Immunoglobulin class of antispermatozoal antibodies and inhibition of sperm penetration into cervical mucus.

Jager, Siemen

IMMUNOGLOBULIN CLASS OF ANTISPERMATOZOAL ANTIBODIES AND INHIBITION OF SPERM PENETRATION INTO CERVICAL MUCUS

S. JAGER

IMMUNOGLOBULIN CLASS OF ANTISPERMATOZOAL ANTIBODIES AND INHIBITION OF SPERM PENETRATION INTO CERVICAL MUCUS

STELLINGEN

behorend bij het proefschrift

IMMUNOGLOBULIN CLASS OF ANTISPERMATOZOAL ANTIBODIES

AND

INHIBITION OF SPERM PENETRATION INTO CERVICAL MUCUS

S. Jager

I

Het schudfenomeen in de SCMC test geeft inzicht in de wijze waarop antispermatozoa antistoffen het transcervicale transport van niet-geagglutineerde spermatozoa kunnen belemmeren.

Dit proefschrift

II

De MAR test op IgG dient een onderdeel van het routine sperma onderzoek te zijn.

Dit proefschrift

III

Antispermatozoa antistoffen, die infertiliteit veroorzaken bij de man, behoren tot de IgA klasse.

Dit proefschrift

IV

Het schudfenomeen is niet specifiek voor een bepaalde immuunglobuline klasse van antispermatozoa antistoffen.

Dit proefschrift

V

Het Fc gedeelte van antispermatozoa antistoffen is betrokken bij het ontstaan van het schudfenomeen.

Dit proefschrift

VI

Cervixslijm bevat waarschijnlijk geen Fc receptoren.

Dit proefschrift

VII

Haploide expressie van HLA antigenen op spermatozoa is niet aangetoond.

VIII

De physisch-chemische eigenschappen van het sperma-plasma beinvloeden de fertiliteit waarschijnlijk niet.

IX

Het gebruik van de term pseudo-agglutinatie bij het semen onderzoek is onjuist.

X

Polyzoospermie is geen oorzaak van infertiliteit.

XI

De waarnemingen van diploide spermatozoa door Frajese et al. berusten waarschijnlijk op een artefact.
Frajese, G., Silvestroni, L., Malandrino, F. & Isidori, A. (1976) Fert. Steril. 27, 14-20.

XII

Indien bij het routine onderzoek geen oorzaak voor de infertiliteit is gevonden dient het fertiliserend vermogen van de spermatozoa te worden nagegaan met behulp van zona-vrije hamster ova (humster test).

XIII

Het kontroleren van de effektiviteit van een behandeling met corticosteroiden ter vermindering van de antispermatozoa antistof aktiviteit bij infertiele mannen dient plaats te vinden door bepaling van het schudfenomeen in de SCMC test en de bepaling van de IgA sensibilisatie in de MAR test.

XIV

Een farmakologisch te weeg gebrachte daling van de HDL-cholesterol koncentratie in serum is niet zonder meer vergelijkbaar met de lage HDL-cholesterol koncentratie die op epidemiologische gronden een verhoogde kans geeft op een ischaemische hartziekte.

XV

Het onderzoek naar stoffen, die voorkomen in lichaamsvloeistoffen en verband houden met de aanwezigheid van maligne ovarium tumoren, dient meer gericht te worden op de herkenning van specifieke tumor-merkstoffen.

XVI

De patient dient recht te hebben op een volledig en schriftelijk verslag van medisch specialistisch onderzoek en behandeling. Dit moet wettelijk worden vastgelegd.

RIJKSUNIVERSITEIT TE GRONINGEN

IMMUNOGLOBULIN CLASS OF ANTISPERMATOZOAL ANTIBODIES AND INHIBITION OF SPERM PENETRATION INTO CERVICAL MUCUS

PROEFSCHRIFT

ter verkrijging van het doctoraat in de geneeskunde
aan de rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. M.R. van Gils
in het openbaar te verdedigen op woensdag 11 maart 1980
des namiddags te 1.30 uur precies

door

SIEMEN JAGER

geboren te Odoorn

I.p.v. 11 maart 1980 dient te worden gelezen: 11 maart 1981.

Druk: VEENSTRA VISSER OFFSET
GRONINGEN

PROMOTOR : Prof. Dr. J. Kremer

CO-PROMOTOR : Prof. Dr. Ph. J. Hoedemaker

CO-REFERENT : Dr. Ph. Rümke

Prof. Dr. T. H. The

This study was supported in part by a grant from the Organization for Health Research TNO.

Aan Truus, Sylvia en Marianne

VOORWOORD

Het in dit proefschrift beschreven onderzoek werd verricht in de Kliniek voor Obstetrie en Gynaecologie van het Academisch Ziekenhuis te Groningen (hoofd: Prof. Dr. J. Janssens).

Aan allen die hebben meegewerkt aan het tot stand komen van dit proefschrift wil ik mijn hartelijke dank betuigen.

In de eerste plaats dank ik mijn promotor Prof. Dr. J. Kremer (buitengewoon hoogleraar aan de Rijksuniversiteit te Utrecht en hoofd van de afdeling Vruchtbaarheidsbeinvloeding van de Kliniek voor Obstetrie en Gynaecologie van het Academisch Ziekenhuis te Groningen) die mij stimuleerde tot het schrijven van dit proefschrift, voor zijn nimmer aflatend enthousiasme en zijn kritische begeleiding.

Prof. Dr. Ph. J. Hoedemaeker dank ik voor de kritische beoordeling van het proefschrift

Dr. Ph. Rümke ben ik zeer dankbaar voor zijn voortdurende belangstelling in het onderzoek, zijn kritische begeleiding en zijn vele waardevolle suggesties.

Prof. Dr. T. H. The dank ik voor de wijze waarop hij het proefschrift beoordeelde.

In het bijzonder wil ik dank brengen aan mijn medewerkers, Tiny van Slochteren-Draaisma en Jaap Kuiken die door hun enthousiasme, hun originele ideeën en de nauwgezette uitvoering van de experimenten in belangrijke mate hebben bijgedragen aan het tot stand komen van dit proefschrift. Ook mijn medewerksters Ina Mulder, mevr. A. M. Schmutzler-de Vries en mevr. I. W. de Wilde-Janssen wil ik danken voor hun bijdrage aan het experimentele werk.

I am grateful to prof. B. Boettcher for correction of the english text of the introduction.

I want also to thank Dr. W. F. Hendry for correction of the english text of some parts of this thesis.

Mevr. Dr. R. Mathias ben ik erkentelijk voor de correctie van de engelse text van chapter 8, chapter 9, de General Discussion en de Summary.

Voorts wil ik dank brengen aan de sperma donoren. Zonder hun bijdragen had dit onderzoek niet kunnen worden verricht.

Tenslotte wil ik dank brengen aan die patienten die door hun vrijwillige medewerking dit onderzoek mogelijk maakten.

CONTENTS

CHAPTER 1

INTRODUCTION

INTRODUCTION

The antigenicity of spermatozoa was discovered independently by Landsteiner and by Metchnikof in 1899. They observed a heterologous sperm immobilizing activity in the peritoneal fluid (Landsteiner, Metchnikof) and in the serum (Metchnikof) of animals immunized previously with semen from the heterologous animal species. Metalnikof (1900) induced homologous and autologous sperm immobilizing activity. These results have been confirmed many times thereafter. Temporary sterility in female animals after injection with spermatozoa has been reported by several investigators (Savini, 1911; Venema, 1916). In 1932 Baskin claimed to have induced infertility in women after injection of human spermatozoa. For an extensive review of the historical papers the reader is referred to Katsh (1959) and Tyler (1961).

The spontaneous occurrence of antispermatozoal antibody activity in women seems to have been observed first by Meaker (1922). For a long time thereafter only a few articles were published on spontaneous sensitization of women against spermatozoa (Ardelt, 1934; Nakabayashi et al., 1961). The investigation of antispermatozoal antibodies in women was strongly stimulated by a publication of Franklin & Dukes (1964a) in which sperm agglutinating activity was found in the sera of a high proportion of women infertile without known cause. Presence of local antibodies, reactive with spermatozoa, was found in cervical mucus first by Parish et al. (1967).

The occurrence of antispermatozoal auto-antibodies in men was demonstated for the first time in 1954 independently by Rümke and by Wilson. Rümke discovered sperm agglutinating activity in the serum of two men with oligozoospermia. Wilson detected sperm agglutinating activity in serum and seminal plasma from two men with partial auto-agglutination of the spermatozoa in the ejaculate.

The relationship between infertility and the presence of antibodies reactive with spermatozoa has been studied by many investigators and by different techniques. Many of the apparent contradictions in results can be ascribed to differences in sensitivity and specificity of the methods. Most studies have been performed with sera. It is obvious, however, that the infertility impairment caused by antispermatozoal antibodies is due to action in the reproductive tract. An essential step in the normal sequence of events leading to fertilization is sperm penetration into cer-

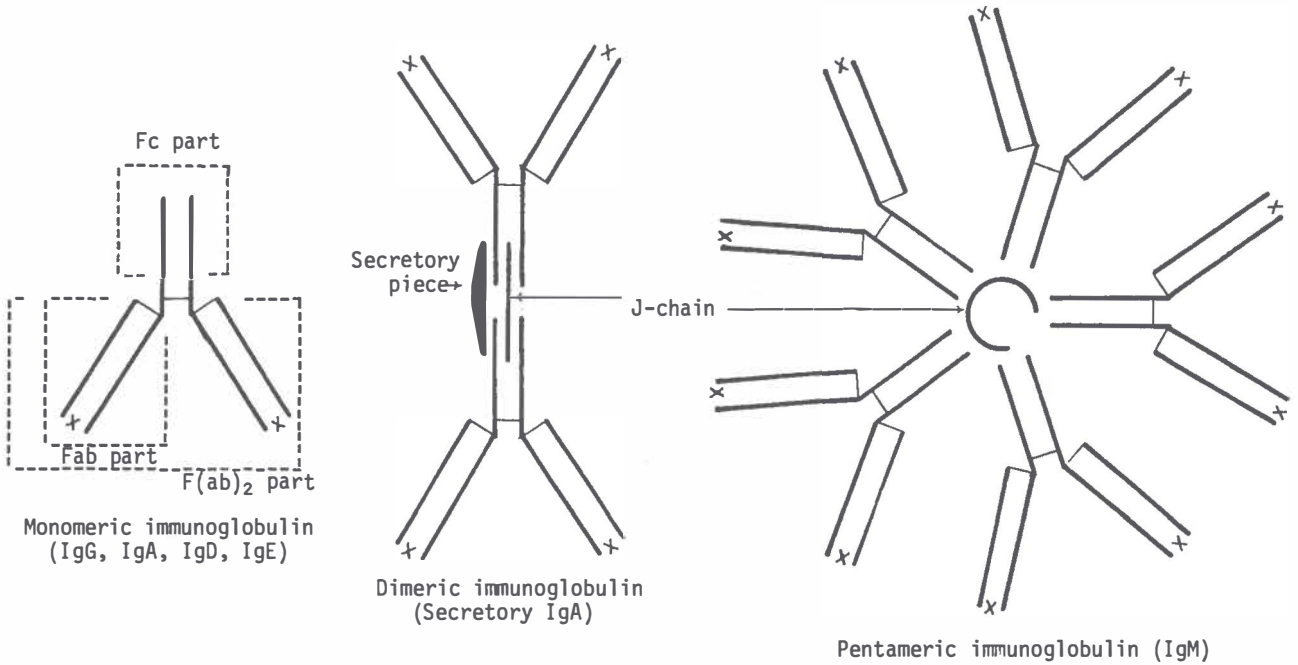


Fig. 1.1. Diagrams of immunoglobulin structure. An immunoglobulin monomer consists of four polypeptide chains connected to each other by disulfide bonds. Each monomer contains two antigen binding sites (X); one on each Fab (Fragment antigen binding) part. On the Fc (Fragment crystalline) part sites can be present for complement fixation and for binding to Fc receptors. Monomeric immunoglobulins are IgG, IgA, IgD and IgE. In secretions IgA occurs in a dimeric form. The two monomers are joined by a short polypeptide called the J-chain. In addition. secrotory IgA contains a polypeptide chain, called the secretory piece. IgM, the largest immunoglobulin, has a pentameric structure and also contains a J-chain.

vical mucus. The mechanism by which antispermatozoal antibodies reduce the capacity of spermatozoa to penetrate cervical mucus is the main subject of this thesis. In this introduction the following topics will be surveyed briefly:

1. Methods for detection of antispermatozoal antibodies.
2. Spermatozoal antigens and effects on fertility of antibodies to these antigens.
3. The penetration and migration of spermatozoa in cervical mucus.
4. Possible sites and mechanisms of fertility impairment by antispermatozoal antibodies.
5. The aims of the present investigations are described in chapters 2-9.

1. METHODS FOR DETECTION OF ANTISPERMATOZOAL ANTIBODIES

This section deals mainly with the commonly used methods. During a WHO sponsored workshop (Rose et al., 1976) proposals were made for standardization of various techniques. The nomenclature used in this workshop is also used in the present studies. A second WHO workshop (Boettcher et al., 1977) was dedicated to an international comparative investigation of results obtained by several laboratories testing a panel sera by the commonly used methods and some newer techniques.

1.1. Agglutination tests

Agglutination tests are sensitive techniques for detection of spermatozoal surface antigens. The test depends on the ability of antibodies to bind more than one antigenic determinant per antibody molecule (fig.1.1). If the antigens bound by the antibody molecules are present on different cells, these cells agglutinate. A drawback of agglutination tests is that agglutination can be caused by agents other than antibodies. For a survey of non-immunoglobulin sperm agglutinating factors see Shulman (1975). For a non-immunoglobulin sperm agglutinating factor in sera from women see also 2.8.

A commonly used procedure is the gelatin agglutination test (GAT) developed by Kibrick, Belding & Merril (1952) with rabbit antisera reactive with human spermatozoa. The GAT has often been referred to as the Kibrick- or KBM-test. In this test, motile spermatozoa are suspended in a gelatin-containing medium and mixed with the fluid under study which has been

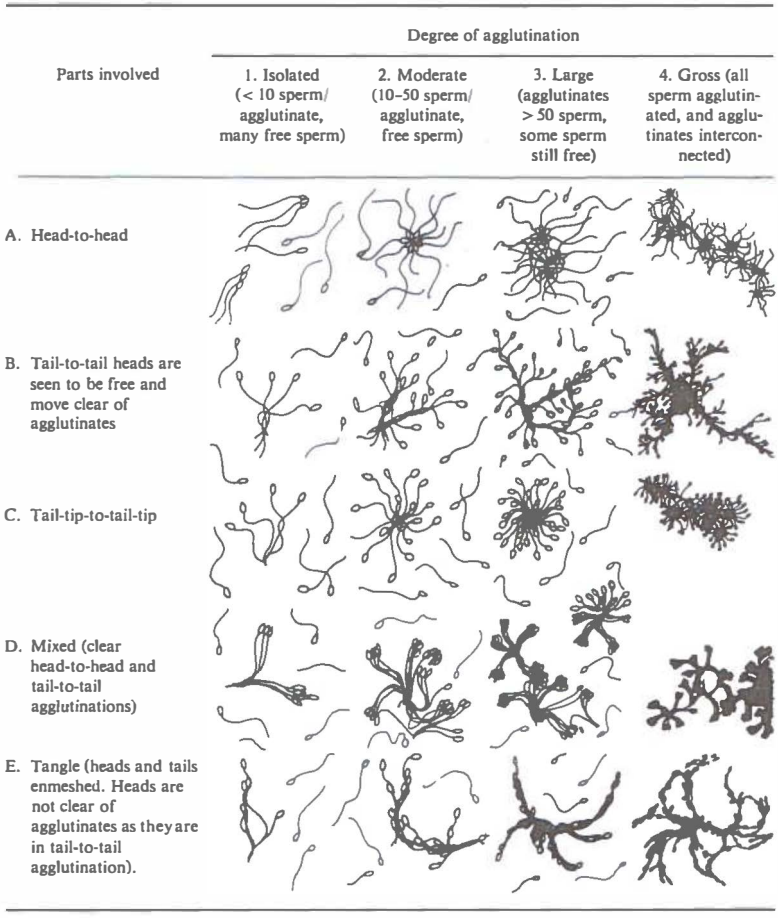


Fig. 1.2. Standardized descriptions of type and degree of sperm agglutination. Descriptions are based on the parts of the spermatozoa involved in the agglutination and the number of spermatozoa involved in the agglutination (from Rose et al., 1976).

serially diluted in small bore culture tubes. The presence of gelatin favors the formation of large agglutinates with low sedimentation rate. The agglutination is observed macroscopically. The agglutination type (fig. 2.1) cannot be determined in the GAT. The GAT was applied first on human sera by Rümke (1954).

In 1964 Franklin and Dukes introduced the tube-slide agglutination test (TSAT), formerly called the Franklin & Dukes or F&D test. In this test 0.05 ml of a suspension of motile spermatozoa is mixed with 0.5 ml serum or a serum dilution in a test tube, and this is incubated at 37 ^{o}C. Samples are transferred from the tube to a microscopic slide and the agglutination is studied microscopically. Comparative investigations have shown that some sera, positive in the GAT, were negative in the TSAT and sera positive in the TSAT could be negative in the GAT (Boettcher et al., 1971; Hansen, 1974; Boettcher et al., 1977). Possibly pipetting can result in disruption of tail-to-tail agglutinates. This agglutination type, easily seen in the GAT is then missed in the TSAT. On the other hand, head-to-head agglutinates, easily observed in the TSAT, are often missed in the GAT. The head-to-head agglutinates are generally much smaller than the tail-to-tail agglutinates. Tail-to-tail agglutination is mostly seen in sera from men and head-to-head agglutination in sera from women. This is apparently the reason why the GAT is frequently used for sera from men and the TSAT for sera from women.

A new method, the tray agglutination test (TAT), was introduced by Friberg (1974). In this method 5 microliters of a serum sample and 1 microliter of a suspension of motile spermatozoa are transferred to a disposable chamber (the so called tray) under liquid paraffin oil. The agglutination is studied with an inverted microscope. Titer, type and degree of sperm agglutination can be determined in the TAT. Tail-to-tail agglutinates are not disrupted, because the reaction mixture is not transferred, and head-to-head agglutinates can easiliy be detected because of the microscopic observation. The TAT is therefore suitable for testing sera from men as well as from women. Comparative investigations have shown that the sensitivity of the TAT for sera from men is comparable with the GAT (Friberg, 1974; Hellema & Rümke, 1976; Boettcher et al., 1977). An additonal advantage of the TAT is that only small volumes of test sample and spermatozoa suspension are needed for the test.

In view of the advantages of the TAT over the GAT and the TSAT, we have used in the present studies the TAT for determination of sperm

agglutinating activity. A detailed description is presented in chapter 4.

1.2. Complement dependent tests

Complement dependent tests for determination of antispermatozoal antibody activity are sperm immobilization tests and spermotoxicity tests. After the antibody molecule has formed a complex with its specific antigen, complement can fix to the Fc part of the antibody molecule (fig.1.1). This only happens if the immunoglobulin is of a complement binding class i. e. IgG (except IgG_4) and IgM, but not IgA. The consequence of complement activation can be damage of the cell membrane with subsequent death of the cell. The complement dependent tests have the advantage over agglutination tests that positive results can more reliably ascribed to antibodies, because of the complement dependency. To be sure that the immobilization is caused by complement a control test must be performed with inactivated complement added to the test sample (Rose et al., 1976). Disadvantages of the complement dependent tests are the lower sensitivity (Boettcher et al., 1977) and the impossibility to detect non-complement binding antibodies.

Sperm immobilization tests are described by Rümke (1954), Fjällbrant (1965) Parish et al. (1967), Isojima & Tzuzuku (1968), Isojima et al. (1968), Jefferry & Parish (1972), Pacheco-Romero (1973), Isojima et al. (1974), Isojima & Koyama (1974), Husted & Hjort (1975a) and Hellema & Rümke (1978a). In the last three papers, tests with small volumes are described. In our studies we also have used a microscale test (chapter 8). To perform a sperm immobilization test, a complement preparation and a suspension of spermatozoa with good motility are mixed with the fluid to be tested. After a suitable incubation time the decrease in the number of motile spermatozoa is determined. Serum samples from humans, rabbits or guinea pigs have been used as complement preparations. The hemolytic activity and sperm immobilizing activity by complement from different species has been compared by Isojima et al. (1977). They found that the sensitivity in both systems were not parallel. Human serum proved to be less efficient than the animal sera in immoblizing human sperm. The highest sensitivity was found with guinea pig serum.

A suspension of washed spermatozoa (i. e. obtained by centrifugation of semen followed by resuspension of the sedimented spermatozoa in another medium) was found to give better results than diluted semen (Rümke, 1954; Isojima et al., 1972). The centrifugation procedure, however,

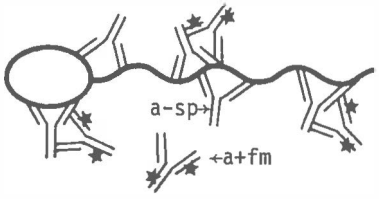
results in a decreased motility. Recently, Hellema & Rümke (1978) introduced a new method for isolation of motile spermatozoa. In this method a buffered medium is layered on a semen sample. Only motile spermatozoa can penetrate into the upper layer. Use of motile spermatozoa, thus obtained, makes reading of the test easier, because fewer immotile spermatozoa and agglutinates are found. In addition, the reduced background of immotile spermatozoa increased the sensitivity of the immobilization test by about two titer steps.

Immobilization tests may be read by measuring the time necessary for immobilization of a fixed number or percentage of spermatozoa (Rümke, 1954; Fjällbrant, 1965; Isojima & Tzuzuku, 1968). But it is more convenient to estimate the decrease of motility after a fixed incubation period, as introduced by Isojima et al. (1968). The sperm immobilization activity in undiluted sera was determined quantitatively by calculation of the sperm immobilization value (SIV). The SIV is the percentage of motile spermatozoa in the control (C) divided by the percentage of motile spermatozoa in the test (T). Thus SIV = C/T. The test is considered positive if the SIV is at least 2. This criterion has now been adopted by most investigators. The sperm immobilizing activity can also be measured quantitatively by determining the immobilization titer. The titer is the highest dilution with an SIV of at least 2.

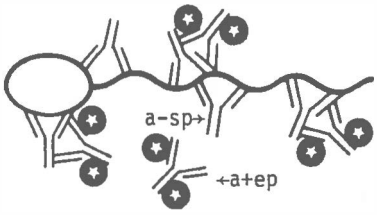
Spermotoxicity tests are described by Parish et al. (1967), Hamerlynck & Rümke (1968), Manarang-Pangan & Behrman (1971), Husted & Hjort (1975a), D'Almeida (in Rose et al., 1976), Sung et al. (1977) and Suominen et al. (1980). In most spermotoxicity tests a vital exclusion dye is added to a test sample after the addition of the complement. After incubation the increase in number of stained, i. e. dead, spermatozoa is determined and compared with the control. The immobilization test was found slightly more sensitive than the spermotoxicity test (Manarang-Pangan & Behrman, 1971; Husted & Hjort, 1975a; Boettcher et al., 1977). Comparison of immobilizing and spermotoxic effects has shown that immobilization precedes staining. Unstained and immotile spermatozoa can be seen but stained, motile spermatozoa are not observed. It is probable, therefore, that with the immobilization test and the spermotoxicity test the same antibodies are determined.

1. 3. Immunoglobulin binding techniques

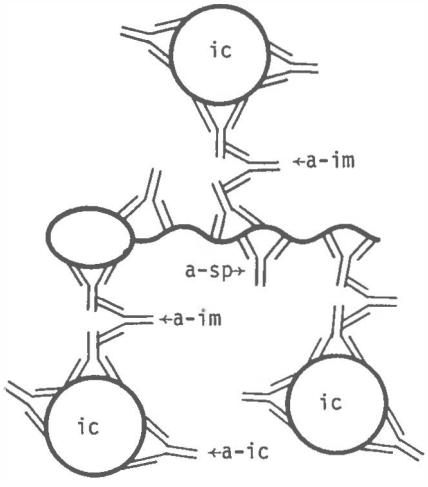
In immunoglobulin binding techniques antibodies to human immunglobulins



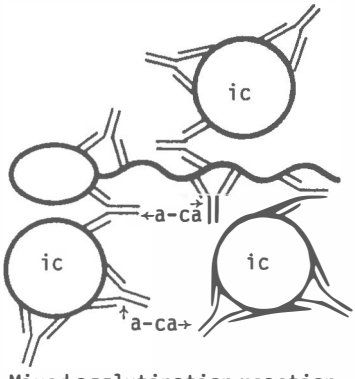
A. Immunofluorescence technique



B. Immunoperoxidase technique



C. Mixed antiglobulin reaction



D. Mixed agglutination reaction

Fig. 1.3. Diagrammatic illustration of techniques for detection of antispermatozoal antibodies using labelled immunoglobulins (A and B) or indicator cells (C). For comparison also the mixed agglutination reaction is shown (D).
a-sp = antispermatozoal antibody,
a+fm = antibody labelled with fluorescing molecule,
a+ep = antibody labelled with (enzyme) peroxidase,
ic = indicator cell (erythrocyte in most cases),
a-im = antibody reactive with immunoglobulin,
a-ic = antibody reactive with antigen on indicator cell,
a-ca = antibody reactive with antigen common to spermatozoon and indicator cell.

are used (fig. 1.3). In one type of binding techniques the antibodies to immunoglobulins are labelled. A second type of binding techniques is based on the mixed antiglobulin reaction (MAR). Labels used for the detection of antispermatozoal antibodies are a radio-active isotope (Fjällbrant, 1979), a fluorescing molecule (the immunofluorescence technique, IFT) and an enzyme (the immunoperoxidase method). The technique is direct if used for detection of auto-antibody on spermatozoa of the patient. Indirect techniques are used for detection of antibodies in fluids with the aid of donor spermatozoa or testicular tissue. Important advantages of binding techniques are the possiblity of antigen localization and determination of the immunoglobulin class of the antibody being detected.

To perform the *immunofluorescence technique* initially most investigators used unfixed spermatozoa or unfixed testicular tissue as antigen preparation (Cruickshank & Stuart-Smith, 1959; Sutherland & Landing, 1961; Feltkamp et al., 1965; Sobbe et al., 1966; Schwimmer et al., 1967a; Parish et al., 1967; Entschev & Momchilov, 1969; Haensch, 1969; Hjort & Hansen, 1971; Wall et al., 1975a, 1975b; Petrunia et al., 1976; Harrison, 1978); Fixed spermatozoa were used first by Beck et al. (1962). Methanol fixation was introduced by Franklin & Dukes (1964). Hamerlynck (1970) found that after methanol fixation the number of positive reactions increased. In addition, the fluorescence patterns changed. Hansen & Hjort (1971) obtained weaker reactions with unfixed spermatozoa with unfixed spermatozoa than with fixed spermatozoa. Moreover, the characteristic staining patterns (fig. 1.4) were more sharply delineated with fixed spermatozoa. The method introduced by Hjort & Hansen (1971) has since been adopted by most other investigators. Treatments other than fixation also can interfere with the results. Kay & Alexander (1978) observed that the fluorescence pattern could change after changing the speed of centrifugation for washing the spermatozoa.

In an international comparative investigation (Boettcher et al., 1977) the IFT was applied with the same set of sera, but with different conjugated antisera. Except where antigens of swollen sperm heads were being detected, considerable differences in incidence and type of positive reactions were obtained. These differences were ascribed to differences in sensitivity and also to differences in interpretation. The interpretation is highly subjective and is therefore difficult to standardize. The interpretation is hindered by the background staining, probably caused by a "non-specific" binding of the labelled immunoglobulins through the Fc

Fig. 1.4. Various patterns of immunofluorescent staining of spermatozoa incubated in sera of infertile patients. A, Anterior region of the head. B, Equatorial area, C. Post-nuclear region. D. Midpiece. E. Tail. F, Tip of tail. G. Nucleus after artificial swellin.

part (Boettcher et al., 1977; Hjort & Poulsen, 1978) or to binding to cross-reacting antibodies primarily directed against bacterial antigens (Tung, 1975). It must be concluded that, for determination of antispermatozoalantibodies, except those against antigens of swollen sperm heads, the IFT is not sufficiently reliable in its presence performance. With antigens of swollen sperm heads, which are protamin 1 and 2, the IFT has been shown to be reliable (2.5).

The immunoperoxidase technique (fig. 1.3b) has also been applied on spermatozoa (Mancini et al., 1971; Morton, in Rose et al., 1976; Storch et al., 1976; Hjort, 1976). In this technique the immunoglobulins are labelled with horseradish peroxidase. The binding of the labelled immunoglobulin is disclosed by anenzymatic staining reaction. The test is basically the same as the IFT and has therefore the same drawbacks.

In *the mixed antiglobulin reaction (MAR)* presence of spermatozoal surface antibodies is demonstrated by agglutination of a different type of cell (indicator cell) to spermatozoa. On the indicator cell immunoglobulin is present belonging to the class to be detected on the spermatozoa (fig. 1.3c). The MAR was used first by Coombs et al. (1956) for demonstration of IgG molecules on thrombocytes. They used as indicator cells group-O, Rh-positive human red blood cells sensitized by incubation in a human serum containing a high titer of incomplete anti-D. The MAR has been applied as an indirect test for the detection of antispermatozoal antibodies present in serum and seminal plasma (Edwards et al., 1964; Jeffery & Parish, 1972; Coombs et al., 1973). The last authors performed the MAR on fresh semen from one patient. The mixed *antiglobulin* reaction must not be confused with the mixed *agglutination* reaction (fig. 1.3d). The mixed agglutination reaction was first described by Wiener & Herman (1939) and is used for detection of common antigenic determinants on two different types of cells. The mixed agglutination reaction has been used for demonstration of blood group antigens on spermatozoa (Edwards et al., (1964).

In view of the insufficient reliablity of the IFT we used the MAR for determination of the immunoglobulin class of antispermatozoal antibodies on spermatozoa. Details of direct and indirect MAR tests developed for use with motile spermatozoa can be found in chapters 5, 6 and 8.

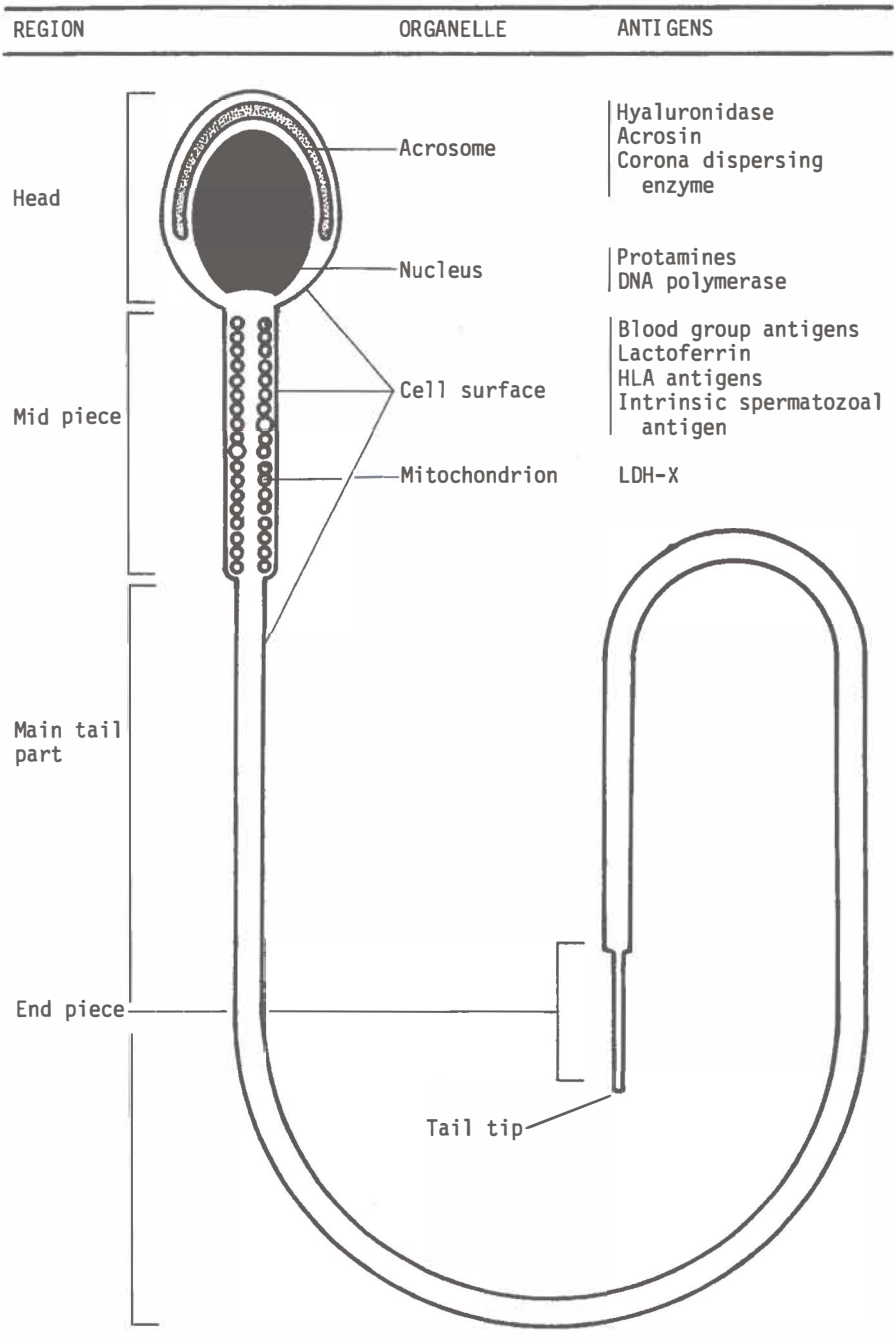


Fig. 1.5. Schematic representation of spermatozoal structures and antigens.

2. *SPERMATOZOAL ANTIGENS AND EFFECTS ON FERTILITY OF ANTIBODIES TO THESE ANTIGENS*

2.1. Sperm coating antigens

Antibodies raised in animals against seminal plasma were found to be immunological indistinguishable from antibodies raised against washed spermatozoa of the same ejaculate (Weil et al., 1956). This was found for semen from rabbits and for semen from men. The antigen, corresponding to these antibodies, was not present on spermatozoa obtained from a rabbit's epididymis or on human spermatocele spermatozoa. On the other hand, the antigen was found present in semen from azoospermic men. It was, therefore, concluded that the antigen is taken up from secretions added to the semen beyond the epididymis. The antigen was called the sperm coating antigen (SCA)(Weil, 1960; Weil & Rodenburg, 1960). The origin of the SCA was found to be the seminal vesicle (Weil & Rodenburg, 1962).

In human seminal plasma up to eight antigens, not detected in blood have been found (Searcy et al., 1964; Bandhauer, 1966; Behrman & Amano, 1967; Quinlivan, 1969; Hekman & Rümke, 1969; Quinlivan & Sullivan, 1972; Isojima et al., 1974; Barnes et al., 1978). Three of these antigens were found to originate in the prostate and two in the seminal vesicle. The prostate antigens were probably acid phosphatase iso-enzymes (Barnes et al., 1978). One of the seminal vesicle antigens could be demonstrated to be lactoferrin (Hekman & Rümke, 1969). After absorption of antiserum to seminal plasma with washed ejaculate spermatozoa only the antibody against lactoferrin disappeared. This suggest the SCA, demonstrated by Weil and coworkers, to be lactoferrin (Hekman & Rümke, 1969). However, two other seminal plasma antigens have been detected on ejaculated spermatozoa (Li & Behrman, 1970).

No antifertility effect of antibodies to lactoferrin has been reported. According to Isojima et al. (1974), sperm immobilizing antibodies in sera from some infertile women can be absorbed out with seminal plasma but not with washed spermatozoa. This seminal plasma antigen was not identical to lactoferrin.

2.2. Blood group antigens

As early as 1926 Yamakani demonstrated the presence of blood group A and B substance in sperm-free seminal plasma, by inhibition of naturally occurring ABO agglutinins with the seminal plasma. In the same year Land-

Table 1.1. Comparison of incidence of ABO incompatibility in fertile and infertile couples.

	Infertile couples		Fertile	Expected	χ^2	P
	Unexplained	Organic cause	couples	incidence*		
Behrman et al. (1960)	89/102(87%)		66/105(39%)		2.73	0.05>P>0.025
Whitelaw et al. (1962)	21/50(42%)		47/136(34%)		1.06	0.40>P>0.30
Schwimmer et al. (1967b)	45/101(45%)	13/46(34%)			1.04	0.40>P>0.30
Boettcher & Hay (1968)	19/63(30%)			22/36(35%)	0.36	0.50>P>0.40
Solish & Gershowitz (1969)	1130/2880(39%)			1131/2880(39%)	0.0018	0.95>P>0.90

* based on observed incidence in comparable control population.

steiner & Levine found that washed spermatozoa could absorb blood group antibodies. The results were highly specific for the donors' blood groups. Blood group antigens can be detected in semen only if the man concerned is a secretor (Friedenreich & Hartman, 1938). ABO antigens are bound very firmly to the spermatozoa. After washing several times spermatozoa still can absorb blood group antibodies. Surprisingly, natural or immune type ABO antibodies mostly do not agglutinate spermatozoa or exhibit complement dependent sperm immobilizing activity. Yet the antigens must be located at the spermatozoal surface, since they can easily be demonstrated with the mixed agglutination reaction (Sjövall, 1949; Gullbring, 1957; Edwards et al., 1964; Isojima & Tzuzuku, 1968; Fernández-Collazo & Thierer, 1972; Kerēk, 1974)(fig. 1.3d).

In 1960 Behrman et al. put forward the hypothesis that conception may be prevented at the cervical level due to agglutination or immobilization of spermatozoa carrying the A or B antigen. Presence of anti-ABO antibodies in cervical mucus was demonstrated by Gershowitz et al. (1958), Solish et al. (1961) and Parish et al. (1967). Behrman et al. (1961) found a significantly higher percentage of blood group incompatibility in unexplained infertile couples than in fertile couples. Results of other investigations could not confirm this conclusion (Boettcher & Hay, 1968; see also table 1.1).

M, N and Tja antigens are probably present on spermatozoa, whereas the Rh-D antigen and the Xga antigen could not be detected (Levine & Celano, 1961; Edwards et al., 1964). No significant difference in frequency of children from compatible and incompatible couples was found for the Rh, MNS, Kell, Duffy and P systems (Solish & Gershowitz, 1969; Krieg et al., 1973).

2.3. HLA antigens

HLA antigens are present on the surface of all nucleated cells and thrombocytes, but not on erythrocytes. The antigens are called human leukocyte antigens (HLA) because they were demonstrated first on leukocytes. The HLA system is the strong transplantation antigenic system in man (for references see Kissmeyer-Nielsen & Thorsby, 1970). Antigens of the HLA system have been detected on human spermatozoa (Fellous, 1969; Piazza et al., 1969, both cited by Fellous & Dausset, 1970; Halim & Festenstein, 1975).

Antibodies against HLA antigens can occur after transfusions, trans-

plantations, planned immunizations and during pregnancies. In 10% to 30% of first pregnancies antibodies to HLA antigens have been detected (Ahrons, 1971; Stastny, 1972; Doughty & Gelsthorpe, 1976). The incidence increases with subsequent pregnancies (Nielsen & Svejgaard, 1972). It is unlikely that antibodies to HLA antigens can be cause of infertility because an increased incidence of infertility proportionate to the increased incidence of anti-HLA antibodies has never been reported. Possibly, women may also become sensitized against HLA antigens by contact with spermatozoa. Stolp et al. (1973) found antibodies, reactive with husband's lymphocytes, in the serum of seven women from primary infertile couples. Hofmann et al. (1978) reported the presence of anti-lymphocyte antibodies in two women from primary infertile couples without an immunization event in the history. On the other hand, Tait et al. (1976) and Mälzer et al. (1979) were unable to find anti-HLA antibodies in women from infertile couples.

2.4. The H-Y (male) antigen

The male specific (H-Y) antigen was discovered with the observation that male mice skin grafts were rejected by recipient female mice of the same strain; whereas male-to-male, female-to-female and female-to-male grafts almost always succeeded (Eichwald & Silmser, 1955). The antigen can be detected on almost all if not all male cells. The antigen has been detectected on mouse spermatozoa (Goldberg et al., 1971; Bennet & Boyse, 1973; Koo et al., 1973). Although not yet investigated it is likely that the H-Y antigen is also present on human spermatozoa. The H-Y antigen does not exist as an integral compound of the plasma membrane but is anchored to the membrane by the ubiquitously expressed β_2-microglobulin major histocompatibity antigen dimers. On gonadal cells H-Y antigen also binds to specific receptors (for review see Ohno, 1976 or 1977). Free H-Y antigen has been found in the epididymal fluid of adult rats (Müller et al., 1978).

Human H-Y antibody is generated only when HLA compatible male donors and female recipients share the HLA-A2 determinant. Human H-Y antibody, so obtained, lyses in the presence of complement only those male targets that carry HLA-2 determinant (Van Leeuwen et al., 1977). An effect on fertility by H-Y antibodies is unknown.

2.5. Spermatozoal specific antigens below the cell surface

Acrosomal antigens. Several enzymes are present in the acrosome (Zaneveld

& Polakoski, 1976). At least some of these enzymes are known to play a key role in the fertilization process. These are the corona dispersing enzyme, hyaluronidase and acrosin. The enzymes enable the spermatozoon to penetrate the corona and the zona pellucida. Kosuda & Bigazzi (1978) reported the occurrence of auto-antibodies to acrosomal enzymes in vasectomized mice. Presence of an anti-hyaluronidase factor was found in serum from one women by Metz (1973). The immunoglobulin nature of the factor has not been established, however, and a relation with impairment of the fertility is uncertain. Immunization of animals with acrosin or hyaluronidase has not resulted in decreased fertility (Morton, 1977; Morton & McAnulty, 1979; Syner et al., 1979; Warwas et al., 1979).

Nuclear antigens. During the last phase of the spermatogenic process the chromatin becomes very compact as a result of the replacement of the histones by protamines. The dense packing of the chromatin can be ascribed to the increased number of disulfide bonds in the nucleoprotein complex (Zamboni, 1971; Kolk & Samuel, 1975).

Men with antibodies to spermatozoal surface antigens can also have antibodies against protamines. The protamines can be detected with antibodies only after artificial decondensation of the sperm nucleus (Kolk et al., 1974; Samuel et al., 1975). The decondensation is accompanied by a visible swelling of the sperm head.

Recently a DNA polymerase has been isolated from sperm nuclei. IgG from sera from two men with strong sperm agglutinating activity also showed a strong inhibitory action on the enzyme (Witkin et al., 1978). The enzyme is therefore probably not identical with DNA polymerase in somatic cell nuclei.

Neck and tail antigens. Very few sperm specific antigens below the surface of the neck and tail of human spermatozoa are known. The sperm specific lactate dehydrogenase iso-enzyme X (LDH-X) is associated with the mitochondria (Machado de Domenech et al., 1972). The high level of LDH in seminal plasma have been considered, at least partially, to be derived from spermatozoa (Roussel & Stallcup, 1965; Murdoch & White, 1968).

With sensitive radio-immuno assays only occasionally weakly positive anti-LDH-X reactions have been found in sera from women (Boettcher, 1980).

2.6. Antigens detected with the IFT on unswollen spermatozoa

In the immunofluorescence technique (IFT) a number of staining patterns have been described (fig. 1.4): staining corresponding to the localization

of the acrosome, of the entire head, of the posterior part of the head, of the equatorial zone of the head, of the neck region, of the midpiece, of the tail and also of the entire spermatozoon (Beck et al., 1962; Feltkamp et al., 1965; Entschev & Momchilov, 1969; Rümke, 1968; Haensch, 1969; Hjort & Hansen, 1971; Tung, 1975). After fixation the fluorescence becomes stronger and the characteristic staining patterns are more sharply defined. Already Hansen & Hjort (1971) have suggested, therefore, that the histological structures detected are situated beneath the cell membrane which is altered in the fixation procedure. The identity of the antigens detected with the IFT is unknown.

