

MICROSCOPIC VASOVASOSTOMY AND SPERMATOGENESIS

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ABSTRACT

Initial clinical experience with a new microscopic 2-layer anastomosis for vasovasostomy shows that normal patency can be achieved almost every time. Histologic and electron microscopic studies on initial specimens and subsequent ejaculates indicate that stricture is a common cause of failure with conventional vasovasostomy techniques, obstruction of the vas deferens inhibits spermatogenesis and relief of obstruction allows return of spermatogenesis in the vast majority of cases.

Many reasons have been offered for the poor results obtained after reversing vasectomy. Certainly the tiny diameter of the lumen of the vas ($\frac{1}{3}$ mm.) has been a formidable obstacle. However, now using a microscopic 2-layer anastomosis with perfect mucosal reapproximation of the lumen (using 9 and 10-zero nylon sutures with a magnification of 16 to 40 \times) there is virtually 100 per cent success in surgical restoration of stricture-free continuity to the vas deferens.¹⁻³ This technique has produced normal sperm counts in 90 per cent of the patients and in every patient who has had sperm in the seminal fluid at the time of vasovasostomy. It is too early to report accurately a final pregnancy figure but it has been 59 per cent within the first year. In Australia, Owen has used the same technique but has a longer followup and has reported a 75 per cent pregnancy rate. Our initial experiments in rats using this technique demonstrated completely normal fertility after bilateral vasectomy and immediate subsequent vasovasostomy.

Herein we will present evidence that conventional methods of vasovasostomy result in a strictured communication (or microchannels in an inflammatory sperm granuloma) that eventually scars down completely. The increased back pressure from this partially obstructed (or rather partially open) system depresses sperm production and results in stasis and poor sperm transfer across the suture line. Electron microscopic studies are revealing exciting new basic information about the mystery of spermatogenesis and may answer one of the most perplexing questions our patients ask us: "What happens to the sperm after vasectomy?"

METHOD

Two groups of patients were subjected to microscopic vasovasostomy—in 1 group of 30 there had been no previous attempt at reversal and in the other group of 6 there had been an apparently successful vasovasostomy in the past by well known and competent urologists (yet no pregnancy had occurred and subsequent sperm counts showed return to near azoospermia). Since this presentation, more than 200 patients have been operated upon with this technique by the author, with similar results.

The technique was standard for all patients, performed by the same surgeon and has been described in great detail.¹⁻³ Six interrupted sutures of 9-zero nylon (35 mu.) were used to create a perfect fluid-tight mucosal anastomosis (fig. 1). The muscularis was then approximated with 10 to 12 interrupted sutures of 9-zero nylon. GS-9 or GS-16 needles were used but the latter was found to be preferable. Instruments were individually designed.† A Zeiss operating microscope was used

with a magnification of 16 to 40 \times . No splints were used. The anastomosis was leak-tight. The proper performance of this technique requires extensive practice in the laboratory on rats and has developed out of the author's (S. J. S.) 4 years of laboratory experience in microsurgery.

Many of the vasectomies had extended well into the convoluted portion, and this in no way hindered an accurate and perfect anastomosis. The scrotal wound was closed with 3 interrupted intracuticular sutures of 3-zero dextron. The patient was allowed to ambulate immediately and was discharged from the hospital 1 day postoperatively in every case.

Semen from the proximal cut end of the vas deferens was examined at the time of vasovasostomy by light and electron microscopy. The ejaculate was similarly examined at monthly intervals postoperatively. No patient was accepted for the operation unless he agreed in advance to allow this careful followup.

Only patients operated upon 3 months or more ago are included. Most patients have been operated on only in the last 6 months.

In patients whose previous conventional vasovasostomies were being redone with the microscopic technique, the area of previous anastomosis was excised and subjected to longitudinal and cross-sectional histologic examination in an effort to determine the degree of patency. Since this presentation more than 40 such patients have been redone by the author after conventional operations had failed.

Testicular biopsy was performed on all patients who had no sperm at the time of vasovasostomy and on a random sample of those who did have sperm at the time of vasovasostomy.

