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Sperm autoantibodies as a consequence of vasectomy

I. WITHIN 1 YEAR POST-OPERATION

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SUMMARY

In a group of fifty-two vasectomized men, 1 year post-vasectomy, 73% showed sperm-agglutinating antibodies in the serum with titres of 8 to 1024 in the tray agglutination test, and 42% showed sperm-immobilizing antibodies with titres of 1 to 128 in a micro-immobilization test. 3 months post-operatively, about 75% of the men who were to develop antibodies within the year already possessed them. With sperm agglutinins there was a gradual increase in incidence in the period from 3 months to 1 year, with titres increasing up to the 9 month stage. At 1 year, 68% of the positive titres were 32 or higher. Although no increase in the incidence of sperm-immobilizing antibodies was seen beyond 6 months post-operatively, titres increased up to 1 year. A strong correlation was found between the titres of sperm-agglutinating and sperm-immobilizing antibodies, and all sera with an agglutination titre of at least 128 also showed sperm immobilization. Tail-to-tail agglutination was the most predominant type of agglutination observed. No correlation between the type of agglutination and the presence of sperm-immobilizing antibodies could be found. The total number of spermatozoa in a pre-vasectomy ejaculate (as a measure of antigen dose) could not be correlated with the incidence or titre of sperm-agglutinating or sperm-immobilizing antibodies.

These results are discussed in the context of possible interference with fertility if vasovasostomy is to be performed.

INTRODUCTION

In the sera of azoospermic men whose vasa deferentia were obstructed, but who otherwise had normal spermatogenesis, Rümke & Hellinga (1959) found a high incidence of sperm-agglutinating antibodies Obstruction and spermatostasis may lead to extravasation of sperm in the epididymis. Phadke (1964) and Alexander (1973a) observed ingestion of sperm by phagocytic cells in the epithelial wall of the tubuli of the epididymis. Both processes are considered as possible mechanisms by which spermatozoal antigens are brought into contact with the blood or lymph system, so being capable of inducing a humoral and/or cellular autoimmune response against spermatozoa. As a result of the obstruction of the efferent ducts, male sterilization by vasectomy would be expected to give rise to the formation of sperm autoantibodies. Sperm autoantibodies after vasectomy have been reported in rats (Rümke & Titus, 1970; Brannen & Coffey, 1974; guinea-pigs (Alexander, 1973b), rabbits (Bigazzi *et al.*, 1976a,b), Rhesus monkeys (Alexander, Wilson & Patterson, 1974; Alexander, 1977) and men (Ansbacher, 1971, 1973, 1974; Ansbacher, Keung-Yeung & Wurster, 1972; Shulman *et al.*, 1972; Alexander *et al.*, 1974; Van Lis, Wagenaar & Soer, 1974; Gupta *et al.*, 1975a; Samuel *et al.*, 1975; Tung, 1975; Linnet & Hjort, 1978). In man, these studies are mainly concerned with sperm-agglutinating and sperm-immobilizing antibodies. Samuel *et al.* (1975) and Tung (1975) reported, however, the occurrence of autoantibodies against

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human protamine, as detected by the indirect immunofluorescence technique on swollen sperm heads (Kolk, Samuel & Rümke, 1974). Moreover, in the indirect immunofluorescence technique, Tung (1975) also found antibodies directed towards the acrosome (speckled distribution) and the main piece of the tail, as a result of vasectomy.

Sperm autoantibodies in men have been commonly investigated with the gelatin agglutination technique (GAT) (Kibrick, Belding & Merrill, 1952), or with the immobilization test (Isojima, Li & Ashitaka, 1968). Recently, micro-techniques have been described for the detection of sperm-agglutinating antibodies (Friberg, 1974) and of sperm-immobilizing antibodies (Husted & Hjort, 1975a). The value of the micro-agglutination technique (also denominated the tray agglutination technique or TAT), as described by Friberg (1974), has been confirmed by Hellema & Rümke (1976), who in an extensive study compared the TAT with the GAT. In a recent publication (Hellema & Rümke, 1978a), the superiority of testing for immobilizing antibodies in a micro-immobilization test with only motile spermatozoa has been pointed out.

It was the aim of the present study to investigate, with the TAT and the micro-immobilization test, the humoral antibody response in vasectomized men. Furthermore, the results are compared with those obtained by Samuel *et al.* (1975), who examined the same sera for antibodies against human protamine.

MATERIALS AND METHODS

Donor semen sample. For sperm agglutination, semen samples containing more than 6×10^7 spermatozoa per ml with a motility of at least 60% and a good progression rate were used. Samples with too many non-spermatozoal cells or cellular debris, or showing pseudo-agglutination, were discarded.

For sperm immobilization, semen samples were used with a sperm count of at least 4×10^7 spermatozoa per ml and with a motility of at least 50%. Samples with too high a viscosity were discarded.

