Autoimmunity Against Sperms in Infertile Men*

Philip Rümke, M.D.

Involuntary infertility is a problem for more than 10 per cent of couples in the western world. Some of the cases where the infertility used to be called "unexplained" are now known to be of immunological origin. If "unexplained" is taken to include cases where postcoital tests show a poor migration or penetration of spermatozoa in the cervical mucus, (without obvious explanations such as bad quality of the semen or unfavourable physico-chemical quality of the mucus), then the infertility is due to immunological factors in husband or wife in 5 to 30 per cent of cases. Men are more often affected than women.

Sperm autoantigens in man

Sperm agglutinating antibodies found in the serum of some infertile men and women, or of vasectomised men reveal different patterns of agglutination, suggesting that distinct surface antigens occur in the head, the main part of the tail and the tip of the tail. De Almeida *et al*[1,2] fractionated washed, previously frozen sperms by grounding, ultracentrifugation and treatment with 8 M urea. Inhibition experiments using sera with high titres of various types of agglutinins revealed that at least three different autoantigens are present on the human sperm membrane. Hjort *et al*[3] question whether separate specific antigens occur in the head or the tail. These authors give evidence for the existence of at least three autoantigens in the membrane, two of which are glycoproteins, but different patterns of agglutination were considered to be due to the class or affinity of antibody rather than to the specificity of the antibodies.

Earlier it was found that two strongly basic nuclear proteins – protamines – isolated from human spermatozoa could be identified as autoantigens.[4] Antibodies to these protamines in sera of some infertile patients and some vasectomised men cross-react with fish protamines, and *vice versa*. Antibodies against salmon protamines found in diabetic patients treated with protamine-Zinc-insulin cross-react with protamine from human spermatozoa,[5] while they do not react with the nuclei of somatic cells. The cross-reactivity between human and fish protamines may have a medical implication, since salmon protamines are being used therapeutically in long-acting insulins and as antagonists against heparin. Allergic reactions to protamines have been described[5] and recently an anaphylactic reaction was observed in a vasectomised man who received protamine sulphate after open heart surgery. This patient appeared to have a high titre of antibodies against protamine in his serum.[6]

Non-organ specific sperm antigens

Histocompatibility and blood-group antigens

Claims that histocompatibility antigens are present on human spermatozoa, even with an indication of a haploid expression[7] have not been confirmed by others.[8-10] Bloodgroup substances A and B, which are present in the seminal plasma of secretors adhere to spermatozoa. Whether weak A and B antigenicity also occurs on the spermatozoa of non-secretors is still controversial, but most investigators could not detect bloodgroup substances A and B as intrinsic antigens on human spermatozoa.[11]

Sperm-coating antigens

In any discussion on the antigens of spermatozoa it must be realised that ejaculated spermatozoa are coated by substances derived from the adnexal glands, which are hard to remove without damaging the spermatozoa. When heterologous antisera are used in the analysis of semen, these sperm-coating antigens (SCAs) can be the cause of cross-reactions between antibodies to ejaculated spermatozoa and to seminal plasma. Apart from the bloodgroup substances A and B, the major SCA in man is lactoferrin which originates from the seminal vesicle.[12] Other SCAs are contributed by the prostate and epididymis.[11] Recently, it has been shown that a monoclonal antibody against one of the SCAs, found in both seminal

*From the Division of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

plasma and in milk, which was different from lactoferrin, could strongly immobilise but not agglutinate spermatozoa.[13] Isojima *et al*[14] described absorption of immobilising antibodies from the sera of infertile women by one of the SCAs. Recently, Vernon *et al*[15] described a SCA originating from the caput epididymis.

Test systems currently in use for the detection of sperm antibodies in men

Technical aspects of the detection of sperm antibodies in men and women can be found in review.[16-19]

