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Sexual Activity, Seminal Characteristics, and Reproductive Organs in Sexually Inexperienced Castrate Rabbits Following Testosterone Implantation (38452)

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Little is known about the sexual activity and other reproductive characteristics of males deprived of testicular androgens before sexual experience and subsequently given testosterone therapy. In mature rabbits trained to ejaculate before castration, injection of 8 mg of testosterone propionate every 48 hr (1) or the implantation of polydimethylsiloxane capsules (430 mm^2 of surface area) filled with testosterone (2) restored libido and seminal characteristics to control levels. Since previous sexual experience can have marked carryover effects on postcastration sexual behavior, testicular androgens were replaced with testosterone implants in immature sexually inexperienced male rabbits in the present study. The effects on libido, seminal characteristics, and reproductive development were assessed.

Materials and Methods. Twenty Dutch-belted male rabbits 73–95 days old were randomly allotted to five groups of four bucks each. One group served as controls (C) and four groups were castrated and received either a 2- or 1-cm implant immediately (I) or after a delay (D) of 26 days. Capsules containing testosterone were prepared from Silastic tubing (Dow-Corning, 3.35-mm i.d., 4.65-mm o.d.) so as to have effective chamber lengths of 1 and 2 cm. The inside surface area was 421 mm^2 for the 2-cm implants and 210.5 mm^2 for the 1-cm implants. The length of chamber used was based on a preliminary experiment with six sexually experienced adult Dutch-belted males in which 1-, 2-, and 4-cm testosterone-filled implants inserted at the time of castration maintained testosterone levels, libido, reproductive organs, and seminal characteristics.

The experiment was conducted for 77 days, with the day of castration being designated as Day 0. Blood (5 ml) was collected weekly into heparinized tubes several hours before semen collection, centrifuged and the plasma frozen until assayed for testosterone using a competitive protein binding assay (3). Standards were prepared in triplicate and unknowns in duplicate. Assay losses were corrected to 100% recovery.

Semen collections with the aid of an artificial vagina (4) were initiated on Day 26 when the delayed implants were inserted. Collections were attempted twice per day at 30 min intervals on Monday, Wednesday, and Friday for 8 weeks. Semen data for Day 77 were discarded because a change in the semen collection schedule on the day animals were sacrificed resulted in atypical responses. The two ejaculates for each male, after removal of any gel plug, were combined and frozen, and a weekly pool for each male was accumulated for fructose assay. Fructose determinations were based on the reaction of fructose with resorcinol (5, 6).

A teaser was placed with a buck for a maximum of 3 min and, if mounting and/or ejaculation did not occur, the teaser was replaced with a second one for up to 3 min. Libido was scored on a scale of eight (rapid mounting and ejaculation) to zero (no mounting) according to a modification of the scheme described by Macmillan *et al.* (1).

On Day 77, animals were sacrificed and their penes and accessory sex glands were removed and weighed. Implants were removed, incubated at 37° and weighed until a constant weight was reached.

Student's t test was used to determine the statistical significance of paired comparisons (7). Honestly significant differences (hsd test) were used for multiple comparisons.

Results. Table I shows the average body and

TABLE I. Average Animal and Organ Weights of Young Rabbits.

	Body wt (g)			% of body wt				
Treatment	Day 0	Day 77	Growth from Day 0–77	Testes[a]	Epididymides[a]	Accessory sex glands[b]	Penis[b]	Adrenals[b]
Control	—	1794	—	0.1945	0.06625	0.1175[d]	0.089	0.202
2 cm I	1212	1708	496	0.0912	0.0956	0.088[d,e]	0.090	0.213
1 cm I	1408	1775	367	0.0791[c]	0.0733[c]	0.038[d,e]	0.057	0.153
2 cm D	1276	1798	522	0.0899	0.0807	0.44[d,e]	0.082	0.188
1 cm D	1356	1845	489	0.0798[c]	0.0756[c]	0.031[e]	0.051	0.174

[a] Expressed as % of body wt when removed at castration (Day 0) excepting the controls, where body wt at sacrifice (Day 77) was used.

