AN ABSTRACT OF THE THESIS OF

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Title: SOME EFFECTS OF DIETHYLSTILBESTROL ON SEXUAL MATURATION AND GROWTH OF MALE CALVES

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Six experiments were conducted to determine the effects of DES implantation on preweaning performance and sexual maturation of male calves when implanted at various stages of development and different sites.

Calves receiving DES implants in Experiments I and II were implanted at birth, at birth and at about three months of age, or at about three months of age. Some of the calves in Experiment I were left intact until slaughter, while the remainder of the calves in the first two trials were castrated two weeks prior to weaning. Statistical analysis of data from the first trial showed that calves implanted (12 mg.) at birth gained significantly ($P < .05$) faster to three months of age than did non-implanted calves and held a slight advantage to weaning. An advantage in preweaning performance was not observed for implanted calves in Experiment II. However, there was a significant ($P < .01$) reduction in the average external diameter of

seminiferous tubules in calves receiving two preweaning implants. In both trials, DES treatment caused a delay in development of secondary sex characteristics, such as crest, increased shoulder development and curly hair about the head.

In Experiment IIIa there were no differences in preweaning performance and sexual maturation between bull calves implanted at birth and at three months of age and those implanted at three months of age. In yearling bulls with the same treatments (Experiment IIIb) there was a delay in the onset of semen production.

Bull calves in Experiment IV received implants at birth, at three months of age, at weaning and after 110 days in the feedlot; the same treatment without the birth implant; or no DES implants. There was a trend toward increased average daily gain with increasing levels of DES. The testes weight was significantly ($P < .01$) decreased by DES treatment. Furthermore, testes weights of the two DES treatments were significantly ($P < .05$) different. There was a significant ($P < .01$) reduction in average external seminiferous tubule diameter with each increase in DES level.

Two levels of scrotal DES implants at birth plus ear implants at three months of age were compared to two levels of ear implants at birth plus ear implants at three months of age. In this experiment (Experiment V) scrotal implants did not cause an increase in gain to three months, but there was an advantage over the controls to weaning.

Gains were similar for scrotal and ear implants to weaning. At weaning there was a significant ($P < .01$) reduction in testes weight and average external seminiferous tubule diameter with DES treatment.

Experiment VI dealt with bull and steer calves implanted with DES at three different stages of development prior to weaning. At slaughter the inside diameters of the urethras were measured at four locations. Urethra diameter was significantly ($P < .01$) reduced by castration. DES implants caused a significant ($P < .01$) increase in the inside diameter of the urethras of steers, but had no effect in bulls.

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

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SOME EFFECTS OF DIETHYLSTILBESTROL ON SEXUAL MATURATION AND GROWTH OF MALE CALVES

INTRODUCTION

The effects of subcutaneous administration of diethylstilbestrol (DES) on the rate of gain, the efficiency of feed utilization and the carcass quality of finishing poultry, sheep and cattle have been extensively investigated.

Administration of DES, as subcutaneous pellet implants, to three week old cockerels, older cockerels and roosters resulted in an increase in the fat content of various tissues (Lorenz, 1943, 1945; Hughes, 1946; Andrews, 1947). The increase in fat deposits resulted in improved carcass quality. It was also found that young cocks, toms, drakes and ganders could be successfully castrated by subcutaneous DES implants (J. Am. Vet. Med. Assn., 1949). This approach to castration has not been extensively investigated in beef cattle.

Numerous investigators have reported marked increases in rate of gain and efficiency of feed utilization, due to DES implants, in beef heifers (Dinusson et al., 1950), beef steers (Andrews et al., 1950) and lambs (Andrew et al., 1949; Jordan, 1950; Perry et al., 1951). Carcasses of treated steers generally grade lower than non-treated steers primarily because of reduced subcutaneous fat (Clegg and Cole, 1954; Andrews et al., 1954). Conversely, it is the

increased subcutaneous fat of treated bulls and excellent muscle development that qualify them for higher grades (Winchester and Andrews, 1953). The reason for this difference in response is not clear.

The reports in the literature on the effects of DES on bull performance are limited and somewhat conflicting. Several workers report increased rate of gain and efficiency of feed utilization in treated bulls (Klosterman et al., 1955; Bailey, Probert and Bohman, 1966a) while others found no advantages from such treatments (Pilkington et al., 1959; Voelker and Dracy, 1956; McArthur, 1964). Therefore, the effects of DES on bull performance are questionable.

Voelker and Dracy (1956) reported that oral DES reduced testes weights in dairy bull calves. Implants in older bulls have been shown to either decrease (Cahill, 1956) or to have no influence on testes weights (Bailey, Probert and Bohman, 1966a). Whether or not early implants will greatly retard testes development and cause histological changes in the testes is still an open question. Likewise, the semen production of early DES implanted bulls has not been extensively investigated.

Although the effects of various estrogens on the accessory glands of wether lambs has been reported (Clegg et al., 1955; O'Mary et al., 1952), the effects of DES on urethra size of lambs and cattle has not been shown. If DES were to reduce the size of the urethra it

could certainly be a factor in the incidence of urinary calculi. To date, this type of relationship has not been demonstrated.

The site of implant apparently has no measurable influence on the performance of wether lambs (Andrews and Beeson, 1953). However, the influence of site of implant on preweaning performance and testicular development of bulls has not been reported.

There is some evidence that secondary sex characteristics are retarded in implanted feedlot bulls (Cahill et al., 1956; Klosterman et al., 1955). The effects of very early DES treatment on secondary sex characteristics have not been set forth. This is a very important aspect, for inhibition of secondary sex characteristics could certainly improve the carcass quality of bulls.

The majority of the DES implant work that has been done to date has been with birds or animals of feeder age. The cockerels in the studies of Lorenz (1943, 1945) were three weeks old when implanted. Furthermore, most of the ruminant work mentioned above was done with animals of feedlot age. No work has been reported on the implantation of calves at birth.

No measurable quantity of androgens can be detected in the urine of bulls at one month of age. However, measurable quantities of androgens are present in the testis of bulls at one and one-half months of age (Nalbandov, 1958). Implanted estrogen could possibly meet with some direct or indirect androgen antagonism if the calves

were more than a month and a half old at the time of treatment. It was of interest in this study to find out if treatment at birth would produce significant advantages in performance over calves treated later.