TAT. The TAT was performed as described by Friberg (1974), with only minor modifications (Hellema & Rümke, 1976).

Micro-immobilization test. This test is a modification of the technique described by Husted & Hjort (1975a). The modifications mainly concern the sperm suspension and the source of complement (Hellema & Rümke, 1978a). The sperm suspension contains almost exclusively progressively motile spermatozoa, which are obtained by the penetration of the motile sperm cells into an overlying buffer layer. As a source of complement a 1:8 dilution of guinea-pig serum in human AB serum was used, being added to the spermatozoa 0·5 hr after the addition of the serum.

Immunofluorescence technique. The indirect immunofluorescence technique (IFT) on swollen sperm heads was performed as described by Kolk *et al.* (1974), with minor modifications described elsewhere (Samuel *et al.*, 1975).

Sera from vasectomized men. Sera from fifty-two vasectomized men were kindly provided by Dr Van Lis. From each man, serum was available from blood specimens obtained before, and 10 days, 6 weeks, and 3, 6, 9 and 12 months post-vasectomy. The sera were kept at −20°C and thawed just before use. For sperm immobilization, the sera were inactivated by heating for 3 0min at 56°C.

RESULTS

The detailed results of the tests for sperm-agglutinating and sperm-immobilizing antibodies, and for antibodies to human protamine as they are detected by the immunofluorescence test on swollen spermatozoa, are given in Table 1.*

Occurrence of sperm-agglutinating autoantibodies

Sperm-agglutinating autoantibodies were found in the sera of forty out of fifty-two vasectomized men (77%), the titres ranging from 8 to 1024 or more (Fig. 1). Sperm agglutinins could already be demonstrated 10 days after the operation in the serum of one man (a titre of 16). The time of first appearance of sperm antibodies after vasectomy is shown in Fig. 2. It can be seen that most men became positive within the first 3 months. For sperm agglutination, 54% of all vasectomized men, or 70% of the men becoming positive at any time, were positive within this period. With time, some increase in incidence can still be observed (Fig. 3). An increase in titre was found up to 9 months post-vasectomy. At 1 year, 68% of the positive men had titres of 32 or higher (Fig. 3). Two men showed sperm-agglutinating activity

* The latter studies were performed by Dr. Talma Samuel in our laboratory and were published earlier (Samuel *et al.*, 1975).

TABLE 1. Titres of sperm agglutinins, sperm immobilizins and antibodies to human protamine as detected by immunofluorescence on swollen spermheads in sera of fifty-two vasectomized men

Patient number	Pre-vasectomy	Time after vasectomy: 10 days	6 weeks	3 months	6 months	9 months	12 months	Type of agglutination*
(01)	< 8†	< 8	64	16	8	8	< 8	T
	0‡	0	2	0	0	0	0	
	0§	0	0	0	0	0	0	
(02)	< 8	< 8	< 8	8 M	16	16	16	T
	0	0	0	0	1	0	0	
	0	0	0	0	0	0	0	
(03)	< 8	< 8	< 8	< 8	< 8	16	32	T
	0	0	0	0	0	0	0	
	0	0	0	0	0	0	0	
(04)	< 8	< 8	< 8	16	16	32	32	T
	0	0	0	0	0	0	0	
	0	0	0	0	0	0	0	
(06)	< 8	< 8	< 8	< 8	32	32	16	M
	0	0	0	0	2	8	4	
	0	0	0	0	0	2	4	
(07)	< 8	n.a.¶	< 8	< 8	16	n.a.	32	T
	0	n.a.	0	0	0	n.a.	0	
	0	0	0	0	0	n.a.	4	
(09)	< 8	< 8	n.a.	8	32	32	16	M
	0	0	n.a.	0	0	0	0	
	0	0	0	0	0	4	4	
(11)	< 8	< 8	< 8	32	32	16	16	T
	0	0	0	0	0	0	0	
	0	0	0	0	0	0	0	
(13)	< 8	< 8	8	< 8	< 8	< 8	< 8	T
	0	0	0	0	0	0	0	
	0	0	0	0	0	0	0	
(14)	< 8	< 8	< 8	< 8	< 8	16	16	T
	0	0	0	0	0	0	0	
	0	0	0	4	0	0	0	
(16)	< 8	< 8	16 M	32	128	512	256	T
	0	0	0	4	⩾1**	8	4	
	0	0	0	0	0	0	0	
(18)	< 8	< 8	< 8	16	n.a.	512	256	T
	0	0	0	4	n.a.	8	8	
	0	0	0	0	2	4	8	
(19)	< 8	< 8	< 8	8 T	32 T	64 M	128 M	T, M
	0	0	0	0	0	0	0	
	0	0	0	0	0	0	0	
(20)	< 8	< 8	32	32	128	128	32	M(H > T)
	0	0	0	4	4	4	n.a.	
	0	0	0	0	0	0	0	
(21)	< 8	< 8	< 8	< 8	< 8	8	8	T
	0	0	0	0	0	0	0	
	0	0	0	0	0	0	0	