[b] Expressed as % of body wt at Day 77.

[c] Low values due to two cryptorchid animals in the 1 cm-I group and one in the 1 cm-D group.

[d,e] Values which have different superscripts are significantly different ($P < 0.05$).

organ weights. The larger values for weight changes with the larger implants were not significant. The only significant difference ($P < 0.05$) was in the size of the accessory sex glands; in the 1-cm delayed testosterone implant group (1 cm-D) glands were smaller than in the controls.

The coded libido values for both the first and second collections are plotted in Fig. 1. Scores for each collection day were tested statistically. Throughout most of the experiment (after Day 37) libido in the controls was higher than in the 2 cm-I group ($P < 0.01$) and libido in the 2 cm-I group was higher than in the 1 cm-I group ($P < 0.05$). Males with delayed implants tended to have lower libido throughout, although these groups were improving near the end of the experiment. Rabbits with the delayed implants required 6 weeks of testosterone therapy to achieve libido scores equivalent to those exhibited by males in the corresponding immediate implant groups after the latter were implanted for 26 days. At first collection delayed implant groups were consistently lower ($P < 0.05$) than the corresponding immediate implant group, but second collection libido values obtained from Day 68 to 75 for both delayed implant groups were similar ($P > 0.05$) to groups receiving the same size implants immediately.

Semen volume for first and second ejaculates combined is portrayed in Fig. 2. From Day 47 on, when males were 120–142 days old, the controls had reached puberty and their semen volume exceeded all other groups ($P < 0.01$). Trends were the same for the gel-free volumes. Little or no semen was collected from implanted castrates excepting the 2 cm-I group.

Fructose concentration in the available semen for each of the 8 weeks is summarized in Table II. There was considerable variation due to individual rabbits, but fructose levels in the 2 cm-I group clearly were at least equivalent to the controls.

Figure 3 shows the plasma testosterone levels. Before the delayed implants were inserted, males that were castrated only were not significantly different from each other but both were significantly lower ($P < 0.01$) than the other three groups. With implants installed, the 2 cm-D treatment average generally was significantly higher than for the 1 cm-D treatment group, and the 2 cm-I group was always higher than the 1 cm-I group. After the delayed implants were inserted, both groups with 2-cm implants tended to have similar high testosterone values which averaged 2.41 ng/ml. The 1-cm implant groups averaged 1.33 ng/ml while the average plasma testosterone value for the controls was 1.52 ng/ml. Differences among animals with similar implants were less than among controls. Approximately 95% of the original testosterone remained in all implants used, indicating that they could supply testosterone for longer periods.

Discussion. Rabbits with 2-cm implants had higher plasma testosterone levels than the controls. For the 2 cm-I group, the continuous supply of testosterone released was sufficient to stimulate development of the accessory sex glands, fructose secretion, and penis growth