The conventional test systems for the detection of antibodies against surface antigens of spermatozoa are the agglutination and the complement-dependent immobilisation and cytotoxicity tests. The agglutination test in gelatin-containing medium in tubes (GAT), which is read macroscopically and the micro-test in trays (TAT), which is read microscopically, are the most widely used agglutination tests. The GAT and TAT, in general, give comparable results, although the chance of non-immune agglutination, as for instance due to β2-macroglobulin in some sera, is greater in the TAT than in the GAT. The advantage of the TAT is the micro-scale application and the possibility of recording the type of agglutination, i.e. head-to-head, tail-to-tail, tailtip-to-tailtip and mixed forms. Fortunately, in spite of the presence of bloodgroup A and B antigens as SCAs, neither anti-A nor anti-B antibodies agglutinate spermatozoa from individuals positive for the crorreponding antigen(s). Therefore, every donor sample of semen of good quality can be used as substrate in these tests. The immobilisation test is applied in various modifications. Whereas the agglutinins are always semi-quantitated by titration, the immobilisation test as well as the cytotoxicity test are sometimes performed with undiluted serum and the strength of the reaction is expressed as an index. The qualitative and quantitative differences between the immobilisation and cytotoxicity tests are minimal. The same antibodies are detected by both these techniques. A particular advantage of the latter tests is that, because of their complement dependency, positive results can be more reliably ascribed to the activity of antibodies. An advantage of the agglutination tests is their higher sensitivity, with few exceptions, immobilisins (including cytotoxic antibodies) are found only when agglutinins are present in the same serum. The higher the agglutinin titres, the more frequently immobilisins are also present. The relationship is irrespective of the agglutination type, with the exception of the rarely occurring tail-tip type that has been found in high titres in the absence of immobilisins. Because of its anti-complementarity it is difficult to detect immobilisins or cytotoxic antibodies in seminal plasma.

Recently, a number of other tests for antispermatozoal antibodies have been developed. The mixed antiglobulin test is feasible for routine testing and has the advantage that the immunoglobulin class of the antibodies is determined at the same time. In particular, the test has been used to detect and classify antibodies bound to spermatozoa in ejaculates of patients,[20-22] but it can also be applied for the detection of serum antibodies.[23] Bronson *et al*[24] used immunobeads for the classification and localisation of sperm antibodies.

Tests with direct biological relevance are the sperm-cervical mucus penetration test (S-CMPT)[25] and the sperm-cervical mucus contact test (S-CMCT).[26] They are both applied in cases of male as well as female infertility. The S-CMPT measures the degree to which spermatozoa, sensitised by autoantibodies from the seminal plasma or by isoantibodies in the cervical mucus, are inhibited in their migration through a capillary filled with cervical mucus. The S-CMCT measures the percentages of spermatozoa that display jerking movements without progressive motility. This type of flagellation-in-situ is called the "shaking phenomenon" and is found to be associated with agglutinating antibodies in high titre. It is caused by adherence of the sensitised spermatozoa to the micelles of the cervical mucus. Since spermatozoa sensitised with $F(ab')_2$ derived from autoantibodies do not adhere, and also since $F(ab')_2$ of antibodies against the Fc part of IgG can inhibit the phenomenon, it is likely that the adherence is due to an interaction of the Fc part with the micelles.[27]

The indirect immunofluorescence test, as applied to sperm smears, has not found a wide application, not only because positive results were often obtained with control sera and the correlation with the result of agglutination and immobilisation tests was poor, but also because of a lack of interlaboratory reproducibility.[17] An exception can be made for the detection of anti-protamine antibodies on swollen spermatozoa. In that case the test is reproducible and does not reveal positive reactions in the controls.

An interesting new technique is based on the inhibition of the penetration of human spermatozoa into zona-free hamster ova.[28-30] If the spermatozoa are coated with antibody, they lose the capacity to penetrate the zona-free hamster ovum.

Another interesting new approach concerns the detection of antibody-coated sperm by "panning" procedures.[31] Wells on plastic plates are coated with anti-immunoglobulin molecules by either one step or two step procedure and the spermatozoa are then incubated in these wells for up to 1 h and the wells washed. Antibody coated spermatozoa remain attached in large numbers while control spermatozoa are washed out. These

procedures have the advantages that they are cheap, simple, do not involve sperm fixation and can be used with relatively diluted cell suspensions and with spermatozoa of low motility.

Most of the tests discussed so far are semiquantitative, subjective and liable to experimental error, therefore there is a need for objective assays such as those in which radiolabeled anti-antibody is quantitatively measured. A test involving nuclear binding of ^{3}H-actinomycin-D after membrane damage by complement-dependent cytotoxic antibodies is advantageous for testing many samples at the same time.[32,33] A test based on the difference in ATP content between damaged and undamaged spermatozoa gave satisfactory results in the assay for cytotoxic antibodies, and appeared simpler and faster than the conventional staining methods, allowing 200 samples to be tested within one day.[34] Nanogram amounts of IgG bound to guinea-pig testicular cells could be detected using radiolabeled staphylococcal protein A[35]

Enzyme-linked immunosorbent assays (ELISA) for the detection of sperm antibodies have been developed by several investigators. Paul *et al*[36] used sperm in PHA-coated-, and solubilised spermatozoal antigens adsorbed on poly-lysine coated microtitre plates with satisfactory results. A study of Lee *et al*[37] suggests that SDS gel/protein blot radioimmuno-binding can be a useful tool for the detection of antibodies against solubilised sperm antigens.

