Vasectomy and vasectomy reversal : development of newly designed nonabsorbable polymeric stent for reconstructing the vas deferens

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Chapter 6

A polymeric mini-stent designed to facilitate the vasectomy reversal operation. A model study in rabbits.

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Introduction

Vasectomy has become widely accepted as a safe and reliable method for contraception[1-3]. Worldwide, more than 40 million couples rely on vasectomy to prevent pregnancy. The procedure is fast and reliable, and the long-term complication rates are low, i.e., in the range 0.08 – 0.10 %. The popularity of vasectomy has induced, however, an increasing demand for its reversal operation, which is known as vasovasostomy. In essence, vasovasostomy is bilateral microsurgical rejoining of the loose ends of the vas deferens. A recent population-based study by Holman et al. [4], which was performed on a large cohort of vasectomy patients (>28,000) in Western Australia, showed that the population rates of vasectomy are stable, while the incidence of seeking a reversal has increased. The most important factors are: age, being single, being divorced, and being separated. Age appears to be an important factor: for men who had the vasectomy between 20 and 24 years of age, it was found that 11 % seek reversal within the first 15 years. For the cohort 25 – 29 years, this percentage was 6 [4].

Compared to vasectomy, vasovasostomy is a technically demanding operation. Modern microsurgical techniques, pioneered by Silber, represent the golden standard [5,6]. From literature, it can be concluded that the rates for patency and maternity are in the range 85–90 % and 50–60 %, respectively. Scarification and stricturing are believed to be the major causes of failure. According to Silber's original concept, the anatomoses are made in two layers. Some urologists prefer a modified one-layer technique, which is easier to perform, quicker, and believed to induce less scarification and stricturing. Fischer and Grandmyre compared the two techniques, and found that the patency rates are comparable [7] . However, the mean operation time was 167 min for the two-layer technique, and 96 min for the modified one-layer procedure. There have been several attempts to improve the technique for vasovasostomy further. For example, Seaman reported preliminary experiments with a laser technique to

facilitate vasovasostomy surgery [8], and Rothman et al. described a clinical trial with an absorbable polymeric stent for vasectomy reversal [9]. The absorbable stent, however, resulted in lower patency rates (81 % vs. 90 %), and lower pregnancy rates (22 % vs. 51 %), as compared to two-layer microscopic vasovasostomy.

Herein, we report our first experience with a new vasovasostomy technique, which is based on the use of a non-absorbable polymeric hollow mini-stent. This device is implanted to keep both vas deferens lumens open and exactly in line. We postulated that use of the ministent will decrease the risk for scarification and stricturing, provided that the biomaterial is sufficiently biocompatible. Furthermore, it was anticipated that the mini-stent will facilitate the operation. Biodegradability was considered to be of minor importance.

At the onset of this study, we decided to use a biomaterial on the basis of NVP. Poly (NVP) is well-known for its excellent biocompatibility and safety. For example, NVP-containing copolymers were found to adhere to porcine intestine to create a hydrophilic surface for cell adhesion and growth. As in our previous work[10], we copolymerized NVP with n-butylmethacrylate (BMA) [11,12]. These copolymers do not dissolve in water, and the NVP/BMA ratio provides a means to control the hydrophilicity and the degree of swelling. Furthermore, the material was cross linked by incorporating tri (ethyleneglycol) dimethacrylate in the reaction mixture. The exact composition was chosen on the basis of two criteria and a series of preliminary experiments. The criteria were:

* The material must have sufficient rigidity in the dry state to allow for precision machining.
* Immersion in water must lead to considerable softening, keeping the dimensions virtually unchanged.