The general lack of information in the aforementioned areas initiated plans for this particular study. Hence, the objectives of this study were to determine:

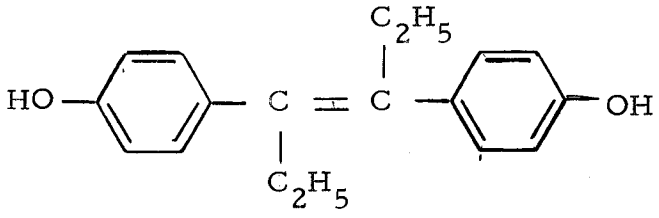
1. The influence of DES on preweaning performance of male calves when implanted early.
2. The influence of DES on secondary sex characteristics of bull calves.
3. The effect of DES on testicular development of bull calves.
4. The effect of DES on testicular development of yearling bulls.
5. The influence of DES on sperm production and semen characteristics of yearling bulls.
6. The effects of the site of DES implantation on suckling performance and sexual maturation of bull calves.
7. The effect of DES on the size of the urethras of bulls and steers.

REVIEW OF LITERATURE

The use of substances, directly or indirectly involved in regulating the endocrine system, to promote growth, fattening or milk production is widespread. Such substances as thyroprotein, various goitrogens, growth hormone, natural estrogens, androgens and progestogens have been used with varying degrees of success (Casida et al., 1959). Many synthetic chemical substances with effects of hormones have also been used. Dienestrol, hexestrol, diethylstilbestrol (Casida et al., 1959) and, recently, melengestrol acetate (Tuco Products Company) are examples of some of these synthetic substances. Of these, DES has been quite successful in promoting increased rate of gain and feed efficiency in ruminants.

A tremendous amount of work has been done with DES and consequently there is a massive amount of literature on the subject. This review will only touch on those papers which are pertinent to this study.

Diethylstilbestrol (DES), 4:4'-dihydroxy-α β-diethylstilbene (see



structure), is a synthetic product which has some three to five times

the estrogenic potency of estrone (White, Handler and Smith, 1959).

The poultry industry was the first to make any extensive use of DES in agriculture. Lorenz (1943) found that when a total of 25 mg. of DES was administered as subcutaneous pellet implants, to three week old cockerels, the birds' tissues had a marked and consistently greater fat content than those of the controls. The fat of the breast muscle, leg muscle, liver and abdomen was significantly increased by treatment. Growth increase was not consistent, but the meat quality was improved by DES over a wide range of age due to increased fat deposits. The effect of DES on depot fat was quickly reversed when pellets were removed (Lorenz, 1945).

Another use found for DES in domestic fowl was for castration of young males. It was found that young cocks, toms, drakes, and ganders could be successfully castrated by DES implants in subcutaneous tissue (J. Am. Vet. Med. Assn., 1949).

Shortly thereafter, DES was tried in growing and fattening ruminants. Andrews, Beeson and Harper (1949) reported that subcutaneous implantation of 12 or 24 mg. of DES or 10 mg. of testerone significantly increased the gains of wether lambs during a 68-day feeding period. All treated groups required significantly less feed per pound of gain than did the controls. Dressing percent was slightly lower in DES groups and the carcass grades were not as high as either the controls or the testosterone groups. The same

general observations were made by Jordan (1950). Andrews et al. (1950) reported on the effects of DES on the growth and fattening of steers. Two levels of DES (60 and 120 mg. implants) showed a .23 lb. and a .44 lb. per head per day advantage, respectively, over controls. The feed required per pound of gain was significantly reduced by DES treatment. In this case, there was no significant difference in the carcass grades of the treated and control animals. Later reports by the same and different workers indicate that the carcass grade of steers is lowered by DES treatment (Andrews, Beeson and Johnson, 1954; Clegg et al., 1955).

Reviews on the use of DES in beef cattle are given by Casida et al. (1959), and Andrews and Clegg (1957).

Early work with swine did not show consistent results. Consequently, work in that area has been limited (Dinusson, Klosterman and Buchanan, 1951).

Bulls: Performance

The reports on the effects of DES on bull performance are somewhat conflicting. Klette and Hueber (1951) reported that beef bulls implanted with 84 and 132 mg. of DES produced daily gains of approximately 0.20 pounds more than untreated bulls and up to 0.64 pounds more than untreated steers. Klosterman et al. (1955) noted that in dry lot fattening trials bulls gained more economically and

significantly faster than did steers. Both steers and bulls responded favorably to DES implants, but the increase was greater for the steers. Non-implanted bulls gained about the same as implanted steers, but implanted bulls gained 0.20 to 0.30 pounds per day more than implanted steers. Bailey, Probert and Bohman (1966a) reported that bulls implanted with 60 mg. DES tended to gain more rapidly and were somewhat fatter than controls.

Implanted bull calves may respond in a different manner. When implanted with 12 mg. of DES at 3.5 months and 24 mg. at 6.5 months of age, Pilkington et al. (1959) reported that bull calves made greater gains than steer calves, but less than untreated bull calves. Voelker and Dracy (1956) fed 2 to 3 mg. of DES to dairy bull calves which caused a slight reduction in weight gains at 88 and 116 days of age. Hendrickson et al. (1957) reported that 5 mg. DES per head per day fed in the creep feed or 12 or 15 mg. implants in suckling calves resulted in an increase in gains. The gain advantages were not as large as would be expected with larger cattle, but they were in the range of 8 to 12 percent. Nelson et al. (1959) reported that 12 mg. implants in suckling steer and heifer calves increased summer gains 14 and 23 pounds, respectively, in a 112-day period. Subsequent wintering gains were not adversely affected by this early implanting. Later trials at the same station substantiated these findings (Nelson and Kuhlman, 1962; Nelson, Pope and Stephens, 1964).

Subsequent Feedlot Performance

The question has arisen as to whether or not early treatment will depress subsequent feedlot performance. For this reason the effect of early DES treatment on subsequent feedlot performance has been looked into more closely. Nelson et al. (1959) concluded that subsequent performance of implanted calves was not adversely affected. Feedlot gains of calves implanted the previous summer were an average of nine pounds greater than for calves receiving no implant previously. Similarly, there was a six pound advantage for calves previously given summer implants and fed wintering rations. Later work at that station showed that feedlot gains did not suffer from early implanting. On a two-year average, 128-day feedlot gains were 267, 262 and 265 pounds for calves receiving no implant, those receiving 6 mg. implants and those implanted with 12 mg. during the suckling period, respectively. All animals received a 24 mg. implant when entering the feedlot. Nelson, Pope and Stephens (1964) fed 10 mg. DES daily to calves previously implanted with 0, 6 and 12 mg. of DES. The feedlot performance was almost identical for all groups regardless of previous treatment. The carcass grade of previously implanted calves was lower than the controls, but the dressing percent of implanted calves was slightly higher than for controls.

