

# MICROSURGICAL VASOEPIDIDYMOSTOMY: A PROSPECTIVE RANDOMIZED STUDY OF 3 INTUSSUSCEPTION TECHNIQUES IN RATS

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## ABSTRACT

**Purpose:** Vasoepididymostomy is a technically challenging but cost-effective treatment for obstructive azoospermia. We evaluated the outcomes of 3 intussusception vasoepididymostomy techniques, namely 3 suture triangulation, 2 suture transverse and a new 2 suture longitudinal technique.

**Materials and Methods:** Male Wistar rats were randomized into 4 experimental and 1 control groups. After 3 weeks of vasal obstruction bilateral vasoepididymostomy was performed. In group I, 3 sutures were placed in triangular fashion. In group II, 2 sutures were placed perpendicular to the tubule. In group III, 2 sutures were placed longitudinal to the tubule. The tubules were then opened in the direction of the needles and anastomosed to the vasa. After 5 months patency was evaluated in blinded fashion.

**Results:** The functional patency rate (presence of motile sperm in the vas) was 64%, 64% and 93% in groups I to III, respectively ( $p < 0.001$ ). As evaluated by methylene blue retrograde vasography toward the epididymis, the mechanical patency rate was similar for the 3 techniques, that is 86%, 86% and 93% in groups I to III, respectively. The sperm granuloma rate was significantly lower in group III (36%, 21% and 0% in groups I to III, respectively,  $p < 0.001$ ).

**Conclusions:** Transverse 2 suture vasoepididymostomy has a patency rate similar to that of the 3 suture technique. Our new 2 suture longitudinal technique, which allows a larger opening in the epididymal tubule for anastomosis, is superior to the 2 and 3 suture techniques with respect to the patency and sperm granuloma rates.

**KEY WORDS:** testis; rats, Wistar; oligospermia; microsurgery; epididymis

Infertility is recognized as a profound and widespread medical and social problem, affecting an estimated 20% of all couples in the United States. Of male infertility cases 10% to 15% are caused by reproductive tract obstruction. Reconstruction of the male reproductive tract using microsurgical vasovasostomy or vasoepididymostomy has been demonstrated to be a more cost-effective treatment option than assisted reproductive technology such as in vitro fertilization or intracytoplasmic sperm injection.<sup>1,2</sup>

Vasoepididymostomy is considered the most technically challenging operation in male reproductive microsurgery. Historically the earliest vasoepididymostomy was attempted in 1903 by creation of a fistulous communication between the multiply incised epididymal tubules and the opened lumen of the vas deferens.<sup>3</sup> In 1918 Lespinasse first attempted precise epididymal tubule anastomosis to the vasal lumen.<sup>4</sup> However, before the introduction of microsurgical technique the success rate of vasoepididymostomy varied but was generally poor in terms of patency and subsequent pregnancy. With the introduction of optical enhancement microsurgical end-to-end single tubule anastomosis was introduced in 1978 by Silber,<sup>5</sup> and end-to-side anastomosis was introduced by Wagenknecht et al<sup>6</sup> and popularized by Thomas<sup>7</sup> using 6 to 8 microsutures. The patency rate of vasoepididymostomy was 50% to 85%.<sup>8,9</sup> However, extreme precision and exquisite microsurgical skills are required to anastomose the delicate epididymal tubule, which has a diameter of 200 to 400  $\mu\text{m}$ .

to the vasal lumen. Hence, the outcomes of these techniques highly depended on surgeon experience.

In an attempt to simplify microsurgical technique we introduced the use of double arm microsutures.<sup>10,11</sup> Stefanovic et al described tubular intussusception for vasoepididymostomy in rats using a single mucosal suture.<sup>12</sup> Berger applied this technique in humans using 3 double armed microsutures placed to an epididymal tubule in triangular fashion.<sup>13</sup> When the tubule is opened, the 3 double armed sutures are placed inside out through the vasal mucosa, forming a 6-point anchor for anastomosis and allowing the epididymal tubule to intussuscept into the vasal lumen. Preliminary results of this 3 suture triangulation intussusception technique were superior to those of previous techniques of vasoepididymostomy. Subsequently Marmar modified this technique by using only 2 microsutures placed perpendicular to the epididymal tubule for anastomosis.<sup>14</sup> While this last modification further simplified the technique of vasoepididymostomy and has gained increasing popularity among microsurgeons, to our knowledge no studies have compared the patency rate of the 2 versus 3 suture intussusception techniques.

