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Animal Experiments on Reanastomosis of the Vas Deferens Using Fibrin Glue

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Summary. In 31 rabbits the stumps of the previously severed vasa deferentes were joined with a fibrin glue and splinted in various ways: silicone T-tube, silk thread and chromium catgut thread. In a fourth group the vasovasostomy was carried out with a suture. The patency of the vasa deferentes was examined by evaluation of of the ejaculate, vasography and histological examination. In almost 50% of the cases, patency of the vas deferens was achieved with fibrin glueing. According to our investigations, temporary splinting with silk thread during the glueing or suture process appears to be the best method. Patency of anastomosed vasa deferentes should be evaluated by examination of the ejaculate. The radiological findings only permit a definitue statement when there is bilateral occlusion.

Key words: Vasovasostomy, Fibrin glueing, Restoration of Fertility Ejaculation in Animals, Vasography.

The increase in the world population by 76 million a year makes clear the importance of birth control. Contraceptive measures have so far been applied mostly to women, although disquieting side effects of the pill and intrauterine pessary have become known (10, 25, 26). Vasectomy is at present the safest and most successful method of controlling fertility in the male. It has been applied in recent years especially in the USA (7 million) and in India (7 million) (27). With the increasing number of vasectomies, the wish is expressed ever more frequently for a later restoration of fertility (11, 18, 19).

The frequency of vasovasostomies is indicated by the retrospective study of Davies and Hulka in which 1109 urologists interviewed had carried out reanastomosis in 6% of the men they had vasectomized (1).

Results of surgical restoration of fertility have varied. Klosterhalfen and Wagenknecht were able observed in 36% of the 270 patients they operated on (11). Data on pregnancy rates was not given. Collected statistics from Derrick et al. contain data on 1630 vasovasostomies: there was patency in 38%, the conception rate was 19.5% (2). On the other hand, Silber achieved a patency of around 80% with a microsurgical method (20). Similar results (patency around 80% and conception rate around 30%) were attained by Schmidt (18) and Lee (12).

Lack of patency must be blamed on the surgical method. The usual suture in anastomosis of the vas deferens can lead to substantial trauma affecting all wall layers. We therefore examined that the use of glue could improve the results of vasovasostomy.

MATERIALS AND METHODS

The experimental animals were male rabbits, which are especially suitable for post-operative control because of the simplicity of ejaculate collection. We operated on 29 animals of the white New Zealand breed aged between 4 to 8 months and 12 animals (crosses) aged between 6 to 8 months. The average body weight was 3440 g. The animals were kept in separate cages.

In 31 rabbits, the stumps of the previously severed vas deferens were joined with a fibrin glue. In five rabbits vasovasostomy was carried

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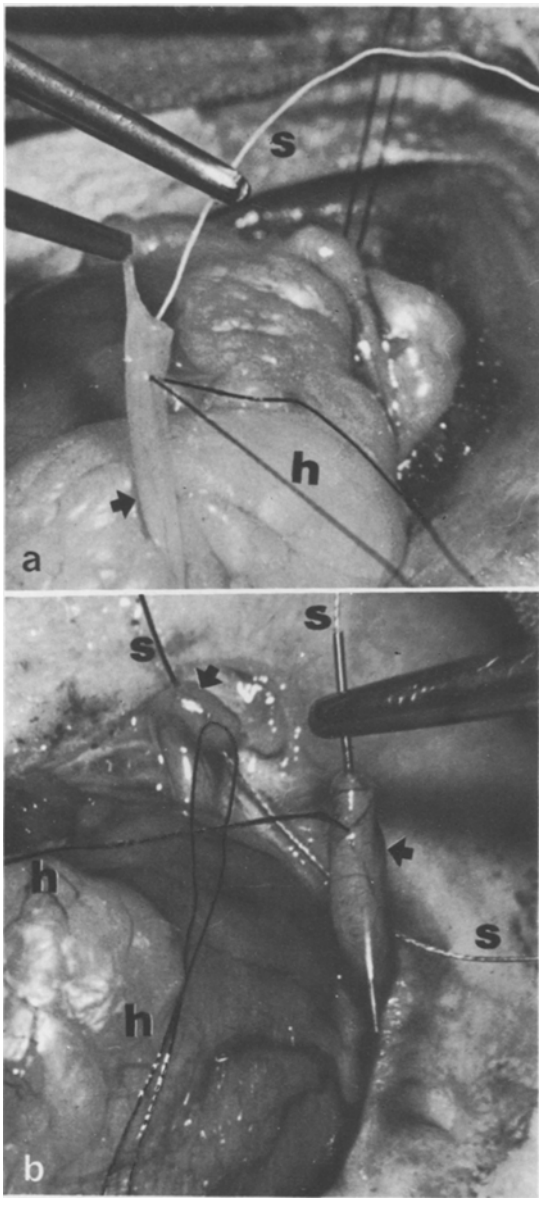