Secondary Sex Characteristics and Side Effects

Secondary sex characteristics are retarded in implanted calves as is evidenced by decreased hide weight (Cahill et al., 1956) and by decreased crest development (Klosterman et al., 1955).

Ralston, Church and Kennick (1966) have summarized the generally reported side effects. "Steers may exhibit raised tail heads, broken loins, and teat development. Heifers may, in addition to increased mammary development, show a hyperemia of the urogenital tract, occasionally prolapse, and in some cases come into milk production." The raised tail head condition was also reported in bulls given high levels of DES as implants (Klette and Hueber, 1951). Such side effects are considered to be due to either too large doses or too rapid absorption of the DES. Some drastic side effects were observed when levels between 60 and 120 mg. of DES were used, but these are minor when up to 36 mg. are administered.

Testicular and Accessory Gland Development

Klosterman et al. (1955) reported that on a fattening trial, bulls initially implanted with 84 mg., and 98 days later with 132 mg. DES, exhibited retarded testicular development. At levels of 84 mg. at 550 pounds and 84 mg. midway through the feeding period, Cahill et al. (1956) noted retarded sex characteristics in bulls. This was

demonstrated by a decrease in testicle and penis weights. Voelker and Dracy (1956) noted inhibition of testicle development in dairy calves fed 2 or 3 mg. DES daily from four to 88 days of age. Testes weights averaged 9.8 g. in treated calves and 21.07 g. in controls. Testes, tunica albuginea, epididymis and vas deferens averaged 18.9 g. in DES calves and 38.1 g. in controls.

Simms (1964) reported a significant ($P < .01$) reduction in external seminiferous tubule diameter of testes of ram lambs at 12 and 24 mg. implant levels when compared to non-implanted lambs. However, the differences in the tubule diameter of lambs treated with 12 and 24 mg. levels were not statistically significant.

The effects of DES on accessory glands and structures of beef cattle has not been extensively reported. However, various types of estrogens and methods of administration have been found to increase the size and weight of the seminal vesicles, prostate, ampulla, bulbourethral glands, prepuce and bladder of wether lambs (O'Mary et al., 1952; Bell, Smith and Erhart, 1954; Clegg et al., 1955; and Hale et al., 1955).

Cahill et al. (1956) reported increased pituitary and adrenal weights for implanted bulls, while thyroid weights were significantly decreased by the same treatment.

Urethra Size

Calculi formation in the urinary bladder is a nutritional problem. These calculi physically obstruct the passage of urine from the bladder. The urine eventually passes through the bladder wall when the bladder becomes distended and creates a toxic condition. This condition causes poor performance and eventually death if it is not corrected. The terms generally used to describe this condition are urinary calculi or urolithiasis.

Castration and administration of DES both tend to reduce development of secondary sex characteristics. It is possible that both of these treatments would also reduce the size of the urethra. This could, in the presence of calculi formation, increase the problem of urethra blockage. Udall and Jensen (1958) reported that 20 percent (60 of 300) of the DES implanted lambs died from urinary calculi during a feeding period. There was no incidence of urinary calculi among the non-implanted group. The first cases occurred only ten days after the lambs were treated. There was impaction of crystals around the urethral process, but only a few stones were found in the bladder. It was felt that an inflammation of the system was more directly to blame than the stones in the bladder in the early cases.

Emerick and Embry (1963) reported lower incidence of urinary calculi in lambs receiving 2 mg. DES orally per day or 3 mg.

implants than in control lambs. It was concluded that these levels of DES did not cause an increase in urinary calculi in lambs.

Urethral measurements were made by Frank et al. (1962) on steers which had urinary calculi and some which did not. They found that the urethra diameters were not significantly different on steers with or without urinary calculi (0.279 inches with urinary calculi; 0.282 inches without urinary calculi).

Semen Production

There had been no work reported on the effects of DES on semen qualities of bulls until recently, when Bailey, Probert and Bohman (1966a) reported on some work done with feedlot bulls. When beef bulls were implanted with 60 mg. DES at about 600 pounds, subsequent semen concentration, percent live sperm and sperm motility were not altered. In this case, semen was collected with an electro-ejaculator at about 15 months of age. Testicle weights for treated and control animals were the same.

When given to adult boars, implants of 2,000, 3,000 or 4,000 mg. of DES had no apparent effect on either the sperm content or volume of the semen. However, DES administration to prepubertal boars caused an increased viscosity of the semen and a reduction in volume (Wallace, 1949).

One may suggest that when DES administration in bulls is such

that it causes a reduction in testicle size and retardation of secondary sex characteristics, an alteration in semen volume and quality might also be expected.

Carcass Characteristics

The effects of DES on carcass characteristics of steers have been well reviewed by Cassida et al. (1959). In treated bulls, an increase of about one-third of a carcass grade over non-implanted controls has been reported (Cahill et al., 1956; Klosterman et al., 1955; Bailey, Probert and Bohman, 1966a). In each case, the increase in carcass grade was attributed to the increased subcutaneous fat, plus the fact that the bulls exhibited excellent muscular development.

The percent edible portion of the bull carcasses was lowered by implants, but was still well above that of steer carcasses (Cahill et al., 1956). However, the percent of chuck from bull carcasses was somewhat higher than from steer carcasses and the tenderness was lower (Bailey, Probert and Bohman, 1966a).

Implant Site

Generally speaking, the site of implantation of DES pellets has been the mid-portion of the ear. The reason for this is the ruling by the Food and Drug Administration that makes it necessary to

implant in an inedible portion of the animal. The ear was immediately recommended since it is not processed for human consumption. There is no doubt that various sites of implantation have been used in ruminants.

