PERFECT ANATOMICAL RECONSTRUCTION OF VAS DEFERENS WITH A NEW MICROSCOPIC SURGICAL TECHNIQUE*

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Early results with a new, two-layer microscopic technique for anastomosis of the vas deferens, using an operating microscope and ultrafine suture, reveal that patency of the vas deferens can be achieved in virtually every case. Normal sperm counts can be achieved in up to 95% of patients. Failure with conventional techniques is usually due to continuing partial obstruction. A poor sperm count after this technique is most likely due to an inherent inability of the patient to produce normal sperm, a likely sequela of chronic obstruction. Thus, while success is also good 10 years after vasectomy, it is not as predictable. Previous failure with a conventional operation does not limit success with a reoperation using the microscopic two-layer technique. Operations on 200 patients since this original study are confirming these early results.

Many reasons have been offered for the poor results obtained after reversing vasectomy. Certainly the tiny diameter of the lumen of the vas (0.333 mm) presents a formidable obstacle. Some assume that the present crude techniques of vas anastomosis are adequate but that other factors such as autoimmunity usually prevent restoration of fertility.[1-5] However, the present methods of vas anastomosis actually create a strictured, partially obstructed, channel, or even a mere fistula. Some, but not enough, sperm may get through. Moreover, the partial obstruction allows a continuation of high pressure and dilatation on the testicular side of the anastomosis, and this in turn inhibits spermatogenesis. Our studies indicate that a major impediment to successful reversal of vasectomy is the quality of the reanastomosis and that a nonstrictured vasovasostomy can only be accomplished with confidence by a two-layer microscopic anastomosis with an operating microscope and ultrafine suture. My initial experiments in rats demonstrated completely normal fertility after bilateral vasectomy and immediate subsequent vasovasotomy using this technique. Owen[6] in Australia has used the same technique on humans for several years and has obtained a pregnancy rate of 75%. This paper describes the early results (3 to 6 months' follow-up) of this approach on patients in the United States. Pregnancy results are not relevant until after 2 years of follow-up and thus will be reported later. However, *early* follow-up indicates over 50% pregnancy.

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IMMUNOLOGIC CONSIDERATIONS

In 1964, Phadke and Padukone[7] and later Rümke[8] and Shulman et al.[9] found that agglutinating antibodies were present in the serum of vasectomized men. In 1971, Ansbacher[1] discovered that 6 months after vasectomy sperm-immobilizing antibodies as well as spermagglutinating antibodies were present in the serum of 33% and 54% of men, respectively. However, he noted that 2% of fertile men also have these antibodies. The high 70% "success" rate for vas reanastomosis, associated with a low 25% rate for pregnancy, led him to speculate that an autoimmune response to sperm, triggered by vasectomy, was responsible for the discrepancy. Some problems with these in vitro assays are that the titers were generally low, between 1:2 and 1:32 (these could represent background activity rather than a specific antibody), and previous HL-A sensitization to the sperm donor with different HL-A antigens was not ruled out.

Halim and Antoniou[10] tested the serum of 100 men before and 6 weeks after vasectomy for the presence of spermagglutinating antibodies. They found that 2% had positive titers before vasectomy, and 6% following vasectomy, a much lower rise than found by other authors. Furthermore, spermatoxic antibodies had only increased

from 1% before to 2% after vasectomy. They concluded that a low background level of antibody activity against sperm—not incompatible with fertility—exists in a small percentage of all men.[10]

Studies on sperm antibody formation after vasectomy were also performed by Alexander et al.[2-4] Serum from rhesus monkeys was measured for spermagglutinating and sperm-immobilizing antibodies at 2 weeks and 6 months after vasectomy. At 2 weeks they found high titers (average, 1:760) of both immobilizing and agglutinating antibodies. However, by 6 months most titers had returned to low levels. More to the point, Alexander found *no correlation* between the fertility of vasovasostomized rhesus monkeys and their antibody levels. Owen[6] noted the same findings in humans.

Therefore, serious questions remained unanswered: What is the in vivo effect of antisperm antibodies? They have been measured solely by in vitro techniques. It is a weak syngeneic system, and results of in vitro assays may not have physiologic importance. Spermatozoa used for testing come from indifferent donors, and so the antibodies measured must be specific for a general antigen found in human sperm (and yet not present in cells other than sperm). Obviously, allogeneic HL-A antigens could cause false-positive reactions in HL-A-sensitized patients' sera, and yet would be of no clinical significance.