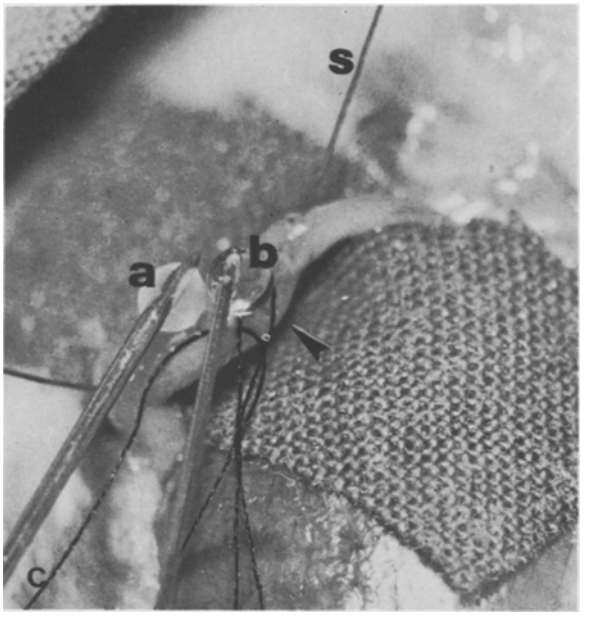


Fig. 1. a Intubation of the vas deferens with chromic catgut 40 (s). Arrow = vas deferens; h = holding suture. b Intubation of the vas deferens with silk thread 40 armed with a straight needle (s). Arrow = vas deferens; h = holding sutures. c Application of the fibrin glue Human Immuno (a) to the anastomosis (arrow) of the two vas deferens stumps. (b = thrombin + factor XIII mixture, s = intravasal splint)

out with sutures. In five control animals a sham operation was performed, simply exposing the vas deferens.

Surgical Technique

The surgical intervention was performed under i.v. pentobarbital anesthesia. We located the two vasa deferentes after lower abdominal incision. The vas deferens was severed between two holding sutures of 7/0 silk 1 cm apart. The stumps were approximated immediately afterwards with fibrin glue using intravasal splints (Fig. 1a and 1e).

The vas deferens was splinted in various ways. Table 1 shows the various experimental arrangements in the individual groups.

a) Group 1 (7 animals): splinting with a silicone T tube (external diameter 0.6 mm and internal diameter 0.3 mm). The proximal stump was intubated for about 3 cm with the tube. It was led out of the lumen 2 cm from the distal stump. The free end was laid under the skin. The splint was left in for 10 or 20 days ("temporary long-term splint").

b) Group II (13 animals): splinting using a silk thread 4/0 armed at both ends with straight needles. The thread was led out of the vas 1 cm proximal and distal to the point of severance. The silk thread only served as a splint during the glueing process ("temporary short-term splint").

c) Group III (11 animals): splinting with a chromic catgut thread 4/0. The thread was in-

Table 1. Group division according to the kind of splinting and anastomosis (n = 41)

Group no.	n	Splint	Operation
I	7	silicon T tube	fibrin glueing
II	13	silk thread	fibrin glueing
III	11	chromium catgut	fibrin glueing
IV	5	silk thread	suture with 70 silk thread
V	5	∅	exposure of vas deferens only

troduced for a distance of about 2 cm into the vas lumen proximal and distal to the severance point and left there ("absorbable permament splint").

