Sperm Retention and Resorption in Sexually Active Rabbits with Epididymal Ligatures* (34065)

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The epididymides serve as important storage organs for sperm (1–6), where simultaneously sperm may mature to maximum potential followed by aging and degeneration. The extent to which the epididymides may remove sperm is uncertain. A review of the quantitative aspects of spermatogenic capacity of the testes (7, 8) indicates that more sperm can be produced than are accounted for by sperm obtained with frequent semen collections, thus implicating epididymal resorption or other sources of loss. Conflicting conclusions from such studies with rabbits have been reported (5, 6), partly due to differences in experimental procedures and methods of calculating results. Other studies after vasoligation (9, 10) and with radioisotopes (11) suggest that epididymal resorption of sperm may be variable. If epididymal resportion is considerable, it potentially could play an important role in removing aged and damaged cells from the epididymis. Conversely, if the epididymis plays a lesser role in sperm resorption than currently is suggested, it may be desirable to minimize the time spent by the older sperm in the cauda epididymidis and ductus deferens. This can be accomplished by frequent ejaculation (1–3, 5, 9, 12, 13). The present study was designed to examine sperm resorption, in sexually active rabbits, by determining the rate of epididymal accumulation as well as retention of sperm in various portions of the epididymis isolated by appropriately placed ligatures.

Materials and Methods. Dutch-belted males, averaging 12 months of age and 2.17 kg in body weight were used in Expt. I and II, and New Zealand males, averaging 14 months of age and 4.29 kg in body weight were used in Exp. III. All males were trained to serve the artificial vagina, and two ejaculates of semen were collected each Monday, Wednesday, and Friday throughout the experiment. Such intensive ejaculation yields approximately the same number of sperm as daily semen collections used earlier in this laboratory(13).

One side was chosen at random to be ligated and the other side served as an untreated control. Ligatures were applied in Exp. I to the ductus deferens near its junction with the cauda epididymidis, in Exp. II to the ductus deferens and to the center of the corpus epididymidis, and in Exp. III to the previous two sites plus the ductuli efferentes (14). The time of applying the ligatures is referred to as Week 0. Sperm output from the untreated side was monitored by continuing the semen collections until animals were castrated at various intervals up to 12 weeks after ligation. Castration was performed immedately after the last semen collection in the particular week.

The epididymides were trimmed free of other tissue, and divided by cutting the middle of the corpus so that each caput and cauda epididymidis included a portion of the corpus. Each part was weighed, cut with small scissors in 0.87% saline, and homogenized in a larger volume of saline for 10 min. The saline contained Dow Corning Anti-Foam C. This effectively prevented foam accumulation during homogenization. The sperm cells were broken by this procedure

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TABLE I. Average Sperm Content of Ligated Epididymides, and Sperm Content and Sperm Ejaculated from the Control Contralateral Side.

Expt. no.	Weeks	No. of rabbits	Control side: Epididymal sperm (10^6) Caput	Cauda	Total	Weekly sperm output (10^6)[a]	Ligated side: Epididymal sperm (10^6) Caput	Cauda	Total
I	4	4[b]	112	294	405	271	85	1132[d]	1218[d]
	8	4[b]	96	250	346	320	124	2638[d]	2762[d]
	12	4[b]	126	396	522	400[c]	111	3636[d]	3747[d]
	Av.	12	111	313	424	330[c]	107	2469[d]	2576[d]
II	4	4	93	213	306	292	14[d]	230	244
	8	2	66	217	283	323	36[c]	254	290
	12	2	132	337	469	315	142	302	444
	Av.	8	96	245	341	310[c]	64	262	326
III	1	2	90	296	386	219	95	322	417
	4	2	116	209	325	285	44[c]	233	277
	8	2	170	542	712	415[c]	24[d]	459	483
	12	3	110	555	665	366[c]	22[d]	198	220
	Av.	9	120	418	538	350[c]	44[d]	291	335

[a] Sperm output equivalent to one side in Week 0 for experiments I, II, and III was 219, 242, and 228 million sperm, respectively.

[b] Two control sides in each group were removed initially in experiment I and the others as specified.

[c] $p<.05$. Epididymal weights are compared with controls and sperm output with Week 0.

[d] $p<.01$.

and sperm heads were counted from duplicate subsamples using a hemocytometer.