The results of many experiments in animals show that immunization with sperm may or may not lead to high levels of circulating antisperm antibodies (depending on the species or the investigator); but fertility at least is not impaired by this procedure unless spermatozoa are injected with Freund's adjuvant.[11-16] These investigators found that a low level of antisperm activity exists in many animals that are normally fertile. These studies led Hulka and Davis[5] in 1972 to question seriously the importance of autoimmunity in preventing successful results from vasovasostomy.

Phadke and Phadke,[17] in their large series of vasovasostomies performed by conventional techniques, showed that fertility (that is, pregnancy of the patients' wives) was closely related to sperm count and sperm quality of the ejaculate, but not to sperm antibody levels. Their results were among the best reported, with a 55% rate of pregnancy and an 83% incidence of sperm in the ejaculate. They used a simple nylon splint with 6-0 arterial silk and three stitches through the seromuscular layer only. It is difficult to understand why they obtained better results than others with this technique; yet the fact remains that the good results correlated with good postoperative sperm counts and did not correlate with sperm antibody titers.

OBSTRUCTED VAS DEFERENS

It has generally been assumed by urologists in evaluating patients for primary infertility that total absence of sperm in the ejaculate is required to indicate congenital obstruction as the cause of infertility. Most men with primary infertility have oligospermia rather than aspermia, and have an intact vas deferens without obstruction. On this basis, urologists have erroneously assumed over the years that the presence of *any* sperm in the ejaculate means "no obstruction." It has thus been thought that, with any degree of patency of the vas, an adequate number of sperm will cross the anastomosis to the ejaculatory duct and be expelled normally. This point of view disregards two factors: (1) The majority of sperm come from the epididymis at the time of ejaculation, and partial obstruction will impede adequate transfer. (2) With partial obstruction, the proximal vas and epididymis are dilated and sperm production itself may be inhibited or defective.

Freund and Davis[18] showed that 70% of the sperm in the ejaculate of a healthy man comes from the part of the vas deferens proximal to the point of vasectomy (the testicular side of the vasectomy) and from the epididymis. At the time of ejaculation, a series of peristaltic waves carries the bulk of the sperm from the epididymis and proximal vas to the ejaculatory duct and out into the seminal fluid. The remarkably thick muscularis of the vas as compared with the minuscule lumen underscores the importance of this peristaltic rush at the time of intercourse. A partial obstruction in the area of an anastomosis would make transfer of sperm into the semen difficult. Thus, like the ureter, the vas deferens usually cannot function properly when partially obstructed.

MICROSURGICAL APPROACH

With these thoughts in mind, we wondered whether better methods of microsurgery could result in greater success with vasectomy reversal.[19] Our results to date with this method indicate that 100% anatomical success can be achieved in microsurgical restoration of the continunity of the vas deferens. This 100% ana-

tomical success has resulted in a higher rate of functional success than the cruder methods reported in the past. Every patient with sperm in the seminal fluid at the time of vasovasostomy has had sperm in the ejaculate postoperatively. Ninety-five per cent have normal sperm counts, including number, motility, and morphology. Only those few with no sperm at all at the time of surgery have lower success rates.

The techniques of microsurgical anastomosis were developed during a 4-year experience with microvascular surgery, both in experimental animals and in humans.[19-27] Nylon suture 10-0 (Ethicon) is used for anastomosing blood vessels as small as 0.25 mm in diameter. An operating microscope with magnification of up to ×40 is necessary.

The instruments used are Barraquer or Silber needle holders, jeweler's forceps that have been carefully polished under a microscope, and various types of neurosurgical aneurysm clips for clamps (V. Mueller).

Under the microscope it is apparent that after vasectomy the lumen of the vas deferens distal to the ligature is approximately 0.25 to 0.333 mm in internal diameter and the lumen proximal to the ligature is approximately 0.50 to 0.75 mm in diameter. Some have suggested the vas lumen diameters to be 1 to 1.7 mm, but their measuring technique involved dilatation with probes. It is really very clear under ×40 magnification and a measuring ruler that the lumen of the vas is 0.5 mm or less. This was also noted by Hulka and Davis.[5] After the scarred segment is removed, a copious amount of fluid containing mostly dead spermatozoa effluxes from the dilated end. The external diameters and proximal vas are essentially the same despite the dilatation of the lumen proximally, which means that the wall of the proximal vas is thinner than that of the distal vas. This fact must be taken into consideration in planning the anastomosis. The technique is no different even in the convoluted portion of the vas. *The method of vasectomy should have absolutely no bearing on subsequent reversibility.*