The objectives of the current study were 2-fold. Using a rat model we compared postoperative outcomes of vasoepididymostomy performed using the Berger 3 suture triangulation intussusception technique<sup>13</sup> versus the 2 suture transverse technique described by Marmar.<sup>14</sup> In addition, we describe a new 2 suture longitudinal vasoepididymostomy technique and compared its surgical outcomes with those of the 2 and 3 suture techniques.

## MATERIALS AND METHODS

**Animal model.** Institutional review board approval of this study was obtained before performing the experiments. All

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animals were handled in accordance with United States Department of Agriculture standards. A total of 28, 6-week old male Wistar rats (Charles River Laboratories, Wilmington, Massachusetts) weighing 250 gm. were randomized into 4 study groups of 7 each, namely group I—3 suture triangulation intussusception vasoepididymostomy, group II—2 suture transverse intussusception vasoepididymostomy, group III—new 2 suture longitudinal vasoepididymostomy and group IV—controls. Anesthesia for all surgical procedures was induced using intraperitoneal injection of xylazine (10 mg./kg.) mixed with ketamine chloride (100 mg./kg.).

**Creation of epididymal obstruction.** Epididymal obstruction was created bilaterally in all animals using modified bilateral vasectomy. With the rat under anesthesia the abdominal cavity was opened to deliver the reproductive organs. The vasa deferentia and epididymides were identified and dissected to expose the vasoepididymal junction, where a 3 mm. titanium Auto Suture Premium Surgiclip S-9.0' (U. S. Surgical, Norwalk, Connecticut) was placed. After the procedure the testes were placed back into the scrotum and the abdomen was closed with 3-zero Ethicon U213 (Ethicon, Inc., Somerville, New Jersey) absorbable sutures.

**Surgical procedure.** Three weeks after the induction of obstruction by modified vasectomy vasoepididymostomy was performed bilaterally in groups I to III under an OPMI1 (Carl Zeiss, Oberkochen, Germany) operating microscope at 15 to 25 $\times$  magnification. The time of each anastomosis was determined by dividing the total operating time per animal from skin incision to the end of wound closure by 2. In the control group sham operation was performed by dissecting and exposing the reproductive organs without forming an anastomosis.

When the reproductive organs were exposed, the epididymides were mobilized. A 2 mm. buttonhole opening was made in the epididymal tunic in the cauda epididymis, where a subjacent dilated epididymal tubule was selected for anastomosis. The vas deferens was then secured to the epididymal tunic proximal to the selected tubule using 2 interrupted 9-zero Sharpoint JA-2565 (Surgical Specialties Corp., Reading, Pennsylvania) nylon sutures.

**Three Suture Triangulation Intussusception Vasoepididymostomy in Group I:** Using a No. 151 Devon (Devon Industrial, Inc., Chicopee, Massachusetts) micromarking pen 6 microdots were placed on the cut surface of the vas deferens at the 1, 3, 5, 7, 9 and 11 o'clock positions, indicating the planned exit points of the sutures for the anastomosis.<sup>15</sup> Three monofilament double armed 10-zero nylon sutures using 70  $\mu$ m. tapered Sharpoint AK-0105 (Surgical Specialties Corp.) micro-needles were placed in a triangular configuration in the select epididymal tubule (fig. 1). To prevent collapse of the epididymal tubule due to fluid leakage from the needle holes, which would make placement of subsequent needles and proper opening of the tubule difficult, all needles were placed in the epididymal tubule without pulling them through. The epididymal tubule was then gently opened with microscissors transverse or perpendicular to the epididymal tubule. The epididymal fluid was examined microscopically at 400 $\times$  magnification by phase contrast microscopy to confirm the presence of sperm. Each needle from the double armed sutures was then pulled through and passed through the lumen of the vas in inside out fashion, exiting at the previously placed microdots and taking no more than a third of the thickness of the muscularis. The sutures were tied sequentially, resulting in invagination or intussusception of the epididymal tubule into the vasal lumen (fig. 2). Second layer closure was performed using 6 to 8 interrupted 9-zero nylon sutures to approximate the sheath of the vas deferens to the epididymal tunic.

