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Testicular Morphology in Rats Vasectomized as Adults

Abstract. *Vasectomy was performed on 63 rats at age 81 to 105 days. Examination 28 to 58 days later disclosed no significant reductions in testis size with respect to preoperative testis length and to testis length and weight in unoperated controls. The fact that the minimal atrophy observed was primarily left-sided sugested that these effects were artifacts of the procedure or systemic left-right differences in the animals.*

Sackler *et al.* (*1*) reported that vasectomy of immature rats results in significant reductions of urinary 17-ketosteroid output and testis weight. As they and others point out, further research is needed on the procedures and consequences of vasectomy (*1, 2*), especially in view of the social importance of this issue. At the same time, however, any conclusions should be based on data derived from an appropriate experimental model. Since my colleagues and I needed vasectomized adult rats in some of our studies (*3*), we developed a surgical procedure and have performed more than 130 operations since late 1970. While testicular atrophy has been found in some of our animals, it appears that these instances can be attributed to artifacts of the surgery, rather than to vasectomy per se.

Surgery was performed on 53 BLU:(LE) hooded rats under pentobarbital sodium anesthesia with clean surgical procedures, care being taken to avoid resection of the deferential blood vessels (*4*). In comparisons of pre- and postoperative measurements of testis length (*5*), no decreases were found (Table 1). Since, in many studies of vasectomy in animals, either the deferential vessels are resected or the status of these vessels is not reported, ten additional rats were vasectomized, this time with ligation and resection of the deferential vessels along with the ductus deferens. While the postsurgical interval in these animals was less than that of Sackler *et al.* (*1*), there is evidence that many complications of vasectomy are at their peak 1 month after surgery (*6*).

This latter group did not differ from ten age-matched, unoperated controls in body weight or testis weight (Table 1). However, the analysis of variance (*7*) for testis length yielded a significant interaction of group by side-of-body ($F = 11.5$; d.f. $= 1, 18$; $P < .01$). Length of right testes of vasectomized rats (2.27 ± 0.06 cm) and controls (2.24 ± 0.04 cm) did not differ, whereas left testes of vasectomized rats (2.17 ± 0.06 cm) were smaller than the left testes of controls (2.31 ± 0.03 cm) ($t = 3.3$; $P < .01$). Furthermore, the within-group comparisons of testis length for the 63 vasectomized rats yielded a significant interaction of surgery by side-of-body ($F = 5.6$; d.f. $= 1, 186$; $P < .05$), reflecting the fact that testes on the right increased in length between surgery (2.16 ± 0.01 cm) and postmortem examination (2.23 ± 0.02 cm) ($t = 2.8$; $P < .01$), whereas presurgical (2.17 ± 0.01 cm) and postsurgical (2.15 ± 0.03 cm) measurements of left testes did not differ. This left-right discrepancy was corroborated by the fact that a greater proportion of left testes (23.8 percent) than right testes (3.2 percent) decreased in length after surgery ($\chi^2 = 15.4$; d.f. $= 1$; $P < .01$). In addition, all adhesions observed in these 63 rats occurred on the left side. The reason for this left-right difference cannot yet be explained. There is the possibility of some subtle difference in technique between the two sides. There is also evidence that disturbances of the circulation of the left testis and scrotum may be more damaging than disturbances on the right. The fact that 98 percent of cases of varicocele (a congested condition of the scrotal blood vessels) in humans occur on the left side has been explained by the relatively inefficient system of venous return from this side (*8*).

It would thus appear that these effects on testis size were related to a compromise of the testicular circulation or to other complications of surgery. The fact that Sackler *et al.* (*1*) found decreases in urinary 17-ketosteroid output in vasectomized rats but not in vasoligated rats suggests that these changes may be explained in a similar way.

Many aspects of vasectomy require further investigation (*1, 2*). It is important, however, that the effects of surgery be clearly distinguished from the effects of vasectomy per se. While the data presented here do not demonstrate that vasectomy is necessarily an innocuous procedure, they do indicate that certain apparently deleterious effects of vasectomy may result from pro-

Table 1. Body weight, testis length, and testis weight (grams per kilogram of body weight) in vasectomized and control rats. Data are mean ± standard error of mean.

Animals (No.)	Age at surgery (days)	Post-surgical interval (days)	Body weight		Testis length		Testis weight (g/kg)
			Final (g)	Gain (%)	Initial (cm)	Final (cm)	
			Vasectomy, deferential vessels intact				
53	90.1 ± 0.6	41.5 ± 1.2			2.16 ± 0.01	2.18 ± 0.02	
			Vasectomy, deferential vessels occluded				
10	102.0 ± 0.8	29.8 ± 0.4	469.9 ± 13.4	16.0 ± 1.2	2.22 ± 0.03	2.22 ± 0.04	3.81 ± 0.12
			Control				
10	100.0 ± 0.0	27.0 ± 2.4	474.4 ± 9.2	14.8 ± 2.1		2.28 ± 0.03	3.82 ± 0.08

cedural artifacts, and that vasectomy performed in the adult rat does not result in the extensive morphological changes reported for the immature rat.

