Clin. exp. Immunol. (1979) 38, 31–36.

Sperm autoantibodies as a consequence of vasectomy

II. LONG-TERM FOLLOW-UP STUDIES

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(*Accepted for publication* 19 *January* 1979)

SUMMARY

Thirty-four out of fifty-two vasectomized men studied previously were studied again about 5 years after vasectomy for sperm-agglutinating and sperm-immobilizing antibodies, and for antibodies to human protamine. Only one man lost his already weak antibody activity, whilst one out of eight men, negative at 1 year, appeared to be positive at 5 years. Slightly higher titres in one of the three sperm antibody tests were found in the sera of about 30% of the men. Only in three (9%) was there a rise of two or more steps in more than one technique. Agglutinins and immobilizins were shown to be strongly correlated, as was the case with antibodies to human protamine and head-to-head agglutinins. Seminal plasma sperm agglutinins were detected in the samples of only four (out of thirty) men, in low titres. Circulating immune complexes tested with various techniques were only found in a few sera and not consistently. This prolonged study shows that sperm autoantibodies formed within 1 year after vasectomy are persistent. Their role in remaining infertility after reanastomosis requires further study.

INTRODUCTION

Resorption of spermatozoa or their products as a consequence of vasectomy can be the cause of sperm autoantibody formation in man and other mammalian species (Hellema & Rümke, 1978a). In a previous study, it was found that 1 year after vasectomy 73% of fifty-two vasectomized men had sperm-agglutinating and 42% also had sperm-immobilizing antibodies, whilst 29% had anti-sperm nuclear antibodies as well (Hellema & Rümke, 1978a). After 1 year sperm autoantibodies were reported to increase with time in incidence and titre (Gupta *et al.*, 1975), but it has also been claimed that they decrease in the second year (Ansbacher *et al.*, 1976). The importance of sperm antibodies in vasectomized men lies mainly in the supposed interference with fertility after a reanastomosis operation. An interference due to sperm antibodies in the seminal fluid that agglutinate spermatozoa or render them otherwise incapable to migrate through the cervical mucus, has been found to exist in some naturally infertile, normospermic men (Hekman & Rümke, 1976; Rümke & Hekman, 1977). In this study we report on the occurrence of sperm antibodies in the serum of thirty-four of the earlier studied fifty-two men who donated blood again about 5 years post-operatively. From thirty of them, a seminal plasma sample was also tested. The serum samples were investigated as well for the presence of circulating immune complexes, since it was claimed that immune complexes are formed as a result of vasectomy in rabbits where they are found to be deposited in the basement membrane of the testis (Bigazzi *et al.*, 1976) and in the monkey where they might exacerbate diet-induced atherosclerosis (Alexander & Clarkson, 1978).

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0099-9104/79/1000-0031$02.00

MATERIALS AND METHODS

Serum and seminal plasma samples. From thirty-four (65%) of the original group of fifty-two men of our prior study (Hellema & Rümke, 1978a) blood was drawn again about 5 years after the vasectomy. Thirty of them provided also a seminal plasma sample. Serum and seminal plasma samples were divided in small portions (1–2 ml) and stored at −20°C.

Along with the new samples all 34 1 year samples were retested. They had been stored for more than 5 years at −20°C and were tested for the first time about 3 years ago (Hellema & Rümke, 1978a). The serum and seminal plasma samples of a patient were examined with the same donor semen sample in order to obtain optimal comparable results.

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

Micro-immobilization test. The immobilization test was performed as described by Hellema & Rümke (1978b). Essential in the technique is the use of a suspension of only progressively motile spermatozoa which is obtained after spermatozoa have penetrated an overlying buffer. The source of complement was a non-spermatotoxic 1:8 dilution of guinea-pig serum in human AB serum. The mixture of sperm and serum was incubated for 30 min at 37°C, after which complement was added for another 2 hr incubation period.

Immunofluorescence on swollen spermheads. The indirect immunofluorescence test (IFT) was performed as described by Samuel *et al.* (1975) and swollen spermheads were prepared as described by Samuel *et al.* (1976).

Circulating immune complexes. Tests for the detection of circulating immune complexes were performed by three independent investigators. Dr I. van Wingerden (The Netherlands Cancer Institute, Amsterdam) used the indirect phagocytosis test in which immune complexes ingested by normal neutrophils are detected by indirect immunofluorescence, using rabbit antisera against IgG and C3 (Van Wingerden *et al.*, 1979). Dr C.L. Cambiaso (International Institute of Cellular and Molecular Pathology, Catholic University of Louvain) used inhibition tests of latex agglutination by C1q and by rheumatoid factor (Cambiaso *et al.*, 1978). Dr C.L. Molenaar (Central Laboratory of the Netherlands Red Cross Bloodtransfusion Service, Amsterdam) used a modified C1q deviation test according to Sobel, Bokisch and Müller-Eberhard (1975).