**Two Suture Transverse Intussusception Vasoepididymostomy in Group II:** With a setup similar to that of the 3 suture technique described 2 double armed 10-zero nylon sutures

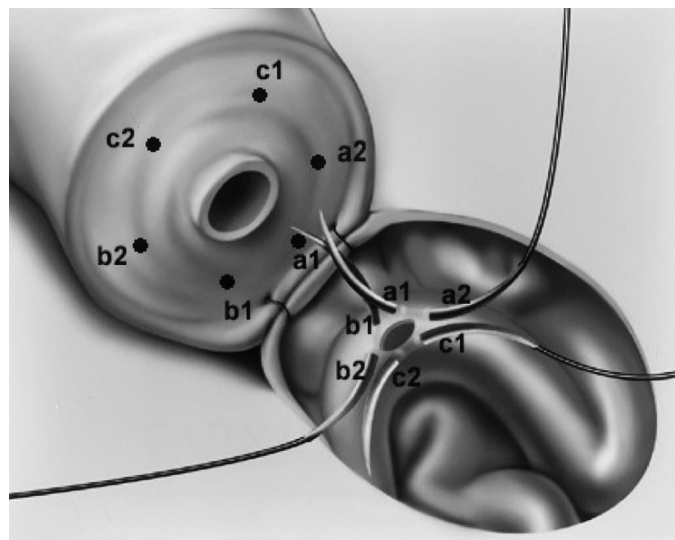


FIG. 1. In group I for triangulation 3 suture intussusception vasoepididymostomy 3 monofilament double armed 10-zero nylon sutures were placed in triangular configuration in epididymal tubule and opening within triangle was made in epididymal tubule.

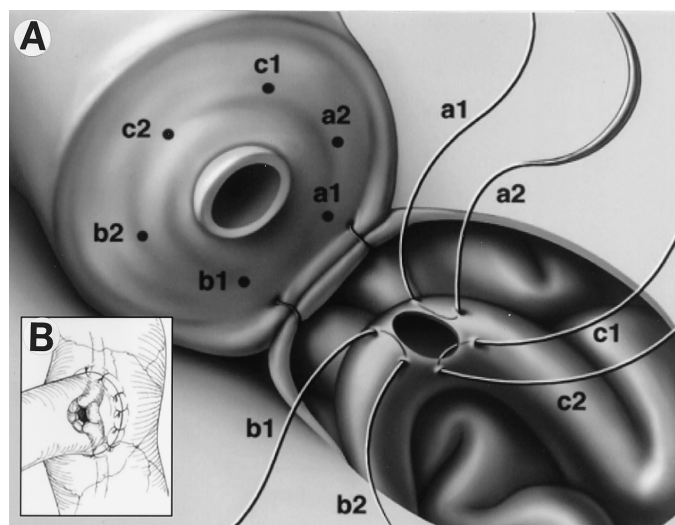


FIG. 2. Triangulation 3 suture intussusception vasoepididymostomy in group I. A, sequential suture placement in inside out fashion through vas lumen at 6 evenly distributed points. B, sutures were tied sequentially, resulting in intussusception of epididymal tubule into vasal lumen.

were placed perpendicular to the select epididymal tubule (fig. 3). To prevent tubule collapse the needles were not pulled through the epididymal tubule until the tubular opening was made. The epididymal tubule was opened with microscissors transversely between the 2 needles. After confirming sperm in the vasal fluid the sutures were passed through the vasal lumen in inside out fashion, exiting at 4 points, and tied sequentially, resulting in intussusception of the epididymal tubule into the vasal lumen. Second layer closure using 9-zero nylon was performed in similar fashion, as described.

**New 2-Suture Longitudinal Intussusception Vasoepididymostomy in Group III:** In this technique 2 double armed 10-zero nylon sutures were placed longitudinally along the select epididymal tubule (fig. 4). Again to prevent tubule collapse the needles were not pulled through the epididymal tubule until the tubular opening was made. The epididymal tubule was opened with microscissors by making a longitudinal incision in the tubule between the 2 sutures. After