Because of the nature of the treatments it was not possible to reutilize the same rabbits. However, the influence of animal variability on treatment was minimized by leaving one side of each animal untreated, so that each animal served as its own control. The *t* test was used to determine the statistical significance of paired comparisons.

Results. The average sperm content of the epididymides, epididymal weights and the average number of sperm cells ejaculated from the control side in the three experiments are shown in Tables I and II.

In Expt. I the sperm could migrate into all portions of the epididymis up to the ligature on the ductus deferens. This they appeared to do, as sperm numbers in the caput remained relatively unchanged. The counts in 6 caput and 6 cauda epididymides removed at Week 0 were 100 and 244 million sperm, respectively. However, on the ligated side the cauda epididymidis significantly increased in size due to an accumulation of sperm cells within 4 weeks after ligation. This accumulation continued throughout the experiment.

TABLE II. Epididymal Weights.

Expt. no.	Weeks	Epididymal weight (g) Controls	Ligated
I	4	721	1117[a]
	8	690	1484[b]
	12	988	2275[b]
	Av.	743	1625[b]
II	4	747	1005[a]
	8	922	1336[b]
	12	784	990
	Av.	781	1084[a]
III	1	1170	1680
	4	1440	1395
	8	1470	1500
	12	1483	1680
	Av.	1401	1577

[a] $p<0.5$ compared to controls.

[b] $p<.01$ compared to controls.

There was no evidence of any back pressure reaching the caput epididymides or the testes.

Sperm numbers ejaculated per week from the control side unexpectedly increased. Body weight changed very little, but testis weights at 4, 8, and 12 weeks were 2.32, 2.53, and 2.96 g. Also, animals may have become more accustomed to the collection procedure during the experiment.

The number of sperm collected per week and sperm accumulated in the epididymides on the contralateral side is compared in Table III. The latter was computed by subtracting the sperm counted in epididymides at the beginning of the experiment from the number found after 4, 8, and 12 weeks (Table I), and dividing by the appropriate number of weeks. The number of sperm collected from the control side was greater than the accumulation on the ligated side. This might have been due to sperm resorption, an underestimate of accumulated sperm, a decrease in spermatogenesis on the ligated side, or an increase in spermatogenesis on the control side. At the same time it should be noted that more sperm accumulated than would have been predicted from sperm output at the time of ligation (Table I).

In Expt. II with ligatures on both the corpus epididymidis and ductus deferens, new sperm could not migrate past the proximal part of the corpus. The caput became edematous and was responsible for an increase in total weight of the epididymis (Table II). Sperm numbers decreased by Week 4 in the caput, and fluid pressure back on the testis disrupted spermatogenesis (15). By Week 12 limited renewal of spermatogenesis was apparent and new sperm cells had moved into the caput. The number of sperm in the caudal portion of the epididymis isolated by ligatures did not decrease during the 12 weeks.

TABLE III. Comparison of Sperm Accumulated Versus Ejaculated in Experiment I.

	Average sperm numbers per week (10^6)	
Weeks	Obtained from the control side by semen collections	Epididymal accumulations on the ligated side
1–4	276	218
1–8	322	302
1–12	385	284

In Expt. III ligatures on the ductus deferens, corpus epididymidis, and ductuli efferentes isolated the sperm present at the time of ligation into two compartments. No additional sperm could enter the epididymis from the testis. Under these conditions sperm numbers in the caput declined significantly ($p<.01$), indicating resorption. Contrary to this, sperm numbers in the caudal segment did not decline significantly ($p>.05$), thus supporting the evidence from Expt. II that sperm cells in the cauda are not resorbed appreciably over a period of 12 weeks. The size of the epididymis remained unchanged under these conditions. The large size throughout this experiment is due to the larger New Zealand rabbits used instead of the Dutch used in Expt. I and II.

Discussion. The count in control caput epididymides averaged 111, 96, and 120 $\times$ 10^6 sperm in the three experiments, which was not different from the sperm content of the caput after vasoligation (Expt. I). In contrast, sperm numbers in the caput declined within 4 weeks ($p<.01$) when sperm were trapped by ligatures on the corpus (Expt. II). Disruption of spermatogenesis (15) prevented a resupply of sperm until effective renewal of spermatogenesis was noted at 12 weeks. Ligatures on the ductuli efferentes, in Expt. III, prevented new sperm from being transported to the caput, and sperm content declined significantly ($p<.01$). These results clearly show that the caput epididymidis is capable of sperm resorption, but it is not likely that large numbers of sperm are resorbed during the usual time required for sperm transport through this segment. Considerable fluid from the testis apparently is absorbed, but not enough to prevent interference with spermatogenesis (15).

