2.33 ± 0.54	6.47 ± 3.15
Control–ovx	85	8.90 ± 0.85	7.49 ± 0.60	2.57 ± 0.50	9.21 ± 2.92
	136	8.64 ± 0.85	7.89 ± 0.60	1.43 ± 0.50	7.66 ± 2.92
Androgenized–intact	85	9.20 ± 0.85	8.11 ± 0.60	2.00 ± 0.50	8.46 ± 2.92
	136	7.84 ± 0.85	7.29 ± 0.60	1.29 ± 0.50	4.71 ± 2.92
Androgenized–ovx	85	8.40 ± 0.92	6.78 ± 0.65	3.17 ± 0.54	8.52 ± 3.15
	136	7.37 ± 0.93	6.50 ± 0.65	1.33 ± 0.54	5.37 ± 3.15

TABLE III. LEAST-SQUARES MEANS AND STANDARD ERRORS FOR PROLACTIN SECRETORY PARAMETERS

Treatment group	Age (d)	Overall mean (ng/ml)	Baseline mean (ng/ml)	Peaks (No./8 hr)	Amplitude (ng/ml)
Control–intact	85	127.4 ± 13.1	120.1 ± 12.8	4.00 ± 0.85	53.82 ± 28.13
	136	96.6 ± 13.1	90.1 ± 12.8	4.67 ± 0.85	26.68 ± 28.13
Control–ovx	85	115.5 ± 12.1	105.1 ± 11.9	4.57 ± 0.78	45.46 ± 26.04
	136	67.5 ± 12.1	59.5 ± 11.9	6.14 ± 0.78	27.84 ± 26.04
Androgenized–intact	85	102.4 ± 12.1	89.3 ± 11.9	3.42 ± 0.78	102.14 ± 26.04
	136	94.6 ± 12.1	82.7 ± 11.9	5.57 ± 0.78	37.81 ± 26.04
Androgenized–ovx	85	116.7 ± 13.1	105.8 ± 12.8	4.33 ± 0.85	44.60 ± 28.13
	136	92.5 ± 13.1	76.5 ± 12.8	4.83 ± 0.85	72.41 ± 28.13

cision of the method for definition of secretory patterns employed by Millard *et al.* (18) and in the present study is superior to that of the method used by Jansson *et al.* (2). Such observations raise the question of whether masculinized patterns of GH secretion are basis for the reported increased growth rate in intact androgenized female rats (12, 13). In the present study, neither treatment nor the interaction of treatment and age had a significant effect on any parameter of GH or PRL secretion. These data imply that the enhanced rate and efficiency of growth observed in the intact androgenized ewes were not mediated through modified GH or PRL secretion.

It has been reported that when parameters of GH and PRL secretion are incorporated into multiple regression models, significant proportions of the variation in measures of growth are explained (8). In the present study, efficiency of gain was analyzed using a model which included the linear terms for baseline concentration, and number and mean amplitude of peaks of GH and PRL at 136 days of age which contributed to a reduction in error mean square. The analysis used the hormone information from 136 days of age because that was midway through the period the growth data was collected. Approximately 52% of the variation in the conversion of food to live weight could be explained with inclusion of the endocrine parameters which contributed to a reduction in the error mean square, i.e., number of GH peaks and PRL baseline concentration and peak amplitude. Only 22% of the variation in feed conversion was explained by treatment alone. The results of this analysis suggest that variations in parameters of GH and PRL secretion are related to variations in efficiency of gain.

Observation of masculine urinary posture in androgenized ewe lambs emphasizes the myriad of physiological modifications induced in females by exposure to androgens during early development. Urinary posture is a sexually dimorphic trait controlled by the central nervous system (19). It is not the result of an indirect effect of modification of genital anatomy. In the bitch it is the consequence of androgen programming of the central nervous system pre- and perinatally which can be amplified by additional androgen treatment in adulthood. This observation, masculine urinary posture in androgenized ewes, which has been reported previously (20) emphasizes the need to expand the list of possible mediators of the treatment beyond alteration of hypothalamic–hypophysial regulation of somatotropic hormone secretion. Gustafsson and coworkers (2, 21–23) have demonstrated differences between male and female rodents with respect to GH-regulated hepatic function and hepatic receptors for PRL. Hepatic response to GH and PRL may partially mediate the growth response observed in the androgenized ewe lambs. A local effect of GH on long bone growth in rats has been reported (24, 25). The local response to GH may also be modified by prenatal androgen exposure and postnatal ovarian secretions.

Exposure of ewe lambs to significant concentrations of androgen during early fetal development altered the processes of differentiation of the external genitalia, some behavioral characteristics, and rate and efficiency of growth in intact ewes. Contrary to what had been hypothesized, treatments which altered rate or efficiency of growth were not mediated through measurable effects on GH and PRL secretion. Regression analysis suggests that variation in estimates of GH and PRL secretory parameters is related to variation in estimates of conversion of feed to body tissue. It may be that subtle changes in control of

secretion of adenohypophysial somatotropic hormones, GH and PRL, and/or response to those hormones are responsible for the changes in growth observed in the present study.

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