Antibodies have been detected with the IFT performed with sera from children, fertile (including pregnant) women, fertile men and infertile men and women. However, compared with other groups, the percentage of sera with high titers was significantly higher for infertile men and women (Hamerlynck, 1970; Hjort & Hansen, 1971; Husted, 1975; Wall et al., 1975a, 1975b; Petrunia et al., 1976). Antigens detected with the IFT are, at least in many cases, not identical with antigens detected in agglutination or immobilization tests. High titers in the IFT are found with low titers or negative results in the GAT, the TAT and the sperm immobilization test (Feltkamp et al., 1965; Hamerlynck, 1970; Hjort & Hansen, 1971; Hansen, 1974; Petrunia et al., 1976; Morgan et al., 1977; Boettcher et al., 1977). Positive reactions have also been obtained with cervical mucus from fertile and infertile women but the incidence in infertile women is higher. The results of the IFT performed with cervical mucus showed no significant correlation with results of other antispermatozoal antibody tests performed with cervical mucus (Parish et al., 1967; Parish & Ward, 1968; Coelingh Bennink & Menge, 1974; Menge et al., 1977; D'Almeida & Eyquem, 1978; Harrison, 1978). Seminal plasma samples with sperm agglutinating activity were found positive with the IFT, using fixed spermatozoa as antigen preparation, in a only a few cases by Husted & Hjort (1975b). In contrast, Harrison (1978), using both fixed or unfixed spermatozoa, obtained positive reactions with one third of seminal plasma samples from a group of infertile (but otherwise unselected) men. With the direct IFT Husted & Hjort (1975b) could detect antibodies on washed and dried but otherwise unfixed, spermatozoa from infertile men with sperm agglutinating activity in serum and seminal plasma in only a few cases.

2.7. Spermatozoa-specific surface antigens

Antibodies to the spermatozoa-specific surface antigens are more significant, in relation to fertility impairment, than antibodies to the antigens dealt with in the previous sections. We will, therefore, deal with the spermatozoa-specific surface antigens and the corresponding antibodies in more detail. Rümke (1959) demonstrated the tissue specificity of antigens detected in the GAT with sera containing sperm agglutinating activity. The author tested the sera against erythrocytes, leukocytes and platelets; the results were uniformly negative. Based on the different sperm agglutination patterns three different antigen locations have been distinguished. The agglutination can involve the head, the main tail region, the tip of the tail or a combination of these regions (Wilson, 1954; Rümke & Hellinga, 1959; Rose et al., 1976)(fig. 1.2). It is supposed that, in most cases, spermatozoal antigens detected with complement dependent sperm immobilizing antibodies are identical to antigens detected with sperm agglutinating antibodies. Differences between results of immobilization tests and results of agglutination tests can be ascribed partly to the lower sensitivity of the immobilization test and partly to the fact that agglutinating antibodies are not always of the complement binding class (Boettcher et al., 1977). However, sera with sperm immobilizing but without sperm agglutinating activity (in the GAT) have been described (Manarang-Pangan & Behrman, 1971; Boettcher & Gruszynski, 1978). Possibly, however, there were head-to-head agglutinates in the test; these agglutinates are often small and, therefore, not noticed in the GAT. Recently D'Almeida et al. (1980) succeeded in fractionating human spermatozoal antigens. Their results suggested that at least three different antigens are responsible for head-to-head agglutination, tail-to-tail agglutination and spermotoxicity respectively. Isolation of human sperm membrane antigens has been reported by Poulsen & Hjort (1980). With some human sera with sperm agglutinating and sperm immobilizing antibodies the authors isolated at least three polypeptide chains.

2.7.1. Circulating antispermatozoal antibodies

2.7.1.1. Antispermatozoal antibodies in sera from men

An important cause of induction of antispermatozoal antibodies in men might be an abnormal resorption of spermatozoa. Antispermatozoal antibody activity in serum has been observed in a high percentage of men with obstructive azoospermia or after vasectomy (Rümke, 1959) see also table

Table 1.2. Incidences of serum sperm agglutinating activity* with titers more than 16† among different groups of men.

Authors	Groups of men					
	Fertile	Infertile	with obstructive azoospermia	post-vasectomy ≤6 months	post-vasectomy ≥1 year	with urogenital tract other inflamation
Rümke & Hellinga (1959)	0/416(0%)	62/1913(3%)				
Cruickshank & Stuart-Smith (1959)						2/14(14%)
Phadke & Padukone (1964)	0/25(0%)		5/28(18%)		8/25(32%)	
Bandhauer (1966)						23/217(11%)
Sobbe et al. (1966)		9/150(6%)				
Fjällbrant (1868a, 1969a)	6/500(1%)	18/400(5%)				11/500(2%)§
Ahlgren (1969)		5/121(4%)				
Hamerlynck (1970)	0/33(0%)					9/105()%)¶
Haensch (1973)		63/1224(5%)				
Ansbacher (1973)	0/106(0%)			8/69(12%)	7/44(16%)	
Halim & Antonionou (1973)	3/100(3%)	21/144(15%)		8/100(8%)		
Van Lis et al. (1974)	0/52(0%)			7/51(14%)	16/52(31%)	
Amelar et al. (1975)			18/29(62%)			
Samuel et al. (1975)				2/27(7%)	9/23(39%)	
Husted (1975)		24/657(4%)				
Gupta & Garg (1975)	0/15(0%)	0/70(0%)				
Gupta et al. (1975)				24/45(53%)	43/50(86%)	
Friberg & Kjessler (1975)						8/59(14%)#
Schoenfeld et al. (1976)		70/406(17%)				
Hunter et al. (1976)	1/114(1%)				9/56(16%)	
Linnet & Hjort (1977)				4/47(9%)	24/47(51%)	
Hendry et al. (1977)		50/591(8%)				
Hellema & Rümke (1978, 1979)				26/52(50%)	20/34(59%)	
Tyler et al. (1979)	0/72(0%)			5/72(7%)	2/10(20%)	
Koskimies (1979)		9/150(6%)				
Girgis et al. (1979)			13/32(40%)			
Kaskarelis et al. (1979)		10/200(5%)				
Fattah et al. (1980)		13/115(11%)				

* Determined in the gelatin agglutination test, except 13, 19 (capillary tube agglutination test; Shulman, 1971), 2 (slide agglutination test; Wilson, 1954), 17, 23, 25 (tray agglutination test) and 26 (also tube slide agglutination test).
† Except 3 (≥80) and 15 (≥64). § Blood donors. ¶ Aged men. # Azoospermic men.

1.2). Rümke & Hellinga (1959) suggested that the antispermatozoal antibodies in these cases were due to exposure of spermatozoal antigens to the immune system after sperm extravasation as a consequence of ruptures of the walls of the epididymal duct, the efferent ducts or the rete testis, due to spermatostasis. Other important causes for antibody formation might be inflammatory processes in the male genital tract region (Fjällbrant & Obrant, 1968; Fjällbrant & Nilsson, 1978; Eyquem et al., 1978; Fattah et al., 1980; table 1.2).

In sera from men sperm agglutinating antibodies, with titers of at least 32, have been identified as mainly IgG. This was found particularly for the tail-to-tail agglutinating antibodies. Head-to-head agglutination activity in sera from men was mostly due to IgM (Boettcher et al., 1971; Friberg, 1974; Husted & Hjort, 1975b). A sperm agglutination titer more than 16 has been found significantly more often in men from infertile couples than in fertile men (table 1.2). Ansbacher (1973) and Jones et al. (1976) observed a sperm immobilization activity only in men from infertile couples. In a retrospective study Rümke et al. (1974) demonstrated the existence of a reverse relationship of fertility grade and titer of sperm agglutinating activity in serum of men. Occasionally, however, men with titers up to 512 could become father after some time, despite persistance of the relatively high agglutination titer.

2.7.1.2. Antispermatozoal antibodies in sera from women

The cause of antispermatozoal antibody formation in women is not known. Also unknown is the reason why women, despite frequent exposure to spermatozoal antigens do not show an immune response. It might be that the amount of antigens is low and a low-dose tolerance exists. Another possibility is that spermatozoa have an immunosuppressive action themselve. For a discussion of various aspects of the unresponsiveness of women to spermatozoa the reader is referred to Hancock (1978) and Rümke (1980).

Franklin & Dukes (1964a,b) found with the TSAT a sperm agglutination activity in the serum of 67% of the women from couples with an unexplained infertility. In sera of women from couples with an explained fertility the incidence was 16%. Other investigators could not reproduce these results. In sera from pregnant women the incidence of sperm agglutinating activity was as high as or even higher than, in sera of women from couples with unexplained infertility (table 1.3). In most positive sera the agglutination factor was probably not an antibody but a high

Table 1.3. Incidences of serum sperm agglutination activity determined with the tube-slide-slide agglutination test* among various groups of women.

Authors	Women from infertile couples		Fertile women	
	Unexplained	Organic cause	Pregnant	Non-pregnant
Tyler et al. (1967)	2/41(5%)			5/61(8%)
Olson (1967)	47/77(61%)			11/46(24%)
Schwimmer et al. (1967b)	40/96(42%)		15/44(34%)	
Dukes & Franklin (1968)	45/67(67%)	44/277(16%)	4/44(9%)	
Boettcher & Hay (1969)	13/56(23%)		67/86(78%)	
Glass & Vaidya (1970)	24/122(20%)	2/46(4%)		
Masson et al. (1970)	17/24(71%)	7/22(32%)		
Kolodny et al. (1971)	11/78(14%)		2/78(3%)	1/35(3%)
Hanafiah et al. (1972)	6/70(9%)	11/54(20%)		
Isojima et al. (1972)	27/72(38%)	18/93(19%)	38/83(46%)	
Pacheco-Romero et al. (1973)	7/23(30%)	9/19(47%)		
Shulman et al. (1975)	24/147(16%)		2/77(3%)	1/35(3%)
Jones et al. (1976)	108/492(22%)	16/119(13%)	9/50(18%)	
Lehman et al. (1977)	27/487(6%)	29/487(6%)		
Mettler (1977)	51/304(17%)	10/550(2%)	0/221(0%)	0/231(0%)

* according to or modified after Franklin & Dukes (1964a).

Table 1.4. Incidences of serum sperm immobilization activity among various groups of women

Authors	Women from infertile couples		Fertile women	
	Unexplained	Organic cause	Pregnant	Non-pregnant
Vaidya & Glass (1971)	1/30(3%)	0/10(0%)		
Pacheco-Romero et al. (1973)	0/23(0%)	0/19(0%)		
Husted & Hjort (1975a)	0/247(0%)			
Petrunia et al. (1976)	5/51(10%)	2/43(5%)	0/48(0%)	
Jones et al. (1976)	40/492(8%)	3/119(3%)	0/50(0%)	
Lehman et al. (1977)	11/487(2%)	6/487(1%)		
Mettler (1977)	7/304(2%)	0/550(0%)	0/221(0%)	0/231(0%)
Isojima et al. (1977)	30/165(18%)	5/411(1%)	1/202(0.5%)	

molecular weight compound with the electrophoretic mobility of a β-globulin (Boettcher, 1974; Ingerslev, 1979). This β-agglutinin shows a weak activity in most cases but a titer of 64 has been reported (Boettcher, 1974). In the GAT no significant difference in incidence was found between sera from fertile and infertile women for low titers (Isojima et al., 1972). However, titers of 16 and higher were seen only in sera from infertile women (Ahlgren, 1969; Ingerslev & Hjort, 1979). With the sperm immobilization test positive results have been obtained with 2% to 18% of the sera from unexplained infertile women. In contrast, only a few sera from pregnant women showed positive results (table 1.4).

The occurrence of pregnancies is not significantly different in patients with and without antispermatozoal antibodies (Lehman et al., 1977; Ingerslev & Ingerslev, 1980; Jones, 1980). However, in women with unexplained infertility or with a minor abnormality, a significant lower pregnancy rate was found if the duration of the infertility was at least three years at the time a positive result in the immobilization test was obtained (Jones, 1980). Moreover, Ingerslev & Ingerslev (1980) found that the cumulative probability of conception per year was significantly lower in women with sperm agglutinating antibodies than in women without such activity.

2.7.1.3. Conclusion

A relation between the presence of antispermatozoal antibodies in serum and infertility has been found. The relationship cannot be a direct one, however. The antibody molecules have to contact the spermatozoa before being effective. Antibody activity in the genital tract, is therefore more relevant to fertility disturbance than antispermatozoal antibodies in serum.

2.7.2. Antispermatozoal antibodies in the male and female genital tracts

2.7.2.1. Local antibodies

Locally present antibodies can be passively derived from the circulation. In addition, several mucous membranes have also a local production and secretion of immunoglobulins (Tomasi, 1972; Brandtzaeg, 1973). Fig. 1.6. shows a schematic representation of an idealized mucoglandular mucosa. Characteristic of a local secretory immune system is the production of dimeric IgA by plasma cells in the lamina propria. During the passage

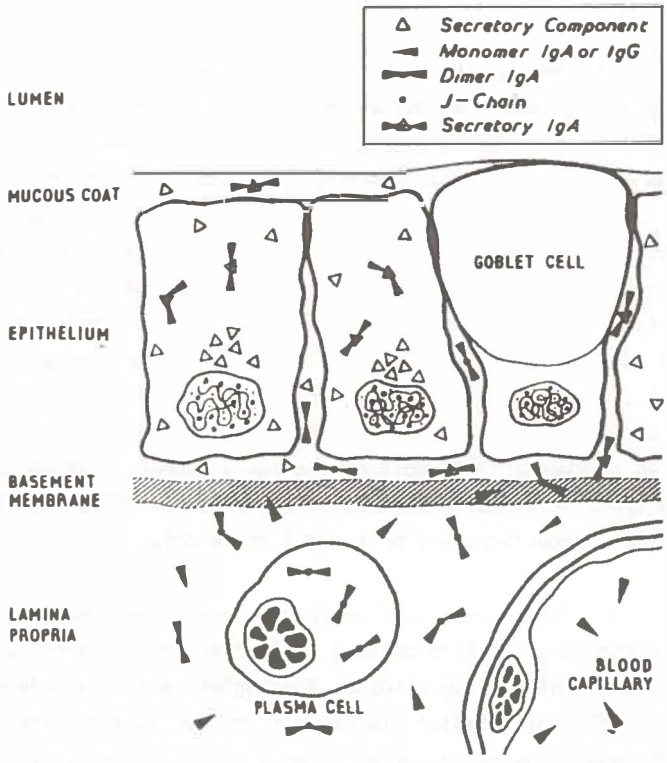


Fig. 1.6. Diagrammatic representation of secretory IgA excretion at an idealized membrane.

through the epithelium the IgA is linked with secretory component synthesized by the epithelial cells. The secretory type IgA (fig. 1.1) is the predominant immunoglobulin in most secretions. The IgA predominance results commonly in an IgA/IgG ratio much higher than the mean ratio of 0.16 in the serum (Chodirker & Tomasi, 1963).

2.7.2.2. The male genital tract

2.7.2.2.1. Immunoglobulins in seminal plasma

In seminal plasma from normal men IgG and IgA are present at approximately 1% of the serum level. The bulk of the IgG is probably derived from the serum and enters the ejaculate via the prostate (Rümke, 1974; Tauber et al., 1975). These athors could not detect IgM in the seminal plasma. Kula et al. (1979) detected IgM in the seminal plasma of men from infertile couples, mainly in cases of azoospermia. IgA and IgG concentrations in seminal plasma from infertile men are not, or are only slightly higher than those in the seminal plasma from normal men (Friberg, 1974; Kövari et al., 1977; Friberg, 1980). IgA/IgG ratios calculated from these reports are close to the IgA/IgG ratio in serum. However, according to Van Munster (cited by Rümke, 1978) most of the IgA in seminal plasma is of the secretory type. This indicates that in the male genital tract there is local antibody production, but on a small scale only.

2.7.2.2.2. Antispermatozoal antibody activity in semen

The presence of antispermatozoa antibodies can be expressed by auto-agglutination of the spermatozoa. The agglutination is seldom complete directly after ejaculation. In semen from most patients the number and size of the agglutinates increases after ejaculation but many freely swimming spermatozoa remain. Few patients produce apparently normal semen without agglutinates despite high titers in the serum (Rümke & Hellinga, 1959). The degree of spontaneous agglutination in semen will depend on several factors such as motility and concentration of the spermatozoa, concentration and avidity of the antibodies and also the observation time. Possibly also the availability of the antigen on the spermatozoa can play a role. In most cases the sperm agglutination titer in the seminal plasma is lower than the titer in the serum (Rümke, 1874a; Frgiberg, 1974; Husted & Hjort, 1975b; Friberg & Friberg, 1977). The agglutination type is, generally, tail-to-tail. Head-to-head agglutination has been reported occasionally (Fjällbrant, 1965). Complement dependent sperm immobilizing activity was found by Husted & Hjort (1975b) in seminal plasma from four men who had a strong sperm immobilizing activity in the serum and high sperm agglutination titers in serum and seminal plasma.

2.7.2.2.3. Origin of antispermatozoal antibodies in semen

Antispermatozoal antibodies in semen can be derived, at least partially, from the serum because some serum immunoglobulins leak into the semen via the prostate (2.7.2.2.1). On the other hand, sperm agglutinating activity in semen is probably mainly due to locally produced antibodies. Whereas circulating sperm agglutinating antibodies are mainly IgG, the agglutinating activity in semen is mainly caused by IgA antibodies (Coombs et al., 1973; Friberg, 1974; Husted & Hjort, 1975b).

The local production of antispermatozoal antibodies, probably, occurs in the epididymis and/or in the rete testis. After vasectomy antispermatozoal antibodies seldom appear in seminal plasma (Hellema & Rümke, 1978b). Following vasovasostomy high levels of antispermatozoal antibody activity can be found in the semen. In addition, high sperm agglutinating activity has been found in the epididymal fluid during vasovasostomy operation (Linnet & Fogh-Anderson, 1979)

2.7.2.3. The female genital tract

2.7.2.3.1. Immunoglobulins and immunoglobulin producing cells

The presence of immunoglobulins and secretory component has been demonstrated in human Fallopian tubes by Tourville et al. (1970) using the immunofluorescence technique. IgG and IgA staining was seen along the basal lamina and in the stroma of the villi. Secretory piece staining was seen in epithelial cells. IgM was rarily detectable. Lippes et al. (1972) found IgG in the oviductal fluid in all samples examined and IgM in half of the cases. IgA was undetectable in some of the investigated specimens. The immunoglobulin concentrations, determined with the radial immunodiffusion method, were 8% to 10% of the serum values. The mean IgA/IgG ratio calculated from 14 specimens was 0.18.

Edwards et al. (1968) using immunoelectrophoresis demonstrated the presence of IgG, IgA and IgM in uterine secretions obtained from diffusion chambers. Schumacher (1980) determined immunoglobulin concentrations in endometrial fluid obtained from surgical specimens after hysterectomy. IgG, IgA and IgM were present in all cases. Only traces of secretory component were found. The immunoglobulin concentrations ranged from 10% to 60% of the serum level. The IgA/IgG ratios were approximately the same as in the corresponding sera.

In the cervical stroma, immunoglobulin producing plasma cells can be

found (Masson & Ferin, 1967). In cervical biopsies from women without evidence of a recent local inflammation the number of plasma cells is generally small. An increased number of plasma cells in the stroma has been found in women with vaginal candiasis, vaginal trichomoniasis, gonorrhoea or gonorrhoea contacts (Chipperfield & Evans, 1972). Using the immunofluorescence technique Hulka & Omran (1969) detected secretory piece in the epithelium and the lumen of the crypts.

The concentration of IgG and IgA in cervical mucus show strong cyclic variations. In the postmenstrual and the early proliferative phase the average concentrations are about 2 g/l for IgG and about 0.5 g/l for IgA. The mid-cycle minima are approximately 0.1 g/l for IgG and 0.04 g/l for IgA. The overall IgA/IgG ratio is 0.37. IgM is found occasionally in traces (Schumacher, 1980). Most of the IgA in cervical mucus is of the secretory type (Waldman et al., 1972; Tjokronegoro & Sirisinha, 1975).

In biopsies from the vagina IgA and IgM plasma cells were absent and no secretory piece was detected in the vaginal epithelium (Vaerman & Ferin, 1974). Biopsy specimens from the vagina do not show *in vitro* IgA and IgM synthesis but IgG synthesis was found (Lai a Fat et al., 1973).

Vaginal secretions are, in normal cases, always contaminated with cervical secretions. However, Waldman et al. (1972) reported that the IgA/IgG ratio in vaginal secretions from three completely hysterectomized women was as high as in cervicovaginal secretions. On the other hand, Jalanti & Isliker (1977) found no significant difference of the IgG concentrations in the vaginal contents of normal and hysterectomized women but the IgA concentration was strongly decreased in the hysterectomized women. In the last group of women secretory IgA could not be detected any longer. Cantuária & Jones (1980) compared the immunoglobulin concentrations in cervicovaginal secretions of women from infertile couples, in whom cervical hostility was the only known cause of infertility, and of fertile women. No significant difference in concentrations of IgG and IgA was found but the IgM level was significant higher in the infertile group. From the ratios of protein levels the authors calculated the local production of immunoglobulins. The authors found local production of IgG and IgM in more of the infertile women and in greater amounts than in the fertile women.

In conclusion: local production and secretion of immunglobulins has been found in the oviducts and the cervix but not in the corpus uteri and the vagina.

2.7.2.3.2. Antispermatozoal antibody activity in cervical mucus

The presence of sperm agglutinating antibodies in cervical mucus has been reported by Shulman & Friedman (1975), Kremer & Jager (1976, see chapter 2), Kremer et al. (1978, see chapter 3), Telang et al. (1978) and Moghissi et al. (1980). In these studies an inverse relationship was found between the presence of sperm agglutinating antibodies and the results of the post coital test. In cervical mucus also complement dependent sperm immobilizing or spermotoxic activity has been found (Parish et al., 1967) Antibodies were found in a high percentage of women with no abnormality except an apparent cervical hostility. No such antibodies were detected in controls (Soffer et al., 1976; Menge et al., 1977; Cantuária, 1977; Moghissi et al., 1980). Waldman et al. (1972) could absorb the sperm immobilizing activity in cervicovaginal fluid with anti-IgM but with anti-IgG antiserum. Cantuária & Jones (1980) found that absorption of immunoglobulins from cervicovaginal secretions could result in a decrease of the inhibition of the *in vitro* sperm penetration into the secretions. Most effective was the removal of IgA.

3. THE PENETRATION AND MIGRATION OF SPERMATOZOA IN CERVICAL MUCUS

Spermatozoa deposited in the vagina during coitus must pass the cervical canal in order to reach the fertilization sites in the oviducts. The passage through the cervical canal depends on the motility of the spermatozoa and the physico-chemical properties of the cervical mucus. Testing the penetration of spermatozoa into cervical mucus has become an integral part in the investigation of the infertile couple. Penetration tests can be performed *in vivo* or *in vitro*. The *in vivo* penetration test, or post coital test (PCT), is an investigation on the presence and the motility of spermatozoa in the cervical mucus after intercourse. With an *in vitro* penetration test the interaction of spermatozoa and cervical mucus is investigated under laboratory conditions. Cross tests with donor mucus and donor spermatozoa can be performed with *in vitro* penetration tests. These cross tests enable a diagnosis wether a reduced penetration or a disturbed migration is caused by a mucus or a semen factor.

3.1. Physico-chemical characteristics of cervical mucus

It is amply documented that the physico-chemical properties of cervical

mucus show cyclic variations determined by the cyclic change of estrogenic and progestagenic influence on the secretory cells of the cervical epithelium. Due to maximum estrogenic stimulation during the pre-ovulatory phase of the menstrual cycle the amount of cervical mucus is maximal at that time. The mucus also then becomes translucent and relatively acellular. The viscosity is minimal and the elasticity maximal.

Cervical mucus is a hydrogel composed of a high viscosity and a low viscosity fraction. The high viscosity fraction determines the visco-elastic properties of cervical mucus. This fraction consists of glycoproteins (Gibbons, 1959). It has been postulated that these proteins are cross-linked to each other. The degree of cross-linking then determines the visco-elasticity (Gibbons & Mattner, 1966). Odeblad (1959, 1973) suggested that the glycoproteins are arranged in interconnected bundles. With electron microscopy filaments have been observed that seem to support the existence of the bundles (Van Bruggen & Kremer, 1970; Chrêtien et al., 1973). The model of a cross-linked macromolecular network has been debated by Lee et al. (1977). By using laser light-scattering spectroscopy they found that the molecular arrangement might more accurately described as an ensemble of entangled macromolecules. The electron microscopically observed filaments were considered to be artifacts.

It is thought that the glycoproteins of the gel component are made up of repeating subunits of polypeptide chains that are linked by disulphide bridges and hydrophobic forces. The visco-elastic properties are rapidly lost by the action of proteolytic enzymes, disulphide bond reducing agents and high concentrations of detergents (Doehr & Moghissi, 1973; Bushana Rao et al., 1976).

The low viscosity fraction of cervical mucus consists of a watery solution of proteins, lipids, carbohydrates, salts and other molecules. Some proteins are typical for external secretions such as lactoferrin (Masson & Ferin, 1969) and secretory IgA (2.7.2.3.1). Other proteins are identical with serum proteins and probably, at least partially, derived from the serum. Among these proteins are albumin, α1-antitrypsin, transferrin and immunoglobulins (Schumacher, 1980). The presence of immunoglobulins in cervical mucus is discussed in section 2.7.2.3.1. In presumably ovulatory menstrual cycles and sequential hormonal contraceptive cycles the concentrations of the soluble proteins change markedly with lowest values at mid-cycle (Schumacher, 1980).

3.2. Penetration of spermatozoa into cervical mucus under normal physiological conditions

During coitus semen is mixed with cervical mucus protruding from the external cervical os. Some spermatozoa will traverse the cervical canal directly towards the uterine cavity and the oviducts. Settlage et al. (1973) detected presence of spermatozoa in the oviduct within five minutes after depositing spermatozoa in the proximal vagina. Most motile spermatozoa will colonize the cervical crypts (Insler et al., 1980) from which spermatozoa are released continuously as long as permitted by the mucus qualities and the longivity of the spermatozoa. At mid-cycle live spermatozoa can normally be found in cervical mucus up to 48 hours after intercourse.

3.3. The post coital test (PCT)

The PCT was originally described by Sims (1886) and popularized by Huhner (1913). Despite its present popularity there is no unanimous opinion about the significance of the results of the PCT in predicting the achievement of a pregnancy of the couple under test. This prediction value is considered to be low by some investigators (Southam & Buxton, 1956; Gibor et al., 1970) and significant by others (Santomauro et al., 1972; Kremer et al., 1975; Moghissi, 1976). The reasons for this disagreement are differences in timing, performance and evaluation of the results of the PCT and in the number of PCT's per couple considered to be sufficient to come to a conclusion. Well known causes for poor results are a wrong coitus technique, semen deficiences and unfavorable properties of the cervical mucus (3.1). A less generally recognized cause is low pH of the cervical mucus (Kroeks, 1976; Kroeks & Kremer, 1977). Finally poor results of the PCT may be due to the presence of antispermatozoal antibodies in semen or in cervical mucus (4.3).

3.4. The in vitro sperm penetration tests

The earliest *in vitro* sperm penetration test is the coverslip test described by Miller & Kurzrok (1932). The test is still used by many investigators because of its simplicity. In the test, cervical mucus and semen are brought into close contact on a microscopic slide covered with a coverslip. The process of penetration of spermatozoa into the cervical mucus is studied under a microscope.

With normal semen and normal pre-ovulatory cervical mucus penetration of the spermatozoa starts after some delay. Groups of spermatozoa form a

kind of spearhead for which the term phalanx has been used. With the test according to Miller & Kurzrok (1932) a good impression of the capacity of spermatozoa to penetrate, and of the penetrability of cervical mucus, is obtained. However, the coverslip test cannot be used to obtain exact information on the migration distance, the penetration density or the duration of spermatozoal motility. These data can be obtained by using a capillary method.

The first capillary sperm penetration test was described by Lamar et al. (1940). Several other capillary test have been described. Among these is the sperm penetration meter (SPM) test according to Kremer (1965).

The capillary tube of the SPM is filled with cervical mucus by aspiration. The aspiration causes the glycoprotein bundles of the high viscosity component and the surrounding spaces, filled with the low viscosity component, to be straightened more or less parallel to the longitudinal axis of the capillary tube. Thus canals are formed forcing the spermatozoa to swimm mainly parallel to the longitudinal axis. This oriented swimming cause the spermatozoa to traverse a distance in the shortest time possible.

The SPM used in the present studies (chapters 3, 4 and 5) is provided with three semen reservoirs and has a level reduction, about 1 cm in length, just in front of the semen reservoirs (chapter 3, fig. 2). This construction prevents the semen from creeping out of the reservoirs into the thin cleft between the slide and the capillary tubes. Using this apparatus it is possible to study different parameters of the sperm-cervical mucus interaction. Detailed descriptions of performance, reading and classification of the readings can be found in chapters 4 and 5 and in Kremer (1980).

4. POSSSIBLE SITES AND MECHANISMS OF FERTILITY IMPAIRMENT BY ANTISPERMATOZOAL ANTIBODIES

4.1. The upper part of the female genital tract

Antispermatozoal antibodies might damage the fertilized egg. O'Rand (1977) using a cytotoxicity test, detected the appearance of spermatozoa specific antigens on rabbit eggs after fertilization. In the same species it has been found that antibodies to spermatozoa can also inhibit attachment of spermatozoa to the corona (Menge & Protzman, 1967; Metz, 1973).

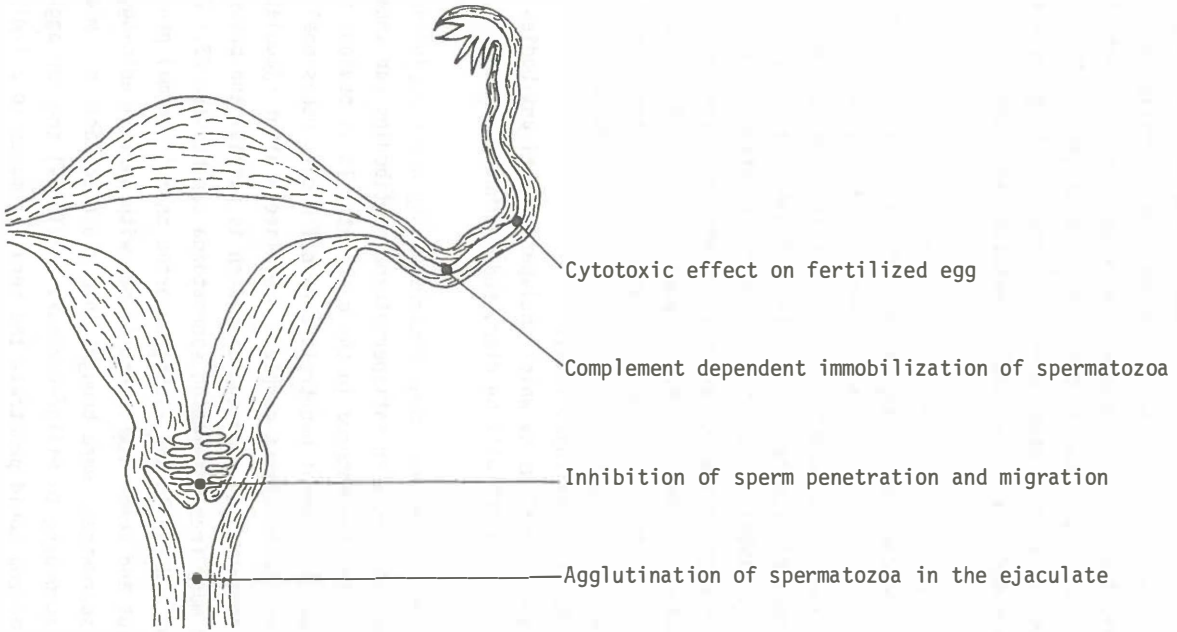


Fig. 1.7. Possible sites and mechanisms of fertility impairment by antispermatozoal antibodies

Another possible impairment of fertility is that antispermatozoal antibodies immobilize spermatozoa in the presence of complement in the oviducts. Ping (1979) determined sperm immobilizing and sperm agglutinating activity in human Fallopian fluid of women from infertile couples and of fertile women. The reported incidences were surprisingly high, but the incidences were not significantly different in both groups. The lack of technical details in this paper makes it impossible to judge wether the factors determined were indeed antispermatozoal antibodies.

4.2. The lower part of the female genital tract.

Complement dependent sperm immobilizing antibodies have been found in cervical mucus from a high percentage of women from infertile couples with no abnormality except an apparent cervical hostility. No such antibodies were detected in controls (Soffer et al., 1976; Menge et al., 1977; Wong, 1978; Moghissi et al., 1980). In addition, an inverse relationship has been found between the results of the PCT and the presence of sperm agglutinating antibodies (Shulman & Friedman, 1975; Kremer et al., 1978, see chapter 3; Moghissi et al., 1980). In infertile couples the results of the PCT have also often been found poor if antibodies to spermatozoa were present in the husband (Rümke & Hellinga, 1959).

The possible mechanisms by which antispermatozoal antibodies can cause a poor result of the PCT will be discussed in the next sections.

4.3. Inhibition of cervical mucus penetration by sperm agglutination

Semen samples from men with antispermatozoal antibodies can show auto-agglutination of the spermatozoa in the ejaculate. It is obvious that aglutinated spermatozoa cannot penetrate cervical mucus. Occasionally, the auto-agglutination is almost complete immediately after ejaculation. In most cases, however, the auto-agglutination is partial and between the agglutinates many freely swimming spermatozoa can be seen (2.7.2.2.2). Wilson (1954) and Fjällbrant (1965) reported that if normal pre-ovulatory cervical mucus and semen from infertile men with partial auto-agglutination of the spermatozoa were brought into close contact on a microscopic slide (test according to Miller-Kurzrok, 1932)(3.4) the non-agglutinated motile spermatozoa could penetrate the cervical mucus to a limited extent only. From these observations we can conclude that auto-agglutination of spermatozoa in the ejaculate can only partially explain the poor results of the PCT due to presence of antispermatozoal antibodies in the husband.

4.4. Complement depending immobilization of spermatozoa as cause of poor results of the PCT

The reduced capacity of cervical mucus penetration by spermatozoa from infertile men with antispermatozoal antibodies is probably not due to complement depending sperm immobilization. The antispermatozoal antibodies in semen were found to belong mostly to the non-complement binding class IgA (Friberg, 1974; Husted & Hjort, 1975b). In addition, Boettcher & Gruszinsky (1978) observed an inhibition of the cervical mucus penetration by spermatozoa pre-incubated in a serum with sperm-agglutinating but without sperm immobilizing activity. Moreover, it was found that a penetration inhibition due to antispermatozoal antibodies can also be observed if the complement in cervical mucus has been inactivated by heat treatment (Kremer & Jager, 1976, see chapter 2; Ikuma et al., 1980). The inhibition of cervical mucus penetration by non-agglutinated motile spermatozoa from infertile men with antispermatozoal antibodies can therefore not be explained by complement depending immobilization of the spermatozoa in cervical mucus.

Antispermatozoal antibodies in cervical mucus might cause an immobilization of spermatozoa in combination with complement also present in cervical mucus. Presence of complement like (heat labile) activity in cervical mucus containing sperm immobilizing antibodies has been reported (Parish & Ward, 1968; Menge et al., 1977). In pooled cervical mucus from two women Price & Boettcher (1979) measured, with a technique involving complement depending lysis of human erythrocytes, a complement activity of 11.5% of the serum level. With fresh human serum diluted to the 11.5% complement level, the authors found that about 50% of spermatozoa, incubated in a serum with an exceptionally high sperm immobilization titer (256) became immobilized after one hour.

4.5. Inactivation of a spermatozoal antigen essential for cervical mucus penetration

Metz & Anika (1970) performed intravaginal insemination in rabbits with rabbit semen pre-incubated with univalent (Fab, fig. 1.1a) antispermatozoal IgG. The univalent antibodies were obtained by treatment of goat IgG antibodies to rabbit semen with the proteolytic enzyme papain. The authors observed a decreased concenption rate in rabbits inseminated with the pretreated semen. Flushings from the genital tract of rabbits inseminated intravaginally with this pretreated semen contained approximately 50

times less spermatozoa than controls inseminated with semen pretreated with univalent goat IgG not reactive with rabbit spermatozoa. Since the spermatozoa were not agglutinated by the univalent IgG and the motility was not visibly affected these two factors did not explain the observed conception failure. The authors suggested that the univalent antibodies blocked some spermatozoal (surface?) antigen that has an important role in cervical mucus penetration.

4.6. Cross-linking of spermatozoa to the high viscosity component of cervical mucus through the antibody molecule

Freely swimming spermatozoa from men with antispermatozoal antibodies change their propulsions into local shaking movements upon contact with cervical mucus (chapter 2). This effect, called the "shaking phenomenon", is the main subject of investigation in this thesis. To explain the shaking phenomenon the hypothesis was put forward that spermatozoa sensitized with antispermatozoal antibodies stick to the gel component of the cervical mucus.

The binding might be due to a cross-linking of the spermatozoa to the gel component through the antibody molecule. Two types of cross-linking are possible. One type of cross-linking occurs if the spermatozoal surface and the gel component have an antigenic determinant in common. The result is a mixed agglutination. The other cross-linking type is binding of the spermatozoa to the gel component by the Fc part of the antibody molecules on the spermatozoa. In this case cervical mucus has an Fc receptor function.

The type of cross-linking involved can be investigated using spermatozoa sensitized with univalent antibodies (Fab fragments, fig. 1.1a) and spermatozoa sensitized with bivalent antibodies without Fc part i. e. $F(ab)_2$ fragments. Isojima et al. (1979) found, with spermatozoa pretreated with Fab fragments of sperm immobilizing IgG, an excellent sperm penetration into cervical mucus. Spermatozoa pretreated with the intact antibodies could not penetrate. On the other hand, Hjort et al. (1978) found that spermatozoa pretreated with $F(ab)_2$ fragments from head-to-head agglutinating antibodies could not penetrate cervical mucus. Spermatozoa sensitized with $F(ab)_2$ fragments from tail-to-tail agglutinating antibodies could penetrate, however. In our opinion the results of Hjort et al. (1978) can be explained, at least partially, by the quick sperm agglutination in the incubation medium and at the semen mucus border in the

presence of the head-to-head agglutinating $F(ab)_2$ fragments.

5. THE AIMS OF THE INVESTIGATIONS DESCRIBED IN CHAPTERS 2 TO 9.

The main object of the present studies is to investigate by which mechanism antispermatozoal antibodies reduce the cervical mucus penetrability of freely swimming spermatozoa sensitized with antispermatozoal antibodies. It will be shown that these spermatozoa change their forward propulsions into local shaking movements after contact with cervical mucus. On this so called " shaking phenomenon" a new test is based: the sperm-cervical mucus contact (SCMC) test (chapter 2).

The results of the SCMC test will be compared with the results of the PCT to see whether the shaking phenomenon can be an explanation of, otherwise, unexplained poor result of the PCT (chapter 3).

The results of the SCMC test will also be compared with the results of the sperm penetration meter (SPM) test (Kremer, 1965). It will be investigated whether the SCMC test is more suitable than the SCMC test for studying the effect of antispermatozoal antibodies on the penetration and migration of spermatozoa into cervical mucus (chapter 4).

For detection of immunoglobulins on motile spermatozoa and determination of the immunoglobulin class the mixed antiglobulin reaction (MAR) (Coombs et al., 1956) will be applied. It will be shown that a MAR test for IgG, performed on untreated fresh ejaculates, can be used for screening men on the presence of antispermatozoal antibodies (chapter 5).

In addition, two studies will be presented concerning the relations between the class of immunoglobulins on motile spermatozoa, the circulating and local sperm agglutinating activity and the shaking phenomenon in the SCMC test (chapter 6 and 8).

Furthermore, some investigations to relate the occurrence of a shaking phenomenon to the immunoglobulin class of antispermatozoal antibodies in cervical mucus will be presented (chapter 7).

Finally it will be shown that the presence of the Fc parts of the antibody molecules on motile spermatozoa is responsible for the shaking phenomenon.

REFERENCES

Ahlgren, M. (1969) Migration to the Fallopian tubes and the abdominal cavity in women. Including some immunological aspects. Thesis, University of Lund.

Ahrons, S. (1971) Leukocyte antibodies: Occurrence in primigravidae. Tissue Antigens 1: 178-183.

Amelar, R.D., Dubin, L. and Schoenfeld, C. (1975) Circulating sperm-agglutinating antibodies in azoospermic men with congenital bilateral absence of the vasa deferentia. Fert. Steril. 26: 228-231.

Ansbacher, R. (1971) Sperm agglutinating and sperm-immobilizing antibodies in vasectomized men. Fert. Steril. 22: 629-632.

Ansbacher, R. (1973) Vasectomy: Sperm antibodies. Fert. Steril. 24: 788-792.

Ardelt, F. (1934) Spermaresorption bei gesunden und genitalkranken Frauen. Arch. Gynäk. 156: 357-361.

Bandhauer, K. (1966) Immunreaktionen bei Fertilitätsstörungen de Mannes. Urol. Int. 21: 247-282.

Barnes, G. W., Lee, L. M. Y., Kenny, M. and Barnes, M. S. (1978) Tissue antigens in seminal secretions. I. Precipitin reactions of rabbit antiserum to human seminal plasma. Immunology 35: 589-600.

Baskin, M. J. (1932) Temporary sterilization by the injection of human spermatozoa. A preliminary report. Am. J. Obst. Gynecol. 24: 892-897.

Beck. J. S., Edwards, R. G. and Young, M. R. (1962) Immune fluorescence technique and the Iso-antigenicity of mammalian spermatozoa. J. Reprod. Fert. 4: 103-110.

Behrman, S. J. and Amano, Y. (1967) Immunochemical studies on human seminal plasma. I. Antigenicity of seminal protein. Int. J. Fert. 12: 291-298.

Behrman, S. J., Buettner-Janusch, J., Heglar, R., Gershowitz, H. and Tew, W. L. (1960) ABO (H) blood incompatibility as a cause of infertility: a new concept. Am. J. Obst. Gynecol. 79: 847-855.

Bennet, D. and Boyse, E. A. (1973) Sex ratio in progeny of mice inseminated with sperm treated with H-Y antiserum. Nature 246: 308-309.

Boettcher, B. (1974) The molecular nature of sperm agglutinins and sperm antibodies in human sera. J. Reprod. Fert., Suppl. 21: 151-167.

Boettcher, B. (1980) Detection of antibodies to lactate dehydrogenase-X in human sera by radio immuno assay. Abstract, First Int. Soc. Immunol. Reprod., Paris

Boettcher, B. and Gruszynski, R. (1978) The passage of human spermatozoa treated with spermantibodies through cervical mucus. In: Immunology of Reproduction, Proc. 3rd Int. Symp. (Bratanov, K., Vulchanov, V. H., Dikov, V., Somlev, B. and Kozhouharova, L. eds.). pp 444-449. Publishing House of the Bulgarian Academy of Sciences, Sofia.

Boettcher, B. and Hay, J. (1968) An investigation of a proposed mechanism for infertility based on ABO blood group incompatibility. Am. J. Obst. Gynecol. 100: 437-441.

Boettcher, B. and Hay, J. (1969) The nature of sperm agglutinins in human sera. In: Immunology of Spermatozoa and Fertilization (Bratanov, K., Vulchanov, V. H., Dikov, V., Dokov, V. K. and Somlev, B. eds.). pp 47-50. Bulgarian Academy of Sciences Press, Sofia.

Boettcher, B., Hjort, T., Rümke, P., Shulman, S. and Vyazov, O. E. (1977) Auto- and Isi-antibodies 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., Sect. C, Suppl. 258: 1-69.

Boettcher, B., Kay, D. J., Rümke, P. and Wright, L. E. (1971) Human sera containing immunoglobulin and non immunoglobulin spermagglutinins. Biol. Reprod. 5: 236-245.

Brandtzaeg, P. (1973) Structure, synthesis and external transfer of mucosal immunoglobulins. Ann. Immunol. (Inst. Pasteur) 124C: 417-438.

Bushana Rao, K. S. P., Barbier, B., Masson, P. L., Heremans, J. F. and Ferin, J. (1976) Susceptibility of cervical mucus to enzymes. In: Human semen and fertility regulation in men (Hafez, E. S. E. ed.). pp 237-242. Mosby Cy., St. Louis.