TABLE 1. (continued)

Patient number	Pre-vasectomy	Time after vasectomy: 10 days	6 weeks	3 months	6 months	9 months	12 months	Type of agglutination
(22)	< 8	< 8	< 8	< 8	32	32	32	M
	0	0	0	2	2	4	8	
	0	0	0	0	2	4	4	
(23)	< 8	< 8	64 H	32 H	32 H	16 M	16 M	H, M
	0	0	4	16	8	4	4	
	0	0	0	0	0	0	0	
(24)	< 8	< 8	< 8	< 8	32 M	32 T	64 T	T
	0	0	0	0	1	2	4	
	0	0	0	0	0	4	8	
(25)	< 8	< 8	16	64	256	256	256	T
	0	0	1	8	8	8	4	
	0	0	0	0	0	0	0	
(27)	< 8	< 8	< 8	< 8	< 8	32	16	T
	0	0	0	0	0	4	n.a.	
	0	0	0	2	0	0	0	
(28)	< 8	< 8	256 T	256 T	256 T	256 M	256 M	T, M
	0	0	4	4	4	4	4	
	0	0	0	8	0	0	0	
(29)	< 8	< 8	16	< 8	32	32	64	T
	0	0	0	0	0	1	2	
	0	0	0	0	0	0	0	
(31)	< 8	< 8	8 M	16 M	16 T	16 T	16 T	M, T
	0	0	0	0	0	0	0	
	0	0	0	0	0	0	0	
(36)	< 8	< 8	< 8	16 H	32 M	256 M	256 M	H, M
	0	0	0	0	⩾4††	32	32	
	0	0	0	0	0	0	0	
(38)	< 8	< 8	16 M	64 M	32 T	16 T	16 T	M, T
	0	0	0	1	0	0	0	
	0	0	0	0	0	0	0	
(39)	< 8	< 8	< 8	< 8	< 8	< 8	8	T
	0	0	0	0	0	0	0	
	2	4	4	4	8	8	4	
(40)	< 8	< 8	< 8	< 8	< 8	< 8	32	T
	0	0	0	0	2	1	2	
	0	0	0	0	0	0	0	
(41)	< 8	< 8	512	256	256	256	256	T
	0	0	16	16	16	n.a.	8	
	0	0	0	0	0	0	0	
(42)	< 8	< 8	< 8	32	64	256	256	T
	0	0	0	4	4	8	8	
	0	0	0	0	2	2	2	
(43)	< 8	< 8	< 8	32	32	32	32	T
	0	0	0	2	2	0	0	
	0	0	0	0	0	0	0	
(44)	< 8	< 8	< 8	8	16	n.a.	16	T
	0	0	0	0	0	n.a.	0	
	0	0	0	0	0	0	0	

TABE 1. (continued)

Patient number	Pre-vasectomy	Time after vasectomy: 10 days	6 weeks	3 months	6 months	9 months	12 months	Type of agglutination
(45)	< 8	< 8	8 H	16 M	64 T	64 T	64 T	H, M, T
	0	0	1	2	2	1	1	
	0	0	0	0	0	0	0	
(47)	< 8	< 8	16 H	64 T	1024 T	1024 T	⩾1024 T	H, T
	0	0	0	16	32	16	32	
	0	0	0	0	0	0	0	
(49)	< 8	16 T	16 T	32 T	128 M	256 M	256 M	T, M
	0	0	0	2	8	4	4	
	0	0	0	0	0	0	0	
(51)	64	32	128	64	64	64	64	T
	0	0	1	0	1	0	0	
	0	0	4	4	4	2	4	
(52)	< 8	< 8	< 8	< 8	< 8	< 8	< 8	
	0	0	0	1	2	?	0	
	0	0	0	0	0	0	0	
(54)	< 8	< 8	64	64	32	32	32	M
	0	0	0	0	0	0	0	
	0	2	4	8	4	4	8	
(56)	< 8	< 8	8	16	64	128	128	T
	0	0	0	4	8	4	4	
	0	0	0	0	4	4	8	
(57)	128	128	⩾1024	1024	512	1024	1024	M
	4	8	128	64	64	32	32	
	0	0	0	0	4	4	4	
(59)	< 8	< 8	< 8	64	n.a.	256	1024	T
	0	0	0	8	n.a.	8	64	
	0	0	0	0	0	0	0	
(61)	< 8	< 8	< 8	16	32	32	32	T
	0	0	0	2	2	1	1	
	0	0	0	0	0	0	0	

Patients number 05, 08, 10, 17, 33, 34, 35, 37, 46, 50, 53 and 60: all samples in all tests negative (i.e.< 8 in agglutination, 0 in immobilization and immunofluorescence).