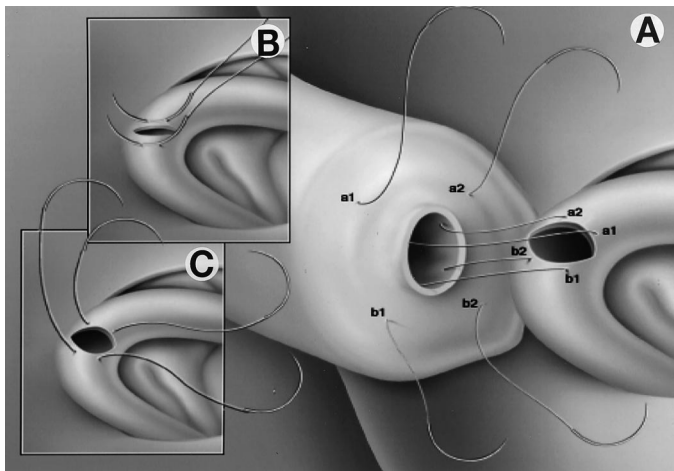


FIG. 3. Transverse 2 suture intussusception vasoepididymostomy in group II. *A*, 2 monofilament double armed 10-zero nylon sutures were placed in perpendicular fashion in unopened epididymal tubule. *B*, epididymal tubule was then opened transversely between 2 sutures. *C*, sutures were placed sequentially in inside out fashion through vas lumen at 4 evenly distributed points.

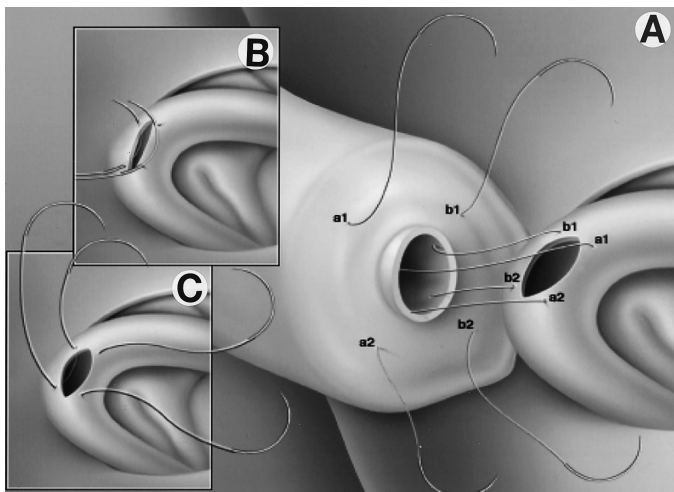


FIG. 4. New 2 suture longitudinal intussusception vasoepididymostomy in group III. *A*, 2 monofilament double armed 10-zero nylon sutures were placed longitudinally in unopened epididymal tubule. *B*, epididymal tubule was opened longitudinally between 2 sutures. *C*, suture was placed sequentially in inside out fashion through vas lumen at 4 evenly distributed points.

confirming sperm in the vaginal fluid the sutures were passed through the vaginal lumen in inside out fashion, exiting at 4 points, and tied sequentially, resulting in intussusception of the epididymal tubule into the vaginal lumen. Second layer closure using 9-zero nylon was performed in similar fashion, as described.

**Patency studies.** At 5 months postoperatively all animals were anesthetized. All evaluations were performed in blinded fashion. The reproductive organs were exposed to evaluate the anastomosis for any complications. Patency was defined

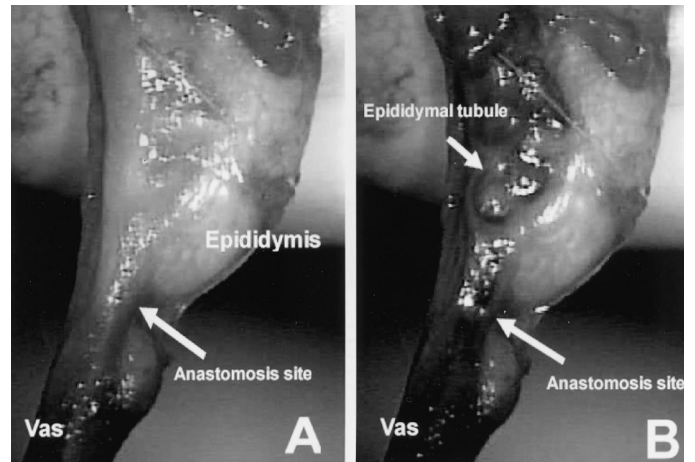


FIG. 5. Retrograde methylene blue vasogram shows mechanical patency of anastomosis. Testicular end of vas was catheterized with 24 gauge angiocatheter sheath. Dye flowed freely through anastomosis and filled epididymal tubules in retrograde fashion. No evidence of dye extravasation was noted.

using 2 criteria. Functional patency was achieved when motile sperm were found in the vaginal fluid microscopically at 400 $\times$  magnification by phase contrast microscopy and mechanical patency was achieved when retrograde methylene blue vasogram using a 24 gauge angiocatheter sheath to inject dye from the vas toward the epididymis demonstrated dye passage through the anastomosis into the epididymal tubules (fig. 5).