Cantuária, A. A. (1977) Sperm immobilizing antibodies in the serum and cervicovaginal secretions of infertile and normal women. Brit. J. Obst. Gynaecol. 84: 865-868.

Cantuária, A. A. and Jones, A. L. (1980) The effect of immunoglobulins on sperm-migration inhibition in the cervicovaginal secretions of infertile women. Brit. J. Obst. Gynaecol. 87: 56-66.

Chipperfield, E. J. and Evans, B. A. (1972) The influence of local infections on immunoglobulin formation in the human endocervix. Clin. Exp. Immunol. 11: 219-223.

Chodirker, W. B. and Tomasi, T. B. (1963) Gamma globulins: Quantitative relationships in human serum and nonvascular fluids. Science 142: 1080-1081.

Chretien, F. C., Gerningon, C., David, G. and Psychoyos, A. (1973) The ultrastructure of human cervical mucus under scanning electron microscopy. Fert. Steril. 24: 746-757.

Coelingh Bennink, H. J. T. and Menge, A. C. (1974) Spermatozoal antibodies in cervical mucus. Europ. J. Obst. Gynecol. reprod. Biol. 4: 147-152.

Coombs, R. R. A., Marks, J. and Bedford, D. (1956) Specific mixed agglutination: Mixed erythrocyte-platelet anti-globulin reaction for the detection of platelet antibodies. Brit. J. Haemat. 2: 84-94.

Coombs, R. R. A., Rümke, P. and Edwards, R. R. G. (1973) Immunoglobulin classes reactive with spermatozoa in the serum and seminal plasma of vasectomized and infertile men. In: Immunology of Reproduction (Bratanov, K., Edwards, R. G., Vulchanov, V. H., Dikov, V. and Somlev, B. eds.). pp 354-359. Bulgarian Academy of Sciences Press, Sofia.

Cruickshank, B. and Stuart-Smith, D. A. (1959) Orchitis associated with spermagglutinating antibodies. Lancet (1959) 708.

D'Almeida, M. and Eyquem, A. (1978) Activité antispermale dans le mucus cervicale chez femmes infertiles. In: Immunology of Reproduction (Bratanov, K., Vulchanov, V. H., Dikov, V., Somlev, B. and Kozhouharova,

L. eds.). pp 432-437. Publishing House of the Bulgarian Academy of Sciencs, Sofia.

D'Almeida, M., Joliy, M. and Voisin, G. A. (1980) Fractionation of human spermatozoa autoantigens. Abstract, Fourth Int. Congr. Immunol., Paris.

D'Almeida, M., Righenzi, S. and Voisin, G. A. (1980) Ultrastructural localization of human spermatozoa surface antigens by immunoperoxidase labelling. Abstract, Fourth Int. Congr. Immunol., Paris.

Doehr, S. A. and Moghissi, K. S. (1973) Human and bovine cervical mucins. In: The Biology of the Cervix (Blandau, R. J. and Moghissi, K. eds.). pp 125-142. University of Chicago Press, Chicago.

Doughty, R. W. and Gelsthorpe, K. (1976) Some parameters of lymphocyte antibody activity through pregnancy and further eluates of placental material. Tissue Antigens 8: 43-48.

Dukes C. D. and Franklin, R. R. (1968) Sperm agglutinins and human infertility; Female. Fert. Steril. 19: 263-267.

Edwards, R. G., Ferguson, L. C. and Coombs, R. R. A. (1964) Blood group antigens on human spermatozoa. J. Reprod. Fert. 7: 153-161.

Edwards, R. G., Talbert, L., Israelstam, D., Nino, B. and Johnson, M. H. (1968) Diffusion chamber for exposing spermatozoa to human uterine secretions. Am. J. Obst. Gynecol. 102: 388-396.

Eichwald, E. J. and Silmser, C. R. (1955) Communication. Transplant. Bull. 2: 148-149.

Entschev, E. M. and Momchilov, B. (1969) Detection of autospermo-antibodies in man by means of immunofluorescence. In: Immunology of Spermatozoa and Fertilization (Bratanov, K., Vulchanov, V. H., Dikov, V., Dikov, V. K. and Somlev, B. eds.). pp 201-204. Bulgarian Academy of Sciences Press, Sofia.

Eyquem, A., Heuze, D., Schwartz, J. and Languillet, G. (1978) Implications of heterophile antigens in immunological infertility in males. Arch. Androl. 1: 241-248.

Fattah, A. A., Azim, A. A., Habeib, M. and Rafih, M. (1980). Studies on infertility in males. Fert. Steril. 33: 157-159.

Fellous, M. and Dausset, J. (1970) Probable haploid expression of HLA-A antigens on human spermatozoa. Nature 225: 191-193.

Feltkamp, T. E. W., Kruyff, K., Ladiges, N. C. J. J. and Rümke, P. (1965) Autospermagglutinins: Immunofluorescent studies. Ann. N. Y. Acad. Sci. 124: 702-708.

Fernandez-Collazo, E. and Thierer, E. (1972) Action of ABO antisera on human spermatozoa. Fert. Steril. 23: 376-382.

Fjällbrant, B. (1965) Immunoagglutination of sperm in cases of sterility. Acta Obst. Gynecol. Scand. 44: 474-490.

Fjällbrant, B. (1968) Sperm agglutinins in sterile and fertile men. Acta Obst. Gynecol. Scand. 47: 89-101.

Fjällbrant, B. (1969) Cervical mucus penetration by human spermatozoa treated with antispermatozoal antibodies from rabbit and man. Acta Obst. Gynecol. 48: 71-84.

Fjällbrant, B. (1970) Localization of human male autoantibodies on sper-

matozoa. Am. J. Obst. Gynecol. 108: 550-556.

Fjällbrant, B. and Nilson, S. (1977) Decrease of sperm antibody titer in males, and conception after treatment of chronic prostatitis. Int. J. Fert. 22: 255-256.

Fjällbrant, B. and Obrant, O. (1968) Clinical and seminal findings in men with sperm antibodies. Acta Obst. Gynecol. 47: 451-468.

Franklin, R. R. and Dukes, C. D. (1964a) Antispermatozoal antibody and unexplained infertility. Am. J. Obst. Gynecol. 89: 6-9.

Franklin, R. R. and Dukes, C. D. (1964b) Further studies on sperm-agglutinating antibody and unexplained infertility. J. Am. Med. Ass. 190: 682-683.

Friberg, J. (1974). Clinical and immunological studies on sperm-agglutinating antibodies in serum and seminal fluid. Acta Obst. Gynecol. Scand. Suppl. 36: 1-76.

Friberg, J. (1980) Immunoglobulin concentration in serum and seminal fluid from men with and without sperm-agglutinating antibodies. Am. J. Obst. Gynecol. 136: 671-675.

Friberg, J. and Tilly-Friberg, I. (1977) Spontaneous sperm-agglutination in ejaculates from men with head-to-head or tail-to-tail spermagglutinating antibodies in serum. Fert. Steril. 28: 658-662.

Friberg, J. and Kjessler, B. (1975) Sperm-agglutinating antibodies and testicular morphology in fifty-nine men with azoospermia or cryptozoospermia. Am. J. Obst. Gynecol. 121: 987-990.

Friedenreich, V. and Hartmann, G. (1938) Ueber die Verteilung der Gruppen Antigene in Organismus der sogenannten "Ausscheider" und "Nichtausscheider. Z. ImmunForsch. 92: 141-151.

Gershowitz, H., Behrman, S. J. and Neel, J. V. (1958) Hemagglutinins in uterine secretions. Science 128: 719-720.

Gibbons, R.A. (1959) Chemical properties of two mucoids from bovine cervical mucin. Biochem. J. 73: 209.

Gibbons, R. A. and Mattner, P. (1966) Some aspects of the chemistry of cervical mucus. Int. J. Fert. 11: 366-372.

Gibor, Y., Garcia, C. J., Cohen, M. R. and Scommegna, A. (1970) The cyclical changes in the physical properties and the results of the post coital tests. Fert. Steril. 21: 20-27.

Girgis, S. M., Ekiadios, E. M., Iskander, R. M. and Gishin, F. K. (1979) Serum sperm antibodies in case of azoospermia: Comparative diagnostic value of separate and combined agglutination, immobilization and cytotoxic serological tests. Andrologia 11: 417-421.

Glass, R. H. and Vaidya, R. A. (1970) Sperm-agglutinating antibodies in infertile women. Fert. Steril. 21: 657-661.

Goldberg, E. H. Boyse, E. A., Bennet, D., Scheid, M. and Carswell, E. A. (1971) Serological demonstration of H-Y (male) antigen on mouse sperm. Nature 232: 478-480.

Gullbring, B. (1957) Investigation on the occurrence of blood group antigens in spermatozoa from man, and serological demonstration of the segregation of character. Acta Med. Scand. 159: 169-172.

Gupta, I., Dhawan, S., Goel, G. D. and Saha, K. (1975) Low fertility rate in vasovasostomized men and its possible immunologic mechanism. Int. J. Fert. 20: 183-191.

Gupta, K. G. and Garg, A. K. (1975) Presence of antisperm antibodies in fertile and infertile persons. Acta Obst. Gynecol. Scand. 54: 407-410.

Haensch, R. (1969) Fluorescenzimmunologische Spermienautoantikörper Befund bei männlichen Fertilitätsstörungen. Arch. Gynäk. 208: 91-102.

Haensch, R. (1973) Autoimmune reactions to spermatozoa in cases of genital trauma and obstructive azoospermia. Andrologia 5: 147-152.

Halim, A. and Antonionou, D. (1973) Autoantibodies to spermatozoa in relation to male infertility and vasectomy. Brit. J. Urol. 45: 559-562.

Halim, K. and Festenstein, H. (1975) HLA-D on sperm is haploid, enabling use of sperm for HLA-D typing. Lancett ii: 1255-1256.

Hamerlynck, J. V. (1970) Cytotoxic and other auto-antibodies against spermatozoa in relation to infertility in the human male. Dissertation University of Amsterdam.

Hamerlynck, J. and Rümke, P. (1968) A test for the detection of cytotoxic antibodies to spermatozoa in man. J. Reprod. Fert. 17: 191-194.

Hanafiah, M. J., Epstein, J. A. and Sobrero, A. J. (1972) Sperm-agglutinating antibodies in 236 infertile couples. Fert. Steril. 23: 493-497.

Hancock, R. J. T. (1978) Sperm antigens and sperm immunogenicity. In : Spermatozoa, Antibodies and Infertility (Cohen, J. and Hendry, W. F. eds.). pp 1-9. Blackwell Scientific Publications, Oxford.

Hansen, K. B. (1974) A comparative study of various methods for the detection of spermatozoal antibodies. Acta Obst. Gynecol. Scand. 53: 69-76.

Hansen, K. B. and Hjort, T. (1971) Immunofluorescent studies on human spermatozoa. II. Characterization of spermatozoal antigens and their occurrence in spermatozoa from the male partners of infertile couples. Clin. Exp. Immunol. 9: 21-31.

Harrison, R. F. (1978) Significance of sperm antibodies in human fertility. Int. J. Fert. 23: 288-293.

Hekman, A. and Rümke, P. The antigens of human seminal plasma. Fert. Steril. 20: 312-323.

Hellema, H. W. J. and 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: 284-292.

Hellema, H. W. J. and Rümke, P. (1978a) The micro-sperm immobilization test: the use of only motile spermatozoa and studies of complement. Clin. exp. Immunol. 31: 1-11.

Hellema, H. W. J. and Rümke, P. (1978b) Sperm antibodies as a consequence of vasectomy. I. Within 1 year post-operation. Clin. exp. Immunol. 31: 18-29.

Hendry, W. F., Morgan, H. and Stedronska, J. (1977) The clinical significance of antisperm antibodies in male subfertility. Brit. J. Urol. 49: 757-762.

Hjort, T. (1976) Iso- and auto-antibodies to human sperm as reactants for the study of immunogenic components of human sperm. In: Developments of Vaccines for Fertility Regulations (WHO) pp 37-62. Scriptor, Copenhagen.

Hjort, T. and Hansen, K. B. (1971) Immunofluorescent studies on human spermatozoa. I. The detection of different spermatozoal antibodies and their occurrence in normal and infertile women. Clin. exp. Immunol. 8: 9-23.

Hjort, T., Hansen, K. B. and Poulsen, F. (1978) The reactivity of F(ab)2 fragments of sperm antibodies and their use in the investigation of antigen-antibody systems. In: Spermatozoa, Antibodies and Infertility (Cohen, J. and Hendry, W. F. eds.). pp 101-115. Blackwell Scientific Publications, Oxford.

Hofmann, R., Rätz, K.-H., Mattheus, A. and Wegener, S. (1978) Sperma- und HLA-Antikörpernachweiss bei Sterilität. Zbl. Gynäk. 100: 122-126.

Huhner, M. Sterility in the male and female and its treatment. Rebman Co., New York, 1913.

Hulka, J. F. and Omran, K. F. (1969) The uterine cervix as a potential local antibody secretor. Am. J. Obst. Gynecol. 104: 440-442.

Hunter, J., Logan, H. and Greer, G. (1976) Incidence of sperm antibodies before and after vasectomy. J. Clin. Pathol. 29: 1127-1129.

Husted, S. (1975) Sperm antibodies in men from infertile couples. Analysis of sperm agglutinins and immunofluorescent antibodies in 657 men. Int. J. Fert. 20: 113-121.

Husted, S. and Hjort, T. (1975a) Microtechnique for simultaneous determination of immobilizing and cytotoxic sperm antibodies. Clin. exp. Immunol. 22: 256-264.

Husted, S. and Hjort, T. (1975b) Sperm antibodies in serum and seminal plasma. Int. J. Fert. 20: 97-105.

Ikuma, K., Kubota, K., Takada, Y., Koyama, K. and Isojima, S. (1980) Inhibitory effect of sperm immobilizing antibody on sperm migration in cervical mucus. Abstract, Tenth World Congr. Fert. Steril, Madrid.

Ingerslev, H. J. (1979) Characterization of sperm agglutinins in sera from infertile women. Int. J. Fert. 24: 1-12.

Ingerslev, H. J. and Hjort, T. (1979) Spermagglutinating antibodies and β-spermagglutinins in sera from infertile and fertile women. Fert. Steril. 31: 496-502.

Ingerslev, H. J. and Ingerslev, M. (1980) Clinical findings in infertile women with circulating antibodies against spermatozoa. Fert. Steril. 33: 514-520.

Insler, V., Glezerman, M., Zeidel, L., Bernstein, D. and Misgav, N. (1980) Sperm storage in the human cervix: A quantitative study. Fert. Steril. 33: 288-293.

Isojima, S., Koyama, K., Ikuma, K. and Kou, S. (1979) Influence of weak antisperm antibody on sperm penetration in women's cervical mucus. In: Immunology of Reproduction (Bratanov, K., Vulchanov, V. H., Dikov, V., Georgieva, R. and Somlev, B. eds.). pp 341-346. Publishing House of the Bulgarian Academy of Sciences, Sofia.

Isojima, S., Kamata, T. and Koyama, K. (1977) The important role of com-

plement in sperm immobilization test. In: Immunological influence on Human Fertility (Boettcher, B. ad.). pp 247-253. Academic Press, Sidney.

Isojima, S. and Koyama, K. (1976) Studies on circulating antibodies to spermatozoa found in women sterile for unknown reason. In: Biological and Clinical Aspects of Reproduction (Ebling, F. J. G. and Henderson, I. W. eds.). pp 178-184. Excerpta Medica, Amsterdam.

Isojima, S., Kpyama, K. and Tsuchija, K. (1974) The effect on fertility in women of circulating antibodies against human spermatozoa. J. Reprod. Fert., Suppl. 21: 125-150.

Isojima, S., Li, T. S. and Ashitaka, Y. (1968) Immunologic analysis of sperm-immobilizing factor found in sera of women with unexplained sterility. Am. J. Obst. Gynecol. 101: 677-683.

Isojima, S., Tsuchiya, K., Koyama, K., Tanaka, C., Nada, O. and Adachi, H. (1972) Further studies on sperm-immobilizing antibody found in sera of unexplained cases of sterility in women. Am. J. Obst. Gynecol. 112: 199 -207.

Isojima, S. and Tzuzuku, O. (1968) Problem of ABO blood group incompatibility and sterility: The effect of blood group antibody on spermatozoa. Am. J. Obst. Gynecol. 102: 304-306.

Jalanti, R. and Isliker, H. (1977) Immunoglobulins in human cervicovaginal secretions. Int. Arch. Allergy Appl. Immunol. 53: 402-408.

Jeffery, W. and Parish, W. E. (1972) Allergic infertility: laboratory techniques to detect antispermatozoal antibodies. Clin. Allergy 2: 261-279

Jones, W. R. (1980) Immunologic infertility-Fact or fiction. Fert. Steril. 33: 577-586.

Jones, W. R., Ing, R. M. Y. and Hobbin, E. R. (1976) Approaches and perspectives in the development of antisperm immunity as a contraceptive principle. In: Development of Vaccines for Fertility Regulations (WHO) pp 17-35. Scriptor, Copenhagen.

Kaskarelis, D., Milingos, S., Kallipolitis, G., Messinis, I. and Lolis, D. (1979) Depistage annuel (1977) sur les anticorps agglutinants du sperme portant sur les patients qui ont visité les consultations de la subfertilité et de gynécologie de la maternité "Alexandria" Athens. In: Immunology of Reproduction (Bratanov, K., Vulchanov, V. H., Dikov, V., Georgieva, R. and Somlev, B. eds.). pp 295-303. Publishing House of the Bulgarian Academy of Sciences, Sofia.

Katsh, S. (1959) Immunology, fertility, and infertility: A historical survey. Am. J. Obst. Gynecol. 77: 946-956.

Kay, R. and Alexander, N. J. (1978) Immunofluorescence titres of sperm centrifuged at different speeds. Clin. exp. Immunol. 32: 299-301.

Kerék, G. (1974) Distribution of the blood group antigens A and B on human spermatozoa. Int. J. Fert. 19: 181-191.

Kibrick, S., Belding, D. L. and Merrill, B. (1952) Methods for the detection of antibodies against mammalian spermatozoa. Fert. Steril. 3: 419-438.

Kissmeyer-Nielsen, F. and Thorsby, E. (1970) Transplantation Reviews, Vol. 4. Munksgaard, Copenhagen.

Kolk, A. H. J. and Samuel, T. (1975) Isolation, chemical and immunochemical characterization of two strongly basic nuclear proteins from human spermatozoa. Biochim. Biophys. Acta 393: 307-319.

Kolk, A. H. J., Samuel, T. and Rümke, P. (1974) Auto-antigens of human spermatozoa. Solubilization of a new auto-antigen detected on swollen sperm heads. Clin. exp. Immunol. 16: 63-76.

Kolodny, R. C., Koehler, B. C., Tora, G. and Masters, W. H. (1971) Sperm agglutinating antibodies in infertile women. Obst. Gynecol. 38: 576-582.

Koo, G. C., Stackpole, C. W., Boyse, E. A., Hämmerling, U. and Lardis, P. M. (1973) Topographical location of the H-Y antigen on mouse spermatozoa by immunoelectron microscopy. Proc. Natl. Acad. Sci. USA 70: 1502-1505.

Koskimies, A. T. (1979) Sperm-agglutinating antibodies in infertile couples. Arch. Androl. 2: 241-245.

Kosuda, L. L. and Bigazzi, P. E. (1978) Autoantibodies to acrosomal antigens of spermatozoa in vasectomized mice. Invest. Urol. 16: 140-141.

Kremer, J. (1965) A simple sperm penetration test. Int. J. Fert. 10: 209-215.

Kremer, J. (1980) In vitro sperm penetration in cervical mucus and AIH. In: Homologous Artificial Insemination (AIH)(Emperaire, J. C., Audebert, A. and Hafez, E. S. E. eds.). pp 30-37. Martinus Nijhoff Publishers, The Hague.

Kremer, J., Bouman, O. and Tjeenk Willink, H. D. (1975) De betekenis van de Sims-Huhnertest voor de fertiliteitsprognose. Ned. T. Geneesk. 119: 653-658.

Krieg, H., Gathof, A. G., Netter, P. and Hedrich, D. (1973) Further investigations on blood group differences and fertility. In: Immunology and Reproduction (Bratanov, K., Edwards, R. G., Vulchanov, V. H., Dikov, V. and Somlev, B. eds.). pp 537-543. Bulgarian Academy of Sciences Press Sofia.

Kroeks, M. V. A. M. (1976). Cervixslijm. Thesis, University of Utrecht.

Kroeks, M. V. A. M. and Kremer, J. (1977) The pH in the lower third of the genital tract. In: The Uterine Cervix in Reproduction (Insler, V. and Bettendorf, G. eds.). pp 109-118. Georg Thieme Publishers, Stuttgart.

Kula, K., Owczarcyk, I. and Reterski, Z. (1979) Serum immunoglobulins IgG and IgM in the seminal plasma of men with normospermia, oligozoospermia and azoospermia. Arch. Androl. 3: 37-41.

Lai a Fat, R. F. M., Suurmond, D. and Van Furth, R. (1973) Clin. exp. Immunol. 14: 377-395.

Lamar, J. K., Shettles, L. B. and Delfs, E. (1940) Cyclic penetrability of human cervical mucus to spermatozoa in vitro. Am. J. Physiol. 129: 234.

Landsteiner, K. Zur Kenntnis der spezifisch auf Blutkörperchen wirkenden Sera. Zbl. Bakt. 25: 546-549.

Landsteiner, K. and Levine, P. On group specific substances in human spermatozoa. J. Immunol. 12: 415-418.

Lee, W. I., Verdugo, P., Blandau, R. J. and Gaddun-Rosse, P. (1977) Mo-

lecular arrangement of cervical mucus: A reevaluation based on laser-light-scattering spectroscopy. Gynecol. Invest. 8: 254-266.

Levine, P. and Celano, M. J. (1961) The question of D (Rho) antigenic sites on human spermatozoa. 6: 720-723.

Li, T. S. and Behrman, S. J. (1970) The sperm- and seminal plasma specific antigens of human semen. Fert. Steril. 21: 565-573.

Lehman, F., Stripling, K., Büdel, B., Krebs, D. and Masson, D. (1977) Sperm antibodies in infertile women: Incidence and clinical significance. In: The Uterine Cervix in Reproduction (Insler, V. and Bettendorf, G. eds.). Georg Thieme Publishers, Stuttgart.

Linnet, L. and Hjort, T. (1977) Sperm agglutinins in seminal plasma and serum after vasectomy. Correlation between immunological and clinical findings. Clin. exp. Immunol. 30: 413-420.

Lippes, J., Ogra, S., Tomas, T. B. and Tourville, D. R. (1970) Immunohistological localization of IgG, IgA, IgM, secretory piece and lactoferrin in the human genital tract. Contraception 1: 163-183.

Machado de Domenech, E., Domenech, C. E., Aoki, A. and Blanco, A. (1972) Biol. Reprod. 7: 136.

Manarang-Pangan, S. and Behrman, S. J. (1971) Spermatotoxicity of immune sera in human fertility. Fert. Ateril. 22: 145-151.

Mälzer, G., Freistedt, B., Berge, H., Felker, A. and Hentschel, W. (1979) Bereichert das HLA-System unsere Möglichkeiten im Rahmen der Sterilitäts-Diagnostik? Zbl. Gynäk. 101: 72-74.

Mancini, R. E., Gutierez, O. and Fernadez-Collazo, E. (1971) Immunihistochemical localization of antigens in human spermatozoa. Fert. Steril. 22: 475-481.

Masson, D., Lehman, F., Breckwoldt, M. and Krebs, D. (1970) Sperma-Antikörper als mögliche Ursacher einer ungeklärten Sterilität. Geburtsh. Frauenheilk. 30: 103-116.

Masson, P. L. and Ferin, J. (1969) Les costituants protêique du mucus du col. Gynêcol. Obst. (Paris) 68: 419-432.

Meaker, S. R. (1922) Some aspects of the aspects of the problems of sterelity. Boston Med. Surg. J. 187: 535.

Menge, A. C., Schwanz, M. L., Riolo, R. L., Greenberg, V. N., and Neda, T. (1977) The role of the cervix and cervical secretions in immunologic infertility. In: The Uterine Cervix in Reproduction Insler, V. and Bettendorf, G. eds.). Georg Thieme Publishers, Stuttgart.

Menge, A. C. and Protzman, W. P. (1967) Origin of the antigens in rabbit semen which induce antifertility antibodies. J. Reprod. Fert. 13: 31-40.

Metalnikoff, S. (1900) Etudes sur la spermotxine. Ann. Inst. Pasteur 14: 577-589.

Metchnikoff, E. (1899) Etudes sur la rêsorption des cellules. Ann. Inst. Pasteur 13: 737-769.

Mettler, L. (1977) Immunologie der Weiblichen Sterilität. Grosse Verlag, Berlin.

Metz, C. B. (1973) Role of specific sperm antigens. Fed. Proc. 32: 2057-2064.

Metz, C. B. and Anika, J. (1970) Failure of conception in rabbits inseminated with nonagglutinating, univalent antibody-treated semen. Biol. Reprod. 2: 284-290.

Miller, E. G. and Kurzrok, R. (1932) Biochemical studies of human semen. III. Factors affecting migration of sperm through the cervix. Am. J. Obst. Gynecol. 24: 19.

Moghissi, K. S. (1967) Postcoital test: Physiological basis, technique, and interpretation. Fert. Steril. 27: 117-129.

Moghissi, K. S., Sacco, A. G. and Borin, K. (1980) Immunologic infertility I. Cervical mucus antibodies and postcoital test. Am. J. Obst. Gynecol. 136: 941-950.

Morgan, H., Stedronska, J., Hendry, W. F., Chamberlain, G. V. P. and Dewhurst, C. J. (1977) Sperm/cervical-mucus crossed hostility testing and antisperm antibodies in the husband. Lancet i: 1228-1230.

Morton, D. B. (1977) Immunoenzymic studies on acrosin and hyaluronidase in ram spermatozoa. In: Immunobiology of Gametes (Edidin, M. and Johnson M. H. eds.). pp 115-155. Cambridge University Press.

Morton, D. B. and McAnulty, P. A. (1979) The effect on fertility of immunizing female sheep with ram sperm acrosin and hyaluronidase. J. Reprod. Immunol. 1: 61-73.

Müller, U., Aschmoneit, I., Zenzes, M. T. and Wolf, U. (1978) Binding studies of H-Y antigen in rat tissues: indications for a gonad-specific receptor. Human Genet. 43: 151-158.

Murdoch, R. N. and White, I. G. (1968) Studies of the distribution and source of enzymes in mammalian semen. Aust. J. biol. Sci. 21: 483-490.

Nakabayashi, N. T., Tyler, E. T. and Tyler, A. (1961) Immunological aspects of human infertility. Fert. Steril. 12: 544-550.

Nielsen, L. S. and Svejgaard, A. (1972) HL-A immunization and HL-A types in pregnancy. Tissue Antigens 2: 316-327.

Odeblad, E. (1959) The physics of the cervical mucus. Acta Obst. Gynecol. Scand. 38, Suppl. 1: 44-57.

Odeblad, E. (1973) Biophysical techniques of assessing cervical mucus and microstructure of cervical epithelium. In: Cervical Mucus in Human Reproduction (Elstein, M., Moghissi, K. S. and Borth, R. eds.). pp 58-74. Scriptor, Copenhagen.

Ohno, S., Nagai, Y., Ciccarese, S. and Iwata, H. (1979) Testis-organizing H-Y antigen and the primary Sex-determining mechanism of mammals. Recent Progr. Hormone Res. 35: 449-476.

Olson, L. E. (1967) Immunologic studies in infertility. Connecticut Med. 31: 703-704.

O'Rand, M. G. (1977) The presence of sperm-specific surface isoantigens on the egg following fertilization. J. exp. Zool. 202: 267-273.

Pacheco-Romero, J. C., Gleich, G. J. Loegering, D. A. and Johnson, C. E. (1973) 849-852.

Parish, W. E., Carron-Brown, J. A. and Richard, C. B. (1967) The detection of antibodies to spermatozoa and the blood group antigens in cervical mucus. J. Reprod. Fert. 13: 469-483.

Parish, W. E. and Ward, A. (1968) Studies of cervical mucus and serum from infertile women. J. Obst. Gynecol. Brit. Commonw. 75: 1089-1100.

Petrunia, D. M., Taylor, P. J. and Watson, J. I. (1976) A comparison of methods of screening for sperm antibodies in the serum of women with otherwise unexplained infertility. Fert. Steril. 27: 655-661.

Phadke, A. M. and Padukone, K. (1964) Presence and significance of auto-antibodies against spermatozoa in the blood of men with obstructed vas deferens. J. Reprod. Fert. 7: 163-170.

Ping, W. W. (1979) Sperm antibody activity in human Fallopian tube fluid. Fert. Steril. 32: 676-680.

Poulsen, F. and Hjort, T. (1980) Human sperm membrane antigens. II Isolation and partial characterization of labelled antigens. Abstract, Fourth Int. Congr. Immunol., Paris.

Price, R. J. and Boettcher, B. (1979) The presence of complement in human cervical mucus and its possible relevance to infertility in women with complement dependent sperm-immobilizing antibodies. Fert. Steril. 32: 61-66.

Quinlivan, W. L. (1969) The specific antigens of human seminal plasma. Fert. Steril. 20: 58-66.

Quinlivan, W. L. and Sullivan, H. (1972) The identity and origin of antigens in human semen. Fert. Steril. 23: 873-878.

Rose, N. R., Hjort, T., Rümke, P., Harper, M. J. K. and Vyazov, O. (1976) Techniques for detection of iso- and auto-antibodies to human spermatozoa. Clin. exp. Immunol. 23: 175-199.

Roussel, J. D. and Stallcup, O. T. (1965) Parallelism between semen characteristics and glutamic oxaloacetatic transaminase, glutamic pyruvic transaminase activities. J. Diary Sci. 48: 1506-1510.

Rümke, P. (1954) The presence of sperm antibodies in serum of two patients with oligozoospermia. Vox Sang. 4: 135-140.

Rümke, P. (1959) Auto-antilichamen tegen spermatozoën als oorzaak van onvruchtbaarheid bij de man. Thesis, Univesity of Amsterdam.

Rümke, P. (1968) Sperm-agglutinating autoantibodies in relation to male infertility. Proc. roy. Soc. Med. 61: 275-278.

Rümke, P. The origin of immunoglobulins in semen. Clin. exp. Immunol. 17: 287-297.

Rümke, P. (1978) Autoantibodies against spermatozoa. In: Spermatozoa, Antibodies and Infertility (Cohen, J. and Hendry, W. F. eds.). pp 67-79. Blackwell Scientific Publications, Oxford.

Rümke, P. (1980) Auto- and iso-immune reactions to antigens of the gonads and genital tract. Proc. 4th Int Congr. Immunol, Paris, Academic Press, In press.

Rümke, P., Van Amstel, N., Messer, E. N. and Besemer, D. (1974) Prognosis of fertility of men with sperm agglutinins in the serum. Fert. Steril. 25: 393-398.

Rümke, Ph. and Hellinga, G. (1959) Autoantibodies against spermatozoa in sterile men. Am. J. Clin. Pathol. 32: 357-363

Samuel, T., Kolk, A. H. J., Rümke, P. and Van Lis, J. M. J. (1975). Auto-

immunity to sperm antigens in vasectomized men. Clin. exp. Immunol. 21: 65-74.

Savini, E. (1911) Immunité spermotoxique et fécodation. Comt. rendue Soc. Biol. 71: 22-23.

Schoenfeld, C., Amelar, R. D. and Dubin, L. (1976) Clinical experience with sperm antibody testing. Fert. Steril. 27: 1199-1203.

Schumacher, G. F. B. (1980) Humoral immune factors in the female reproductive tract and their changes during the cycle. In: Immunological Aspects of Infertility and Fertility Regulation (Dhinda, G. and Schumacher, G. F. B. eds.). pp 93-141. Elsevier North Holland, Amsterdam.

Schwimmer, W. B., Ustay, K. A. and Behrman, S. J. (1967a) Sperm-agglutinating antibodies and decreased fertility in prostitutes. Obst. Gynecol. 30: 192-200.

Schwimmer, W. B., Ustay, K. A. and Behrman, S. J. (1967b) An evaluation of immunologic factors of infertility. Fert. Steril. 18: 167-180.

Searcy, R. L., Craig, R. G. and Bergquist, L. M. (1964) Immunochemical properties of normal and pathologic seminal plasma. Fert. Steril. 15: 1-8.

Settlage, D. S. F., Motoshima, M. and Tredway, D. R. (1973) Sperm transport from the external cervical os to the Fallopian tubes in women: A time and quantitative study. Fert. Steril. 24: 655-661.

Shulman, S. (1975) Reproduction and antibody response. CRC Press, Cleveland.

Shulman, S. and Friedman, M. R. (1975) Antibodies to spermatozoa. V. Antibody activity in human cervical mucus. Am. J. Obst. Gynecol. 122: 101-105.

Shulman, S., Jackson. and Stone, M. L. Antibodies to spermatozoa, VI. Comparative studies to sperm agglutinating activity in groups of infertile and fertile women. Am. J. Obst. Gynecol. 123: 139-144.

Sims, J. M. (1866) Clinical Notes on Uterine Surgery. Wood, New York.

Sjövall, H. (1949) An attempt to differentiate between homozygotes and heterozygotes of the ABO-system by examining the spermatozoa. Acta genet. (Basel) 1: 245-251.

Sobbe, A., Haferkamp, O. and Doepfmer, R. (1966) Serologische und immunhistologische Untersuchungen an Sperma und Seren von Männern steriler Ehen. Dtsch. med. Wschr. 91: 1234-1236.

Soffer, Y., Marcus, Z. H., Bukovsky, I. and Caspi, E. (1976) Immunological factors and post coital test in unexplained infertility. Int. J. Fert. 21: 89-95.

Solish, G. I. and Gershowitz, H. (1969) Distribution of ABO blood types among fertile and infertile women. Am. J. human Genet. 21: 23-35.

Solish, G. I., Gershowitz, H. and Behrman, S. J. (1961) Occurrence and Titer of isohemagglutinins in secretions of the human uterine cervix. Proc. Soc. exp. Biol. Med. 108: 645-649.

Southam, A. L. and Buxton, L. (1956) Seventy postcoital tests made during conception cycle. Fert. Steril. 7: 133-140.

Santomauro, A. G., Sciarra, J. J. and Varma, A. O. (1972) A clinical in-

vestigation of the role of the semen analysis and postcoital test in the evaluation of male infertility. Fert. Steril. 23: 245-251.

Stastny, P. (1972) Tissue typing antisera from immunization by pregnancy. Tissue Antigens 2: 123-127.

Stolp, W., Bachner, U., Beck, K. J., Kessler, M. and Schneider, J. (1973) Occurrence of HL-A antibodies in sera of sterile women. Int. J. Fert. 18: 141-144.

Storch, H., Piskazesk, K. and Krishnan, S. (1976) Detection of sperm antibodies: A new method for the detection of immunological problems of infertility. Int. J. Fert. 21: 251-254.

Sung, J. S., Shizuya, H., Black, D. D. and Mumford, D. M. (1977) A radiomicroassay for cytotoxic antibody to human spermatozoa. Quantification by tritiated actinomycin D. Clin. exp. Immunol. 27: 469-477.

Suominen, J. J. O., Multamäki, S. and Djupsund, B. M. (1980) A new method for measurement of cytotoxic antibodies to human spermatozoa. Arch. Androl. 4: 257-264.

Sutherland, J. M. and Landing, B. H. (1961) Failure to demnostrate antibody to sperm in serum of prostitutes. Lancet ii: 56.

Syner, F. N. (1979) Active immunization of female rabbits with purified rabbit acrosin and effect on fertility. Fert. Steril. 32: 468-473.

Tait, B. D., Barrie, J. U., Johnston, I. and Morris, P. J. (1976) Cellular immunity to lymphocyte antigen in human infertility. Fert. Steril. 27: 389-396.

Tauber, P. F., Zaneveld, L. J. D., Propping, D. and Schumacher, G. F. B. (1975) Components of human split ejaculates. I. Spermatozoa, fructose, immunoglobulins, albumin, lactoferrin and other plasma proteins. J. Reprod. Fert. 43: 249-267.

Telang, M., Reyniak, J. V. and Shulman, S. (1978) Antibodies to spermatozoa. VIII. Correlation of sperm antibody activity with postcoital tests in infertile couples.

Tomasi, T. B. (1972) Secretory immunoglobulins. New Engl. J. Med. 287: 500.

Tourville, D. R., Ogra, S. S., Lippes, J. and Tomasi, T. B. (1970) The human female reproductive tract: Immunohistological localization of γA, γG, γM, secretory "piece", and lactoferrin. Am. J. Obst. Gynecol. 108: 1102-1108.

Tung, K. S. K. (1975) Human sperm antigens and antisperm antibodies. I. Studies on vasectomy patients. Clin. exp. Immunol. 20: 93-104.

Tyler, A. (1961) Approaches to the control of fertility based on immunological phenomena. J. Reprod. Fert. 2: 473-506.

Tyler, A., Tyler, E. T. and Denny, P. C. (1967) Concepts and experiments in immunoreproduction. Fert. Steril. 18: 153-166.

Tyler, J. J. P., Richardson, D. W. and Newton, J. R. (1979) The hormonal and immunological status of vasectomized men. Contraception 19: 599-611.

Vaidya, R. A. and Glass, R. H. (1971) Sperm-immobilizing and agglutinating antibodies in the serum of infertile women. Obst. Gynecol. 37: 546-549.

Van Bruggen, E. F. J. and Kremer, J. (1979) Electron microscopy of bovine

and human cervical mucus. Int. J. Fert. 15: 50-57.

Van Leeuwen, A., Goulmy, E., Bradley, B. A. and Van Rood, J. T. (1979) The immunogenicity in women of male cells bearing H-Y and HLA-A2. Tissue Antigens 10: 249.

Van Lis, J. M. J., Wagenaar, J. and Soer, J. R. (1974) Sperm-agglutinating activity in serum of vasectomized men. Andrologia 6: 129-134.

Waldman, R. H., Gruz, J. M. and Rowe, D. S. (1972) Immunoglobulin levels and antibody to Candida albicans in human cervicovaginal secretions. Clin. exp. Immunol. 10: 427-434.

Wall, J. R., Harrison, R. F., Stedronska, J. Lessof, M. H. (1975a) Antibodies against spermatozoa in infetile women with poorly invading spermatozoa on postcoital tests. Am. J. Obst. Gynecol. 121: 198-201.

Wall, J. R., Stedronska, J., David, R. D., Harrison, R. F., Goriup, D. and Lessof, M. H. (1975b) Immunologic studies of male infertility. Fert. Steril. 1035.

Warwas, M., Wendt, V., Shams-Borhan, G. and Dietl, Th. (1979) Anti-acrosin antibodies in sera, colostrum and genital organs of vaccinated ewes: Estimation and correlation with fertility. Andrologia 11: 367-378.

Weil, A. J. (1960) Immunological differentiation of epididymal and seminal spermatozoa of the rabbit. Science 131: 1040-1041.

Weil, A. J., Kotsevalov, O. and Wilson, L. (1956) Antigens of human seminal plasma. Proc. Soc. exp. Biol. Med. 92: 606-610.

Weil, A. J. and Rodenburg, J. M. (1960) Immunological differentiation of human testicular (spermatocele) and seminal spermatozoa. Proc. Soc. exp. Biol. Med. 105: 43-45.

Weil, A. J. and Rodenburg, J. M. (1962) The seminal vesicle as the source of the spermatozoa-coating antigen of seminal plasma. Proc. Soc. exp. Biol. Med. 109: 567-570.

Whitelaw, M. J., Grams, L. and Antone, M. (1962) Blood groups in fertility and sterility. Obst. Gynecol. 20: 317-319.

Wilson, L. (1954) Sperm agglutinins in human semen and blood. Proc. Soc. exp. Biol. Med. 85: 652-655.

Wiener, A. S. and Herman, M. (1939) The second stage of the agglutination reaction. J. Immunol. 36: 255-259.

Witkin, S. S., Higgins, P. J. and Bendich, A. (1978) Inhibition of viral reverse transcriptase and human sperm DNA polymerase by anti-sperm antibodies. Clin. exp. Immunol. 33: 244-251.

Wong, W. P. (1978) Sperm antibodies in the cervical mucus and sera and postcoital tests in infertile women. Europ. J. Obst. Gynecol. Reprod. Biol. 8: 363-367.

Yamakani, K. (1926) The individuality of semen, with reference to its property of inhibiting specifically iso-hemo-agglutination. J. Immunol. 12: 185-189.

Zaneveld, L. J. D. and Polakoski, K. L. (1976) Biochemistry of human spermatozoa. In: Human Semen and Fertility Regulation in Men (Hafez, E. S. E. ed.). pp 167-175. Mosby Cy, St Louis.

Zamboni, L. (1971) Fine morphology of mamalian fertilization. Harper and Row, New York.

CHAPTER 2

THE SPERM-CERVICAL MUCUS CONTACT TEST: A PRELIMINARY REPORT

FERTILITY AND STERILITY
Vol. 27, No. 3, March 1976

Printed in U.S.A.

THE SPERM-CERVICAL MUCUS CONTACT TEST: A PRELIMINARY REPORT

JAN KREMER, M.D., AND SIEMEN JAGER, M.Sc.

Fertility Unit of the Department of Obstetrics and Gynaecology, University Hospital, Groningen, The Netherlands

Infertility in men and women with spermagglutinins is the result of disturbed penetration and migration of spermatozoa in the cervical mucus. In ejaculates with partial spermagglutination caused by autoimmunization, the progressive propulsion of the sperm was changed into stationary, shaking movement the moment the sperm came into contact with cervical mucus. The same alteration in spermatozoal motility pattern also occurred when spermatozoa from a normal, fertile ejaculate came into contact with cervical mucus of a woman whose serum contained sperm antibodies. This shaking phenomenon was visualized in a simple test, the sperm-cervical mucus contact test.

We demonstrated that sensitized spermatozoa exhibit the shaking phenomenon after contact with the glycoprotein fraction of the cervical mucus and not after contact with the aqueous fraction. Therefore, the hypothesis is introduced that the shaking phenomenon is due to an interaction between sensitized spermatozoa and the glycoprotein micelles in cervical mucus.

In 1954 Rümke[1] and Wilson,[2] independently of each other, described the occurrence of spermagglutinating immune antibodies in men whose ejaculates showed spermagglutination. In his report, Wilson[2] drew attention to the fact that there was little spermatozoal penetration and rapid loss of motility in cervical mucus, both in vivo and in vitro, in the two couples described by him.

As a result of sperm penetration tests in vitro, Fjällbrant[3,4] found a negative correlation between the spermagglutinin titer in blood serum and the depth of penetration and duration of motility in cervical mucus. Not entirely in accord with the above findings are the results of an investigation by Ahlgren,[5] who, in postcoital tests, found progressively moving spermatozoa both in the cervical mucus and in the uterine cavity in two of five women whose husbands had blood serum spermagglutinin titers of 1024 and 2048, respectively. However, he did not say whether in these two cases the spermagglutinins were present in the seminal fluid. Presumably, the antibody activity in seminal plasma is more relevant to the fertility of a man than is the activity in the blood serum. However, Rümke et al.[6] found a negative correlation between the chances for pregnancy and the spermagglutinin titer in the blood serum of the husband. From our own investigation it appears that the presence of spermagglutinins in the husband's blood serum strongly coincides with an unfavorable postcoital test.

Received July 11, 1975.

It has been postulated that motile, nonagglutinated spermatozoa in an ejacu-

late with partial autoagglutination lose their motility soon after penetration into cervical mucus, due to the presence of complement in cervical mucus which is not found in seminal plasma.[7] The results of our investigations are not in accord with this hypothesis, since decomplementation of cervical mucus by heating did not change its capacity to inhibit the progressive movement of sensitized spermatozoa. Moreover, our results show that sensitized spermatozoa are not completely immobilized after penetration into cervical mucus. They lose only their progressive motility and retain a locally active motility pattern for several hours. On the basis of results of a new test, the sperm-cervical mucus contact (SCMC) test, we introduce the following hypothesis: *The interaction between the antisperm antibody-coated spermatozoa and the long glycoprotein micelles in cervical mucus is the cause of the subfertility (or infertility) of men with sperm-agglutinins.* This hypothesis can also be used to explain the subfertility of women with spermagglutinins.