RESULTS

Effect of long-term storage at −20°C on sperm antibody containing sera

In order to obtain an optimal comparison in the 1 year with the 5 year post-vasectomy serum sample from the same man, both samples were titrated at the same time with the same donor semen sample. The usefulness of this procedure depends on the quality of the older serum samples, i.e. that they had not deteriorated during their longer storage. Table 1 (left part) shows the earlier found and published titres together with the titres found when the sera were retested approximately 3 years later. For agglutination, 85% of the titres did not differ more than one two-fold dilution step. One serum showed a titre two steps lower when retested, and two weak titres of sixteen could not be confirmed, but two other sera were first found negative (i.e. titre <8) and weakly positive after retesting with titres of eight and sixteen. For immobilization, 88% of the titres did not differ more than 1 two-fold dilution step. Two sera decreased and two other sera increased two steps in titre.

The data thus show that there is no evidence of deterioration of sperm antibody activity during storage of about 3 years at −20°C. The slight variability is likely to be due to differences in the qualities of the donor semen samples and in the preparation of the two-fold dilution series.

Comparison of serum titres 1 and 5 years post-vasectomy. The titres of the 1 and 5 years post-vasectomy serum samples are shown in Table 1. Declines of antibody titres were hardly seen. Only one man (04) lost an already weak antibody activity. In one man (49) the agglutinin but not the immobilizin titre decreased with two steps, and in two other men (06 and 59) the immobilizin titre decreased with 2–3 steps but without a change of the agglutinin titre. Rises in titres between 1 and 5 years were seen in some cases. Of the eight men having no agglutinins at 1 year only one (10) developed them later, and of the twenty-two having no immobilizins at 1 year five had them after 5 years. Four of these men already had agglutinins at 1 year. Rises of 2 steps or more in the titre of at least one of the three different kinds of antibodies occurred in eight men (07, 11, 19, 21, 22, 42, 52, 57), but only in two of them (22, 52) were these rises in at least two of the techniques detectable. The overall tendency is that in 30% of the men a slight rise of sperm antibody formation occurred.

The mode of agglutination mostly seen was the tail-to-tail type, though in 30% of the agglutinating sera there was also some head-to-head agglutinating activity. Head-to-head agglutination was pre-

TABLE 1. Antibodies to spermatozoal antigens detected in serum samples obtained 1 and 5 years post-vasectomy, and in seminal plasma samples obtained 5 years post-vasectomy

	Titres 1 year post-vasectomy					Titres 5 years post-vasectomy				
	Sera tested 3 years ago			Sera recently retested		Sera			Seminal plasma	
Men's code nr.+	Agg.	Immob.	IFT	Agg.	Immob.	Agg.	Immob.	IFT	Agg.	Immob.
02	16T	0	0	< 2	0	32T	1	< 8	< 2	0
03	32T	0	0	16M	0	16M	0	< 8	< 2	0
04	32T	0	0	8T	0	< 2	0	< 8	< 2	0
06	16M	4	4	32M	8	64M	1	8	< 2	0
07	32T	0	4	16T	0	64T	4	8	< 2	0
09	16M	0	4	8M	0	4M	0	8	< 2	0
10	< 8	0	0	< 2	0	64T	2	< 8	< 2	0
11	16T	0	0	< 2	0	16T	4	< 8	< 2	0
14	16T	0	0	16T	0	16T	0	< 8	na	na
16	256T	4	0	128T	2	64T	2	< 8	< 2	0
19	128M	4	0	128H	8	128M	4	16	< 2	0
21	8T	0	0	16T	0	64T	0	< 8	< 2	0
22	32M	8	4	64H	2	256H	4	16	2M	0
24	64T	4	8	64T	2	128T	2	< 8	< 2	0
27	16T	< 4*	0	32T	< 4*	32T	0	< 8	< 2	0
38	16T	0	0	8T	0	4T	0	< 8	< 2	0
40	32T	2	0	16T	0	64T	2	< 8	< 2	
42	256T	8	2	256T	4	1024T	8	< 8	8T	0
44	16T	0	0	32T	0	32T	0	< 8	< 2	0
45	64T	1	0	64T	0	128T	1	< 8	< 2	0
49	256M	4	0	128M	16	32M	16	< 8	2T	0
50	< 8	0	0	16T	0	16T	0	< 8	< 2	0
52	< 8	0	0	8T	0	512T	8	< 8	< 2	0
54	32M	0	8	32T	0	32M	0	16	< 2	0
56	128T	4	8	128T	2	64T	1	16	na	na
57	1024M	32	4	1024H	128	1024H	128	16	32M	0
59	1024T	64	0	512T	64	1024T	16	< 8	< 2	0
01, 05, 33, 34, 35, 37, 60	< 8	0	0	< 2	0	< 2	0	< 8	< 2	0 (2× na)

