Vasocystostomy in the Rabbit: Surgical Technique and Sperm Outflow

RUSSELL H. HOOKER

From the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts

The surgical technique employed for construction of bilateral vas-to-bladder fistulae (vasocystostomy) in the male rabbit is described. The method of collection and results of examination of sperm from 15 of these animals are presented. The average daily number of intact sperm (185×10^6) passed in the urine of vasocystostomized rabbits was consistent with previous estimates of sperm production and slightly exceeded previous measurements of sperm output in ejaculated semen.

Key words: vasocystostomy, surgery, rabbit, sperm, urine.

Diversion of sperm from the vasa deferentia into the urinary bladder (vasocystostomy) has been found to be a satisfactory model for sperm output measurement in the rat (Vreeburg et al, 1974; Kort et al, 1975) and in the rat and mouse (Urry and Hill, 1979). Recently, vasocystostomy was found to be technically feasible in the rabbit and to be a more "physiologic" method of male fertility control, with fewer side effects than conventional vasectomy (Hooker, 1979, 1980). However, its use for measuring the outflow of spermatozoa in the urine of rabbits has not been described. This report describes a successful operative technique and a method for measurement of sperm outflow.

Materials and Methods

Sperm fistulae between the vasa deferentia and urinary bladder (vasocystostomy) were created in 15 mature male rabbits, of mixed breeds, weighing between 2.9 and 5.2 kg. Thirteen animals were observed for 12 months, one for five months, and one for four months. Urine specimens and ejaculates were collected at random times (see below) and examined for the presence and number of sperm, using a light microscope and hemocytometer. At the end of the period of study, the status of each fistula was verified by injecting saline into the surgically exposed vas deferens and observing its flow into the bladder.

Anesthesia and Surgical Technique

The technique of vasocystostomy was modified from the procedure of Vreeburg et al (1974). Chlorpromazine (Thorazine; Smith, Kline and French Laboratories) was given intramuscularly (3.5 mg/kg) 1 hour preoperatively, and food and water were removed at this time. Anesthesia was induced by intravenous administration of sodium pentobarbital (Diabutal; Diamond Laboratories), 20 mg/kg body weight. In case of respiratory depression, mouth-to-mouth rebreathing via plastic tubing was instituted and continued until spontaneous respirations resumed. Under sterile conditions, a 5-cm suprapubic midline abdominal incision was made through skin and subcutaneous tissue. The abdominal wall at the operative site then was infiltrated with 2 or 3 ml lidocaine HCl (Xylocaine; Astra Pharmaceutical). The incision was deepened and the bladder was elevated from the peritoneal cavity and emptied by manual pressure. Using a magnifying (1 ×) loupe, the left vas near the ampulla was gently elevated with nerve hooks and cleared of its mesentery for approximately 1 cm (Fig. 1). Deferential blood vessels were preserved. A single ligature of 6-0 Prolene (Ethicon) was tied about the vas near the center of the cleared zone, and the vas was incised transversely on the proximal side of the ligature (Fig. 2). An 8-cm length of Silastic tubing (OD 1.19 mm) with a wire obturator formed into a sharp tip was passed into the afferent vas and punctured through the vas wall 2–3 cm proximally (Fig. 3). The vas then was transected completely, allowing the Silastic tube to project from its cut end (Fig. 4).

Reprint requests: Dr. Russell H. Hooker, Visiting Scientist, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545.

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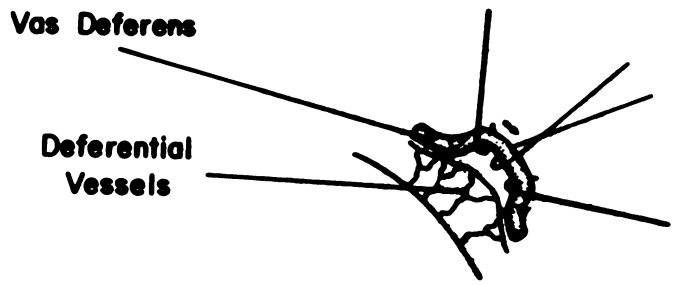
Vas Deferens
Deferential Vessels
Bladder Serosa