MATERIALS AND METHODS

Performance of the Postcoital Test

An investigation was carried out to determine the correlation between the presence of antisperm antibodies in the husbands and the results of the postcoital tests of 19 couples who had had a routine fertility examination. The blood sera of all of the husbands contained spermagglutinins. In this group, postcoital tests of the endocervical mucus had been performed between 2 and 8 hours after intercourse, during the preovulatory phase of the menstrual cycle. The result of the test was considered positive if one or more spermatozoa were found in the endocervical mucus and negative if no spermatozoa were detected. Classification of a positive test was based on the grade of sperm motility; the concentration of spermatozoa in the cervical mucus and the percentage of motile spermatozoa were not considered.

Method for the Separation of Nonagglutinated Spermatozoa in Ejaculates with Partial Autoagglutination

Semen was pretreated by filling a test tube (7 cm, internal diameter 8 mm) to within 0.5 cm of the rim and leaving it upright for 30 minutes at room temperature in order to allow agglutinated spermatozoa to settle to the bottom. Approximately 0.1 ml of the semen was then withdrawn from a level approximately 0.5 cm under the fluid surface, using a disposable tuberculin syringe fitted with a very fine needle. With this method freely swimming spermatozoa and only a few tiny groups of agglutinated spermatozoa were drawn into the sample.

The Sperm-Cervical Mucus Contact Test

The SCMC test was performed by one of the following methods:

Method 1 (Fig. 1A). A drop of preovulatory cervical mucus with signs of good estrogenic stimulation and a pH greater than 7.0 was placed on a glass slide next to a drop of the pretreated semen. Both drops were then thoroughly mixed with the aid of a cover slip and the cover slip was then placed on the mixture. A second drop of the pretreated semen was placed on the same glass slide and also covered with a cover slip. Immediately thereafter the motility of the spermatozoa under both cover slips was compared. The slide was then placed in a moist Petri dish, left standing for half an hour at room temperature, and examined again.

Method 2 (Fig. 1B). A large drop of normal preovulatory cervical mucus was placed on a slide and its surface was spread to a diameter of approximately

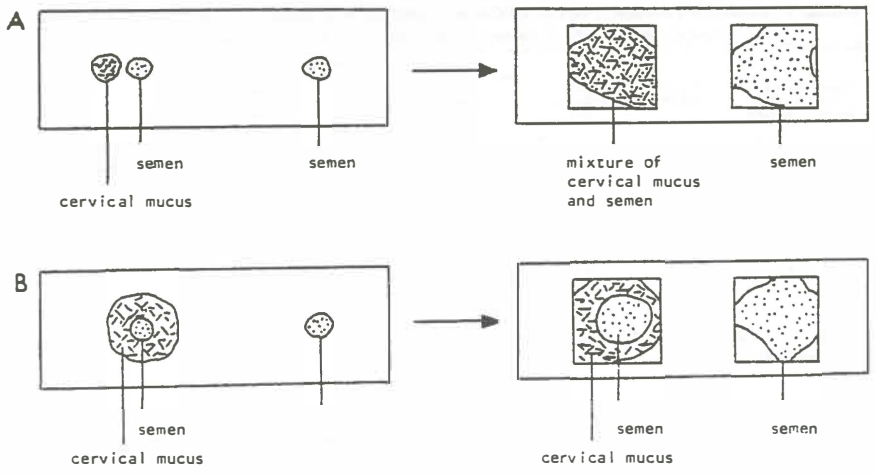


FIG. 1. Preparation of the SCMC test. *A*, Method 1; *B*, method 2.

1 cm. A small drop of pretreated semen was mounted on the center of the mucus. Mucus and semen were covered with a cover slip and this was lightly pressed in such a way that the semen spread in a thin layer over the mucus. A second drop of pretreated semen was placed on the same glass slide and also covered with a cover slip. The examination procedure thereafter was that described for method 1.

Examined in parallel were SCMC tests of: (1) patient sperm and preovulatory cervical mucus of his wife, (2) patient sperm and normal preovulatory cervical mucus, (3) normal sperm and cervical mucus of the patient's wife, and (4) normal sperm and normal preovulatory mucus. Normal spermatozoa with good motility were obtained from volunteers or from fertile donors for artificial insemination. Normal preovulatory cervical mucus was obtained from wives of infertile men visiting the fertility clinic.

Both methods of the SCMC test were suitable for our purposes since the results of each were identical. However, method 1 was more advantageous than method 2 because it could be performed more easily and provided better contact between both kinds of test material; moreover, less cervical mucus was required. Method 2, however, causes no dilution of the semen and therefore may be useful in cases of oligospermia. In addition, method 2 is sometimes easier to perform than method 1 in cases where mixing of semen and mucus proves difficult.

Separation of Cervical Mucus into Two Fractions

Separation of the cervical plasma and the mucous glycoprotein fraction of cervical mucus was achieved by centrifugation for 60 minutes at 100,000 × *g* or for 30 minutes at 200,000 × *g*.

RESULTS

In a group of 19 couples, 52 of the 61 postcoital tests performed did not reveal any progressively moving spermatozoa (Table 1), although all of the men were normospermic (apart from partial sperm-agglutination in their ejaculates) and the

TABLE 1. *Results of Postcoital Tests in 19 Couples[a] with Normospermia, Normal Preovulatory Cervical Mucus, and History of Normal Intravaginal Ejaculatio Durante Coitum*

Spermagglutinin titer in blood serum of the male	No. of couples	No. of postcoital tests	Results of postcoital tests[b]				
			Negative	Positive without motility	Positive with local motility	Positive with sluggish progressive motility	Positive with fair progressive motility
1:8	1	3	3				
1:16	1	3	1			1	1
1:32	1	2		2			
1:64	1	2		2			
1:128	5	22	8	4	6	4	
1:256	5	15	6	6		3	
1:512	5	14	5	6	3		
Total	19	61	23	20	9	8	1

[a]The sera of all of the husbands contained spermagglutinins (Kibrick test).
[b]Classification described under "Materials and Methods."

physicochemical properties of the wives' cervical mucus met the criteria for normal preovulatory cervical mucus. In eight tests, the spermatozoa in the cervical mucus showed poor progression, and in only one case was the progression qualified as fair; in this case the spermagglutinin titer in the blood serum was low. Five husbands had spermagglutinin titers of 512 in their blood sera, and no progressively moving spermatozoa were found postcoitum in the endocervical mucus of their wives.

In six men having a spermagglutinin titer of 128 or more in their blood sera, the interaction between the nonagglutinated spermatozoa in their ejaculates and normal preovulatory cervical mucus was examined with the aid of the SCMC test. In each of these six men, 80 to 100% of the progressively moving spermatozoa in the semen appeared to acquire a local, shaking motility pattern as soon as contact between sperm and cervical mucus had taken place. The spermatozoa exhibited the same intense "pulling" movements that can be observed at the edge of the clumps of agglutinated spermatozoa in an ejaculate. This complete inhibition of progressive movement occurred after contact of each of the semen samples with normal preovulatory cervical mucus of five different women and also with cervical mucus obtained during the estrogenic phase of a cycle of a woman using a sequential oral contraceptive.

The number of spermatozoa whose progressive motility changed into a stationary, shaking pattern varied from 80 to 100%. The rest became immotile or continued to move progressively. The number of shaking spermatozoa increased slightly in some cases during the first 30 minutes of the sperm-cervical mucus contact. After that there was no further increase. The intensity of the shaking movements decreased after a few hours at room temperature; however, in three of the six cases the movements lasted for more than 8 hours.

To determine which fraction of the cervical mucus caused the shaking phenomenon, we separated the glycoprotein fraction from the aqueous fraction by means of ultracentrifugation. We used normal preovulatory cervical mucus from a fertile woman. After mixing semen from a man carrying spermagglutinins with the aqueous fraction of the cervical mucus, the motility pattern of the spermatozoa was unaltered. However, in testing the glycoprotein fraction with both methods 1 and 2 of the SCMC test, the shaking phenomenon was seen when pretreated semen from a man with spermagglutinins was used; it was not seen when nor-

mal semen was used. With normal semen the spermatozoa showed slowly progressive movements with method 1 of the SCMC test (due to the high viscosity of the material) but normal progressive movements with method 2 of the SCMC test.

The shaking phenomenon appeared to be complement-independent, since it occurred in all six cases to the same extent when the cervical mucus was heated to 56° C for 30 minutes prior to testing. Normal spermatozoa remained progressively motile in heated cervical mucus.

When the spermatozoa of these six men were washed by allowing them to migrate through a 3-cm capillary tube filled with 1% bovine albumin in 10% TC Earle's solution, they exhibited the shaking phenomenon after contact with cervical mucus, as they had before the washing procedure.

A strong shaking phenomenon was also observed after normal semen had been incubated for 15 minutes with blood serum containing spermagglutinins in titers of 128 to 512. Furthermore, incubation of normal semen with seminal plasma containing spermagglutinins revealed the shaking phenomenon in the SCMC test.

The shaking phenomenon was also seen in fully developed form in the SCMC test of a couple in which *the husband's semen was normal but the wife's blood serum contained spermagglutinins.* By using the mixed-cell agglutination test[8] and the microagglutination test,[9] spermagglutinins could also be detected in the aqueous fraction of the wife's cervical mucus. The spermagglutinin titer in her blood serum, using the microagglutination test, was 128 (head-to-head) and in the cervical mucus 32 (head-to-head). When mixed with the normal semen of three other men, this otherwise normal preovulatory cervical mucus also caused a shaking phenomenon in fully developed form, with both method 1 and method 2 of the SCMC test.

DISCUSSION

The shaking phenomenon described above can be explained by the following hypothesis: *Progressively moving spermatozoa, coated with antisperm antibodies, probably stick to the network of the long glycoprotein micelles in normal cervical mucus described by Odeblad and Rudolfsson.*[10] The cause of this sticking is not clear, but possibly is due to an alteration of the surface of the spermatozoa by the antibodies. It is unlikely that immobilizing antibodies play a role in the shaking phenomenon, because the phenomenon is complement-independent. If sperm antibodies are already present on the spermatozoa in the semen, then it may be assumed that these spermatozoa stick to the glycoprotein micelles, directly after the first contact (Fig. 2*A*).

If antisperm antibodies are present in the cervical mucus, the spermatozoa will first be sensitized while swimming in the aqueous fraction, between the glycoprotein micelles (Fig. 2*B*). However, we cannot exclude the possibility that the glycoprotein micelles are also coated with antisperm antibodies and that the sper-

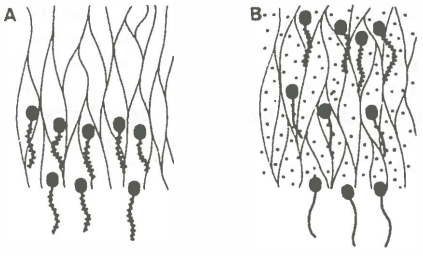


FIG. 2. *A*, Spermatozoa "loaded" with spermagglutinins stick to the glycoprotein filaments as soon as they come into contact with cervical mucus. *B*, Cervical mucus containing spermagglutinins provides the penetrating spermatozoa with the spermagglutinins and afterwards the spermatozoa stick to the glycoprotein filaments.

matozoal shaking phenomenon develops thereby.

For diagnostic purposes the SCMC test is useful in cases of a negative or poor postcoital test while the cervical mucus and seminal characteristics appear normal and coitus is technically normal. In a relatively large number of these cases one will find a positive SCMC test, i.e., an immunologic cause of the infertility.

A second indication for the SCMC test is the presence of partial agglutination in an ejaculate. The SCMC test can differentiate between immunologic and nonimmunologic agglutination; the cervical mucus necessary for the test can be stored frozen.

The ratio between locally shaking spermatozoa, progressively moving spermatozoa, and immobile spermatozoa in the SCMC test is probably relevant to the fertility prognosis.

Spermatozoa coated with antisperm antibodies are probably capable of mediating a conception. We have treated the six couples with agglutinins in the male partner and the couple with sperm antibodies in the female partner by intrauterine inseminations (0.1 to 0.2 ml of semen) for 6 months. In one of these couples in which the male partner had agglutinins (serum titer, 512; seminal plasma titer, 32) and the SCMC test showed only locally shaking and immotile spermatozoa, the wife became pregnant during the first treatment cycle (three inseminations). This couple had been infertile for 8 years and the postcoital test had always been negative or poor.

Acknowledgments. We would like to thank Dr. Ph. Rümke, who kindly read the manuscript and gave valuable comments; Miss Nicolien Boon, who collected the data for Table 1; and Mrs. Van Slochteren-Draaisma, who performed the laboratory work.

REFERENCES

1. Rümke Ph: The presence of sperm antibodies in the serum of two patients. Vox Sang 4:135, 1954
2. Wilson L: Sperm agglutinins in human semen and blood. Proc Soc Exp Biol Med 85:652, 1954
3. Fjällbrant B: Immunoagglutination of sperm in cases of sterility. Acta Obstet Gynecol Scand 44:474, 1965
4. Fjällbrant B: Antibodies affecting fertility in males. In Proceedings of the VII World Congress on Fertility and Sterility, Tokyo and Kyoto, 1971. Amsterdam, Excerpta Medica Foundation, 1973, p 99
5. Ahlgren M: Migration of spermatozoa to the fallopian tubes and the abdominal cavity in women, including some immunological aspects. Lund, Studentlitteratur, 1969
6. Rümke Ph, Van Amstel N, Messer EN, Bezemer PD: Prognosis of fertility of men with sperm-agglutinins in the serum. Fertil Steril 25:393, 1974
7. Rümke Ph: Personal communication
8. Coombs RRA, Rümke Ph, Edwards RG: Immunoglobulin classes reactive with spermatozoa in the serum and seminal plasma of vasectomised and infertile men. In Proceedings of the Second International Symposium, Varna, Bulgaria, 1971, Edited by K Bratanov. Sofia, Bulgarian Academy of Sciences Press
9. Friberg J: Clinical and immunological studies on sperm agglutinating antibodies in serum and seminal fluid. Acta Obstet Gynecol Scand [Suppl] 36:1, 1975
10. Odeblad E, Rudolfsson C: Type of cervical secretions: biophysical characteristics. In The Biology of the Cervix, Edited by RJ Blandau, KS Moghissi. Chicago, University of Chicago Press, 1973, p 267

CHAPTER 3

THE "UNEXPLAINED" POOR POSTCOITAL TEST

Int. J. Fertil., 23(4), 1978, p. 277–281

The "Unexplained" Poor Postcoital Test

J. KREMER, S. JAGER, and TINY VAN SLOCHTEREN-DRAAISMA

Fertility Unit, Department of Obstetrics and Gynecology,
University Hospital, Groningen, The Netherlands

ABSTRACT

Kremer, J., Jager, S., and van Slochteren-Draaisma, Tiny (Fertility Unit, Department of Obstetrics and Gynecology, University Hospital, Groningen, The Netherlands). *The "unexplained" poor postcoital test.*

Int. J. Fertil. **23**: 277–281, 1978.

In 30 of 32 infertile couples with an unexplained negative or bad in vivo and in vitro sperm penetration test, we obtained a strongly positive Sperm Cervical Mucus Contact test (SCMC-test) and demonstrated the presence of antisperm antibodies in the male or female partner. In these 30 couples 25 of the male partners had a sperm-agglutination titre of at least 32 in the serum and at least 4 in the seminal plasma. In the five remaining couples the female partner showed a minimum sperm-agglutination titre of 16 in the serum and a cervical mucus titre of at least 128.

In 48 couples with a fair or good sperm penetration in cervical mucus, in vivo and in vitro, we never found a strongly positive SCMC-test. In 43 of these couples the SCMC-test was negative. Only one man in the latter group had sperm-agglutinating activity in the semen. In a group of 32 couples, with a negative SCMC-test, there was no or only weak sperm-agglutinating activity in the cervical mucus, although 2 women had moderate sperm-agglutinating activity in the blood serum. Based on these data we conclude that the so called "unexplained" poor postcoital test is almost always due to the presence of antisperm antibodies in the semen or in the cervical mucus.

We consider the SCMC-test not only to be a simple and reliable technique for detecting the presence of these antisperm antibodies, but also a method of demonstrating the mechanism by which antisperm antibodies decrease the chance of conception.

INTRODUCTION

There are three conditions necessary for obtaining a good result of the postcoital test (PCT) (Table 1). If, despite supposedly optimal conditions, the result is unfavorable, the generalized term "unexplained poor postcoital test" is often resorted to.

The aim of this paper is to show that the majority of these so called "unexplained" poor postcoital tests has an immunological basis, easily demonstrable by performing a simple test, the Sperm Cervical Mucus Contact test or SCMC-test. This test is based on a new approach to a controversial problem: the correlation between antisperm antibodies and infertility.

In 1976 we published a preliminary report about six infertile couples with antisperm antibodies in the male partner, and one infertile couple with antisperm antibodies in the female. All these couples had a negative or poor PCT, in spite of normospermia—apart from partial autoagglutination—and normal physicochemical properties of the preovulatory cervical mucus.

We found, in each of these seven couples, the same peculiar disturbance in the interaction between spermatozoa and cervical mucus: more than 80% of the progressively moving spermatozoa in the semen sample acquired a local, shaking motility pattern, as soon as contact between sperm and cervical mucus had taken place (6).

We devised a hypothesis to explain this "shaking phenomenon": (Fig. 1)

> "Antibodies against sperm surface antigens have a strong affinity to spermatozoa and to the glycoprotein network in cervical mucus. When spermatozoa, coated with antisperm

Table I. *Conditions necessary to obtain an optimal result of the postcoital test*

1. Ejaculation deeply in the vagina.
2. Normospermia.
3. Clear, thin cervical mucus with good "spinnbarkeit" and pH $>$ 6.5.

antibodies, come into contact with normal cervical mucus, a cross linkage develops between the spermatozoa and the glycoprotein network of the cervical mucus (Fig. 1A). The same cross linkage arises if the glycoprotein network, instead of the spermatozoa, is coated with antisperm antibodies (Fig. 1B). In both situations spermatozoa with potentially good progressive motility, move with local quickly shaking movements without any progression."

MATERIALS AND METHODS

Eighty infertile couples were selected, according to the results of the in vivo and in vitro sperm penetration tests. In each couple the semen had a sperm density of at least 10 million (mln)/ml and a sperm motility of at least grade 2 ($\geq$ moderately progressive). The cervical mucus was clear, thin, with good threadability and pH $\geq$ 6.5. In each couple at least two postcoital tests (PCT) and at least one in vitro sperm penetration test (SPMT) were performed. The PCT was classified as negative when, within 8 hours after intercourse no spermatozoa, and as bad when less than five progressively moving spermatozoa were seen in 10 high power fields (400 $\times$). The in vitro sperm penetration test, performed with the sperm penetration meter (Fig. 2) as described by Kremer (4, 5) and by Kremer and Kroeks (7), was classified as negative, when no sperm penetration occurred; when less than 5 progressively moving spermatozoa had passed the 5-cm mark after 2 hours, the test was classified as bad. When a better result was obtained the in vivo and in vitro sperm penetration tests were classified as fair or good.

The SCMC-test (Fig. 3) was performed at least once in each couple, during the estrogenic phase of a sequential pill cycle, according to the technique described by Kremer and Jager (6). Each SCMC-test was performed in a cross test system (Table II) using the four combinations possible, with husband's and donor's semen, and wife's and donor's cervical mucus. The result of the SCMC-test was only accepted if combination 2 or combination 3 gave about the same percentage of shaking spermatozoa as combination 1 did. This combination of cross testing offers the opportunity for checking the reproducibility, and for tracing the causative factor for a positive result, specifically to husband's semen or wife's cervical mucus.

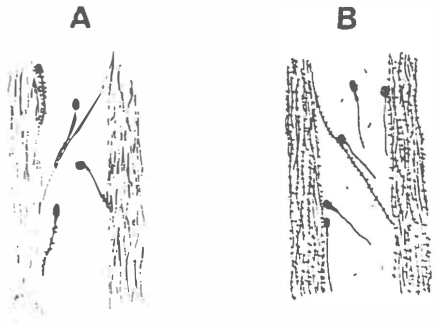


Fig. 1. Interaction between spermatozoa and cervical mucus in the presence of antisperm antibodies.

Combination 4 of the test is a check to be sure that the donor's semen and the donor's cervical mucus are not the source of the factor responsible for the shaking phenomenon.

The results of the SCMC-tests were classified in four groups (Table III). A classification, based on quarterly division, is convenient when approximate estimation is used instead of an exact numeral count. When less than one quarter of the viable, active spermatozoa in a SCMC-test showed locally shaking movements, the test was considered to be negative. The reason is that in 98 out of 100 SCMC-tests, with normal donor semen and donor cervical mucus with normal preovulatory characteristics, the percentage of locally shaking spermatozoa was found to be less than 25. In the two other cases the shaking phenomenon was only slightly more than 25%. For this reason 0–25% shaking phenomenon was considered to be nonspecific.

Auto- and isoimmunization against spermatozoa was examined by means of the so-called Tray Ag-

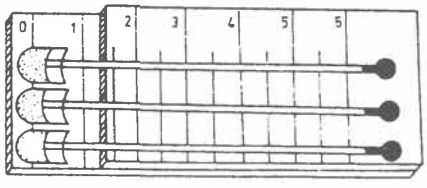


Fig. 2. Sperm penetration meter (SPM).

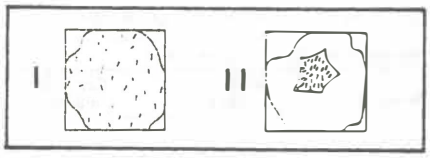


Fig. 3. – The Sperm Cervical Mucus Contact Test (SCMC-test): A. Contact by mixing the two materials (Type I); B. Contact by means of a contact layer (Type II).

glutination Test, a micro-agglutination technique developed by Friberg in 1974. Blood serum and seminal plasma were obtained by normal centrifugation. Ultracentrifugation of cervical mucus, a generally accepted method to obtain the watery fraction, which is a suitable medium for a sperm-agglutination test, was abandoned by us. We found that during the ultracentrifugation process the bulk of the antibodies settled at the bottom of the tube, together with the high viscosity fraction of the cervical mucus. Therefore we now liquefy the cervical mucus by means of incubation with the proteolytic enzyme bromelin. Bromelin is a fruit enzyme, prepared from the pineapple. It belongs to the same group as papain (from the paw-paw) and ficin (from the fig). As far as we know bromelin has never been used for liquefaction of cervical mucus. We found that, under our conditions, liquefaction of cervical mucus with bromelin does not alter the sperm-agglutination titre within 2 hours of incubation.

In this way we could determine the sperm-agglutination titre of the whole mucus sample in a quick and simple manner (Jager, Kremer, Kuiken, and van Slochteren-Draaisma; publication in preparation).

RESULTS

A strong correlation was present between the result of the postcoital test and the in vitro sperm penetration test on the one hand, and the result of the SCMC-test on the other (Table IV). In the group of the 32 couples with an unexplained negative or bad sperm penetration in cervical mucus, the SCMC-test was strongly positive in 30, and moderately positive in 2 couples. In 25 of the 30 couples, with a strongly positive SCMC-test, the cause of the shaking phenomenon was found in the semen and in the 5 other couples in the cervical mucus. In the 48 couples, with at least one fair or good sperm penetration test, none had a strongly positive SCMC-test and in 43 the SCMC-test was negative.

There was also a high correlation between the result of the SCMC-test and signs of antisperm antibody activity in the male or in the female (Table V). In the 25 couples with a strongly positive SCMC-test due to a semen factor, the sperm-agglutination titre in the serum of the husband was always at least 32 and in the seminal plasma always at least 4. In the five couples with a strongly positive SCMC-

Table II. *SCMC-test, performed in a cross testing system*

Combination 1:	Semen husband + cervical mucus wife;
Combination 2:	Semen husband + cervical mucus donor;
Combination 3:	Semen donor + cervical mucus wife;
Combination 4:	Semen donor + cervical mucus donor.

Table III. *Classification of the result of the SCMC-test**

Percentage of locally shaking sperm in the group spermatozoa with signs of good activity	Score
0–25%	–
26–50%	+
51–75%	++
76–100%	+++

* Sperm cervical mucus contact test.

Table IV. *Correlation between the result of the PCT*/SPMT† and the SCMC‡ test (80 infertile couples)*

	Shaking phenomenon in SCMC-test			
Result PCT/SPMT	+++	++	+	neg.
Both tests neg. or bad	30	2	0	0
At least one test fair or good	0	1	4	43

* PCT = Postcoital test; † SPMT = Sperm penetration meter test; ‡ SCMC = Sperm Cervical Mucus Contact Test.

Table V. *Correlation between result SCMC-test and presence of antisperm antibodies*

Result SCMC-test	No. of couples	Sperm-agglutination titre in male or female			
		Serum ♂	Serum ♀	Seminal plasma	Cervical mucus
+++, Due to cause in semen	25	32–4,096(25×)	<16(25×)	4–512(25×)	*
+++, Due to cause in cerv. mucus	5	<4 (5×)	16–128(5×)	<4 (5×)	128–2,048(5×)
Neg.	43	<32(39×) 32–256 (4×)	<16(40×) 16–64 (3×)	<4(42×) 8 (1×)	*
Neg.	32	*	<16(30×) 16–128(2×)	*	≤4(32×)

* Not tested.

test, due to a factor in the cervical mucus, each of the five wives had a sperm-agglutination titre in her serum of at least 16 and in her cervical mucus of at least 128.

In the 43 couples with a negative SCMC-test four men and three women had moderate to high sperm-agglutination titres in the serum but only one man had sperm-agglutinating activity in his seminal plasma. In this group the cervical mucus was not tested but this was done in a comparable group of 32 couples with a negative SCMC-test. Two of these women had a moderate sperm-agglutination titre in the blood serum (16 and 128), but none had sperm-agglutinating activity in the cervical mucus with a titre more than 4.

DISCUSSION

In our series of 30 infertile couples, with a strongly positive SCMC-test, sperm-agglutinating antibodies, with a moderate to high titre were always demonstrable in the serum of the male or the female partner, and in the seminal plasma or in the cervical mucus. A negative SCMC-test almost always means absence or a low titre of sperm-agglutinating activity in seminal plasma and cervical mucus. The correlation between a negative SCMC-test and the absence of sperm-agglutinating activity of the blood serum is less definite. One reason is that sperm-agglutinating activity in sera of women may have a nonimmunological cause (1). Another reason is that immunological sperm-agglutinating activity in blood serum of men may be accompanied by absence of a sperm-agglutination titre in seminal plasma. This is demonstrated in Table VI. However, absence of a sperm-agglutination titre in seminal plasma does not always mean absence of antisperm antbodies on spermatozoa. In five men with a moderate to high sperm-agglutination titre in the blood serum we found no or a low sperm-agglutinating activity in the seminal plasma. However, with a direct mixed antiglobulin reaction (3) we could demonstrate the presence of antisperm antibodies of the IgG

Table VI. *Discrepancy between the level of the sperm-agglutination titre in the serum and the shaking phenomenon in the SCMC-test of five men*

Name	Sperm-agglutination titre		Sperm penetration tests		Result SCMC test
	Blood serum	Seminal plasma	In vivo	In vitro	
St-O	128	<4	Good	Good	Negative
K-G	512	<4	Good	Good	Negative
Fr-Z	256	<4	Fair	Fair	Negative
de J	128	<4	Good	Good	Negative
de R	256	4	Good	Good	Weakly pos.

class on at least 90% of the motile spermatozoa in the ejaculates of each of these five men. Antisperm IgA could not, or only to a low extent, be demonstrated on the spermatozoa.

In all these cases the sperm penetration tests in vivo and in vitro gave a good result. In the SCMC-test there was no or only a weakly positive shaking phenomenon. It is unlikely that these men are infertile due to immobilization of their spermatozoa in the uterine cavity or in the tubes, although in the lumina of these organs complement is present and IgG antibodies can bind complement. However, we found the same duration of sperm motility after the spermatozoa had penetrated human blood serum with and without complement. Moreover, one of these couples achieved a pregnancy without any treatment.

CONCLUSIONS

1. The so-called "unexplained" poor PCT is mostly due to the presence of antisperm antibodies in semen or in cervical mucus.

2. The SCMC-test is a reliable technique for detecting antisperm antibodies in semen or in cervical mucus. It also demonstrates the mechanism by which antisperm antibodies decrease the chance of conception.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Prof. B. Boettcher and Dr. Ph. Rümke for reading the manuscript and for valuable advice. Mrs. R. Mathias was so friendly as to correct the English text.

REFERENCES

1. Boettcher, B. & Kay, D. J.: Fractionation of a human spermagglutinating serum. Nature **223**: 737, 1969.
2. Friberg, J.: Clinical and immunological studies on spermagglutinating antibodies in semen and seminal fluid. Acta Obstet. Gynecol. Scand. Suppl. **36**: entire issue, 1974.
3. Jager, S., Kremer, J., & van Slochteren-Draaisma, T.: A simple method of screening for antisperm antibodies in the human male. Int. J. Fertil. **23**: 1, 12, 1978.
4. Kremer, J.: A simple sperm penetration test. Int. J. Fertil. **10**: 3, 209, 1965.
5. Kremer, J.: The in vitro spermatozoal penetration test in fertility investigations. Thesis. Groningen, The Netherlands. 1968.
6. Kremer, J. & Jager, S.: The Sperm Cervical Mucus Contact test: a preliminary report. Fertil. Steril. **27**: 3, 335–340, 1976.
7. Kremer, J. & Kroeks, M. V. A. M.: Modifications of the in vitro spermatozoal penetration test by means of the sperm penetration meter. Acta Eur. Fertil. **6**: 377–380, 1975.

CHAPTER 4

PRESENCE OF SPERM AGGLUTINATING ANTIBODIES IN INFERTILE MEN AND INHIBITION OF IN VITRO SPERM PENETRATION INTO CERVICAL MUCUS

INTERNATIONAL JOURNAL OF ANDROLOGY
2 (1979) 117–130

Fertility Unit of the Department of Obstetrics and Gynaecology, University Hospital, Groningen, The Netherlands

Presence of Sperm Agglutinating Antibodies in Infertile Men and Inhibition of *in vitro* Sperm Penetration into Cervical Mucus

By

S. Jager, J. Kremer and Tiny van Slochteren-Draaisma

The relation between presence of antispermatozoal antibodies in infertile men and the inhibition of the in vitro sperm penetration into cervical mucus (CM) was studied with the sperm cervical mucus contact (SCMC) test. The tests were performed with semen from infertile men and from semen donors. The CM used permitted good penetration of normal spermatozoa. The so called "shaking phenomenon", the result of a specific interaction of spermatozoa and CM, was expressed in the shaking percentage (S %).
The S % did not change beyond the experimental error within 30 min after mixing semen and CM. The S % was 30 at the most in 194 out of 198 SCMC tests with normal donor semen and normal pre-ovulatory CM. Significant negative correlations ($P < 0.005$) were found between the readings of the sperm penetration meter (SPM) test on one hand and the S %, the sperm agglutination titer in the serum and the sperm agglutination titer in the seminal plasma (SP) on the other hand. Significant positive correlations ($P < 0.005$) were found between the S % and the sperm agglutination titer in the SP. The sperm agglutination titer in serum and in SP correlated significantly better ($P < 0.02$) with the S % in the SCMC test than with the readings of the SPM test.
It was concluded that: 1. A high S % is highly specific for the presence of antispermatozoal antibodies in infertile men, 2. The SCMC test is more suited than the SPM test for studying the effect of antispermatozoal antibodies on the penetration and migration of spermatozoa into CM

Key words: Cervical mucus – infertile men – shaking phenomenon – SCMC test – sperm agglutination – sperm penetration – SPM test.

Received on March 9th, 1979.

Post coital tests often show poor or negative results if in the husband antispermatozoal antibodies are present (Wilson 1954, 1956; Rümke & Hellinga 1959; Fjällbrant 1965, 1968; Kremer & Jager 1976). These poor or negative results can partly be ascribed to auto-agglutination of the spermatozoa in the ejaculate. Obviously, agglutinated spermatozoa are unable to penetrate into cervical mucus (CM). However, the auto-agglutination of ejaculated spermatozoa is almost never complete. In general, freely swimming spermatozoa can be seen between the agglutinates. It has been reported that the normally motile spermatozoa from men with antispermatozoal antibodies penetrate into CM only to a limited extent. Once they have penetrated, these spermatozoa show local movements in spite of previous good motility in the ejaculate (Wilson 1954; Fjällbrant 1965). Spermatozoa from normal men penetrate after a short period into CM and spread rapidly through the entire mucus mass.

The change from progressive motility to local movements after contact with CM, probably, is a phenomenon, typical for the presence of antispermatozoal antibodies. This was supposed by one of us (J.K.) who investigated the semen-CM mixture immediately after an intra-cervical artificial insemination in a couple where the husband had sperm agglutinating antibodies in the serum and in the seminal plasma (SP). The spermatozoa in the mixture showed quickly shaking movements *in loco*. This so called "shaking phenomenon" could also be observed *in vitro*, immediately after mixing semen and CM from the couple. A new test, the sperm cervical mucus contact (SCMC) test was based on this phenomenon. In this test semen and CM are mixed or brought into close contact by means of a thin layer of semen on a layer of CM. Subsequently the percentage of locally shaking spermatozoa is estimated. The SCMC test allows a quantification of this characteristic interaction of spermatozoa and CM if antispermatozoal antibodies are present (Kremer & Jager 1976). It could be shown that the shaking phenomenon offers a good explanation for the poor or negative result of the post coital test if antispermatozoal antibodies are present in the couple under study (Kremer et al. 1978).

The relation between the presence of antispermatozoal antibodies in men and the inhibition of the sperm penetration into CM has also been studied *in vitro* with the glass capillary tube sperm penetration meter (SPM) as described by Kremer (1965). Also with this apparatus Fjällbrant (1968) found a negative correlation between the sperm agglutination titer in the serum from infertile men and the penetration ability of their spermatozoa.

The aim of the present study is to examine the relation between the shaking phenomenon in the SCMC test, the *in vitro* sperm penetration into CM and the presence of sperm agglutinating activity in men. The time dependence of the shaking phenomenon and its specificity wil be discussed and a comparison will be made between the results obtained with the SCMC test and the SPM test. Finally the results of the SCMC test and the SPM test will be correlated with the sperm agglutinating activity in serum and in SP.

Materials and Methods

Samples for testing

Investigated were sera and semen samples from 169 men from infertile couples. These couples were selected because during the fertility investigations the post coital test and the SPM test showed unexpected poor or negative results and/or a positive result was obtained in the mixed antiglobulin reaction (MAR) test for IgG, performed as a routine part of the semen analysis (Jager et al. 1978). SP was obtained from semen samples by centrifugation at 3000 g for at least 10 min.

Donor semen

Normal semen was obtained from young, apparently healthy men, without known fertility problems. The semen was considered normal if it contained per ml at least 6×10^7 spermatozoa with at least 50 % good forward motility and at least 50 % normal morphology.

Cervical mucus (CM)

In order to obtain CM with signs of excellent estrogenic stimulation the women were prescribed a sequential pill cycle (0.100 mg ethinylestradiol from day 2 to day 17 of the menstrual cycle, in combination with 5 mg lynestranol from day 18 to day 22). The test was performed during the second week of the artificial cycle, when generally the amount of mucus produced was sufficient. The couple was asked to abstain from intercourse during at least 7 days before the performance of the SCMC test to avoid presence of spermatozoa in the CM. Presumably normal pre-ovulatory CM was obtained from women visiting the fertility unit. Collection and investigation of the CM was described previously (Jager et al. 1978).

Tray agglutination test (TAT)

The sperm agglutinating activity was determined in serum and in seminal plasma with the TAT according to Friberg (1974) with some modifications (Hellema & Rümke 1976; Jager et al. 1978).

The sperm penetration meter (SPM) test

The *in vitro* sperm penetration into CM was investigated with the SPM introduced by Kremer (1965) and modified by Kremer & Kroeks (1975) and Jager et al. (1978). The SPM test was performed on the same day as the SCMC test. The test was read by estimating the number per low power field (10×10 magnification) and the motility grade of the spermatozoa in the capillary tube

Table 1.
Classification of the readings of the sperm penetration meter (SPM) test.

Migration distance		Migration density at 4.5 cm		Best motility	
Reading (cm)	Classi-fication	Reading (sperm/LPF)*	Classi-fication	Reading (grade)	Classi-fication
< 1	negative	0	negative	immotile	negative
$\geqq 1$ and < 3	poor	> 0 and $\leqq 10$	poor	sluggish	poor
$\geqq 3$ and < 4.5	fair	> 10 and $\leqq 50$	fair	fair	fair
$\geqq 4.5$	good	> 50	good	good	good

* LPF = low power field (10 x 10 magnification).

at distances of 1, 3 and 4.5 cm from the semen reservoir. From these estimations were determined 1. the *migration distance* i. e. the maximum distance from the semen reservoir where spermatozoa were seen, 2. the *migration density* at 4.5 cm i. e. the estimated number of spermatozoa per low power field, 3. the *best motility* i. e. the highest velocity seen at any one of the three distances. Each individual reading was classified as indicated in Table 1.

The sperm cervical mucus contact (SCMC) test

The SCMC test type 1, where semen and CM are thoroughly mixed with each other, was performed as described by Kremer & Jager (1976) and Kremer et al. (1978). Only SCMC type 1 tests were carried out, because the semen-CM mixture shows a much more homogeneous view than the preparation obtained with a SCMC type 2 test. With test type 1 quantification is therefore easier.

The test was read 30 min after mixing of semen and CM. A number of tests was also read directly. Determined by estimation were the percentage of progressively motile spermatozoa (% PM) and the percentage of locally motile spermatozoa with *quickly* shaking movements (% LQ). From these estimations the shaking percentage S % was calculated: $S\,\% = \frac{\%\ LQ}{\%\ PM + \%\ LQ} \times 100$

All percentages were estimated as multiples of 10. A few tests showed motile spermatozoa combining quick shaking movements and sluggish forward motility. This motility variety was considered to belong to the shaking phenomenon. Independent simultaneous estimations by different persons almost always showed S % values differing one multiple of 10 at the most.

The SCMC test was performed in the four combinations possible with husband semen and donor semen and CM from the wife and presumably normal pre-ovulatory CM. From the two test combinations of husband semen and CM from the wife and presumably normal pre-ovulatory CM the lowest S % value was chosen in order to eliminate inhibitory influences of the CM. For determination of the specificity of the S % the results with normal pre-ovulatory CM and donor semen are presented.

The results of the SCMC test were included in this study only if: the semen contained per ml at least 10^7 spermatozoa with at least 20 % fair or good motility and in the SCMC test at least 10 % of the spermatozoa was progressively motile or quickly shaking.

Statistical analysis

Results were compared after calculation of correlation coefficients. Significance of differences between correlation coefficients was determined after performance of z-transformation.

Table 2.
Time dependence of the shaking percentage (S %) in 38 SCMC tests with semen from infertile men and 24 SCMC tests with semen from normal donors.
Difference in S % determined directly ($S\%_{00}$) and after 30 min ($S\%_{030}$).

S % estimated directly	Number of tests (*)	$S\%_{030} - S\%_{00}$			
		− 10	0	+ 10	+ 20
≧ 90	10	0	10	0	0
80	2	0	1	1	0
70	1	0	1	0	0
60	1	1	0	0	0
50	1	0	0	1	0
40	0	0	0	0	0
30	4 (2)	0	3	0	1
20	8 (3)	1	5	2	0
10	7 (3)	0	4	3	0
0	28 (16)	0	28	0	0

(*) = result with donor semen.

Results

Time dependence of the S % (Table 2)

In SCMC tests with semen from 38 infertile men and 24 SCMC tests with semen from nine donors the S % was obtained directly after mixing the semen and the CM. After 30 min the S % was determined again. The difference of the S % between the first and the second reading was 20 in one test with semen from an infertile man and 10 in nine tests with semen from seven infertile men and two semen donors. In the other tests no difference was found.

Comparison of the S % in the SCMC test with the results of the SPM test (Table 3)

The S % could be determined with the semen from 124 of the 169 infertile men. In the other 45 infertile men the concentration or the motility of the spermatozoa in the semen was insufficient for a reliable result of the SCMC test. With semen from 121 men results of both the SCMC test and the SPM

Table 3.
Comparison of the shaking percentage (S %) in the sperm cervical mucus contact (SCMC) test and the results of the sperm penetration meter (SPM) test obtained with semen from 121 infertile men.

Shaking percentage (*)	Number of men	Readings of the sperm penetration meter test §											
		Migration distance				Migration density at 4.5 cm				Best motility			
		n	p	f	g	n	p	f	g	n	p	f	g
≧ 80 (3)	31	3	6	7	15	16	11	4		4	22	2	3
60–70 (2)	7			1	6	1	4	1	1		3	2	2
30–50 (1)	14				14		4	8	2		4	4	6
≦ 20 (0)	69		1	4	64	5	9	22	33	1	5	19	44
Correlation coefficient		− 0.48 ($P < 0.005$)				− 0.62 ($P < 0.005$)				− 0.62 ($P < 0.005$)			

† Log_2 values used for calculation of correlation coefficients, for titer < 4 chosen value of 1.

§ SPM (see Table 1): n = negative (0), p = poor (1), f = fair (2), g = good (3); values in parenthesis used for calculation of correlation coefficients.

* S %: for calculation of correlation coefficients chosen values of: 0 (≦ 20), 1 (30–50), 2 (60–70) and 3 (≧ 80).

test were obtained in 73 cases with CM from the wife and in 48 cases with donor CM. With semen from three men no SPM test was performed for practical reasons.

Significant negative correlations were found for the S % compared with the readings of the SPM test. The correlation coefficient with the migration distance was -0.48 ($P < 0.005$). With the migration density at 4.5 cm and with the best motility a correlation coefficient of -0.62 ($P < 0.005$) was found.

The S % obtained with donor semen and donor CM (Table 4)

In 198 SCMC tests with normal donor semen and presumably normal pre-ovulatory CM the S % was found to be 0 in 82 % of the cases. In 98 % of the test the S % was 30 at the most. On three occasions a high S % of 90, 80 and 70 respectively was obtained. In two cases the cause of the shaking phenomenon could be located in the donor CM because with other semen samples a high S % was also found. One of these two mucus samples could be tested in the TAT. The agglutination titer was < 4. In the third case the origin of the high S % could not be traced.

Comparison of the sperm agglutination titers in serum and in seminal plasma with the S % in the SCMC test and the results of the SPM test

The TAT was performed on both the serum and the SP from 134 infertile men (Table 5). The titers varied from < 4 to 16384 in the serum and from

Table 4.
Estimation of the shaking percentage (S %) in 198 SCMC tests with semen from 45 normal donors and presumably normal pre-ovulatory cervical mucus.

S %	Number of tests	percentage
> 90	0	0
90	1	0.5
80	1	0.5
70	1	0.5
60	0	0
50	0	0
40	1	0.5
30	8	4.0
20	14	7.1
10	4	2.0
0 < 10	6	3.0
0	162	81.8

Table 5.
Comparison of the sperm agglutination titers* in serum and in seminal plasma from 134 infertile men.

Sperm agglutination titer in seminal plasma	Number of men with serum sperm agglutination titers of:													
	< 4	4	8	16	32	64	128	256	512	1024	2048	4096	8192	16384
512										1				
256										2		1		
128						1		1	1	1	1	1		
64								2	1	1				
32	1					1	1	3	4	1				
16					1	3	4	3	2		2			1
8					2	1	4	1	1					
4					2	1		3	1					
< 4	44	4	11	2	3	6	4	2	1					

* determined with the tray agglutination test (TAT) (Friberg 1974).

Table 6.

The sperm agglutination titers in serum from 95 infertile men compared with the results of the sperm penetration meter (SPM) test and the shaking percentage (S %) in the sperm cervical mucus contact (SCMC) test.