* If not stated separately (H = head, T = tail, M = mixed agglutination).
† Agglutination.
‡ Immobilization.
§ IFT on swollen sperm heads.
¶ n.a. = Serum sample not available.
** The serum is positive; no more serum available for titration.
†† No more serum was available for higher titration series.

before vasectomy. A booster effect was observed in one of them (No. 57, see Table 1). The agglutination titre increased from 128 to 1024.

Occurrence of sperm-immobilizing autoantibodies

Twenty-nine out of the fifty-two vasectomized men (56%) showed sperm-immobilizing antibodies in any of their seven serum samples (Fig. 4). Within 3 months after the operation, 37% of the vasectomized men had sperm-immobilizing activity, or 66% of all men who were becoming positive (Fig. 5). Still some

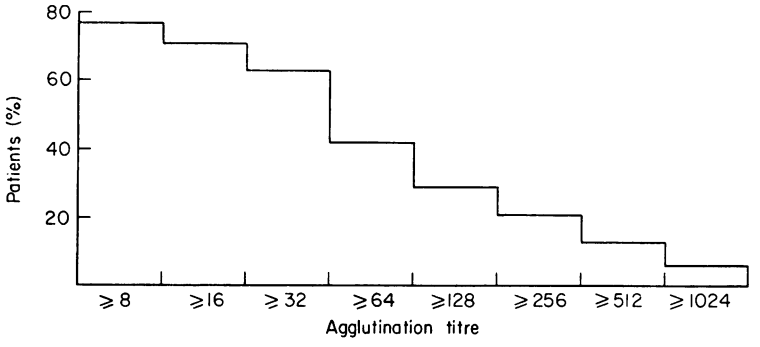

FIG. 1. Occurrence of sperm agglutinins in any of the six serial serum samples per patient obtained after vasectomy.

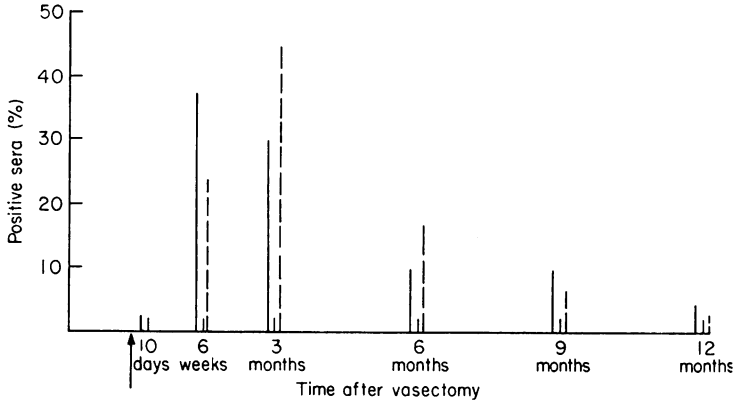

FIG. 2. First appearance of sperm autoantibodies after vasectomy. (———) Sperm-agglutinating antibodies; (– – – –) sperm-immobilizing antibodies. Arrow shows when vasectomy took place.

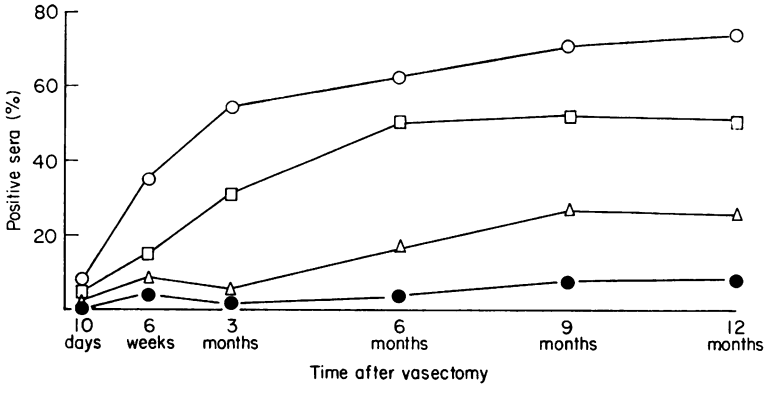

FIG. 3. Incidence of sperm agglutinins in post-vasectomy sera at different serum dilutions. (○) Titre⩾8; (□) titre⩾32; (△) titre⩾128; (●) titre⩾512.

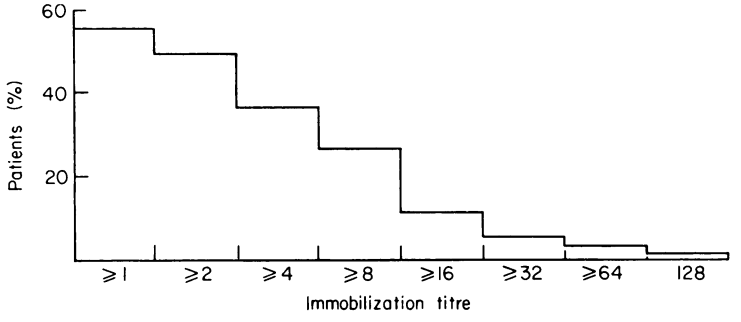

FIG. 4. Occurrence of sperm immobilizins in any of the six serial serum samples per patient obtained after vasectomy.