In the Groups I to III, glueing was carried out as specified below.

d) Group IV (5 animals): vasovasostomy by suture with 7/0 silk atraumatically via a "temporary short-time splint".

e) Group V (5 animals): Control group. In these 5 animals, the vasa deferentes were merely exposed and the wound afterwards closed again by layers.

Glueing Technique

The principle of the glueing technique used here was developed by Matras and Spängler which has been used in various organs (7, 13, 16, 17, 21, 22, 23). It consists of 2 components:

a) "Human fibrin glue Immuno" consisting of 90 mg of coagulable protein/ml solution (Immuno Co., Vienna, Austria).

b) Thrombin and factor XIII.

500 IU thrombin and 14 IU factor XIII are dissolved in 1 ml of Ringer solution with a double concentration of calcium ions.

1 drop of fibrinogen and factor XIII/thrombin mixture is applied to the anastomosis site. After about 1 minute, the glueing process has been completed. In this kind of tissue glueing with fibrinogen, the second phase of blood coagulation, the conversion of fibrinogen to fibrin, is immitated. The endopeptidase thrombin converts fibrinogen into fibrinomer with cleavage of fibrin peptides A and B. The unstable fibrin polymer which is stabilized by the action of activated factor XIII is formed by polymerisation. This activation of factor XIII takes place under the influence of thrombin and calcium. The activated factor XIII brings about a covalent crosslinking of the fibrin network.

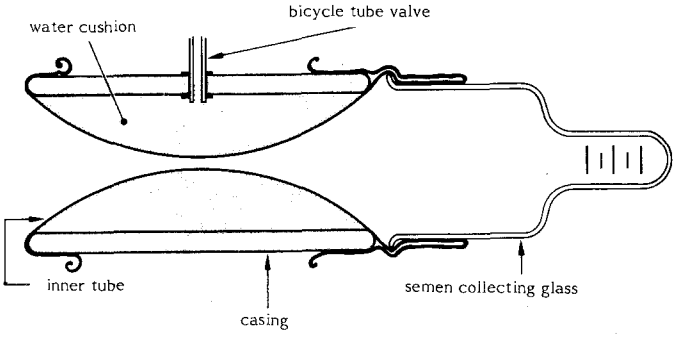


Fig. 2. Artificial vagina (after Grove)

Götze (1949)	100 - 2000 x 10^6/ml
Huhn (1952)	440 - 2700 x 10^6/ml
Hafez (1970)	200 - 400 x 10^6/ml
Own study (1977)	20 - 900 x 10^6/ml

Fig. 3. Normal values of spermatozoal density in rabbits

Followup Studies

The patency of the vasa deferentes was examined by evaluating the ejaculate, vasography and microscopic examination of the section preparations.

a) Ejaculate Investigation. The ejaculate was obtained using an artificial vagina (5), consisting of a coating tube, inner tube and semen collecting glass (Fig. 2). By filling the space between the coating tube and the rubber tube with hot water at 60°C, the internal temperature can be brought up to the 45°C required for ejaculation. After normal ejaculates had been confirmed before operation in the individual animals, the first collection of ejaculate was made 4 weeks after glueing or suture and afterwards every 2 weeks for 4 months.

Activity and Density of Spermatozoa. To evaluate the motility, the spermatozoa were classified microscopically according to an estimate method into forwardly motile, stationary and immobile cells (proportion stated in %). Calculation of the density (spermatozoa per unit of volume in millions/ml) was carried out according to the formula:

Table 2. Macroscopic findings in the vasa deferentes, the testicles and epididymes four months after fibrin glueing or suture