RESULTS

Light microscopy—first vasovasostomy. At the time of vasovasostomy the effluent from the proximal cut vas contained predominantly non-motile sperm. In most instances there was no motile sperm at all. The more recently the vasectomy had been done the more likely there was to be motile sperm in the fluid at the time of vasovasostomy. There was some sperm present in the seminal fluid at the time of operation in 90 per cent of patients whose vasectomy had been done less than 10 years ago. However, if the vasectomy had been done more than 10 years ago only 3 of 8 patients had sperm present at the time of vasovasostomy. Seminal fluid was present in every patient without exception.

The ejaculate of patients at 1 and 2 months postoperatively demonstrated low counts, ranging from a few dead sperm per high power field to 5,000,000 per cc. Motility at 1 and 2 months postoperatively remained low (1 to 30 per cent). Occasionally, the sperm count was normal and the motility was more than 50

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per cent prior to 3 months postoperatively. In most patients sperm counts and motility did not increase to normal until 3 months postvasovasostomy. Counts usually continued to improve during a 6-month period.

All 22 men whose vasectomies had been done less than 10 years ago had sperm in the ejaculate and 90 per cent had normal sperm counts by 3 months. A normal sperm count by light microscopy was considered to be greater than 20,000,000 per cc, greater than 60 per cent motility with good action and greater than 80 per cent normal forms. Most normal counts were actually much more than 30,000,000 per cc. Of the 2 patients who were oligospermic 1 had had no sperm whatsoever at the time of operation. The other had a normal sperm count at 2 months but then showed a decline in count and motility. A stricture was found at reoperation and corrected. His sperm count then returned to normal. Thus, 95 per cent of patients who had had vasectomy less than 10 years ago should be able to achieve a consistently normal sperm count after vasovasostomy and virtually all should have some sperm in the ejaculate.

Of 8 patients who had had vasectomy more than 10 years ago only 3 had normal sperm counts and 4 had no formed sperm at all as late as 4 months postoperatively. Longer followup and a larger number in this group indicate 50 per cent normal sperm counts when the vasectomy has been done more than 10 years ago.

All patients who failed to have formed sperm in the ejaculate at 3 months also had had no sperm in the seminal fluid at the time of vasovasostomy. All patients with formed sperm in the seminal fluid at the time of vasovasostomy had sperm in the ejaculate postoperatively and 95 per cent of them eventually had normal sperm counts. In several cases normal sperm counts occurred even when there was no formed sperm initially but this was less likely.

Light microscopy—reoperations on conventional vasovasostomies that failed. We learned a great deal about stricture formation after conventional vasovasostomies by reoperating on 6 patients who had had 1 attempt at reversal done elsewhere. Five of them were originally considered successes by their urologists when checked for sperm at 1 to 2 months postoperatively. However, all patients progressed to oligospermia and poor motility by 6 months, rather than showing the steady improvement with time that should be seen. They were all subjected to a reoperation using the microscopic technique. The entire segment of the previous anastomosis was excised and subjected to histologic examination.

All patients reoperated on had sperm postoperatively in the ejaculate. Five of the 6 patients have been followed more than 3 months and all have normal sperm counts. One patient is only 1 month postoperative and has a typical finding for that

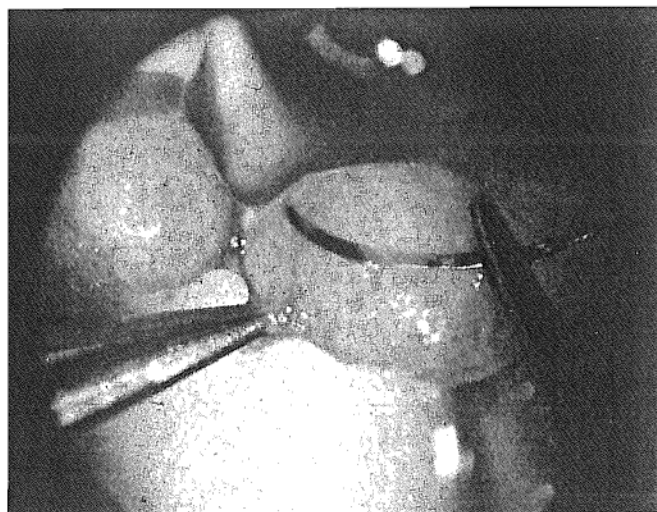


FIG. 1. First 9-zero mucosal suture being placed (microscopic view)

interval of 3,000,000 sperm per cc with no motility, and he also should have a normal count by 3 months. This patient subsequently did have a normal sperm count.