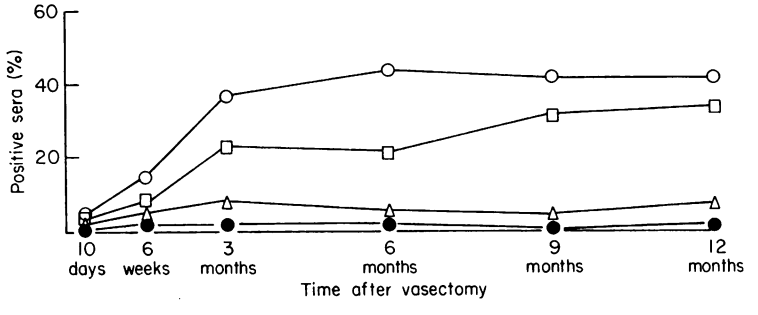

FIG. 5. Incidence of sperm immobilizins in post-vasectomy sera at different serum dilutions. (○) Titre≥1; (□) titre≥4; (△) titre≥16; (●) titre = 64–128.

increase in incidence can be seen up to 6 months. At 1 year post-vasectomy, 42% of the men were positive, compared with the overall incidence of 56%. Some men had lost their sperm-immobilizing activity at the 12 month stage. The immobilization titres ranged from 1 to 128, increasing with time (Fig. 5). One man had sperm-immobilizing antibodies pre-vasectomy. After the operation the titre increase from 8 to 128 (No. 57, Table 1).

Correlation between the occurrence of sperm-agglutinating and sperm-immobilizing antibodies

A significant correlation was found between the agglutination titre and the occurrence of sperm-immobilizing antibodies (Fig. 6). All sera with sperm agglutinins in titres of 128 or higher showed sperm immobilizing activity. The titre of sperm-immobilizing antibodies was also significantly correlated with

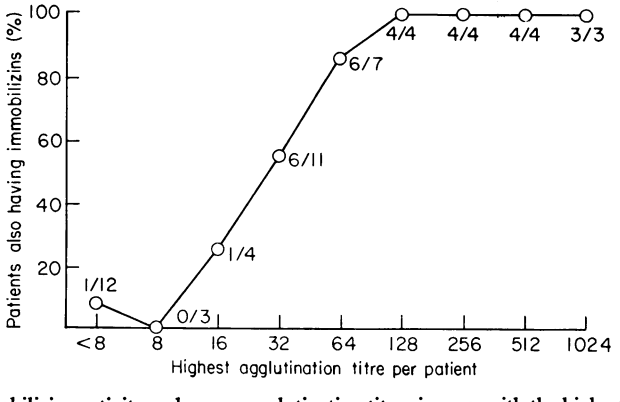

FIG. 6. Sperm-immobilizing activity and sperm-agglutination titres in sera, with the highest agglutination titre from each patient.

the sperm agglutination titre ($r = 0{\cdot}74$, $P<0{\cdot}001$). Out of 160 sera showing no sperm agglutination, at a minimum dilution of 1:8, only five (3%) were positive for sperm immobilization, with titres of 1 or 2 (Table 1). Comparing the time of appearance of sperm-agglutinating and sperm-immobilizing antibodies in the sera of men positive for both types of autoantibodies during the 1 year period, it was found that in 53% of the men they appeared in the same serum sample, in 39% agglutination was found prior to immobilization and in 7% immobilization was detected first. The agglutination titres of the initial positive sera were lower in that group in which sperm agglutination was found before sperm-immobilizing activity than in the group where both activities were detected in the same serum sample.

Types of agglutination and the occurrence of sperm-immobilizing antibodies

When the types of agglutination are characterized as head-to-head (H), tail-to-tail (T), mixed (M) and tail tip-to-tail tip, as described by Rümke & Hellinga (1959) (see also Rose *et al.*, 1976), predominant in the sera of the vasectomized men was the T agglutination (78%); 5% of the men had mainly H-agglutinating sera and 18% M-agglutinating (Table 1). Not all vasectomized men had sera agglutinating only according to one type. When a man develops agglutinins of more than one type, H agglutination is always demonstrated in a serum sample preceding those with either M or T agglutination. The incidence of sperm-immobilizing antibodies in the three categories is 53%, 64% and 60%, for T, H and M agglutination respectively, indicating that there is no prevalence of immobilization with any of the agglutination types.

Relation between sperm autoantibody formation and the number of spermatozoa

Antibody response is commonly related to the dose of the antigenic material which is administered. When the total number of spermatozoa in an ejaculate, i.e. sperm count per ml × volume in ml, is taken as a quantitative measure of antigenic stimulation, a relation might well be expected between the total number of spermatozoa in a pre-vasectomy ejaculate, and antibody response after vasectomy. However, as is shown in Fig. 7, no relationship between total number of spermatozoa and increase in incidence and/or titre of antibodies could be found.