Spermatozoa agglutination titer (log_2) †		Number of men	Readings of the sperm penetration meter test §												(S %) in the SCMC test*			
			Migration distance				Migration density at 4.5 cm				Best motility							
			n	p	f	g	n	p	f	g	n	p	f	g	≦ 20	30–50	60–70	≧ 80
4096	(12)	1		1			1					1						1
2048	(11)	0																
1024	(10)	4	1			3	1	3			1	3						4
512	(9)	4		2		2	2	1	1		1	1	2		1			3
256	(8)	12	1	1	3	7	5	1	4	2	1	6		5	3		2	7
128	(7)	12	1	2	2	7	6	4	2		1	6	2	3	2		1	9
64	(6)	10		1	1	8	2	5	1	2		7	2	1	1	1	2	6
32	(5)	6		1		5	1	1	2	2		2	3	1	2	2	1	1
16	(4)	2				2		1	1			1		1		2		
8	(3)	9				9			2	7			3	6	7	2		
4	(2)	2				2			2				1	1	1	1		
< 4	(1)	33			3	30	3	8	10	12		6	8	19	26	6	1	
correlation coefficient				– 0.41 ($P < 0.005$)				– 0.45 ($P < 0.005$)				– 0.49 ($P < 0.005$)				0.73 ($P < 0.005$)		

For legend see Table 3.

Table 7.

The sperm agglutination titers in seminal plasma from 95 infertile men compared with the results of the sperm penetration meter (SPM) test and the shaking percentage (S %) in the sperm cervical mucus contact (SCMC) test.

Spermatozoa agglutination titer (log_2) †		Number of men	Readings of the sperm penetration meter test §												(S %) in the SCMC test*			
			Migration distance				Migration density at 4.5 cm				Best motility							
			n	p	f	g	n	p	f	g	n	p	f	g	≦ 20	30–50	60–70	≧ 80
512	(9)	1				1		1				1						1
256	(8)	1				1		1				1						1
128	(7)	3	1	1		1	2	1			1	2					1	2
64	(6)	4		1	1	2	2	1	1			4						4
32	(5)	6	1	2	1	2	4	1	1		2	2	1	1			1	5
16	(4)	7		2	2	3	5		1	1		4	1	2	1		1	5
8	(3)	9		1	1	7	2	4	2	1		5	2	2		2	1	6
4	(2)	5	1	1	1	2	3	1	1		1	2	2		1		2	2
< 4	(1)	59			3	56	3	15	19	22		11	15	33	41	12	1	5
correlation coefficient				− 0.40 ($P < 0.005$)				− 0.50 ($P < 0.005$)				− 0.52 ($P < 0.005$)					0.73 ($P < 0.005$)	

For legend see Table 3.

< 4 to 512 in the SP. In only two cases was the titer in the SP greater than in the serum.

The results of the TAT could be compared with the results of the SPM test and the S % values of the SCMC test in 95 infertile men (Tables 6 & 7). For calculation of the correlation coefficients $\log_2$ values of the titers were used. For titers < 4 the $\log_2$ value was assumed to be 1. The results of the SPM test and the estimations of the S % have been expressed in values from 0 to 3. Significant negative correlations were found between the agglutination titers and the results of the SPM test. Significant positive correlations were obtained between the sperm agglutination titers and the S % values. The sperm agglutination titers correlated significantly better ($P < 0.02$) with the results of the SCMC test than with the results of the SPM test.

Discussion

In our preliminary paper on the SCMC test we stated that the number of shaking spermatozoa increased slightly in some cases during the first 30 min of the sperm-CM contact (Kremer & Jager 1976). Now we have tested a larger series it appears that the S % does not change beyond the experimental error of one multiple of ten in the first 30 min (Table 2).

The SCMC test is a new type of test in which the interaction of spermatozoa and CM can be studied *in vitro*. The earliest test of this type is the slide test introduced by Miller & Kurzrok (1932). In this test mainly the passage of spermatozoa through the semen-mucus border and the ability of the spermatozoa to swim in the CM can be studied. In tests of this type it is difficult to obtain reproducible quantitative data about number and motility of the penetrated spermatozoa. Therefore Kremer (1965) introduced a glass capillary tube test, the SPM test. In this test the spermatozoa are observed after the passage of the semen-mucus border. However, the number and the motility of the spermatozoa in the glass capillary tube will depend on the concentration and the motility of the spermatozoa in the semen. In our opinion the S % in the SCMC test is less dependent on the semen qualities than the results of the SPM test, because the S % is based on the ratio of motile and quickly shaking spermatozoa. This explains why the correlations between the results of the SPM test and the SCMC test were not strong (Table 3). Good or fair results of the SPM test were found together with S % values of 80 and more and poor or negative results of the SPM test were seen with S % values of 20 at the most. This is due to the fact that a good result in the reading of the migration distance and the best motility can already be obtained with a few spermatozoa. The migration density at 4.5 cm can be positively influenced by a high concentration of spermatozoa with good motility in the semen. On the other hand

a low concentration of the spermatozoa and a less good motility may affect the reading of the SPM in an unfavourable sense.

Locally shaking spermatozoa are mostly absent in mixtures of normal semen and presumably normal pre-ovulatory CM although S % values of 30 are sometimes found (Table 4). We, therefore, consider a S % value up to 30 (98 % of the cases) as normal. Previously, we considered a S % up to 25 % as normal (Kremer et al. 1978). This conclusion was based upon 100 tests included in the present study. The difference is within the experimental error. Occasionally we found a high S % without evidence of antispermatozoal antibodies. The cause of the shaking phenomenon in these cases is unknown. Perhaps parts of the CM possessed a high consistency which was not noticed in the examination of the mucus prior to the performance of the SCMC test. CM can be heterogeneous (Odeblad 1966; Rudolfsson 1971) and spermatozoa might be "trapped" in the small parts with a high consistency. It must be emphasized that for a good interpretation of the SCMC test CM with signs of excellent estrogenic stimulation and a pH above 6.5 is required.

Presence of antispermatozoal antibodies could be demonstrated in the serum from all 31 infertile men with a S % of at least 80 (Tables 6 & 7). It can be concluded that a high S % is highly specific for the presence of antispermatozoal antibodies in men. The reverse, however, is not always true. The S % can be low even with sperm agglutination titers in the serum from 32 to 512 (Table 6). The presence of antispermatozoal antibodies on the spermatozoa was proven in these cases with the aid of the MAR test for IgG (Jager et al. 1978). However, the S % is rarely low when sperm agglutinating activity is present in the SP (Table 7). It is likely that in the SP the sperm agglutinating activity is caused by antispermatozoal IgA whereas in the serum the sperm agglutinating activity is mostly due to antispermatozoal IgG (Husted & Hjort 1975).

The finding of significant negative correlations between the sperm agglutination titers and the results of the SPM test is in agreement with the results of Fjällbrant (1968) who found a value of -0.55 for the correlation coefficient between the sperm agglutination titer in the serum and the migration distance against a value of -0.41 in this study. Compared to the results of the SPM test significantly higher values for the correlation coefficients were found for the S % in the SCMC test and the sperm agglutination titers (Tables 6 & 7). In our opinion this difference is due to the fact that the results of the SPM test are more influenced by concentration and motility of the spermatozoa in the semen than the results of the SCMC test.

Presence of sperm agglutinating antibodies in men apparently seldom completely inhibits the *in vitro* sperm penetration and migration into CM. With serum titers up to 4096 and SP titers up to 512, spermatozoa were seen in the glass capillary tube in almost all SPM tests (Tables 6 & 7). Good and fair

results were obtained despite high S % values (Table 4). Probably, in the semen from most men in our series spermatozoa were present which combined relatively good motility with a low degree of sensitization with antibody molecules.

The relation between the sperm agglutination titers in the serum and in the SP in our series (Table 5) is in agreement with the results of Rümke (1974), Friberg (1974), and Husted & Hjort (1975). These investigators also found titers in the SP to be in general lower than the titers in the serum. We found serum titers up to 512 in men with SP titers lower than 4. This indicates the absence of a direct relation between circulating antispermatozoal antibodies and inhibition of the sperm penetration and migration into CM. The inhibition will be more directly related to antibodies in the semen. However, the correlation coefficient between the sperm agglutination titers and the S % values in the SCMC test is not higher with SP than with serum (Tables 6 & 7). This is due to the fact that S % values of a least 80 were found with titers of less than 4 in the SP. It must be realized that the antibody activity in the SP is only a residual one after antibody absorption by the spermatozoa. For comparison we found a decrease of one titer step (from 128 to 64) by addition of 5×10^7 washed donor spermatozoa to one ml serum (unpublished results). It is likely that the factor more directly related to inhibition of sperm penetration and migration is the presence of antibody molecules on the spermatozoa.

Although a good correlation coefficient was found between the serum sperm agglutination titers and the S % values, in nine men with titers from 32 to 512 the S % values were $\leqq 20$ (Table 6). In these cases the agglutination titers in the SP were also low. This indicates low or non existent production of local antibodies in these men. In a retrospective study Rümke et al. (1974) demonstrated the existence of a reverse relationship of fertility and presence of sperm agglutination titers in the serum. However, it appeared that occasionally men with titers up to 512 could become father. Possibly, the spermatozoa from these men did not have penetration inhibition into CM, due to the absence of local antibody production.

Acknowledgements

The authors are greatly indebted to Dr. Ph. Rümke for his continuous advice and criticism. We thank Professor Ph. Hoedemaeker for commenting on the manuscript. Dr. V. Fidler for statistical advice and Dr. F. von der Walde for revision of the English text.

References

Fjällbrant B. (1965) Immunoagglutination of sperm in cases of sterility. *Acta obstet. gynec. scand. 44.* 474.

Fjällbrant B. (1968) Interrelation between high levels of sperm antibodies. reduced penetration of cervical mucus by spermatozoa, and sterility in men. *Acta obstet. gynec. scand. 47.* 102.

Friberg J. (1974) A simple and sensitive micro-method for demonstration of sperm-agglutinating activity in serum from infertile men and women. *Acta obstet. gynec. scand. Suppl. 36.* 21.

Hellema H. W. J. & P. Rümke (1976) Comparison of the tray agglutination technique with the gelatin agglutination technique for the detection of spermagglutinating activity in human sera. *Fertil. Seril. 27.* 284.

Husted S. & T. Hjort (1975) Sperm antibodies in serum and seminal plasma. *Int. J. Fertil. 20.* 97.

Jager S.. J. Kremer & T. van Slochteren-Draaisma (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. Fertil 23.* 12.

Kremer J. (1965) A simple sperm penetration test. *Int. J. Fertil 10.* 209.

Kremer J. & S. Jager (1976) The sperm cervical mucus contact test: a preliminary report. *Fertil. Steril. 27.* 335.

Kremer J.. S. Jager & T. van Slochteren-Draaisma (1978) The "unexplained" poor postcoital test. *Int. J. Fertil. 23.* 277.

Kremer J. & M. V. A. M. Krocks (1975) Modifications of the *in vitro* spermatozoal penetration test by means of the sperm penetration meter. *Acta Eur. Fertil. 6.* 377.

Miller E. G. & R. Kurzrok (1932) Biochemical studies of human semen III. Factors affecting migration of sperm through the cervix. *Amer. J. Obstet. Gynec. 24.* 19.

Odeblad E. (1966) Micro-NMR in high permanent fields. *Acta obstet. gynec. scand. 45.* 2.

Rudolfsson G. (1971) Nuclear magnetic resonace and cytometric studies on mucus from single cervical glands. *Int. J. Fertil. 16.* 147.

Rümke P. (1974) Autoantibodies against spermatozoa in infertile men. *J. Reprod. Fertil. Suppl. 21.* 169.

Rümke P. & G. Hellinga (1959) Autoantibodies against spermatozoa in sterile men. *Amer. J. clin. Path. 32.* 357.

Rümke P.. N. van Amstel. E. N. Messer & P. D. Bezemer (1974) Prognosis of fertility of men with spermagglutinins in the serum. *Fertil. Steril. 25.* . 393

Wilson L. (1954) Sperm agglutinins in human semen and blood. *Proc. Soc. exp. Biol.. N.Y. 85.* 652.

Wilson L. (1965) Sperm agglutination due to autoantibodies. A new cause of sterility. *Fertil. Steril. 7.* 262.

Author's address:

S. Jager. Fertility Unit of the Department of Obstetrics and Gynaecology. University Hospital. Groningen. The Netherlands.

CHAPTER 5

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

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

S. JAGER, J. KREMER and TINY VAN SLOCHTEREN-DRAAISMA

Fertility Unit of the Department of Obstetrics and Gynecology, University Hospital, Groningen, the Netherlands

ABSTRACT

Jager, S., Kremer, J., and van Slochteren-Draaisma, Tiny. (Fertility Unit of the Department of Obstetrics and Gynecology, University Hospital, Groningen, the Netherlands). *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 23: 12–21, 1978.

A simple and rapid test for the detection of antisperm antibodies of the IgG class on freely swimming spermatozoa in fresh human semen is described. The test is based on the formation of motile mixed agglutinates between erythrocytes sensitized with incomplete anti-Rh-antibodies and freely swimming spermatozoa with surface antisperm antibodies, after mixing both cell types together with anti-IgG antiserum. Agglutination of the red blood cells serves as an internal control. The test can be applied on ejaculates with spermatozoa concentrations down to one million per ml, provided the motility is sufficient.

The percentage of motile spermatozoa found to be coated with antisperm antibodies of the IgG class, and the extent of the coating, proved to be correlated with the agglutination titer of circulating antisperm antibodies and with the inhibition of sperm penetration into cervical mucus.

The test can be used as a screening for the presence of antisperm autoantibodies in serum and semen.

INTRODUCTION

Sperm agglutinating autoantibodies in men can be determined semiquantitatively by assaying the titer in the blood serum. Although this value correlates with infertility, pregnancies in spite of high sperm agglutination titers in the serum of the husband have been reported. The impairment of the fertility might therefore be more related to the amount of antisperm antibodies in the semen than in the serum (1). Though a correlation between sperm agglutination titers in the serum and in seminal plasma (SP) exists (2, 3, 4), sperm agglutination may be slight in the ejaculates from infertile men with serum antisperm antibodies. On the other hand, in ejaculates from fertile men, aggregates similar to the agglutination present in the semen from infertile men with antisperm antibodies can be seen.

Many freely swimming spermatozoa can often be seen between the agglutinates of spermatozoa in semen from infertile men with autoagglutination. This indicates that sperm agglutination is not the only cause of the infertility. The presence of agglutinating antisperm antibodies in the serum correlates with reduced penetration of spermatozoa into cervical mucus (CM) (5, 6). This lack of penetration ability is due to the fact that the freely swimming spermatozoa of an ejaculate with autoagglutination change their progressive motility into local shaking movements after contact with CM, probably because the spermatozoa become attached to the glycoprotein micelles of the CM, due to the presence of antibody molecules on these spermatozoa

(7). The aim of this study is to examine whether these freely swimming spermatozoa are coated with immunoglobulins.

Presence of antibodies on untreated spermatozoa could be demonstrated by direct immunofluorescence in only a few patients with sperm agglutinating activity in the serum (4). Therefore the mixed antiglobulin reaction (MAR) (8) was chosen. With this reaction the presence of surface antibodies is demonstrated by coupling a different type of cell to spermatozoa. The reaction has been applied as an indirect test for the detection of antisperm antibodies present in serum (9, 10, 11) and in SP (11). The reaction has been previously reported as a direct test on fresh semen from only one patient, who appeared to have IgG molecules on his spermatozoa (11).

We will describe here the use of a direct MAR as a simple and rapid test for the detection of IgG surface antibodies on spermatozoa, that can be applied directly on fresh semen, and that can be incorporated into the routine semen analysis as a screening for the presence of antisperm antibodies. It will be shown that a strong correlation exists between the presence of IgG on spermatozoa and the agglutination titer in the serum. There is also a correlation between the presence of IgG on spermatozoa and the inhibition of sperm penetration into CM and, therefore, with infertility. This correlation, however, is weaker than the former one.

MATERIAL AND METHODS

Patient Groups

The MAR test was carried out on ejaculates from three groups of husbands from infertile couples:

Group I. Unselected patients. All men, whose ejaculates were received for a routine semen analysis during the period from August 5, 1975 to October 31, 1975. There were 253 patients of whom 158 presented one ejaculate, 74 brought two ejaculates, 20 brought three ejaculates and one patient brought four ejaculates.

Group II. Thirty-four patients with sperm agglutinating activity in the serum, including 16 patients from group I.

Group III. Nineteen patients without sperm agglutinating activity in the serum (titer < 4), including four patients from group I.

Semen Analysis

Patients were asked to abstain from sexual intercourse for 3 to 5 days immediately prior to semen collection. The semen was collected at home or in the clinic in a dry plastic jar and was received at the laboratory within 2 hours after ejaculation. Soon thereafter the semen was thoroughly mixed and the motility of the spermatozoa was estimated in a drop of undiluted semen. Presence of agglutination was noted. Aggregation around cells or in gelatinous parts was considered as pseudoagglutination.

Examination of Cervical Mucus (CM)

After removal of the mucus at the external os, the pH of the endocervix was measured in situ with the aid of a thin glass electrode (12). The CM was then sucked up with a disposable plastic tuberculin syringe (Gilette scimitar). Examination of CM included determination of volume, aspect, consistency, and ferning.

Sperm Penetration Meter (SPM) Test

The in vitro penetration test was performed with the aid of the SPM as described by Kremer (13) with some minor modifications. Cervical mucus of the woman under test was sucked up in a flat capillary tube (Vitro Dynamics, Rockaway, New Jersey, USA) with an internal optical path length of 0.3 mm. For comparison donor CM was used in an identical capillary tube. After sealing one end of the capillary tube with modelling clay, spermatozoa were allowed to penetrate into the tubes from the reservoir at 37°C. Presence of spermatozoa and their motility was noted at distances of 1, 3 and 5 cm from the reservoir after 2 hours.

Sperm Cervical Mucus Contact (SCMC) Test

This test was carried out according to Kremer and Jager (7). Semen was pretreated by leaving it for half an hour in a narrow test tube at room temperature in order to allow the agglutinated spermatozoa to settle out. The semen to be used was drawn from about half a cm below the fluid surface. On a microslide a drop of the CM was then mixed with a drop of the pretreated semen. A drop of the pretreated semen on the same microslide served as a comparison of the motility pattern. In parallel were performed SCMC tests of: semen of the husband and CM of his wife; husband semen and normal preovulatory CM; normal donor semen and CM of the wife; and normal donor semen and normal preovulatory CM. For practical reasons the CM of the

female partner of the couple was obtained during the estrogenic phase of a sequential pill cycle. Normal preovulatory CM was obtained from women attending the fertility unit. Normal donor sperm with good motility was obtained from volunteers with unknown fertility. After 30 minutes the percentages of progressively motile, locally motile and immotile spermatozoa were estimated.

Tray Agglutination Test (TAT)

This test was performed as described by Friberg (14), with some minor modifications (15). Onto donor semen, kept in a narrow test tube, was pipetted about 1 ml of Earls solution, pH 7.4, containing 1% bovine serum albumin. After 1 hour the layer just above the semen was pipetted off (16). This layer contained a high percentage of spermatozoa with a good motility. The spermatozoa concentration was adjusted to about 40 million per ml by dilution with the Earls solution. Seminal plasma, obtained by centrifugation of semen, or serum to be tested was diluted twofold serially on Cooke microtiter plates (Dynatech Companies) with V-shaped wells by means of 0.025 ml diluters, with a starting dilution of 1:4. Five microliters of each diluted sample were then transferred, with the aid of a micro-syringe with a disposable tip, to the disposable test trays (Møller-Coates AS, Moss, Norway) under liquid paraffin oil. To each 5 microliters of diluted sample 1 microliter of the donor spermatozoa suspension was added. The test was read after incubation at 37°C for 2 hours. A positive and a negative control were always included. Each serum or SP was tested with spermatozoa from at least two donors.

Mixed Antiglobulin Reaction (MAR) Test

I. Preparation of the indicator cells. This was carried out as described by Chalmers et al. (17). Group-O, Rh-positive red blood cells of the R_1R_2 type were washed three times with phosphate buffered saline (PBS), pH 7.5. Five volumes of this suspension were mixed with one volume of a strong incomplete anti-D serum (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands), for 30 minutes at 37°C. The cells were then washed three times with PBS and resuspended in the PBS to an hematocrit of about 5–10%. The cell suspension was prepared for us by the Blood Grouping Laboratory of the University Hospital of Groningen.

II. Performance of the test. Onto a microslide were pipetted separately but close to each other: 1. a drop of fresh semen, 2. a drop of undiluted monospecific anti-IgG antiserum (Behring ORCM), and 3. a drop of the sensitized R_1R_2 erythrocyte suspension. The three drops were thoroughly mixed and the mixture covered by a coverslip. The reaction was then studied at once under the microscope. Generally the motility of the spermatozoa ceased after 15 to 30 minutes, probably because of a detrimental factor present in the IgG antiserum. In general this gave no problems as the test could be read within this period.

In positive MAR tests we noticed that the greater the percentage of motile spermatozoa participating in mixed agglutinates, the greater the number of erythrocytes participating in mixed agglutinates with the individual spermatozoa (Table III).

In strongly positive reactions directly after mixing the three drops, some motile spermatozoa with an adhering erythrocyte could already be seen. Subsequently erythrocytes adhered to more spermatozoa and the number of erythrocytes per spermatozoon increased. The progressive motility of these mixed agglutinates changed then into local "snaky" movements. The mixed aggregates then grew larger because of the attachment of more erythrocytes until all free red blood cells had been taken up into agglutinates and no, or almost no, freely swimming spermatozoa could be seen anymore, although their presence was indicated by the local movements of the mixed agglutinates. If some freely swimming spermatozoa remained, they were covered with small particles of unknown identity.

In addition to the mixed agglutination and agglutination of erythrocytes alone, an increase of the sperm agglutination could be observed in ejaculates with a sufficiently high concentration of freely swimming spermatozoa. These newly formed agglutinates had a looser appearance than the already existing ones. These agglutinates also became covered with red blood cells in the direct MAR. Increase of sperm agglutination also occurred if only anti-IgG antiserum was added to the semen. If the erythrocytes were used unsensitized, then mixed agglutinates were also observed. This effect is probably due to the absorption of IgG from the SP onto the erythrocytes. After washing the spermatozoa three times in PBS, mixed agglutinates were formed with sensitized red blood cells only.

III. Classification of the results. No interpretation of the test was given unless agglutination of the red blood cells and the presence of motile spermatozoa were observed. If the red blood cells did not agglutinate, then the amount of anti-IgG was increased. In cases of low sperm concentrations a higher concentration of red blood cells was applied in order to increase the chance of the formation of mixed agglutinates.

The reaction was considered to be negative if no motile mixed agglutinates were seen, freely swim-

ming spermatozoa could be observed, and the erythrocytes showed good agglutination.

The reaction was considered to be positive if motile mixed agglutinates were observed. The results were noted as the precentages of the motile spermatozoa incorporated into mixed agglutinates, and according to the site of attachment of the red blood cells to the spermatozoa. The accuracy of the estimation is probably not better than 10% at intermediate values: with 10% and less, or with 90% and more of the progressively moving spermatozoa bound to erythrocytes, the estimations, are in our experience, more accurate. The results are less accurate if the reaction is applied to ejaculates with only sluggishly moving spermatozoa or to ejaculates with only few progressively motile spermatozoa.

Three types of positive reactions were distinguished:

1. Doubtfully positive, if in a test only occasionally a mixed agglutinate, involving a motile spermatozoon with an adhering, red blood cell was seen;

2. Positive, with the percentage of spermatozoa involved in mixed agglutinates expressed in multiples of ten, ranging from <10% (but more than doubtfully positive) to >90% (but less than almost 100%) of the motile spermatozoa attached to erythrocytes:

3. Strongly positive, if 100% or almost 100% of the motile spermatozoa were incorporated into mixed agglutinates.

The types of attachment of red blood cells to the spermatozoa distinguished were: 1. to the end part of the tail (type e); 2. to the main part of the tail and the midpiece (type m) and 3. to the head (type h). Attachment to more than one region was indicated as: e,m, and e.m,h etc.; e(m) indicates attachment mainly to the end part of the tail and to the middle region to a minor extent. A more precise indication of the attachment localization is not possible, because of the magnitude of the red blood cells compared to the dimensions of the spermatozoa. The result of the estimation of the percentage of motile spermatozoa incorporated into mixed agglutinates may depend on the time of reading the test after the reaction has been started. The reaction rate may also be related to the final result of the estimation. With strongly positive reactions, the reaction may be completed within 5 minutes, with less positive results the reaction may sometimes not be completed until after 10 to 15 minutes.

RESULTS

Patient Group I

No estimations could be obtained from 122 ejaculates from 91 patients. The reasons for failure of the test are given in Table I. It is seen, that most of the ejaculates involved in these cases had no or very few motile spermatozoa. The lack of motility in the MAR test could in a few cases be explained by the delay between ejaculation and investigation at the laboratory. On only two occasions did the sensitized red blood cells fail to agglutinate. From 23 of these patients an estimation could be made on another ejaculate, and no result was obtained from 68 patients.

Table I. *Survey of the reasons for failure of interpretation of the direct mixed antiglobulin reaction test on IgG present on spermatozoa in 122 fresh human ejaculates from 91 patients*

Reasons for failure	No. of ejaculates	No. of patients**
Azoospermia	19	13
Extreme oligozoospermia (<$1 \cdot 10^6$ spermatozoa/ml)	41	29
Bad motility in the ejaculate	31	27
Loss of motility in the test	26*	24
Hypospermia (no material left for the test)	3	2
No agglutination of the erythrocytes	2	2

* Consisting of 16 ejaculates $\leqslant 10 \cdot 10^6$:
5 ejaculates $> 10 \cdot 10^6 \leqslant 20 \cdot 10^6$;
5 ejaculates $> 20 \cdot 10^6$ (four of these ejaculates had less than 10% motile spermatozoa).

** With 6 patients the MAR tests failed for a different reason on another ejaculate.

From the 95 patients, who brought more than one ejaculate, no estimation could be obtained with the ejaculates from 22 patients. Only one estimation could be made on the ejaculates from 19 patients and from 54 patients an estimation was obtained on more than one ejaculate. The results were the same with 24 patients; negative and doubtfully positive respectively with 27 patients; doubtfully positive and positive respectively with two patients; and negative, doubtfully positive, and positive (<10%) respectively with one of these patients.

From the 253 patients (100%) in this group, as mentioned before, no estimation could be obtained in the MAR test on the ejaculates from 68 patients (27%). The test showed only a negative result in 80 patients (32%); with 75 patients (30%) the result was doubtfully positive, at the most; and, on the ejaculates from 30 patients (12%) a positive result was obtained.

In the doubtfully positive results a type e adherence was seen in 90% and types m and e,m in 10% of the tests. Adherence to the head of the spermatozoa was not seen in this patient group, although we observed it a few times on other occasions.

In the positive cases the results ranged from <10% to 100% of the motile spermatozoa incorporated into mixed agglutinates. A further investigation on the presence of antisperm antibodies was carried out on some patients with a MAR test which was <50% positive at the most, and on all patients with a MAR test >50% positive. These patients were incorporated into groups II and III.

Patient Group II. Men with Sperm Agglutinating Activity in the Serum

The MAR tests carried out on the ejaculates from these men resulted in estimates ranging from 0% to 100% of the motile spermatozoa involved in mixed agglutinates (Table II). With 31 patients MAR tests were performed on more than one ejaculate. The results of the estimations on ejaculates from the same patients commonly were similar. With patients Vlt, Bap, and Tib the results varied more widely. The MAR tests on the ejaculates from these patients were of the slow reacting type.

The sperm agglutination titers in the serum of these patients ranged from 4 to 4,096. With serum titers of 32 and higher, MAR tests were 80% or more positive.

Patients with serum titers lower than 32 had on their ejaculates MAR tests with estimates ranging from 0% to >90% positive. No strongly positive reaction was found.

In the SP the sperm agglutination titer varied from <4 to 256 (Table III, upper part). With titers <4 in the SP, estimations in the MAR test varied from 0% to 100% positive. With titers of 4 and higher the estimation in the MAR test was 0% at least.

The agglutination observed in the ejaculates from 19 patients with a titer in the SP of 4 and higher was classified as 3+ in 12 patients, as 2+ in two patients, and as 1+ in three patients. With one patient (Vld, titer in SP: 8) no agglutination was observed. From one patient no data were available. The patients without a sperm agglutination titer found in the SP had no or only 1+ agglutination in the semen, except for one patient (Pom) with 2+ agglutination.

If in the SCMC test, the percentage of local shaking spermatozoa exceeded 80%, then no progressively motile spermatozoa were found at 5 cm in the SPM test. In all these cases the MAR tests showed strongly positive reactions. On the other hand three patients (Hor, Pom, and Sta) had a strongly positive reaction in the MAR test yet progressively motile spermatozoa were found at 5 cm in the SPM test, and the percentage of locally shaking spermatozoa in the SCMC test did not exceed 80.

Patient Group III. Men Without Sperm Agglutination Activity in the Serum (Titer: <4)

The sperm agglutination titer found in the SP was <4 for all patients (Table III, lower part). In the MAR tests the estimations were always 10% or less positive, although on other ejaculates from two patients higher estimations were made (patient Kse: 60%; patient Wei: 20%). From the patients with a negative result in the MAR test seven patients had a doubtfully positive result on other ejaculates and three patients had a positive result of 10% in the MAR test on other ejaculates. One patient presented here with a doubtfully positive result had a negative result in the MAR test on another ejaculate. Spontaneous agglutination was not seen in the ejaculates except for one patient with 1+ agglutination.

Table II. *Reproducibility of the estimations obtained from the direct mixed antiglobulin reaction test for IgG, carried out on different fresh ejaculates from the same patient. Comparison with sperm agglutination titer in serum*

	Sperm-agglutination activity in serum		Results of the estimations in the direct mixed antiglobulin reaction test, expressed in % of motile spermatozoa taken up into mixed agglutinates														
Patient	Titer	Type	0	dbt	<10*	10	20	30	40	50	60	70	80	90	>90**	≈100	100
Wer	4.096	M															xxx
Gen	1,024	T-T													x	x	
Lcr	1,024	M														x	x
Lch	512	M														xx	xxxx
Kaa	512	T-T															xx
Pae	512	T-T														x	xxxx
Kup	512	T-T														x	xxxx
Kog	512	H-H												xx			
Grt	256	T-T												x	x	xxx	
Klo	256	H-H															xx
Vrs	256	T-T													x	x	x
Von	256	T-T															xxx
Voo	256	T-T												x			
Pyl	128	T-T													x		xx
Grv	128	M															x
Ven	128	T-T											x			x	xxx
Hor	128	T-T														x	x
Jga	128	H-H											xx		xx		
Sta	128	T-T														xxx	x
War	64	T-T														xxx	x
Hus	64	T-T													x		
Pom	64	T-T												xxx		xx	
Jod	32	T-T													xxxx		xx
Ros	32	T-T												xx			
Vlt	16	T-T						x		x		x					
Vld	8	T-T											x	x	xxx		
Gro	8	T-T										x		x			
Ter	8	T-T											xx	xx			
Bap	8	T-T						x					x	x			
Tib	8	T-T				x							xxx				
Zyl	8	H-H		xx	x												
Bow	8	T-T	xxx														
Wih	4	T-T	xx	x													
Vrw	4	T-T		xx													

x = one estimation: M = mixed; H-H = head to head: T-T = tail to tail: ≈ = almost: dbt = doubtfully.
* = dbt <10%.
** = 90 < ≈100%.

In the SCMC tests the spermatozoa that were locally shaking were 50% or less of the motile spermatozoa, and in the SPM test progressively motile spermatozoa were observed at 5 cm, apart from one patient with progressively motile spermatozoa at a maximum penetration depth of 3 cm.

DISCUSSION

The direct mixed (erythrocyte spermatozoa) antiglobulin reaction can be applied directly on fresh untreated ejaculates. As the presence of motile spermatozoa is required, the MAR test has to be carried out soon after ejacula-

Table III. *Comparison of sperm agglutinating activity in serum and seminal plasma, agglutination grade in semen, direct mixed antiglobulin reaction test for IgG, sperm cervical mucus contact test, spermatozoa penetration meter test. Group I: 34 patients (upper part) and Group III: 19 patients (lower part)*

	Agglutination activity					Motile* spermatozoa in mixed agglutinates			Sperm-penetration* into cervical mucus	
	Serum		Seminal plasma		Aggl. grade in semen			% local* shaking spermatozoa in SCMC test		
Patient	Titer	Type	Titer	Type		%	Type		Cm	Type
		(1)		(1)	(2)	(3)*	(4)*			(5)
Wer	4.096	M	256	T-T	+++	100	e, m, h	100	1	loc
Gen	1.024	T-T	512	H-H	+++	100	e, m, h	>90	5	loc
Ler	1.024	M	4		+++	100	e, m, h	>80	5	loc
Lch	512	M	64	T-T	+++	100	e, m, h	100	1	loc
Kaa	512	T-T	32	T-T	++	100	e, m, h	90	1	imm
Pae	512	T-T	8		++	100	e, m, h	100	5	loc
Kup	512	T-T	4	T-T		100	e, m, h	60	3	loc
Kog	512	H-H	<4		+	90	e, m, h	0	5	prog
Grt	256	T-T	32		+	≈100	e, m(h)	50	3	imm
Klo	256	H-H	16	H-H	−	100	e, m, h	100	1	loc
Vrs	256	T-T	4	M	++−	>90	e, m, h	>90	5	loc
Von	256	T-T	4		+−+	100	e, m, h	100	0	
Voo	256	T-T	4	T-T	+−−	90	e, m, h	50	1	prog
Pyl	128	T-T	128	T-T	++−	100	e, m, h	100	3	prog
Grv	128	M	32		−	100	e, m, h	90	0	
Ven	128	T-T	8	T-T	++−	100	e, m, h	100	1	loc
Hor	128	T-T	4	T-T	+++	100	e, m, h	80	5	prog
Jga	128	H-H	<4		−	>90	e, m, h	20	5	prog
Sta	128	T-T	<4		−	≈100	e, m, h	10	5	prog
War	64	T-T	16	T-T	++−	≈100	e, m, h	90	1	loc
Hus	64	T-T	8		+++	>90		>90	5	loc
Pom	64	T-T	<4		++	≈100	e, m(h)	70	5	prog
Jod	32	T-T	16	T-T	+++	100	e, m, h	>90	3	prog
Ros	32	T-T	<4		−	90	e, m, h	30	5	loc
Vlt	16	T-T	<4		−	50	e	0	5	prog
Vld	8	T-T	4	T-T	−	>90	e	0	5	prog
Bap	8	T-T	<4		−	90	e(m)h	10	5	prog
Gro	8	T-T	<4		+	80	e, m	50	5	prog
Ter	8	T-T	<4		+	80	e, m, h	10	5	prog
Tib	8	T-T	<4		−	80	e	0	5	prog
Zyl	8	H-H	<4		−	dbt	m, h	0		
Bow	8		<4		−	0		0	5	prog
Wih	4	T-T	<4		−	dbt	e	0	5	prog
Vrw	4	T-T	<4		−	dbt	m	30	5	prog
Str	<4		<4		−	10	e		3	prog
Vwk	<4		<4		−	0		0	5	prog
Ton	<4		<4		−	0		0	5	prog
Elz	<4		<4		−	0		30	5	prog
Wrk	<4		<4		−	0		20	5	prog

1: M = mixed, H-H = head to head, T-T = tail to tail.
2: − = no, + = slight, ++ = moderate, +++ = much.
3: ≈ = almost, dbt = doubtfully.
4: e = end of the tail, m = main part of the tail and or mid piece, h = head.
5: loc = local, imm = immotile, prog = progressive.
* carried out on same day.

Table III. Continued

Patient	Agglutination activity: Serum Titer	Serum Type	Seminal plasma Titer	Seminal plasma Type	Aggl. grade in semen	Motile* spermatozoa in mixed agglutinates: %	Type	% local* shaking spermatozoa in SCMC test	Sperm-penetration* into cervical mucus: Cm	Type
Grk	<4		<4		–	0		0	5	prog
Jbd	<4		<4		–	dbt	e, m	0	5	prog
Bkm	<4		<4		–	0		30	5	prog
Bre	<4		<4		–	dbt	e	30	5	prog
Huj	<4		<4		–	0		10	5	prog
Hou	<4		<4		–	0		0	5	prog
Grv	<4		<4		–	0		30	5	prog
Vtl	<4		<4		–	0		50	5	prog
Hls	<4		<4		–	0		30	5	prog
Vak	<4		<4		–	dbt	e, h	10	5	prog
Sti	<4		<4		–	dbt	(e)m	0	5	prog
Kse	<4		<4		–	10	e(m, h)	10	5	prog
Hum	<4		<4		–	0		0	5	prog
Wei	<4		<4		–	10	(e, m)h	30	5	prog

tion, because the spermatozoa in some ejaculates lose their motility rather quickly.

The test can be applied on ejaculates with spermatozoa concentrations down to 1 million per ml, provided the motility is sufficient. By estimation a reasonable quantitation of the percentage of motile spermatozoa adhering to erythrocytes can be obtained. The results are, in general, acceptably reproducible with patients with strongly positive results in the MAR test, and with patients with negative results. If quantitation becomes doubtful, because of a very low concentration of spermatozoa, then the type of attachment of the erythrocytes can show whether the reaction is of the strongly positive type or not.

The result of the estimation can mostly be obtained within a few minutes. Only in case of a slow reaction more time, up to 10 minutes, is needed for an accurate estimation. A negative reaction is easily recognized, because in the method described here, the agglutination of the erythrocytes is used as an internal control on the reaction. Only in a few cases was it impossible to read the test because of failure of the agglutination of the erythrocytes. Possibly, these ejaculates contained a high amount of IgG, completely neutralizing the anti-IgG. This could be due to the presence of blood. Blood has a much higher concentration of IgG than SP (19, 20). Admixture of a small amount of blood to the semen will therefore prevent attachment of the erythrocytes to each other, and to the spermatozoa.

The MAR test appears to be a sensitive test, as direct immunofluorescence on spermatozoa from ejaculates with antisperm antibodies showed no or only weakly positive reactions (4). Adherence of red blood cells is apparently a more sensitive test than immunofluorescence labelling. The sensitivity of the MAR test is, in our opinion, also due to the ease with which motile mixed agglutinates are recognized. In addition, it seems that motile spermatozoa react much better than immotile spermatozoa. The mixed antiglobulin reaction thus seems well suited for the detection of antibodies on motile spermatozoa.

In our series we found a high correlation between the results of the MAR test and the sperm agglutination titers in the serum. Strongly positive reactions in the MAR were found only in ejaculates from patients with sperm agglutination titers in the serum of 32 and higher.

This relation may be explained by the as-

sumption, that the antisperm IgG is derived from the serum. It has been reported that both albumin and IgG are present in the SP in a concentration of about 1% of the serum level. The distribution of these compounds over different fractions of split ejaculates closely resembles the distribution of zinc, transferrin, and acid phosphatase. This probably means that IgG in the SP is derived from the serum and enters the SP via the prostate (18, 19, 20). With two patients (Kog and Jga, Table II) the MAR test was repeatedly relatively low, despite high sperm agglutination titers (512 and 128 respectively). The agglutination was of the H-H type, which is generally due to IgM antibodies (12). However, in the serum of these patients some IgG antisperm antibodies are probably also present.

There is not a strong correlation between the results of the MAR tests and sperm agglutination titers in the SP. Strongly positive reactions in the MAR test were observed in ejaculates with a sperm agglutination titer <4. These observations are in agreement with results obtained by Friberg (21) after fractionation of serum and SP by gelfiltration and anion exchange column chromatography. Sperm agglutinating activity was eluted in fractions where IgA was expected to be present, apart from one patient, where some activity in the IgG fraction was also found, and who had in the serum a very high sperm agglutination titer (4,096). Moreover it was found that absorption of the SP with anti-Fc-serum did not alter the sperm agglutination titer in the SP, whereas the titer was lowered 3 to 5 titer steps after absorption with anti-free-secretory-component-serum. In the serum the sperm agglutination activity was generally found in the IgM or in IGA fraction, and in the serum from some patients the sperm agglutination activity was probably also due to IgA antisperm antibodies (21). The conclusion, that sperm agglutination activity in the SP is mainly due to IgA, is affirmed by the observation that sperm agglutinating activity is absorbed from the serum but not, or only a little, from the SP with suspensions of *S. aureus* containing protein A, which specifically absorbs IgG (4). As far as we know there has been no report of infertility in men due to antisperm antibodies without sperm agglutinins in serum. The direct MAR test on IgG present on freely swimming spermatozoa can therefore be used as a method for screening for the presence of antisperm antibodies in men.

Sperm agglutination of the H-H type is generally due to antisperm antibodies of the IgM class and IgG antisperm antibodies generally cause the spermatozoa to agglutinate tail to tail (21). However, it appears from the results of the MAR tests, that IgG is present on the heads of the spermatozoa of all our patients with a tail to tail agglutination type in serum, and with a strongly positive reaction in the MAR test.

Comparison of the results of the MAR tests on IgG, and the results of the SCMC and SPM tests, indicate that spermatozoa from ejaculates with a result in the MAR test of 90% or less positive will show little or no inhibition of the sperm penetration and sperm migration into cervical mucus due to antisperm antibodies.

A strongly positive reaction was found in the MAR tests on the ejaculates from all patients who had a shaking phenomenon of at least 80% in the SCMC test, and less than five progressively motile spermatozoa at 5 cm in the SPM test. However we have found one patient (Sta, Table II, III), who had a repeatedly strongly positive MAR test; yet, the shaking phenomenon in the SCMC test was only 10% and many progressively motile spermatozoa were found at 5 cm in the SPM test. It is possible, therefore, that the cause of the incompatibility between cervical mucus and spermatozoa coated with antisperm antibodies, is not due to the presence of antibodies of the IgG class, despite the high correlation of the presence of IgG molecules on spermatozoa with decreased ability of sperm penetration into cervical mucus.

ACKNOWLEDGMENTS

We wish to thank Dr. P. H. Kaars Sypesteyn for supplying the sensitized erythrocytes and for valuable advice concerning the agglutination reaction, and Dr. B. Boettcher for reading through the manuscript. We also gratefully acknowledge the continuous help, advice, and criticism of Dr. Ph. Rümke.

REFERENCES

1. Rümke, Ph., Van Amstel, N., Messer, E. N. & Bezemer, P. D.: Prognosis of fertility of men with sperm agglutinins in the serum. Fertil Steril **25**: 393, 1974.
2. Rümke, Ph.: Autoantibodies against spermatozoa in infertile men: some unsolved problems. Pages 26–35 *in* A. Centaro and N. Caretti, eds. Immunology in obstetrics and gynecology. Proc 1st Int Congr, Padua, 1973. Excerpta Med Found, Amsterdam, 1974.
3. Friberg, J.: Relation between sperm-agglutinating antibodies in serum and seminal fluid. Acta Obstet Gynecol Scand (Suppl) **36**: 73, 1974.
4. Husted, S. & Hjort, T.: Sperm antibodies in serum and seminal plasma. Int J Fertil **20**: 97, 1975.
5. Fjällbrant, B.: Interrelation between high levels of sperm antibodies, reduced penetration of cervical mucus by spermatozoa, and sterility in men. Acta Obstet Gynecol Scand **47**: 102, 1968.
6. Manarang-Pangan, S. & Behrman, S. J.: Spermatotoxicity of immune sera in human infertility. Fertil Steril **22**: 145, 1971.
7. Kremer, J. & Jager, S.: The sperm cervical mucus contact test: a preliminary report. Fertil Steril **27**: 335, 1976.
8. Coombs, R. R. A., Marks, J. & Bedford, D.: Specific mixed agglutination: mixed erythrocyte-platelet anti-globulin reaction for the detection of platelet antibodies. Br J Haematol **2**: 84, 1956.
9. Edwards, R. G., Ferguson, L. C. & Coombs, R. R. A.: Blood group antigens on human spermatozoa. J Reprod Fertil **7**: 153, 1964.
10. Jeffery, W. & Parish, W. E.: Allergic infertility: laboratory techniques to detect antispermatozoal antibodies. Clin Allergy **2**: 261, 1972.
11. Coombs, R. R. A., Rümke, Ph. & Edwards, R. G.: Immunoglobulin classes reactive with spermatozoa in the serum and seminal plasma of vasectomized and infertile men. Pages 354–358 *in* K. Bratanov, ed. Immunology of Reproduction. Proc 2nd Int Symp, Varna, 1971. Bulgarian Academy of Science, Sofia, 1973.
12. Kroeks, M. V. A. M.: pH-meting in situ bij het fertiliteitsonderzoek. Ned Tijdschr Geneeskd **118**: 1244, 1974.
13. Kremer, J.: A simple sperm penetration test. Int J Fertil **10**: 209, 1965.
14. Friberg, J.: A simple and sensitive micromethod for demonstration of sperm-agglutinating activity in serum from infertile men and women. Acta Obstet Gynecol Scand (Suppl) **36**: 21, 1974.
15. Hellema, H. W. J. & Rümke, Ph.: Comparison of the tray agglutination technique with the gelatin agglutination technique for the detection of spermagglutinating activity in human sera. Fertil Steril **27**: 284, 1976.
16. Hellema, H. W. J.: Isolation of only motile spermatozoa from donor semen samples. Abstracts, 3rd European Immunology Meeting, Copenhagen, 1976.
17. Chalmers, D. G., Coombs, R. R. A. & Gurner, B. W.: The mixed antiglobulin reaction in the detection of human iso-antibodies against leucocytes, platelets and HeLa cells. Br J Haematol **5**: 225, 1959.
18. Lindholmer, C., Carlström, A. & Eliasson, R.: Occurrence and origin of proteins in human seminal plasma with special reference to albumin. Andrologia **6**: 181, 1974.
19. Rümke, Ph.: Autoantibodies against spermatozoa in infertile men. J Reprod Fertil (Suppl) **21**: 169, 1974.
20. Tauber, P. F., Zaneveld, L. J. D., Propping, D. & Schumacher, G. F. B.: Components of human split ejaculates. I. Spermatozoa, fructose, immunoglobulins, albumins, lactoferrin and other plasma proteins. J Reprod Fertil **43**: 249, 1975.
21. Friberg, J.: Immunological studies on sperm-agglutinating sera from men. Acta Obstet Gynecol Scand (Suppl) **36**: 43, 1974.