na = Not available.
\+ = Code nr. is the same as in Table 1 of Hellema & Rümke (1978a).
T = Tail-to-tail agglutination.
H = Head-to-head agglutination.
M = Mixed agglutination, i.e. both T and H agglutination.
* = Serum in dilutions 1:1 and 1:2 toxic, i.e. immobilization also without complement.

dominant only twice (22 and 57). No correlation exists between the mode of agglutination and the presence of immobilizins. However, there is a clear relationship between the presence of antibodies against the sperm nucleus as detected in the IFT on swollen spermheads and the presence of head-to-head agglutination (M or H). (For the 1 year post-vasectomy series $X^2 = 4{\cdot}95$, $P = 0{\cdot}032$; for the 5 years post-vasectomy series $X^2 = 11{\cdot}06$, $P = 0{\cdot}001$).

Immobilizins were never seen without the presence of agglutinins. Nearly all sera with agglutinin titres of 64 and all with titres of 128 and higher had also immobilizing activity. Even so, all sera positive in the IFT on swollen spermheads contained agglutinins and 75% had also immobilizins.

Occurrence of sperm antibodies in seminal plasma 5 years post-vasectomy. Agglutination was found with the samples of only four men, in titres of 32, 8, 2 and 2 (Table 1). Their serum titres were 1024, 1024, 256 and 32, respectively. Immobilizins were never found.

Circulating immune complexes. Sera of twenty-nine men were available to be tested for circulating immune complexes. Sera of five men gave scores considerably higher than normal in the indirect phagocytosis test, suggesting the presence of immune complexes. However, men with the highest scores were found to be normal when tested again a few months later. The inhibition tests of latex agglutination by C1q and rheumatoid factor were entirely negative. In the modified C1q deviation test four sera were found positive, but in two this was probably due to the presence of fibrinogen. The positive results of the two laboratories did not correlate.

DISCUSSION

It was earlier shown in a study on fifty-two men that 73% of them possessed sperm-agglutinating and 42% also sperm-immobilizing antibodies 1 year after vasectomy (Hellema & Rümke, 1978a). The present 5 year follow-up study comprised thirty-four of these fifty-two men. Only one man with an already weak antibody titre at 1 year lost antibody activity at 5 years. The other men kept their titres or had (in 30% of the cases) somewhat higher titres 5 years post-operatively. Those men who had no antibodies within one year did not (with one exception) form them thereafter. Although there was some increase in incidence of immobilizing activity, this increase rather reflected a slight rise of sperm antibody formation in general, since immobilizins and agglutinins are strongly related (all sera with agglutinins in titres of 128 or higher also contained immobilizins). So far, it seems that the immobilization techniques cannot detect any antibody that cannot be detected by the tray agglutination technique.

Our results are in contrast to those of Ansbacher *et al.* (1976) who found in their long-term study a decrease in incidence and titre of both agglutinins and immobilizins, 1–2 years post-vasectomy. Sperm-immobilizing antibodies could not even be detected in the sera of the seventeen men returning 5 years after the operation. Alexander & Schmidt (1977), however, found similar levels of sperm agglutinins and immobilizins before and after 5 years post-vasectomy. Higher titres and long-term incidences after vasectomy were reported by Gupta *et al.* (1975) in a group of fifty men vasectomized 1–12 years earlier.