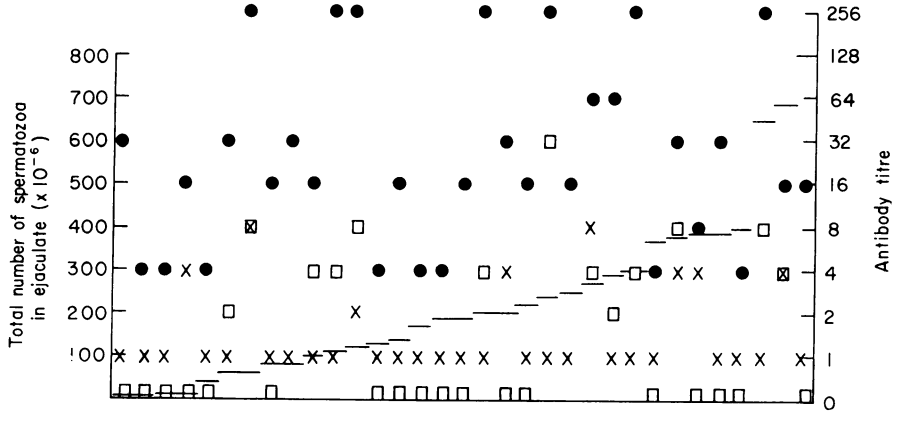

FIG. 7. Comparison of sperm autoantibody titres in the serum of thirty-three patients 1 year after vasectomy with total number of spermatozoa in a pre-vasectomy ejaculate. (●) Titre in the tray agglutination test; (□) titre in the micro-immobilization test; (×) titre in the immunofluorescence test on swollen spermheads.

DISCUSSION

Both the tray agglutination (Friberg, 1974; Hellema & Rümke, 1976) and the micro-immobilization test (Husted & Hjort, 1975a; Hellema & Rümke, 1978a) involve sensitive techniques for the detection of sperm antibodies in human sera, and are more convenient than other tests, the more so when a large series of sera has to be tested. The prerequisite for the TAT is that semen should have a high proportion

of motile spermatozoa and few non-spermatozoal cells or cell fragments, which can cause pseudo-agglutination. While examining sera for agglutination, one has at the same time the opportunity to determine the type of agglutination. This gives information about the site of the antigen, and about the likely type of immunoglobulin involved (Friberg, 1974). Testing 200 to 300 serum samples simultaneously (as stated by Friberg) could, however, not be performed in our laboratory. One person could screen fifty to sixty samples with one donor semen sample, or read the titres of twenty to thirty positive sera, titrated in two-fold serial dilution steps. The micro-immobilization test was performed with a modification, the use of nearly only motile spermatozoa, which were prepared by a method described earlier (Hellema & Rümke, 1978a). As a consequence, many more semen samples can be used than if whole semen is used for the test, while the reading of the immobilization is much easier; both features increase the suitability of the test for large scale studies. Simultaneous reading of agglutination and immobilization was not possible, because with a suspension of only motile spermatozoa, T agglutination was reduced markedly (Hellema & Rümke, 1978b).

Though vasectomy or vasoligation is now a widespread male contraceptive method, and considered to be harmless, the immunological consequences are not yet fully understood. As a result of the obstruction of the vas deferens, sperm is either extravasated into the interstitium of the epididymis (Rümke & Hellinga, 1959) or phagocytosed by epithelial cells of the tubuli of the epididymis (Phadke, 1964; Alexander, 1973a), both processes being able to elicit an autoimmune response. Humoral sperm autoantibodies have been found in mammals undergoing vasoligation or vasectomy, as was reported for rats (Rümke & Titus, 1970; Brannen & Coffey, 1974), guinea-pigs (Alexander, 1973b), rabbits (Bigazzi *et al.*, 1976a,b) and Rhesus monkeys (Alexander *et al.*, 1974; Alexander, 1977). In men, sperm autoantibodies have been found by various investigators (Ansbacher, 1971, 1973, 1974; Ansbacher *et al.*, 1972; Shulman *et al.*, 1972; Alexander *et al.*, 1974; Van Lis *et al.*, 1974; Gupta *et al.*, 1975a; Samuel *et al.*, 1975; Tung, 1975; Linnet & Hjort, 1978). In the present study of fifty-two vasectomized men, 73% showed sperm-agglutinating and 42% sperm-immobilizing antibodies 1 year post-vasectomy. The presence of sperm autoantibodies after vasectomy could not be correlated with the antigenic dose, as far as this could be roughly estimated by the total number of sperm cells in a pre-vasectomy ejaculate.