Autoimmunity against spermatozoa in infertile men

The prevalence of sperm antibodies in different reports varies considerably and depends on the selection of patients, the technique used and the subjective appreciation of the lowest serum dilution in which a positive result can still be recorded, the incidence of sperm antibodies in different reports varies considerably. Roughly estimated, 10 per cent of the male partners in infertile couples in whom no organic cause can be found (so called "unexplained" infertile couples) have sperm agglutinating (mainly of the tail-to-tail type) and often also immobilising antibodies. Sera containing such antibodies do not react in the immunofluorescence test with other organs, and absorption studies have shown that only testis or spermatozoa, and not seminal fluid, can absorb the antibodies. Therefore it may be concluded that in the majority of the cases agglutinating and immobilising antibodies in men are directed against testis-specific sperm antigens and not against SCA. They are most often of the IgG class, although IgM antibodies can also occur. (If agglutinin titres are low, i.e. less than 20, and immobilisins are not present, there is often no proof of the antibody nature of the agglutinating factor).

The higher the titre in the serum, the higher the chance that the seminal plasma also contains agglutinating antibodies. With a serum titre of 64, half of the men, and with a titre of 512, nearly 95% had seminal plasma agglutinins.[38] The agglutinins in seminal plasma can be of the IgG and IgA or sIgA type. Although 1 or 2% of circulating IgG transudates via the prostate into the seminal compartment,[39] most of the antibodies are locally formed somewhere in the genital tract. Since the concentrations of IgA and secretory component in seminal plasma of normal and vasectomised men are not significantly different, it has been concluded that the bulk of IgA originates in the part of the genital tract distal to the vasa deferentia.[40] Agglutinins are hardly ever found in the seminal plasma if they are not present in the serum. The levels of immunoglobulins in the seminal plasma of patients with sperm antibodies in the seminal plasma are not increased.[41]

Patients can be divided into two groups so far as the relationship between autoantibodies and infertility is concerned. In the first group belong the men in whom spermatogenesis is apparently not impaired, because ejaculates contain a normal number of spermatozoa. A normal morphology and a normal initial motility show moreover that in these patients there is no obvious defect in the function of the genital organs. The only problem is the presence of autoantibody in the seminal plasma. The other group comprises a minority of patients with sperm antibodies in the serum in whom the infertility can be explained by oligo- or azoospermia, due to whatever cause. The normospermic men will be discussed first.

A parallel between the inability to invade cervical mucus and serum agglutination and immobilising titres are found, as judged by post-coital and cervic mucus invasion tests *in vitro*.[42] The relationship of serum agglutinin titres to prolonged infertility was shown in a follow-up study comprising 254 men in whom titre of serum agglutinin ranging from 4 to over 1024 had been found.[43] In the subsequent years 36 men became fathers. Thirty of them belonged to a group of 137 normospermic men. An inverse relationship with the titre was evident, since 48% of the men with titres of 4-16 became fathers, whereas 16% of those with a titer of 32-128, and 12% of those with a titre of 128-512 fathered a child. Occasionally men become fathers despite a serum agglutinin titre as high as 1024. The seminal plasma titres have better predictive value. The author has rarely seen a man who became fertile when his seminal agglutinin titre was higher than 16. In a series of 26 patients Husted and Hjort[44] did not see any paternity in 15 patients with titres of 64 or higher, whereas the wives of 4 of 11 patients with titres of 4 to 16 became pregnant. In a later report from the same group[45] no influence of the serum titre upon the prognosis was found, but the seminal plasma titires had a predictive

value. With titires of 4 or lower the pregnancy rate was 55%, with titires of 4-256 22%. A direct relationship has been found between autoagglutination in the ejaculates of the normospermic men and the seminal titre of agglutinins.[20,46] Also, the chance of the remaining non-agglutinated spermatozoa becoming immobilised after penetration in the cervical mucus increases with the seminal plasma titres. Jagar *et al*,[47] found that with titres of 32 and higher, the non-agglutinated spermatozoa did not have a normal migration pattern as measured in the sperm penetration meter test, whilst in the sperm-cervical mucus contact test the vast majority of the spermatozoa showed the "shaking phenomenon", indicating adherence of sensitised spermatozoa to the glycoprotein micelles in the cervical mucus.