**Statistical methods.** All data in each group are expressed per the number of anastomoses (14 per group). All percent data were converted from binomial to normal distribution using square root arcsine transformation before analysis. Chi-square contingency table analysis was performed to compare the proportion of patent anastomoses for the 3 surgical techniques. Modified Turkey's wholly significant difference test was used for multiple sample comparisons with significance considered at  $p = 0.05$ .

## RESULTS

**Patency study.** All animals survived vasectomy and vasoepididymostomy. The table lists the operative time per anastomosis, mechanical and functional patency rates, and incidence of sperm granuloma. The overall mechanical patency rate was comparable in surgical groups I to III (86%, 86% and 93%, respectively, fig. 6). When comparing the functional patency rate, as indicated by the presence of motile sperm in the vaginal fluid, the results of the 3 and 2 suture transverse techniques were also comparable (64% and 67% in groups I and II, respectively). The functional patency rate of the 2 suture longitudinal technique in group III of 92% was significantly higher than that of the other 2 techniques ( $p < 0.001$ ).

**Operative time.** Mean operative time for the 3 suture technique (group I) was 50 minutes, which was significantly longer than that of the 2 suture techniques ( $p < 0.05$ , fig. 7). There was no significant difference in operative time for the

### Postoperative outcomes of the 3 vasoepididymostomy techniques

	Group I	Group II	Group III	Group IV	p Value
No. anastomoses	14	14	14	0	—
Operating time/anastomosis (mins.)	50	42.6	38.2	15	<0.05
No. mechanical patency (%)	12 (86)	12 (86)	13 (93)	0	Not significant
No. functional patency (%)	9 (64)	9 (64)	13 (93)	0	<0.001
No. anastomosis granuloma (%)	5 (36)	3 (21)	0	0	<0.001
No. testicular atrophy complications (%)	2 (14)	2 (14)	2 (14)	1 (7)	Not significant

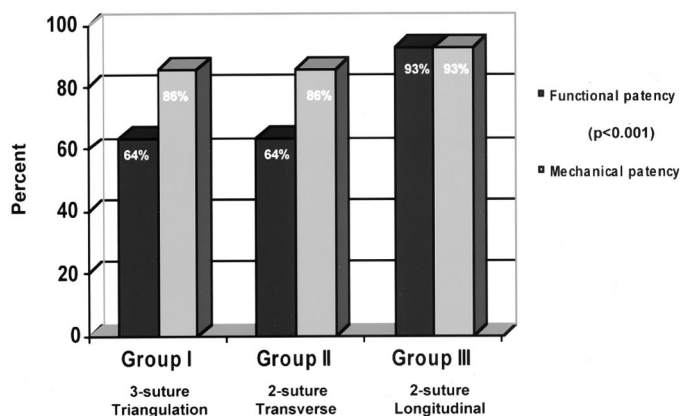


FIG. 6. Functional and mechanical patency rates of 3 vasoepididymostomy techniques.

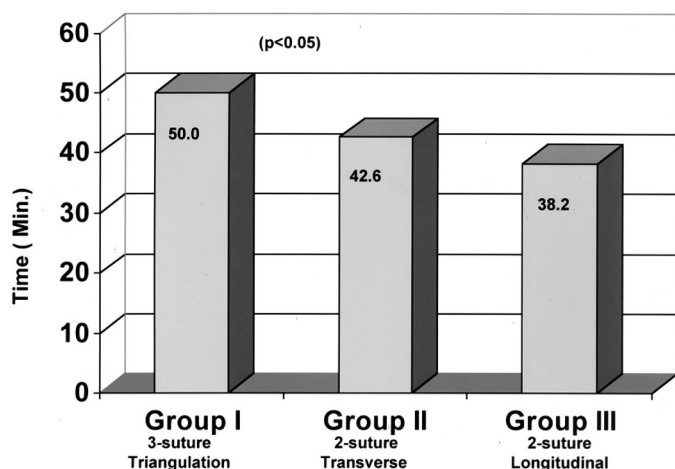


FIG. 7. Mean operative time of 3 vasoepididymostomy techniques.