	Group I		Group II		Group III		Group IV		Total No.	
	uni-lateral	bi-lateral	uni-lateral	bi-lateral	uni-lateral	bi-lateral	uni-lateral	bi-lateral	uni-lateral	bi-lateral
Fusions of the vas deferens with the surrounding tissue	1	3	5	1	2	3	2	3	10	10
Stenosis of the anastomosis with/without congestion of the seminal fluid	2		3	2	3	2			8	4
Testicular or epididymal atrophy	2		3	1	1	2			6	3
Dehiscence and abscess liquefaction of the anastomosis			2		2				4	
Chromium catgut splint still in vas deferens					7	3			7	3

$$\text{Density} = \frac{\text{Counted number of spermatozoa}}{\text{counted surface x height of chamber x dilution x } 10^6}$$

Normal values (Fig. 3) are: 110 x 10^6 to 2000 x 10^6 spermatozoa/ml (4, 6, 8, 15).

b) Vasogram. The vasa deferentes were removed after killing the animals; the lumina were made visible by means of Telebrix® ; radiological examination was carried out with mammography film with a focal distance of 1 m (pure focus). Exposure 40 kV, 12 mAs.

c) Histological Investigation. After vasography, serial cross and longitudinal sections of the anastomosis site were prepared and examined histologically after staining with hematoxylin eosin.

RESULTS

Irrespective of the kind of splinting, unilateral dehiscence of the glued vas deferens occurred in 3 animals (Table 2). As can be further seen from Table 2, fusions of the anastomosis with the surroundings and siphon formation or kinking were found at the time of removal of the preparation after 4 months in 20 out of 72 vasa deferentes in the experimental animals.

1. Investigation of the Ejaculate (Table 3)

In group 1 with the temporary permanent splint, the first ejaculate to contain spermatozoa after glueing was found after 4 to 10 weeks in 4 out of 7 animals. The mean values of the spermatozoal density were below normal in 3 animals and within the normal range in 1 animal. The spermatozoa were mostly immotile forms. Permanent splinting did not influence the result.

In group II with a temporary short-term splint, after 4 to 12 weeks the anastomoses were patent in 6 out of 13 animals. The average spermatozoal density was within the normal range in 4 animals. The motility was good.

In group III with an absorbable permanent splint, the vas deferens was patent in 5 out of 11 animals after 4 to 12 weeks. The average spermatozoal density was in the normal range with one exception. The motility was poor. The chromium catgut threads had not dissolved in 10 animals from this group (see also Table 2).

In group IV, the vas deferens was approximated by a suture. In 4 out of 5 animals, we were able to demonstrate ejaculate containing spermatozoa after 4 weeks. The average spermatozoal density was normal apart from one exception. The motility was poor.

In group V (control group), an unremarkable ejaculate could be demonstrated both before and after the simulated operation.

2. Radiological Findings

The vasogram was evaluated as to whether the vas was freely patent or whether there was occlusion or stenosis. If an occlusion could be demonstrated, the vas deferens was opacified on

Table 3. Synoptic representation of the patency rate of the vasa deferentes and time of the first postoperative demonstration of spermatozoa in relation to the method of operation and intravasal splinting (n = 41)

	Group no.	Splint type	n	Spermatozoa for the first time postoperatively ($\bar{x}$)	Patency of the vasa deferentes	
					No of animals	%
Fibrin glueing	I	silicon T tube	7	6 weeks	4	57
	II	silk thread 40	13	6 weeks	6	46
	III	chromium catgut 3 x 0	11	7 weeks	5	45
Suture with 7 x 0 silk thread	IV	silk thread 40	5	4 weeks	4	80
Simulated operations	V	control group	5	immediately	5	100

both sides of the anastomosis. The criterion of stenosis was the passage of contrast medium through a narrowed area. We regarded the vas deferens as having uninterrupted patency when only a trace of a constriction ring could be detected (Fig. 4a-c).

We found complete patency of the lumen on one side in one animal of group I. There was a bilateral stenosis in two animals, and in one animal bilateral occlusion. The other animals differed on the two sides (stenosis and occlusion).

In group II unilateral uninterrupted patency was demonstrated radiologically in 4 animals. Three animals were occluded on both sides, and two further animals had stenosis on both sides in the other animals, the findings differed from one side to the other (stenosis and occlusion).