Andrews and Beeson (1953) used three locations on wether lambs for DES implants. The three sites were subcutaneously in the neck, in the scrotal cavity and subcutaneously approximately 3 cm. below the margin of the lower eyelid. There were no significant differences in the performance of these groups of lambs. Presumbaly, pellets in different locations of the body would be absorbed at different rates due to the difference in blood supply at various locations. In turn, this could result in varied effects on the animal. However, the effect of implant site on preweaning performance and sexual maturation of bulls has not been reported. Likewise, there is no information available on subsequent performance of ruminants implanted at different sites.

EXPERIMENTAL

Tissue Collection and Evaluation

As the testicles were removed from the animals they were trimmed of all fat and all but one inch of the cord. The epididymis was left on the testis of the adult bulls, but was removed from the calf testis before weighing. Weights were taken on both testicles to the nearest one-half gram.

The tissue samples were taken from the same position on all of the testis. A deep incision was made longitudinally down the midline of the testicle with a scalpel as the testicle lay on its side. This exposed the tissue and subsequent cuts yielded a piece of tissue which was immediately trimmed, cut into two rectangular pieces of tissue, and fixed in Bouin's fixative. The epididymis was sampled by first cutting the entire tail of the epididymis off the distal end of the testicle and then taking a smaller amount of tissue from this. Again, the tissue was fixed in Bouin's solution. The Bouin's fixative was prepared according to the formula of Weesner (1965).

For histological study, the tissue sections were prepared according to the methods described by Weesner (1965), with several modifications. The tissue samples (.30 × .30 × .30 cm.) were dehydrated in an Autotechnicon set on a ten hour cycle. The samples

were washed for two hours before being placed in the first dehydration alcohol. The washing stepwas followed by two one-hour dehydrations in 50% ethanol saturated with lithium carbonate and two hours in 95% ethanol. The ethanol was removed by two one-hour steps in dioxane. From the dioxane the tissue was placed in melted paraffin. Two one-hour changes in paraffin were used. With a large volume of paraffin, as is used in an Autotechnicon, the two long periods seem to allow the same infiltration of the tissue as do many shorter periods.

The tissue blocks were imbedded in a paraffin with a melting point of 56-58° C. This was done by using paper boats as outlined in Weesner (1965). In successive work, the bottoms and tops of micro cover-glass boxes were found to be very useful for "boats".

Again, the procedures of Weesner were used for sectioning the tissue on a microtome and affixing it to microscope slides.

Two staining procedures were used in this work. Iron Hematoxylin stain was used for preliminary work only, since effects are not repeatable with this natural stain. The second staining procedure made use of hematoxylin and eosin and it was used in all experimental work.

Slides were used to evaluate the development or degeneration of the testicular tissue. The presence or absence of sperm in the epididymis was checked in the adult bulls. For the calf testicles

the external diameters of the seminiferous tubules, indications of testicular development, or signs of degeneration were the criteria for evaluation. The external diameters of five seminiferous tubules were measured in the testicular sections of each bull. Only tubules that appeared very round were measured, since they were presumed to be as near representative of the average diameter as possible. The measurements were made with an ocular micrometer in a microscope.

The above criteria and procedure were modified somewhat for evaluation of the testicular activity of adult bulls. The presence or absence of sperm in the epididymis was substituted for the stage of spermatogenesis in the seminiferous tubules used previously.

Statistical Analysis

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

Experiment I

An experiment was designed to study the effect of implantation of DES at birth or at about 3 months of age on preweaning performance, feedlot gains, feed efficiency and carcass characteristics of male calves.

Forty-eight bull calves from the 1964 OSU commercial herd calf-crop were stratified as to age and assigned to three DES treatments. Group I received no implants and were used as controls; Group II received a 12 mg. implant at birth and; Group III was implanted with 12 mg. at about three months of age. Implants were made at the base of the ear. The environment for all calves was similar. All calves were left intact until two weeks prior to weaning, at which time untreated calves were castrated. Testicular development was measured by taking caliper measurements of the widest portion of the testicles in situ before castration. Both calves to be castrated and those to be left intact were measured. The correlation between the testicular measurement on the intact animal and weight of the testicle after castration was determined. The calves were graded by a committee of three at weaning. Average daily gain was determined for from birth to three months of age and from birth to weaning.

Forty of the 48 calves received 24 mg. implants and were placed on a feeding trial at the time of weaning. All animals were individually fed a concentrate finishing ration. The animals were slaughtered at a local plant and carcass data was collected.

Results and Discussion

The calves implanted with 12 mg. of DES at birth made significantly ($P < .05$) greater gains to three months of age than did the other two groups (Table 1). Gains from three months until weaning were similar for all groups, but the early implanted group held an advantage of 0.11 pounds per head per day from birth to weaning. DES implantation, either at birth or at three months of age, reduced the size of the testis. The testicular measurement on the intact calf and the weight of the testicle after castration were significantly ($P < .01$) correlated ($r = .974$). Secondary sex characteristics, as evidenced by crest development, curly hair about the head and muscular shoulder development, were delayed with DES treatments. The weaning grade was higher in early treated calves than in controls. The delay in the onset of secondary sex characteristics and the higher weaning grades of the implanted bull calves may have some advantages in the marketing of bull calves. The increase in weaning grades was attributed to the increased fat deposition and additional bloom carried by the implanted calves.

The average daily gain from weaning to slaughter was greater in the treated groups than in the control group. Similarly, the feed per pound of gain and the cost of finish gain were less for the implanted groups. This advantage was probably not due to the DES

treatment, but to the castration of the control calves at the beginning of the feeding period.

Carcasses of all intact males were graded as bulls; therefore, the official grades were not comparable to those of the control group. The marbling scores, although not significantly different, did favor the castrates. This would indicate leanness in the bull carcasses.

Table 1. Effect of DES implants upon suckling gain and feedlot performance.

	Implant at birth	Implant at 3 mos.	Bull to weaning
Av. daily gain to 3 mos., lbs.	2.48[a]	2.26	2.25
Av. daily gain to wean, lbs.	2.01	1.90	1.90
Testicle dia., mm.	32	30	41
Wean grade[b]	2.5	3.0	2.8
Av. daily gain wean to slaughter, lbs.	2.94	2.74	2.63
Feedlot efficiency[c]	5.70	6.04	6.34
Cost of finish gain, $	18.40	19.52	20.44
Marble score[d]	7.8	8.2	9.9

[a] Significantly ($P < .05$) greater than other two groups.

[b] 1 = fancy, 2 = high choice, 3 = average choice.