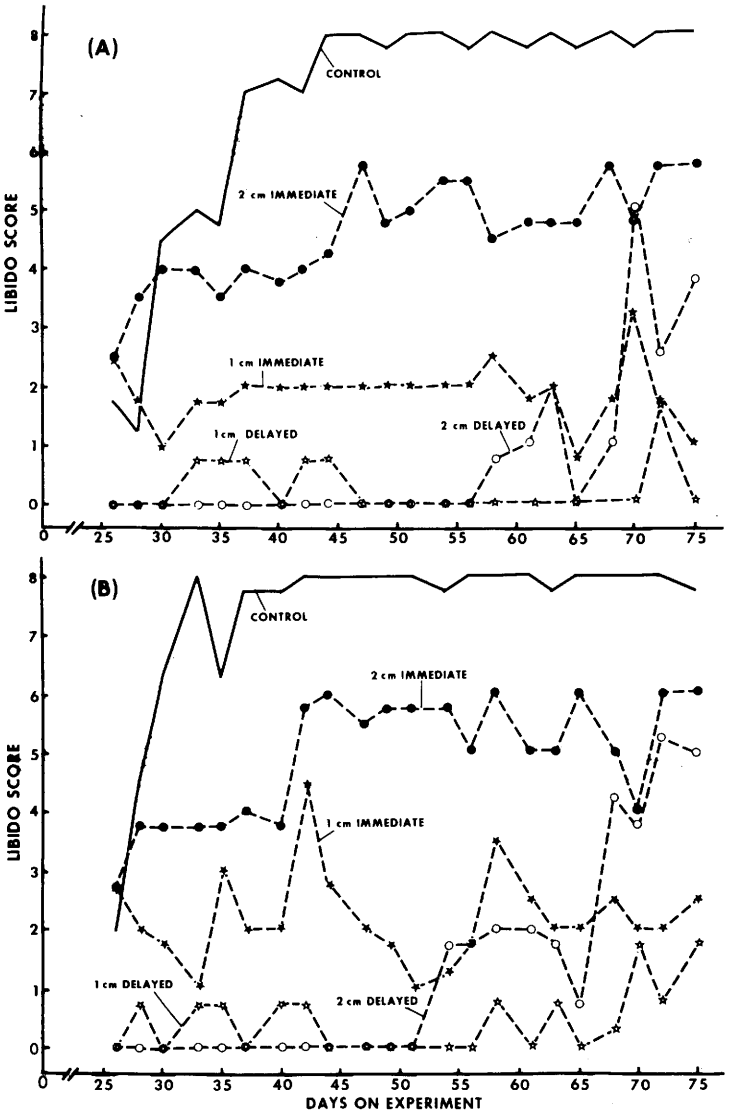

FIG. 1. Average libido score on the first attempted semen collection (A) and the second attempted semen collection (B). For an explanation of libido scores, see text.

equivalent to the controls (Tables I and II). Stratton *et al.* (2) also found that accessory sex glands and fructose secretion in castrated rabbits with 430-mm^2 (inside surface area) implants were comparable to intact rabbits. In one report (2) plasma testosterone levels were slightly higher than our values for implants of similar size. Wall thickness of the implants in the two studies was approximately inversely proportional to body-weights of the rabbits used. Contrary to our results, these workers and Macmillan *et al.* (1) found that libido and semen volume were maintained by testosterone therapy, but their rabbits were castrated after sexual experience, and libido was not abolished by castration (2). Therefore, part of the postcastration sexual activity of the males observed previously may have resulted from prior sexual experience and not merely from hormone therapy.

Semen volume was lower in all implanted groups than in the controls despite the fact that accessory sex gland weights were maintained,

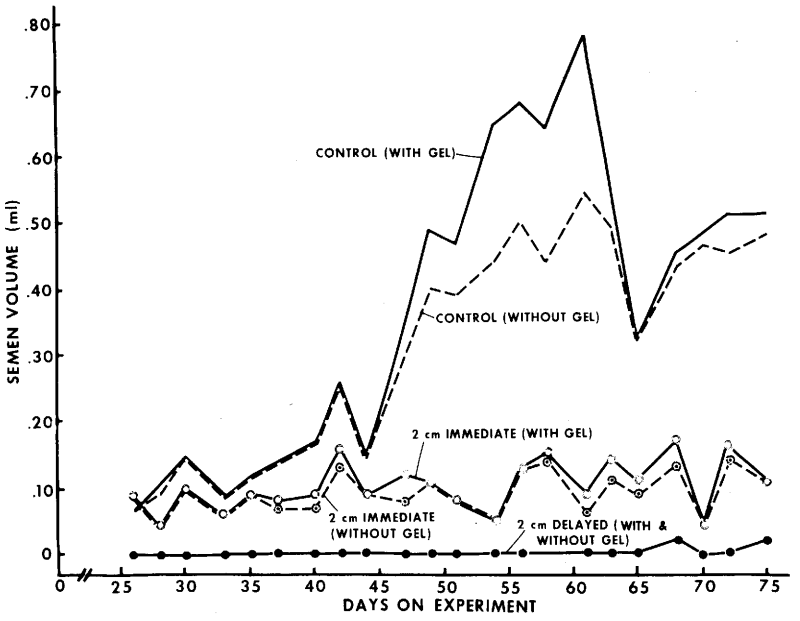

FIG. 2. Total semen volume with and without gel for the two ejaculates combined on each day of semen collection.

particularly by the 2 cm-I group. Part of this difference is due to elimination of the epididymal contribution following castration which accounts for about 10% of the ejaculate volume (8). Also, lower libido in implanted groups could have resulted in less complete emptying of the accessory glands at ejaculation.

