Testicular and Epididymidal Sperm Numbers in Unilaterally Vasoligated Rabbits* (33972)

L. V. SWANSON AND H. D. HAFS

Animal Reproduction Laboratory, Dairy Department, Michigan State University
East Lansing, Michigan 48823

Early workers assumed that nonejaculated sperm are lost in the urine, thus maintaining a viable supply of sperm in the cauda epididymis. However, Simeone and Young (1) reported that nonejaculated sperm in guinea pigs undergo regressive changes which eventually lead to liquefaction within the epididymis and the deferent duct. And on the basis of an exchange of isotopic nucleic acid from sperm to cauda epididymidal epithelium, Orgebin-Crist (2) suggested that some sperm are normally resorbed from the rabbit epididymis, but the amount of exchange was not quantified. In contrast to these reports, Lino *et al.* (3) reported that the number of sperm (av 6.15 billion daily) in the urine of rams during sexual rest approached sperm output (av 7.01 billion daily) during exhaustive ejaculation.

Resorption of sperm from the epididymis often has been studied in vasectomized males. But the influence on spermatogenesis of blockade of the excurrent sperm ducts is controversial. Moore and Quick (4) and Amann (5) found no influence of vasectomy on spermatogenesis in rabbits and bulls, whereas Steinach (6) and Macmillan *et al.* (7) concluded that vasectomy or vasoligation of rabbits induces at least a temporary decline in spermatogenesis. The present paper describes more extensively the fate of spermatogenesis and of epididymidal spermatozoa after a 10-week period of unilateral vasoligation with and without ejaculation.

Materials and Methods. Eleven rabbits of mixed breeds were sexually rested for at least 1 month and unilaterally vasoligated on the left deferent duct within 3 mm of the epididymis. Then five rabbits were sexually rested for a 10-week experimental period and, commencing 4 days after vasoligation, the remaining six rabbits were ejaculated twice every Monday, Wednesday, and Friday, a frequency considered to maximize daily sperm output (8). These rabbits were stimulated sexually with one false mount followed by a 3-min interval before each ejaculation (9). Additional controls consisted of five sexually rested rabbits and three of these were sham vasoligated. Samples of blood serum were collected from the central ear artery of each rabbit after 0, 5, and 10 weeks of the experimental period.

At the end of the 10-week period, rabbits were killed by cervical dislocation, the ejaculated rabbits immediately after the last ejaculation. Testicular parenchymae, capita-corpora epididymides and caudae epididymides were weighted and homogenized, and sperm numbers in these tissues were determined (10). Homologous sperm antibody titers were determined by the micro-titer complement fixation technique (11). In this method, heat-inactivated sera were used at 1:10 dilutions and the antigen consisted of rabbit sperm, washed three times with saline. Homologous sperm and seminal plasma precipitins were determined by agar-gel double diffusion (12).

Results. Sham vasoligation of three control rabbits had no significant influence on any of the criteria measured. Consequently, data from these rabbits were combined with data from the other two control rabbits in the following results. Average weights of the testes and capita-corpora epididymides (Table I) were not influenced significantly by vasoligation. The smaller body weight of the vasoligated ejaculated group accounted for their smaller testicular weights. Vasoligation resulted in dramatically increased cauda epididymidal weight, but we found a 26% reduction in the weight of unligated cauda epididy-

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TABLE I. Body, Testicular Parenchymal and Epididymidal Weights after Unilateral Vasoligation.

		Wt			
			Testicular parenchyma	Epididymides (mg)	
Treatment	Side	Body (kg)	(g)	Caput-corpus	Cauda
Control[a]	Right	2.95 ± 0.24[c]	2.00 ± 0.23	368 ± 57	607 ± 103
	Left		2.07 ± 0.26	357 ± 42	626 ± 93
Vasoligated[a]	Right	3.08 ± 0.29	2.02 ± 0.33	286 ± 40	456 ± 67
	Ligated		2.14 ± 0.40	321 ± 32	1258 ± 184
Vasoligated, ejaculated[b]	Right	2.61 ± 0.30	1.62 ± 0.12	276 ± 31	422 ± 91
	Ligated		1.65 ± 0.13	286 ± 21	1211 ± 237

[a] Averages for five sexually rested rabbits.

[b] Averages for six rabbits ejaculated twice every Monday, Wednesday, and Friday (2 × MWF).

[c] Mean ± standard error.

mides of sexually rested rabbits relative to caudae epididymides of control rabbits. The comparable reduction in the ejaculated vasoligated rabbits was 32%.