Histologic examination revealed a severely scarred down lumen at the site of the previous anastomosis (fig. 2). In all instances there was sperm granuloma noted at the site of scarring, indicating that there must have been a great deal of sperm leakage at the site of the previous anastomosis. Most of these cases were considered a success by the original surgeons, and the perplexing decrease in sperm motility and number was explained as an autoimmune reaction. However, in each case there was purely an anatomic cause.

Electron microscopy. Some astounding findings have been reported (abstract) on electron microscopic observation of specimens using thin sections and freeze fracture techniques. To summarize:

1) Sperm found at the time of vasovasostomy had degenerating membranes and appeared in the process of autolytic disin-

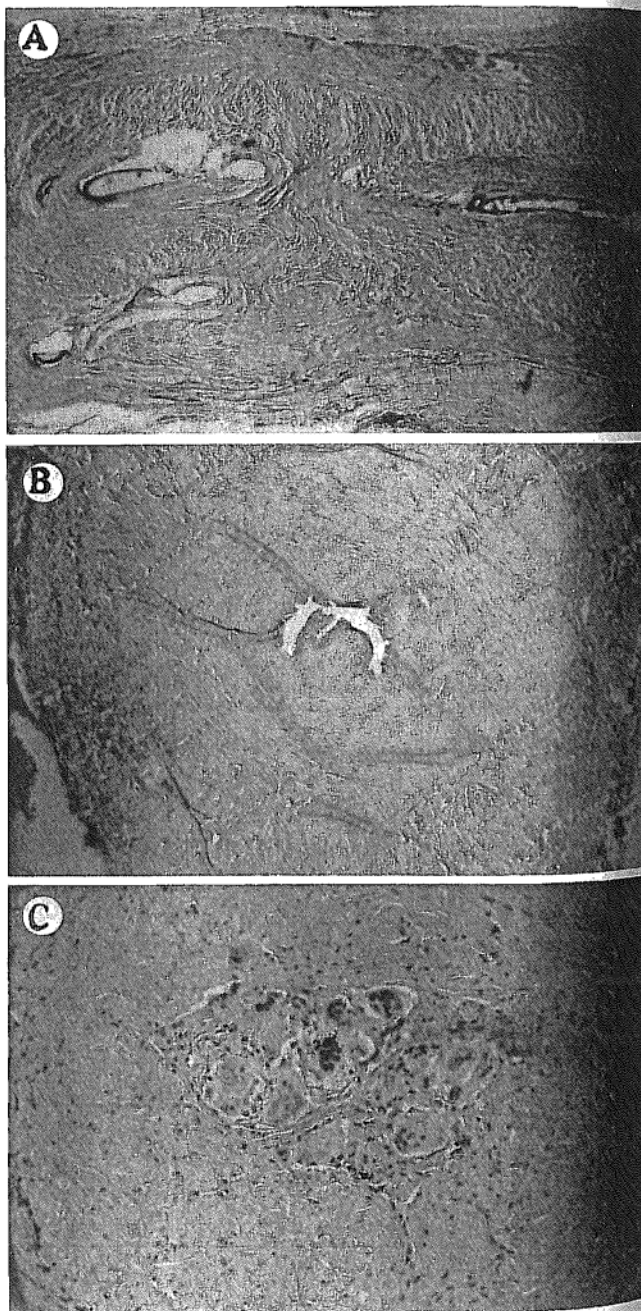


FIG. 2. Histologic sections of vas deferens in area of previous conventional anastomosis show stricture and sperm granuloma obstructing channel. Patient had oligospermia.

tegration (fig. 3). There was no cellular engulfment of formed sperm except in sperm granulomas that were extraluminal. Intraluminally, the sperm were apparently dying of old age with total dissolution of the usual freeze fracture topography.

2) The tight junctions remained intact in the vas deferens after vasectomy. The blood-testis barrier (the Sertoli cell junction) also remained intact in the testis. If there is a breakdown in tight junction function, it is only in the sperm granuloma, at the site of the vasectomy.

3) There were multiple, large, racquet-shaped crystals in lysosomal vacuoles of the vas epithelium that were easily

mistaken for engulfed sperm on light microscopy. However, in truth there was no sperm engulfment whatsoever in the wall of the vas.

4) Basement membranes were somewhat thickened.

5) By 2 to 3 months post-vasovasostomy sperm appeared in the ejaculate that were obviously a fresh new crop, with completely normal freeze fracture patterns (fig. 4).

