Comparison of Different Autogenous Graft Materials for Reconstruction of Large Segment Vas Deferens Defect: Experimental Study in Rat

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Purpose: Vasectomy is one of the most common urological operations performed, and provides permanent contraception. Many vasectomized men ultimately seek vasectomy reversal because of unforeseen changes in lifestyle. Vasovasostomy has varying rates of success. In this study, we utilize vas deferens (VD), artery, and vein grafts to reconstruct 30% and 50% defects of the total vas deferens length.

Materials and Methods: Forty two male Wistar rats were divided into three groups as VD graft, carotid artery and external jugular vein transplantations. Each group was equally divided into 2 different subgroups according to the length of transplant material as 1.0 cm (n = 7) and 1.5 cm (n = 7). To evaluate whether these materials may be used for long segment vas deferens reconstruction, the patency rate, partial or total graft occlusion, and histologic examination of all specimens were examined.

Results: No patency was found in any of the grafts and many of them suffered destructive changes in anatomic structure. Sperm granulomas were determined around the testicular side anastomosis due to accumulated semen fluid which was in our belief, a result of aperistaltic zone caused by the grafts

Conclusion: When the poor results obtained in our study are put into perspective, vasoepididymostomy is the only treatment method to date for reconstruction of large segment vas deferens defects.

Keywords: vas deferens; vasovasostomy; rats; vasectomy; transplantation; graft survival; animals.

INTRODUCTION

The safest and most cost-effective treatment option for the reversal of the vasectomies remains micro-surgical reconstruction, which also allows natural conception.[1] The success rate of this procedure depends on a myriad of factors, including the performing surgeon's skill, presence of antisperm antibodies, high intravasal and epididymal pressure that develops after vasectomy, and the obstruction interval between the vasectomy and reversal.[2,3] Technical failure of human vas deferens (VD) reconstruction mainly occurs several weeks to months after surgery, usually as a result of stricture of the anastomosis. The narrowing and obliteration of the lumen takes place due to granuloma formation at the site of anastomosis, and traction on or devascularization of the VD wall, which eventually leads to sperm leakage after the reversal. Prosthetic stents have been used to simplify the procedure and prevent this sperm leakage. Autodilating stents have been put forth as a possible solution for preventing the secondary stricture of the anastomosis. Also, by preventing sperm extravasation, there is less perivasal inflammation, reducing secondary stricture at the site of the anastomosis.[4,5]

Wald and colleagues evaluated a biodegradable graft for reconstruction of rat vasa deferentia with long obstructed or missing segments with or without some medical therapy. They found that potential role for biodegradable grafts in the reconstruction of VD with long obstructed segments.[4,6]

Rothman and colleagues[7] carried out a randomized clinical trial comparing the usual 2-layer microsurgical vasectomy reversal against a procedure using the new stent. As interesting result, they found that the microscopic vasovasostomy (VV) results in greater pregnancy rates than VV using the absorbable stent. They did not recommend absorbable stent due to poor measured patency ratio and sperm motility[8] were preferred for reconstruction of vasectomy.[7]

On the other hand, large sections of the VD may be affected after some surgical operations such as hernia repair, hydrocelectomy, or complications of vasectomy. In the majority of such cases, the length of vas defects renders direct vasovaso anastomosis either impossible or too risky, due to increased tension in the anastomosis area. To overcome this obstacle, extra anatomical (sub- and suprapubic) vas rerouting was performed to allow shortening of the necessary vas length for anastomosing. This technique has been established to be one of the most technically challenging type of surgery of the male reproductive system. Another interesting possibility for resolution of a vas defect might be the use of a vascular transplant.

The aim of this study presented was to determine experimentally if a vas defect could be repaired either by vas transplantation or by transplantation of an artery and vein graft. Another objective was to determine critical maximum length that can be transplanted while still achieving acceptable patency for sperm transport.