From these studies it can be concluded that, dependent on the titre in the seminal plasma, spermatozoa agglutinate each other, or adhere to the glycoprotein micelles of the cervical mucus, thus explaining why these men with higher titres have a higher chance of remaining infertile. It is difficult to explain why the patient remains infertile although some spermatozoa may escape from agglutination and adherence. In animal experiments it has been show that spermatozoa coated with antibody fragments were not able to adhere to ova.[48] Haas *et al*[29] found that human sperm antibodies of the IgG class inhibited penetration of human spermatozoa in zona-free hamster eggs. Therefore, even if spermatozoa escape from being agglutinated or trapped in the cervical mucus, they might not be able to penetrate the egg. However, it is not known whether spermatozoa that escape do so in spite of being coated by antibody or because of a lack of antibody coating. It is therefore not certain whether inhibition of egg penetration plays a role *in vivo*.

Theoretically it is also possible that spermatozoa escaping from the trap in the cervical mucus become immobilised when they enter a compartment where complement is present, if they are still coated with antibodies. In cervical mucus little or no complement is present but in the fallopian tubes where serum proteins are present in higher concentrations than in the cervical mucus, enough complement may be present to immobilise sensitised spermatozoa.

The second group of patients, i.e. those with an insufficient number of spermatozoa in the ejaculate, can be divided in two subgroups: one subgroup in whom there is complete azoospermia with an anatomical obstruction of the efferent pathways, and the other which comprises the majority of oligozoospermic patients, in whom anatomical obstruction is not evident. In the first subgroup, biopsies of the testis most often show normal spermatogenesis. These patients are comparable to vasectomised men. From longitudinal studies in vasectomised men it is known that 60-80% of them form sperm antibodies within one year after the operation and therefore it can be concluded that pathological resorption of spermatozoa or antigenic fragments as a result of obstruction in the efferent pathways, is a major cause of sperm autoantibody formation. The fact that high-titred sperm autoantibodies occur on the one hand, in the group of normospermic men already discussed and, on the other hand, in azoospermic men with normal spermatogenesis, shows that sperm antibodies as such do not necessarily interfere with spermatogenesis. As discussed earlier, the same has been found in animals: after immunisation with the use of incomplete Freund's adjuvant sperm antibodies are formed, but there is no indication of the development of autoimmune aspermatogenic orchitis.

The last subgroup of infertile men with sperm autoantibodies to be discussed, comprises patients with a low output of spermatozoa, or those azoospermic men in whom a testis biopsy shows abnormalities of spermatogenesis. These patients represent less than 10% of the total of oligozoospermic patients, indicating that an autoimmune syndrome of tesis and/or epididymis is uncommon. Nevertheless, from the point of view of immunopathology, they form an intriguing group of patients. In spite of the fact that the testis was one of the first target organs in experimental animal work in which mechanisms of autoimmunopathology were studied, in the clinical situation autoimmune diseases of the testis and/or epididymis are ill-defined and their very existence is still in doubt. Immunological studies in suspected patients are scantly and incomplete. One of the difficulties in recognising autoimmune orchi-epididymitis by a testis biopsy is that no pathognomonic histological criteria exist other than early clustering of macrophages and lymphocytes.[49] This stage might easily be missed, especially since Leydig cells are not affected, so that patients will have no hormonal deficiency which might lead to earlier presentation.

Because of the importance of T lymphocytes and delayed hypersensitivity to sperm antigens in the animal model, several investigators attempted to demonstrate cellular immunity against sperm antigens. Various techniques have been used, and in several disorders of the testis indications of cellular immunity were found. However, there is no formal proof that the reactivity was directed against sperm antigens. Boettcher *et al*,[50] showed that whole semen could stimulate allogeneic lymphocytes, whereas the same amount of isolated motile spermatozoa from the same semen sample did not stimulate the lymphocytes. Lucas and Rose[51] mention non-specific stimulatory activity or sometimes spontaneous lymphocyte toxicity by whole semen or sperm extracts. Nevertheless, in several studies patients had indications of cell-mediated immunity to

sperm much more often than controls.[52,53] Autologous sperm has sometimes proved to be stimulatory in lymphocyte transformation tests.[54] Since, however, there are indications of cell-mediate autoimmunity in some normospermic men, a positive reaction cannot be considered to be pathognomonic or diagnostic for autoimmune orchitis. A positive finding, such as the presence of humoral sperm antibodies, only indicates that the patient at one time became immunised against his own sperm.

Further immunohistological studies of the testis are needed to prove an effect of autoimmunity at the level of the testis. There are several reports on tissue bound immunoglobulin or C3 in the testis, but most often these concern immunoglobulins bound to the interstitium and the tubular walls and seldom to their contents. In the few cases in which germ cells sealed off from the walls showed immunohistological staining, no attempts were undertaken to relate the findings with the various types of sperm-antibodies.[55-57] No comprehensive studies on such cases have ever been carried out.