6) When no sperm is seen on light microscopy at the time of vasovasostomy, or in the subsequent ejaculates, electron microscopy reveals sloughed, immature forms, dense clumps of nucleoprotein and other sperm elements that demonstrated the

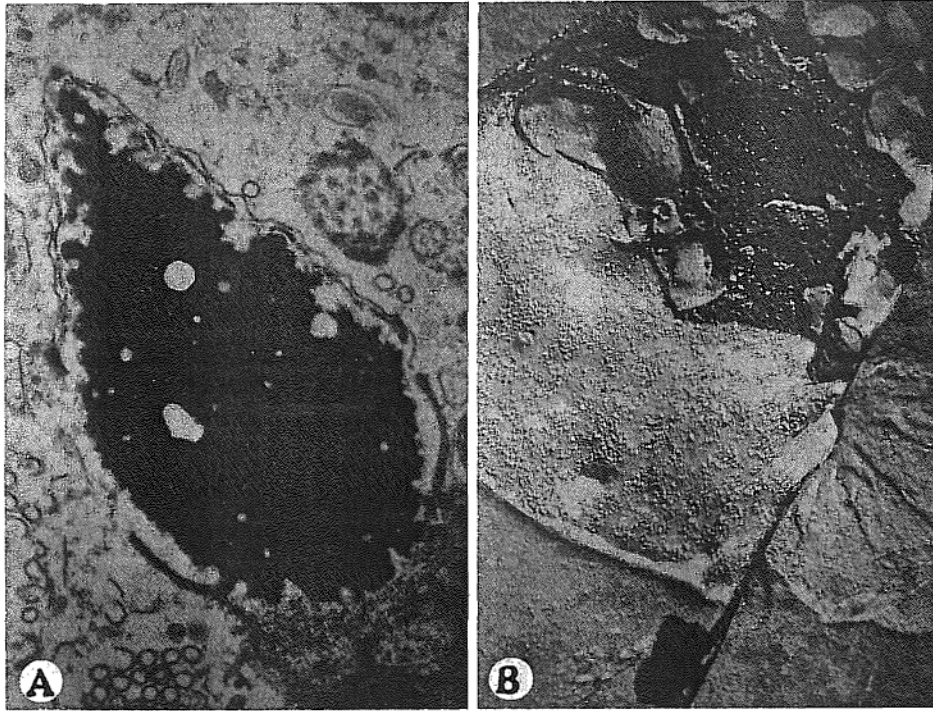


FIG. 3. A, thin section electron micrograph of sperm obtained at time of vasovasostomy. Membranes are degenerating with no white cell engulfment. B, freeze fracture electron micrograph shows loss of normal membrane characteristics of sperm obtained at time of vasovasostomy.