Seminal plasma agglutinins were found in only four men, in titres of 2, 2, 8 and 32. Also Linnet and Hjort (1977) seldom found them in the seminal plasma of vasectomized men, while Ansbacher (1973) was even unable to find sperm antibodies in seminal plasma 1 year post-vasectomy. As can be seen from Fig. 1 in which data from the literature and the present study are compiled, there is a contrast between natural immune-infertility and the status after vasectomy. With similar serum agglutinin titres, naturally immune-infertile men far more often have agglutinins in their ejaculates than vasectomized men. This difference is likely to be the result of local sperm antibody production in natural infertility, since the sperm antibodies in seminal plasma were often of the IgA type (Friberg, 1974; Jager, Kremer & Van Slochteren-Draaisma, 1978), or at least could be proved not to be of the IgG type, while the serum antibodies were of this type (Husted, 1975). Since IgG is present in seminal plasma in concentrations of about 1% of the serum concentration and since transudation mainly occurs via the prostate (Rümke, 1974b), it is likely that in vasectomized men with high serum titres the presence in seminal plasma is due to transudation of serum IgG antibodies. Indeed, only IgG and not IgA sperm antibodies could be detected in the four seminal plasma samples of this study, as determined with the mixed agglutination reaction (kindly performed by Dr S. Jager). Although, so far, there is no indication of locally produced sperm antibodies in vasectomized men, it may be possible that local production occurs proximal of the occlusion. In that case, a higher proportion of vasectomized men should possess sperm antibodies in their seminal plasma after reanastomosis while serum levels would remain the same. This has recently proved to be the case (Linnet & Fogh-Andersen, 1979).

Antibodies to human protamine (as detected with the IFT on swollen spermheads) have been reported to develop after vasectomy (Samuel *et al.*, 1975; Hellema & Rümke, 1978a). The titres were low at 1 year, and though they seemed to rise slightly by 5 years, the titres remained low (i.e. never higher than

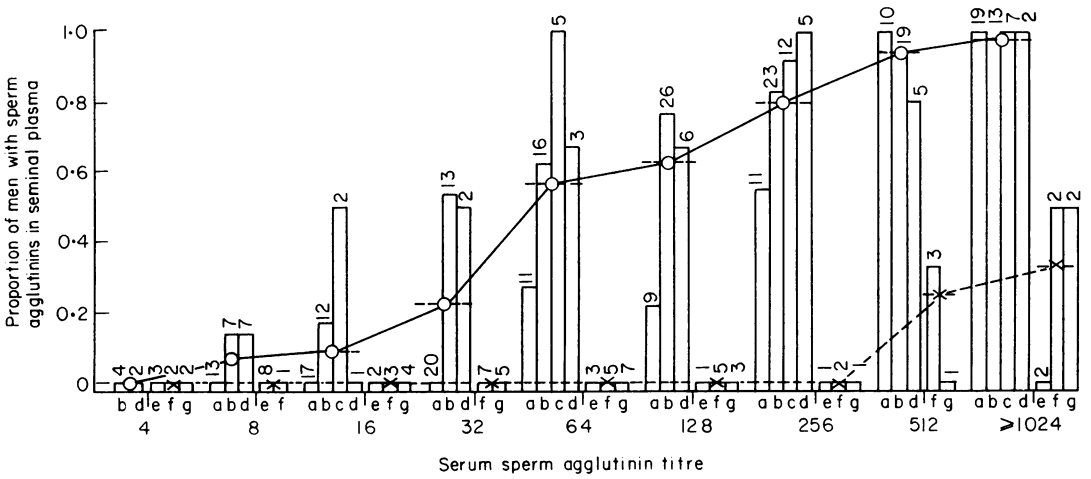

FIG. 1. Occurrence of sperm agglutinins (titres ⩾4) in the seminal plasma of naturally infertile and vasectomized men in relation to the serum agglutinin titre. The data are collected from the literature and this report. For naturally infertile men: (a) Friberg, 1974; (b) Rümke, 1974a; (c) Husted & Hjort, 1975; (d) Jager *et al.*, 1978. For vasectomized men: (e) Ansbacher, 1973; (f) Linnet & Hjort, 1977; (g) results presented in this report.

Report (c) gives only titres in four-fold dilution steps, all the others in two-fold dilution steps. This means that some of the titres of report (c) could have been 1 two-fold dilution step higher than indicated. The agglutination techniques used in the various reports differed: (b), (c), (e) and (f) employed the gelatin agglutination test (Rose *et al.*, 1976); (a), (d) and (g) employed the tray agglutination test (Friberg, 1974). The number of patients examined by each investigator is shown on the top of each column. Mean values for naturally infertile men are represented by circles connected by solid lines; mean values for vasectomized men are represented by crosses connected by broken lines.

The lowest titre which has been considered as positive in most cited publications, except (a), is a titre of 4. The two seminal plasma samples described in the present report as having a titre of 2, are not included as positives in this Figure.