2 suture transverse and 2 suture longitudinal techniques in groups II and III (42.6 and 38.2 minutes, respectively).

**Complications.** The rate of postoperative sperm granuloma at the anastomosis sites was significantly different among surgical groups I to III (36%, 21% and 0%, respectively,  $p < 0.001$ , fig. 8). The only other complication in the study was testicular atrophy (20% to 40% lower volume than that of the contralateral testis) after vasoepididymostomy, which developed in 2 of 14 cases (14%) per group.

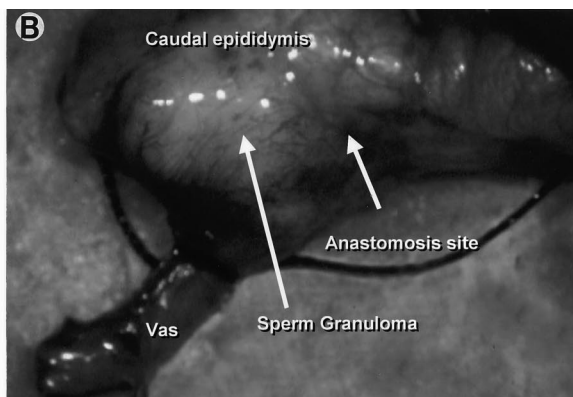
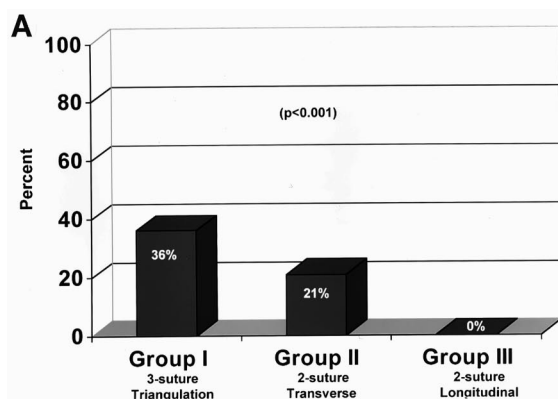


FIG. 8. A, anastomosis granuloma rate of 3 vasoepididymostomy techniques. B, large sperm granuloma at vasoepididymal anastomotic site.

## DISCUSSION

Microsurgical vasoepididymostomy is the procedure of choice for treating male infertility due to epididymal obstruction. In rats Stefanovic et al originally described tubular intussusception for vasoepididymostomy using only a single mucosal suture.<sup>12</sup> This concept was applied clinically by Berger using 3 double armed sutures placed in triangular fashion in a distended epididymal tubule before opening it.<sup>12</sup> This maneuver greatly improves the accuracy of suture placement compared with end-to-end and end-to-side techniques, in which sutures are placed in a collapsed tubule after the opening is made. Furthermore, the Berger technique results in intussusception of the epididymal tubule into the vasal lumen, creating a more leakproof anastomosis. The technical challenges of this 3 suture technique led to the introduction of the 2 suture modification of Marmar.<sup>14</sup> While preliminary results of this simplified 2 suture technique were encouraging, a valid comparison of 2 and 3 suture intussusception vasoepididymostomy was lacking.

In this study using a rat model we compared postoperative outcomes of 2 versus 3 suture intussusception vasoepididymostomy. Our results indicate that the success rate of the 2 suture transverse technique is comparable to that of the 3 suture technique. In addition, operative time per anastomosis is significantly shorter for the 2 suture technique. This information is important to help surgeons decide which technique to use. The cost of the double armed 10-zero microsuture is approximately \$30 each. Decreasing the number of sutures used per anastomosis without compromising the outcome is certainly an advantage. More importantly the 2 suture procedure requires a shorter time to complete. Clinically it translates into short operative and anesthesia times. Hence, it is potentially a safer and more cost-effective surgical option. Furthermore, the 2 suture technique is easier to acquire and perform. The 3 suture technique requires the organization of 6 needles compared with only 4 with the 2 suture technique. Therefore, technical errors are less likely in the 2 suture technique.