Vasoligation also resulted in a significant decrease of testicular sperm concentration relative to that in contralateral control testes ($p<0.025$, Table II). This measure of the rate of spermatogenesis is less variable than total testicular sperm because it ignores differences in testicular weight which are associated with differences in body weight (7, 13). Theoretical daily sperm production, a calculation based on the duration of the stages of the cycle of the seminiferous epithelium represented by the sperm counted in testicular homogenates (14, 15), was reduced 19% by vasoligation (Table II). However, average testicular sperm concentration on the unoperated right side of the vasoligated rabbits did not differ significantly from controls.

As reflected by the increased cauda epididymidal weights, vasoligation caused accumulation of sperm in caudae epididymides (Table III). Although the unligated side of the vasoligated sexually rested rabbits had normal testicular sperm concentrations, the comparable caudae epididymides contained only 82% as many sperm as controls. And ejaculation further reduced unligated cauda epididymidal sperm numbers to 86 million.

Daily sperm output of the ejaculated vaso-

TABLE II. Gonadal Sperm after Unilateral Vasoligation.

			(millions)	
Treatment	Side	Testicular sperm concentration (10^6/g)	Total testicular sperm	Daily sperm production[c]
Control[a]	Right	86 ± 6[d]	172 ± 23	50.2 ± 6.8
	Left	75 ± 4	156 ± 22	45.6 ± 6.4
Vasoligated[a]	Right	89 ±12	171 ± 27	49.7 ± 7.9
	Ligated	68 ± 6	140 ± 25	40.7 ± 7.2
Vasoligated, ejac.[b]	Right	87 ± 9	139 ± 15	40.5 ± 4.5
	Ligated	69 ± 6	111 ± 7	32.3 ± 2.1

[a] Averages for five sexually rested rabbits.

[b] Averages for six rabbits ejaculated 2 × MWF.

[c] Calculated from total testicular sperm ÷ 3.43 (20).

[d] Mean ± standard error.

TABLE III. Epididymidal Sperm after Unilateral Vasoligation.

Treatment	Side	Epididymidal sperm (millions)		
		Caput-corpus	Cauda	Total
Control[a]	Right	113 ± 21[c]	466 ± 70	579 ± 87
	Left	109 ± 17	528 ± 93	637 ± 109
Vasoligated[a]	Right	113 ± 18	407 ± 135	519 ± 149
	Ligated	126 ± 16	2568 ± 479	2694 ± 494
Vasoligated, ejaculated[b]	Right	84 ± 12	86 ± 17	3053 ± 158[d]
	Ligated	81 ± 9	1809 ± 179	1890 ± 182

[a] Averages for five sexually rested rabbits.
[b] Averages for six rabbits ejaculated 2 × MWF.
[c] Mean ± standard error.
[d] Includes 2883 million ejaculated sperm.

ligated rabbits averaged 80 million during the first week and did not deviate significantly from 36 million during the last 9 weeks. Total sperm output for the 10-week experimental period averaged 2883 million. These sperm output values are corrected for the 10% of ejaculated sperm which adhere to the artificial vagina (8). The decline in sperm output during the first week reflected reduction of extra gonadal sperm from the maximal level attained during sexual rest before unilateral vasoligation to a level characteristic of rabbits after frequent ejaculation (10).

Sperm antibody titers (Table IV) were not affected significantly by ejaculation or interval of vasoligation, but varied considerably among individuals. Complement fixing activity against washed rabbit sperm existed in each serum sample from each of these 16 rabbits. Examination of the same sera with the agar-gel double-diffusion technique failed to reveal any sperm or seminal plasma precipitins.

Discussion. Testicular sperm numbers for control rabbits confirm previous observations that right and left testes produce approximately equivalent numbers of sperm (7). However, rate of spermatogenesis (sperm per gram of testis) of rabbits in both experiments suggested that, on the average, right testes may produce slightly more sperm than the left testes. The significant reduction in numbers of testicular spermatozoa on the vasoligated side of both the ejaculated and the sexually rested rabbits indicates that vasoligation for 10 weeks interfered with spermatogenesis, a conclusion also reported by Macmillan *et al.* (7) who studied changes in testicular sperm numbers during a 5-week period after vasoligation. Periodic examination of the rabbits in the present experiment confirmed that the testes remained within the scrotum following vasoligation. These observations suggest that increased hydrostatic pressure within the ligated epididymis may partially inhibit spermatocytogenesis or possibly an early stage of spermiogenesis, but not sperm transport from the testis to the epididymis.

The decreased weight (Table I) and total sperm content (Table III) of the caudae epididymides on the unoperated side from sexually rested unilaterally vasoligated rabbits follows the trend reported by Macmillan *et al.* (7). Thus, changes induced by unilateral vasoligation cause increased loss of sperm from the epididymis on the con-

TABLE IV. Average Rabbit Antisperm Titer[a] in Sera before and after Unilateral Vasoligation.