However, studies in which oligozoospermic patients were treated with corticosteroids do give evidence for the existence of the syndrome in man. Four of 13 oligo- or azoospermic patients, all having indications of cellular immunity against sperm, showed a considerable increase of the sperm output during treatment, whereas 11 such patients, who did not have a positive blastoid transformation test did not benefit from the therapy.[58] Hendry *et al*[59] found a considerable improvement of the sperm output during or after treatment with corticosteroids in some oligozoospermic patients with sperm antibodies, while at the same time the sperm antibodies in the serum and seminal plasma decreased in titre. It was remarkable that, in 3 oligozoospermic men with high titres of anti-sperm antibodies, testis biopsies showed an adequate spermatogenesis. The authors pointed out that testicular biopsies are always taken from the body of the testis, and thus they are unlikely to detect forms of autoimmune orchitis which cause oligozoospermia by affecting transport in the efferent passages, such as can be induced experimentally. Since corticosteroids have no influence on hormonal factors related to spermatogenesis, it may tentatively be concluded that, when sperm counts rise and sperm antibody titres fall, it is likely that the patient had an autoimmune orchi-epididymitis. For further immunological studies related to oligozoospermia the reader is referred to a recent review.[60]

Therapy of infertile men with sperm autoantibodies

High dose corticosteroid therapy, e.g. 96 mg methylprednisolone per day for one week, sometimes synchronised with the last week of the menstrual cycle of the wife, has been advocated by Shulman. Shulman and Shulman[61] reported 31 pregnancies within one year after the treatment of 71 patients (44% success rate). Hendry *et al*,[62] treated 45 men with serum sperm agglutinins in titres of more than 32 and demonstrable impaired sperm penetration of cervical mucus. With 32 mg methylprednisone per day, taken mostly also during the last week of the wife's menstrual cycle, 14 wives (31%) became pregnant. The pregnancies were always associated with a marked drop in sperm immobilising titre and usually with disappearance of antibodies from seminal plasma (but sometimes without a drop in serum agglutinin titres). Hargreave and Elton[63] compared the high dose methylprednisolone scheme with betamethasone, also in 7 day courses (2 mg daily for 3 days, 1 mg daily for 2 days and 0.5 mg daily for 2 days) on alternate weeks for up to 6 months. The pregnancy rate was 11/27 (41%). The results were better with the comparatively lower dose of betamethasone. As pointed out by Hjort[64] in an editorial, even though none of the above studies included properly matched control groups, the results seem to indicate that treatment is effective in a proportion of patients, although the way in which it works is not clear. In these couples, in whom there is a relatively long duration of involuntary infertility, pregnancies occurred immediately following treatment, antibody titres in serum and seminal plasma decreased, and improvements in semen quality were observed in a high proportion of men with poor semen quality before treatment. However, side effects in the treated men were common. Even bilateral aseptic necrosis of the femoral heads was observed late after the treatment period.[61,65] Lower doses should therefore be tried first. De Almeida and Jouannet[66] treated 14 patients with 2 or 3 mg dexamethasone for 13 or 9 weeks with a gradual decrease of the dose in the 7 weeks thereafter. They saw 3 (21%) pregnancies during or shortly after the treatment, when serum-spermatotoxic antibodies and seminal sperm agglutinins had become negative, but while agglutinin titres in the serum remained high.

Antibiotic treatment has been tried in cases where the antibody formation was believed to be related to prostatitis. Fjallbrant and Niisson[67] noted a considerable decrease or even complete disappearance of sperm antibodies in 8 men of whom 5 became fathers.

An interesting new approach was recently reported by Boettcher *et al*.[68] These authors argue that IgG antibodies are derived from testicular and/or epididymal lesions, while sIgA antibodies originate from the prostate. Since the latter antibodies are believed to be the most effective in inhibiting progression of spermatozoa in the cervical mucus, treatment aimed at preventing the binding of the sIgA antibodies to the spermatozoa by letting

patients ejaculate directly into Tyrode's solution. The spermatozoal portion of the ejaculate was collected, suspended in a large volume of Tyrode's solution, centrifuged at 200g for 20 minutes and resuspended in sterile Tyrode's medium to produce a suspension in which more than 90% of spermatozoa were motile and no autoagglutination of spermatozoa was observed. As reported at the 2nd International Congress of Reproductive Immunology, in all 13 patients treated in this way IgA coating of spermatozoa was less than on spermatozoa of the untreated ejaculate, in 5 cases IgA coating could be prevented completely, whereas IgG coating could not be prevented. Four pregnancies were achieved in 3 patients. The authors recommend that this treatment should be tried before immunosuppressive treatment.