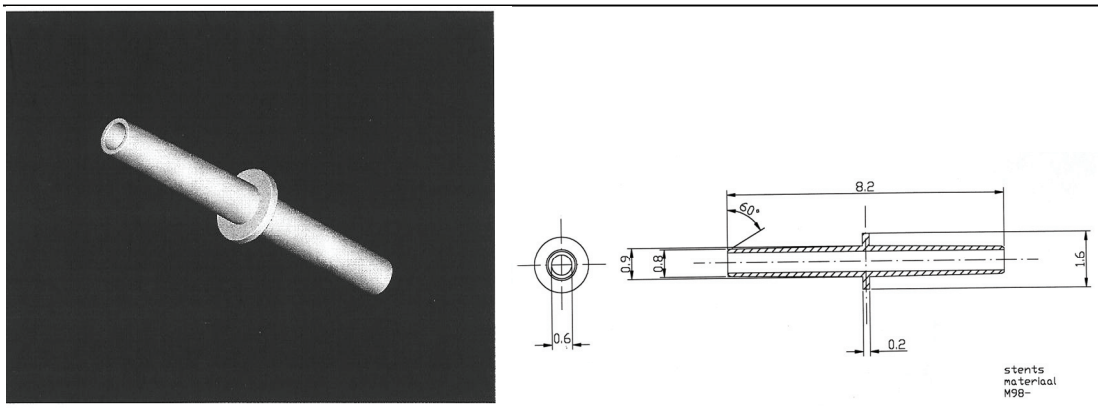

Fig 1. *Design of the polymeric mini-stent.* ***a,*** *Computer-generated picture, showing the tapered ends and the ridge in the middle of the mini-stent. The ridge is sandwiched between both ends of the vas deferens, thus preventing migration of the mini-stent.* ***b****, Exact geometry (millimetres).*

We postulated that the phenomenon of softening and swelling could be utilized, especially to facilitate implantation of the mini-stent. A procedure consisting of three steps was developed:

* First, one half of the mini-stent is carefully inserted into the lumen of the vas deferens, i.e, till the end of the vas reaches the ridge. The rigidity of the biomaterial is advantageous for this step; softening of the stent-half starts after its positioning in the vas lumen.
* Secondly, the other stent half is inserted into the lumen of the other vas end. Again, this is facilitated by the hardness of the dry stent-half. It is essential to perform this step immediately after the first one, in order to avoid premature softening.

* Thirdly, sutures are placed, such that the ridge is sandwiched between both vas ends. The third step should be performed 2 - 3 minutes later than the second one, to enable softening of the ridge prior to suturing.

We verified, in a small number of ex vivo pilots that preceded the animal experiments, that this three-step implantation technique is feasible in practice. Vas material from both rabbit and human origin was used in these pilots; size and structure of human and rabbit vas deferens are highly comparable.

Materials and Methods

Polymere synthesis and mini-stent manufacture.

All reactive monomers were purchased from Aldrich, and were purified by distillation in vacuo. 1-Vinyl-2-pyrrolidinone (25.83 g, or 232.4 mmol), n-butyl methacrylate (14.22 g, or 100.0 mmol), and tri(ethyleneglycol) dimethacrylate (191 mg, or 666 μmol) were transferred into a 100-mL round bottom flask. The radical initiator 2, 2'-azobis (2-methylpropionitrile) (AIBN, 24.0 mg, or 146 μmol) was added and the contents of the flask were thoroughly mixed. The reaction mixture was transferred into several Teflon® tubes, and the polymerization was executed as described [11]. The polymerization reaction proceeded smoothly and reproducibly. A specimen of the material (weight: 0.86 g) was incubated in distilled water (25 mL, 25 ^{o}C) for a period of 1 week. Water uptake and swelling were observed during the first 12 h; no further changes occurred afterwards. After the week of incubation, the aqueous medium was separated and lyophilized. The residue was dissolved in 1 mL of D_2O, and subjected to analysis by ^{1}H NMR spectroscopy at 400 MHz. Only a trace of residual NVP was detected; back-calculation revealed that the original sample contained less than 10 mg of NVP, which is approximately 1 % of the monomer that was present originally. It was established in a separate control

experiment that no free NVP evaporates during lyophilization. A conversion rate of approximately 99 % is not exceptional for laboratory-scale bulk polymerizations. The biomaterial was obtained as cylindrical rods, which were slightly opaque. The rods were cut in pieces of approximately 20-mm length, which were machined on a computer-controlled lathe/mill system (Boley, Eslingen, Germany; type: BDN 160 R). A 1.00-mm hole was first drilled along the cylindrical axis of the specimen. A straight steel wire was fitted into the hole, to reinforce the delicate material during machining toward the desired shape.