In group III unilateral uninterrupted patency could only be demonstrated in only one case. Bilateral occlusion of the vas deferentes was found in one animal and bilateral stenosis in four animals. Because of the remaining chromium catgut thread, two animals could not be X-rayed on both sides and in one animal on one side.

In group IV uninterrupted patency could only be demonstrated on one side in one animal. Four animals had stenoses or occlusions.

3. Histological Findings

The sections of radiologically demonstrated occlusions showed flattening of the lumen, narrowing, destruction of epithelium and multiple incisions (Fig. 5a and b).

In longitudinal section (Fig. 6a and b), however, total obstruction can be recognised by the spermatozoa-containing proximal stump, from the scar in the lumen and from the empty distal stump.

DISCUSSION

In almost 50% of the cases, we were able to achieve patency of the vas deferens with fibrin glueing (Table 2). The success rate corresponds to the result communicated by Pflüger (16). In the sutured vasa deferentes, the success rate was indeed 80%. This result also corresponds to communications from the literature (12, 18, 20), and was attained with muscularis sutures of 70 atraumatic silk, as also applied by Dorsey (3) as well as Klosterhalfen (11) and Kaufmann (9). Schmidt (18) and Silber (20) adapt mucosa and muscularis separately and attain excellent results with this method.

Intraluminal splinting of the vasa deferentes is a great problem and has not been solved. Montie et al. compared Dexon and chromic catgut (14). Urrey et al. compared silastic and chromium catgut (24). The absorbable materials could no longer be demonstrated after six months and had not occluded the lumen of the vas as was unfortunately the case in 10 of our rabbits either unilaterally or bilaterally. Four months after the operation, the chromic catgut lay unaltered in the lumen and there was complete blockage of spermatozoal transport in half of these cases. According to the investigations of Urrey et al. (24), who carried out their operations like Montie et al. (14) on dogs, silastic splints have proved better than chromic catgut splints. The postoperative spermiogram become normal earlier in the dogs splinted with silastic than in animals splinted with chromic catgut and the failures of vasovasostomy were also to be found without exception in this experimental group.

According to our investigations, temporary splinting with a silk thread during the glueing or suture process appears to be the best method.