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4. In this technique, a 1.0-cm midline incision is made 0.5 cm posterior to the penis, followed by a laterally adjacent 0.5-cm incision in the testicular sac. The testis is eased through the retracted incision and its length measured. The ductus deferens is then carefully dissected from its sheath and from the deferential blood vessels and ligated with silk at two points 0.6 cm apart, and 0.4 cm of the duct is removed from between the sutures. The testis is replaced and the incision closed with individual silk sutures, as is the skin incision after the procedure is repeated on the contralateral side. Intramuscular injections of penicillin and streptomycin are administered once a day for 3 days to reduce the probability of infection.
5. Testis length rather than weight was used as the primary measure of testis size because it allows for pre- and postsurgical comparisons. Pearson product moment correlations, calculated for the 20 animals from which both measures were obtained, were .71 for vasectomized rats ($P < .01$) and .54 for controls ($P < .05$). Since the ratio of length to width varies in these animals, these correlations, although significant, might be improved by use of size measurements that account for both length and width.
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Sperm Penetration of Housefly Eggs: Evidence for Involvement of a Female Accessory Secretion

Abstract. *Removal of the paired sex accessory glands associated with the posterior reproductive tract of female houseflies inhibited penetration of the eggs by sperm, but the insemination of females without these glands appeared to be unaffected. The results indicate that either the sperm are "activated" or the permeability of the egg membrane is altered by the secretion of the accessory glands before fertilization occurs.*

Some form of accessory gland is associated with the posterior region of the reproductive tracts of most species of female insects. These glands vary widely in structure, location, and product, but they generally produce secretions that aid in the attachment of the eggs to various substrates, or form protective coatings and egg cases. However, modifications of this usual function are known to occur. For example, venom is produced by an accessory gland in aculeate hymenopteran insects (*1*), and some viviparous dipterans provide nourishment for the larvae from glands associated with the uterine chamber (*2*). It has been further speculated that the secretion of the accessory glands of some insects may facilitate fertilization of the egg during oviposition (*3*).

Previous investigations of the internal genitalia of the female housefly, *Musca domestica*, established that paired accessory glands and the sperm storage organs (spermathecae) discharge their products into the vagina through ducts that have a common opening (*4*). This arrangement suggested that mixing of the sperm with secretion emitted from the glands would be inevitable, if the glands were functional during oviposition. The study reported here indicates that the accessory glands are functional during oviposition and that a mechanism involving the secretory product with sperm penetration does exist in the housefly.

Table 1. Effect of removal of accessory glands on the fertility of female houseflies. In each treatment 118 females were used. The figures in parentheses represent the total number of eggs deposited and examined.

Treatment	Eggs hatched (%)	Eggs fertilized (%)
Sham operation	74.03 (2569)	77.40 (600)
Glands removed	0.68 (2106)	4.20 (208)

Table 2. Effect of removal of accessory glands on insemination of female houseflies. The females were considered inseminated only if sperm were observed in the spermathecae.

Treatment	Females tested (No.)	Females inseminated (%)
Sham operation	147	64.6
Glands removed	112	68.8

Light microscopic examination of histological preparations of the reproductive tracts of houseflies taken at various stages of egg deposition established that sperm penetration of the egg occurs within an anterior chamber located just posterior to the junction of the vagina and common oviduct (*5*). Just before an egg leaves the common oviduct and enters the vaginal canal, several sperm traverse the short distance from the vaginal opening of the spermathecal ducts to the anterior chamber. As the egg enters the vagina, it is thrust into the anterior chamber and held there for several seconds before deposition while one to four sperm penetrate the micropyle and enter the ooplasm.

The participation of the secretion of the female accessory glands in the process of fertilization was investigated in part by surgically removing the glands from 3- to 7-day-old adult females that had mated previously. The glands were excised through a ventral midline incision made in the membrane located between the fifth and sixth abdominal segments. Other females were similarly treated, except that the glands were merely grasped with a forceps and not detached. Only females that appeared to have full control over their ovipositors after either procedure were retained for testing. The next day the females were allowed to oviposit, and most of the eggs deposited were used for determination of hatch; however, a small portion was collected immediately after oviposition, stained by the method of Leopold and Palmquist (*6*), and microscopically examined for the presence of sperm.

As Table 1 shows, fertility was reduced to less than 1 percent by removing the glands, but fecundity was only slightly affected. Further, the microscopic examination revealed that sperm were rarely present within eggs deposited by females lacking accessory glands. Thus, the secretion of these glands is apparently involved in the actual penetration of eggs by sperm, or aids the sperm in reaching the site of penetration.

To rule out the possibility that dam-