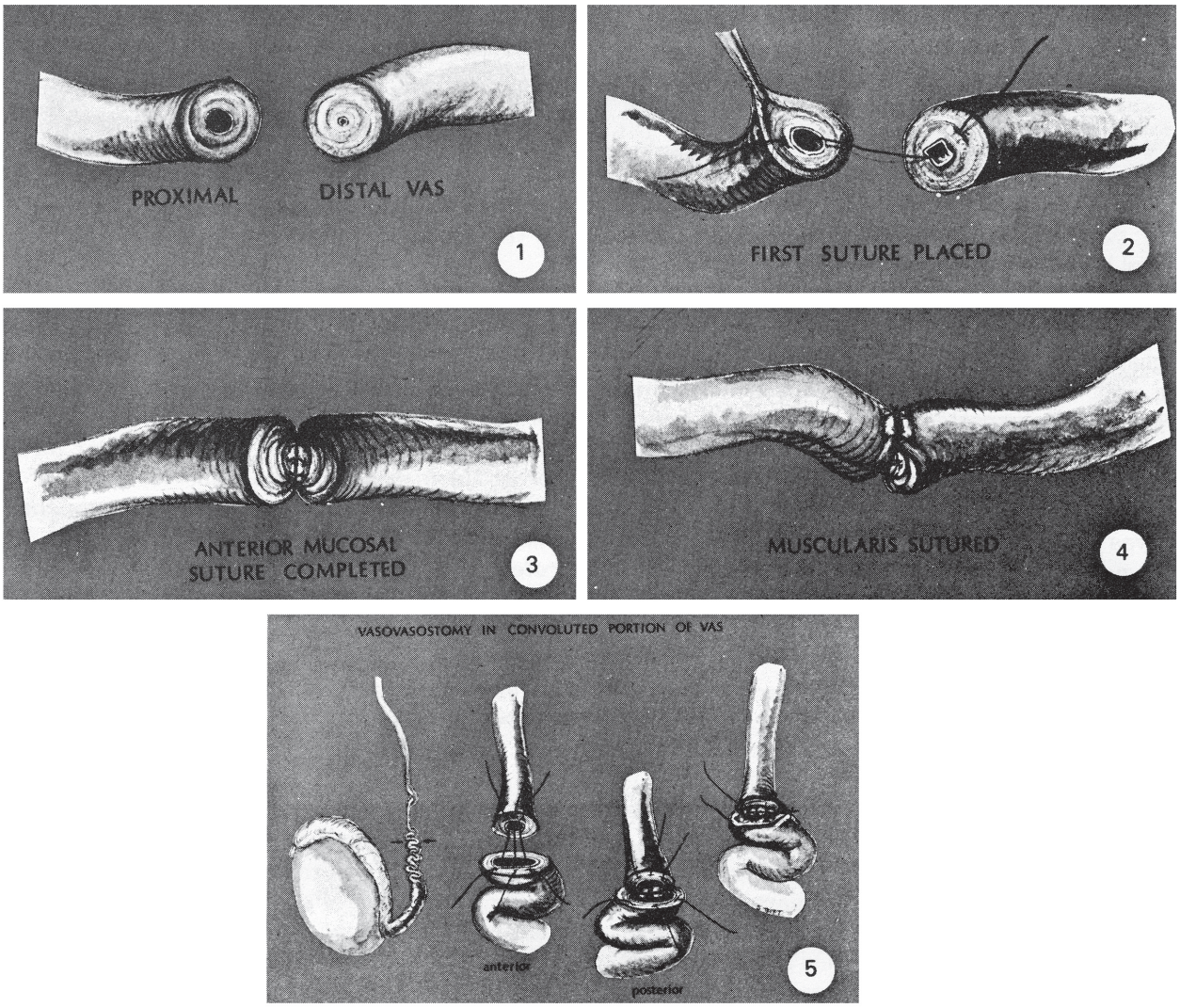
Splints are clearly inapplicable when the convoluted portion is involved. But even in the straight portion, splints create problems which are avoided with the microscopic technique. The mucosa is a separate, delicate layer of pseudostratified columnar epithelium that is easily stripped and damaged by the insertion of a splint unless careful microscopic control is observed. Indeed, this is often what happens with conventional techniques of vas anastomosis when a nylon, wire, or polyethylene splint is inserted into the lumen. The splint is often placed within a false intramural channel in the midst of the musculature. Even if the splint is accurately placed in the lumen, the necessary pulling through in two separate locations causes damage and sperm leakage which eventually result in a stricture. In addition, the splint does not bring about accurate mucosa-to-mucosa approximation. High-power microscopic visualization demonstrates these dangers clearly.