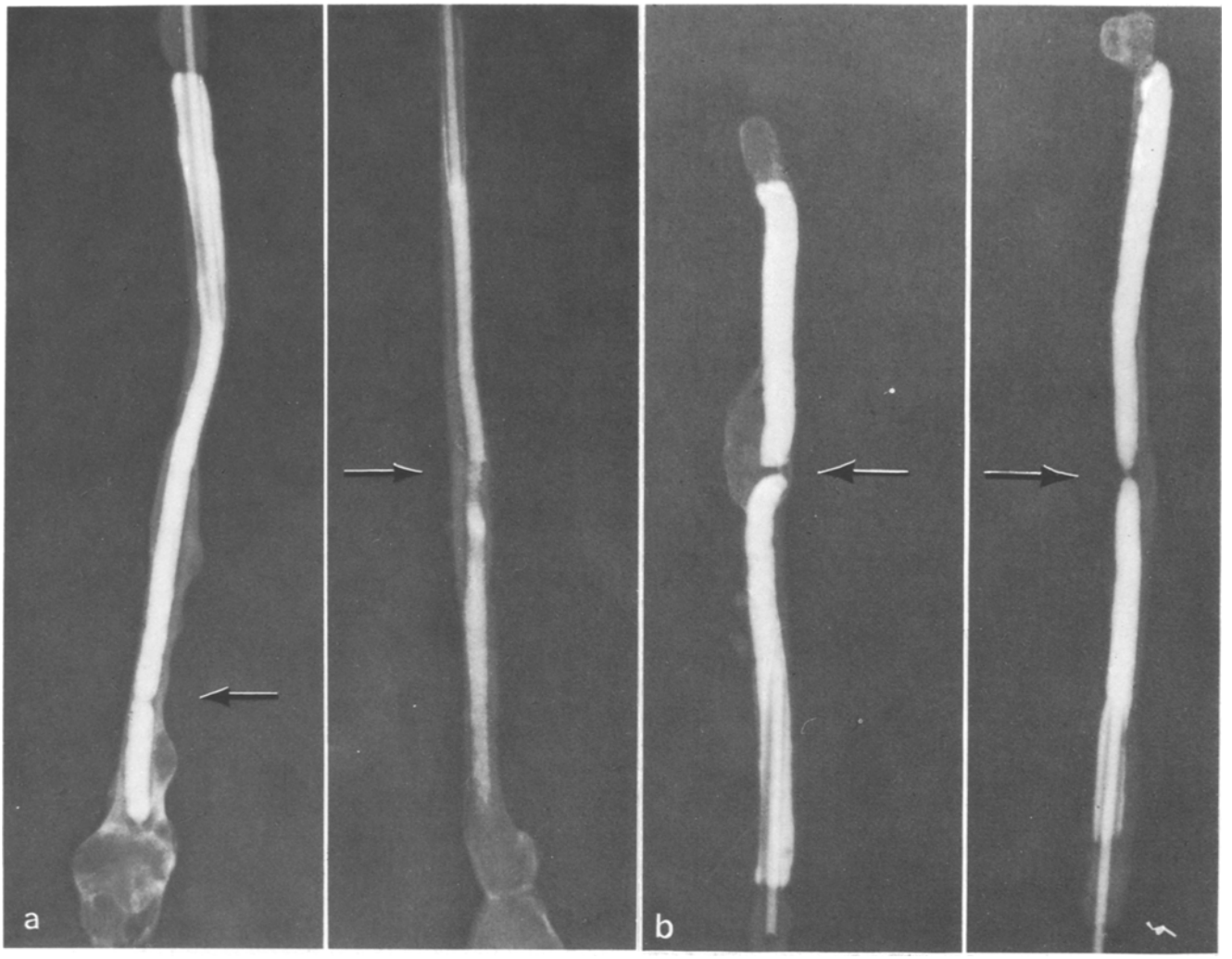


Fig. 4. Vasography after glueing. The glueing points are marked by arrows. a Smooth patency of the anastomosis, b marked stenosis in the anastomosis region. The contrast medium can still pass.