Furthermore, we describe placement of all needles during vasoepididymostomy in the epididymal tubule without pulling them through. This maneuver prevents tubule collapse due to fluid leakage from the needle holes since the 10-zero microsuture is smaller in diameter than the needle, making it easier to place subsequent needles and make a proper opening in the tubule.

Another objective of our current study was to describe a new 2 suture technique, in which we modified the way the epididymal tubule is opened by incising it longitudinally. Our results indicate that this new technique is superior to the 3 suture triangulation and 2 suture transverse techniques

with respect to patency and the complication rate. The advantage of this method is that a relatively larger epididymal tubular opening can be made, in contrast to the 3 suture triangulation and 2 suture transverse methods, in which the epididymal tubules are opened transversely or perpendicularly. The size of the opening in this situation is limited by tubule width (fig. 9). The maximal diameter of the opening is achieved by cutting into a depth equal to half the size of the tubule (fig. 10, A). Beyond half the size of the tubule the back wall of the tubule is weakened, leading to fluid leakage and less secure anastomosis (fig. 10, B).

We believe that the larger tubular opening achieved with our new 2 suture longitudinal technique is significant in contributing to the higher patency rate that we observed in this study. When performing this new 2 suture technique, longitudinal placement of the microneedles may initially seem awkward. However, with practice we think that this technique is as easy to perform as the 2 suture transverse technique. As our study shows, operative time per anastomosis is not significantly different from that of the 2 suture transverse technique.

When measuring postoperative outcomes in this study, patency was assessed by the presence of motile sperm distal to the anastomosis (functional patency) and by retrograde vasogram with injection of methylene blue dye across the anastomosis (mechanical patency). Although it was not as quantitative as semen analysis, which is difficult to perform in an animal model and subject to a high level of variability, objective evaluation of patency postoperatively can be achieved by these 2 methods. Our results show that the mechanical patency rate on retrograde methylene blue vasography was similar for the 3 microsurgical techniques compared (86% to 93%). A similar patency rate was achieved in our previous study of the 3 suture triangulation intussusception technique in a rat model.<sup>16</sup>

However, the functional patency rate, as determined by motile sperm in the vas deferens, was significantly higher for our new 2 suture longitudinal technique. Clearly with the transverse 2 and 3 suture intussusception techniques some anastomoses were patent on vasography but sperm were not able to go through to the vas. It may have been because the anastomosis was too narrow for the viscous epididymal outflow. Hence, with our new longitudinal technique, which allows a larger opening in the epididymal tubule in the anastomosis, the observed functional patency rate was significantly higher.

Alternatively the discrepancy in mechanical and functional patency rates can be explained by leakage of sperm containing epididymal fluid from the anastomotic site, where sperm granuloma formed (fig. 8, B). This latter observation highlights the importance of a leakproof anastomosis, which is

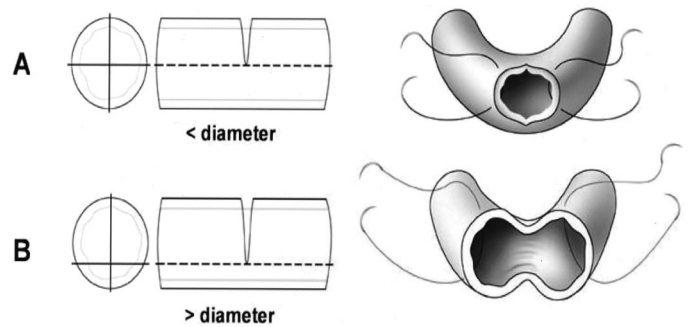


FIG. 10. Limitation of luminal size of transverse tubular incision. A, maximal diameter of tubular opening is achieved by transverse incision when incision depth is half tubule size. B, when incised beyond half tubule size, back wall is weakened, resulting in fluid leakage and less secure anastomosis.

best achieved by successful tubular intussusception and second layer closure of the vasal sheath to the epididymal tunic. Further studies are required to evaluate if a more secure second layer closure or perhaps an additional third layer closure between the vasal sheath and tunic would decrease the granuloma rate and increase the functional patency rate.