MATERIAL AND METHODS

In our experimental setting, we used male adult inbred Wistar rats, weighing 250-300 g, mean 270 g. Females of the same
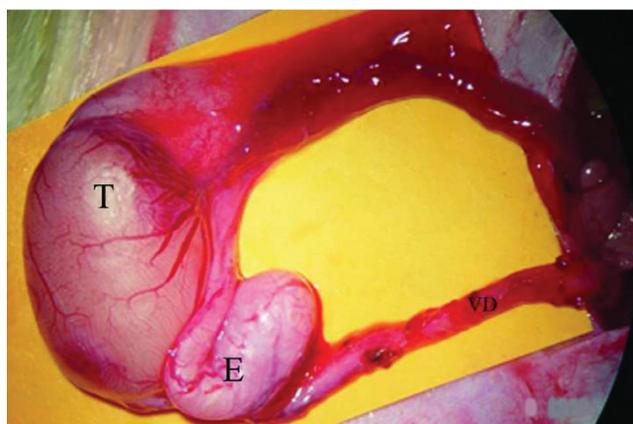

Figure 1. View of scrotal contents of rat.
Keys: T, testis; E, epididymis; VD, vas deferens.

Figure 2. Completed anastomosis of vas deferens autograft.
Keys: TS, testicular side anastomosis; AS, abdominal side anastomosis.

Table . Results of anastomosis condition in all groups.

Variables	Autotransplantation Groups		Artery Graft Groups		Vein Graft Groups		Total
Testicular site anastomosis	1 cm	1.5 cm	1 cm	1.5 cm	1 cm	1.5 cm	------
Normal	2/7	1/7	2/7	0/7	0/7	0/7	5/42
Partial stenosis	2/7	3/7	1/7	2/7	1/7	1/7	10/42
Occluded	3/7	3/7	4/7	5/7	6/7	6/7	27/42
Abdominal site anastomosis	1 cm	1.5 cm	1 cm	1.5 cm	1 cm	1.5 cm	------
Normal	1/7	0/7	1/7	1/7	0/7	0/7	3/42
Partial stenosis	2/7	3/7	1/7	4/7	0/7	0/7	10/42
Occluded	4/7	4/7	5/7	2/7	7/7	7/7	29/42

race and standards were used as vascular donors in the experiment. The study was conducted in accordance with the Guidelines for Animal Care and Research of the university. The animals were kept in a room with standard environmental conditions and fed ad libidum. The male rats (n = 42) were divided into three groups as VD graft, carotid artery and external jugular vein transplantations. Each group was equally divided into 2 different subgroups according to the length of transplant material as 1.0 cm (n = 7) and 1.5 cm (n = 7).

Operative Technique

Operations took place under ketamine (90 mg/kg) and xylazine (10 mg/kg) anesthesia. Supplementary doses were given as necessary. A surgical operation microscope (M 651 Surgi-

cal Microscope; Leica, Sweden), standard microvascular instruments, and 8-0 nylon suture with 75 μm needle were used. The surgical procedure and postoperative observations were performed by the leading author.

The left side was preferred for the experimental groups. An abdominal midline incision was used to explore the VD followed by the opening of the internal spermatic fascia and the VD, which was lying loose next to the funiculus, which was easily exposed leaving the scrotal contents in situ. Two different lengths of VD segment (1.0 cm and 1.5 cm) were resected to create defects in VD and these segments represented 30% and 50% of the total VD length respectively. These defects were reconstructed using VD, carotid artery and external
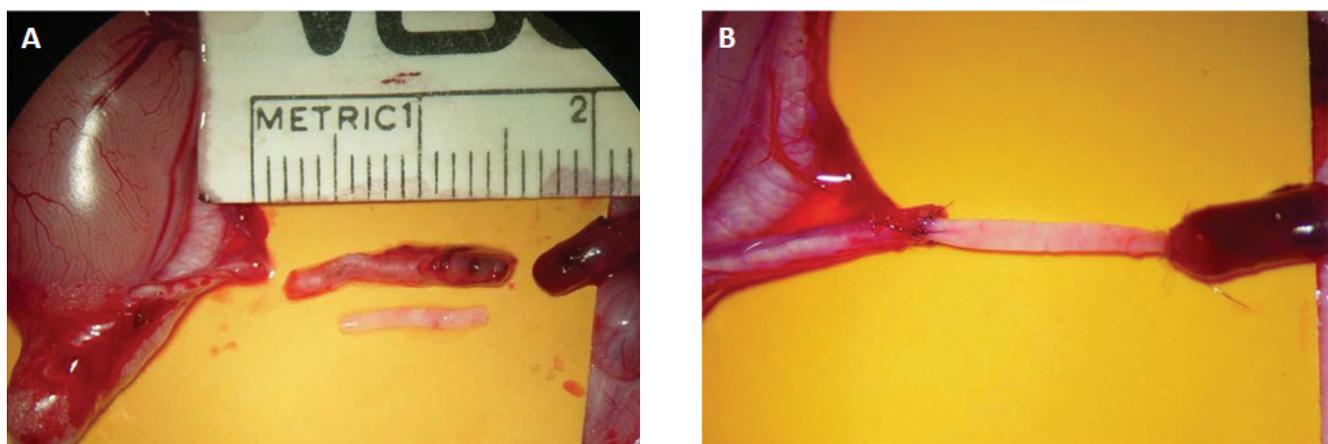