16). Their presence correlated with the presence of head-to-head agglutinins. None of the men with anti-protamine antibodies had antibodies against somatic nuclear antibodies (Hellema *et al.*, 1978), so that false positive reactions due to antinuclear factors can be excluded.

Circulating immune complexes 5 years after vasectomy could be demonstrated in only a few out of twenty-nine men, but not consistently. Also other investigators had either negative (Hess *et al.*, 1977) or controversial (Tung *et al.*, 1978) findings. Possibly, the discrepancies can be attributed to differences in detection principles and sensitivity and in a lack of insight in what the various test systems actually measure. Though it is tempting to conclude that circulating immune complexes do not occur in vasectomized men, it cannot be ruled out that after vasectomy a transient presence of circulating immune complexes might sometimes occur.

In conclusion, sperm antibodies were shown to develop after vasectomy and were found to be persistent. Their ultimate relevance to the rather low fertility rate after an anatomically successful reanastomosis operation (Bradshaw, 1976) has yet to be determined, as well as their possible role in forming immune complexes.

We wish to thank the staff of the Department of Urology, Free University of Amsterdam (Head: Prof. Dr. R.J. Scholtmeyer), for their help in collecting serum and seminal plasma samples of vasectomized men. We are grateful to Dr I. van Wingerden (Division of Immunology, The Netherlands Cancer Institute, Amsterdam), to Dr C.L. Cambiaso (Unit of Experimental Medicine, International Institute of Cellular and Molecular Pathology, Université Catholique de Louvain, Brussels, Belgium), Dr C.L. Molenaar (Department of Immunochemistry, Central Laboratory of the Netherlands Red Cross Bloodtransfusion Service, Amsterdam) for examining the sera for circulating immune complexes, and to Dr S. Jager (Fertility Unit of the Department of Obstetrics and Gynaecology, University Hospital, Groningen) for performing the mixed antiglobulin reaction with some seminal plasma samples.

This study was supported by contract NIH-NICHD-75-2834 with the National Institute of Child Health and Human Development, Department of Health, Education and Welfare, USA, and by a grant from the Dutch Foundation 'De Drie Lichten'.

REFERENCES

ALEXANDER, N.J. & SCHMIDT, S.S. (1977) Incidence of antisperm antibody levels and granulomas in men. *Fert. Steril.* **28**, 655.

ALEXANDER, N.J. & CLARKSON, T.B. (1978) Effect of vasectomy on diet-induced atherosclerosis in monkeys. *Science* **201**, 538.

ANSBACHER, R. (1973) Vasectomy: sperm antibodies. *Fert. Steril.* **24**, 788.

ANSBACHER, R., HODGE, P., WILLIAMS, A. & MUMFORD, D.M. (1976) Vas ligation: humoral sperm antibodies. *Int. J. Fert.* **21**, 258.

BIGAZZI, P.E., KOSUDA, L.L., HSU, K.C. & ANDRES, G.A. (1976) Immune complex orchitis in vasectomized rabbits. *J. exp. Med.* **143**, 382.

BRADSHAW, L.E. (1976) Vasectomy reversibility—A status report. *Population Reports, Sterilization Series D. Population Information Program, George Washington Univ. Med. Center, Washington, D.C.*, p. 41.

CAMBIASO, C.L., RICCOMI, H., SINDIC, C. & MASSON, P.L. (1978) Particle counting immunoassay (PACIA). II. Automated determination of circulating immune complexes by inhibition of the agglutinating activity of rheumatoid sera. *J. Immunol. Methods*, **23**, 29.

FRIBERG, J. (1974) Clinical and immunological studies on sperm-agglutinating antibodies in serum and seminal fluid. *Acta obstet. gynec. scand.*, suppl. 36, pp. 1–76.

GUPTA, J., DHAWAN, S., GOEL, G.D. & SAHA, K. (1975) Low fertility in vasovasostomized males and its possible immunologic mechanism. *Int. J. Fert.* **20**, 183.

HEKMAN, A. & RÜMKE, P. (1976) Auto- and isoimmunity against spermatozoa. *Textbook of Immunopathology.* 2nd edn (ed. by P.A. Miescher and H.J. Müller-Eberhard), p. 947. New York, Grune & Stratton.

HELLEMA, H.W.J. & RÜMKE, P. (1976) Comparison of the tray agglutination technique with the gelatin agglutination technique for the detection of sperm agglutinating activity in human sera. *Fert. Steril.* **27**, 284.