Animal experiments.

All animal experiments were done according to the Principles of Laboratory Animals Care [prepared by the National Institutes of Health (NIH Pub. No. 85-23 rev. 1995]. The study was approved by the Animal Experimental Committee of the University of Maastricht. All animals were male New Zealand white rabbits, aged between 6 and 12 months at the start of the experiments. Animals were anesthetized through intramuscular injection of ketamine (0.5 mL/kg) and xylazine (0.5 mL/kg). During the operation, a mixture of ketamine and xylazine (2 : 1) was administered intravascularly at a rate of 0.2 mL per 30 min. Animals were placed in a dorsal position, shaved, and sterilized. A transverse skin incision was made over both spermatic funiculi. The spermatic internal fascia was opened, and the vas deferens, lying loose within the funiculus, was easily luxated leaving the scrotal contents in situ. The vas was cut in a transverse way, and reconstructed immediately. Mini-stents were implanted bilaterally in the first group, after which both vas ends were approximated with three sutures of one-layer Prolene® 8.0 overlying the stent (a microscope was used). The second group underwent microscopical reconstruction with 4 to 5 one-layer Prolene® 8.0 sutures. For both groups, the vas was replaced within its funiculis and the spermatic internal fascia and overlying skin were closed

with 4.0 Vicryl®. Postoperatively, the animals received Temgesic (0.1 mL/kg) for a maximum of 48 h.

Semen samples were collected several days before the operation, and at regular time intervals after the operation, vide infra. The samples were obtained with the help of an artificial vagina system, as is normally used in agricultural artificial insemination stations for rabbits. At the time of sacrifice, the animals were deeply anesthetised, and then euthanised by an overdose injection of pentobarbital. Explanted *vas deferens* specimens (with or without mini-stent) were subjected to standard histopathological analysis (light microscopy, staining with hematoxylin and eosin).

Results and Discussion

Thirthy-two male rabbits were included. First, semen analyses of all animals were performed; bundant numbers of sperm cells were found in almost every case (Tables 1 and 2). Vasectomy was performed on a small control group of 4 rabbits, with the aim to determine how much time is needed to obtain azoospermia. To the best of our knowledge, this information is not available in the literature. Semen analyses after 6 weeks and after 3 months showed complete azoospermia in all four cases. The four animals were then sacrificed. The remaining 28 rabbits were randomized over two equal groups. The first group underwent vasovasostomy with the mini-stent. One animal in this group died unexpectedly, soon after the operation, because of a gastric hair-ball. The second group underwent conventional vasovasostomy, i.e. using one-layer microscopic surgery. We also lost one animal in this group, because of pneumonia. At least 4 post-operative semen analyses were performed for each animal; in each case the last sample

was obtained just prior to sacrifice and autopsy. The results of these analyses are compiled in Tables 1 and 2.

Table 1. *Sperm cell concentrations measured for the rabbits with two implanted mini-stents.*

Number	Sperm cells per mL, preoperative (millions)	Postoperative semen analysis at weeks	Mean number of sperm cells per mL, post-operative (millions)	Final number of sperm cells per mL (millions)
Mini-Stent Group				
1	160	4, 6, 9, 12	115	209
2	107	2, 4, 10, 13	403	710
3	1400	8, 12, 16	445	871
4	316	7, 13, 25, 37, 42	774	770
5	261	7, 17, 31, 36, 41	454	520
6	205	7, 17, 17, 31, 36, 41	540	1268
7	300	14, 31, 33, 38, 41	439	375
8	54	7, 22, 28, 31, 33	361	423
9	755	17, 24, 27, 28	663	425
10	845	17, 24, 27, 29	505	690
11	193	13, 23, 26, 28	395	371
12	125	13, 23, 26, 28	327	187
13	980	11, 21, 25, 26	569	76
Average	439	-	461	530
s.d.	418	-	162	326

Table 2. *Sperm cell concentrations measured for the rabbits in the control group, on which conventional end-to-end vasovasostomy was performed.*

