

Vasovasostomy in rabbits after vasectomy or vas occlusion by tantalum clip

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Summary. Vasovasostomy was performed in rabbits that had been vasectomized or had the vas occluded by a tantalum clip for 6 or 15 months. Vasovasostomy restored fertility to 40–50% in vasectomized rabbits and 60–70% in the tantalum clip-occluded animals. Any changes that occurred due to vasectomy and tantalum clip occlusion (e.g. testicular weight, enzyme concentrations in the testis) returned to normal within 3–6 months of vasovasostomy. We suggest that vas occlusion with tantalum clips is a good method of surgical male sterilization and has a high success rate for restoration of fertility.

Introduction

The major limitation in the use of vasectomy is that its reversibility can not be assured and there is a need for another method of vas occlusion with higher success rate of reversibility. During the past decade the technique of vas re-anastomosis has been used (see Sciarra, Zatuchni, Speidel & Osborn, 1978). The anatomical success rate, i.e. patency of the duct, ranges from 38 to 100%, but the functional success rate (i.e. pregnancy) is about 10–71% (Roy & Taneja, 1974; Silber, 1981). Although the exact cause of the low fertility after vasovasostomy is not known, a number of factors, such as the technique used for recanalization (Silber, 1978), development of immunological response (Ansbacher, 1971; Shulman, Zappi, Ahmed & Davis, 1972; Alexander, Wilson & Patterson, 1974) and impaired testicular (Singhal, Kinson & Tsang, 1977; Lepow & Crozier, 1979) and accessory reproductive organ (Pardanani, Patil & Pawar, 1976) function after vasectomy, may be involved. In the present investigation we have studied testicular and accessory sex organ functions in rabbits before and after vasovasostomy to assess the relative suitability of vasectomy and vas occlusion by tantalum clip for contraception and its reversal.

Materials and Methods

New Zealand White rabbits of proven fertility and weighing 1.5–1.7 kg were used. The 70 males were housed individually in wire mesh cages and fed a commercial diet (Hindustan Lever Ltd). Water was provided *ad libitum*. The animals were allocated to groups for bilateral vasectomy (Group V, N = 30), tantalum clip occlusion (Group TC, N = 30) and sham operation (Group C, N = 10). Before surgery semen samples were collected from all animals, using an artificial vagina (Walton, 1958). Animals were anaesthetized with thiopentone sodium (20 mg/kg body wt, i.v.) for surgery. Vasectomy was performed by the removal of a 0.5–1 cm piece of each vas and double ligations. Tantalum clip occlusion involved application of one clip of medium size on each vas. Sham operation involved exteriorization of the vas but no cutting or ligations.

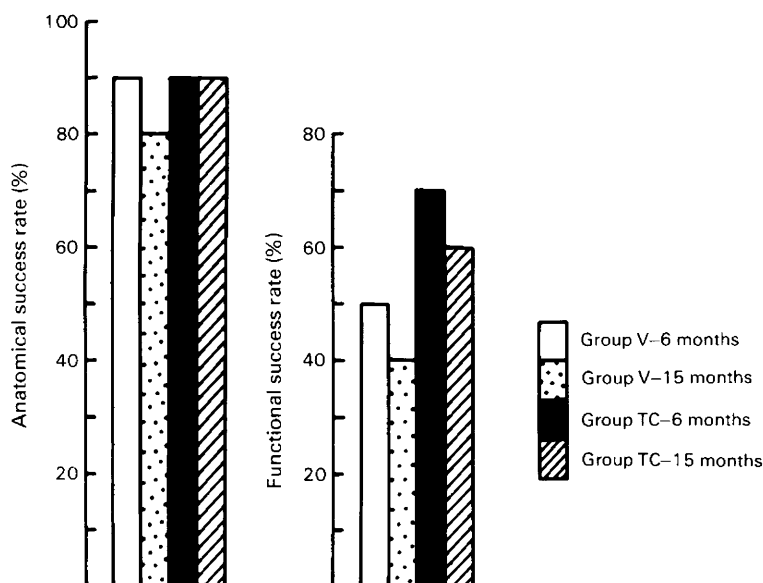
At 6 and 15 months after surgery 10 animals from Groups V and TC were bilaterally

vasovasostomized. The vasal ends were freshened by removal of scar tissue and were anastomosed: a single layer of 6–8 mattress sutures of 7·0 chromic cat-gut was placed with the support of Silastic tubing (1·19 mm o.d.) and the support was removed through a puncture of the vasal ampulla after completion of anastomosis. Semen samples were collected at different times after vasovasostomy and were examined for seminal characteristics (WHO Method Manual, 1980). When the number and motility of spermatozoa returned to the control range, the animals were allowed to mate. Mating was confirmed by the presence of spermatozoa in the vaginal smear and functional success rate was assessed by the occurrence of pregnancy.

