

## Comparison of Vasovasostomy with Conventional Microsurgical Suture and Fibrin Adhesive in Rats

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### ABSTRACT

*Objective:* Microsurgical procedures are currently the gold standard for vasovasostomy with excellent results, but it takes an increased operative time demanding special training and experience in microsurgery. The objective of this study is to reach the same results with reduced operative time.

*Materials and Methods:* Male adults Wistar rats were divided into 3 groups: I) 20 rats in control group, II) 20 with conventional one-layer microsuture and III) 20 with fibrin glue. After four weeks each rat was left for two weeks with two female rats.

*Results:* The fertility rates were 80% in group I, 70% in group II and 65% in group III ( $p > 0.05$ ). In group II granuloma was found in 75% of the rats, while in group III in 85% ( $p < 0.05$ ). Anastomosis was considered with patency in 75% and 80% of the rats in groups II and III ( $p > 0.05$ ). Overall operative time was  $41.7 \pm 2.49$  and  $28.55 \pm 1.14$  minutes in groups II and III ( $p < 0.05$ ) and the time to anastomosis of  $24.6 \pm 1.8$  and  $9.35 \pm 0.78$  minutes ( $p < 0.05$ ), respectively.

*Conclusions:* We can conclude that vasovasostomy with fibrin adhesive has the same results of the conventional microsurgery technique but with a reduced operative time and a simplified procedure.

**Key words:** rats; vasovasostomy; fibrin tissue adhesive; microsurgery; infertility  
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### INTRODUCTION

Currently, more than 30 million couples worldwide use vasectomy as a method of birth control. It is estimated that up to 6% of men who undergo vasectomy will request reversal, and 3.5% will in fact receive a vasovasostomy (1,2). In the United States, from 600,000 to 1 million vasectomies are performed annually, which results in the expectation of 21,000 to 35,000 reversals (3,4).

A number of techniques have been described to restore vas deferens patency, but no consensus has been reached as to which technique is the best (5). Several techniques using microanastomosis show good results, with respect to both anastomosis patency and pregnancy rate, between 80 to 90%, and 50 to 80%, respectively (5-11). Thus, the main objective of this study is to devise a new technique or to improve an existing one that would allow a faster procedure (12,13).

Advances in welding of human tissues have been incorporated in experimental animal studies (14-16). This study was undertaken to prospectively compare the use of conventional suture with the fibrin glue technique in vas deferens reanastomosis, and to determine if these techniques present similar results but with a reduced procedure time and ease of execution.

## MATERIALS AND METHODS

Sixty male Wistar rats (*Rattus norvegicus* Albinus, Rodentia, Mamalia) initially weighing between 220 to 230 grams and aged 90 days were included in the experiments. To confirm fertility, 120 90-days-old female Wistar rats in proestrus, determined by the analysis of vaginal secretions, were used. The ethical guidelines on animal experimentation of the National Council for Control of Animal Experimentation of Brazil were observed. The animals were grouped as follows:

Group I - 20 (twenty) male rats that underwent exposure of the left vas deferens for 5 to 10 minutes. Group II - 20 (twenty) male rats presenting left vasovasostomy with a conventional one layer suture with 10-0 nylon and Group III - 20 (twenty) male rats that underwent a left vasovasostomy with fibrin glue.

**Surgical Technique** - The animals were anesthetized intraperitoneally with sodium pentobarbital and the procedures were performed under aseptic conditions. The surgery started after the loss of corneal reflex. A bilateral incision was made in the scrotum, the vasa deferentia were identified and isolated in all rats. In the three groups, the vasectomy was performed on the right side.

In group I, the vas deferens was released and the skin was sutured using 4-0 chromic catgut. In group II, the left side vas deferens was divided, followed by a one-layer anastomosis with five sutures using 10-0 nylon, according to the technique described by Leonard & Thomas (17), with surgical microscope at 16 to 25X magnification. In group III, on the left side the cut ends were reattached through a transmural suture with one anchor points using 8-0 nylon suture and application of 1 to 2 drops of fibrin glue..

The animals were kept in individual cages for 30 days. At the end of this period, every male was put in a cage together with two females for 2 weeks. After this period the males were anesthetized and underwent a scrotal exploration. At the completion of the experiment, the animals were euthanized with sulphuric ether in a bell jar.