REFERENCES

1. DeAlmeida M, Lefroit-Jolly M, Voisin G. Studies on human spermatozoa antigens. I. Fractionation of sperm membrane anigens: evidence of three antigenic systems. Clin Exp Immunol 1981;4:359-67.
2. DeAlmeida M, Lefroit-Jolly M, Rignenzi S. Human sperm autoantigens: Fractionation and analysis of sperm membrane antigenic system. In: Shulman S, Dondero F, eds, Immunological factors in human contraception. Field Educational Italia. Rome: Acta Medica, 1983:39-46.
3. Hjort T, Ahuja SP, Poulson F. Studies on sperm membrane antigens. In:Shulman S, Dondero F, Nicotra M, eds, Immunological factors in human reproduction. New York: Academic Press, 1982:77-90.
4. Kolk AHJ, Samuel T. Isolation, chemical and immunological characterisation of two strongly basic nuclear proteins from human spermatozoa. Biochim Biophys Acta 1975;393:307-9.
5. Samuel T. Antibodies reacting with salmon and human protamines in sera from infertile men and from vasectomised men and monkeys. Clin Exp Immunol 1977;30:181-7.
6. Watson RA, Ansbacher R, Barry M, Deshon GE, Agree RE. Allergic reaction to protamine: A late complications of elective vasectomy? Urology 1983; 22: 493-5.
7. Festenstein H, Halim K, Aranaiz-Villena R. HLA antigens on human spermatozoa. In: Cohen J, Hendry WF, eds, Spermatozoa, antibodies and infertility. Oxford: Blackwell Sci Pub, 1978:11-6.
8. Law HY, Bodmer WF. Use of microimmobilisation and micro-agglutination assays for attempted detection of HLA antigens and beta 2 microglobulin on human sperm. Tissue Antigens 1978; 12: 249-69.
9. Brodsky FM, Patham P, Barnstable CJ, Crumpton MJ, Bodmer WF. Monoclonal antibodies for analysis of the HLA system. Immunol Rev 1979;47:3-61.
10. Anderson DJ, Bach DL, Yunis EJ, DeWolf WC. Major histocompatibility antigens are not expressed on human epididymal sperm. J Immunol 1982;129:452-4.
11. Hekman A, Rumke P. Auto- and isoimmunity against spermatozoa. In: Miescher PA, Muller-Eberhard HJ, eds, Textbook of immunopathology. New York: Grune & Stratton, 1976:947-62.
12. Hekman A, Rumke P. The antigens of human seminal plasma. With special reference to lactoferrin as a spermatozoa-coating antigen. Fertil Steril 1969; 20: 312-23.
13. Shigeta M, Watanabe T, Maruyama S, Koyama K, Isojima S. Sperm immobilising monoclonal antibody to human seminal plasma antigens. Clin Exp Immunol 1980;42:458-62.
14. Isojima S, Koyama K, Tsuchija K. The effect on fertility in women of circulating antibodies against human spermatozoa. J Reprod Fert 1974;21 (Suppl):125-50.
15. Vernon RB, Muller CH, Herr JC, Feuchter FA, Eddy EM. Epididymal secretion of a mouse sperm surface component recognised by a monoclonal antibody. Biol Reprod 1982;26:523-35.
16. Rose NR, Hjort T, Rumke Ph, Harper MJK, Vyazolv O, eds, Techniques for detection of iso- and auto-antibodies to human spermatozoa. Clin Exp Immunol 1976;23:175-99.
17. Boettcher B, Hjort T, Rumke Ph, Shulman S, Vyazov OE (eds). Auto- and isoantibodies to antigens of the human reproductive system. 1. Results of an international comparative study of antibodies to spermatozoa and other antigens detected in sera from infertile patients deposited in the WHO Reference Bank for reproductive immunology. Acta Pathol Microbiol Scand 1977;258 (Suppl):1-69.
18. Shulman S. Agglutinating and immobilizing antibodies to spermatozoa. In: Cohen J, Hendry WF, eds, Spermatozoa, antibodies and infertility. Oxford: Blackwell Sci Pub, 1978:81-99.
19. Menge AC. Clinical immunologic infertility: diagnostic measures, incidence of antisperm antibodies, fertility and mechanisms. In Immunological aspects of infertility and fertility regulation. Dhindsa DS, Schumacher GFB. New York: Elsevier/North-Holland, 1980;205-24.
20. Jager S, Kremer J, Van Slochteren-Draaisma T. 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 Fertil 1978;23:12-21.
21. Jager S, Kremer J, Kuiken J, Van Slochteren-Draaisma T. Immunoglobulin class of antispermatozoal antibodies from infertile men and inhibition of *in vitro* sperm penetration into cervical mucus. Int J Androl 1980;3:1-14.
22. Stedronska J, Hendry WF. The value of the mixed antiglobulin reaction (MAR Test) as an addition to routine seminal analysis in the evaluation of the subfertile couple. Am J Reprod Immunol 1983; 3: 89-91.
23. Mathur S, Williamson HO, Landgrebe SC, Smith Ch L, Fedenberg HH. Application of passive hemagglutination for evaluation of antisperm antibodies and a modified Coombs' test for detecting male autoimmunity to sperm antigens. J Immunol Methods 1979;30:381-93.
24. Bronson R, Cooper G, Rosenfeld D. Correlation between regional specificity of antisperm antibodies to the spermatozoan surface and complement-mediated sperm immobilization. Am J Reprod Immunol 1982;2:222-4.
25. Kremer J. A simple sperm penetration test. Int J Fertil 1965;10:209-15.
26. Kremer J, Jager S. Characteristics of antispermatozoal antibodies responsible for the shaking phenomeon with special regard to immunoglobulin class and antigen-reactive sites. Int J Androl 1980; 3:143-52.
27. Jager S, Kremer J, Kuiken J, Mulder I. The significance of the Fc part of antispermatozoal antibodies for the shaking phenomenon in the SCMC test. Fertil Steril 1981;36:792-7.
28. Menge AC, Black CS. Effects of antisera on human sperm penetration of zona-free hamster ova. Fertil Steril 1979;32:214-8.
29. Haas GG, Sokoloski JE, Wolf DP. The interfering effect of human IgG antisperm antibodies on human sperm penetration of zona-free hamster eggs. Am J Reprod Immunol 1980; 1:40-3.
30. Riedel HH, Baukloh V, Steinberg R, Wahe-Rohrbach T, Mettler L. Inhibitory effect of antisperm-antibodies on the penetration rate of human spermatozoa studied in the zona free hamster egg cell system. In: Shulman S, Dondero F, eds, Immunological factors in human contraception. Field Educational Italia, Rome: Acta Medica, 1983:113-9.
31. Hancock RJT, Faruki S. Detection of antibody-coated sperm by "panning" procedures. J Immunol Methods 1984; 66:149-