Semen weight, volume and pH were measured. Seminal plasma concentrations of fructose (Mann, 1964), phosphatases (Gutman & Gutman, 1940), lactate dehydrogenase (Cabaud & Wroblewski, 1958), magnesium (Neill & Neely, 1956) and citric acid (Lindner & Mann, 1960) were estimated in samples collected before and 1, 3 and 6 months after vasovasostomy or sham operation.

Animals subjected to vasectomy or tantalum-clip occlusion were killed before ($N = 5$ of each group) and at 3 ($N = 5$ of each group) and 6 ($N = 5$ of each group) months after vasovasostomy. Sham-operated animals were killed 6 and 15 months after surgery. Body and testicular weights were recorded. A small piece of testis from each animal was fixed immediately in Bouin's fluid for routine histology. Sections were examined under the light microscope. To evaluate testicular degeneration quantitatively, tubules showing spermatogenesis at various stages were counted. Seminiferous tubule diameter, Sertoli cell nuclear diameter and Leydig cell nuclear diameters were also measured. Glycogen (Montgomery, 1957), total lipid (Folch, Lees & Sloane-Stanley, 1957), total cholesterol (Zlatkis, Zak & Boyle, 1953), phospholipid (Zilversmit & Davis, 1950), sialic acid (Svennerholm, 1960), total protein (Lowry, Rosebrough, Farr & Randall, 1951), phosphatases (Fiske & Subbarow, 1925), fructose (Mann, 1964), citric acid (Lindner & Mann, 1960) and RNA (Ceriotti, 1955) values in the testicular tissue were determined.

Student's *t* test was applied for statistical analysis.



Text-fig. 1. Anatomical (sperm positive) (a) and functional (pregnancy positive) (b) success rates of vas re-anastomosis in rabbits in relation to the duration and techniques of vas occlusion.

Results

Success rate

As shown in Text-fig. 1, anatomical patency was similar in Groups V and TC but functional success was higher ($P = 0.1$) in Group TC animals.

Semen quality

Motile spermatozoa re-appeared in the ejaculate 28–45 days after vasovasostomy (Table 1) and sperm motility and the percentages of live and abnormal spermatozoa returned to normal values by 60–75 days after surgery.

Biochemical findings

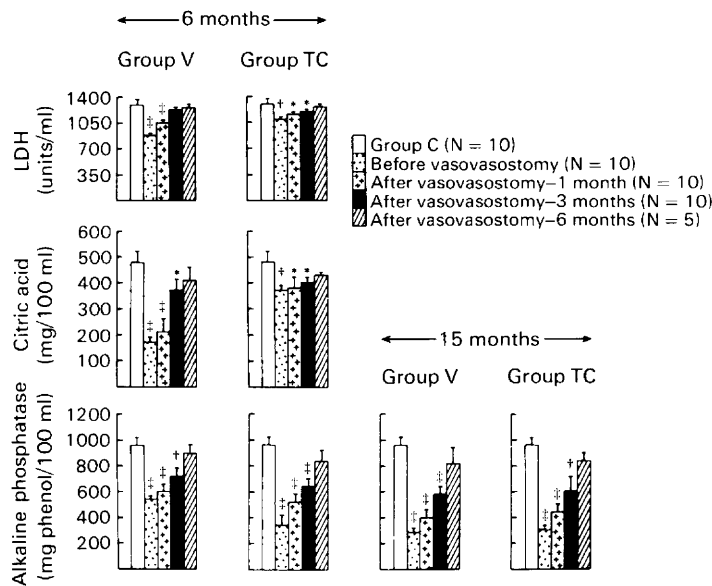
Semen. Alkaline phosphatase, LDH and citric acid values were decreased at 6 and 15 months after surgery in Groups V and TC but values returned to normal 3–6 months after vasovasostomy (Text-fig. 2). Semen weight, volume, pH and seminal plasma fructose, acid phosphatase and magnesium did not change during the period of study when compared with values for the sham-operated controls.