The criteria for data collection and analysis were fertility rate determined by the pregnancy of at least one female, granuloma formation at the site of the anastomosis, amount of neutrophilic exudate at the cut edges of the anastomosis, epithelial alterations (denaturation and tearing), duration of surgery and anastomosis, and anastomosis patency. The last one was evaluated immediately after the removal from the anastomosis site in three ways: 1) observation through surgical microscope of intravasal fluid produced by manual compression (milking) next to the anastomosis, 2) microscopic examination at 40 X 3.3 magnification of the fluid collected from the distal end of the vas deferens with a glass slide to determine the presence of sperm, and 3) catheterization of the proximal end at the anastomosis site with 24-gauge cannula and gravity injection (70 cm height) of methylene blue diluted in saline to 50% (18). In order to consider the anastomosis patent to the passage of sperm, at least two criteria were met.

The Z-test with 95% significance level was used in the statistical analysis of the fertility rate, the granuloma formation and the patency rate. The standard deviation was calculated for the duration of the surgery and anastomosis. The exact Fischer's test was used in the correlation between the presence of granulomas and the patency rate, and in the evaluation of neutrophilic infiltrate. The  $\chi^2$  test incorporating Yates correction for continuity was used in the analysis of the reepithelialization and collagen reorganization.

## RESULTS

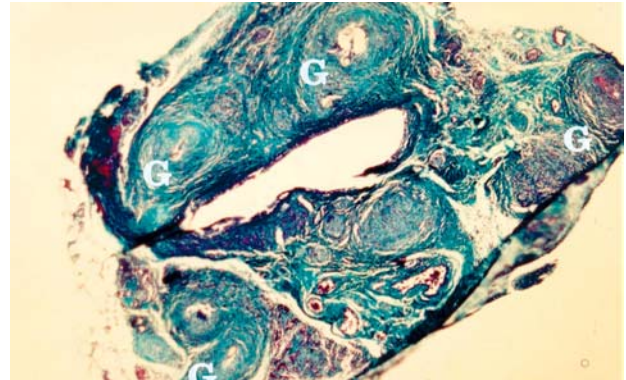
Four animals presented infection of the surgical wound, determined by the direct inspection of the suture line; two animals belonged to group I, one to group II and another to group III. One of the rats from group I showed dehiscence of the skin suture,

without infection. This rat (rat G1-4) impregnated two females.

**Fertility rate** - Some females became pregnant before four weeks were completed, the others were removed on the thirtieth day and their pregnancies were confirmed thereafter. Seventeen female rats (85%) from group I, 14 (70%) from group II and 13 (65%) from group III became pregnant ( $p > 0.05$ ).

**Granulomas** - Only the rats from group II and III were evaluated. The total amount of macro- and microscopic granulomas found in every rat was larger in the group that was vasovasostomized with fibrin glue (Figure-1). Fifteen rats (75%) from group II had from 1 to 7 granulomas with an average of 3.6 granulomas/rat. Seventeen rats (85%) from group III had from 2 to 8 granulomas with an average of 4.2 granulomas/rat ( $p < 0.05$ ). The anastomotic patency was not correlated with the presence of granulomas in group II (Table-1) and group III (Table-2).

**Histology** - The evaluation of neutrophilic exudate related to an inflammatory process at the anastomosis cut edges can be seen in Table-3. The statistical comparison showed no significant differences ( $p > 0.05$ ) between vasovasostomy with conventional suture and with fibrin glue. Intense neutrophilic exudate did not alter significantly anastomosis patency, which suggests that the neutrophilic inflammatory reaction is not an obstructive factor to the reanastomosed lumen (Figure-1).



**Figure 1** – Anastomotic cut edge in vasovasostomy with fibrin glue. Presence of some peripheral granulomas (G), foreign body (anchor point) and intense inflammatory reaction (Gomori trichrome, X40).

The reepithelialization process of the vas deferens lumen for groups II and III showed no significant difference ( $p > 0.05$ ). In same way, collagen fiber reorganization showed no significant difference ( $p > 0.05$ ).

**Patency** - Anastomotic patency was demonstrated in 50 rats. Of these, 19 (95%) were in the control group, 15 (75%) in the vasovasostomy-with-conventional-suture group, and 16 (80%) in the fibrin-glue group (Table-4).

**Surgery Duration** - There was a statistical significant difference in operative time when the dif-

**Table 1** – Comparison between spermatic granuloma in vasovasostomy site and fertility rates in vasovasostomy with microsuture.