The onset of antibody formation was found predominantly within 3 months after the operation. 6 months post-vasectomy 62% of the men had sperm agglutinins, an incidence comparable with the 57% found by Ansbacher (1973). In the same period, Gupta *et al.* (1975a) recorded an incidence of 54%, considering a result only positive when the titre was >32, an incidence which is quite similar to 50% at that level found by us. An increased incidence was found in our study up to 1 year after the operation. This is in accordance with the observations of Ansbacher (1973), who found an increase up to 18 months, and by Gupta *et al.* (1975a) who found, in a group of 141 vasectomized men, a higher incidence related to the time post-vasectomy. An increase in agglutination titre was observed in our group only up to 9 months post-operatively; by Ansbacher (1973) up to 18 months; by Alexander *et al.* (1974) in a group of thirty vasectomized men up to more than 5 years; and by Gupta *et al.* (1975a) up to 12 years.

Sperm-immobilizing autoantibodies were detected at 6 months in 44% of the vasectomized men, a percentage quite similar to the 37% found by Gupta *et al.* (1975a). In 1973, Ansbacher presented data from a group of vasectomized men where the incidence at 6 months was slightly lower (28%) and where there was no increase in incidence after 1 year, as was described in a previous paper (Ansbacher *et al.*, 1972). An increase in the occurrence of sperm-immobilizing antibodies was found by Alexander *et al.* (1974) up to 5 years post-vasectomy and by Gupta *et al.* (1975a), who found a higher incidence after, rather than before, 1 year post-vasectomy. Our results agree with those of Ansbacher (1973), in that we found no increase in incidence beyond 6 months. The highest titre of immobilizins in our study was 128, which is considerably lower than reported by Ansbacher (1973), who found titres up to 1024 1 year post-vasectomy.

In most cases sperm-immobilizing antibodies were only found in sera which also contained sperm agglutinins (Ansbacher, 1971; Ansbacher *et al.*, 1972; Alexander *et al.*, 1974; Gupta *et al.*, 1975a; Husted & Hjort, 1975a). However, in a later paper (1973) Ansbacher described four out of fourteen men who possessed immobilizins without agglutinins. And more recently, Sullivan & Howe (1977) found in

the serum of a vasovasostomized man sperm immobilizins at a titre of 128, but who possessed no sperm agglutinins. In our series, five out of 160 sera showed only sperm immobilization, however, in dilutions below the minimum dilution tested for agglutination, i.e. less than 1:8. We are therefore not able to confirm that immobilizins can occur apart from agglutinins. Our results indicate that there is only a difference in sensitivity between both agglutination and immobilization tests.

As to the presence of other sperm autoantibodies as a result of vasectomy, Samuel *et al.* (1975) and Tung (1975) found antibodies to human protamine, a strongly basic nuclear protein (Kolk & Samuel, 1975), although in low titres ($\leqslant 10$). These antibodies were detected by the indirect immunofluorescence technique (IFT) on swollen spermheads (Kolk *et al.*, 1974). In our series of fifty-two vasectomized men, fifteen individuals (29%) were positive in the IFT on swollen spermheads. This is in accordance with the 33% incidence found by Tung (1975), 6 to 9 months post-vasectomy. All fifteen men with autoantibodies to human protamine have sperm agglutinins, ten of them (67%) also have sperm immobilizins; although not always in the same serum samples. A strong correlation thus exists between anti-protamine antibody formation and the formation of antibodies to the spermatozoal surface as detected by agglutination and immobilization. In the IFT, Tung (1975) also detected antibodies directed to the acrosome (speckled distribution) and to the main piece of tail as a result of the vasectomy. No antibodies could be detected directed to the antigens of the Leydig cells of the testis nor to those of the adrenal cortex in the sera of 150 vasectomized men (Bigazzi & Rose, 1974). Howard & James (1973) could find no evidence for an increased state of autoimmune disease when they looked for the presence of rheumatoid factor, anti-nucleoprotein factor, syphilitic reagin and C-reactive protein, 3–6 months post-operatively. No detectable significant increase in the occurrence of autoantibodies after vasectomy against a variety of seven antigens could be found by Crewe *et al.* (1976) in a group of 346 men vasectomized about 6 months before, nor by Mathews *et al.* (1976), who tested the sera of 100 men before vasectomy, and another 188 men up to 6 years after the operation, for autoantibody activity against ten different antigens.

Sperm autoantibodies in seminal plasma as a result of vasectomy were found in a low incidence by Ansbacher (1974) and by Linnet & Hjort (1978). Gupta *et al.* (1975a) found autoagglutination in the ejaculates of men who had undergone vasovasostomy, but this could also be a result of the reanastomosis, as was shown by Ansbacher *et al.*. (1972).