The failure of testosterone to support fully normal libido and seminal plasma volume ejaculated by our rabbits, which were castrated as young virgins, contrasts with the apparent capability of this hormone to maintain fully these functions in adult rabbits castrated after sexual experience. We interpret this difference to indicate that testosterone alone is insufficient to induce normal sexual development and sexual activity in young virgin rabbits. Plasma testosterone levels with the larger implants in the present experiment were at least equivalent to the controls, ruling out inadequate levels of testosterone as being responsible for the effects observed. From these results we suggest that other substances (probably steroids) are produced by the intact testes which affect the hypothalamus and other target organs in a manner not entirely duplicated by testosterone.

Correlations among the various characteristics studied computed for the control and 2 cm-I groups were similar. Libido at first and second collections was highly correlated ($r = 0.90$). There was also a high positive correlation between the semen volume with and without the gel

TABLE II. Seminal Fructose in Young Rabbits.

Treatment group[a]	Fructose (mg/rabbit/week)							
	1	2	3	4	5	6	7	8
Control	0.40	0.33	0.77	1.33	2.10	2.13	1.78	1.23
2 cm-I[b]	2.30	2.89	3.60	2.35	4.55	3.63	2.14	5.42
2 cm-D	—	—	—	—	—	—	0.06	—

[a] Treatment groups did not differ significantly.

[b] Only one rabbit contributed semen except during weeks 4 and 7, when there were two rabbits.

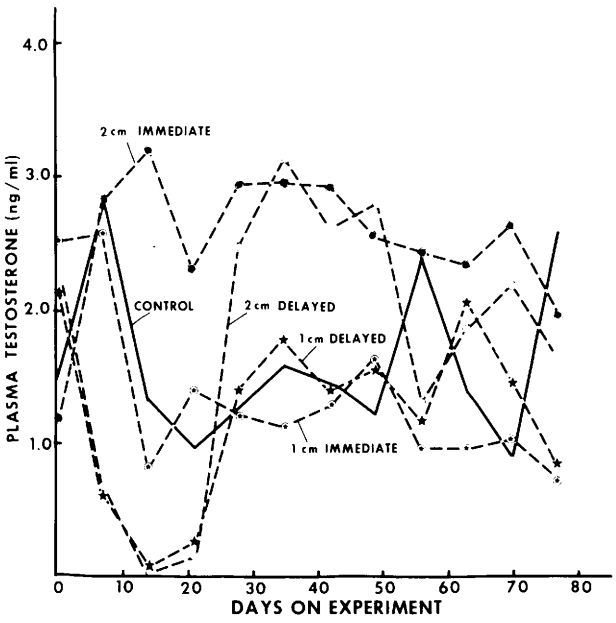

FIG. 3. Plasma testosterone concentration, with levels in implanted males adjusted for increasing body weight.

for both ejaculates and treatment groups (r = 0.94–0.96) partly reflecting correlating a part with the whole.

Summary. Males castrated at 2.5 mo of age were supplied immediately with testosterone-filled implants (210.5-mm^2 vs 421-mm^2 inside surface area). Plasma testosterone levels, libido, and sexual development tended to parallel implant size. Other males castrated for 26 days before receiving testosterone implants were delayed in development, but otherwise responded similarly to testosterone therapy. Libido and semen volume were higher in control males than in other groups despite the fact that plasma testosterone in controls averaged 1.52 ng/ml compared to 2.41 ng/ml for bucks with 2-cm implants and 1.33 ng/ml for bucks with 1-cm implants. This result indicates that testicular products, in addition to testosterone in controls, were involved in producing normal sexual development and activity.

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