HELLEMA, H.W.J. & RÜMKE, P. (1978a) Sperm autoantibodies as a consequence of vasectomy. I. Till 1 year post-operation. *Clin. exp. Immunol.* **31**, 18.

HELLEMA, H.W.J. & RÜMKE, P. (1978b) The micro-sperm immobilization test: the use of only motile spermatozoa and studies of complement. *Clin. exp. Immunol.* **31**, 1.

HELLEMA, H.W.J., SONDAG-TSCHROOTS, I.R.J.M., RÜMKE, P. & FELTKAMP, T.E.W. (1978) Autoantibodies to non-spermatozoal antigens as a consequence of vasectomy. *Immunological consequences of vasectomy in man, a thesis* (ed. by H.W.J. Hellema), p. 97. University of Amsterdam.

HESS, E.V., HERMAN, J.H. & HOUK, J.L. (1977) Immunological consequences of human vasectomy. *Immunological influence on human fertility.* (Ed. by B. Boettcher) *Proceedings of the Workshop on Fertility in Human Reproduction, Newcastle, Australia.* p. 329. Academic Press, Sydney.

HUSTED, S. (1975) Sperm antibodies in men from infertile couples. *Int. J. Fert.* **20**, 113.

HUSTED, S. & HJORT, T. (1975) Sperm antibodies in serum and seminal plasma. *Int. J. Fert.* **20**, 97.

JAGER, S., KREMER, J. & VAN SLOCHTEREN-DRAAISMA, T. (1978) A simple method of screening for antisperm antibodies in the human male. Detection of spermatozoal surface IgG with the direct mixed antiglobulin reaction carried out on untreated fresh human semen. *Int. J. Fert.* **23**, 12.

LINNET, L. & FOGH-ANDERSEN, P. (1979) Vasovasostomy: spermagglutinins in operatively obtained epididymal fluid and in seminal plasma before and after operation. *J. clin. lab. Immunol.* **2**, 3.

LINNET, L. & HJORT, T. (1977) Sperm agglutinins in seminal plasma and serum after vasectomy: correlation between immunological and clinical findings. *Clin. exp. Immunol.* **30**, 413.

ROSE, N.R., HJORT, T., RÜMKE, P., HARPER, M.J.K. & VYAZOV, O. (1976) Techniques for the detection of iso- and autoantibodies to human spermatozoa. *Clin. exp. Immunol.* **23**, 175.

RÜMKE, P. (1974a) *Autoantibodies against spermatozoa in infertile men: some unsolved problems. Proceedings of the First International Congress on Immunology in Obstetrics and Gynaecology, Padua* 1973 (eds. A. Centaro and N. Carretti), p. 26. Excerpta Medica, Amsterdam.

RÜMKE, P. (1974b) The origin of immunoglobulins in semen *Clin. exp. Immunol.* **17**, 287.

RÜMKE, P. & HEKMAN, A. (1977) Sterility: An immunologic disorder? *Clin. Obst. Gynec.* **20**, 691.

SAMUEL, T., KOLK, A.H.J., RÜMKE, P. & VAN LIS, J.M.J. (1975) Autoimmunity to sperm antigens in vasectomized men. *Clin. exp. Immunol.* **21**, 65.

SAMUEL, T., KOLK, A.H.J., RÜMKE, P., AARDEN, L.A. & BUSTIN, M. (1976) Histone and DNA detection in swollen spermatozoa and somatic cells, by immunofluorescence. *Clin. exp. Immunol.* **24**, 63.

SOBEL, A.T., BOKISCH, V.A. & MÜLLER-EBERHARD, H.J. (1975) C1q deviation test for the detection of immune complexes, aggregates of IgG, and bacterial products in human serum. *J. exp. Med.* **142**, 139.

TUNG, K.S.K., BRYSON, R.K., HAN, L.P.B. & WALKER, L.C. (1979) *Circulating immune complexes in vasectomy. Immunologic and immunophysiologic effects of vasectomy. Proceedings of NICHD workshop* (ed. I. Lepow), Airlie, Virginia, Academic Press (in press).

VAN WINGERDEN, I., THE, T.H., VAN DER GIESSEN, M. & RÜMKE, P. (1979) Demonstration of circulating immune complexes in melanoma patients by means of a granulocyte phagocytosis test. *Protides of the Biological Fluids.* Proceedings 26th Colloquium, 1978 (ed. by H. Peters), p. 345. Pergamon Press, Oxford.