	Fertility	No Fertility	Total	Statistics
Granuloma	11	4	15	$p > 0.05$
No granuloma	3	2	5	
	Patency	No Patency	Total	Statistics
Granuloma	10	5	15	$p > 0.05$
No granuloma	5	0	5	

**Table 2** – Comparison between spermatic granuloma in vasovasostomy site and patency rates in vasovasostomy with fibrin glue.

	Fertility	No Fertility	Total	Statistics
Granuloma	9	7	16	
No granuloma	4	0	4	p > 0.05

	Patency	No Patency	Total	Statistics
Granuloma	12	4	16	
No granuloma	4	0	4	p > 0.05

ferent surgical procedures were compared. Anastomosis with fibrin glue took less time to be performed when compared to the conventional suture technique (Table-5).

### COMMENTS

There is no uniformity as to which is the best vasovasostomy technique (19,20). Initially, the con-

troversy was about the use of sutures in one or two layers. The analysis of these two possibilities in the literature shows similar results with respect to fertility and patency rates (8,10,21,22). The fundamental difference is related to operative time, required training and costs. In this study, the reanastomosis were performed immediately after vasotomy and in vivo experience usually reveals a significant discrepancy in lumen sizes, hence, the theoretical reason for 2-layer closures. When both methods are compared, one

**Table 3** – Comparison of histological findings in vasovasostomy site with microsurgical anastomosis and fibrin glue.

Neutrophilic Exudate	Microsurgical Anastomosis	Fibrin Glue Anastomosis	Statistics
Intense	2	3	p > 0.5
Moderate	18	17	p > 0.5
Absence	0	0	
	20	20	
<b>Reepithelization</b>			
Absence of epithelium	12	10	p > 0.05
Thin epithelium	5	5	
Similar to original	3	5	p > 0.05
	20	20	
<b>Collagen Fibers</b>			
Ragged and unnatural	15	13	p > 0.05
Reorganized e dense	4	6	p > 0.05
Reorganized with thickness similar to adjacent tissue	1	1	
	20	20	

**Table 4** – Anastomotic patency after 45 days vasovasostomy.

Anastomotic Patency	Controls	Microanastomosis	Fibrin Glue	Statistics
Fluid at distal duct (i)	19	18	16	
Presence of spermatozoa (ii)	15	15	16	
Methylene blue (iii)	20	18	16	
Total [(i+ii+iii):(i+ii):(i+iii):(ii+iii)]	19 (95%)	15 (75%)	16 (80%)	p > 0.05

would opt for the faster and easier method to be performed (11).

Based on these reasons, anastomosis with fibrin glue was compared to the one-layer technique with mucosa-to-mucosa alignment. However, it has been questioned the need of a few stitches before the fibrin glue application in order to keep the cut ends in alignment. It was decided to use two transmural anchor points in order to improve the vasovasostomy outcome, as previously described (23).

Conventional anastomoses that use microsurgical techniques show high rates of success, with patency rates of about 90% and fertility rates between 40 and 80% (12). Küçükaydin et al. (24) obtained fertility rates of 70% for the fibrin glue or laser vasovasostomy and 80% in control groups. In this experiment, we obtained a fertility rate of 70% for the conventional suture group and 65% for the fibrin glue group.

The presence of granulomas at the vasovasostomy site still does not have a conclusive meaning (25). Granulomas are described in 20 to 35%

of the ducts in vasovasostomies (26). Silverstein et al (12) report granulomas in 28% of the fibrin glue vasovasostomies, but they observed a higher rate (61%) with conventional suture technique. These results are similar to the ones found in this study where 30% of the rats with anastomosis with fibrin glue and 15% with conventional suture presented macroscopic spermatic granulomas. The most plausible explanation for a larger number of granulomas when the fibrin glue was used is the amount of glue that was applied. The excess of glue surrounding the anastomosis can lead to granuloma formation. An experiment in dose titration could clarify this question.

Some authors have emphasized the importance of a patent anastomosis that prevents sperm leakage (23,24,26). The use of fibrin glue guarantees the anastomotic patency, since the glue contacts the full circumference of the vas deferens and not only 4 or 6 points as in the conventional suture technique. However, it is important to note that negative results can occur if the glue is applied to the two ends of the vas deferens before they are reconnected.