CHAPTER 6

IMMUNOGLOBULIN CLASS OF ANTISPERMATOZOAL ANTIBODIES FROM INFERTILE MEN AND INHIBITION OF IN VITRO SPERM PENETRATION INTO CERVICAL MUCUS

INTERNATIONAL JOURNAL OF ANDROLOGY
3 (1980) 1–14

Fertility Unit of the Department of Obstetrics and Gynaecology, University Hospital, Groningen, The Netherlands

Immunoglobulin Class of Antispermatozoal Antibodies from Infertile Men and Inhibition of *in vitro* Sperm Penetration into Cervical Mucus

By

S. Jager, J. Kremer, J. Kuiken and Tiny van Slochteren-Draaisma

The presence of IgG and IgA on motile spermatozoa from normal semen donors and men from infertile couples was studied with mixed antiglobulin reaction (MAR) tests.

The percentage of motile spermatozoa with IgG (IgG MAR %) was found to be related to the circulating antispermatozoal IgG. No direct relation could be detected between the IgG MAR % and the sperm agglutinating activity in seminal plasma (SP) or the percentage of motile spermatozoa showing the shaking phenomenon (S %) in the sperm cervical mucus contact (SCMC) test. The percentage of motile spermatozoa with IgA (IgA MAR %) showed no direct relation to the sperm agglutinating activity in serum and SP, but was roughly proportional with the S %.

It was discerned that the shaking phenomenon in the SCMC test was probably due to presence of IgA on the motile spermatozoa. Previously it had been demonstrated that also the sperm agglutinating activity in SP is caused by IgA that is probably locally produced in the male genital tract. In conclusion it was thought that the reduced ability of penetration into cervical mucus by spermatozoa from infertile men is caused by locally produced IgA.

Key words: antispermatozoal antibodies – cervical mucus – MAR test – shaking phenomenon – SCMC test – sperm agglutination – sperm penetration.

Received on October 4th, 1979.

Human antispermatozoal antibodies can cause male infertility by inhibition of the sperm penetration into cervical mucus (CM) (Fjällbrant 1968). The inhibition is not only due to agglutination of spermatozoa in the ejaculate, but also to a characteristic progression inhibition (shaking phenomenon) of the spermatozoa after contact with CM (Kremer & Jager 1976). These effects are obviously due to antibodies locally present. Local antibodies, therefore, will be more directly related to the inhibition of the sperm penetration into CM than circulating antibodies. It is also obvious that antibodies on spermatozoa are more directly related to penetration inhibition than antibodies in seminal plasma (SP).

An immunofluorescence technique is generally employed to study the antibodies on the surface of cells, but this technique produced unsatisfactory results with spermatozoa (Boettcher et al. 1977). We therefore used the mixed antiglobulin reaction (MAR) (Coombs et al. 1956). Our modification of the MAR is a simple and rapid test that can be applied to fresh untreated ejaculates for the determination of IgG present on motile spermatozoa. In a previous study we used the MAR test for IgG and found immunoglobulins on more than 90 % of the motile spermatozoa from 21 men with sperm agglutination titers in their serum of at least 64. The spermatozoa from most of these men showed a highly reduced penetrability into the CM. The spermatozoa of one man, however, repeatedly showed good penetration into the CM despite the presence of IgG on more than 90 % of his motile spermatozoa. Therefore, we supposed that the reduced penetrability of spermatozoa from men with antispermatozoal antibodies was not due to sensitization with IgG but with IgA (Jager et al. 1978).

In the present study we introduce the direct MAR test for IgA. This new technique will be compared with the IgG MAR test. The percentage of motile spermatozoa with IgG (IgG MAR %) and with IgA (IgA MAR %) is compared with the sperm agglutination titer in serum and SP and with the percentage of motile spermatozoa exhibiting the shaking phenomenon (S %) after contact with CM in the sperm cervical mucus (SCMC) test (Kremer & Jager 1976). In addition the IgG MAR %, the IgA MAR % and the S % are compared with the degree of auto-agglutination of the spermatozoa in the ejaculate. Finally, concentrations of IgG and IgA in SP are determined.

Materials and Methods

Semen samples

Semen samples from two groups of men were investigated.

Normal semen donors. This group consisted of 15 donors from whom the semen was used for artificial insemination purposes (all husbands with children) and nine donors whose semen was routinely employed in immunological tests

(three husbands with children and six men with no known fertility problems). The ejaculates from the normal semen donors were tested in the MAR tests for IgG and IgA. By using a radial immunodiffusion method, the IgG and IgA concentrations were determined in the SP of the nine donors whose semen was normally used for immunological tests.

Men from infertile couples (95 men). In these couples the presence of antispermatozoal antibodies was investigated in the male and female partner. The reason for the investigation was an unexpected poor or negative results of the post coital test in combination with a poor or negative result of the sperm penetration meter (SPM) test (Kremer 1965) or a positive result in the MAR test for IgG, performed as a routine part of the semen analysis. A SCMC test and an IgG MAR test performed with the semen of these 95 men, who were also studied in a previous paper (Jager et al. 1979). By using the tray agglutination test (TAT) (Friberg 1974) the sperm agglutination titers in the sera and SP from all the men in this group were also determined. Semen samples from 51 men were also tested in the IgA MAR test. The concentrations of IgG and IgA were determined in the SP from 33 of the 51 men.

Collection and routine investigation of the ejaculates has been described by Jager et al. (1978). SP was obtained by centrifugation of the semen at 3000 *g* for at least 10 min.

Cercival mucus (CM)

Normal pre-ovulatory CM was obtained from women visiting the fertility unit. For collection and investigation of the CM see Jager et al. (1978).

Antisera

The following antisera were used: rabbit antihuman IgG (Behring), goat antirabbit IgG (Behring), rabbit antihuman IgA (Behring) and rabbit antihuman colostral IgA (Dako). The antisera used, were either untreated or treated by one of the following methods:

Dialysis: One to 2 ml antiserum were dialyzed (Visking dialysis tube, 3/32" diameter) at 4°C against 1 l phosphate buffered saline (PBS) pH 7.4. The PBS was changed two or three times.

Precipitation. For isolation of the immunoglobulin fraction the antisera were precipitated by addition of four volumes of 1.75M $(NH_4)_2SO_4$. The precipitate was washed twice with the $(NH_4)_2SO_4$ solution and dissolved in the PBS to the original antiserum volume and dialyzed for the removal of the $(NH_4)_2SO_4$.

Gel filtration. For isolation of the monomer immunoglobulin fraction the antiserum was fractionated over Sephadex G-200 (Pharmacia). Column dimen-

sions: 2.6 x 100 cm; flow rate: 14 ml/h. The proteins were eluted with a 0.1 M Tris-HCl buffer pH 8.0 containing 0.002 M EDTA and 0.02 % sodium azide. The peak containing the monomer immunoglobulin fraction was concentrated by ultrafiltration (Minicon B15, Amicon) and dialyzed.

The "neutralizing capacity" of the antisera, with the exception of the goat antirabbit IgG antiserum, was determined. One volume of antiserum was mixed with an equal volume of serum. The concentration of IgG and IgA in the mixture and in the serum was then determined with radial immunodiffusion. The "neutralizing capacity" was defined as the difference between the concentrations of IgG or IgA in the serum and in the mixture (after correction for the dilution). The neutralizing capacities were found to be 48 mg/dl for the rabbit antihuman IgG antiserum, 62 mg/dl for the rabbit antihuman IgA antiserum and 120 mg/dl for the rabbit antihuman colostral IgA antiserum.

Colostrum

Colostrum obtained on the first or second day after delivery was pretreated by removing fat and casein (Tomasi et al. 1965). The immunoglobulin fraction was prepared by precipitation and dialysis.

Serum IgA

IgA was isolated from serum by affinity column chromatography. The column (0.9 x 4.5 cm) was prepared by coupling the monomer immunoglobulin fraction (obtained by gelfiltration) of 11 ml of the rabbit antihuman IgA antiserum to CNBr activated Sepharose 4B (Pharmacia). The coupling was performed by a modification of the procedure recommended by the manufacturer (Boettcher, personal advice).

The monomer immunoglobulin fraction of 1 ml serum was obtained by gelfiltration and afterwards applied to the column. The unbound proteins were washed out with a solution of 0.1 M sodium acetate + 0.3 M NaCl, pH 7.2 (washing buffer) and the bound IgA was eluted with a solution of 0.16 M acetic acid + 0.3 M NaCl, pH 2.4. The flow rate was 14 ml/h. The pH of the eluate was neutralized immediately with 1 or 2 N NaOH and concentrated to 0.5 ml by ultrafiltration. The IgA concentration, determined by radial immunodiffusion, was 9 mg/dl.

Indicator cells and particles

The following indicator cells and particles were used:

Blood group O, Rh-positive (R_1R_2) erythrocytes sensitized with a strong anti-D serum (for details see Jager et al. 1978).

Latex particles (Dow latex, dry weight 10 %, particle diameter 5.7 ± 1.5 μm). A volume of 0.5 ml of the latex particles suspension was centrifuged at 200 *g* for 10 min. The sediment was incubated for at least 1½ h at room temperature with 1 ml of the solution of proteins to be coated on the particles. After the suspension was centrifuged, the sediment was washed with saline and resuspended in Earle balanced salt solution, pH 7.4, containing 1 % bovine serum albumin. The particles were coated with proteins from one of the following solutions:

a. goat antirabbit IgG diluted with an equal volume of saline,
b. the immunoglobulin fraction of the goat antirabbit IgG antiserum. The fraction was obtained by precipitation and dialysis,
c. colostrum diluted 30 times,
d. the immunoglobulin fraction of colostrum, obtained by precipitation and dialysis.

Blood group O, Rh-negative erythrocytes pretreated with the coating procedure of Wide & Gemzell (1960). To the erythrocytes was, after the treatment with formalin and tannin, absorbed the immunoglobulin fraction from colostrum or the purified serum IgA diluted to 1 mg/dl.

The effectivity of the coating was tested by mixing equal amounts of particle or cell suspension and antiserum. The coating was considered sufficient if the microscopic evaluation of the agglutination reaction revealed a notably increased reaction in the preparation with antiserum compared with the reaction in the preparation without the antiserum.

Mixed antiglobulin reaction (MAR) tests

MAR tests were performed by mixing equal volumes of 1. fresh semen, 2. sensitized or coated cells or coated particles and 3. corresponding monospecific antiglobulin antiserum. The performance of the MAR test for IgG has been previously described in detail (Jager et al. 1978).

For the IgA MAR test the reaction mixture was aspirated into a flat capillary tube with an internal optical path length of 0.05 mm (Vitro Dynamics) which reduced the evaporation thereby lengthening the reaction time. In order to stimulate the motility of the spermatozoa and the reaction rate a warm stage (35°C) microscope was used.

MAR tests were evaluated when the percentage of motile spermatozoa, to which erythrocytes or latex particles adhered (MAR %), became stabilized. MAR % estimations were expressed as 0, 0 < 10, 10, multiples of 10 to 90 and > 90. In cases where MAR % estimations were obtained with different ejaculates from the same man the mean value of these estimations, rounded upwards, was presented. In more than 90 % of the MAR tests performed, the

Table 1.

The percentage of motile spermatozoa with IgG (IgG MAR %) present in fresh untreated ejaculates from 95 infertile men compared with:

a. the sperm agglutination titer (SAT)** in serum,
b. the SAT in seminal plasma,
c. the percentage of motile spermatozoa showing the shaking phenomenon (S %) after contact with cervical mucus.***

SAT or S %	Percentage of motile spermatozoa with IgG*****											
	0	0<10	10	20	30	40	50	60	70	80	90	>90
a. (compared with SAT in serum)												
4096												1
2048												
1024											1	3
512						1						3
256										1	2	9
128												12
64											1	9
32							2	1		1	1	1
16	2											
8	5	2	1							1		
4	1		1									
<4	22	7		2					2*			
b. (compared with SAT in seminal plasma)												
512												1
256												1
128												3
64											1	3
32									1			5
16										1	1	5
8								1		1		7
4						1	1					3
<4	30	9	2	2			1		1*	1	3	10
c. (compared with S %)												
>90											1	12
90											1	12
80												5
70									1			4
60							1					1
40	1		2					1				
30	5	1								1		
20	3									1	1	
10	2	1				1				1		1
0<10				1							1	
0	18	6		1			1		1*		1	2

* IgG MAR % decreased to 20 in 20 min with spermatozoa from one man.
** Determined in the tray agglutination test (Friberg 1974).
*** Determined in the SCMC test (Kremer & Jager 1976).
**** Correlation coefficients: a. 0.870; b. 0.646; c. 0.760. $P < 0.005$.

Table 2.

The percentage of motile spermatozoa with IgA (IgA MAR %) present in fresh untreated ejaculates from 51 infertile men compared with:

a. the sperm agglutination titer (SAT)* in serum,
b. the SAT in seminal plasma,
c. the percentage of motile spermatozoa showing the shaking phenomenon (S %) after contact with cervical mucus**.

SAT or S %	Percentage of motile spermatozoa with IgA***											
	0	0<10	10	20	30	40	50	60	70	80	90	>90
a. (compared with SAT in serum)												
4096												1
2048												
1024									1	1		
512		1								1	1	1
256		1	2	1						1	3	4
128		1								3	1	4
64	1	1				1		4			1	
32		1	1			1	1				1	
16	1											
8	1											
4												
<4	7											1
b. (compared with SAT in seminal plasma)												
256									1			
128												1
64										1	2	1
32										2		3
16			1	1		1		1				3
8			1							2	2	2
4		1				1	1				1	1
<4	10	4	1					3		1	2	
c. (compared with S %)												
>90								2			3	5
90						1	1			4		5
80								2	1	1	1	
70				1						1	1	1
60						1					1	
50		1										
40											1	
30	1		1									
20		1	1									
10		2	1									
<10												
0	9	1										

* Determined in the tray agglutination test (Friberg 1974).
** Determined in the SCMC test (Kremer & Jager 1976).
*** Correlation coefficients: a, 0.559; b, 0.628; c, 0.886. $P < 0.005$.

duplicate MAR % estimations did not reveal a difference of more than 10. Also with different ejaculates from the same man, the IgG MAR % values did not differ by more than 10 in 90 % of the cases. The differences found amongst the IgA MAR % values were more than 10 in about 40 % of the cases with a mean difference of 20 (± 20). In order to exclude false positive results estimations from tests where the motility of the spermatozoa was not better than sluggish were ignored.

In most of the cases the time limit for the evalution of the IgG MAR % did not exceed 10 min. The IgA MAR test, usually, proceeded much slower. Sometimes the maximum IgA MAR % was obtained after 60 min. On the other hand, the motility of the spermatozoa often decreased too rapidly for a reliable assessment. By using dialyzed antiserum the motility of the spermatozoa was prolonged. Only in few patients did the reaction time decrease, by changing from the anti-IgA antiserum to the anticolostral IgA antiserum. A possible explanation for the slow reaction is weak coating of the indicator particles or cells. Therefore, we made a comparison by using uncoated red blood cells and erythrocytes coated with colostral immunoglobulins in tests with ejaculates from five infertile men. With the coated erythrocytes IgA MAR % values of 80 to $>$ 90 were found whereas with the uncoated cells only a few of the freely swimming spermatozoa adhered to the erythrocytes. These five ejaculates were also tested with erythrocytes coated with antihuman colostral IgA. Antihuman colostral IgA antiserum was not added in the test. The MAR % was $0 < 10$ with four ejaculates and 90 with one semen sample.

The use of particles or cells and the different types of coating did not change the reaction speed or the IgA MAR % values. However, with erythrocytes the test was more easily evaluated than with the latex particles. The modification whereby the erythrocytes were coated with colostral immunoglobulins and performed with rabbit antihuman colostral IgA was finally considered to be the most convenient one.

Tray agglutination test (TAT)

The TAT was performed as originally described by Friberg (1974) and as modified by Hellema & Rümke (1976) and Jager et al. (1978).

The sperm cervical mucus contact (SCMC) test

The SCMC test was carried out according to Kremer & Jager (1976), Kremer et al. (1978) and Jager et al. (1979).

Radial immunodiffusion

Radial immunodiffusion (Mancini et al. 1965) was performed with Partigen plates (Behring). LC plates were used for the determination of IgG and IgA

in SP and Tri plates for the determination of the "neutralizing capacity" of the antisera. The concentrations of IgG and IgA were calculated by comparison with a standard serum (Behring).

Results

The IgG MAR % and IgA MAR % values obtained with semen samples from men from infertile couples were compared with the sperm agglutination titers in serum and in SP and with the S % in the SCMC test (Tables 1 and 2). A comparison of IgG MAR % and IgA MAR % values is shown in Table 3. In this table the results with semen samples from donors are included.

With motile spermatozoa from one patient the IgG MAR % decreased from 70, shortly after the preparation has been made, to 20 in approximately 20 min. With IgG MAR tests performed on four other ejaculates from this man, a similar decrease of the MAR % was observed. No auto-agglutination of the spermatozoa was seen in his ejaculate.

Table 3.
The percentage of motile spermatozoa with IgA (IgA MAR %) compared with the percentage of motile spermatozoa with IgG (IgG MAR %). Results with fresh untreated ejaculates from 51 infertile men and 24 normal semen donors.

IgA MAR %	Percentage of motile spermatozoa with IgG**											
	0	0<10	10	20	30	40	50	60	70	80	90	>90
<90									1			10
90								1				6
80											1	5
70												1
60											3	1
50												1
40							1					1
30												
20												1
10										2	1	
0<10	10(10)	4(4)				1					2	2
0	14(8)	3(1)	1(1)						1*			1

In parenthesis number of normal semen donors.
* IgG MAR % decreased to 20 in 20 min.
** Correlatient coefficient: 0.768. $P < 0.005$.

In nine cases an IgG MAR % of at least 90 was found with a S % of not more than 20. The semen of five of the six men revealed an IgA MAR % value of 10 or less. The IgA MAR % value of the sixth patient was not determined. In all six cases the agglutination titer in the SP was < 4 and only a slight degree of auto-agglutination was observed.

The degree of auto-agglutination of the spermatozoa was noted for 50 of the 51 men whose ejaculates were also tested in the IgA MAR test. A comparison with IgG MAR % values, IgA MAR % values and S % values is shown in Table 4.

The concentrations of IgG in SP were 10 ± 3.7 mg/dl (range 5–18 mg/dl)

Table 4.

The degree of auto-agglutination of the spermatozoa in ejaculates from 50 men from infertile couples. Comparison with:

a. the percentage of motile spermatozoa with IgG (IgG MAR %),
b. the percentage of motile spermatozoa with IgA (IgA MAR %),
c. the percentage of motile spermatozoa showing the shaking phenomenon after contact with cervical mucus**.

MAR % or S %	Degree of auto-agglutination***			
	–	+	++	+++
a. (compared with IgG MAR %)				
≧ 80	5	16	6	11
30–70	3*	1		
≦ 20	7	1		
b. (compared with IgA MAR %)				
≧ 80	2	9	3	9
30–70	1	4	1	2
≦ 20	12	5	2	
c. (compared with S %)				
≧ 80	1	11	4	10
30–70	3	3	2	1
≦ 20	11	4		

* IgG MAR % decreased from 70 to 20 in 20 min with spermatozoa from one patient.

** Determined in the SCMC test (Kremer & Jager 1976).

*** Correlation coefficients: a. 0.534; b. 0.552; c. 0.639. $P < 0.005$.

for men from infertile couples and 10 ± 3.8 mg/dl (range 4–14 mg/dl) for donors. The IgA concentrations in SP were 2.6 ± 1.7 mg/dl (range 0.6–8.7 mg/dl) for men from infertile couples and 1.7 ± 1.1 mg/dl (range < 0.6–8.7 mg/dl) for semen donors.

Discussion

The present results of the IgG MAR test are an extension of a previously published series (Jager et al. 1978) and confirm our former observation that the percentage of motile spermatozoa with IgG was more directly related to the sperm agglutination titer in the serum than to the SP titer. A high (≧ 90) IgG MAR % was always found together with a serum titer of at least 32 and a low (≦ 20) IgG MAR % corresponded in all cases with a serum titer of 16 or less. On the other hand, an IgG MAR % of ≧ 90 could be found with SP titers from < 4 to 128. The fact that the IgG MAR % showed a better correlation with the serum titer compared with the titer in the SP, seems logical, as in the serum of infertile men the sperm agglutinins are mainly IgG antibodies, whereas the sperm agglutination activity in the SP is mainly due to IgA, which is probably locally produced (Fjällbrant 1969b; Friberg 1974; Husted & Hjort 1975).

An unexpectedly low (< 90) IgG MAR % was found in the semen from six men with a sperm agglutination titer in their serum of at least 32 (Table 1a). Possible, in these cases the serum sperm agglutination activity was caused by antibodies of the IgG class for a small part only. High serum sperm agglutinating activity, probably not caused by IgG, was also reported by Husted & Hjort (1975). On the other hand, we found relatively high (70 and 80) IgG MAR % values in the ejaculates from three men with serum sperm agglutination titers of 8 in one case and < 4 in two cases. With spermatozoa from one patient the reaction was probably not a specific antigen-antibody reaction as the IgG MAR % decreased to a low value in about 20 min, indicating a weak reaction. In addition, no other evidence of the presence of antispermatozoal antibodies in this man could be found. With spermatozoa from the two other men the IgG MAR % did not decrease in time. Possible in these men the circulating IgG had an abnormal transudation to the SP or some antispermatozoal IgG was locally produced.

No direct relation was found between the IgG MAR % and the percentage of motile spermatozoa showing the shaking phenomenon in the SCMC test. Although a high S % was always found with a high IgG MAR %, the reverse was not true. With semen from six men a low S % was combined with a high IgG MAR % (Table 1c). It therefore seems unlikely that the shaking phenomenon is due to the presence of IgG on the spermatozoa.

There was no direct relation between the IgA MAR % and the sperm agglutination titer in the serum (Table 2a). This can easily be explained as the agglutination titer in the serum is mostly due to antispermatozoal IgG. However, there was also no direct relation between the IgA MAR % and the titer in the SP (Table 2b). Low IgA MAR % values (20 or less) were found together with sperm agglutination titers up to 16. Possible, the agglutination activity in the SP was, in these cases, not caused by IgA. On the other hand, IgA MAR % values of 80 and higher were found with a SP titer < 4. The antispermatozoal antibodies may have been absorbed from the SP by the spermatozoa. Variation of absorption of antispermatozoal IgA, due to variation of the concentration of spermatozoa in the ejaculate, may also be the reason for the relatively poor correlation between the degree of auto-agglutination and the IgA MAR % (Table 4). In addition, the degree of auto-agglutination will depend on the motility of the spermatozoa and, possibly, on the avidity of the antispermatozoal IgA.

It is seen from Table 2c that the IgA MAR % is roughly proportional to the S %. However, the scattering is rather wide. One explanation for this, is the variation in the estimation of both the IgA MAR % and the S %. Possibly, in some cases the IgA MAR % was underestimated due to a slow reaction rate in the MAR test for IgA. It is unlikely that unexpectedly low IgA MAR % values were due to inactivation of the anti-IgA antiserum as a result of a high IgA concentration in the semen. The concentration of IgA in SP from normal and infertile men was found by us to be maximal 8.7 mg/dl. This is in aggreement with the reports of Rümke (1974), Friberg (1974), Tauber et al. (1975), and Husted & Hjort (1975). This IgA concentration is much lower than the "neutralizing capacity" of the antisera we used. From the absence of a direct relation between the S % and the IgG MAR % on one hand and the presence of a roughly proportional relationship between the S % and the IgA MAR % on the other hand, it was concluded that *the shaking phenomenon in the SCMC test, with spermatozoa from infertile men with antispermatozoal antibodies, probably is due to the presence of IgA molecules on the motile spermatozoa.*

In these infertile men also the agglutination activity in the semen is caused by probably locally produced IgA, as mentioned before. Sperm agglutination and shaking phenomenon are the two activities by which antispermatozoal antibodies reduce the ability of spermatozoa to penetrate into CM. Therefore, we can also conclude that *the reduced ability of penetration into CM by spermatozoa from infertile men with antispermatozoal antibodies, is caused by IgA that is produced locally in the male genital tract.* This does not mean that IgG cannot induce a shaking phenomenon. Fjällbrant (1969a) showed that incubation in sperm agglutinating sera could reduce the ability of spermatozoa to penetrate into CM. As the sperm agglutinating activity in serum is mostly due to IgG,

it is probable that also the presence of IgG on motile spermatozoa can cause a shaking phenomenon. However, the concentration of antispermatozoal IgG in semen is probably too low to produce a shaking phenomenon.

Acknowledgments

The authors are grateful to Dr. Ph. Rümke for his continuous advice and to Prof. Ph. J. Hoedemaeker for commenting on the manuscript. We also wish to thank Dr. D. R. Franken for the correction of the English text.

References

Boettcher B.. T. Hjort, P. Rümke, S. Shulman & O. E. Vyazov (1977) Auto- and iso-antibodies to antigens of the human reproductive system. I 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 path. microbiol. scand. Sect. C. Suppl. 258*, 1.

Coombs R. R. A., J. Marks & D. Bedford (1956) Specific mixed agglutination: Mixed erythrocyte-platelet anti-globulin reaction for the detection of platelet antibodies. *Brit. J. Haemat. 2*, 84.

Fjällbrant B. (1968) Interrelation between high levels of sperm antibodies, reduced penetration of cervical mucus by spermatozoa, and sterility in men. *Acta obstet. gynec. scand. 47*, 102.

Fjällbrant B. (1969a) Cervical mucus penetration by human spermatozoa treated with anti-spermatozoal antibodies from rabbit and man. *Acta obstet. gynec. scand. 48*, 71.

Fjällbrant B. (1969b) Studies on sera from men with sperm antibodies. *Acta obstet. gynec. scand. 48*, 131.

Friberg J. (1974) Clinical and immunological studies on sperm-agglutinating antibodies in serum and seminal fluid. *Acta obstet. gynec. scand. Suppl. 36*, 1.

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

Husted S. & T. Hjort (1975) Sperm antibodies in serum and seminal plasma. *Int. J. Fertil. 20*, 97.

Jager S., J. Kremer & T. van Slochteren-Draaisma (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. Fertil. 23*, 12.

Jager S., J. Kremer & T. van Slochteren-Draaisma (1979) Presence of sperm agglutinating antibodies in infertile men and inhibition of in vitro sperm penetration into cervical mucus. *Int. J. Androl. 2*, 117.

Kremer J. (1965) A simple sperm penetration test. *Int. J. Fertil. 10*, 209.

Kremer J. & S. Jager (1976) The sperm cervical mucus contact test: a preliminary report. *Fertil. Steril. 27*, 335.

Kremer J., S. Jager & T. van Slochteren-Draaisma (1978) The "unexplained" poor post-coital test. *Int. J. Fertil. 23*, 277.

Mancini G., A. O. Carbonara & J. F. Heremans (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochem.* 2, 235.
Rümke P. (1974) The origin of immunoglobulins in semen. *Clin. exp. Immunol.* 17, 287.
Tauber P. F., L. J. D. Zaneveld, D. Propping & G. F. B. Schumacher (1975) Components of human split ejaculates. I. Spermatozoa, fructose, immunoglobulins, albumin, lactoferrin, transferrin and other plasma proteins. *J. Reprod. Fertil.* 43, 249.
Tomasi T. B., E. M. Tan, A. Solomon & R. A. Prendergast (1965) Characteristics of an immune system common to certain external secretions. *J. exp. Med.* 121, 101.
Wide L. & C. A. Gemzell (1960) An immunological pregnancy test. *Acta endocrin. (Kbh.)* 35, 261.

Author's address:

S. Jager, Fertility Unit of the Department of Obstetrics and Gynaecology,
University Hospital, Groningen, The Netherlands.

CHAPTER 7

IMMUNOGLOBULIN CLASS OF SPERM AGGLUTININS IN CERVICAL MUCUS

IMMUNOGLOBULIN CLASS OF SPERM AGGLUTININS IN CERVICAL MUCUS

S. Jager, J. Kremer, J. Kuiken and T. van Slochteren-Draaisma

Fertility Unit, Department of Obstetrics and Gynaecology
University Hospital, Groningen, The Netherlands

The presence in cervical mucus of antisperm antibody correlated to inhibition of the in vivo or in vitro sperm penetration has been reported by Parish et al. (1967), D'Almeida & Eyquem (1975) and Soffer et al. (1976), Recently Kremer et al. (1977) reported the presence of sperm agglutinins in cervical mucus of five women with repeatedly poor results of the in vivo and in vitro sperm penetration tests. From table 1 it is seen that, in spite of the good qualities of the husband's semen (at least 60 million spermatozoa per ml and at least 50% good progressive motility), the results of the in vitro sperm penetration tests with the cervical mucus of these women were very poor, compared to the results obtained with normal donor cervical mucus. The cervical mucus of the five women tested showed signs of excellent estrogenic stimulation, while the pH was in the normal range (6.3 to 7.2). The poor sperm penetration can be ascribed to the so called shaking phenomenon as has been demonstrated in the SCMC test (Kremer & Jager, 1976). In this test at least 80% of the motile spermatozoa were seen locally shaking after the husband's semen has been mixed with cervical mucus from the wife.

Table I. Comparison of the results of the sperm cervical mucus contact (SCMC) test (Kremer & Jager, 1976) and of the results of the sperm penetration meter test (Kremer, 1965) in couples with sperm agglutinins in the cervical mucus

Couple	% locally shaking sperms in SCMC test	Penetration into cervical mucus					
		of wife			of donor		
		cm	sperms per LPF	motility grade	cm	sperms per LPF	motility grade
A	80	3	0 - 5	1-	5	20- 50	2+
B	>80	3	0 - 1	1	5	100-200	1+
P	>90	5	0 - 1	1	5	100-200	2
S	100	5	0 - 1	<1	5	>200	2+
Z	>80	3	0 - 1	1	5	100-200	2+

Table II. Comparison of the results of the sperm cervical mucus contact (SCMS) test (Kremer & Jager, 1976) on cervical mucus and of the results of the tray agglutination test (Friberg, 1974) on cervical mucus and serum.

Number of women	% locally shaking sperms in SCMC test	Distribution of results in relation to sperm agglutination titer in:							
		cervical mucus				serum			
		≤4	8	16	≥32	≤4	8	16	≥32
40	0 - 25	39	1	-	-	34	3	2	1
1	26 - 50	1	-	-	-	1	-	-	-
0	51 - 75	-	-	-	-	-	-	-	-
5	76 -100	-	-	-	5	-	-	-	5

The shaking phenomenon, observed in the SCMC test, was compared to the sperm agglutination titer in the cervical mucus (table II). Cervical mucus samples from the women in a group of 46 infertile couples were investigated. The mucus was aspirated from the endocervix with a disposable plastic syringe without a needle, mostly, during the estrogenous phase of a sequential pill cycle.

The cervical mucus employed in the test was first treated with a bromelin preparation (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service) in order to liquify it before determining the sperm agglutination titer. The cervical mucus was weighed and a same amount of the bromelin solution was added. In general, the duration of the incubation period varied from one to two hours. In the last stage of this investigation it was found that "solubilization" of the mucus could be obtained within about five minutes at room temperature by repeatedly sucking up and expelling the mixture with the aid of a syringe.

The sperm agglutination titer was determined in a two-fold dilution series with the tray agglutination test according to Friberg (1974) as modified by Hellema & Rümke (1976). Spermatozoa with good progressive motility were obtained from donor semen.

Table II shows that the shaking phenomenon observed in the SCMC test correlated with the presence of a high sperm agglutination titer in the cervical mucus. From the 46 women tested only the five women with a shaking phenomenon of more than 75% showed a sperm agglutination titer of at

Table III. Immunoglobulin class of sperm agglutinins present in cervical mucus treated with bromelin. Effect of absorption with monospecific rabbit anti-human-immunoglobulin antiserum on sperm agglutinating activity.

Patient	Agglutination titer and type			
	Before absorption	Absorbed with		
		anti-IgA	anti-IgG	anti-IgM
	titer type	titer type	titer type	titer type
A	64	<4	nt	nt
B	4096 H-H	<4	4096 H-H	4096 H-H
P	512 H-H	<4	nt	nt
S	128 H-H	<4	128 H-H	64 H-H
Z	4096 H-H	<4	2048 H-H	2048 H-H

nt = not tested

least 32, whereas the women with a shaking phenomenon of less than 26% had no sperm agglutinins or only a low titer in the cervical mucus. In the latter group three women had a titer of at least 16 in the serum. All five women with high titers in the cervical mucus also had moderate to high titers in the serum, at the time the cervical mucus being tested was collected. These five women were treated with condom therapy and intrauterine inseminations. The results of this treatment will be dealt with elsewhere (Kremer & Jager, 1977).

The immunoglobulin class of the sperm agglutinating antibody was determined by absorption with monospecific rabbit anti-human-immunoglobulin antisera (table III). The antisera were fractionated before by gelfiltration over Sephadex G-200. The protein peak containing the 7S immunoglobulins was concentrated thereafter by ultrafiltration to 3/4 of the original volume of the antiserum. Five microliters of the concentrated antiserum fraction were added to 25 microliters of "solubilized" cervical mucus. The sperm agglutination titer was then determined in a twofold dilution series as described before.

The sperm agglutination titers in the cervical mucus were found ranging from 64 in patient A to a titer of 4096 in patients B and Z. The agglutination was of the head-to-head type and could be absorbed with anti-IgA antiserum but mot with anti-IgG and anti-IgM.

Table IV. Immunoglobulin classes of antisperm antibodies present in cervical mucus treated with bromelin. Results of indirect mixed antiglobulin reaction tests

Patient	% of the motile spermatozoa incorporated into mixed agglutinates in tests for:			
	IgA	secretory component	IgG	IgM
A	50	<10	50	0
B	60	20	70	<10
P	>90	60	100	<10
S	90	50	30	0
Z	90	20	80	<10

The presence of antisperm immunoglobulins in cervical mucus could also be demonstrated with indirect mixed antiglobulin reaction tests (Coombs et al., 1956). Cervical mucus treated with bromelin was mixed with an equal amount of fresh donor semen, the sperm suspension was incubated at 37 ^{o}C for 15 to 60 minutes. One drop of the sperm suspension was then mixed with one drop of a suspension of indicator particles and one drop of a monospecific rabbit antiserum reactive with human immunoglobulins or secretory component.

For demonstration of antisperm IgG the indicator particles were group O, Rh-positive erythrocytes (R_1R_2 type) sensitized with incomplete anti-D antibodies. In the tests for IgA, IgM and secretory component the indicator particles were Dow latex spheres (mean diameter 5.7 micron) coated according to Bercks as described in Kwapinsky (1972), with the gamma globulin fraction of a goat anti-rabbit IgG antiserum in the test for IgM and with the gammaglobulin fraction of colostrum in the tests for IgA and secretory component.

Table IV shows that antisperm IgA and antisperm IgG were present in the cervical mucus of the five fertile women. With four women also secretory component could be demonstrated. The reaction was negative or very low for IgM.

An attempt has been made to relate the shaking phenomenon to the immuno-globulin class of the antisperm antibodies. Bromelin treated cervical mucus from patients A and P was mixed with anti-IgA, anti-IgG or anti-IgM

antiserum. Fresh donor semen was added too, and the resulting sperm suspension incubated at 37 ^{o}C for one hour. In an SCMC-test type II (Kremer & Jager, 1976) 3 microliters of the sperm suspension were laid upon fresh untreated cervical mucus spread out upon a microslide.

After absorption with anti-IgA antiserum 40% of the motile spermatozoa remained progressively motile. When anti-IgA and anti-IgG antiserum were both added the same result was obtained. After absorption of IgG or IgM almost 100% of the motile spermatozoa were seen locally shaking. These results, which were found reproducible, indicate that antisperm IgA is responsible for the shaking phenomenon in the SCMC test. However, the limited number of observations does not yet enable the reaching of a definite conclusion.

SUMMARY

Cervical mucus obtained from five infertile women, in which sperm penetration was inhibited due to a strong shaking phenomenon, was investigated. After pretreatment of the cervical mucus with bromelin, which greatly facilitates the determination of antisperm antibodies, high sperm agglutination titers were found in the cervical mucus of these five women. The sperm agglutination activity could be absorbed completely with anti-IgA antiserum. With indirect mixed antiglobulin reaction tests we could demonstrate the presence of antisperm IgA, at least partly of the secretory IgA subclass, and antisperm IgG. Preliminary experiments indicate that the shaking phenomenon and therefore the inhibition of the sperm penetration, might be due to the action of antisperm antibodies of the IgA class.

REFERENCES

Coombs, R. R. A., Marks, J. and Bedford, D. (1956) Brit. J. Haematol. 2: 84.

D'Almeida, M. and Eyquem, A. (1975) Abstract, Third Int. Symp. Immunol. Reprod., Varna, p. 83.

Friberg, J. (1974) Acta Obst. Gynecol. Scand., Supple. 36:1.

Hellema, H. J. W. and Rümke, Ph. (1975) Fert. Steril. 27: 284.

Kremer, J. (1965) Int. J. Fert. 10: 209.

Kremer, J. & Jager, S. (1976) Abstract, Ninth World Congr. Fert. Steril. Miami Beach, p. 316.

Kremer, J., Jager, S. and Van Slochteren-Draaisma, T. (1977) Abstract, Ninth World Congr. Fert. Steril., Miami Beach, p. 295.

Kwapinsky, J. B. G. (1972) Methodology of Immunochemical and Immunological Research, New York, p. 387.

Parish, W. E., Carron-Brown, J. A. and Richards, C. B. (1967) J. Reprod. Fert. 13: 469.

Soffer, Y., Marcus, Z. H., Bukovsky, I. and Caspi, E. (1976) Int. J. Fert. 21: 89.

CHAPTER 8

INDUCTION OF THE SHAKING PHENOMENON BY PRETREATMENT OF SPERMATOZOA WITH SERA CONTAINING ANTISPERMATOZOAL ANTIBODIES

INDUCTION OF THE SHAKING PHENOMENON BY PRETREATMENT SPERMATOZOA WITH SERA CONTAINING ANTISPERMATOZOAL ANTIBODIES

S. Jager, J. Kremer, J. Kuiken, Tiny van Slochteren-Draaisma, Ina Mulder and Irma W. de Wilde-Jansen

Fertility Unit of the Department of Obstetrics and Gynaecology, University Hospital, Groningen, The Netherlands

ABSTRACT

Sera, containing sperm agglutinating and/or complement depending sperm immobilization activity, were tested for their ability to induce a shaking phenomenon in the SCMC test. Donor spermatozoa were treated with the sera in a one step incubation and washing procedure. The percentage of motile pretreated spermatozoa, showing the shaking phenomenon (S%), was determined in the sperm cervical mucus contact test. In addition, the immunoglobulin class of antibodies present on the pretreated spermatozoa was determined in mixed antiglobulin reaction tests for IgG, IgM and IgA. A S% of at least 80 was always observed with spermatozoa pretreated in sera with a sperm agglutination titer of at least 32 and provided that IgG was detected on more than 90% of the motile spermatozoa. The high S% was also obtained with spermatozoa pretreated in purified IgG from sera containing antispermatozoal antibody activity. We concluded that the reduced cervical mucus penetration capacity of spermatozoa pretreated with sera containing antispermatozoal IgG can be ascribed, at least partially, to the occurrence of a shaking phenomenon.

INTRODUCTION

Incubation of spermatozoa in sera with antispermatozoal antibody activity can result in a reduced ability of the spermatozoa to penetrate cervical mucus (Fjällbrant, 1969; Manarang-Pangan & Behrman, 1971; Boettcher & Gruszynski, 1978). The reduced sperm penetrability is partly due to agglutination and/or complement depending immobilization of the spermatozoa during the incubation in the serum. Inhibition of the sperm penetration might also be due to complement present in the cervical mucus. Complement has been found in pooled cervical mucus from two women at approximately 11% of the serum level. This complement level would be sufficient to immobilize the majority of the pretreated spermatozoa within a few hours (Price & Boettcher, 1979). The reduced penetration capacity of spermatozoa pretreated with sera containing antispermatozoal antibody activity might also be due to the occurrence of the so called "shaking phenomenon". This phenomenon has been observed previously with freely swimming spermatozoa from infertile men with antispermatozoal antibodies. These spermatozoa changed their forward motility into local shaking movements immediately after contact with normal pre-ovulatory cervical mucus. The shaking phenomenon could not be ascribed to complement depending immobilization of the spermatozoa, because vigorous shaking movements were seen to persist for many hours. In addition, preceding inactivation of the complement by heat treatment of the mucus did not prevent the shaking phenomenon (Kremer & Jager, 1976). Moreover, the shaking phenomenon was found to correlate with presence of IgA and not with presence of IgG on the motile spermatozoa from the infertile men with antispermatozoal antibodies (Kremer et al., 1977; Jager et al., 1977; Jager et al., 1980). On the other hand, antispermatozoal antibodies in sera from men were found to be predominantly IgG and in sera from women mostly IgM (Boettcher et al., 1971; Friberg, 1974; Husted & Hjort, 1975).

The aim of this study is to demonstrate that motile spermatozoa pretreated with sera containing antispermatozoal antibodies can show a shaking phenomenon upon contact with cervical mucus. It will also be demonstrated that the shaking phenomenon can be caused by purified antispermatozoal IgG.

MATERIALS AND METHODS

Samples for testing

Sera from 22 men and 32 women from infertile couples visiting the fertility unit, sera from five vasectomized men and 23 sera from the WHO Reference Bank for Reproductive Immunology at Aarhus were investigated. The WHO sera were studied previously with several antispermatozoal antibody tests in an international cooperative investigation (Boettcher et al., 1977). WHO serum number 2 was replaced by another serum sample (number 120) from the same man. The IgG fractions from five sera (three men and two women from infertile couples were also tested. In addition, we studied a solution of heat aggregated IgG (0.1 g/l) and sera from two men and five women with systemic disaeses who had circulating immune complexes. These samples were kindly provided by Dr M. van der Giessen (Department for Clinical Immunology, University Hospital, Groningen).

Spermatozoa

Spermatozoa with good motility were obtained from normal semen samples from young, apparently healthy men. Semen was considered normal if it contained per ml at least 60 x 10^6 spermatozoa with at least 50% good forward motility and at least 50% normal head forms.

Cervical mucus

Cervical mucus permitting good sperm penetration was obtained from women visiting the fertility unit. Collection and investigation of the cervical mucus was described previously (Jager et al., 1978).