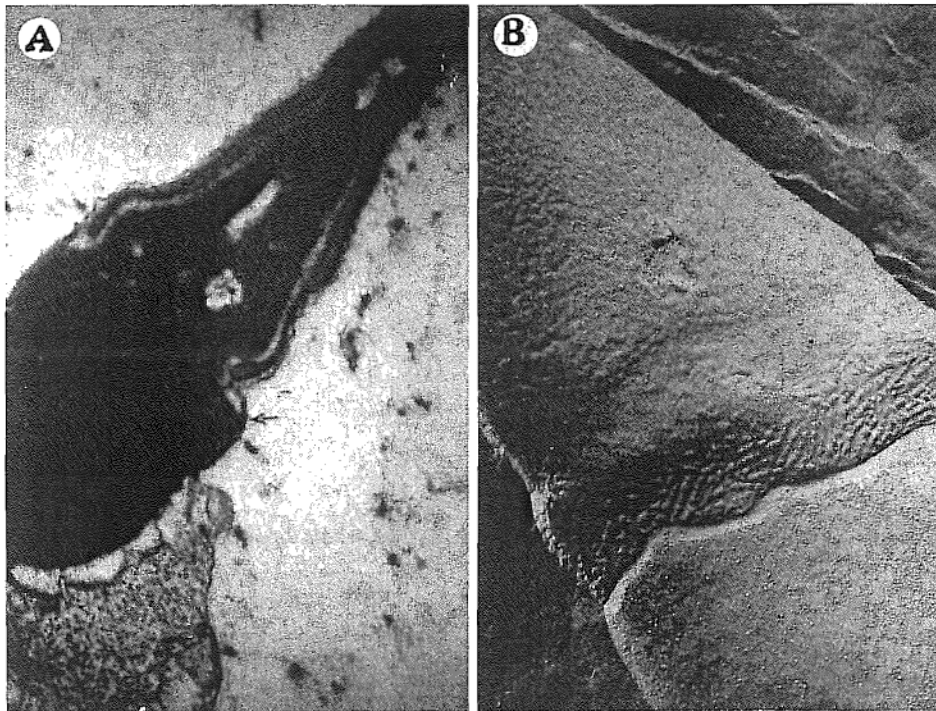


FIG. 4. A, thin section electron micrograph of sperm in ejaculate 3 months after microscopic vasovasostomy. It is perfectly normal. B, freeze fracture electron micrograph of sperm in ejaculate 3 months after microscopic vasovasostomy. Membrane pattern is perfectly normal.

anastomosis was patent. Early stages of spermatogenesis appeared normal in the testicular biopsies of these patients. Of course the rate of spermatogenesis could not be determined without sophisticated kinetic experiments but the delay in appearance of new sperm indicates inhibition of sperm production in the vasectomized man.

DISCUSSION

Therefore, we answer the question, "What happens to the sperm after vasectomy?" with the following hypothesis. Normal sperm continue to form but at a slower rate than normal. They disintegrate in the lumen of the vas. Epithelial cells show evidence of increased (endocytosis) pinocytosis but do not phagocytose sperm. They phagocytose broken down basic polypeptides. Cell junctional integrity is fully maintained in the vas deferens and testes (except in areas of sperm granuloma formation at the site of the ligature). Leukocytes are not involved in the scavenging process within the lumen of the ducts (only in sperm granuloma). There is increased intraluminal pressure and blockage of the normal vas deferens epithelium secretory function (crystals in endoplasmic reticulum). With long-term obstruction spermatogenesis appears to continue but it is minimal and the later maturational stages are more severely curtailed than the earlier stages. After relief of obstruction spermatogenesis usually recovers.

When the sperm count decreases rather than increases every month after a vasovasostomy, and motility decreases rather than improves, probably the anastomosis is stricturing down. It is true that in recanalization after vasectomy a relatively narrow channel can allow normal sperm counts and fertility but the type of stricture that usually follows vasovasostomy or is associated with the average post-vasectomy recanalization is so tiny as to truly preclude normal counts. Marshall and Lyon showed that the majority of men who do recanalize after vasectomy have a poor count and eventually scar down to aspermia, and are not fertile.⁴ In fact a histologic section of the usual post-vasectomy recanalization does not show a distinct channel but rather consists of sperm working their way through an extensive granuloma to reach the other lumen, usually resulting in a low sperm count and eventually scarring down completely in many instances.⁵ This is also the typical picture for stricture as a cause of conventional vasovasostomy failures. Sperm counts in our patients remained high and showed no tendency to diminish except in the 1 patient who had a documented stricture.

The commonly reported failure to achieve pregnancy despite finding sperm in the ejaculate postoperatively can be explained by 2 factors: 1) with conventional techniques most patients remain oligospermic and 2) even those with good counts initially will often scar down later, before the wife manages to become pregnant.

It appears from careful followup of sperm counts in these patients that this technique of anastomosis is virtually 100 per cent successful in restoring continuity to the vas deferens and at least 95 per cent successful in providing a communication free of stricture. There are 2 reasons why this may be important. Since the majority of sperm come from the epididymis at the time of ejaculation partial obstruction will impede adequate transfer.⁶ It also appears that the obstructive effect of the vasectomy inhibits spermatogenesis considerably, so that 3 months are required for the appearance of a healthy crop of new sperm whose genesis began after release of the chronic obstruction. The non-motile sperm seen at the time of vasovasostomy most probably had died of old age and the paucity of motile sperm indicates a slow rate of production of new sperm under the effects of obstruction. Once the obstruction is relieved spermatogenesis resumes in most cases. There probably is some residual loss of spermatogenic capacity but in the cases that were done within 10 years after vasectomy this loss was not great enough to prevent normal sperm counts from being achieved 90 per cent of the time, and some sperm in virtually every case. In cases done more than 10 years after vasectomy it is hoped that longer followup may demonstrate return of a normal rate of spermatogenesis in more than the 50 per cent that we now see.

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