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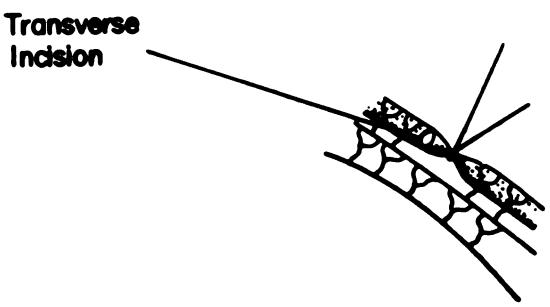
Transverse Incision

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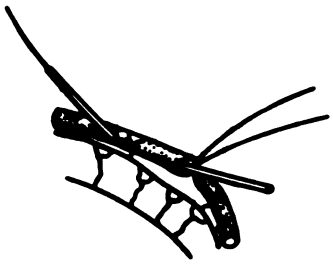

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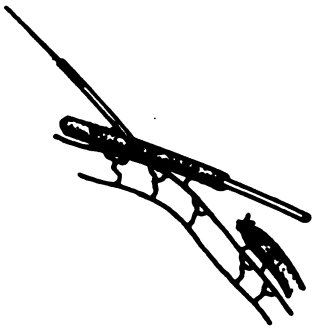

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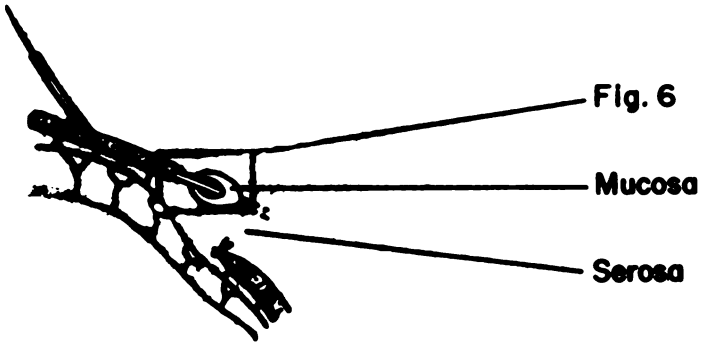
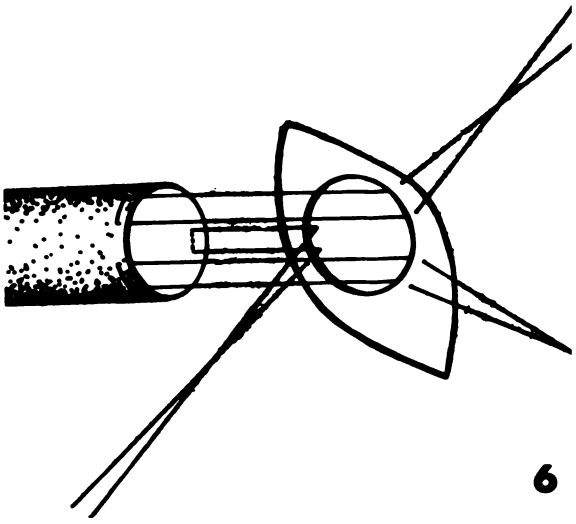


Fig. 6
Mucosa
Serosa

5



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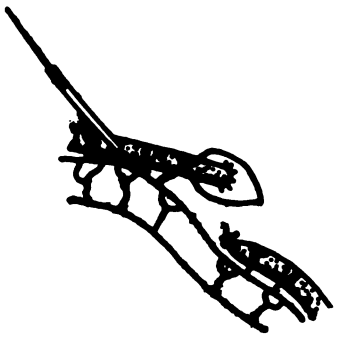

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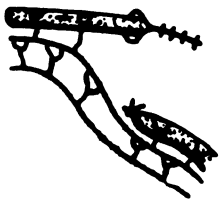