Number	Sperm cells per mL, preoperative (millions)	Postoperative semen analysis at weeks	Mean number of sperm cells per mL, post-operative (millions)	Final number of sperm cells per mL (millions)
End-to-end Group				
1	850	8, 14, 26, 38, 42	410	651
2	760	8, 14, 26, 38, 42, 44	300	233
3	324	7, 13, 25, 37, 41	312	360
4	785	7, 13, 26, 37, 42	651	960
5	414	7, 23, 37, 42, 47	531	258
6	625	7, 13, 25, 37	570	570
7	766	13, 30, 37, 40	437	450
8	249	13, 31, 37, 40	380	439
9	889	16, 23, 26, 28	420	620
10	324	16, 23, 26, 28	641	520
11	477	15, 22, 26, 29, 30	441	1267
12	283	15, 25, 28, 30	270	215
13	325	15, 22, 28, 40	492	430
Average	544	-	450	536
s.d.	240	-	123	298

The mini-stent remained patent in all 13 cases (Table 1). There was generally a high concentration of living sperm cells in the semen samples which were taken prior to autopsy. Preoperatively, the average sperm cell concentration in this group was 439 million / mL with

a very high spread (s.d. = 418 million / mL). Prior to autopsy, this average was 530 million / mL (s.d. = 326 million / mL). Using the Student's t-test (two-tailed distribution, paired), it followed that $p = 0.525$, i.e. the sperm counts in the two series are not significantly different. Histopathology of the explants showed that mini-stent is well-tolerated in the rabbit vas deferens. Signs of a mild inflammatory response were usually seen in the surrounding tissue. Most likely, this is a normal reaction to the presence of a foreign polymeric biomaterial. Remarkably, the epithelium that constitutes the highly curved lumen of the normal vas deferens, adapts to the presence of the mini-stent (Figure 2). None of the implants were surrounded by a fibrous capsule. In many cases, sperm cells were observed inside the lumen of the mini-stent (Figure 2d). Rabbit # 13 was matched with a female partner, in order to establish whether the mini-stent allows actual propagation. The female became pregnant, and gave birth to eight young rabbits after 4 weeks of pregnancy.