Treatment	Weeks of vasoligation		
	0	5	10
Control[b]	38	27	33
Vasoligated[b]	70	58	58
Vasoligated, ejac.[c]	49	39	60

[a] Expressed as reciprocal of the 50% hemolytic end point.
[b] Averages for five sexually rested rabbits.
[c] Averages for six rabbits ejaculated 2 × MWF.

tralateral unligated side. One possible explanation for this increased loss of sperm is that the dramatic accumulation of sperm in the caudae epididymides on the ligated side may have induced isoimmunization against sperm and thereby increased resorption of sperm from the epididymis on the unligated side. But, the sperm antibody titers (Table IV) indicate that vasoligation did not induce circulating sperm antibodies. The sperm antibody activity which was found in all rabbits and varied in titer from 1:8 to 1:128, is apparently the same nonspecific activity found by others (16, 17). Edwards (16) reported that this activity was present in male and female rabbits after 70 days of age.

Another possible explanation for the increased loss of sperm from the cauda epididymidis on the unligated side is that distention of the epididymidal tubule with accumulated sperm on the vasoligated side may enhance contractility of the excurrent sperm ducts on both sides. This hypothesis was not tested in the present experiment. If it is true, the increased loss of sperm from the unligated side may be due to a gradual and rather continuous escape of sperm via the urethra. Previously, researchers have tested the possibility that sperm may be lost through the urethra in intact rabbits by looking for sperm in the urine, and found none (18). We suggest that sperm may ooze from the uretha and be ingested in the same manner as coprophagy occurs in rabbits. Individually caged rats are known to ingest "spontaneous ejaculations" which occur in the absence of any exteroceptive sexual stimulation (19).

Total extra gonadal sperm (av 3053 million) on the unligated side of ejaculated rabbits is considerably greater than the total (av 1890 million) on the ligated side of the same rabbits. In part, this reflects reduced numbers of sperm entering the epididymis on the ligated side, because when the numbers of extra gonadal sperm are adjusted to a uniform rate of spermatogenesis (extra gonadal sperm per 100 million testicular sperm), the average of 2270 for the unligated side is more similar to, but significantly greater ($p \cong 0.04$) than 1735 for the ligated side of the ejaculated rabbits. The comparable average for the ligated side of sexually rested rabbits is 1904.

The daily sperm production of these rabbit testes (Table II, av 43.2 million/testis) was calculated on the basis that the sperm counted in testicular homogenates represent 3.43 days (32%) of the 10.7-day cycle of the seminiferous epithelium (20). We used this value rather than 4.93 days (10) or 5.44 days (18) because it is based upon more extensive data. For comparison with the daily sperm production data in Table II, estimates of numbers of epididymidal sperm at the beginning were subtracted from the totals found at the end of the 10-week experimental period and the remainder divided by 70 days to give an estimate of the number of sperm which entered the epididymis daily (daily extra gonadal sperm). The relationship between calculated daily sperm production and calculated daily extra gonadal sperm is shown in Fig. 1. Based upon these limited data, the correlation coefficient between these two variables is 0.71 ($p < 0.01$), and the regression of daily extra gonadal sperm on daily sperm production ($b = 0.78$) did not differ significantly from the theoretical value of 1.00 expected if no sperm were resorbed from the epididymis.

Inspection of the data in Fig. 1 suggests that the epididymis on the ligated side of ejaculated rabbits (open circles) had lower daily extra gonadal sperm than would have been predicted from daily sperm production. When the data for the ligated side of ejaculated rabbits were separated from the others in Fig. 1, the regression associated with them ($b = -0.03$) did not approach the ideal value, but the regression associated with the remaining 11 observations ($b = 1.14$) in Fig. 1 was even closer to the ideal of 1.00. The latter calculation supports the conclusion that few sperm were lost from these epididymides for the 10-week experimental period and the low regression of −0.03 for ligated sides of ejaculated rabbits indicates that ejaculation enhanced resorption of sperm from the ligated epididymides.

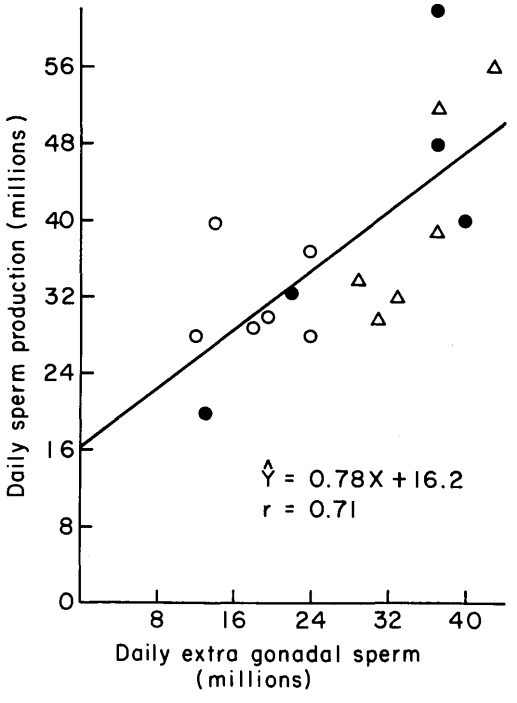

FIG. 1. Relationship of calculated daily sperm production to calculated daily extra gonadal sperm: (●) vasoligated rested rabbits, ligated side; (○), vasoligated ejaculated rabbits, ligated side; (△), vasoligated ejaculated rabbits, unligated side.