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Attention was now directed to the bladder adjacent to the divided vas. A 1-cm incision was made through the serosal and muscular layers, and the bladder was manually compressed, causing the mucosal layer to herniate slightly. The mucosa was grasped with two mosquito clamps and opened approximately 3 mm. The Silastic tube protruding from the end of the vas was inserted into the bladder lumen to serve as a stent during the anastomosis (Fig. 5). Five or six everting absorbable mattress sutures of 5-0 Vicryl (Ethicon) were positioned (Fig. 6) and then tied and trimmed (Fig. 7). If necessary, the mucosa was closed with additional sutures. Serosa and muscularis were closed about the vas until there was no further leakage of urine, after which the Silastic tube was pulled out and removed (Fig. 8). Closure of the orifice in the vas wall was found to be unnecessary. The right vas was similarly transplanted. The bladder was replaced in the abdominal cavity, and the incision was closed in two layers with interrupted sutures of 6-0 silk.

Urine Collection and Examination

The method of collecting and processing urine was a modification of that of Holtz and Foote (1972). After a minimum of eight days of postoperative healing, routine urine collection was carried out as described below.

In the late afternoon, the buck was allowed to mate with a doe, to mount several times, or an ejaculate was collected by means of an artificial vagina. A clean metal pan covered with a screen to exclude solid droppings was placed under the cage. A baffle was also used to minimize urine loss and to prevent possible contamination from neighboring animals. At approximately 11 A.M. the following day, using a bulb syringe, the accumulated urine was collected from the pan. Any residue was washed down with aspirated urine.

Urine Processing

The volume of urine was measured, and a 10-ml aliquot was passed through two layers of mesh gauze into a graduated tube, which was centrifuged for 10 minutes at 2000 rpm. The supernatant was decanted, and two or more drops of concentrated HCl was added to the residue, the volume of HCl varying with the quantity of extraneous organic matter requiring oxidation. After frothing had subsided, the remaining volume was brought up to 1, 5 or 10 ml until the dilution was sufficient for clear visualization of spermatozoa in the counting chamber.

Sperm Counting

The concentration of intact sperm (fragments of sperm were not included) in the original sample was determined, and the total number of intact sperm passed in the urine during the 18-hour period was calculated.

Sperm counts were made of 61 urine specimens collected from 15 different animals during a 12-month period. Initial collections and counts were made as follows: one week postoperative, five animals; two to four weeks postoperative, four animals; seven to ten weeks postoperative, six animals. Thereafter, the interval between collections varied from minimums of one to four weeks, ten animals, and six to 12 weeks, five animals, to maximums of nine to 18 weeks, eight animals, and 19 to 29 weeks, seven animals.

The proportion of intact sperm to tails (fractured sperm) was calculated in four instances.

Results

All vas-to-bladder fistulae were proven by saline flush to be patent at the end of the period of study; the health and libido of rabbits was unaffected by vasocystostomy; and all animals were infertile except one whose ejaculates contained urine (Hooker 1979).

Urine specimens collected from the vasocystostomized rabbits contained large numbers of fractured sperm heads and tails as well as whole or intact sperm. The proportion of intact sperm to tails in four samples of urine from different bucks was 1/7, 1/4, 3/1, and 1/1.

The total number of intact sperm per 18-hour sample from each individual animal and from 15 different animals varied widely (Table 1). The total number of intact sperm (mean ± SE) per 18-hour sample (n = 61) was $140 \pm 24 \times 10^6$.

Discussion

The surgical technique used for vasocystostomy in this study was more complex than that previously used in rats (Vreeburg et al, 1974; Kort et al, 1975). It was felt that a mucosa-to-mucosa anastomosis between vas deferens and bladder in the rabbit would provide greater assurance of long-term fistula function and less danger of leakage. Preservation of deferential vessels added slightly to the duration of the procedure and may not have been necessary since the vas deferens is known to have a double blood supply composed of intercommunicating branches from both the testicular and vesical arteries (Hamilton, 1978).

In comparison with the vas in a number of other mammals (Mather, 1975), the vas deferens of the

Figs. 1–8. The steps taken to construct a sperm fistula between the vas deferens and the urinary bladder in the rabbit. **Fig. 1.** Vas elevated. Arrow indicates direction of sperm flow. **Fig. 2.** Ligature tied; vas opened transversely. **Fig. 3.** Silastic tube inserted. **Fig. 4.** Transection of vas completed. **Fig. 5.** Bladder opened; silastic tube inserted. **Fig. 6.** Enlarged view of rectangle in Fig. 5. Placement of sutures. (Silastic "stent" is not shown.) **Fig. 7.** Anastomosis of vas to bladder mucosa completed. **Fig. 8.** Bladder serosa and muscularis closed; silastic tube (stent) removed.

