Some Effects of Diethylstilbestrol on Sexual Maturation and Growth of Male Calves

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Abstract

Six experiments involving 300 calves were conducted to determine the effects of subcutaneous administration of diethylstilbestrol (DES) at birth on growth and maturation of the intact male calf. Calves implanted at birth and again at three months of age grew at least equal to or faster than the controls. The greatest advantage in growth was from birth to three months of age. Five levels of DES were used (0 mg, 6, 12, 15, and 24 mg). Direct comparisons of all levels were not made within years, but the 12-mg implant seemed to produce the most consistent results. Testicular development was retarded and seminiferous tubules were significantly smaller in the implanted calves. Average testes weights and average seminiferous tubule diameters were significantly ($P<.01$) correlated ($r=.875^{**}$). Spermatogenesis was retarded in the treated male. The animals also lacked in libido and produced semen of inferior quality having more abnormal sperm and sperm of lower motility. The use of DES increased the size of the urethra in steers but had no effect upon urethral development in the intact male.

Introduction

The effects of subcutaneous administration of diethylstilbestrol (DES) on rate of gain, efficiency of feed utilization and carcass quality in poultry, sheep, and cattle have been extensively investigated. Increased rate of gain and improvement of feed efficiency due to DES implants were reported in beef heifers (Dinusson *et al.*, 1950), beef steers (Andrews, *et al.*, 1950), and lambs (Andrews, *et al.*, 1949; Jordan, 1950; Perry, *et al.*, 1951).

Nonsignificant but generally consistent reductions in quality grades in DES-treated steers were reported (Clegg and Cole, 1954; Andrews *et al.*, 1950). Conversely, Winchester and Andrews (1953) reported increased subcutaneous fat in DES-treated bulls.

Reports on the response of bulls to DES treatment are more limited and somewhat conflicting. Improved rate of gain and improved efficiency of feed utilization have been reported by some workers (Klosterman *et al.*, 1955; Bailey, *et al.*, 1966), while others have found no advantage to such treatments (Pilkington *et al.*, 1959; Voelker and Dracy, 1956).

Voelker and Dracy also reported that oral DES reduced testes weights in dairy bull calves. Implants of DES on older bulls have been shown to decrease (Cahill *et al.*, 1956) or to have no effect upon testes weights (Bailey *et al.*, 1966). Some researchers (Cahill *et al.*, 1956; Klosterman *et al.*, 1955) have reported reduced secondary sex characteristics in implanted feedlot bulls. Estrogenic effects upon the accessory glands have been reported (Clegg *et al.*, 1955; O'Mary *et al.*, 1952), but effects upon urethra size in lambs or cattle have not been shown.

Since cattle in the reported experiments were implanted at two months of age or older, the effects of DES implanted at birth were investigated. The parameters involved included level of implant, site of implant, preweaning and postweaning gains, testicular development, sperm production, semen characteristics, and urethra development.

Methods

In Experiments 1, 2, 3, and 4, male calves were stratified as to age and randomly allotted to the hormonal treatments (Table 1). Implants were

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Station Bulletin 616 October 1974

Agricultural Experiment Station
Oregon State University, Corvallis

subcutaneous, close to the base of the ear unless otherwise specified. All calves were left intact until two weeks prior to weaning (190 days), at which time they were castrated. Tissue samples were prepared for histological study according to the method of Weesner (1965) with minor modifications. Average daily gain (ADG) was established on full weights and weaning grade by a committee of three.

The number of calves per treatment were 16, 9, 15, and 13 for experiments 1 through 4, respectively. In Experiment 3, half the calves from each treatment were left intact after seven months of age to study the effects of DES implantation on semen production and quality. An additional bull with a 15-mg implant at birth and one nontreated bull calf were used for comparative purposes. Semen collections were attempted with an electroejaculator at 10 months of age or when each animal acted sexually mature. The semen collected was evaluated according to the method of Perry (1963).

In Experiment 5, 25 bull calves were randomly allotted to three hormonal treatments and were slaughtered at a weight of a approximately 500 kilograms. The effects of DES on total growth and testicular development were the parameters measured.

Ninety-three male calves were used in Experiment 6 to determine the effects of castration and DES implants on urethra development. At time of slaughter the inside diameter of the urethras of steers and bulls was measured at four locations: (1) directly behind the head of the penis; (2) midway from the head of the penis to the first curvature of the sigmoid flexure; (3) at the first curvature of the sigmoid flexure; and (4) at the second curvature of the sigmoid flexure.

Analysis of variance for a completely randomized design was performed on all data. Treatment means were compared using Kramer's (1956) extension of Duncan's method (Li, 1965).

Results and Discussion

Implanting of intact male calves with DES did not decrease and in most cases increased ADG at three months of age or at weaning, with an accompanying reduction in sexual development (Table 1). The fact that a somewhat greater increase in gain occurred in the first three months may be due to less androgenic antagonism modifying the effectiveness of DES. Nalbandov (1958) reported that there is no detectable androgen in bull urine prior to 6 weeks of age.