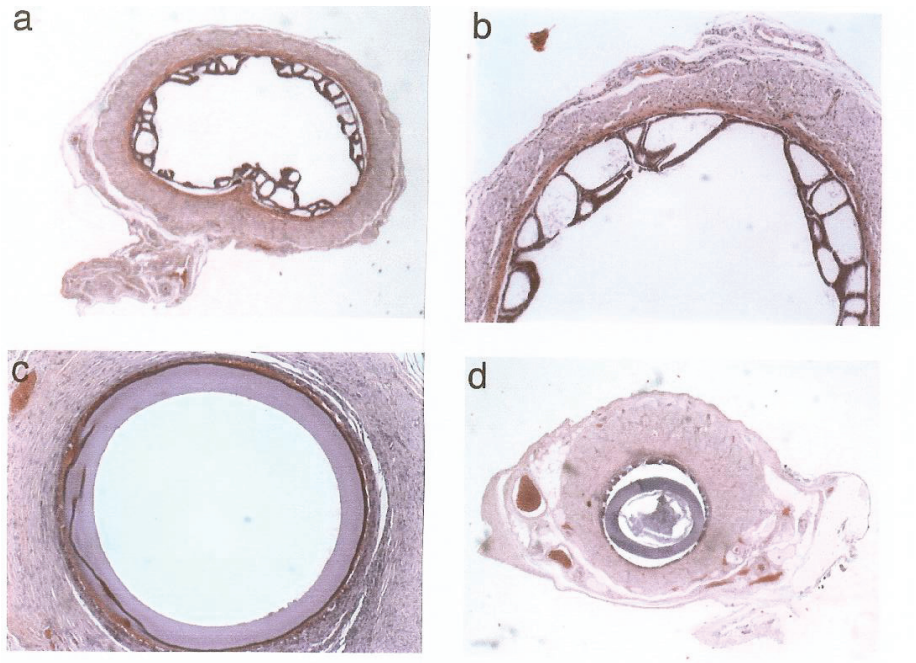

Fig 2 *Histology of the vas deferens explants (light microscopy, staining with haematoxylin and eosin).* ***a, b,*** *Normal rabbit vas deferens (transverse view at two different enlargements), showing the pronounced curvature of the epithelium. All cell nuclei, especially those of the smooth muscle cells in the outer layers of the vas deferens, are clearly visible.* ***c,*** *Transverse view of a representative explant that contains a mini-stent. This section was cut in the central part; note that the epithelium lies as a circular band adjacent to the exterior surface of the mini-stent.* ***d,*** *View as in c, but now cut close to the proximal end. Here, the vas lumen is wider than the stent, thus leaving an interstitial space around the stent. Spermatozoa were observed in the lumen of the mini-stent, as well as in the interstitial space*

The data in Table 2 show that all end-to-end operations were also successful. In this group, the average sperm cell concentration prior to the operation was 544 million / mL (s.d. = 241 million / mL). Prior to the different autopsies this average was 536 million / mL (s.d. = 298 million / mL). These data reflect that conventional end-to-end vasovasostomy is a well-established technique. The operation is associated with a very

high rate of success, provided that the skilled surgeon can work in a suitable infrastructure and with adequate equipment.

Both methods led to 100 % patency, and the ultimate sperm counts were generally very high. Using the Student's t-test (two-tailed distribution, paired) to compare the arrays of sperm counts (prior to autopsy) for two groups, a p-value of 0.961 was found. This indicates that the outcome of both groups is not statistically different at all.

It was experienced that vasovasostomy with use of the mini-stent was faster and easier to perform. The data in Table 3 show that the bilateral operation is accelerated by approximately 30 min, due to the use of the mini-stent. For the mini-stent group, an average operation time of 98 min was found (s.d. = 16 min). For the end-to-end group, this average was 132 min (s.d. = 19 min). Based on the Student's t-test (two-tailed distribution, paired), it can be concluded that the operation times for the two groups are statistically different ($p < 0.0005$).

Table 3. *Operation times measured during the vasovasostomy operations of this study*.

End-to-end group		**Mini-stent group**	
Rabbit number	Operation time (min)	Rabbit number	Operation time (min)
1	142	7	98
2	148	8	92
3	131	9	110
10	168	11	111
13	123	12	62
14	131	15	111
16	142	17	75
21	139	18	83
23	111	25	105
24	99	26	113
29	121	27	98
30	149	28	107
31	112	32	109
Average	132	-	98
s.d.	19	-	16

The patency rate for microsurgical vasovasostomy in humans is approximately 85-90 % [4,13,14]. Most probably, the higher patency rates in our model can be ascribed to the fact that the rejoining of the vas deferens was performed immediately after dissectioning; the time interval between vasectomy and vasovasostomy in clinical practice is usually in the order of several years.

Recently, it has become clear that vasovasostomy is the therapy of choice for obstructive azoospermia after vasectomy [15]. Kolettis et al. showed that the cost per living birth after vasovasostomy is approximately 31,000 US $, while the cost per living birth after epididymal sperm aspiration / intracytoplasmic sperm injection is around 51,000 US $ [16]. More recently, Heidenreich et al. also concluded that vasovasostomy is preferred over its alternatives [17,18]. Our next step is to investigate the utility of the mini-stent in humans. The two questions to be answered are, evidently:

1. Is implantation of the mini-stent in humans as successful as conventional vasovasostomy, in terms of short-term and long-term complication rates?
2. Is the use of the mini-stent cost-effective because of shorter operation time?

Moreover, the mini-stent may provide an alternative option in those cases where conventional vasovasostomy has failed, e.g. as a result of stricturing at the anastomosis. The mini-stent may also offer new possibilities to execute vasovasostomies under less stringent circumstances (a microscope is not necessary), e.g, in clinics in developing countries.

Conclusion

This animal study indicates that the vasovasostomy operation can be simplified and accelerated by using a polymeric hollow mini-stent. From the perspective of biomaterials science, it is encouraging that the novel NVP-based hydrophilic biomaterial can, apparently, enable transport of spermatozoa, which are notoriously vulnerable, through the anastomosis.

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