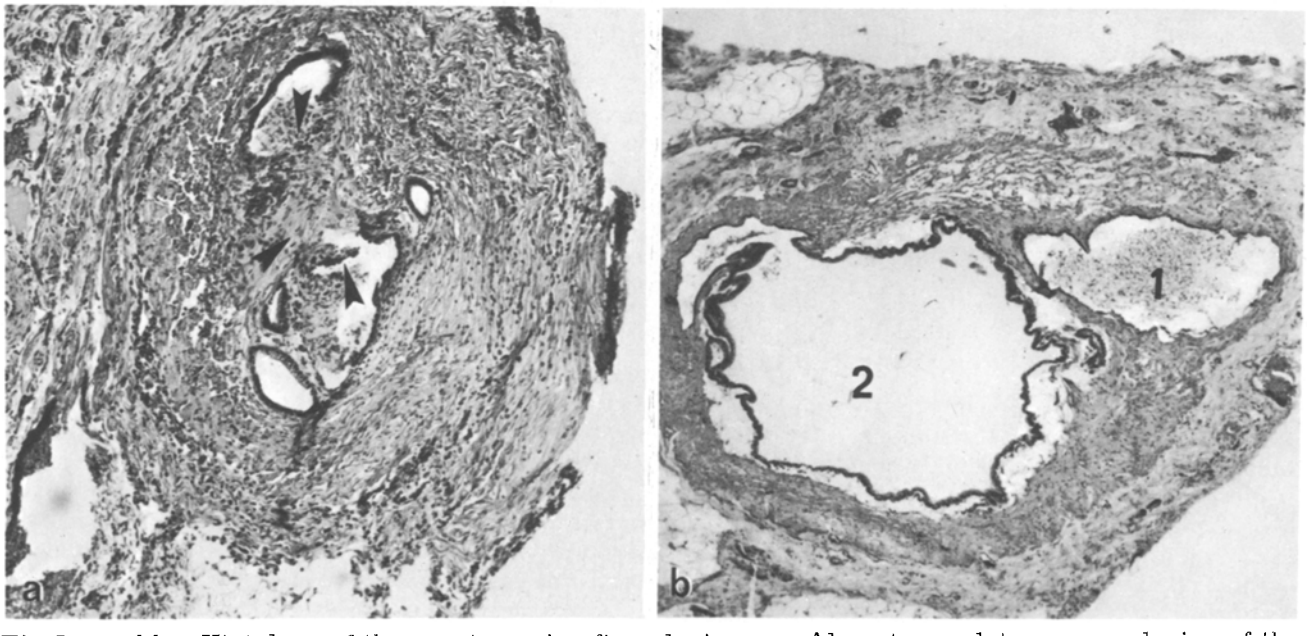
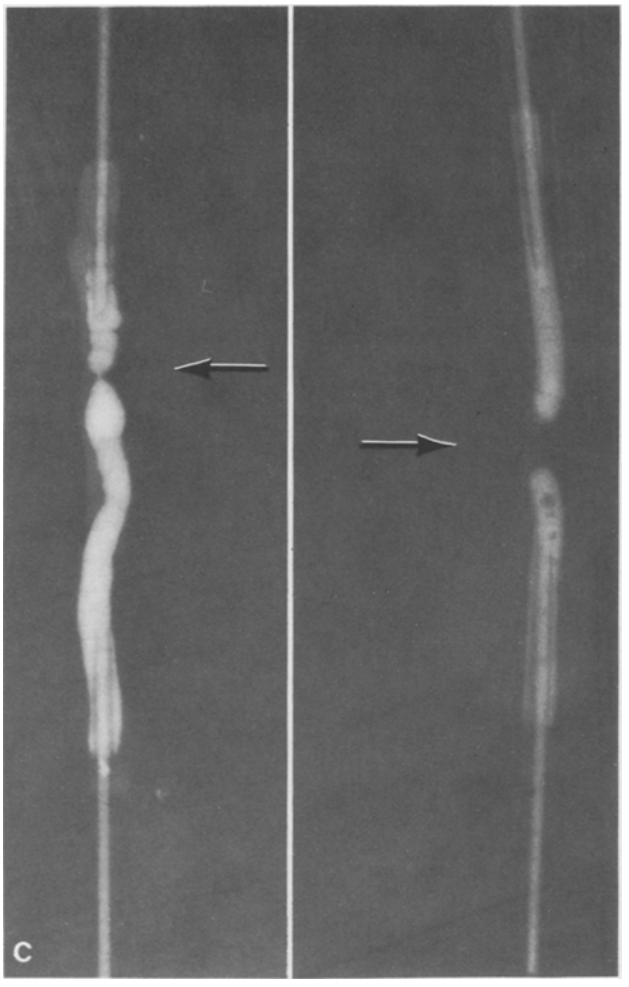


Fig 5 a and b. Histology of the anastomosis after glueing. a Almost complete scar occlusion of the lumen (arrows). Epithelial destruction. Hematoxylin-eosin staining, magnification x 60. b Several sections of an obstructed anastomosis after glueing. In the proximal stump (1) spermatozoa. Empty lumen in the distal stump (2). Hematoxylin-eosin staining, magnification x 60



c Occlusion of the anastomosis. Vasography distal and proximal to the obstruction

We were unable to observe any particular advantages with silastic and chromic catgut as splinting material. The postoperative results hardly differ in the two groups. Besides, fixation of the silastic tube and finding it again in a second operation was problematic. The time for which it was left in (10 or 20 days) did not have any influence on the result. Our results show that the patency of anastomosed vasa deferentes should be evaluated by examination of the ejaculate. The radiological finding only permits a definite statement when there is bilateral occlusion.

In these cases, the ejaculate never contains spermatozoa. On the other hand, stenoses are functionally insignificant, since most ejaculates of these animals contained spermatozoa.