59.
32. Sung JS, Shizuya H, Black DD, Mumford DM. A radiomicroassay for cytotoxic antibody to human spermatozoa. Quantification by tritiated actinomycin D. Clin Exp Immunol 1977; 27:469-77.
33. Boettcher B, Boettcher MJ. Spermotoxic antibodies in sera from infertile couples determined with ^{3}H-actinomycin D. In: Bratanov K, et al, eds, Immunology of reproduction. Sofia: Bulgarian Academy of Sciences, 1979; 2:331-5.
34. Suominen JJO, Multamaki S, Djupsund BM. A new method for measurement of cytotoxic antibodies to human spermatozoa. Arch Androl 1980; 4:257-64.
35. Han B LP, Tung KSK. A quantitative assay for antibodies to surface antigens of guinea pig testicular cells and spermatozoa. Biol Reprod 1979; 21:99-108.
36. Paul S, Baukloh V, Mettler L. Enzyme-linked immunosorbent assays for sperm antibody detection and antigenic analysis. J Immunol Methods 1983; 56:193-9.
37. Lee CY G, Lum V, Wong E, Mengse AC, Huang YS. Identification of human sperm antigens to antisperm antibodies. Am J Reprod Immunol 1983; 3:183-7.
38. Rumke P. Autoantibodies and infertility. In Spermatozoa, antibodies and infertility. Cohen J, Hendry WF (eds.). Oxford: Blackwell Scientific Publications. 1978: 67-79.
39. Rumke P. The origin of immunoglobulins in semen. Clin Exp Immunol 1976; 17: 287-97.
40. Kay DJ. Clinical significance of antibodies to antigens of the reproductive tract. In Immunological influence of human fertility. Boettcher B. (Ed.) Sydney: Academic Press, 1977:119-23.
41. Friberg J. Immunoglobulin concentration in serum and seminal fluid from men with and without sperm-agglutinating antibodies. Obstet Gynecol 1980; 136:671-5.
42. Fjallbrant B. Sperm antibodies and sterility in men. Acta Obstet Gynecol Scand 1968; Vol. XLVII, suppl. 4.
43. Rumke P, Van Amstel N, Nesser EN, Bezemer PD. Prognosis of fertility of men with spermagglutinins in the serum. Fertil Steril 1974; 25:393-8.
44. Husted S, Hjort T. Sperm antibodies in serum and seminal plasma. Int J Fertil 1973; 20:97-105.
45. Hjort T, Brogaard Hansen K. Seminal antigens in man with particular regard to possible immunological contraception. In: Shulman S, Dondero F, eds, Immunological factors in human contraception. Field Educational Italia, Rome: Acata Medica 1983:47-56.
46. Fribert J. Autoagglutination in ejaculates caused by spermagglutinating antibodies. Am J Reprod Immunol 1980; 1:44-8.
47. Jager S, Kremer J, Van Slochteren-Draaisma T. Presence of sperm agglutinating antibodies in intertile men and inhibition of *in vitro* sperm penetration into cervical mucus. Int J Androl 1979; 2:117-30.
48. Metz CB, Anika J. Failure of conception in rabbits inseminated with nonagglutinating, univalent antibody treated semen. Biol Reprod 1970; 2:284-90.
49. Tung KSK. Autoimmunity of the testis. In: Immunological aspects of infertility, and fertility regulation. Dhindsa DS, Schumacher GFB (eds.). New York: Elsevier/North-Holland, 1980:33-91.