Table 1. Semen quality after vasovasostomy of rabbits in Groups V (vasectomy) and TC (tantalum clip occlusion) at 6 and 15 months

Months of treatment	Days after vasovasostomy	No. of animals	Sperm count ($\times 10^6/\text{ml}$)		Motility		Spermatozoa (%)			
			Group V	Group TC	Group V	Group TC	Live		Abnormal	
							Group V	Group TC	Group V	Group TC
6	0	10	179 \pm 24	189 \pm 21	4	4	96.3 \pm 4.6	98.11 \pm 3.8	9.2 \pm 2.3	3.0 \pm 0.7
	7	10	0	0	0	0	0	0	0	0
	15	10	0	0	0	0	0	0	0	0
	21	10	0	0	0	0	0	0	0	0
	28	10	0	20 \pm 6	0	1	0	74.8 \pm 5.5	0	3.4 \pm 0.3*
	45	10	8 \pm 4	60 \pm 17	1	2	68.1 \pm 9.2	86.7 \pm 4.4*	7.6 \pm 0.9*	2.1 \pm 0.5
	60	10	50 \pm 19	120 \pm 20*	2	4	80.0 \pm 7.3*	92.4 \pm 3.9	12.2 \pm 0.7	1.8 \pm 0.7
	75	10	120 \pm 9*	187 \pm 25	1	4	92.1 \pm 4.5	96.6 \pm 8.2	10.0 \pm 1.2	3.5 \pm 0.4
	90	10	190 \pm 32	221 \pm 10	3	3	93.3 \pm 5.4	95.1 \pm 2.4	9.0 \pm 2.4	4.2 \pm 0.5
	120	5	180 \pm 26	200 \pm 11	4	4	94.7 \pm 7.7	97.7 \pm 3.3	6.8 \pm 1.4	2.0 \pm 0.7
	180	5	186 \pm 41	195 \pm 33	3	4	91.4 \pm 3.3	96.3 \pm 4.5	4.7 \pm 0.7	2.5 \pm 0.6
15	0	10	139 \pm 27	152 \pm 24	4	4	93.7 \pm 5.8	95.2 \pm 4.2	8.6 \pm 2.0	5.2 \pm 0.8
	7	10	0	0	0	0	0	0	0	0
	15	10	0	0	0	0	0	0	0	0
	21	10	0	0	0	0	0	0	0	0
	28	10	0	30 \pm 5	0	1	0	57.0 \pm 7.9	0	8.5 \pm 0.3*
	45	10	5 \pm 3	60 \pm 7	1	2	49.0 \pm 10.1	62.0 \pm 6.4	10.2 \pm 0.9*	7.0 \pm 0.2
	60	10	45 \pm 10	122 \pm 13*	1	3	84.0 \pm 8.8*	79.4 \pm 8.8*	12.4 \pm 0.4	9.4 \pm 0.5
	75	10	95 \pm 20*	198 \pm 36	2	4	82.5 \pm 4.5	80.0 \pm 2.3	8.9 \pm 0.3	4.7 \pm 0.6
	90	10	113 \pm 15	165 \pm 29	3	4	84.3 \pm 6.8	91.9 \pm 3.3	15.1 \pm 0.4	3.9 \pm 0.3
	120	5	170 \pm 31	140 \pm 21	2	3	89.4 \pm 5.4	89.9 \pm 4.1	9.7 \pm 0.7	4.4 \pm 0.7
	180	5	163 \pm 39	190 \pm 50	4	4	87.2 \pm 6.4	93.4 \pm 1.8	5.5 \pm 0.5	6.3 \pm 0.4

Values are mean \pm s.e.m.

* First value not significantly different from the presurgery value.