Isolation of IgG

IgG was isolated from sera by affinity chromatography over a column of protein A-Sepharose CL-4B (Pharmacia) as recommended by the manufacturer. For removal of contaminating IgA and IgM the eluate from the protein A-Sepharose CL-4B column was passed twice through columns of antihuman IgA (Behring) resp. antihuman IgM (Nordic) immobilized by coupling to CNBr-Sepharose-4B (Pharmacia) as described previously (Jager et al., 1980). The purified IgG was concentrated by ultrafiltration through Amicon 25 CF centriflo membrane cones by centrifugation at 750 g for at least 10 minutes to between 50% and 100% of the original serum volume. The purity of the concentrated IgG solution was checked by double diffusion against

antihuman colostral IgA (Dako) and antihuman IgM (Dako).

The tray agglutination test (TAT)
The tray agglutination test was performed according to Friberg (1974) but modified as described previously (Jager et al., 1978).

The tray immobilization test (TIT)
The TIT was performed on a microscale with the same equipment as used for the TAT. Sera to be tested were pretreated by heating at 56 ^{o}C for 30 minutes to inactivate complement. Five microliters of the sample were transferred to the disposable microchamber under parafin oil. Subsequently one microliter of undiluted guinea pig serum (stored at -70 ^{o}C until used) and one microliter of a spermatozoa suspension in Earle's balanced salt solution (carbonate free), containing 1% bovine serum albumin, pH 7.4 were added. The spermatozoa suspension with a high percentage of motile spermatozoa was prepared as described by Hellema & Rümke, 1978) and diluted to 60 x 10^{6} spermatozoa per ml. For the control one microliter of heat inactivated guinea pig serum and one microlter of the spermatozoa suspension were added to another five microliters of the sample. A negative and a positive control serum were always included. For determination of the sperm immobilization titer the samples were diluted with human serum, without sperm immobilizing activity in a twofold dilution series. The test was read after one hour incubation at 37 ^{o}C. The result was considered positive if the sperm motility decreased to 50% or less of the sperm motility in the heat inactivated guinea pig serum. The sample was considered positive only if a positive result was obtained with spermatozoa from two different donors. The differences were never more than two titersteps. Mean titer values, rounded off upwards to an exponent of 2, will be presented in this paper.

Incubation and washing of spermatozoa for SCMC and MAR tests
Spermatozoa were incubated and washed in one step by a density gradient centrifugation method modified after Koo et al. (1973). In Kibrick tubes (internal dimensions 2 x 37 mm) three layers were pipetted:
Bottom layer: Bovine serum albumin (12.5%) prepared by mixing equal amounts of 25% bovine serum albumin in sterile Tyrode solution (Sigma) with Earle's balanced salt solution.

Intermediate layer: A mixture of 40 microliters of serum or IgG solution and 4 microliters of a 1% Trypan blue solution. The addition of the dye ensured an easy recognition of the three layers. To increase the density of the IgG solution bovine serum albumin was added to a final concentration of 6%.

Top layer: A suspension of spermatozoa with good motility diluted to about 20 x 10^6 per ml with the Earle's balanced salt solution.

The height of each layer was about 1 cm. Directly after preparation of the density gradient the tube was centrifuged for 10 minutes at 200 g at room temperature. The top and intermediate layer were carefully pipetted off and the bottom layer was tested in the SCMC and MAR tests.

Mixed antiglobulin raction (MAR) tests

Indicator particles. Blood group O, Rh-positive erythrocytes sensitized with a strong anti-D serum as described previously (Jager et al., 1978), were used in the MAR test for IgG. The IgA MAR test was performed with blood group O, Rh-negative erythrocytes coated with colostral IgA (Jager et al.,1980). IgM coated red blood cells were prepared in a similar procedure as used for IgA erythrocytes.

IgM, for coating of erythrocytes, was isolated from serum of a patient with Waldenström's macroglobulinemia. Five volumes of 1.75 M $(NH_4)_2SO_4$ were added to the serum. The precipitate was washed once with the $(NH_4)_2SO_4$ solution and dissolved in a half volume of 0.1 sodium acetate + 0.3 M NaCl, pH 7.2. To remove contaminating IgG and IgA, the dissolved precipitate was applied to the protein A-Sepharose CL-4B column, to which was connected a second column of immobilized antihuman IgA. The peak of unbound proteins was concentrated six times by ultrafiltration. The final protein concentration, determined with the biuret reaction, was 13 g/l. With radial immunodiffusion the IgM concentration was found to be 11.7 g/l. With double diffusion a faint precipitation line against antihuman IgG was seen. IgA could not be detected.

Antisera used were monospecific rabbit antihuman IgG antiserum (Behring), rabbit antihuman colostral IgA antiserum (Dako) and rabbit antihuman IgM antiserum (Dako). The antisera were pretreated by dialyzing against phosphate buffered saline.

Performance of the test. MAR tests were performed by transferring two microliters respectively of, the incubated spermatozoa suspension, the sensitized or coated erythrocytes suspension and the corresponding anti-

serum. Each addition was followed by thoroughly mixing. The suspension was then sucked up into a flat capillary tube (Vitro Dynamics) by capillary force and the reaction was studied under a warm stage microscope at 35 °C. The optical path length of the capillary tube was 0.05 mm or 0.1 mm.

Reading of the test. Immediately after mixing the spermatozoa were immotile but gradually the motility was regained. The test was read only if the motility was good and the percentage of motile spermatozoa attached to the erythrocytes (MAR%) did not increase any longer. Strongly positive IgG MAR% values (>90) were always obtained within five minutes. Strongly positive IgA MAR% values and weakly positive MAR% values could increase during up to 60 minutes. With some sera the MAR% decreased from a high value, soon after mixing, to a low value in about 20 to 30 minutes. We considered this effect as non-specific for antigen-antibody reaction as it was also observed if no other evidence for presence of antispermatozoal antibodies was found. In these cases the lowest MAR% was noted. The results of the MAR tests were expressed as <10, 10, a multiple of 10 to 90 or >90. Each sample was tested with spermatozoa from two different donors. In this paper mean values rounded off upwards to a multiple of 10 are presented. If one of the two MAR% values was <10 or >90 the other one was never more than 30 resp. less than 80. With weakly positive MAR% values differences up to 50 were observed. Previously we found a similar high variability for weakly positive MAR% values with the direct IgG MAR test performed on fresh ejaculates (Jager et al., 1978).

The sperm-cervical mucus contact (SCMC) test

The SCMC test type 1, where spermatozoa and cervical mucus are thoroughly mixed, was performed as described by Kremer & Jager (1976), Kremer et al. (1978b) and Jager et al. (1979). In the SCMC test the percentage of motile spermatozoa showing quickly shaking movements (S%) is estimated. Spermatozoa showing quickly shaking movements combined with slow propulsion were included in the S%. The shaking movements observed with pretreated donor spermatozoa were somewhat slower than seen with untreated spermatozoa from infertile men with antispermatozoal antibodies. Probably the incubation and washing procedure results in a slight damage of the spermatozoa. With each mucus sample also a serum sample, without sperm agglutinating activity was tested. Each sample was tested with

spermatozoa from at least two different donors. Expression and presentation of the results is the same as for the MAR% values. The differences of the S% values with the two donors on the same sample was 30 at most except for WHO serum number 11 (table 3) with S% values of 40 and 80.

Determination of immune complexes

The determination of immune complexes was performed by Dr M. van der Giessen (Department for Clinical Immunology, University Hospital, Groningen) with three different techniques: 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); a solid phase C1q-binding assay (Van der Giessen et al., 1980) and a poly-ethyleneglycol precipitation test.

RESULTS

Sperm agglutination titers, sperm immobilization titers, IgG MAR% values, IgM MAR% values, IgA MAR% values and S% values obtained with sera from men and women from infertile couples and with sera from the WHO Reference Bank for Reproductive Immunology are presented in tables 1, 2 and 3. Table 4 shows the antispermatozoal antibody activity found in the sera containing the immune complexes and in the solution of heat aggregated IgG. In some IgG MAR tests the MAR% decreased from a high or intermediate value soon after mixing, with more than 20 after 10 to 30 minutes. Duplicate tests mostly showed only the low value. Serum IC-7 was from a 63 years old man with a severe rheumatoid arthritis. He also had a necrotizing vasculitis in one leg. He had children from two wifes and was not vasectomized. Results with purified IgG are presented in table 5. Sperm immobilization titers are not presented in this table because in most tests the spermatozoa in the inactivated controls also showed strongly decreased motility

DISCUSSION

Comparison of sperm agglutination and immobilization titers in our series (tables 1, 2 and 3) reveales essentially the same feature found previous-

Table 1

Antispermatozoal antibody activities in sera from 22 men from infertile couples and in sera from five vasectomized men

Patient number	Sperm agglutination		Sperm immobilization	% of motile spermatozoa with			% of shaking spermatozoa
	Titer	Type	titer	IgG	IgM	IgA	
(1)	(2)	(3)	(3)	(5)	(6)	(7)	(8)
M1	16384	T-T/Tt-Tt	1	>90	<10	<10	>90
M2	8192	M	16	>90	40↓	90	>90
M3 v	4096	M	32	>90	40	70	>90
M4 v	4096	T-T	16	>90	<10	<10	>90
M5	2048	T-T	2	>90	<10	70	>90
M6	1024	M	8	>90	<10	70	>90
M7	1024	T-T	8	>90	<10	10	70
M8	1024	T-T	8	>90	<10	<10	70
M9	512	T-T	4	>90	<10	<10	>90
M10	512	Tt-Tt	0	>90	20	<10	50
M11	256	T-T	2	>90	<10	10	80
M12	256	T-T	8	>90	<10	<10	80
M13	256	T-T	8	>90	<10	<10	80
M14	256	T-T	2	>90	<10	<10	90
M15	256	M	1	>90	<10	<10	70
M16	128	T-T	2	>90	<10	<10	90
M17	128	T-T	1	>90	<10	<10	60
M18	128	H-H	2	90	10	30	70
M19	64	T-T	2	>90	<10	20	70
M20	64	T-T	0	>90	<10	<10	30
M21 v	32	T-T	1	>90	<10	<10	40
M22	<4	-	1	<10	<10	<10	20
M23	<4	-	1	<10	<10	<10	20
M24	<4	-	1	<10	<10	<10	10
M25	<4	-	0	<10	<10	<10	20
M26 v	<4	-	0	<10	<10	<10	20
M27 v	<4	-	0	<10	<10	<10	10

(1) v = vasectomized man.
(2) determined in the tray agglutination test according to Friberg (1974) with slight modifications (Jager et al., 1978).
(3) determined in the tray agglutination test; T-T = tail-to-tail, Tt-Tt = tail tip-to-tail tip, M = mixed; H-H = head-to-head.
(4) determined in the tray immobilization test (see methods).
(5),(6),(7) determined in indirect mixed antiglobulin reaction tests (see methods) ↓ = a high value initially seen in at least one test (see text).
(8) determined in the indirect sperm cervical mucus contact test (see methods).

Table 2

Antispermatozoal antibody activities in sera from 32 women from infertile couples

Patient number	Sperm agglutination		Sperm immobilization titer	% of motile spermatozoa with			% of shaking spermatozoa
	Titer	Type		IgG	IgM	IgA	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
W1	512	H-H	2	>90	<10	<10	>90
W2	512	M	2	>90	<10	10	70
W3	256	H-H	2	>90	<10	>90	>90
W4	256	T-T	1	<10	50	<10	30
W5	128	H-H	16	>90	<10	90	>90
W6	128	H-H	0	60	<10	<10	40
W7	128	H-H	32	>90	<10	10	>90
W8	128	H-H	0	>90	50	<10	30
W9	64	M	2	>90	<10	10	>90
W10	64	T-T	1	50	50	<10	70
W11	64	H-H	0	>90	<10	<10	40
W12	64	H-H	0	<10	<10	<10	20
W13	32	H-H	4	>90	<10	<10	>90
W14	32	H-H	0	<10	<10	10	10
W15	32	M	8	>90	<10	10	50
W16	8	H-H	2	>90	<10	<10	50
W17	8	H-H	1	<10	<10	<10	40
W18	4	T-T	2	70	<10	<10	20
W19	4	H-H	1	<10	<10	<10	30
W20	<4	-	2	>90	<10	<10	50
W21	<4	-	1	<10	<10	nt	50
W22	<4	-	0	50	<10	nt	40
W23	<4	-	1	<10	<10	<10	30
W24	<4	-	1	<10	<10	<10	30
W25	<4	-	1	40	<10	<10	30
W26	<4	-	0	<10	<10	<10	20
W27	<4	-	0	<10	<10	<10	10
W28	<4	-	0	20	<10	<10	20
W29	<4	-	0	<10	<10	<10	20
W30	<4	-	0	30	<10	<10	10
W31	<4	-	0	<10	<10	<10	20
W32	<4	-	0	<10	<10	<10	20

nt = not tested. For further legend see table 1.

Table 3

Antispermatozoal antibody activities in 23 sera from the WHO Reference Bank for Reproductive Immunology

	Serum number	Sperm agglutination Titer	Type	Sperm immobilization titer	% of motile spermatozoa with IgG	IgM	% of shaking spermatozoa
	(1)	(2)	(3)	(3)	(5)	(6)	(8)
Sera from men	120	2048	M	128	>90	<10	>90
	18	512	T-T	32	>90	<10	80
	28	512	M	8	>90	<10	90
	30	512	T-T	8	>90	<10	90
	15	512	M	2	40	<10	30
	17	256	T-T	64	>90	<10	>90
	16	256	M	32	>90	20	90
	21	256	M	8	>90	<10	80
	11	128	H-H	4	>90	10	60
	25	<4	-	0	70↓	<10	20
	26	<4	-	0	20	<10	30
Sera from women	1	256	H-H	2	>90	10	40
	9	256	T-T	0	30↓	10	40
	7	128	H-H	2	>90	<10	90
	13	64	H-H	2	>90	<10	30
	19	64	T-T	1	10	30↓	30
	23	16	H-H	2	10↓	<10	30
	6	8	T-T	0	30↓	<10	30
	12	8	H-H	1	<10	<10	20
	10	4	T-T	0	<10	<10	20
	4	<4	-	0	30	<10	20
	8	<4	-	0	80	<10	40
	20	<4	-	0	<10	<10	30

For legend see table 1.

Table 5

Antispermatozoal antibody activities of purified IgG from sera of three men and two women from infertile couples

Patient	Sperm agglutination Titer	Type	% of motile spermatozoa with IgG	% of shaking spermatozoa
(1)	(2)	(3)	(5)	(8)
M1	1024	T-T	>90	>90
M2	4092	M	>90	>90
W3	128	H-H	>90	90
W5	256	H-H	>90	70
M24	<4	-	20	40

For legend see table 1.

Table 4

Antispermatozoal antibody activity of seven sera containing immunecomplexes and of a solution of 0.1 g/l heat aggregated IgG

Sample	Sperm agglutination		Sperm immobilization titer	% of motile spermatozoa with			% of shaking spermatozoa	Neutrophil phagocytosis (IF score)	C1q binding titer	PEG precipitaion (Ext)	Sex	Age (years)	Disease
	Titer	Type		IgG	IgM	IgA							
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
IC-1	<4	-	0	80	<10	<10	30	296	6	182	male	69	AA
IC-2	<4	-	0	40+	<10	<10	30	488	3	114	female	30	SLE
IC-3	4	T-T	0	30	<10	<10	30	14	4	835	female	24	SLE
IC-4	4	T-T	0	20	<10	<10	20	640	2	407	female	11	SLE
IC-5	16	H-H	0	30+	<10	<10	20	146	3	177	female	59	SLE
IC-6	32	T-T	0	30+	<10	<10	20	>1000	5	242	female	15	SLE
IC-7	>512	M	0	>90	30+	>90	70	37	4	327	male	63	RA
ag-IgG	<4	-	0	10+	<10	<10	30	>1000	7	pos	-	-	-

(1) IC = immune complexes containing serum, ag-IgG = solution of heat aggregated IgG.
(2)-(8) for legend see table 1.
(9) Phagocytosis test according to Van Wingerden et al. (1979); IF = immunofluorescence; normal value $\leq$20.
(10) C1q binding test according to Van der Giessen et al. (1980); normal value $\leq$1.
(11) Ext = extinction; normal value 189±44.
(14) AA = allergic alveolitis; SLE = systemic lupus erythrmatosis; RA = rheumatoid arthritis.

ly in the WHO report (Boettcher et al., 1977); a high sperm immobilization titer generally corresponds with a high agglutination titer but the reverse correlation is much weaker. In sera from women sperm agglutination activity without sperm immobilization activity can be due to a non-antibody factor (Boettcher, 1974). In six sera (W12, W14, W17, WHO-10, WHO-12), all from women, we found sperm agglutinating activities presumably not due to antispermatozoal antibodies. With MAR tests no antispermatozoal antibodies were detected. The weak sperm immobilization activities found in three of these sera (W17, W19, WHO-12) are probably not caused by antibodies specific for spermatozoal antigens; a weak immobilization activity was also present in six sera (M22, M23, M24, W23, W24) without positive results in the other antispermatozoal antibody tests. This might mean that in our series only an immobilization titer of at least 2 is immunologically significant. We found in sera from two men a weak (M1) or an absent (M10) sperm immobilizing activity, despite a high agglutination titer. The agglutination type was partly (M1) or completely (M10) tail tip-to-tail tip. A similar case was presented in the WHO report. The high IgG MAR% values confirmed the presence of antispermatozoal antibodies. Apart from these two sera no relation was found between the agglutination type and the sperm immobilization activity.

A remarkedly high incidence of sperm agglutinating activity was found in the immune complexes containing sera. It is uncertain, however, whether the sperm agglutination activity is due to the immune complexes (table 4). The series is too small to conclude whether the high number of sperm agglutinating sera is accidental or not. In serum IC-7, no immobilizing activity was found despite a high agglutination titer. The high IgA MAR% suggests that a high proportion of the antispermatozoal antibodies in this serum might belong to the IgA class. The antibodies might be the consequence of a testicular or epididymal vasculitis. We could not obtain a serum sample from this man for testing.

An IgG MAR% of at least 90 is probably specific for the presence of antispermatozoal antibodies. We found this high MAR% always with sperm agglutination titers of at least 32 or with sperm immobilization titers of at least 2. IgG MAR% values from 10 to 80 may indicate the presence of antispermatozoal antibody concentrations too low for agglutination or immobilization. Another explanation for these weakly positive IgG MAR% values might be binding of IgG containing immune complexes to the spermatozoa through the Fc part. Witkin et al. (1980) reported rosette

formation of antibody coated ox erythrocytes with human spermatozoa. In addition, the authors found binding of aggregated human gamma globulin to spermatozoa, proportional to the aggregated globulin concentrations. Monomeric IgG bound to a much lesser extent. In the present study, however, we found only weak or temporarily high IgG MAR% values in the IgG MAR test with the aggregated IgG solution and with the immune complexes containing sera. Binding of monomeric IgG is not detected in our IgG MAR test; we found binding of erythrocytes to only less than 10% of the motile spermatozoa with all 14 sera from men and women from infertile couples with sperm agglutination titers less than 4 and sperm immobilization titers less than 2. The difference between our results and those of Witkin et al. (1980) may be due to the fact that our readings were restricted to the *motile* mixed agglutinates in our test.

The IgM MAR test showed, compared to the IgG MAR test, in relatively few sera only a weakly positive result, despite strong agglutination of the IgM coated erythrocytes. For sera from men, this preponderance of antispermatozoal IgG is in agreement with previous reports (Boettcher et al., 1971; Coombs et al., 1973; Friberg, 1974; Husted & Hjort, 1975). For sera from women, the predominance of antispermatozoal IgG is unexpected. In previous reports (Boettcher et al., 1971; Friberg, 1974) the antispermatozoal antibody activity in sera was mainly located in the IgM fraction. The strong agglutination of the erythrocytes in the IgM MAR test makes it unlikely that the test is insensitive.

The IgA MAR test is less precise than the IgG MAR test due to the slow reaction rate combined with the decrease of the motility of the spermatozoa (Jager et al., 1980). Similar difficulties have already been described by Coombs et al., 1973). The fact that only a few strongly positive results are obtained in the IgA MAR test is in agreement with the lack of predominance of antispermatozoal IgA in previous reports (Boettcher et al., 1971; Coombs et al., 1973; Friberg, 1974).

A high (at least 80) S% value can be considered as specific for antispermatozoal antibodies. We found this high S% only with a sperm agglutination titer of at least 32, a sperm immobilization titer of at least 2 (except one case: M1) and an IgG MAR% more than 90. Intermediate (40-70) S% values mostly correspond with the presence of antibodies reactive with spermatozoa. With serum W21 we ascribe the intermediate S% value to a non-immunoglobulin factor because the results of all tests for antispermatozoal antibodies were negative. A low (30 or less) S% value was

found mostly with a sperm agglutination titer less than 32, a sperm immobilization titer of 1 at most and IgG MAR% values lower than 90. However, with three sera (M20, W8, WHO-13) the S% was low despite a high IgG MAR% combined with a sperm agglutination titer of at least 64. With these sera the antibody coating of the motile spermatozoa was probably too weak for the induction of a shaking phenomenon. Pretreatment of donor spermatozoa with sera containing antispermatozoal antibodies can thus result in a shaking phenomenon involving almost all motile spermatozoa upon contact with cervical mucus. We conclude that the reduced cervical mucus penetration by spermatozoa pretreated in sera containing antispermatozoal antibodies, can be ascribed, at least partially, to the occurrence of a shaking phenomenon after contact of the sensitized spermatozoa with the cervical mucus.

Previously we found in the SCMC test, with spermatozoa from men with antispermatozoal antibodies, that the S% was roughly proportional to the IgA MAR% but no direct relation was seen between the S% and the IgG MAR%. We, therefore, supposed that the shaking phenomenon with spermatozoa from infertile men was due to, locally produced, IgA (Jager et al., 1980). The IgG coating of the spermatozoa from these men was probably too sparse for the occurrence of a shaking phenomenon. We ascribe the sparse coating with IgG to the low antispermatozoal activity of IgG in the semen from men with antispermatozoal antibodies as demonstated by Husted & Hjort (1975). We found in the present investigation that also spermatozoa pretreated with sera containing antispermatozoal IgG can show a shaking phenomenon in the SCMC test. In all cases with a S% of at least 80 we found IgG on more than 90% of the motile spermatozoa. An high IgA MAR% was found only with three sera (M2, W3, W5). Results with purified IgG confirm that antispermatozoal IgG can cause a shaking phenomenon. It thus appears that the occurrence of a shaking phenomenon is not specific for a particular immunoglobulin class.

ACKNOWLEDGMENTS

This investigation was supported by a grant of the Organization for Health Research TNO. The authors are grateful to Dr Ph. Rümke for valuable advice and criticism, to prof. Ph Hoedemaeker for commenting on the manuscript, to Dr M. van der Giessen for supplying the heat aggregated IgG and the immune comlexes containing sera and to Dr R. Mathias for correction of the english text.

REFERENCES

Boettcher, B. (1974) The molecular nature of spermagglutinins and sperm antibodies in human sera. J. Reprod. Fert., Suppl. 21, 151-167.

Boettcher, B., Hjort, T., Rümke, P., Shulman, S. and Vyazov, O. E. (1977) Auto- and iso-antibodies 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., Sect. C, Suppl. 258, 1-69.

Boettcher, B. and Gruszynski, R. (1978) The passage of human spermatozoa treated with spermantibodies through cervical mucus. In: Immunology of Reproduction (Bratanov, K., Vulchanov, V. H., Dikov, V., Somlev, B. and Kozhouharova, L. eds.). pp 444-449. Publishing House of the Bulgarian Academy of Sciences, Sofia.

Boettcher, B., Kay, D. J., Rümke, P. and Wright, L. E. (1971) Human sera containing immunoglobulin and non-immunoglobulin spermagglutinins. Biol. Reprod. 5, 236-245.

Coombs, R. R. A., Rümke, P. and Edwards, R. G. (1973) Immunoglobulin classes reactive with spermatozoa in the serum and seminal plasma of vasectomized and infertile men. In: Immunology of Reproduction (Bratanov, K., Edwards, R. G., Vulchanov, V. H., Dikov, V. and Somlev, B. eds.). pp 354-358. Bulgarian Academy of Sciences Press, Sofia.

Fjällbrant, B. (1969) Cervical mucus penetration by human spermatozoa treated with antispermatozoal antibodies from rabbit and man. Acta Obst. Gynec. Scand. 48, 71-84.

Friberg, J. (1974) Clinical and immunological studies on sperm agglutinating antibodies in serum and seminal fluid. Acta Obst. Gynec. Scand. Suppl. 36, 1-76.

Hellema, H. W. J. and Rümke, P. (1978) The micro-sperm immobilization test: the use of only motile spermatozoa and studies of complement. Clin. exp. Immunol. 31, 1-11.

Husted, S. and Hjort, T. (1975) Sperm antibodies in serum and seminal plasma. Int. J. Fert. 20, 97-105.

Jager, S., Kremer, J. and 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, 12-21.

Jager, S., Kremer, J. and Van Slochteren-Draaisma, T. (1979) Presence of sperm agglutinating antibodies in infertile men and inhibition of in vitro sperm penetration into cervical mucus. Int. J. Androl. 2, 117-130.

Jager, S., Kremer, J., Kuiken, J. and Van Slochteren-Draaisma, T. (1980) Immunoglobulin class of antispermatozoal antibodies from infertile men and inhibition of in vitro sperm penetration into cervical mucus. Int. Androl. 3, 1-14.

Koo, G. C., Stackpole, C. W., Boyse, E. A., Hämmerling, U. and Lardis, M. P. (1973) Topographical location of H-Y antigen on mouse spermatozoa by immunoelectronmicroscopy. Proc. Nat. Acad. Sci. USA 70, 1502-1505.

Kremer, J. and Jager, S. (1976) The sperm-cervical mucus contact test: A preliminary report. Fert. Steril. 27, 335-340.

Kremer, J., Jager, S. and Kuiken, J. (1977) The clinical significance of antibodies to spermatozoa. In: Immunological influence on human fertility (Boettcher, B. ed.). pp 47-66. Academic Press, Sydney.

Kremer, J., Jager, S. and Van Slochteren-Draaisma, T. The "unexplained" poor postcoital test. Int. J. Fert. 23, 277-281.

Manarang-Pangan, S. and Behrman, S. J. (1971) Spermatotoxicity of immune sera in human infertility. Fert. Steril. 22, 145-151.

Price, R. J. and Boettcher, B. (1979) The presence of complement in human cervical mucus and its possible relevance to infertility in women with complement-dependent sperm-immobilizing antibodies. Fert. Steril. 32, 61-65.

Van der Giessen, M., Dokter-Fokkens, J. and The, T. H. (1980) A solid phase C1q-binding assay for measuring circulating immune complexes (C1q ELISA). In: Immuno enzymatic assay techniques (Malvano, R. ed.). pp 223-231. Martinus Nijhoff Publishers, The Hague.

Van Wingerden, I., The, T. H., Van der Giessen, M. and Rümke, P. (1979) Demonstration of circulating immune complexes in melanoma patients by means of a granulocyte phagocytosis test. In: Protides of the Biological Fluids (Peters, H. ed.). Proc. 26th Coll. pp 345-348. Pergamon Press, Oxford.

Witkin, S. S., Shahani, S. K., Gupta, S., Good, R. A. and Day, N. K. (1980) Demonstration of IgG Fc receptors on spermatozoa and their utilization for the detection of circulating immune complexes in human serum. Clin. exp. Immunol. 41, 441-452

CHAPTER 9

THE SIGNIFICANCE OF THE Fc PART OF ANTISPERMATOZOAL ANTIBODIES FOR THE SHAKING PHENOMENON IN THE SCMC TEST

THE SIGNIFICANCE OF THE Fc PART OF ANTISPERMATOZOAL ANTIBODIES FOR THE SHAKING PHENOMENON IN THE SCMC TEST

S. Jager, J. Kremer, J. Kuiken and Ina Mulder

Fertility Unit, Department of Obstetrics and Gynaecology, University Hospital, Groningen, The Netherlands

ABSTRACT

Donor spermatozoa with good motility were pretreated with four sera containing high titers of sperm agglutinating IgG, one serum without sperm agglutinating activity, the IgG fractions from these five sera, F(ab)2 and Fab fragments from these sera and from the IgG fractions, and one seminal plasma sample with a high titer of sperm agglutinating IgA. We determined with mixed antiglobulin reaction tests the percentage of motile pretreated spermatozoa sensitized with IgG Fab or IgG Fc parts. Spermatozoa sensitized with intact antispermatozoal IgG, showed a strong reduction in their capacity to penetrate cervical mucus. The reduction of the penetration capacity was determined by estimating the percentage of motile spermatozoa rapidly shaking (S%) in the sperm cervical mucus contact (SCMC) test. Removal of the Fc parts resulted in a decreased S%. Treatment of spermatozoa, on which Fab fragments were present, with intact antibodies to IgG Fab fragments, resulted in a recurrence of a high S%. A decrease of the S% was also found if Fab fragments from antibodies to IgG Fc fragments were added to spermatozoa sensitized with intact antispermatozoal IgG. Similarly, we found a decrease of the S% if IgA sensitized spermatozoa were treated with Fab fragments from antibodies to human IgA. In the sperm penetration meter test the IgA sensitized spermatozoa, treated with Fab fragments from antihuman IgA antibodies showed a better penetration than untreated IgA sensitized spermatozoa.

INTRODUCTION

Antispermatozoal antibodies can inhibit the in vitro sperm penetration of cervical mucus by two well known mechanisms: sperm agglutination and complement depending sperm immobilization in the antibody containing medium. However, also in the absence of complement freely swimming spermatozoa can show a reduced capacity to penetrate cervical mucus (Boettcher & Gruszynski, 1978). These sensitized spermatozoa change their propulsions into local shaking movements after contact with the cervical mucus (Kremer & Jager, 1976). To explain this so called shaking phenomenon these authors put forward the hypothesis that spermatozoa sensitized with antispermatozoal antibodies stick to the gel component of cervical mucus. This binding might be due to a cross-linking of the spermatozoa through the antibody molecule. Two types of cross-linking are possible. One type of cross-linking occurs if the spermatozoal surface and the gel component of cervical mucus have an antigenic determinant in common. The other type of cross-linking is binding of the spermatozoa to the gel component by the Fc parts of the antibody molecules on the spermatozoa. The type of cross-linking involved has been investigated by using spermatozoa sensitized with univalent antibodies (Fab fragments) and spermatozoa sensitized with bivalent antibodies without Fc part i. e. F(ab)2 fragments. Isojima et al. (1979) found that spermatozoa, pretreated with Fab fragments of sperm immobilizing IgG, showed an excellent sperm penetration of cervical mucus. Spermatozoa pretreated with the intact antibodies could not penetrate the cervical mucus. On the other hand, Hjort et al. (1978) found a reduced penetration with spermatozoa sensitized with F(ab)2 fragments. These results suggested that antispermatozoal antibodies cause the reduced sperm penetrability by F(ab)2 mediated cross-linking.

In this study we will show that presence of Fc parts of antispermatozoal antibody molecules on spermatozoa, is involved in the reduction of the penetration capacity. In addition, we will demonstrate that the reduction of the penetration capacity of donor spermatozoa, sensitized by antispermatozoal IgG or IgA, can be reversed by treatment with Fab fragments from antibodies to human IgG or IgA.

MATERIALS AND METHODS

Materials

We tested four sera containing sperm agglutinating IgG, one serum without sperm agglutinating activity, the IgG fractions from these five sera and one seminal plasma sample containing a high titer of sperm agglutinating IgA. The preparation of the IgG fractions and results of antispermatozoal antibody tests performed on the sera and the IgG fractions were described previously (chapter 7). We tested intact antibodies, F(ab)2 fragments and Fab fragments both from full sera and from the IgG fractions. The seminal plasma was obtained by centrifugation of a semen sample at 3000 g for about 20 minutes. The supernatant was dialyzed against phosphate buffered saline (PBS), pH 7.4. The semen sample was obtained from the male partner of an infertile couple. The sperm agglutination titer of the seminal plasma sample was 8192. Absorption of the seminal plasma sample with antihuman IgG antiserum did not affect the sperm agglutination titer whereas the agglutination activity disappeared completely after absorption with antihuman IgA antiserum.

The antisera used were raised in rabbits. For absorption of the sperm agglutination activity in the seminal plasma sample we used antihuman IgG (Dako, 10-090) and antihuman IgA (Dako, 10-MAT). For MAR tests we used antiserum to Fab fragments from human IgG (Behring, OTNY), antiserum to Fc fragments from human IgG (Behring, OTOB), antihuman IgG (Behring, ORCM), antihuman IgA (Behring, ORCI). For blocking Fc parts of antispermatozoal IgG on spermatozoa we used concentrated Fab fragments from antihuman IgG (Dako, 10-090). The anti-IgG antiserum reacted with intact IgG on spermatozoa but not with F(ab)2 and Fab fragments on spermatozoa. For blocking of antispermatozoal IgA we used concentrated Fab fragments from antihuman colostral IgA (Dako, 10-MAT).

Spermatozoa with good motility were obtained from normal semen samples from young, apparently healthy men. Semen was considered normal if it contained per ml at least 60 x 10^6 spermatozoa with at least 50% good forward motility and at least 50% normal head forms

Cervical mucus permitting good penetration was obtained from women visiting the fertility unit. Collection and investigation of the cervical mucus was described previously (Jager et al., 1978).

Preparation of F(ab)2 and Fab fragments

F(ab)2 fragments were produced according to the method described by Nisonoff et al. (1960) slightly modified by Hjort & Poulsen (1978). The immunoglobulin containing fluid was dialyzed against 0.1 M sodium acetate buffer, pH 7.0 and 0.1 M HCl was added until a pH of 4.3 was reached. The digestion was performed by addition of crystallized pepsin (Boehringer) to a pepsin/protein ratio of 4/100. The digestion was carried out by incubation at 37 ^{o}C for 24 hours. The precipitate was removed by centrifugation at 12,000 g for 30 minutes and solid TRIS salt was added to the supernatant until a pH of 8.0 was reached. The fluids were dialyzed against PBS, pH 8.0 and concentrated by ultrafiltration with the aid of centriflo membrane cones (Amicon CF 25) by centrifugation at 750 g for at least 10 minutes. The preparations were concentrated to between 50% and 100% of the original sample volume.

Fab fragments were prepared by the method of Porter (1959) slightly modified. The immunoglobulin containing fluid was dialyzed against PBS, pH 7.0. To the fluids was added papain (Boehringer) to a papain/protein ration of 4/100, cystein to a final concentration of 0.02 M and EDTA to a final concentration of 0.005 M. The mixture was kept at 37 ^{o}C for 16 hours and subsequently dialyzed against PBS, pH 7.4. For removal of Fc fragments and unsplit IgG molecules the digested antisera were passed through a Protein A-Sepharose CL-4B (Pharmacia) column. The digested sera and IgG fractions were concentrated about five times.

Incubation of donor spermatozoa for MAR and SCMC tests

Donor spermatozoa were pretreated with the sera or the IgG fractions and washed in one step by a density gradient centrifugation method described previously (chapter 7). For pretreatment with antispermatozoal IgA a suspension of 20 x 10^{6} spermatozoa per ml in Earle's balanced salt solution containing 1% bovine serum albumin was mixed with an equal amount of seminal plasma. The spermatozoa were incubated at room temperature for at least five minutes. For a second incubation the pretreated spermatozoa were mixed with an equal amount of antiserum, containing intact antibodies or Fab fragments from the antiserum, during ten minutes.

Tests for determination of antispermatozoal antibody activity

Sperm agglutination titers and types were determined with the tray agglutination test according to Friberg (1974) modified as described by Jager et al. (1978)(see chapter 5). Sperm immobilization titers were determined with the tray immobilization test described in chapter 7. The percentage of motile spermatozoa with IgG (IgG MAR%) was determined with the IgG MAR test (Jager et al., 1978; Jager et al., 1980). The percentage of motile spermatozoa with F(ab)2 or Fab fragments (Fab MAR%) was determined with the IgG MAR test with the antiserum to Fab fragments from human IgG. The Fc MAR% was also determined with the IgG MAR test but with antiserum to Fc fragments from human IgG. The IgA MAR% was determined with the IgA MAR test described by Jager et al. (1980). The percentage of motile spermatozoa shaking after contact with cervical mucus (S%) was determined in the SCMC test type 1, where semen and cervical mucus are thoroughly mixed, was performed according to Kremer & Jager (1976) and Jager et al. (1979). The sperm penetration test was performed with the penetration meter (SPM) described by Kremer (1965) gradually modified (Kremer, 1968, 1980).

The results presented were obtained with spermatozoa from at least two different donors.

RESULTS

The sperm agglutination titers and types and the sperm immobilization titers of the sera and the IgG fractions before and after treatment with pepsin or papain are shown in table 1. In this table are also shown the Fab MAR% values, the Fc MAR% values and the S% values (fig. 1a, 1b and 1c).

Table 2 shows the S% values obtained with spermatozoa incubated first with intact antispermatozoal IgG, with Fab fragments from antispermatozoal IgG or with antispermatozoal IgA from seminal plasma. The so treated spermatozoa were tested in the SCMC test without further treatment or after a second incubation with Fab fragments from antihuman IgG (specific for Fc parts, with Fab fragments from antihuman IgA, with intact antibodies to Fc fragments from human IgG, with intact antibodies to Fab fragments from human IgG or with Fab fragments from antibodies to human IgA (see also fig. 1d and 1e).

Table 1

Effect of pretreatment of donor spermatozoa with Fab and F(ab)2 fragments from antispermatozoal IgG on the shaking phenomenon. Results with full sera and with purified IgG fractions treated with papain and pepsin.

Serum from patient	Sample	Treatment of sample	Sperm agglutination* Titer	Type	Sperm immobilization titer**	% of motile *** spermatozoa with Fab	Fc	% of shaking spermatozoa in SCMC test
M24 (control serum)	Serum	None	<4	-	1	<10	<10	20
		Pepsin	<4	-	0	<10	<10	20
		Papain	<4	-	1	<10	<10	20
	IgG fraction	None	<4	-	0	<10	<10	30
		Pepsin	<4	-	0	<10	<10	30
		Papain	<4	-	1	20	<10	20
M1	Serum	None	4096	T-T/Tt-Tt	1	>90	>90	>90
		Pepsin	4096	T-T/Tt-Tt	0	>90	<10	30
		Papain	16	T-T	1	>90	<10	10
	IgG fraction	None	1024	T-T/Tt-Tt	2	>90	>90	90
		Pepsin	2048	T-T/Tt-Tt	0	>90	<10	30
M2	Serum	None	4096	M	16	>90	>90	90
		Pepsin	4096	M	0	>90	<10	50
		Papain	16	M	0	>90	<10	30
	IgG Fraction	None	8192	M	16	>90	>90	90
		Pepsin	8192	M	0	>90	<10	70
		Papain	<4	-	0	>90	<10	10
W3	Serum	None	128	H-H	16	>90	>90	>90
		Pepsin	128	H-H	nt	>90	<10	20
		Papain	<4	-	nt	>90	<10	10
	IgG fraction	None	128	H-H	16	>90	>90	90
		Pepsin	64	H-H	0	>90	20	20
W5	Serum	None	128	H-H	16	>90	>90	80
		Pepsin	64	H-H	0	>90	<10	30
		Papain	<4	-	1	>90	<10	20
	IgG fraction	None	128	H-H	8	>90	>90	70
		Pepsin	128	H-H	nt	>90	<10	30

* Determined in the tray agglutination test (Friberg, 1974). T-T = tail-to-tail, Tt-Tt = tail tip-to-tail tip, M = mixed, H-H = head-to-head. ** Determined in the tray immobilization test (see methods). nt = not tested. *** Determined in MAR tests (see methods).

Table 2

Induction and reversal of the shaking phenomenon in the sperm cervical mucus contact (SCMC) test.

Treatment of donor spermatozoa		% of motile spermatozoa shaking in the SCMC test
First incubation of spermatozoa with:	Second incubation of spermatozoa with:	
Fab fragments of anti-spermatozoal IgG from serum of patient M2	None	10
	Intact anti-IgG Fab	>90
	Intact anti-IgG Fc	20
Intact antispermatozoa IgG from serum of patient M2	None	90
	Fab/anti-IgG Fc	30
	Fab/anti-IgA	90
Seminal plasma with sperm agglutination titer of 8192	None	>90
	Fab/anti-IgG Fc	>90
	Fab/anti-IgA	30

Table 3

Concentration and motility of spermatozoa, incubated with seminal plasma containing antispermatozoal IgA, after in vitro penetration of cervical mucus*. Effect of second incubation with Fab fragments from antihuman IgG and with Fab fragments from antihuman IgA.

First incubation of spermatozoa with:	Second incubation of spermatozoa with:	Concentration and motility grade of spermatozoa in SPM test at:		Motility percentage and motility grade of spermatozoa in SPM test after: (hours)			
		1cm	2 cm	2½	4	5½	21
Seminal plasma with sperm agglutination titer <4**	Fab/anti-IgG Fc	21-50/g	11-20/g	90%g	80%g	70%f	0
	Fab/anti-IgA	21-50/g	11-20/g	90%g	80%g	60%f	0
Seminal plasma with sperm agglutination titer of 8192**	Fab/anti-IgG Fc	1- 5/p	0	<10%p	<10%p	0	0
	Fab/anti-IgA	21-50/f	11-20/p	40%g	20%f	10%f	0

* Determined in the sperm penetration meter (SPM)(Kremer, 1965). Estimated were the concentration (number of spermatozoa per low power field i. e. 10 x 10 magnification) and the motility grade of the spermatozoa in the capillary tube filled with cervical mucus. Motility grades: g = good, f = fair, p = poor.

** Determined with the tray agglutination test (Friberg, 1974).

Scheme	Sensitization with:	Second antibody	S%
a 	Intact antisperma-tozoal IgG	No	70->90
b 	F(ab)2 fragments from antisperma-tozoal IgG	No	20-70
c 	Fab fragments from antispermatozoal IgG	No	10-30
d 	Fab fragments from antispermatozoal IgG	Intact antibodies to Fab fragments from human IgG	>90
e 	Intact antisperma-tozoal IgG	Fab fragments from anti-bodies to Fc fragments from human IgG	30

Fig. 1. Schematic representation of sensitization of donor spermatozoa with anti-spermatozoal antibodies, binding of second antibodies and percentage of motile spermatozoa shaking (S%) in the SCMC test.

Table 3 shows the results of a typical experiment demonstrating the effect of treatment of IgA sensitized motile spermatozoa with Fab fragments from antibodies to IgA on the number of motile spermatozoa after penetration into cervical mucus, the motility grade and the duration of the motility of the spermatozoa penetrated into the cervical mucus.

DISCUSSION

The effectivity of digesting the antispermatozoal antibodies by pepsin and papain is demonstrated by the decrease of the sperm immobilizing activity. The retention of the antibody activity after pepsin digestion is demonstrated by the retention of the sperm agglutinating activity and the high Fab MAR%. After papain digestion the retention of the antibody activity is detected by the high Fab MAR% alone (table 1). The simultaneous decrease to low or non-specific levels of the S% and the Fc MAR% values in most cases indicates that presence of the FC parts of antibody molecules on motile spermatozoa is involved in the occurrence of a shaking phenomenon and thus also in the reduction of the capacity of spermatozoa to penetrate cervical mucus (see also fig. 1a, 1b and 1c). Incubation of spermatozoa in IgG F(ab)2 fragments from patient M2 resulted in an unexpectedly high S%. This high S% is probably not due to an incomplete digestion of the antispermatozoal IgG as Fc parts could not be detected on the motile spermatozoa. Possibly, the small piece of Fc fragment remaining attached to Fab parts after pepsin digestion but removed with papain still plays a role in the occurrence of a shaking phenomenon. Another possibility is that the antispermatozoal antibodies from patient M2 are also reactive with the gel component of cervical mucus. The role of the Fc part in the occurrence of the shaking phenomenon is confirmed by a strong decrease of the S%, using spermatozoa sensitized with antispermatozoal IgG to which were added Fab fragments from antibodies to Fc parts of human IgG. In addition, a shaking phenomenon was observed using spermatozoa sensitized with Fab fragments from antispermatozoal IgG to which were added intact antibodies to these Fab fragments; new Fc parts were added in this way to the Fab fragments (table 2, fig. 1d and 1e).