Figure 3. (A) View of resected vas deferens segment (1.5 cm) with similar length of the arterial graft (1.5 cm). Arterial graft is seen shortly compared to vas deferens defect due to elastic shrinkage of vessel wall. (B) Completed vas deferens- arterial graft anastomoses.

Figure 4. Sperm granulomas in the vein graft. This collection populated neighbor areas of testicular side anastomosis while abdominal side anastomosis did not occupied this structure.

jugular vein grafts and grafts used for reconstruction were the same length as the resected vas segment.

Autotransplantation Group (n = 14)

In this group, resected segment of VD were anastomosed in the same place. Anastomoses were performed by applying 4 stitches and only seromuscular suture was placed using 8-0 non-absorbable nylon. The first two sutures were placed at opposite ends 180 degrees apart precisely aligning mucosa of two ends of VD. One suture was placed between these stitches on each of the front and back wall.

Artery and Vein Groups

Carotid artery and external jugular vein grafts were harvested the same length (1.0 cm and 1.5 cm) as the vas defect from the female Wistar rats. For the anastomosis of VD with artery and vein grafts, the same operative technique described as above was used with exception that only full-thickness sutures were applied through the vascular wall. The skin was closed with 4-0 silk sutures in every rat after the application pf the procedure.

Final Examination

Four weeks postoperatively the rats were euthanized. Occurrence of sperm granulomas[9] was recorded. The abdominal end of the VD after both anastomosis zones in all groups were transected and the intraluminal fluid was microscopically examined for functional patency with the occurrence of sperm at 400 × magnification. The VDs were transected distal from the anastomoses and functional patency checked by smear examination to see the presence of sperm at the distal portion. The transplanted segment was then incised longitudinally in order to explore if there are any occlusions or partial stenosis. Equal segments of the VD, artery and vein grafts with the anastomoses included were excised. For the control specimen, ductus deferens from one male animal, carotid artery and external jugular vein from one female animal were harvested. All specimens were fixed in neutral buffered 4% formalin and paraffin embedded for further slicing. Multiple tissue segments of each specimen were taken from anastomosis area and proximal and distal to the anastomosis. All samples were cut at 4 μm thickness and slides were stained with hematoxylin and eosin. All the samples were examined by the same pathologist.
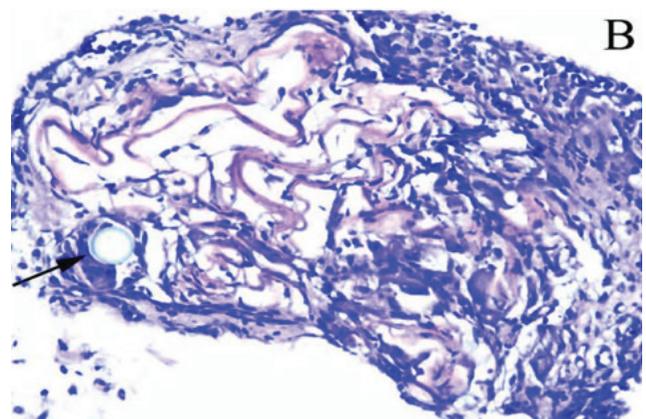