**Table 5** – Comparison of anastomotic and operative time in vasovasostomy with conventional microsuture and fibrin glue.

Time	Microsurgical Anastomosis	Fibrin Glue Anastomosis	Statistics
Operative time			
Mean (minutes)	41,7	28,55	F = 21.76
Standard deviation (SD)	±2.49	± 1.14	p = 0.0001
Time to anastomosis			
Mean (minutes)	24.6	9.35	F = 57.26
Standard deviation (SD)	± 1.8	± 0.78	p = 0.0001



At present, the majority of studies demonstrate that anastomosis patency is not altered by the presence of granulomas (18,27), this data is similar to what was observed in this experiment, since no correlation was found between the presence of granulomas or their number with an obstruction of the anastomosis.

It was observed that the presence of sperm in the distal vas segment occurred in 75% of the anastomosis with conventional suture and in 80% of the ones with fibrin glue. These results are similar to those reported in literature, which ranges from 80 to 90% (12,16,23,24).

The vas deferens is lined internally by a pseudostratified epithelium, composed mainly of small basal hemispherical cells and columnar principal cells with microvilli (28). The exact physiological role of the vasa deferentia in men is not completely understood, but it is expected that after a reconstruction of the vasa one obtains a histological evaluation as similar as possible as it was observed before the vasectomy. We observe that the majority of vasa deferentia from the conventional suture and fibrin glue groups showed lack of or initial epithelialization, 60 and 50% respectively. In spite of the fact that there was no statistical difference showing unequivocally that the highest pregnancy and patency rates are related to the ducts that showed a higher degree of reepithelialization, it was observed at least a trend in this direction. It is noteworthy to mention that the evaluation at 45 days may partially explain these results.

Although approximately 20% of the original mass of fibrin glue is dissolved by fibrinolysis within 72 hours following the application, a local inflammatory reaction may be observed. This tissular reaction is a result of the combination of trauma and the physical and chemical properties of the materials used in the synthesis (29). The permanence of neutrophils indicates a persistent inflammatory process. It was detected a predominance of a moderate inflammatory reaction in the anastomosis with conventional suture and in the ones with fibrin glue, 90 and 85% respectively. Similar results have been reported in the literature related to the evaluation of inflammatory reactions in surgical wounds (30). Also, the number of neutrophils at about six weeks may represent a response to the surgical trauma

(12,23,24,29,31). The inflammatory process, as a result of the surgical manipulation and of the synthesis material, did not seem to affect the anastomosis patency. Few studies in the literature make this correlation, but those that evaluate it do not believe in cause-effect relationship either (28).

In the functional analysis of vasovasostomy we need to keep in mind that there are several factors that may alter the patency and not only the quality of the anastomosis itself. The denervation of the vas deferens during the vasectomy may halt the peristaltic propulsion of sperm through the anastomosis. Our results, with a 75% patency rate for the vasectomy with conventional suture and an 80% patency rate for the vasectomy with fibrin glue, agree with the literature that reports a 80 to 90% patency rate (5-8,10-12,26).

The results from different techniques are equivalent in terms of patency and pregnancy rates, but it is expected a reduction in the operative time. There is no clear definition in literature about surgery duration. Some authors do not mention how time was measured in methods (12,24,32), others start time measurement at the incision (16,20), and there are those who measure time from the first vasovasostomy stitch (13). In spite of these differences, average operative time for a conventional suture vasovasostomy ranges from 24 to 96 minutes, while in the fibrin glue or laser technique it is reduced to 10 to 35 minutes (12,13). The results of this experiment are  $24.6 \pm 1.8$  minutes for the conventional anastomosis and  $9.35 \pm 0.78$  minutes for the one with fibrin glue, and they are comparable to literature. The experiments of longer duration refer to the total duration of surgery. In this case, our time measurements of  $41.7 \pm 2.49$  minutes for conventional suture and  $28.55 \pm 1.14$  minutes for the fibrin glue technique still agree with literature.

The fibrin glue technique is simpler, requires less microsurgery experience and may transform the vasovasostomy a procedure within reach of a general urologist (12), since it shows results similar to the conventional microsuture technique, but with reduction of operative time. It is expected that the reduction in surgery time validates this technique, but caution needs be taken when the surgeon encounters no fluid and needs to perform vasopididymostomy.

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## CONFLICT OF INTEREST

None declared.

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