[c] Pounds of feed to produce a pound of beef.

[d] 6 = traces, 9 = slight, 12 = small.

Experiment II

Experiment II was designed to determine the effects of DES treatment on preweaning gains and testicular development.

Thirty-six bull calves from the 1965 OSU commercial herd

calf-crop were randomly alloted to four treatment groups. Group I received 6 mg. DES in the ear at birth; Group II received 6 mg. in the ear at birth and 15 mg. in the ear at about three months of age; Group III were implanted with 15 mg. in the ear at three months of age and; Group IV calves received no implants and were controls. All implants were made at the base of the ear. The calves were weighed at birth, at about three months of age and at weaning. Environment was similar for all groups.

The above four groups of bull calves were castrated two weeks before weaning. The testes were trimmed, weighed and treated as described in the section on Tissue Collection and Evaluation. The calves were allowed to remain with the cows for about two weeks while they recovered from castration. They were then put on concentrate rations containing various ratios of protein and energy. All calves received 15 mg. implants when they entered the feeding period and again midway through the trial. They were finished out on these rations and carcass data were collected at the time of slaughter.

Results and Discussion

Gain to three months of age was non-significantly greater for calves implanted at birth (Group I) than for non-implanted calves (Groups III and IV) (Table 2). Calves (Group II) receiving two implants showed an advantage in weight gain to three months and to

weaning. The average daily gain to weaning, although not significantly different, did favor the early implanted calves.

The weaning grades favored the calves that had received two implants of DES, while the controls and those implanted only at birth graded the same. The calves receiving an implant at three months of age graded somewhat below the other three groups. In general, the grades reflected the amount of fat carried by the calves.

Implanted calves, particularly Group II, were slower to develop secondary sex characteristics than were the non-implanted controls. This was evidenced by a slowness to develop a crest, curly hair and masculinity about the head and shoulder musculature. The testis were smaller, surrounded by more fat and were harder to remove than those of control calves.

Table 2. Effect of DES implants upon suckling gain and testis development.

	Group I (Birth, 6 mg. in ear)	Group II (Birth, 6 mg. ear +3 mo., 15 mg. in ear	Group III (3 mo., 15 mg. in ear)	Group IV (Controls, no implants)
Av. daily gain to 3 mos., lbs.	2.22	2.32	2.16	2.21
Av. daily gain to weaning, lbs.	1.90	2.04	1.87	1.94
Weaning grade [c]	7.15	7.50	6.93	7.20
Seminiferous tubule dia., μ	114.36[a]	72.00[b]	108.81[a]	123.44[a]

[a, b] Values on the same line bearing different superscript letters are significantly ($P < .01$) different.

[c] A score from 1 (least desirable) to 10 (most desirable).

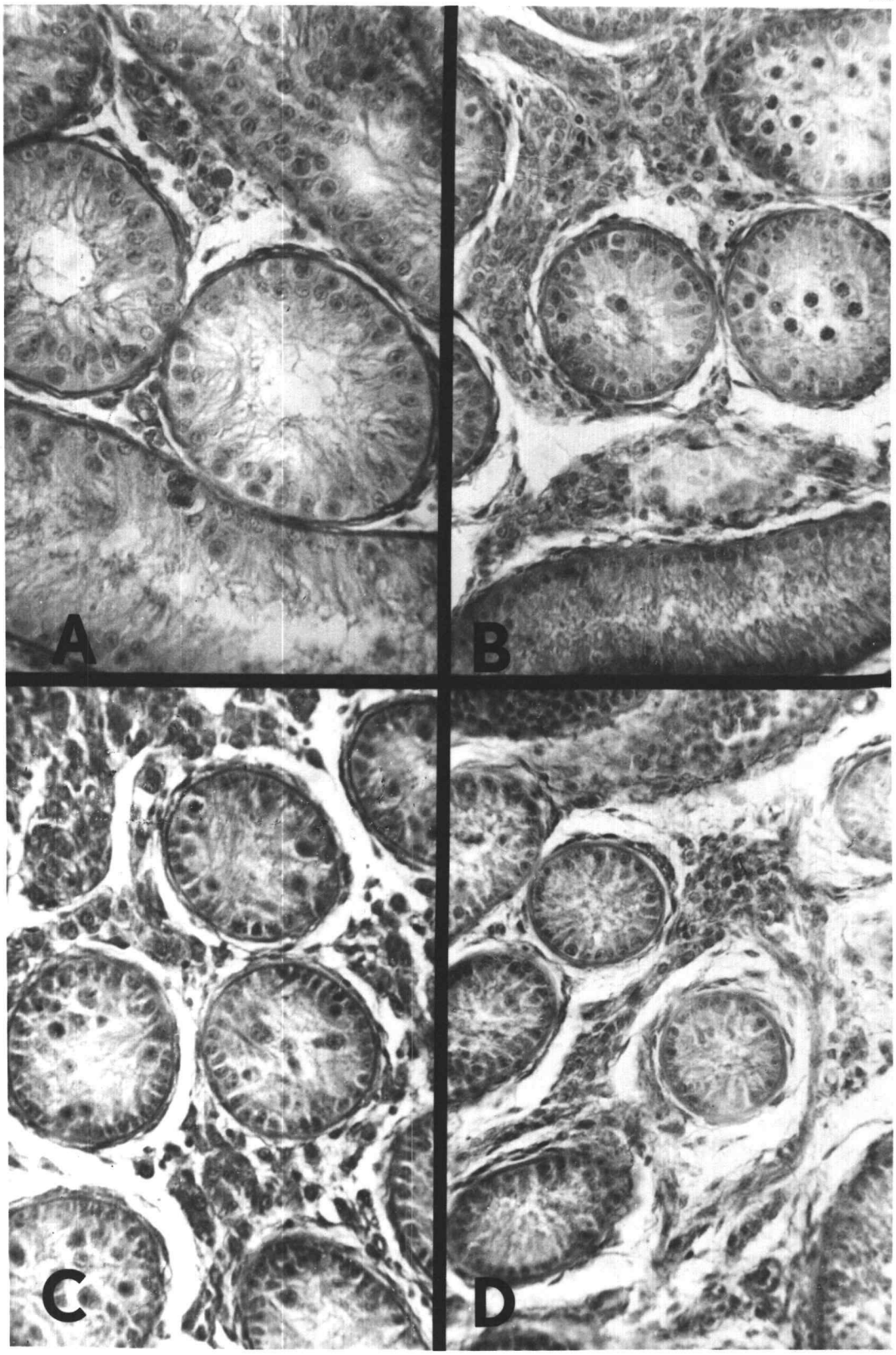
Figure 1. Microphotographic comparison of seminiferous tubule development of non-treated and of DES treated bull calves (× 340).