The calves implanted at birth and again at three months of age were fatter at weaning than the controls. Greater amounts of scrotal fat were apparent during castration, supporting the work of Winchester and Andrews (1953).

The size of the testicles was reduced by as much as 25%, with the body of the testicle poorly developed and flaccid to the touch. Implants at

Table 1. Effect of DES on Growth, Grade, and Seminiferous Tubule Size

Experiment	Treatment	ADG to 3 months		ADG to wean		Wean grade[a]	Seminiferour tubule dia. μ
		lb.	(kg)	lb.	(kg)		
1	12 mg at birth	2.48[b]	(1.13)	2.01	(0.91)	7.5	.....
	12 mg at 3 mo.	2.26	(1.03)	1.90	(0.86)	7.0	.....
	0 mg (control)	2.25	(1.02)	1.90	(0.86)	7.8	.....
2	6 mg at birth	2.22	(1.01)	1.90	(0.86)	7.2	114
	6 mg at birth + 15 mg at 3 mo.	2.32	(1.05)	2.04	(0.93)	7.5	72[c]
	15 mg at 3 mo.	2.16	(0.98)	1.87	(0.85)	6.9	109
	0 mg (control)	2.21	(1.00)	1.94	(0.88)	7.2	123
3	15 mg at birth + 15 mg at 3 mo.	2.39	(1.09)	1.90	(0.86)	7.2	129
	15 mg at 3 mo.	2.35	(1.07)	1.96	(0.89)	7.7	137
4	6 mg at birth[d] + 12 mg at 3 mo.	2.35	(1.07)	1.92	(0.87)	7.6	102
	12 mg at birth[d] + 12 mg at 3 mo.	2.38	(1.08)	1.97	(0.90)	7.6	94
	6 mg at birth + 12 mg at 3 mo.	2.46	(1.12)	1.90	(0.86)	7.5	96
	12 mg at birth + 12 mg at 3 mo.	2.55	(1.16)	1.97	(0.90)	7.5	94
	0 mg (control)	2.36	(1.07)	1.84	(0.84)	6.9	168[c]

[a] A score from 1 (least desirable) to 10 (most desirable).
[b] Significantly ($P < .05$) greater than others within that column and experiment.
[c] Significantly ($P < .01$) different than others in that experiment.
[d] Initial implant was in the scrotum and the second one in the ear.

birth and at three months reduced seminiferous tubule size (Figure 1).

The implanted intact males retained from Experiment 3 lacked in masculinity. In addition to reduced testis size, they were "steer-headed," showed little crest with straight hair, and lacked in libido. Consistent semen collections were obtained only from the control bull and from one bull implanted at birth only (Table 2). When successful collections were made from the treated bulls, the semen quality was inferior with more abnormal sperm and somewhat fewer live sperm that generally lacked in motility.

Average daily gains to three months of age were similar for animals with scrotal implants and the controls and less than the gains for animals initially implanted in the ear (Table 1). This may have been due to some infection at the site of implantation. Because of this infection, subsequent implants were made in the ear, which was not as susceptible to bacterial proliferation. Gains were greater for all implanted calves from birth to weaning. The testes weights and diameters of seminiferous tubules were significantly greater ($P<.01$) for the controls. Average testes weights and average seminiferous tubule diameters were significantly ($P<.01$) correlated ($r=.875^{**}$).

Increased gains to slaughter resulted when calves were implanted initially at birth or at 90 days of age and reimplanted at 90-day intervals (Table 3). There was an accompanying significant reduction in the testis weight and diameter of seminiferous tubules ($P<.01$).

There was a trend toward larger urethral lumen with delayed castration (Table 4). When implants of either 12 or 24 mg were administered to late castrates, the urethral lumen were significantly ($P<.01$) larger. The use of DES implants on intact males failed to reduce urethral lumen diameter regardless of the age at implant (Table 5). Although urethral blockage at the sigmoid flexure seems to cause the greatest problem, locations 3 and 4 were significantly ($P<.01$) larger than locations 1 and 2 in all animals measured. The implanting of intact males with DES had no effect upon urethra size.