The histological picture only admits of a limited statement as to the success or failure of glueing. Pflüger has shown that despite a radiologically patent anastomosis, a stenosis could always be demonstrated by serial histological sections (16). Only unequivocal occlusions can be evaluated histologically with confidence when accumulations of spermatozoa are seen.

According to our studies, an end-to-end anastomosis of the vas deferens is possible using fibrin glue. There is no doubt that the patency rate can be appreciably improved if splinting is carried out uniformly with a temporary splint. A requirement for a potentially successful outcome is exact approximation of stumps. In our experimental series, there was no development of semen granulomas, which according to Silber

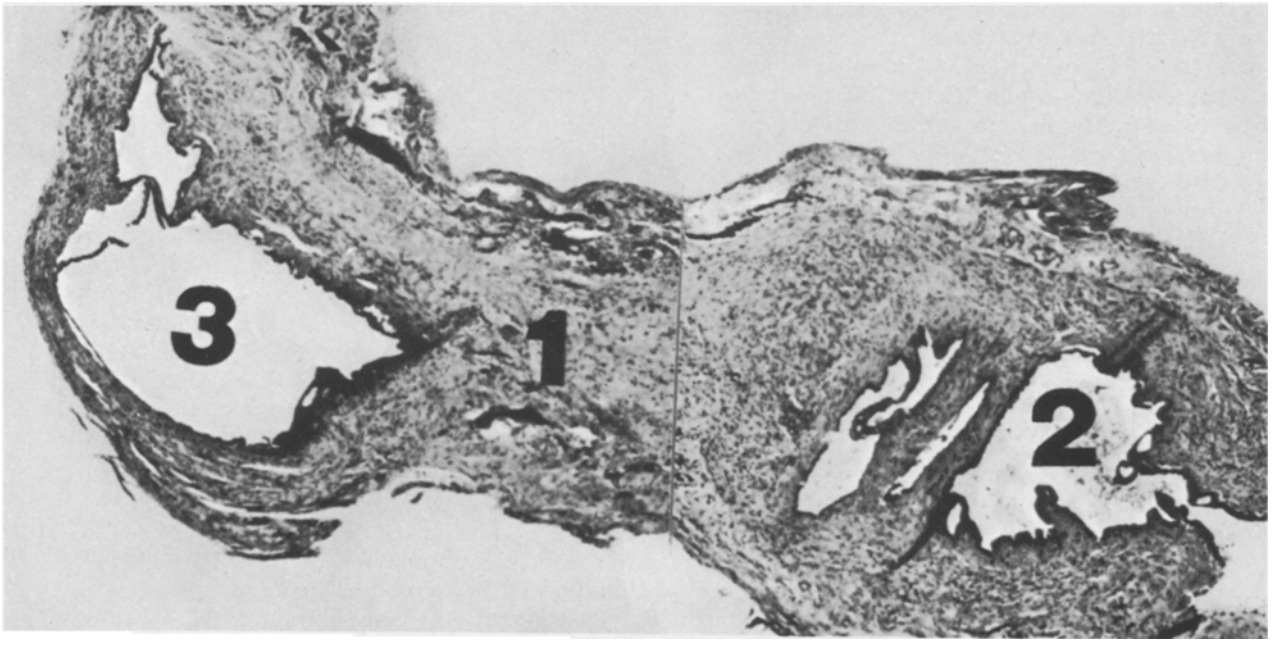


Fig. 6. Longitudinal section of an obstructed anastomosis. Long scar (1), proximal stump with spermatozoa (2), empty lumen in the distal stump (3). Hematoxylin-eosin staining, magnification x 60

(19) have a favourable influence on the semen quality after reanastomosis. The three cases of anastomosis dehiscence may be attributable to errors in replacement of the spermatic ducts. On the other hand, it is conceivable that the rate of dehiscence can be reduced by addition of antifibronolytics, as is usual in the hospital. Suture of the vas deferens with the surgical microscope using a temporary short-term splint appears to be the method of choice so far in vasovasostomy. Trials of glueing, which has the advantage of simplicity, should therefore be repeated under the conditions of microsurgery.

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