Figure 6. (A) Control artery tissue (hematoxylin and eosin × 40), (B) vascular wall was fragmented in artery graft, and mix inflammatory cells and also suture material (arrow) were seen (hematoxylin and eosin × 200).
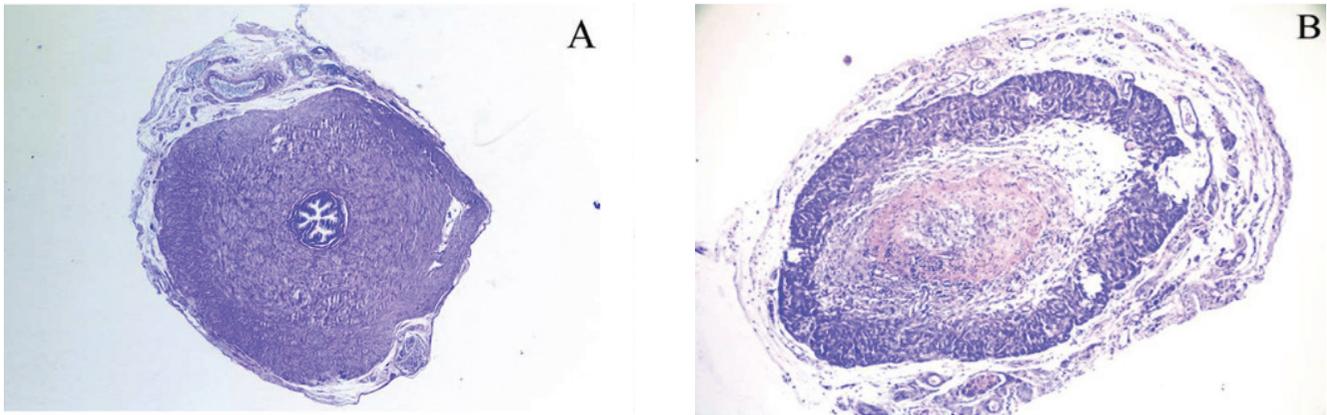
Figure 5. (A) Control vas deferens (hematoxylin and eosin × 40), (B) the lumen was obliterated and the thickness of muscle layer was reduced, and subepithelial area was expanded by fibrous tissue growing and inflammatory cells in the vas deferens graft (hematoxylin and eosin × 100).

RESULTS

Autotransplantation Group

Anatomical patency was not preserved in any of the segments. In the 1 cm-long graft group, normal, partial stenosis, and occlusion were observed as 2/7, 2/7 and 3/7 in testicular side (TS) anastomosis, and 1/7, 2/7 and 4/7 in abdominal side (AS) anastomosis, respectively. In the 1.5 cm long graft group, normal, partial stenosis, and occlusion were observed as 1/7, 3/7, and 3/7 in TS anastomosis, and 0/7, 3/7, and 4/7 in AS anastomosis respectively. Sperm granulomas (SG) occurred in all 14 segments and they were situated 12/14 and 2/14 TS and AS anastomoses, respectively.

Artery Graft Group

Anatomical patency was not observed in any of the segments. In the 1 cm- long graft group, normal, partial stenosis, and occlusion were observed as 2/7, 1/7 and 4/7 in TS anastomosis, and 1/7, 1/7 and 5/7 in AS anastomosis, respectively. In the 1.5 cm long graft group, normal, partial stenosis, and occlusion 0/7, 2/7, and 5/7 were observed as in TS anastomosis, and 1/7, 4/7, and 2/7 in AS anastomosis, respectively. SG occurred in all 12 segments and they were situated 11/12 and 1/14 TS and AS anastomoses, respectively.

Vein Graft Group

Anatomical patency was not recorded in any of the segments. In the 1 cm- long graft group, normal, partial stenosis, and occlusion were observed as 0/7, 1/7 and 6/7 in TS anastomosis,
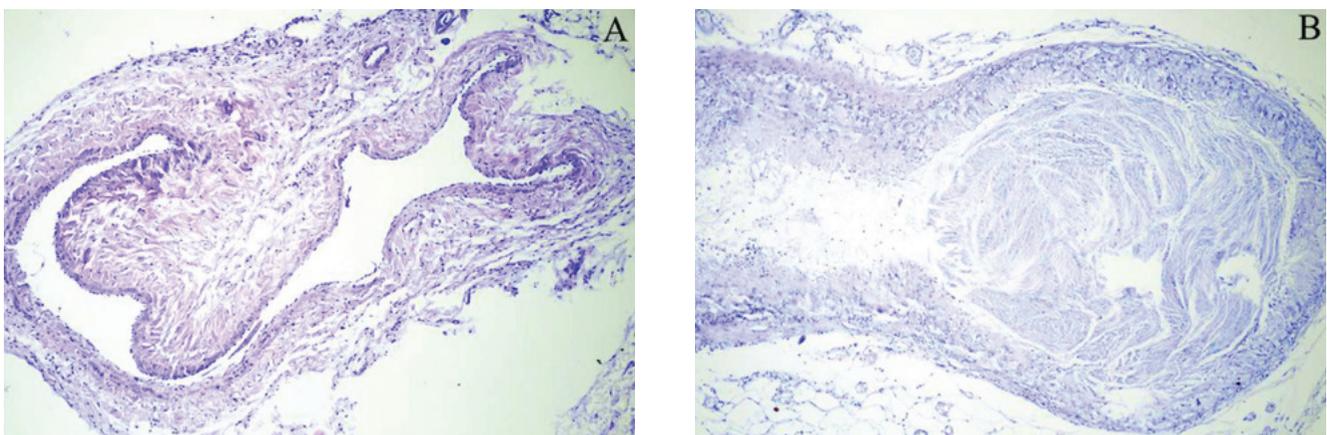