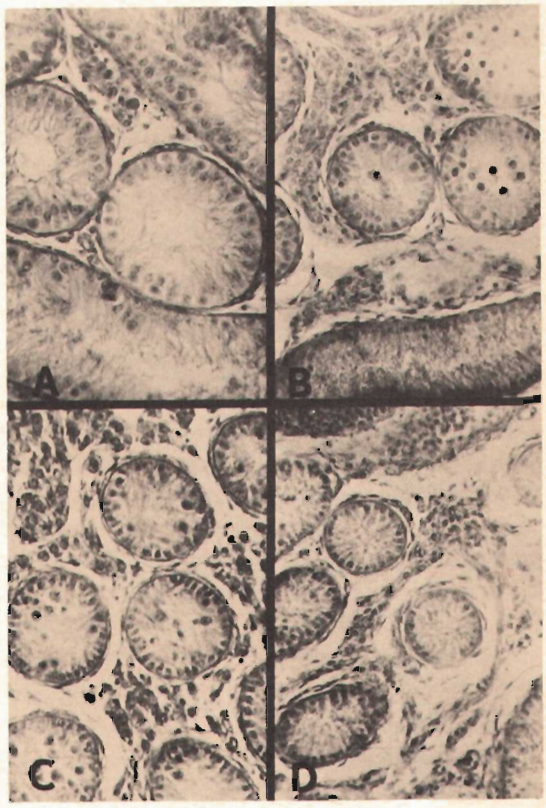

Figure 1. Microphotographic comparison of seminiferous tubule development of nontreated and of DES-treated bull calves (X 340). A. Control: An average external seminiferous tubule diameter of 158 μ. B. Implanted with 6 mg od DES at birth: An average external seminiferous tubule diameter of 103 μ. C. Implanted with 15 mg of DES at three months of age: An average external seminiferous tubule diameter of 98 μ. D. Implanted with 6 mg of DES at birth plus 15 mg of DES at three months of age: An average external seminiferous tubule diameter of 67 μ.

Table 2. Effects of DES Upon Semen Production and Testes Development

	Group 1[a] (birth, 15 mg ear + 3 mo., 15 mg. ear)	Group 2[b] (3 mo., 15 mg ear)	Group 3 no DES	Group 4 (Birth, 15 mg ear)
Av. no. of collection atempts	10.0	9.0	8.0	10.0
Av. no. of successful attempts	2.0	3.0	8.0	9.0
Av. concentration of sperm, million/cc	400.0	275.5	406.5	454.5
Av. percent live sperm	30.0	25.0	31.86	31.78
Av. percent abnormal sperm	23.50	27.44	17.42	17.22
Av. motility score[c]	3.50	3.00	5.14	4.00
Av. testes weight, g	241.25[e]	270.70[e]	342.00[d]	301.00[e]
Av. seminiferous tubule dia., μ	301.50[e]	289.80[e]	357.00[d]	309.00[e]

[a] Only one of the group was collected from the collection at the tenth period was the only one of high concentration.
[b] Only two bulls could be collected from and then only at the later collection periods.
[c] A score from 1 (least motile) to 10 (most motile).
[d, e, f] Means in the same line bearing different superscripts are significantly ($P < .01$) different.

Table 3. Effect of DES Upon Feedlot Gain and Testes Development of Bulls (Experiment 5)

Treatment[a]	ADG to slaughter		Av. testis wt. (g)	Av. seminiferous tubule dia. μ
	lb.	(kg)		
1	3.00	(1.36)	136	245
2	2.94	(1.34)	189[b]	266[d]
3	2.86	(1.30)	291[c]	294[e]

[a] Treatment 1. Implant of 12 mg DES at birth and 3 mo. and 15 mg at 7 mo. and 11 mo.
2. Implant 12 mg DES at 3 mo. and 15 mg at 7 mo. and 11 mo.
3. No DES (control)

[b] Significantly ($P<.05$) greater than 1.
[c] Significantly ($P<.01$) greater than 1 and 2.
[d,e] Values bearing different superscripts in the same column are significantly ($P<.01$) different.

Table 4. Effect of Castration and DES Upon Urethra Size

	Time of castration		
	Birth dia., mm	3 mo. dia., mm	7 mo. dia., mm
Steer treatment[a]			
0 mg DES	4.8	5.2	5.2
12 mg DES	5.1	5.4	6.5[b]
24 mg DES			6.5[b]

[a] Steers were implanted at 7 months of age.
[b] Significantly ($P<.01$) larger than other steers.

Table 5. Effect of DES Implants on Urethra Size in the Intact Male

	Time of implant		
	Birth dia., mm	3 mo. dia., mm	7 mo. dia., mm
0 mg DES			8.6
12 mg DES	8.6	7.9	
23 mg DES			8.5

Summary

Six experiments were conducted to determine the effects of subcutaneous administration of diethylstilbestrol (DES) at birth on growth and maturation of the intact male calf. Calves implanted at birth and again at three months grew at least equal to or faster than the controls. The greatest advantage in growth was from birth to three months of age. The 12-mg implant produced the greatest gains and most consistent results. Testicular development was retarded and seminiferous tubules were significantly ($P<.01$) smaller in the implanted calves. Spermatogenesis was retarded in implanted males, and collections indicated the treated animal lacked in libido and produced semen of inferior quality. The use of DES in steers increased the size of the urethra but had no such effect upon the treated bull.

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