50. Boettcher B, Misko IS, Roberts TK, Kay DJ, Hicks L, Gruszynski R. Allogeneic cellular immune reaction to human seminal cells. In: Bratanov K, et al, eds, Immunology of reproduction. Sofia: Bulgarian Academy of Sciences, 1979:201-4.
51. Lucus PL, Rose NR. Immunological consequences of vasectomy. I Prospective study through one-year postvasectomy. Arch Androl 1978; 1:249-55.
52. Dondero F, Lenzi A, Picardo M, Pastore R, Valesini G. Cell-mediated antisperm immunity in selected forms of male infertility. Andrologia 1980; 12:25-9.
53. Polidori R, Ambrogi F, Izzo PL, Mariani M, Grassi B, Menchini Fabris GF. Cell-mediated immunity in male fertility. Immunological infertility in men. Andrologia 1980; 12:141-5.
54. Thestrup-Pedersen K, Husted S, Hjort T. Lymphocyte-transformation test with spermatozoal antigens in men from infertile couples. I A methodological study of different spermatozoal preparations as antigen. Int J Fertil 1976; 21:218-25.
55. Wall JR, Stedronska J, David RD, Harrison RF, Goriup D, Lessof M. Immunologic studies of male infertility. Fertil Steril 1975; 26:1035-41.
56. Morenz J, Donat H. Immunological factors in human male infertility: Immunopathological studies of testes. Int J Fertil 1981; 26:94-100.
57. Jadot-van de Casseye W, de Bled G, Gepts W, Schoysman R. An immunohistochemical study for testicular biopsies in cases of male infertility. Andrologia 1980; 12:199-2.
58. Bassili F, El-Alfia OS. Immunological aspermatogenesis in man. II. Response to corticosteroids in cases of non-obstructive azoospermia with a positive blastoid transformation. J Reprod Fert 1970; 21:29-35.
59. Hendry WF, Stedronska J, Hughes L, Cameron KM, Pugh RCB. Steroid treatment caused by antisperm antibodies. Lancet 1979; 2:498-502.
60. Rumke P. Can oligozoospermia be induced by autoimmunity? In: Frajese G, et al, eds, Oligozoospermia: Recent progress in andrology, New York: Raven Press 1981: 185-97.
61. Shulman JF, Shulman S. Methylprednisolone treatment of immunologic infertility in the male. Fertil Steril 1982; 38:591-9.
62. Hendry WF, Stedronska J, Parslow J, Hughes L. The results of intermittent high dose steroid therapy for male infertility due to antisperm antibodies. Fertil Steril 1981; 36:351-
63. Hargreave TB, Elton RA. Treatment with intermittent high dose methylprednisolone or intermittent betamethasone for antisperm antibodies: preliminary communication. Fertil Steril 1982; 38:586-90.
64. Hjort T. Treatment of infertility associated with autoantibodies to sperm. Int J Androl 1983; 6:113-5.
65. Hendry WF. Bilateral aseptic necrosis of femoral heads following intermittent high-dose steroid therapy. Fertil Steril 1982; 38:120.
66. De Almeida M, Jourannet P. Dexamethasone therapy of infertile men with sperm autoantibodies: immunological and sperm follow up. Clin Exp Immunol 1981; 44: 567-75.
67. Fjallbrant B, Nilsson S. Decrease of sperm antibody titer in males, and conception after treatment of chronic prostatitis. Int J Fertil 1977; 22:255-6.
68. Boettcher B, Kay DJ, Fitchett SB. Successful treatment of male infertility caused by antispermatozoal antibodies. Med J Aust 1982; 2:471-4.