OPERATIVE TECHNIQUE (FIGS. 1 TO 5)

Finely polished jeweler's forceps are used to dilate the lumen of the distal vas temporarily to make the anastomosis easier. A separate mucosal anastomosis is performed with six to eight interrupted stitches of 9-0 or 10-0 nylon. In view of the extremely small lumen size and the delicacy of the tissue, heavier suture creates a risk of sperm leakage, granuloma formation, and obstruction. Under ×25 to ×40 magnification, nylon suture heavier than 9-0 appears much too large. Indeed, 6-0 suture is thicker in diameter than the inner diameter of the vas, and would be obstructive within the lumen.

If, instead of doing a separate mucosal layer, one uses full-thickness stitches that traverse muscularis and mucosa, so much muscle is pulled into the anastomosis that the next stitch is impossible to place accurately. In addition, with a through-and-through stitch, one is less apt to obtain a watertight anastomosis, and sperm leakage and granuloma may occur. Finally, bunching of tissue by such huge bites can produce further obstruction of the lumen and less-perfect mucosa approximation, resulting in stricture. *When the mucosal layer is sutured separately, it allows the shrunken mucosa of the distal side to be matched with the dilated mucosa of the normal side.* The small lumen dilates to the diameter of the larger lumen on the previously obstructed side.

After the mucosal anastomosis has been performed, a separate muscularis layer is closed also with 9-0 nylon sutures. This ensures a watertight closure and ensures adequate approximation of muscularis. This latter point is also of importance since normal conduction of peristalsis is essential for propulsion of the sperm from the epididymis into the ejaculate at the time of intercourse.



FIGS. 1 TO 5. The steps in performing the microscopic two-layer anastomosis of the vas deferens with 9-0 or 10-0 nylon sutures.

If the original vasectomy included the convoluted portion of the vas, the technique is no different. I am totally opposed to attempts at vasoepididymostomy, since this procedure essentially eliminates chances for successful fertility with the microscopic technique. One cut in the epididymis (which is just one long coiled tube) means that the only chance for fertility is an improbable fistula. Vasoepididymostomy makes a proper subsequent vasovasostomy futile.

SERIAL OBSERVATION OF SPERM COUNTS IN 28 CONSECUTIVE CASES

In an effort to determine the effect of obstruction and relief of obstruction on spermatogenesis, semen from the proximal cut end of the vas deferens was examined at the time of vasovasostomy, and the ejaculate was examined at monthly intervals afterward. Only the patients operated upon 3 months or more prior to this presentation are included. The interval between vasectomy and vasovasostomy was correlated with ultimate sperm counts. A normal sperm count was considered to be greater than 20 million/ml and to have greater than 60% motility with good action and greater than 70% normal forms. Most normal counts were actually above 30 million/ml. The patient's original sperm count prior to vasectomy was, of course, unknown, but would probably be of considerable interest in any such future study.

The results are summarized in Table 1. Of 22 men whose vasectomies had been performed less than 10 years before vasovasostomy, *all had sperm in the ejaculate after vasovasostomy* and 90% had

TABLE 1. *Relation of Sperm Counts after Microscopic Vasovasostomy to Time after Initial Vasectomy*[a]

	Years after vasectomy					
	0–2	3–4	5–6	7–8	9	>10
No. with normal sperm count[b] (>20 million/ml, 60% motile)	4	5	9	2	1	3
No. with poor sperm count (<20 million/ml, 60% motile)	0	1 (oligospermic)	1 (oligospermic)	0	0	4 (no sperm) 1 (oligospermic)

[a]Of 22 patients with vasectomy not greater than 10 years ago, 100% (22) had sperm in ejaculate, 90% (20) had normal sperm count, 5% (1) had partial obstruction causing oligospermia, and 5% (1) had primary testicular failure causing oligospermia.
[b]Most had a count greater than 30 million/ml.

normal sperm counts. Of the two who were oligospermic, a stricture was clearly the cause in one and was corrected with reoperation. Thus 95% could be expected to have a normal sperm count.