The caput epididymidis is known to be the site of resorption of most of the large quantity of testicular fluid which accompanies sperm entering the epididymis (*22*, *23*). An undetermined quantity of ^{3}H-thymidine from epididymidal sperm has been localized within the epididymidal epithelium (*2*), but epididymidal resorption of sperm may be quantified from the data in the present paper. The percentage of daily sperm production which could not be accounted for in daily extra gonadal sperm (probable epididymal sperm resorption) was 42 ± 8% for the ligated side of ejaculated rabbits, considerably greater than the comparable average of 26 ± 7% for the ligated side of sexually rested rabbits ($p \cong 0.16$). But both were significantly greater than the average of 11 ± 5% for the unligated side of ejaculated rabbits ($p < 0.01$). These calculations indicate that vasoligation for 10 weeks resulted in increased epididymidal resorption of sperm, but at rates much less than the 96% reported for vasectomized bulls (*21*).

Comparison of the 2694 million sperm which accumulated in the vasoligated epididymis of sexually rested rabbits with the 608 million found in unoperated control rabbits indicates that nearly 80% of the sperm produced during the 10-week experimental period were lost from the control rabbits. Since maximal sperm resorption (in ejaculated vasoligated rabbits) amounted to only 42%, we conclude that at least 40% of an unoperated rabbit's sperm production may be lost via the urethra during periods of sexual inactivity.

Summary. Gonadal and extra gonadal sperm were determined in sexually rested and in ejaculated rabbits 10 weeks after unilateral vasoligation. Vasoligation depressed testicular sperm numbers by about 24% on the ligated side only, without altering testis weight. Sperm resorption from epididymides on unligated sides of ejaculated rabbits amounted to 11% of sperm production. Comparable averages for vasoligated sides of sexually rested rabbits and for vasoligated sides of ejaculated rabbits were 26 and 42%, respectively. These increments in epididymidal sperm resorption were not associated with increased sperm antibody titers. Epididymidal sperm numbers on the unoperated side of sexually rested unilaterally vasoligated rabbits were only 85% of those in sexually rested control rabbits, indicating that unilateral vasoligation increased the loss of sperm from the contralateral unoperated side. The data support the conclusion that at least 40% of an unoperated rabbit's sperm production may be lost via the urethra during periods of sexual inactivity.

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Separation of Peptide Components of Urinary Kinin (Substance Z)* (33973)

I. MIWA,[1] E. G. ERDÖS, AND T. SEKI[1]

Department of Pharmacology, University of Oklahoma Medical Center, Oklahoma City, Oklahoma 73104

Werle and Erdös (1, 2) described the existence of a biologically active substance in normal human urine and named the partially purified peptide Substance Z. The substance was hypotensive and contracted isolated smooth muscle preparations of various laboratory animals. Some subtle differences such as stability in alkali and action on rat colon seemed to distinguish this material from kallidin. Gomes (3) indicated that the peptide might be identical with bradykinin, and afterwards it was frequently referred to as urinary kinin. (4) Subsequently, during chromatography of the peptide on a cellulose column, two different components were separated and named Z_1 and Z_2 (5, 6). It was also indicated that the major component was identical with bradykinin and the minor one with kallidin (7). Other investigators attempted to link changes in excretion of the urinary peptide or peptides to various pathological conditions (8). Because of the heterogeneity of the substance and the possible clinical importance of its presence in urine, we undertook to separate and identify its various components. A preliminary report on the subject was published recently (9).

Materials and Methods. The glassware used in the purification process was coated with silicone and treated with 0.1% hexadimethrine (10). Urine was collected in plastic containers from normal male volunteers and extracted as fresh as possible at 4°. The pH of the urine was between 4 and 5 after 1 ml of 0.002 *M* *p*-toluenesulfonic acid was added per 100 ml (11). Subsequently, 100 mg of Amberlite IRC-50 resin in the H^+ form was suspended in each 100 ml of urine. The pH was adjusted to 4 with 10 *M* formic acid and the suspension was stirred for 1 hr (12). The peptides adsorbed on the resin were eluted

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