Figure 7. (A) Control vein tissue (hematoxylin and eosin × 100), (B) necrotic debris and semen material filled the vein lumen and covered the inner surface, and also inflammatory cells were seen in the vascular wall and the lumen of graft (hematoxylin and eosin × 100).
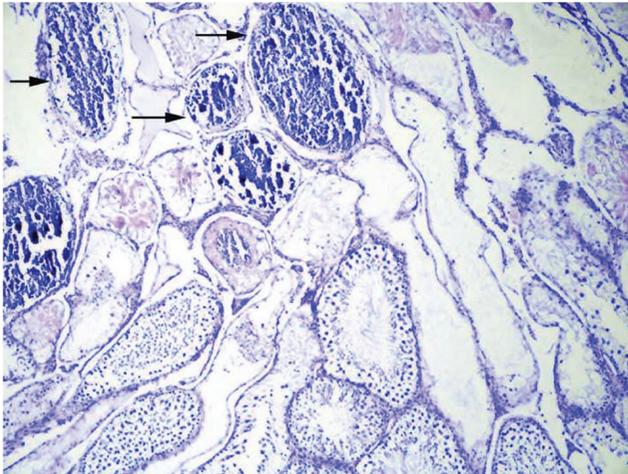
Figure 8. Normal seminiferous tubules were seen at the lower area, but some tubules at the upper side (arrows) were degenerated and filled with necrotic and cellular debris (hematoxylin and eosin × 100).

and 0/7, 0/7 and 7/7 in AS anastomosis, respectively. In the 1.5 cm long graft group, normal, partial stenosis and occlusion were observed as 0/7, 1/7, and 6/7 in TS anastomosis, and 0/7, 0/7, and 7/7 in AS anastomosis, respectively. SG occurred in all 12 segments and they were situated 12/12 and 0/12s TS and AS anastomoses, respectively.

Intraluminal Fluid Examination

No motile sperm were found in intraluminal fluid microscopically. Only necrotic cells and debris were observed upon smear examination.

Histological Examination

VD Grafts

The muscle layer of the graft was reduced, to between 10 and 35% of the original thickness in most of the cases. For some cases especially in the longer grafts, the whole wall was replaced by fibrous tissue. Atrophic epithelial layer was observed with intact epithelium in short grafts while longer grafts had only remnants of an atrophic epithelium.

Artery Grafts

The thickness of the muscular layer was slightly or moderately reduced. Inflammatory changes were present, especially with massive SG. Intimal layer was destroyed and detached from basal layer and protruded into the vascular lumen.

Vein Grafts

The whole walls of vein grafts were invaded by inflammatory cells. The lumens of many vein grafts were occluded by pannus-like tissue, which is granulation and fibrous tissues. Intimal layer was not found in many grafts and it was de-

tached from basal layer into the lumen.

DISCUSSION

There are a lot of techniques described for the reversal of vasectomies and the reconstruction of defects in the vas deference area. It was reported that one-layer VV and two-layer VV seem to be equal with regard to vasal patency.[9] Furthermore we preferred one layer VV as our microsurgical skills with our clinical cases. We believed that one layer approximation of vasal ends is easier, quicker and safer compare to two layer technique.