TABLE 1. Total Number of Intact Sperm in Urine Specimens Collected for 18 Hours from Male Rabbits at Varying Intervals Following Vasocystostomy

Rabbit No.	No. of Urine Specimens	Volume (ml, mean ± SE)	No. of Sperm × 10^6 (mean ± SE)
1	7	156 ± 34	181 ± 42
2	4	128 ± 26	54 ± 2
3	5	213 ± 34	123 ± 47
4	5	218 ± 45	85 ± 35
5	5	157 ± 52	222 ± 67
6	4	155 ± 61	85 ± 37
7	6	150 ± 53	29 ± 11
8	4	91 ± 21	112 ± 97
9	4	29 ± 14	105 ± 65
10	3	103 ± 51	20 ± 12
11	3	338 ± 18	496 ± 127
12	3	178 ± 112	183 ± 29
13	2	325 ± 125	76 ± 59
14	3	65 ± 18	50 ± 25
15	3	93 ± 17	367 ± 339
	61	160 ± 14	140 ± 24

rabbit is thin and easy to dilate, but when opened it collapses and becomes edematous so that visibility is impaired. However, it is easily cannulated (Holtz and Foote, 1974), and a cannula in the lumen of the vas can serve as a stent and hence assist in the accurate placement of sutures. Silastic tubing is suitable for this procedure because of its inertness and pliability (Pardanani et al, 1973). In the rabbit, such tubing can be removed through a puncture in the vas wall without complication. This step of the method might not be applicable for species more prone to granuloma and stricture formation.

The principal purpose for the collection and examination of an occasional urine specimen from each animal was to determine the condition of the fistula between the vas deferens and the urinary bladder. The presence of sperm in the urine was considered proof that a vas-to-bladder anastomosis was open and functioning—unilaterally and, perhaps, bilaterally. The number of sperm was of secondary importance; no attempt was made to measure actual sperm production by the testes. Some voided sperm were surely lost in the process of urine collection, and on microscopic examination, many sperm were seen to be fractured. This was perhaps due to their age and to the unphysiologic environment. Also, the treatment with HCl probably accounted for some sperm disruption. Presumably, sperm continuously passed into the bladder from the vasa deferentia, and some remained in the bladder after urination. Thus, the population of sperm at any one time was made up of individual spermatozoa of different ages, some of which were old and degenerated (Martin-Deleone et al, 1973).

A large number of sperm present in the urine of vasocystostomized rabbits were intact. These were presumably fresh, ie, recently discharged from the ductus deferens into the urinary bladder. The average number of intact sperm in 61 urine samples collected over an 18-hour period from 15 vasocystostomized rabbits and adjusted for a 24-hour period was 185 × 10^6. This correlated well with previous estimates of daily sperm production for this species (147 × 10^6, Orgebin-Crist, 1968; 250 × 10^6, Amann, 1970; 187 × 10^6, Holtz and Foote, 1972) and in fact exceeded previous measurements of sperm output based on collected ejaculates from New Zealand white rabbits (Amann, 1970) and collected ejaculates and urine from Dutch Belted Rabbits (Holtz and Foote, 1972).

Although the *p*H and isotonicity of urine are not suitable for prolonged sperm survival (Emmens, 1947), sperm can survive in urine for at least 10 to 15 minutes (Chang and Thorsteinsson, 1958). One vacocystostomized rabbit with urine-contaminated ejaculates in the present study actually fathered a litter.

It is concluded that vasocystostomy in the male rabbit is a safe and effective method for diverting the transport of spermatozoa and that the number of spermatozoa passed in the urine can be measured with reasonable accuracy.

In considering the possible application of vaso-

cystostomy to the human, it is an intriguing thought that a second operation to restore fertility might perhaps not be necessary; that fertile sperm might be obtainable from the urine for artificial insemination.

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