Our results are in agreement with the results of Isojima et al. (1979)

who found with spermatozoa, pretreated with Fab fragments from sperm immobilizing IgG, an excellent sperm penetration into cervical mucus. Spermatozoa, pretreated with the intact antibodies, could not penetrate. on the other hand, Hjort et al. (1978) found that spermatozoa, pretraeted during 30 minutes with F(ab)2 fragments from head-to-head agglutinating IgG could not pentrate cervical mucus. Spermatozoa, sensitized with F(ab)2 fragments from tail-to-tail agglutinating antibodies, however, could penetrate cervical mucus; although the penetration was still reduced compared to the penetration observed with untreated spermatozoa. In our opinion the results of Hjort et al. (1978) can be explained, at least partially, by agglutination of the spermatozoa in the incubation medium. Head-to-head agglutination is a rapid process; within half an hour almost all spermatozoa can be bound in agglutinates in the presence of a high titer of antispermatozoal antibodies. Possibly, F(ab)2 fragments can reduce the capacity of spermatozoa to penetrate cervical mucus to some extent as we also found a moderate positive S% with F(ab)2 fragments from antispermatozoal IgG in the serum of patient M2.

Our conclusion that the Fc parts of antispermatozoal antibodies are involved in the reduction of the capacity to penetrate cervical mucus is in conflict with the conclusion of Metz & Anika (1970). These authors reported a decreased conception rate in rabbits inseminated with rabbit semen pretreated with Fab fragments from goat IgG reactive with rabbit semen. Flushings from the genital tract of rabbits, inseminated intravaginally with the pretreated semen, contained approximately 50 times less spermatozoa than controls inseminated with Fab fragments from goat IgG not reactive with rabbit spermatozoa. Since the spermatozoa were not agglutinated and the motility was not visibly affected, these two factors did not explain the observed conception failure. The authors suggested that the Fab fragments blocked some spermatozoal (surface?) antigen that has an important role in cervical mucus penetration. We have no explanation for the conflicting results.

The involvement of the IgG Fc part in the occurrence of a shaking phenomenon does not automatically imply the presence of specific receptors in cervical mucus. If these Fc receptors would be present they also react with IgA, or the mucus contains IgA receptors as well as IgG Fc receptors. Preliminary (unpublished) results of experiments to demonstrate the presence of Fc receptors were negative until now. In these experiments we investigated binding of IgG sensitized erythrocytes and *non-motile*

IgG sensitized spermatozoa to fresh untreated cervical mucus, to "diluted" cervical mucus, or to fragmented cervical mucus. The fragmentation was achieved by digestion with bromelin, by disulphide bond reduction, or by sonication. Results with sensitized cells did not differ from results with non-sensitized cells.

Our results demonstrate the importance of the Fc part for the occurrence of the shaking phenomenon only for IgG. Whether the Fc part of IgA has the same importance remains to be shown. The anti-IgA antiserum used (table 2, table 3) is specific for α-chains present in both the Fc and Fab parts of the IgA molecule.

The reduction of the capacity of spermatozoa to penetrate cervical mucus is one of the effects of antispermatozoal antibodies leading to infertility of men. We found that treatment of spermatozoa, on which antispermatozoal IgA is present, with Fab fragments from antibodies to human IgA improved the concentration and motility of spermatozoa in the cervical mucus in the capillary tube of the SPM (table 3). This result might mean that one of the antifertility effects of antispermatozoal antibodies in man can, at least partially, be reversed.

ACKNOWLEDGEMENTS

This investigation was supported by a grant from the Organization for Health Research TNO. The authors are grateful to Dr. Ph. Rümke for continuous advice, to prof. Ph. Hoedemaeker for commenting on the manuscript and to Dr. R. Mathias for correction of the english text.

REFERENCES

Boettcher, B. and Gruszynski, R. (1978) The passage of human spermatozoa treated with spermantibodies through cervical mucus. In: Immunology of Reproduction (Bratanov, K., Vulchanov, V. H., Dikov, V., Somlev, B. and Kozhouharova, L. eds.). pp 444-449. Publishing House of the Bulgarian Academy of Sciences, Sofia.

Friberg, J. (1974) Clinical and immunological studies on sperm agglutinating antibodies in serum and seminal fluid. Acta Obst. Gynec. Scand. Suppl. 36, 1-76.

Hjort, T., Hansen, K. B. and Poulsen, F. (1978) The reactivity of F(ab)2 fragments of sperm antibodies and their use in the investigation of antigen-antibody systems. In: Spermatozoa, Antibodies and Infertility (Cohen, J. and Hendry, W. F. eds.). pp 101-115. Blackwell Scientific Publications, Oxford.

Hjort, T. and Poulsen, F. (1978) Reactivity of F(ab)2 fragments of sperm antibodies in agglutination, immobilization and immunofluorescence tests. Arch. Androl. 1, 83-88.

Isojima, S., Koyama, K., Ikuma, K. and Kou, S. (1979) Influence of weak antisperm antibody on sperm penetration in women's cervical mucus. In: Immunology of Reproduction (Bratanov, K., Vulchanov, V. H., Dikov, V., Georgieva, R. and Somlev, B. eds.). pp 341-346. Publishing House of the Bulgarian Academy of Sciences, Sofia.

Jager, S., Kremer, J. and 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, 12-21.

Jager, S., Kremer, J. and Van Slochteren-Draaisma, T. (1979) Presence of sperm agglutinating antibodies in infertile men and inhibition of in vitro sperm penetration into cervical mucus. Int. J. Androl. 2, 117-130.

Jager, S., Kremer, J., Kuiken, J. and Van Slochteren-Draaisma, T. (1980) Immunoglobulin class of antispermatozoal antibodies from infertile men and inhibition of in vitro sperm penetration into cervical mucus. Int. J. Androl. 3, 1-14.

Kremer, J. (1965) A simple sperm penetration test. Int. J. Fert. 10, 209-215

Kremer, J. (1968) The in vitro spermatozoal penetration test in fertility investigations. Thesis, University of Groningen.

Kremer, J. (1980) In vitro sperm penetration in cervical mucus and AIH. In: Homologous Artificial Insemination(AIH)(Emperaire, J. C., Audebert, A. and Hafez, E. S. E. eds.). pp 30-37. Martinus Nijhoff Publishers, The Hague.

Kremer, J. and Jager, S. (1976) The sperm-cervical mucus contact test. A: preliminary report. Fert. Steril. 27, 335-340.

Metz, C. B. and Anika, J. (1970) Failure of conception in rabbits inseminated with nonagglutinating, univalent antibody-treated semen. Biol. Reprod. 2, 284-290.

Nisonoff, A., Wissler, F. C., Lipman, L. N. and Woernly, D. L. (1960) Separation of univalent fragments from the bivalent rabbit antibody molecule by reduction of disulfide bonds. Arch. Biochem. Biophys. 89, 230-244.

Porter, R. R. (1959) The hydrolysis of rabbit gamma-globulin and antibodies with crystalline papain. Biochem. J. 73, 119-126.

GENERAL DISCUSSION

GENERAL DISCUSSION

Antispermatozoal antibodies can cause infertility in men by reducing the capacity of spermatozoa to penetrate cervical mucus. In infertile couples the results of the post coital test (PCT) have often been unexpectedly poor if antibodies to spermatozoa were present in the husband. The reduced penetration capacity can be ascribed, partially, to agglutination of the spermatozoa in the ejaculate. The auto-agglutination is, however, seldom complete. In most cases many freely swimming spermatozoa can be observed between the agglutinates. We observed that freely swimming spermatozoa from infertile men with antispermatozoal antibodies changed their propulsion into local shaking movements after contact with cervical mucus. The shaking phenomenon was also observed if the antibodies were present in cervical mucus. The antibodies involved in the shaking phenomenon, observed with spermatozoa from such infertile men or with cervical mucus containing antispermatozoal antibodies, belong to the IgA class. However, spermatozoa pretreated with antispermatozoal IgG also showed a shaking phenomenon. A new test was based on the shaking phenomenon: the sperm cervical mucus contact (SCMC) test. A high percentage of motile spermatozoa showing the shaking phenomenon (shaking percentage) in the SCMC test was found to be highly specific for the presence of antispermatozoal antibodies. Only occasionally we found a high shaking percentage without evidence of antispermatozoal antibodies.

The shaking phenomenon could not be ascribed to complement depending immobilization in the cervical mucus. The shaking phenomenon was observed in cervical mucus heat-treated to inactivate complement. In addition, intense shaking movements, starting immediately after contact of the spermatozoa with cervical mucus, have been seen to last unaltered for up to eight hours; in case of a complement depending immobilization a gradual decrease of the movements is to be expected. Moreover, the antispermatozoal antibodies involved in the shaking phenomenon, observed with spermatozoa and cervical mucus from infertile couples, were of the non-complement fixing class IgA.

Our results are not consistent with the suggestion of Metz & Anika (1970)(chapter 1: 4.5) that Fab fragments from antispermatozoal IgG inhibit cervical mucus penetration by blocking some spermatozoal (surface?) antigen essential for penetration. We found that human spermatozoa

sensitized with IgG Fab fragments did not show a shaking percentage higher than control values. In addition, attachment of new Fc parts to the IgG Fab fragments on the spermatozoa induced a recurrence of the high shaking percentage in the SCMC test. Moreover, blocking of the Fc part of antispermatozoal IgG on spermatozoa with Fab fragments from antibodies to Fc fragments from human IgG (chapter 9: fig. 1e) prevented the occurrence of a high shaking percentage in the SCMC test. We concluded that the presence of the Fc parts of antispermatozoal IgG on spermatozoa is a factor in the production of a shaking phenomenon in indirect SCMC tests.

To explain the shaking phenomenon we put forward the hypothesis that spermatozoa sensitized with antispermatozoal antibodies stick to the gel component of cervical mucus. This binding was thought to be due to a cross-linking of the motile spermatozoa with the gel component through the antibody molecules. The concept that the shaking phenomenon is caused by a cross-linking of spermatozoa with the gel component is supported by results obtained with Concanavalin A (Con A). The bivalent lectin, Con A, binds specifically to a variety of polysaccharides and glycoproteins (Goldstein et al., 1965; Yariv et al., 1968). Spermatozoa, incubated with Con A, agglutinate. When Con A-sensitized erythrocytes are mixed with spermatozoa, a strong mixed agglutination (chapter 1: fig. 1.3D) occurs (Van Lis & Kalsbeek, 1972). We found a high shaking percentage after fresh, normal semen was brought into close contact with a mixture of cervical mucus and Con A. These shaking movements could not be distinguished from the shaking observed with antispermatozoal antibodies (Kremer et al., 1977).

The involvement of the IgG Fc part in the production of the shaking phenomenon in the indirect SCMC test does not automatically imply the presence of specific Fc receptors in cervical mucus. If these Fc receptors are present they must also react with IgA, or else the cervical mucus contains both IgA receptors and IgG Fc receptors. The preliminary (unpublished) results of experiments to demonstrate the Fc receptors were negative until now. In these experiments we investigated binding of IgG sensitized erythrocytes and *non-motile* spermatozoa to fresh untreated cervical mucus, to "diluted" cervical mucus, or to fragmented cervical mucus. The fragmentation was achieved by digestion with bromelin, by disulphide bond reduction or by sonication. Results with sensitized cells did not differ from results with non-sensitized cells. Additional expe-

riments are necessary before the presence of Fc receptors in cervical mucus can be excluded.

Another possible explanation for the shaking phenomenon could be a decrease in the negative surface charge of the spermatozoa. This negative charge is present on all mammalian cells (Weiss, 1974). In physiological circumstances spermatozoa and the cervical mucus glycoproteins as well carry a coat of negative electrical charge (Gottschalk, 1960; Yanagimachi; et al., 1972). Antibodies might neutralize this charge and cause the spermatozoa to attach to the gel component of cervical mucus; however, spermatozoa treated with neuraminidase to remove the negatively charged sialic acid residues did not show a shaking phenomenon in the SCMC test. The effectiveness of the neuraminidase treatment was demonstrated by the disappearance of the sperm agglutinating activity of a Sendai virus suspension (unpublished result).

The reduction of the capacity of spermatozoa to penetrate cervical mucus is one of the effects of antispermatozoal antibodies leading to infertility of men. We found that treatment of IgA sensitized spermatozoa with Fab fragments from antibodies to human IgA improved the sperm penetration into cervical mucus in the SPM test. We hope that it will be possible to increase the fertility of men with antispermatozoal antibodies by treatment of their spermatozoa with F(ab)2 or Fab fragments from antibodies to human IgA. It has been demonstrated, however, that human spermatozoa pretreated in plasma containing antispermatozoal IgG have a reduced capacity to penetrate zona-free hamster eggs (Haas et al., 1980). It remains, therefore, to be shown that treating IgA sensitized spermatozoa with IgA F(ab)2 or IgA Fab fragments also can reverse the reduction of the capacity to penetrate the human egg.

REFERENCES

Goldstein, I. J., Hollerman, C. E. and Smith, E. E. (1965) Protein-carbohydrate interaction. II. Inhibition studies on the interactions of concanavalin A with polysaccharides. Biochemistry 4: 876-883.

Gottschalk, A. (1960) Correlation between composition, structure, shape and function of a salivary mucoprotein. Nature 186: 949.

Haas, G. G., Sokoloski, J. E.and Wolf, D. P. (1980) The interfering effect of human IgG antisperm antibodies on human sperm penetration of zona-free hamster eggs. Am. J. Reprod. 1: 40-43.

Kremer, J., Jager, S. and Kuiken, J. (1977) The clinical significance of antibodies to spermatozoa In: Immunological Influence on Human Fertility (Boettcher, B. ed.). pp 47-66.

Metz, C. B. and Anika, J. (1970) Failure of conception in rabbits inseminated with non-agglutinating, univalent antibody treated semen. Biol. Reprod. 2: 284-290.

Van Lis, J. M. J. and Kalsbeek, G. L. (1973) The interaction between human spermatozoa and the lectin Concanavalin A. Andrologie 5: 45-47.

Weiss, L. (1974) The topography of cell surface sialic acids and their possible relationship to specific cell interactions. Behring Inst. Mitt. 55: 185-193.

Yanagimachi, R., Noda, Y. D., Fujimoto, M. and Nicholson, G. L. (1972) The distribution of negative surface charges on mammalian spermatozoa. Am. J. Anat. 135: 497-520.

Yariv, J., Kalb, A. J. and Levitzki, A. (1968) The interaction of concanavalin A with methyl α-D-glucopyranoside. Biochem. Biophys. Acta 165: 303-305.

SUMMARY

SUMMARY

The main object of the present study was to investigate the mechanism by which antispermatozoal antibodies reduce the capacity of spermatozoa to penetrate cervical mucus. We demonstrated that non-agglutinated spermatozoa from infertile men with antispermatozoal antibodies changed their propulsion into local shaking movements after contact with cervical mucus. the shaking phenomenon was also observed if the antibodies were present in cervical mucus. A new test was based on the shaking phenomenon: the sperm-cervical mucus contact (SCMC) test. In this SCMC test the percentage of motile spermatozoa showing the shaking phenomenon (S%) is estimated. By performing the SCMC test as a cross test, using spermatozoa and mucus from normal semen donors, it could be decided whether the antibodies were present in the semen or in the cervical mucus (chapter 2).

We investigated with the SCMC test, spermatozoa and cervical mucus from infertile couples with negative or poor results of the PCT and the sperm penetration meter test. These negative or poor results could not be explained by the qualities of the semen or the cervical mucus or by a poor coitus technique in 32 couples. In 30 of these 32 couples a strongly positive result of the SCMC test was obtained and presence of antispermatozoal antibodies was demonstrated in the male or the female partner (chapter 3).

We studied the relationship between sperm agglutinating activity in infertile men and the S% in the SCMC test and with the readings of the SPM test. The SCMC tests and the SPM tests were performed with cervical mucus permitting good penetration of normal spermatozoa with good motility. We found significant negative correlations between the readings of the SPM test on one hand, and the S% and the sperm agglutination titers in serum and seminal plasma on the other hand. In addition, we found a significant positive correlation between the S% and the sperm agglutination titers in serum and seminal plasma. The sperm agglutination titers correlated significantly better with the S% than with the readings of the SPM test. We also concluded that the SCMC test was more suitable than the SPM test for studying the effect of antispermatozoal antibodies on the in vitro sperm penetration and migration in cervical mucus. (chapter 4).

The immunoglobulin class of antispermatozoal antibodies on spermatozoa

was determined with the mixed antiglobulin reaction (MAR). The MAR tests were based on the occurrence of *motile* mixed agglutinates between motile spermatozoa and erythrocytes sensitized or coated with immunoglobulins of the class to be detected. The results were expressed in the percentage of motile spermatozoa bound in motile mixed agglutinates (MAR%). The MAR test for IgG, performed on fresh untreated ejaculates, appeared to be a simple and rapid screening method for the detection of antispermatozoal antibodies in men (chapter 5).

We determined with MAR tests the presence of IgG and IgA on the motile spermatozoa from infertile men and compared the MAR% values with the S% values in the SCMC test and with the sperm agglutination titers in serum and seminal plasma. The IgG MAR% appeared to be related with the circulating sperm agglutinating antibodies. We did not find a direct relation between the IgG MAR% and sperm agglutination titers in the seminal plasma or the S%. The IgA MAR% was not directly related with the sperm agglutination titers in the serum and seminal plasma but was roughly proportional with the S%. We concluded that the shaking phenomenon in the SCMC test with spermatozoa from infertile men was due to the presence of IgA on the motile spermatozoa. Previously it had also been found that the sperm agglutinating activity in semen is caused by IgA (Husted & Hjort, 1975) and that this is probably produced locally in the male reproductive tract. It thus appears that the reduced ability to penetrate cervical mucus by spermatozo from infertile men is probably caused by locally produced IgA (chapter 6).

We investigated cervical mucus from five infertile women, in which sperm penetration was inhibited due to a strong shaking phenomenon. After pretreatment of the cervical mucus with bromelin, which greatly facilitates the determination of sperm agglutinating activity, high sperm agglutination titers were found in the cervical mucus of these five women. The sperm agglutinating activity could be absorbed completely with anti-IgA antiserum but not with anti-IgG or anti-IgM antiserum. This indicates that also in women antispermatozoal antibodies causing, a shaking phenomenon in the SCMC test, are locally produced IgA (chapter 7).

The shaking phenomenon in the SCMC test could also be obtained with donor spermatozoa pretreated in sera containing antispermatozoal antibodies. The pretreatment was performed in a one step incubation and washing procedure by centrifugation of the spermatozoa on a density

gradient. The sensitization of the spermatozoa was determined with MAR tests. A S% of at least 80 was observed only with spermatozoa pretreated in sera from men with a tail-to-tail sperm agglutination titer of at least 512 or from women with a head-to-head sperm agglutination titer of at least 32, and provided that IgG was detected on more than 90% of the motile spermatozoa. The high S% was also obtained with spermatozoa pretreated in purified IgG from sera containing antispermatozoal antibody activity. We concluded that a shaking phenomenon can also be induced with antispermatozoal IgG. The shaking phenomenon is thus not specific for a particular immunoglobulin class (chapter 8).

By comparing the S% obtained with donor spermatozoa pretreated with intact antispermatozoal IgG, with F(ab)2 fragments or Fab fragments from antispermatozoal IgG we demonstrated involvement of the Fc part in the production of a shaking phenomenon. Presence or absence on spermatozoa of the Fab and Fc parts was demonstrated with MAR tests. Removal of the Fc parts of antispermatozoal IgG resulted in a decreased S%. Treatment of spermatozoa, on which Fab fragments were present, with intact antibodies to Fab fragments from human IgG resulted in a recurrence of a high S%. A decrease of the S% was also found if Fab fragments from antibodies to Fc parts of human IgG were added to spermatozoa sensitized with intact antispermatozoal IgG. We concluded that (after sensibilization of the spermatozoa with antispermatozoal IgG) the Fc part is involved in the production of a shaking phenomenon. However, cross-reactivity between spermatozoal surface antigen and the gel component of cervical mucus cannot be excluded. We found also a decrease of the S% if spermatozoa, sensitized with antispermatozoal IgA, were treated with Fab fragments from antibodies to human IgA. In the SPM test the IgA sensitized spermatozoa treated with Fab fragments from antihuman IgA antibodies showed a better penetration than untreated IgA sensitized spermatozoa (chapter 9).

REFERENCE

Husted, S. and Hjort, T. (1975) Sperm antibodies in serum and seminal plasma. Int. J. Fert. 20: 97-105.

SAMENVATTING

SAMENVATTING

Het doel van het onderzoek, beschreven in dit proefschrift, was na te gaan op welke wijze antistoffen gericht tegen spermatozoa, het vermogen van spermatozoa om te penetreren in cervixslijm verminderen. Niet-geagglutineerde spermatozoa van mannen met antispermatozoa antistoffen in het semen, tonen na kontakt met normaal pre-ovulatoir cervixslijm een lokaal schuddende beweging in plaats van de normale voortbeweging. Dit zogenaamde "schudfenomeen" werd ook waargenomen als normaal bewegende spermatozoa in kontakt kwamen met cervixslijm waarin antispermatozoa antistoffen aanwezig waren. Op het schudfenomeen werd een nieuwe test gebaseerd: de sperma-cervix mucus contact (SCMC) test. Het kontakt tussen spermatozoa en cervixslijm wordt in deze test tot stand gebracht door op een objektglas semen en cervixslijm te mengen of door een dunne laag semen aan te brengen op een laag cervixslijm. Met de SCMC test wordt een schatting gemaakt van het percentage van de bewegende spermatozoa dat het schudfenomeen toont (S%). De SCMC test wordt uitgevoerd als een gekruiste test in vier kombinaties met donor spermatozoa en donor cervixslijm. Hierdoor kan worden vastgesteld of de faktor, die het schudfenomeen veroorzaakt aanwezig is in het semen of in het cervixslijm van het onderzochte echtpaar (chapter 2).

Wij onderzochten met de SCMC test semen en cervixslijm van 32 infertiele echtparen bij wie negatieve of slechte resultaten in de post coitale test en in de spermatozoa penetratie meter (SPM) test waren verkregen. Deze negatieve of slechte resultaten konden niet worden verklaard met de uitslagen van de gebruikelijke methoden voor onderzoek van semen en cervixslijm. Bij 30 van deze 32 echtparen werd een sterk positief resultaat in de SCMC test verkregen en konden antistoffen worden aangetoond bij de man of bij de vrouw (chapter 3).

Wij onderzochten bij infertiele mannen het verband tussen de spermatozoa-agglutinatie aktiviteit in serum en in sperma plasma met de resulvan de SCMC test en met de resultaten van de SPM test. De SCMC en de SPM test werden uitgevoerd met cervixslijm waarin normale spermatozoa met een goede motiliteit goed konden penetreren. Wij vonden een signifikant negatieve korrelatie tussen de uitslagen van de SPM test enerzijds en de uitslagen van de SCMC test en de spermatozoa-agglutinatie titers in serum en in sperma plasma anderzijds. Verder vonden wij een signifikant positieve korrelatie tussen het S% en de spermatozoa-agglutinatie titers

in serum en in sperma plasma. De spermatozoa-agglutinatie titers korreleerden signifikant beter met de uitslagen van de SCMC test dan met uitslagen van de SPM test. Wij konkludeerden dat de SCMC test geschikter was dan de SPM test voor het bestuderen van het effekt van antispermatozoa antistoffen op de penetratie en migratie van de spermatozoa in het cervixslijm in vitro (chapter 4).

De immuunglobuline klasse van antistoffen op de spermatozoa werd bepaald met de "mixed antiglobulin reaction (MAR)". De MAR tests zijn gebaseerd op het waarnemen van *bewegende* gemengde agglutinaten die ontstaan tussen erythrocyten, die gesensibiliseerd zijn met immuunglobulinen van de klasse waarop wordt onderzocht, en gesensibiliseerde bewegende spermatozoa. De resultaten worden uitgedrukt in het percentage van de bewegende spermatozoa dat gebonden is in de bewegende gemengde agglutinaten (MAR%). De MAR test voor IgG, uitgevoerd op verse, onbehandelde ejakulaten, bleek een eenvoudige en snelle "screenings" methode te zijn voor de aanwezigheid van antispermatozoa antistoffen bij mannen (chapter 5).

Wij bepaalden met MAR tests de aanwezigheid van IgG en IgA op de bewegende spermatozoa van infertiele mannen en vergeleken de MAR% waarden met de S% waarden verkregen in de SCMC test en met de spermatozoa-agglutinatie titers in het serum en in het sperma plasma. Het IgG MAR% toonde een verband met de cirkulerende spermatozoa-agglutinerende antistoffen. Wij vonden geen direkt verband tussen het IgG MAR% en de spermatozoa-agglutinatie titers in het sperma plasma of het S%. Er was geen direkt verband tussen het IgA MAR% en de spermatozoa-agglutinatie titers in het serum of in het sperma plasma. Daarentegen was het IgA MAR% globaal evenredig met het S%. Wij konkludeerden daarom dat het schudfenomeen in de SCMC test met spermatozoa van infertiele mannen waarschijnlijk veroorzaakt wordt door IgA op de bewegende spermatozoa. Reeds eerder was gevonden dat de spermatozoa-agglutinatie aktiviteit in het semen wordt veroorzaakt door IgA (Husted en Hjort, 1975) dat vermoedelijk wordt geproduceerd in de tractus genitalis van de man. Het is dus waarschijnlijk dat het verminderde vermogen van spermatozoa om te penetreren in cervixslijm wordt veroorzaakt door lokaal geproduceerd antispermatozoa IgA (chapter 6).

Wij onderzochten cervixslijm van vijf vrouwen bij wie in de SCMC test een sterk positief schudfenomeen werd gevonden. Het cervixslijm werd behandeld met bromeline. Door deze behandeling wordt de bepaling van de spermatozoa-agglutinatie in cervixslijm sterk vergemakkelijkt. In het

behandelde cervixslijm van deze vijf vrouwen werden hoge spermatozoa-agglutinatie titers gevonden. Deze agglutinatie aktiviteit kon volledig worden geabsorbeerd met anti-IgA antiserum, maar niet met anti-IgG of anti-IgM antisera. Dit wijst er op dat ook bij vrouwen lokaal geproduceerd antispermatozoa IgA de oorzaak is van het schudfenomeen in de SCMC test (chapter 7).

Het schudfenomeen in de SCMC test kon ook worden verkregen met donor spermatozoa die voorbehandeld waren met sera die antispermatozoa antistoffen bevatten. De voorbehandeling geschiedde met een gekombineerde inkubatie- en was-methode, door de spermatozoa te centrifugeren door een dichtheids gradient. Het percentage gesensibiliseerde spermatozoa werd bepaald met MAR tests. Een S% van tenminste 80 werd alleen waargenomen met spermatozoa die voorbehandeld waren met sera van mannen met een staart-staart agglutinatie titer van tenminste 512 of met sera van vrouwen met een kop-kop agglutinatie titer van tenminste 32 en als tevens IgG was aangetoond op meer dan 90% van de bewegende spermatozoa. Dit hoge S% werd ook verkregen met spermatozoa die voorbehandeld waren met gezuiverd IgG van sera met antispermatozoa antistof aktiviteit. Wij konkludeerden dat een schudfenomeen ook kon worden geinduceerd met antispermatozoa IgG. Het schudfenomeen is dus niet specifiek voor een bepaalde immuunglobuline klasse (chapter 8).

Door vergelijking van het S%, verkregen met spermatozoa, die voorbehandeld waren met intakt antispermatozoa IgG, met F(ab)2 fragmenten of Fab fragmenten van antispermatozoa IgG, onderzochten wij of het Fc gedeelte betrokken is bij het ontstaan van het schudfenomeen. Aanwezigheid of afwezigheid van Fab en Fc gedeelten werd vastgesteld met behulp van MAR tests. Verwijdering van het Fc gedeelte van het antispermatozoa IgG resulteerde in een verlaging van het S%. Behandeling van spermatozoa (waarop Fab fragmenten aanwezig waren) met intakte antistoffen gericht tegen Fab fragmenten van humaan IgG, resulteerde in een terugkeer van een hoog S%. Een verlaging van het S% werd ook gevonden als Fab fragmenten van antistoffen tegen Fc fragmenten van humaan IgG, werden toegevoegd aan spermatozoa, gesensibiliseerd met intakt antispermatozoa IgG. Wij konkludeerden dat (na sensibilisatie van spermatozoa met antispermatozoa IgG) de aanwezigheid van het Fc gedeelte noodzakelijk is voor het ontstaan van het schudfenomeen. Een tevens bestaande kruis-reaktiviteit tussen oppervlakte antigenen van spermatozoa en cervixslijm kon echter niet worden uitgesloten. Wij vonden ook een afname van het S% als sperma-

tozoa, die gesensibiliseerd waren met antispermatozoa IgA, werden behandeld met Fab fragmenten van antistoffen tegen menselijk IgA. In de SPM test toonden deze spermatozoa een betere penetratie dan onbehandelde spermatozoa, gesensibiliseerd met antispermatozoa IgG (chapter 9).

LITERATUUR

Husted, S. en Hjort, T. (1975) Sperm antibodies in serum and seminal plasma. Int. J. Fert. 20: 97-105.

APPENDIX TO CHAPTERS 4 AND 6

RESULTS OF TESTS WITH SEMEN AND SERUM OF 121 MEN FROM INFERTILE COUPLES

RESULTS OF TESTS WITH SEMEN AND SERUM OF 121 MEN FROM INFERTILE COUPLES

Column Legend

1 Sperm-cervical mucus contact (SCMC) test number.

2 Percentage of motile spermatozoa shaking in the SCMC test (S%).
In seven tests the shaking percentage included a percentage of spermatozoa combining rapid shaking with slow propulsion (α: 40%, β: 40%, γ: 40%, δ: 20%, ε: 40%, ζ: 40%, η: 30%)

3 Migration density and motility grade of spermatozoa at 1 cm in the sperm penetration meter (SPM) test. n = no motile spermatozoa, p = poor motility, f = fair motility, g = good motility.

4 Same as column 3 but at 3 cm.

5 Same as column 3 but at 4.5 cm.

6 Concentration of spermatozoa in the semen sample used for the SCMC test.

7 Percentage of motile spermatozoa in the semen sample. The motility grade was at least fair.

8 Grade of agglutination of the spermatozoa in the semen sample.

9 Percentage of motile spermatozoa with IgG in the semen sample determined with the mixed antiglobulin reaction (MAR) test for IgG (IgG MAR%).
(θ: IgG MAR% decreased to 20 in 20 minutes)

10 Percentage of motile spermatozoa with IgA in the semen sample determined with the MAR test for IgA (IgA MAR%).
(ne = no estimation possible)

11 Sperm agglutination titer, determined with the tray agglutination test (Friberg, 1974), in the serum.
(κ: titer with one donor 128)

12 Sperm agglutination type in the serum.

13 Sperm agglutination titer in the seminal plasma.

14 Sperm agglutination type in the seminal plasma.

nt = not tested

SCMC test		Migration density and motility grade in sperm penetration meter test at:			Spermatozoa in semen sample					Sperm agglutination in			
Test nr.	% of shaking sperms	1 cm	3 cm	4.5 cm	Concentration x10^6/ml	% of motile sperms	Agglutination grade	% of motile sperms with IgG	% of motile sperms with IgA	Serum Titer	Serum Type	Seminal plasma Titer	Seminal plasma Type
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
7-040	>90	0	0	0	81	20	++	>90	nt	1024	T-T	128	T-T
6-016	>90	6- 10/p	0	0	173	50	+++	>90	90	512	T-T	64	T-T
6-010	>90	21- 50/f	1- 5/p	1- 5/g	35	30	nt	>90	>90	512	M	8	T-T
6-109	>90	1- 5/p	1- 5/p	0	46	60	+	>90	90	256	T-T	64	T-T
6-078	>90	>100/p	21- 50/p	11- 20/p	91	50	nt	>90	>90	256	T-T	8	T-T
6-011	>90	0	0	0	12	40	nt	>90	>90	256	T-T	4	T-T
7-007	>90	1- 5/f	1- 5/p	0	83	50	+++	>90	>90	128	T-T	16	T-T
6-092	>90	1- 5/p	0	0	22	20	nt	>90	>90	128	T-T	16	T-T
6-053	>90	1- 5/p	0	0	38	30	nt	>90	>90	128	T-T	8	T-T
8-030	>90	11- 20/p	1- 5/p	0	14	30	+	>90	nt	128	T-T	8	T-T
7-037	>90	11- 20/p	1- 5/p	0	196	60	+++	90	60	64	T-T	16	T-T
6-003	>90	21- 50/p	6- 10/p	1- 5/p	25	30	+++	>90	ne	64	T-T	8	T-T
6-108	>90$_\alpha$	>100/p	51-100/p	21- 50/p	36	30	+	>90	60	64	T-T	<4	
8-007	90	1- 5/p	0	0	17	30	++	>90	>90	4096	T-T	128	M
6-009	90	21- 50/p	1- 5/p	1- 5/p	12	40	nt	>90	nt	1024	T-T	512	H-H
7-002	90	>100/p	21- 50/p	1- 5/p	167	30	+++	90	80	1024	T-T	64	T-T
6-059	90	1- 5/n	0	0	12	50	+	>90	80	512	T-T	32	T-T
6-113	90$_\beta$	>100/p	51-100/p	11- 20/p	108	30	+	>90	>90	256	M	64	T-T
8-009	90	6- 10/p	1- 5/p	0	48	30	+++	>90	>90	256	T-T	32	T-T
6-063	90$_\gamma$	21- 50/g	1- 5/f	1- 5/f	96	60	+	>90	80	256	T-T	32	T-T
8-023	90	11- 20/p	0	0	53	50	-	90	90	64	T-T	128	T-T
6-062	90	0	0	0	47	30	nt	>90	nt	128	M	32	T-T
6-081	90$_\delta$	21- 50/p	1- 5/p	1- 5/n	30	40	+	>90	>90	128	M	16	T-T
6-114	90	51-100/p	6- 10/p	1- 5/p	27	30	+	>90	80	128	T-T	<4	
6-056	90	1- 5/p	0	0	38	30	+++	>90	40	64	T-T	16	T-T

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
7-008	90	1- 5/p	0	0	19	30	+++	>90	50	32	T-T	4	T-T
7-043	80	>100/p	>100/p	6- 10/p	62	70	+	>90	70	1024	M	256	T-T
6-099	80ε	>100/p	21- 50/p	11- 20/p	98	60	+	>90	80	128	T-T	8	T-T
7-041	80	51-100/f	11- 20/g	1- 5/g	30	60	+	>90	90	128	T-T	<4	
8-013	80	>100/p	51-100/p	6- 10/p	36	70	+	>90	60	64	T-T	<4	
6-064	80ζ	21- 50/f	21- 50/f	6- 10/f	34	30	nt	>90	60	64	T-T	<4	
6-111	70η	>100/g	>100/g	51-100/f	43	60	+	>90	20	256	T-T	16	T-T
6-094	70	11- 20/p	1- 5/n	0	200	40	-	>90	90	256	T-T	4	T-T
6-013	70	51-100/p	21- 50/g	6- 10/g	93	50	+++	>90	80	128	T-T	8	T-T
8-026	70	21- 50/p	6- 10/p	1- 6/p	156	40	+	>90	ne	64	T-T	128	T-T
8-035	70	21- 50/f	11- 20/f	11- 20/f	18	60	++	70	>90	<4		32	H-H
6-042	60	21- 50/p	11- 20/p	1- 5/p	34	50	+	>90	90	64	T-T	<4	
7-009	60	>100/f	1- 10/f	6- 10/p	54	30	+	50	40	32	T-T	4	T-T
6-079	50	>100/p	>100/f	>100/f	91	50	nt	>90	<10	64	T-T	<4	
6-106	50	51-100/p	21- 50/p	6- 10/n	15	20	+	<10	nt	<4		<4	
6-055	50	51-100/f	51-100/g	20- 50/g	45	20	-	<10	nt	<4		<4	
8-049	40	>100/p	51-100/p	21- 50/p	147	60	nt	70	90	32	T-T	8	T-T
6-068	40	21- 50/g	51-100/g	21- 50/g	24	20	-	10	nt	8	T-T	<4	
6-044	40	>100/f	51-100/f	11- 20/p	32	40	nt	10	nt	4	T-T	<4	
6-036	40	>100/p	>100/p	21- 50/n	47	50	nt	<10	nt	<4		<4	
8-032	30	>100/f	>100/f	51-100/f	49	60	++	80	10	32	T-T	8	T-T
6-084	30	51-100/p	11- 20/n	1- 5/n	69	50	-	<10	nt	16	T-T	<4	
8-041	30	51-100/g	51-100/g	21- 50/g	16	60	-	<10	<10	16	T-T	<4	
6-069	30	51-100/g	51-100/g	21- 50/g	61	50	-	<10	nt	8	T-T	<4	
6-022	30	51-100/p	51-100/g	11- 20/f	114	40	nt	<10	nt	<4		<4	
6-040	30	21- 50/g	21- 50/g	6- 10/g	17	40	nt	<10	nt	<4		<4	
6-012	30	11- 20/f	1- 5/f	1- 5/p	12	50	nt	<10	nt	<4		<4	
7-045	20	>100/g	>100/g	51-100/g	76	40	++	>90	10	256	T-T	<4	
6-024	20	>100/p	21- 50/f	6- 10/f	16	70	nt	80	nt	8	T-T	<4	
6-093	20	51-100/g	51-100/g	51-100/g	39	20	-	<10	nt	8	H-H	<4	

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
6-041	20	>100/p	21- 50/p	0	74	70	nt	<10	nt	<4		<4	
6-014	20	51-100/g	21- 50/g	21- 50/g	50	40	+	<10	nt	<4		<4	
6-098	20	>100/n	>100/p	6- 10/f	67	50	-	<10	nt	nt		nt	
6-107	20	>100/f	>100/n	21- 50/p	58	50	-	<10	nt	nt		nt	
7-012	20	51-100/g	21- 50/g	51-100/g	35	30	-	<10	nt	nt		nt	
8-036	20	51-100/g	51-100/g	51-100/g	48	60	-	<10	<10	nt		nt	
7-044	10	>100/f	51-100/f	21- 50/f	108	60	-	40	<10	512	T-T	4	T-T
8-020	10	51-100/f	51-100/g	21- 50/f	146	30	+	80	10	256	T-T	16	T-T
6-045	10	51-100/f	21- 50/f	11- 20/g	60	20	-	>90	<10	128	T-T	<4	
7-017	10	>100/g	>100/g	11- 20/g	81	50	-	<10	nt	<4		nt	
6-058	10	>100/f	>100/g	>100/g	12	50	+	<10	nt	<4		<4	
6-061	10	51-100/f	21- 50/f	21- 50/p	15	50	-	10	nt	<4		nt	
6-065	10	>100/p	51-100/p	21- 50/p	84	60	nt	<10	nt	<4		<4	
6-091	10	>100/f	11- 20/f	21- 50/f	35	40	-	<10	nt	<4		<4	
6-103	10	>100/f	>100/f	6- 10/f	178	50	-	<10	nt	nt		nt	
6-105	10	>100/n	>100/n	11- 20/g	108	60	+	<10	nt	nt		nt	
6-110	10	>100/g	>100/g	>100/g	85	50	-	<10	nt	nt		nt	
7-003	10	>100/g	>100/g	51-100/g	39	60	-	<10	nt	nt		nt	
8-012	10	51-100/g	21- 50/g	21- 50/g	50	50	-	<10	nt	nt		nt	
6-070	<10	>100/g	>100/g	21- 50/g	27	40	+	90	<10	256	H-H	<4	
6-029	<10	21- 50/p	21- 50/p	6- 10/f	12	30	nt	>90	nt	128	H-H	<4	
7-014	<10	>100/g	>100/g	>100/g	30	60	-	>90	<10	64	T-T	<4	
6-031	<10	>100/f	21- 50/f	21- 50/f	86	60	nt	50	nt	32	T-T	<4	
8-003	<10	51-100/g	51-100/g	21- 50/g	12	40	-	90	<10	32	M	<4	
6-034	<10	11- 20/f	21- 50/g	11- 20/g	16	40	nt	<10	nt	8	T-T	<4	
6-077	<10	>100/n	>100/f	>100/f	200	70	nt	<10	nt	8	T-T	<4	
6-083	<10	>100/n	>100/p	>100/g	151	60	nt	<10	nt	8	T-T	<4	
7-032	<10	>100/g	>100/g	>100/g	85	70	-	<10	nt	8	H-H	<4	
8-047	<10	>100/g	>100/g	>100/f	136	60	-	<10	<10	8	T-T	<4	
5-054	<10	>100/p	>100/g	51-100/g	87	40	nt	10	nt	<4	T-T	nt	

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
6-017	<10	>100/g	51-100/g	21- 50/g	26	40	-	<10	nt	4	T-T	<4	
6-032	<10	>100/g	>100/f	>100/f	61	70	nt	20	nt	<4		<4	
6-043	<10	11- 20/g	1- 5/g	1- 5/g	15	50	nt	<10	nt	<4		<4	
6-071	<10	>100/f	>100/p	>100/g	100	70	-	<10	nt	<4		<4	
8-004	<10	51-100/f	51-100/f	51-100/f	31	20	++	70θ	<10	<4		<4	
5-052	<10	21- 50/g	11- 20/g	6- 10/g	26	40	nt	<10	nt	<4		<4	
5-053	<10	21- 50/f	11- 20/p	6- 10/g	14	20	nt	<10	nt	<4		<4	
6-002	<10	>100/p	>100/n	51-100/g	54	60	nt	<10	nt	<4		<4	
6-015	<10	51-100/f	51-100/f	51-100/f	25	60	-	<10	nt	<4		<4	
6-051	<10	21- 50/p	1- 5/p	0	11	40	nt	<10	nt	<4		<4	
6-054	<10	11- 20/p	11- 20/f	6- 10/p	38	30	nt	<10	nt	<4		<4	
6-060	<10	>100/n	>100/n	>100/f	154	70	nt	<10	nt	<4		<4	
6-066	<10	21- 50/p	21- 50/p	21- 50/f	18	40	-	<10	nt	<4		<4	
6-067	<10	>100/f	>100/g	>100/g	70	70	-	<10	nt	<4κ		<4	
6-074	<10	21- 50/p	11- 20/p	0	174	60	-	<10	nt	<4		<4	
6-075	<10	>100/n	>100/p	>100/g	>200	80	-	<10	nt	<4		<4	
6-076	<10	>100/g	>100/p	>100/g	51	70	nt	20	nt	<4		<4	
8-005	<10	>100/g	>100/g	21- 50/g	53	60	-	<10	<10	<4		<4	
8-017	<10	>100/g	51-100/g	51-100/g	136	70	-	<10	<10	<4		<4	
8-018	<10	51-100/g	51-100/g	51-100/g	25	50	-	<10	<10	<4		<4	
8-022	<10	51-100/g	>100/g	21- 50/g	83	50	-	<10	<10	<4		<4	
8-025	<10	>100/g	>100/g	>100/g	61	70	+	<10	<10	<4		<4	
8-014	<10	>100/g	>100/g	>100/g	152	60	-	<10	<10	<4		<4	
6-082	<10	51-100/n	1- 5/n	0	172	60	-	<10	nt	nt		nt	
6-095	<10	>100/g	>100/g	51-100/g	57	40	-	<10	nt	nt		nt	
6-097	<10	>100/f	>100/p	>100/g	96	70	+	<10	nt	nt		nt	
7-006	<10	>100/g	>100/g	>100/g	114	70	-	<10	nt	nt		nt	
7-010	<10	>100/n	>100/p	>100/g	>200	80	-	<10	nt	nt		nt	
7-013	<10	21-50/f	11- 20/p	1- 5/n	13	20	-	<10	nt	nt		nt	
7-018	<10	>100/g	>100/g	>100/g	97	70	-	<10	nt	nt		nt	

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
7-030	<10	>100/f	>100/g	>100/g	65	50	-	<10	nt	nt		nt	
7-036	<10	5- 11/p	0	0	14	20	-	<10	nt	nt		nt	
7-039	<10	>100/f	>100/f	>100/f	98	60	-	<10	nt	nt		nt	
7-042	<10	>100/g	51-100/g	21- 50/g	27	30	-	<10	nt	nt		nt	
8-010	<10	21- 50/g	11- 20/g	11- 20/g	42	40	-	<10	ne	nt		nt	
8-034	<10	51-100/g	51-100/g	21- 50/g	25	40	-	<10	<10	nt		nt	