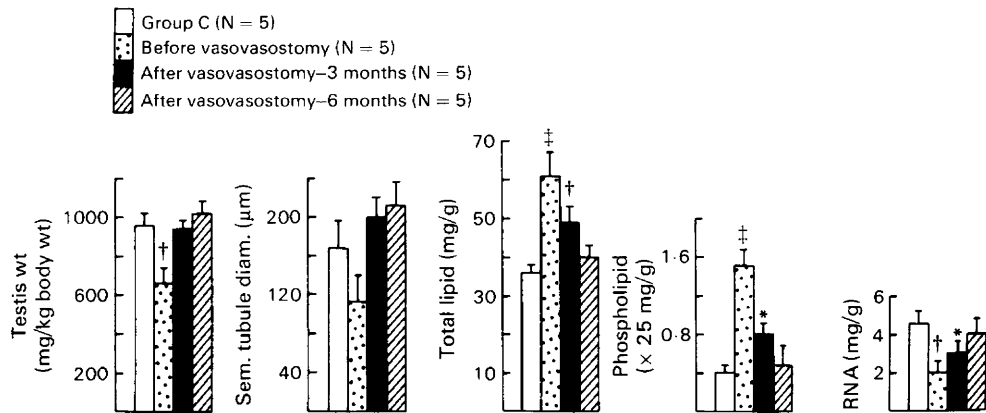


Text-fig. 2. Seminal plasma concentrations of alkaline phosphatase (ALP), LDH and citric acid in rabbits after sham operation (Group C), vasectomy (Group V), tantalum clip occlusion (Group TC) at various times after vasovasostomy. Values are mean \pm s.e.m. Compared with Group C values: * P < 0.05, † P < 0.01, ‡ P < 0.001.

Testes. Total lipid, phospholipid and RNA constituents were altered 6 months after vasectomy but returned to the normal range by 6 months after vasovasostomy (Text-fig. 3). There were no appreciable changes in glycogen, citric acid, acid phosphatase, alkaline phosphatase, total protein, sialic acid or fructose contents. None of these biochemical constituents changed detectably in the rabbits in Group TC at 6 and 15 months or in those in Group V at 15 months.

Testicular histology

The reproductive tract of vasovasostomized animals appeared normal. A decrease in testicular weight in Group V rabbits at 6 months was regained by 6 months after vasovasostomy (Text-fig. 3).



Text-fig. 3. Various characteristics of the testes in sham-operated rabbits (Group C) and rabbits vasectomized for 6 months (Group V). Values are mean \pm s.e.m. (N = 5). Compared with Group C values: * P < 0.05, † P < 0.01, ‡ P < 0.001.

All other Group V and TC animals had testicular weights within the control range. Quantitative degenerative changes occurring after 6 months in Group V were recovered by 6 months after vasovasostomy. All other animals had histologically normal testes. Leydig cell and Sertoli cell nuclear diameters were within the control range.

Discussion

The restoration of fertility after successful reanastomosis has been reported to be influenced by pressure-related changes in the testis (Silber, 1978, 1981). The pathological changes observed in the testes of long-term vasectomized rabbits did not recover after vasovasostomy (Hooker, 1980). However, Chapman *et al.* (1978) reported fine structural changes (infolding and duplication of the basal lamina, presence of late spermatids and sperm tails in the basal and perinuclear Sertoli cell cytoplasm) in the seminiferous tubules of unilateral vasoclasped and vasoligated rhesus monkeys and related these changes to the restoration of fertility after recanalization. Urry, Thompson & Cockett (1976) observed testicular histopathological and biochemical changes after vasovasostomy of dogs vasectomized for 6 months but suggested that these changes did not interfere with the reversibility of the procedure. The changes in seminal plasma values observed in the present study confirm previous observations on men (Naik, Pardanani, Joshi & Sheth, 1979; Medappa, 1981; Joshi, 1981) and suggest that accessory sex organ function which decreases after vasectomy returns to normal after vasovasostomy.

Nevertheless, the present results indicate that vas occlusion by a tantalum clip is similar to, if not better than, vasectomy in terms of lack of effects on biochemical measurements and the success rate for fertile matings.

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References

- Alexander, N.J., Wilson, B.J. & Patterson, J.C. (1974) Vasectomy: immunological effects in rhesus monkeys and men. *Fert. Steril.* **25**, 149–156.
- Ansbacher, R. (1971) Sperm agglutinating and sperm immobilizing antibodies in vasectomized men. *Fert. Steril.* **22**, 629–632.
- Cabaud, P.G. & Wroblewski, F. (1958) Colorimetric measurement of lactic dehydrogenase activity of body fluids. *Am. J. clin. Path.* **30**, 234–236.
- Cerioti, G. (1955) Determination of nucleic acids in animal tissues. *J. biol. Chem.* **214**, 59–70.
- Chapman, E.S., Heidger, P.M., Harrison, R.M., Roberts, J.A., Domingue, G.J. & Schlegel, J.U. (1978) Vasectomy in rhesus monkeys. IV. Electron microscopic studies of the seminiferous epithelium. *Anat. Rec.* **192**, 41–58.
- Fiske, C.H. & Subbarow, Y. (1925) Colorimetric determination of phosphorus. *J. biol. Chem.* **66**, 375–400.
- Folch, J., Lees, M. & Sloane-Stanley, G.H.S. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.* **226**, 497–504.
- Gutman, A.B. & Gutman, E.B. (1940) Determination of acid phosphatase in serum. *J. biol. Chem.* **136**, 201–209.
- Hooker, R.H. (1980) Changes in the testes and epididymides of rabbits following long-term vasectomy or vasocystostomy: correlation with results of vasovasostomy. *Biol. Reprod.* **22**, 297–306.
- Joshi, U.M. (1981) Endocrine and accessory sex organ function after vasectomy and vasovasostomy. *Archs Androl.* **7**, 187–191.
- Lepow, I.H. & Crozier, R. (Eds) (1979) *Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man*. Academic Press, New York.
- Lindner, H.R. & Mann, T. (1960) Relation between the content of androgenic steroids in the testis and the secretory activity of the seminal vesicle in the bull. *J. Endocr.* **21**, 341–360.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193**, 265–275.
- Mann, T. (1964) *The Biochemistry of Semen and the Male Reproductive Tract*, p. 239. Methuen, London.
- Medappa, N. (1981) Long-term effects of vasectomy: hypothalamopituitary-gonadal function. *ICMR Bull.* **11**, 37–41.
- Montgomery, R. (1957) Determination of glycogen. *Archs Biochem.* **67**, 378–389.
- Naik, V.K., Pardanani, D.S., Joshi, U.M. & Sheth, A.R. (1979) Seminal plasma concentration of glyceryl-