A. Control: An average external seminiferous tubule diameter of 158 μ.

B. Implanted with 6 mg. of 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.


A
B
C
D

The average seminiferous tubule diameter of the controls (Group IV) was significantly ($P < .01$) larger than in the calves receiving two implants (Group II). Furthermore, the seminiferous tubules of the calves receiving only one implant were significantly ($P < .01$) larger than those implanted twice. The average diameter of the seminiferous tubules for the control groups was non-significantly larger than for the two groups receiving only one implant. The reduction in the average seminiferous tubule diameter agrees with the work done by Simms (1964) with lambs. The microphotographs in Figure 1 represent the relative size of the seminiferous tubules of each treatment in this work.

The effect of DES on spermatogenesis was not determined in this work since the calves were castrated before there was significant cell division in the seminiferous tubules.

From these results it seems evident that DES can cause a reduction in the size of the testis and the seminiferous tubules of early implanted calves. These results would also indicate that the level and time of DES treatment do have an effect on preweaning performance, weaning grade and seminiferous tubule diameter.

Experiment IIIa

The purpose of Experiment IIIa was to compare the effects of two different levels of DES and time of implant on preweaning

performance and testicular development.

Thirty bull calves from the 1966 OSU commercial herd calf-crop were used in this trial. The calves were divided into two groups. Group I was given 15 mg. DES in the ear at birth and another 15 mg. implant at about three months of age. Group II received a 15 mg. implant in the ear at about three months of age. All implants were made at the base of the ear. The calves were weighed at birth, at about three months of age and at weaning. Both groups were handled in a similar manner throughout the experiment.

Two weeks before weaning about half of each group was castrated and both the testes and the castrated calves were treated as in Experiment II. The intact bull calves were weaned with the rest and were used in Experiment IIIb.

Results and Discussion

The average daily gain to three months of age and to weaning were not significantly different in the two groups (Table 3). Previous work showed an increase in gain to three months for implanted calves (Experiment I). Although calves implanted at birth (Group I) gained as well as non-implanted calves (Group II) to three months of age, it was felt that the level of DES was too high for optimum response. The grades at weaning were not significantly different for the two groups, although they favored Group II. This too would

indicate that the 30 mg. treatment was more detrimental than helpful as far as performance was concerned. In the calves of both groups the onset of the development of the secondary sex characteristics was delayed. This resulted in bull calves that lacked masculinity.

The testis weights appeared to be less than would be expected in non-treated calves, but they were very similar for the two treated groups.

Seminiferous tubule diameters were non-significantly larger for Group II than for Group I. This would indicate that the seminiferous tubules had developed to some extent before the three month DES treatment, or that the larger level given to Group II was responsible for the reduction in seminiferous tubule size. The fact that the largest testes had the smallest average seminiferous tubule diameter cannot be explained except on the basis of inherent differences due to sample size and/or individual variation.

Table 3. Effect of DES implants upon suckling gain and testis development.

	Group I (Birth, 15 mg. ear + 3 mo., 15 mg. ear)	Group II (3 mo., 15 mg. ear)
Av. daily gain to 3 mos., lbs.	2.39	2.35
Av. daily gain to weaning, lbs.	1.90	1.96
Weaning grade	7.20	7.67
Testis weight, g.	29.9	27.1
Seminiferous tubule, dia., μ	129.00	137.14

Experiment IIIb

Experiment IIIb was carried out to determine the effects of DES level and time of administration on semen production, semen quality and secondary sex characteristics of bulls. At the time of weaning the bulls were put in dry lot and fed a concentrate ration and grass hay free choice. Two main groups of bulls were used. Group I bulls had received 15 mg. DES at birth and another 15 mg. implant at about three months of age. Group II received a 15 mg. implant at about three months of age. Two additional bulls were used in this experiment. One of these bulls had received no DES implants (Group III) and the other received 15 mg. at birth (Group IV) but no later implant. Group numbers were small for all groups in this experiment.

Semen collection was first attempted when each of the bulls had reached about ten months of age, or when each appeared and acted sexually mature. An electroejaculator was used for collections which were attempted every two weeks.

The semen collected was evaluated according to the procedure of Perry (1963) with some modifications. A small drop of semen was placed on a slide and the slide was placed on a microscope stage warmer at 38° C. While on the stage warmer the motility was graded with a score from one to ten. The lower scores

indicated less motility and the higher scores greater motility. Sperm smears were stained with an eosin-fast-green stain (Meyer et al., 1957). These smears were used for live-dead and abnormal sperm counts. The live sperm are not stained by the eosin while the dead ones are, making it easy to differentiate between them. A live-dead percentage was derived on the basis of 200 sperm counted. Abnormality categories consisted of tailless, coiled tails and those with protoplasmic tufts or bulges on the tail. Abnormality percentages were based on 100 sperm counted. Semen concentration was estimated by the conventional techniques for counting red blood cells.

All bulls were slaughtered in June, 1967 at weights between 950 and 1,250 pounds. Samples of the testis and epididymides of each bull were taken at the time of slaughter and treated according to the procedure outlined in the section on Tissue Collection and Evaluation.

Results and Discussion

In general, the implanted bulls lacked masculinity throughout. The bulls' heads took on the look of steers, they lacked libido and the testis were small and under-developed. Semen collection was difficult in most cases. The only bulls that gave consistent results were those that had received no DES or only one implant at birth. Natural collections were completely unsuccessful.

Table 4. Effects of DES upon semen production and testes development.

	Group I[b] (Birth, 15 mg. ear + 3 mo., 15 mg. ear)	Group II[c] (3 mo., 15 mg. ear)	Group III (No DES)	Group IV (Birth, 15 mg. ear)
Av. no. of collection attempts	9.5	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[a]	3.50	3.00	5.14	4.00
Av. testes weight, g.	241.25	270.70	342.00	301.00
Av. seminiferous tubule dia., μ	301.50[e]	289.80[e]	357.00[d]	309.00[e]

a A score from 1 (least motile) to 10 (most motile).

b Only one of the group was collected from and the collection at the tenth period was the only one of high concentration.

c Only two bulls could be collected from and then only at the later collection periods.

d, e Means in the same line bearing different superscripts are significantly ($P < .01$) different.