Testicular atrophy was observed at a similar frequency (14%) in all 3 surgical groups. A similar complication rate was noted in our previous study.<sup>16</sup> In humans postoperative testicular atrophy is not as common. This complication may be due to ischemic injury to the fine testicular blood vessels during dissection. Alternatively since during vasoepididymostomy the testes were dissected free from the scrotal attachment, testicular torsion may be more likely to occur, leading to ischemic injury. When establishing our animal model, we attempted to simulate the clinical situation in humans by iatrogenically inducing epididymal obstruction in all animals through vasal obstruction. The resulting dilatation of the epididymal tubule provides a suitable model of epididymal obstruction.<sup>16</sup>

A limitation of our study is that we did not determine the patency rate at earlier or later postoperative periods. In this study we chose the time point of 5 months postoperatively based on experience in similar studies that anastomotic healing is complete after 12 weeks of recovery, as indicated by a lower rate of granuloma formation and tissue fibrosis on histological sections of the anastomotic sites.<sup>12, 17, 18</sup> Whether the patency rates at earlier postoperative and more importantly at longer periods are similar remains to be determined.

Another limitation of our study is that the animal model may not replicate the clinical setting in humans. The wall of the vas deferens in rats is softer than in the human vas. Complemented by the fact that the vasal lumina are relatively smaller than in humans the precise placement of sutures in the rat vasal lumen is more difficult to achieve. In fact, at the age of the animals (6 to 8 weeks at a weight of approximately 200 gm.) when we performed vasoepididymostomy, the epididymal tubules were significantly smaller than those in humans. As shown in animal and clinical studies, the success rate of vasoepididymostomy generally decreases at more proximal levels when tubular size is progressively smaller and, hence, it is technically more difficult to form a precise anastomosis.<sup>17, 18</sup> To overcome this discrepancy we chose to perform the anastomosis only at the caudal epididymis, where tubular diameter is greatest. This approach eliminated the confounding effects of luminal diameter differences on anastomotic success and allowed more valid comparison of the effects of the 3 suture placement methods on surgical outcomes.

Epididymal tubule diameter is an additional factor that may influence surgeons when choosing which techniques to

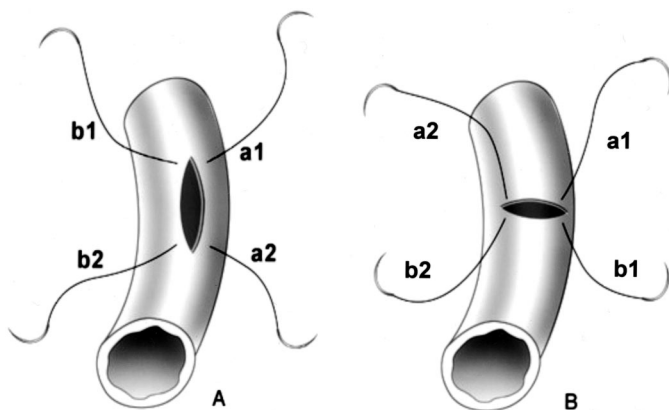


FIG. 9. Longitudinal cut on epididymal tubule provides larger opening than transverse cut. A, longitudinal cut. B, transverse cut.

use for vasoepididymostomy. Clinically when treating more proximal epididymal obstruction or obstruction at the efferent ductule level, a 3 suture technique is difficult to perform on such small tubules. In this setting we believe that 2 suture techniques are most appropriate, as supported by another recent study.<sup>19</sup>

The results of our animal model demonstrate that 2 suture techniques are at least comparable to 3 suture techniques and potentially require a shorter time to perform. Our preliminary clinical results using the various intussusception techniques are encouraging and consistent with our findings in this study.<sup>19,20</sup> Further clinical investigations are required to evaluate fully the different techniques with respect to short-term and long-term patency, pregnancy and complication rates in humans.

#### CONCLUSIONS

Since its recent description, intussusception vasoepididymostomy using the 2 or 3 suture technique has been gaining in popularity. Using a rat model we noted that the outcomes of microsurgical vasoepididymostomy using previously described 2 or 3 suture techniques were comparable with respect to the patency and complication rates. Operative time for the 2 suture technique appears to be shorter. We also describe placement of all microneedles before pulling the suture through and opening the epididymal tubule, preventing premature collapse of the tubule due to fluid leakage through the needle holes. Furthermore, we describe a new 2 suture technique in which the epididymal tubule is opened longitudinally, forming a larger opening for anastomosis. Preliminary results 5 months postoperatively indicate a superior patency rate and a lower rate of granuloma formation. Further investigations are required to determine if similar results can be achieved in humans.

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