- phosphocholine before and after vasectomy and vasereanastomosis. *Fert. Steril.* **32**, 685–686.
- Neill, D.W. & Neely, R.A. (1956) The estimation of magnesium in serum using titan yellow. *J. clin. Path.* **9**, 162–163.
- Pardanani, D.S., Patil, N.G. & Pawar, H.N. (1976) Some gross observations of epididymides following vasectomy. *Fert. Steril.* **27**, 267–270.
- Roy, S. & Taneja, S.L. (1974) Vasectomy, vas occlusion and vasanastomosis, a critical appraisal, pp. 1–41. National Institute of Family Planning, Green Park, New Delhi.
- Sciarra, J.J., Zatuchni, G.I., Speidel, J.J. & Osborn, C.K. (Eds) (1978) *Reversal of Sterilization*. Harper & Row, Hagerstown.
- Shulman, S., Zappi, E., Ahmed, U. & Davis, J.E. (1972) Immunologic consequences of vasectomy. *Contraception* **5**, 269–278.
- Silber, S.J. (1978) Vasectomy and vasectomy reversal: microsurgical approach. In *Reversal of Sterilization*, pp. 34–39. Eds J. J. Sciarra, G. I. Zatuchni, J. J. Speidel & C. K. Osborn. Harper & Row, Hagerstown.
- Silber, S.J. (1981) Reversal vasectomy and the treatment of male infertility: role of microsurgery, vasoepididymostomy and pressure induced changes of vasectomy. *Urol. Clin. N. Am.* **8**, 53–62.
- Singhal, R.L., Kinson, C.A. & Tsang, B.K. (1977) The endocrine and biochemical consequences of vasectomy in mammalian systems. In *Advances in Sex Hormone Research*, pp. 259–307. Eds R. L. Singhal & J. A. Thomas. University Park Press, Baltimore.
- Svennerholm, L. (1960) Isolation and determination of neuraminic (sialic) acid by resorcinol reaction. In *Methods of Biochemical Analysis*, vol. 8, pp. 214–215. Ed. D. Glick. Interscience, New York.
- Urry, R.L., Thompson, J. & Cockett, A.T.K. (1976) Vasectomy and vasovasostomy. II. A comparison of two methods of vasovasostomy: silastic versus chromic stents. *Fert. Steril.* **27**, 945–950.
- Walton, A. (1958) Improvement in the design of an artificial vagina for the rabbit. *J. Physiol., Lond.* **143**, 26 p–28 p.
- WHO Method Manual (1980) Collection and examination of human semen. In *Laboratory Manual for the Estimation of Human Semen and Semen-Cervical Mucus Interaction*, pp. 9–32. Eds N. A. Belsey, R. Eliason, A. J. Gallegos, K. S. Moghissi, C. A. Paulsen & M. R. N. Prasad. Press Concern, Singapore.
- Zilversmit, D.B. & Davis, A.K. (1950) Microdetermination of plasma phospholipids by trichloro acetic acid precipitation. *J. Lab. clin. Med.* **35**, 155–160.
- Zlatkis, A., Zak, B. & Boyle, H.J. (1953) A new method for the direct determination of serum cholesterol. *J. Lab. clin. Med.* **41**, 486–498.

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