It should be emphasized that, at the time of vasovasostomy, the effluent from the proximal cut vas contains mostly dead sperm. There were often no motile sperm at all, but even when motile sperm were present, nonmotile sperm vastly predominated. At 1 month and 2 months postoperatively, sperm counts were still very low, generally between "a few dead sperm" and 5 million sperm/ml, although an occasional patient had high counts immediately. By 3 months in most cases sperm counts were normal, with motility greater than 60%. Counts (and often motility also) continued to increase over the next 5 months.

Of the eight patients whose vasectomies had been performed more than 10 years previously, only 50% had formed sperm at 3 months after vasovasostomy, and only 37% had a normal count. With longer follow-up it is possible that sperm counts will improve in more of these cases. Subsequent follow-up since this presentation indicates that 50% of these patients develop normal sperm counts. All patients who failed to have formed sperm in the ejaculate 3 months postoperatively had no sperm originally in the fluid coming from the proximal vas at the time of vasovasostomy. *All patients with formed sperm in the fluid coming from the proximal vas at the time of vasovasostomy had sperm in the ejaculate postoperatively, and 95% of these had normal sperm counts by 3 to 5 months. Those patients without formed sperm in the ejaculate did have sperm with detached heads and tails, spermatids, or bizarre forms* (identified with assurance by electron microscopy) *that could have easily been misinterpreted as "white blood cells" or debris, and indicated patency of the anastomosis even in these cases.*[28]

Thus, it appears from careful follow-up of sperm counts in these patients that this technique of anastomosis is virtually 100% successful in restoring continuity to the vas deferens, and at least 95% successful in providing a stricture-free communication. 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 nonmotile sperm seen at the time of vasovasostomy most probably had died of "old age," and the paucity of motile sperm indicates a very 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 vasovasostomies performed less than 10 years after vasectomy, this loss was not great enough to prevent normal sperm counts from being achieved 95% of the time.

TABLE 2. *Early Results with Reoperation Using Two-Layer Microscopic Technique*

Case	Time from original vasectomy	Time from original reversal attempt	Sperm count after original reversal	Sperm count at time of reversal	Subsequent sperm count
	yr	*yr*			
1	2	1	"Many motile sperm"	0	25 million/ml at 3 mo, 70% motile
2	17	3½	50 million/ml, 10% motile	4 million/ml, 5% motile	108 million/ml, 70% motile
3	4	2	0	0	45 million/ml, 70% motile
4	6	1	19 million/ml at 3 wk, 0 at 8 wk	0	65 million/ml, 80% motile
5	5	2	3/HPF[a]	0	40 million/ml, 50% motile

[a]HPF, High-power field.

RESULTS OF MICROSCOPIC VASOVASOSTOMY AFTER CONVENTIONAL VASOVASOSTOMY FAILED

Ten additional patients were operated upon who had previously had vasovasostomies performed that had failed with conventional techniques by excellent and well-known urologists elsewhere. In most of these cases, an initial presence of sperm had indicated success, but the sperm later disappeared or decreased. In most instances, the patients' urologists attributed the drop in count to "sperm antibodies." Nine now have normal sperm counts and one has sperm present, after reoperation with the two-layer microscopic technique. Table 2 summarizes the findings in the first five of these ten patients.

In all patients, a scarred lumen was demonstrated histologically at the site of the previous anastomosis. In all instances a sperm granuloma was noted at the site of scarring. This appeared to indicate that extensive sperm leakage had occurred at the site of the previous anastomosis. In fact, it appears that the sperm granuloma, rather than an intact lumen, was the vehicle for transfer of sperm to the other side and thus into the ejaculate after the first operation.

We know that recanalization can occur all too frequently after a vasectomy if sperm leakage and granuloma formation are allowed. In rare instances enough motile sperm get through to result in pregnancy. However, in the great majority of recanalization instances, only a few sperm are seen in the ejaculate because it is *not* a normal channel. For vasovasostomy this sort of result is not adequate.

It appears that when reconnection of the vas is requested by the patient, a direct mucosa-to-mucosa approximation is necessary to prevent leakage and secondary stricture formation. Once one is committed to mucosa-to-mucosa anastomosis, it is then important not to use suture so large that it is relatively obstructive to the 0.333-mm diameter lumen of the distal vas. Use of anything larger than 9-0 nylon invites that risk. A truly perfect microscopic surgical technique is necessary to ensure a proper reconnection.

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