The bulls in Groups III and IV appeared to be sexually mature and collections of viable semen were obtained in the first and second collection periods (Table IV). However, the bulls of the other two groups did not appear to be sexually mature at the same age. Semen could not be collected from these bulls until near the end of the trial. Even then the semen was not of high quality in most cases. This would tend to indicate that the DES treated bulls were beginning to recover from the effects of the DES by the end of the experiment. At the end of the experiment the average testes weights of Groups III and IV were non-significantly larger than those of Groups I and II. The average seminiferous tubule diameter of Group III was significantly ($P < .01$) larger than that of Groups I or II.

Experiment IV

An experiment was carried out to determine effect of different levels of DES on testicular development and sperm production of bulls kept intact until slaughter age.

Twenty-five Hereford bulls were randomly alloted to three groups. Group I bulls received 12 mg. DES at birth, 12 mg. at about three months of age, 15 mg. upon entering the feedlot and 15 mg. after 110 days in the feedlot. Group II bulls were given 12 mg. at about three months of age, 15 mg. upon entering the feedlot and 15 mg. after 110 days in the feedlot. Group III bulls

received no DES and were used as controls. All implants were made subcutaneously in the ear.

At the time of slaughter testicular and epididymal samples were taken and evaluated as described in the section entitled Tissue Collection and Evaluation.

Results and Discussion

The average daily gain was not significantly different, but there was a noticeable trend with increasing levels of DES (Table 5). There was a significant difference between testes development at the various levels of DES administration. Group III had significantly ($P < .01$) larger testis weights than did the other two groups. Group II had significantly ($P < .05$) larger testis weights than did Group I. The fact that testes weights were reduced by DES treatment supports the findings of Klosterman et al. (1955) and Cahill et al. (1956). Similarly, the average seminiferous tubule diameter of Group III was significantly ($P < .05$) larger than in either of the DES treated groups. Group II had a significantly ($P < .01$) larger average seminiferous tubule diameter than did Group I. Testes weight and average seminiferous tubule diameter were significantly ($P < .01$) correlated ($r = .659^{**}$).

From histological sections of the epididymides it could be seen that the tissues of Group III bulls were more highly developed than those of the other groups. In Group III the lumen of the epididymis

was clear and contained large amounts of sperm, while in DES treated groups the lumen was smaller and there were fewer sperm present.

With increasing levels of DES, results of this experiment showed an increase in average daily gain, a decrease in testes weight, a decrease of average external seminiferous tubule diameter and less development of the epididymal tissue.

Table 5. Effect of DES upon feedlot gain and testis development of bulls.

	Group I (Birth, 12 mg. ear + 3 mo., 12 mg. ear + into feedlot, 15 mg. ear + 110 days, 15 mg. ear)	Group II (3 mo., 12 mg. ear + into feedlot, 15 mg. ear + 110 days, 15 mg. ear)	Group III (Control, no DES)
Av. daily gain to slaughter, lbs.	3.00	2.94	2.86
Av. testes weight, g.	135.75	188.56[b]	290.67[a]
Av. seminiferous tubule dia., μ	244.71[e]	266.14[d]	293.67[c]

[a]Significantly ($P < .01$) greater than Groups I and II.

[b]Significantly ($P < .05$) greater than Group I.

[c, d, e]Values bearing different superscript letters on the same line are significantly ($P < .01$) different.

Experiment V

The purpose of this experiment was to determine the effects of the location of DES implants on preweaning performance and sexual maturation of bull calves.

Sixty-seven bull calves from the 1967 OSU commercial herd calf-crop were randomly allotted to five groups of about equal numbers. Group I received 6 mg. DES in the scrotum; Group II received 12 mg. DES in the scrotum; Group III was given 6 mg. DES in the ear; Group IV received 12 mg. DES in the ear and; Group V received no DES and were used as controls. All treated calves received 12 mg. DES implants at about three months of age in addition to the above birth implants. A few calves were reimplanted in the scrotum; the remainder were reimplanted in the ear. All five groups were weighed at birth, at about three months of age and at weaning. The environment was similar for all groups throughout the experiment.

All calves in the five groups were castrated two weeks before weaning. Testicular weights were recorded and tissue samples were taken and treated according to the procedure given under Tissue Collection and Evaluation. The calves were also given a live grade at the time of castration.

Results and Discussion

The average daily gain to three months of age for the scrotal implants were similar to that of the control group (Table 6). However, both ear implant groups made non-significantly greater gains to three months of age than did the other groups. The average daily gain to weaning was in favor of the implanted calves; the calves

receiving 6 mg. implants (scrotal and ear) gained similarly and gained 0.07 pounds per head daily more than the controls. However, this was not significant at the ten percent level. The calves treated with 12 mg. implants gained 0.13 pounds per head daily more than the controls. Average weaning grades for all DES implanted groups were similar and slightly greater than those of the controls. This reflected the increased fat carried by the implanted calves.

The average testes weight of the control group was significantly ($P < .01$) greater than that of any of the DES treating groups. The average testes weight of Group IV was significantly ($P < .05$) greater than that of the 12 mg. scrotal implant group. The average seminiferous tubule diameter of the control group was significantly ($P < .01$) greater than that of all the treated groups. The average seminiferous tubule diameter of the DES treated groups was similar. In this experiment the average testes weight and the average seminiferous tubule diameter was significantly ($P < .01$) correlated ($r = .875^{**}$).

The above work would tend to indicate that there was no measurable advantage in gain to three months of age caused by scrotal implants at birth. This was probably caused by a setback the calves suffered from being implanted in the scrotum. However, they equaled the ear implanted calves in average daily gain to weaning. The average testes weight was smaller for scrotal implanted calves

than for the ear implants (12 mg.). There was no apparent reduction in average seminiferous tubule diameter caused by the scrotal implants over the ear implants.

Table 6. Effect of level and site of implant upon performance and testes development.