With vasoligation spermatogenesis continued, and sperm output was accommodated, primarily by a dramatic increase in sperm stored in the cauda epididymidis. With this distention some resorption may have oc-

curred. In one male rupture of the cauda epididymidis had occurred at 12 weeks. Accumulated sperm (Table I) accounted for 76–89% of the ejaculated sperm at the different time intervals. This suggests that sperm resorption was low in sexually active rabbits. If sperm resorption normally were high, large numbers likely would be resorbed during periods of prolonged retention. In both Expt. II and III the number of sperm remaining in the cauda after periods up to 12 weeks showed little more than random fluctuation from initial counts. Consequently, the evidence supports the hypothesis of no appreciable sperm resorption in the nondistended cauda epididymides, even during periods considerably longer than sperm normally are retained in this segment by sexually active males. Thus, it is unlikely that there was extensive resorption of sperm on the control side, and sperm collected would be a reasonable indication of sperm entering the contralateral epididymis. From these results it would appear that resorption is of much less importance than in the bull (9), even if all differences noted were due to resorption.

Other reports (10, 16) show that gonadal reserves in the rabbit are reduced after vasoligation. Atrophy of the testes has been observed in males vasectomized for several years (15). Thus, reduced spermatogenesis also may account for some of the difference. However, a quantitative histological analysis of the testes (17) showed no effects of vasoligation on the proportion of spermatids, primary spermatocytes, tubular tissue, diameter of the seminiferous tubules, or frequency of the stages of spermatogenesis (15).

It is possible to estimate sperm losses by predicting sperm produced, and comparing this with the number of sperm collected. The number of sperm collected, on the basis of intact animals, was about 91 $\times$ 10^6/day for Dutch-belted and 100 $\times$ 10^6/day for New Zealand rabbits. This is comparable to values previously reported for rabbits ejaculated frequently (3, 5, 13, 18, 19). The number collected is more than was reported produced in one study with Dutch and New Zealand rabbits (5) and is less than was found in other studies with New Zealand males (6, 20). Gonadal reserves were not estimated in the present study so no direct comparison of production with sperm output is possible. Furthermore, differences calculated by this method may represent losses other than resorption (7), although sperm losses in the urine have not been found (6).

The increase in sperm output from the control side, as the experiments progressed, appears primarily due to an increase in testis size. This was unexpected, since the males should have been sexually mature at the age used and they grew little during the experiments. It is possible that the sexual activity (frequent semen collections) during the experiment influenced spermatogenic activity.

The average epididymal sperm content of the control side was 424, 341, and 538 $\times$ 10^6 sperm in the three experiments. Frequent ejaculation has a considerable effect in depleting the cauda (1–3, 5, 9, 12). Since the rabbits had been ejaculated twice just prior to slaughter, and six times within the preceding 5 days, ejaculated sperm should be added to estimate normal epididymal contents. On a whole-animal basis epididymal reserves ranged from 888 to 1308 $\times$ 10^6 sperm, when only the last two ejaculates were added. Adding all six ejaculates, which might approximate the reserves in sexually rested males, gave total sperm counts ranging from 1302 to 1776 $\times$ 10^6. These values are similar to others reported for various breeds of rabbits (3, 5, 6, 12). Lambiase and Amann (12) reported higher values when Triton X-100 was used during tissue homogenization.

Summary. Possible epididymal sperm resportion in sexually active rabbits (six ejaculates of semen collected per week) was examined under conditions allowing for sperm accumulation in distended epididymides versus retention of sperm in various segments isolated by appropriately placed ligatures. As much as 85% of the sperm in the isolated caput underwent dissolution and resorption within 4 weeks ($p<.01$). In contrast there was no significant decline in the number of sperm retained in cauda epididymides by ligation over a 12-week period. Seventy-six to 89% of the sperm ejaculated from the control nonligated side could be accounted for by

sperm accumulated in contralateral epididymides with vasoligations. These results suggest that epididymal resorption may be minimal in the sexually active rabbit.

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