However, in large defects, when the VV is not possible due to technical reasons, hollow structures such as vessels might come up as an idea for grafting. However, since there is contractility present in VD, especially due to parasympathetic stimulation,[8] it is extremely hard to demonstrate that contractility with graft materials. In human, the VD epididymis and efferent ducts has proximodistal increase in the muscle layer, and also the thickness of the muscle layer is greater than any structure in human body, compared to its lumen. VD and distal epididymis propel the sperm with their rhythmic contractions, and if this peristalsis is disrupted, a sperm granuloma might occur.

It was reported thirty years ago that microsurgical VV technique for vasectomy reversal has resulted in significantly improved outcomes compared to older techniques.[10] Patency rates after microsurgical VV using non-absorbable sutures have reached 99%. The semen can pass through the anastomosis zone as well as an aperistaltic segment in anastomosis zone shortly after VV. The obstructive interval after vasectomy is a significant determinant of the patency and pregnancy rates. Which can be exemplified by the patency and pregnancy rates decreased 88% and 53% between 3-8 years after vasectomy.[11] Those poor results may be related to long segment fibrosis in VD following a long term interval after vasectomy. Shandling and Janik found that simply clamping the vas of rats could produce muscle disruption and fibrosis.[12] The thick muscle layers can easily get damaged with an insult minor than vasectomy which triggers fibrosis. Inflammation and fibrosis worsening the whole scenario by causing more extensive damage with vasectomy.[13] The problem associated with vasectomy or iatrogenic injury to the VD include a long obstructive interval, unpredictable length of occlusion, injury to the testicular blood supply and these factors may cause long segment injuries to VD.[14] Vasoepididymos-

tomy may be performed to resolve large VD defects.[15] But advanced microsurgical techniques are necessary and it has a lower pregnancy and patency rate compared to a VV.[16] Also, prosthetic stents are not long enough to use for microsurgical VV reconstruction of VD defect. Although experimental VD auto-transplantation is not applicable for clinical operations, Carringer evaluated VD and vascular grafts to repair large VD defect in rats.[17] However, low patency rates were reported for these grafts and this result was explained with poor graft viability and absent neural innervations. Carringer postulated that graft neovascularization occurred from the margins of the transplant and the outcome depended on the "bridging phenomenon". This phenomenon, to elaborate, is the growth of vessels anastomosing with the vessels of the transplant.[17]

The total length of rat VD is 3-3.5 cm. In our study, we aimed to create VD defects at 30% and 50% of the total length, making 1 and 1.5 cm segment resections from VD respectively. After the defects were created, we examined the results comparing various grafts which had different muscle layer thicknesses. We found no patency in either auto-transplantation or vascular grafts groups. However, rates of vein graft occlusion were higher compared to its artery groups. We hypothesized that this result was due to the thick wall of the artery graft preventing lumen from collapsing, thus creating lower graft occlusion, although they still yielded poor total patency outcomes. We have some differences from Carringer's neovascularization hypothesis.[17] Main neovascularization mechanism of all grafts is provided by sprouting new vessels from donor site or formation of anastomoses between graft and host vessels. Graft's nutrition is dependent on plasma diffusion until new vessels form. It is known that thin tissues gain enough neovascularization providing graft nutrition in a shorter time than thick tissues.[18] Although we did not conduct detailed examinations in order to determine the difference of neovascularization quality, all groups displayed the same histological views of new vessels sprouting under light microscopic study. Artery and vein grafts which have thin walls may cause mechanical obstruction in their lumen as a result of collapse and kinking. Mechanical blockage invites inflammation and fibrous tissue growing into luminal space as a result, permanent lumen obstruction. We have observed intraluminal fibrous tissue in some histological sections during light microscopic study. In our opinion, the main problem of lower patency rates were related to long VD defects which create aperistaltic zones during semen transport. Although there is no evidence of VD contraction except during the ejaculation period, thick muscle and mucosal layer of VD may propel semen during asexual period as well.

CONCLUSION

We concluded that vein and VD grafts are not useful for long segment defect reconstructions which are 30-50% of the VD length in rats. We think that any material used for large VD defect reconstruction must have peristaltic movement in order to push the semen forward. Furthermore, we concluded that there is no autogenic or prosthetic material has this special function to perform semen transportation. For this reason, vasoepididymostomy seems to be only indisputable solution for long segment VD reconstruction.

CONFLICT OF INTEREST

None declared.

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