	Group I (Birth, 6 mg scrotum + 3 mo., 12 mg ear)	Group II (Birth, 12 mg scrotum + 3 mo., 12 mg ear)	Group III (Birth, 6 mg ear + 3 mo., 12 mg ear)	Group IV (Birth, 12 mg. ear + 3 mo., 12 mg. ear	Group V (Control, 0 mg)
Av. daily gain to 3 mos., lbs.	2.35	2.38	2.46	2.55	2.36
Av. daily gain to weaning, lbs.	1.92	1.97	1.90	1.97	1.84
Weaning grade[a]	7.55	7.58	7.54	7.50	6.86
Av. testes wts. g.	15.77[c]	12.71[d]	15.35[d]	17.25[c]	53.61[b]
Av. seminiferous tubule dia., μ	101.85	93.90	96.00	94.20	168.15[e]

[a] A score from 1 (least desirable) to 10 (most desirable).

[b] Significantly ($P < .01$) different than all other groups.

[c, d] Values on the same line bearing different superscript letters are significantly ($P < .05$) different.

[e] Significantly ($P < .01$) different than all other groups.

Experiment VI

Ninety-three male beef calves were used in three trials conducted to determine the effects of castration and DES implants on urethra development. Both steer and bull calves were used in these trials. Three levels of DES and three times of castration were used

for the steers. Levels of DES implants for steers were 0, 12 and 24 mg. Time of castration was at birth, at three months, or at seven months of age. Bulls were either left as controls (no implants), implanted at birth or at three months of age with 12 mg. DES, or were implanted at seven months of age with 24 mg. of DES.

At the time of slaughter the inside diameter of the urethras of steers and bulls were measured at four locations: (1) Directly behind the head of the penis; (2) midway from the head of the penis to the first curvatures of the sigmoid flexure; and (3) and (4) the first and second curvatures of the sigmoid flexure.

Results and Discussion

Although Frank et al. (1962) found no difference in the size of urethras of steers with or without urinary calculi, the size of the urethra was significantly ($P < .01$) reduced by castration (Table 7). When steers were implanted with DES the size of the urethra was significantly ($P < .01$) increased over that of non-implanted steers. In all treatments the urethra was significantly ($P < .01$) larger at locations 3 and 4 than at locations 1 and 2. The implantation of bulls with DES caused no significant change in urethra measurements.

If urethral blockage by calculi formation is dependent on a small urethral lumen, DES treatment should reduce the incidence of urinary calculi in feedlot steers.

Table 7. Effect of castration and DES upon urethra size.

	Time of castration		
	Birth	3 months	7 months
	Dia. in.	Dia. in.	Dia. in.
Castrate + 0 DES	.187	.205	.204
Castrate[a] + 12 mg. DES	.200	.214	.256[b]
Castrate[a] + 24 mg. DES			.257[b]

	Bulls implanted at		
	Birth	3 months	7 months
0 DES			.340
12 mg. DES	.339	.309	
24 mg. DES			.336

[a]Steers were implanted at seven months of age.

[b]Significantly ($P < .01$) larger than other groups.

GENERAL RESULTS AND DISCUSSION

The advantage in gain to three months of age in calves implanted at birth was not as pronounced in Experiment II as it had been in Experiment I. For that reason there was question as to the repeatability of the results of Experiment I. However, the results of the ear implants in Experiment V strongly supported the findings of the first experiment. The less than expected gain to three months in Experiment II was thought to be due to a poor feed supply during the preweaning period. Average daily gain to weaning was in the favor of the implanted calves in Experiments I and V. Even in Experiment II the implanted calves gained as well as the non-implanted controls. The level of DES implant was felt to be too high for optimum performance in Experiment IIIa. Average daily gain to slaughter was increased in bulls in Experiment IV.

There was a reduction in development of secondary sex characteristics, testes size and seminiferous tubule diameter in all implanted bulls studied. The onset of semen production of yearling bulls was apparently delayed by DES implants given prior to weaning. The site of implantation did not alter the beneficial effect of DES on gain to weaning, although scrotal implantation caused less gain to three months of age than did ear implantation.

The size of the urethra was decreased by castration. In steers

the urethra size was increased by DES treatment, but in bulls the urethra was unaffected by DES implants.

SUMMARY

The objective of this study was to determine the effects of DES implantation on preweaning performance and sexual maturation when given at various stages of development and at different sites of implantation.

In Experiment I, statistical analysis of the data showed that calves implanted (12 mg.) at birth gained significantly ($P < .05$) faster to three months of age than did non-implanted calves. Also, they held a slight advantage to weaning. The weaning grade was improved by DES treatment. Any preweaning DES implant caused a delay in the onset of secondary sex characteristics and a reduction in testes size.

Results of Experiment II showed no significant difference in preweaning performance of implanted and non-implanted calves. However, implanted calves had small testes and there was a delay in secondary sex characteristic development. Average seminiferous tubule diameter was significantly ($P < .01$) decreased in calves that had received two preweaning implants.

The two DES implanted groups in Experiment IIIa were similar in preweaning performance, testes weight and average seminiferous tubule diameter. In Experiment IIIb the onset of semen production was apparently delayed by previous DES implantation. At slaughter

the average external seminiferous tubule diameter was significantly ($P < .01$) larger in the control than in the bulls receiving early implants.

Average daily gain in the feedlot of bulls in Experiment IV was increased with each level of DES treatment. The average external seminiferous tubule diameters were significantly ($P < .01$) different for all treatment groups. The testes weight of the control group was significantly ($P < .01$) greater than that of the other two groups. The testes weight of the DES treated groups were significantly ($P < .05$) different.

Calves implanted with DES in the scrotum at birth (Experiment V) gained no better than the controls to three months of age. Those calves receiving ear implants outgained all other groups to three months of age. At weaning the scrotal implanted calves equaled the gains of the ear implants, which still held an advantage over the control calves. At each DES level the calves lacked the secondary sex characteristics exhibited by the control group. Testes weight and seminiferous tubule diameter were very significantly ($P < .01$) correlated and significantly ($P < .01$) reduced by DES treatment.

The inside diameter of the urethra of castrated calves was significantly ($P < .01$) smaller than calves left intact in Experiment VI. DES treatment of castrates resulted in a significant ($P < .01$) increase in inside urethral diameter. The size of the urethras of

bull calves were not influenced by DES implants.

Although results of this work have shown measurable effects of DES on performance and sexual maturation, the precise mode and site of action of DES warrants further investigation.

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