In recent years, the immunological consequences of vasectomy have been reconsidered with regard to vasovasostomy and subsequent fertility. Most investigators agree that spermatogenesis is hardly affected by vasoligation or vasectomy, as is reported for rats (Johnson, 1972; McGlynn & Erpino, 1974; Neaves, 1974; Bedford, 1976), rabbits (Flickinger, 1975a,b; Bedford, 1976), Rhesus monkeys (Alexander, 1972) and for men (Johnson, 1972; Gupta *et al.*, 1975b). A transient depression of spermatogenesis (for 3 to 10 months) was reported by Derrick *et al.* (1974) for both dogs and men, and for dogs by Urry *et al.* (1976), most probably due to the increase in intra-tubular hydrostatic pressure. In the guinea-pig, however, an autoimmune aspermatogenesis was found after vasectomy, similar to that observed when animals were immunized with washed sperm in Freund's complete adjuvant (Alexander, 1973b). More recently, Tung & Alexander (1977) concluded that the early lesions in vasectomized guinea-pigs are probably not due to immunological mechanisms, although lesions resembling experimental autoimmune orchitis may occur at longer intervals (12–24 months) after vasectomy. Bigazzi *et al.* (1976b) found in a group of twenty-four vasectomized rabbits four with immune complex orchitis, along with high and persistent levels of circulating sperm antibodies, of which two also developed a mild glomerulonephritis. It is generally agreed that the restoration of fertility is far less than may be expected from the success rate of the operation itself. It is tempting to presume that the sperm autoantibodies elicited after vasectomy would interfere adversely with subsequent fertility.

From studies on infertile men it appeared that the fertility of normospermic men (sperm count $> 2 \times 10^7$ spermatozoa per ml) showed a reverse relation with the titre of sperm-agglutinating antibodies in their serum (Rümke *et al.*, 1974). A relationship between the serum titre and the presence of antibodies in seminal plasma has been found (Friberg, 1974; Husted, 1975; Husted & Hjort, 1975b; Rümke, 1974a). Circulating antibodies can diffuse, dependent on the type of immunoglobulin involved, from the blood into the seminal compartment (Rümke, 1974b). Apart from a systemic antibody production, sperm agglutinins can also be produced locally in the genital tract. The clinical importance of sperm agglutinins

in the seminal plasma was emphasized by Husted & Hjort (1975b). Men remained infertile when the seminal plasma titres were 64 or higher. In spite of the proven local production of sperm antibodies, no cases have been reported of local production only. Serum antibodies were always present. The role of sperm-immobilizing antibodies in the serum of infertile men has been investigated less often. Fjällbrant (1968) found a somewhat higher (though not significantly higher) correlation between the degree of penetration into cervical mucus and sperm immobilizins than between the penetration ability and sperm agglutinins. But, when both antibody activities were divided into low and high levels, the distribution of fertile and infertile men among these groups was similar. Additionally, Fjällbrant & Obrant (1968) found a highly significant difference between the sperm motility rate in the semen from men with a high (titre $\geqslant 64$) and men with a low sperm agglutination titre in their serum. Ansbacher, Keung-Yeung & Behrman (1973) claimed that the presence of sperm-immobilizing antibodies is more relevant to infertility than agglutinating antibodies. In a group of thirteen infertile men they found eight with sperm agglutinins of whom four fathered children, but none of the five men with sperm immobilizins had become a father. No data, however, were presented in this study with regard to the titres of the agglutinating antibodies.

To date only Gupta *et al.* (1975a) and Sullivan & Howe (1977) reported the possible immunological influences after vasovasostomy, as far as it concerned man. In a group of twenty-five vasovasostomized men, Gupta *et al.* (1975a) found thirteen (52%) normospermic men (sperm count $> 2 \times 10^7$ spermatozoa per ml). Of these thirteen men, the wives of only three of them had become pregnant. Though all men possessed sperm agglutinins in titres of 32 to 128, those three men lacked sperm-immobilizing antibodies in their serum, as well as sperm agglutinins in their seminal plasma as revealed by autoagglutination. Sullivan & Howe (1977) found a significant difference in the occurrence of sperm-agglutinating antibodies between the fertile and infertile group of patients after vasovasostomy (all of the men had a technically successful reanastomosis, as judged by sperm in the ejaculate). They also found a difference in the occurrence of sperm immobilizins between both groups, but this difference was not significant. They did not consider, however, autoagglutination as the mechanism involved in the reduced incidence of functionally successful reanastomosis (as judged by pregnancy). Besides that in the fertile group 48% of the men possessed sperm agglutinins (titres 4 to 256), in none of the ejaculates of the forty-five (fertile and infertile) men was autoagglutination observed. They pointed to antibodies to sperm enzymes as more revelant to reduced fertility after vasovasostomy. In the Rhesus monkey, Alexander (1977) found no correlation between sperm-immobilizing antibodies and fertility rate. She emphasized the importance of a high sperm count. It is evident that sperm autoantibodies as a consequence of vasectomy can play a role in post-vasovasostomy infertility. More studies are, however, needed for a more reliable evaluation of their significance after reanastomosis.

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