[Frontiers in Bioscience 1, e96-108, 1 September 1996]

ASSESSMENT OF SPERM FUNCTION AND CLINICAL ASPECTS OF IMPAIRED SPERM FUNCTION

Dana A. Ohl, M.D.[1, 3] and Alan C. Menge, Ph.D.[2]

[1] Section of Urology, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-0330 & [2] F4826 Mott Hospital, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0272

TABLE OF CONTENTS

1. ABSTRACT

Fertility is dependent on a complex set of events, involving both male and female components. Normal sperm function involves many steps, including motility, capacitation, acrosome reactivity and, ultimately, fertilization of the oocyte. While male fertility is most often assessed by means of gross semen parameters, infertility may also be caused by abnormal sperm function, and only by performing specific tests of this function, may the reasons for infertility become evident. Specific tests which may be helpful include semen analysis, detailed sperm motility assessment, motility longevity, hypo-osmotic swelling test, mucus penetration assay, acrosome reactivity, antisperm antibody tests, sperm penetration assay and *in vitro* fertilization. Relatively well-defined syndromes of abnormal sperm function include immunologic infertility, immotile-cilia syndrome, anejaculation and nifedipine-associated infertility.

Received: 7/31/96; Accepted: 7/31/96.

[3] To whom correspondence should be addressed, at Section of Urology, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-0330, Tel: (313)936-5770, Fax: (313)936-9127, E-mail: dana.ohl@umich.edu

2. INTRODUCTION

Infertility affects approximately 15% of couples in the United States. In about half of these cases, the male factor is the sole or contributing cause of the infertility (1). Some cases of male infertility are due to a specific cause such as that seen in men who are azoospermic following vasectomy, loss of testes due to cancer or injury, and untreated undescended testes. The infertility of some patients, however, is due to less obvious etiologies and results in less drastic changes in the semen parameters. Despite comprehensive testing, no abnormalities can be identified in a subset of infertile couples (2). In some couples with unexplained infertility, some abnormalities in sperm function may be detected. When the sperm is subjected to functional testing, some men with minor deviations of raw semen

parameters may later be found to have more significant sperm abnormalities (3-6).

The production of mature sperm cells is a complex process consisting of spermatogenesis, spermiogenesis, sperm maturation, transport and storage. The formative steps include cell division of spermatogonia, meiosis, mitosis, morphogenesis, maturational changes during transport through the epididymis and finally mixing with seminal fluids upon ejaculation (7). All these steps require interaction of germinal cells, Sertoli cells, specialized epithelial cells, secretory components and the sperm cells.

In the era of assisted reproductive technologies the evaluation of semen and the functional capacity of its main cellular component, spermatozoa, has become quite detailed. Attempts to predict the fertility potential of sperm has largely failed for several reasons: 1, the number of sperm in ejaculate is variable; 2, there is no simple, accurate and reliable assay to predict the fertilization potential of sperm and 3, unless *in vitro* fertilization (IVF) is utilized, evaluating fertility in human couples is very difficult because of potential male fertility factors. To date there is no single assay available to assess the *in vivo* or *in vitro* fertilizing potential of sperm cells as the process leading to that point is composed of numerous steps.

In this review we will attempt to give an overview on available methodologies for the assessment of sperm function and the rationale for possible therapies.

3. SUMMARY OF NORMAL SPERM FUNCTION

Following sexual intercourse during the female partner's fertile period, there are a great many functions that sperm cells must perform to be able to initiate a pregnancy. Motility, or the ability to move in a progressive fashion, is necessary to allow transit through the female reproductive tract to encounter the oocyte. The attributes of motility and fertilization capability are usually gained during the sperm cells course through the epididymis (8). The first boundary to traverse is the cervical mucus, which allows sperm survival and penetration in the periovulatory time period (9). The cervical mucus acts as a reservoir to allow continuous slow release of motile sperm into the higher reproductive tract, but also allows separation of spermatozoa from the constituents of the seminal fluid.

Upon separation from the seminal plasma, and transit into the uterus, the process of capacitation begins (10), in which complex changes in the plasma membrane occur and new motility patterns are seen. Hyperactivated motility is seen, in which the sperm move in a vigorous, but non-progressive pattern (11). Capacitation culminates in the acrosome reaction, when acrosomal contents are released and the inner acrosomal membrane is exposed (12). The enzyme acrosin, contained in non-reacted acrosome, is thought to have a role in digestion of the sperm path through the zona pellucida or in the zona binding process (13, 14). The acrosome reaction occurs following tight binding to the ZP3 receptor located on the zona pellucida of the oocyte and is a prerequisite for the fertilization process (15). Sperm which acrosome react prematurely will be unable to bind to the zona pellucida. Sperm which are unable to bind to the oocyte and/or unable to acrosome react, will also be unable to fertilize the oocyte (16). Next, there is fusion of the plasma membranes of the oocyte and spermatozoa, depositing the male-derived DNA into the oocyte. Sperm derived antigens may also have a role in early embryo development (17).

4. ASSESSMENT OF SPERM FUNCTION

Evaluation of semen has been long been a means for assessment of the infertile state. Measurement of sperm numbers, motility and morphology have been used with some degree of success in the assessment of sperm function particularly when low extremes of these parameters are used as the criteria for defining the abnormal sperm. Within “normal limits”, however, the ability to discern the functionality of sperm cells is also of clinical significance. Normal sperm function is being broadly defined as the ability of sperm cells to negotiate the obstacle course of the female reproductive tract and egg investments and to be able to fertilize the egg and in not delivering any stealth detrimental effects.

4.1 Semen analysis

The cornerstone of the evaluation of male fertility is the semen analysis. This test does, however, have limitations. While routine semen analysis can many times allow a definitive diagnosis of infertility, such as in a man with azoospermia, in many cases there remains a question of the impact of more minor semen abnormalities on the clinical status of the couple.

Typical measurements done in a semen analysis include: semen volume, semen pH, viscosity, liquefaction, sperm concentration, sperm motility, progressive motility, sperm morphology and foreign cell contamination (18). The referenced World Health Organization manual gives an excellent overview of the techniques and normal values for routine semen analysis (18). These values are noted in Table 1. In addition to the parameters listed in the table, further evaluation of raw semen may involve computer

Table 1: World Health Organization normal semen values

Volume	≥2.0 ml
Sperm Concentration	≥20 million per ml
Total sperm count	≥40 million per ejaculate
Motility	≥50% with forward progression or ≥25% with rapid progression
Morphology	≥30% (lower if strict criteria employed)

assisted determination of sperm motion characteristics (11), morphology assessment by strict criteria (19, 20), and quantification of the number of leukocytes, which may be able to damage sperm by generation of reactive oxygen species (21).

4.1.1 Sperm concentration

The most important aspect of semen analysis is detecting an adequate number of motile sperm in the ejaculate (22). Although sperm counts for fertile males range from 60-120 million per ml of semen (1), these values do not represent the minimum number of sperm necessary for fertility. The WHO lower "limit of adequacy", below which fertility begins to drop, is > 20 million/ ml and 40 million/ejaculate (18). Men with counts lower than the limit of adequacy may be fertile, but are statistically less likely to initiate a pregnancy (22). There has been a recent controversy regarding whether mean sperm concentrations have been declining over time (23, 24). Assisted reproductive technologies, such as *in vitro* fertilization (IVF), can readily overcome infertility caused by the low sperm count, if other aspects of sperm structure and function are intact.

4.1.2 Sperm motility

Since only progressively motile sperm are capable of migrating through cervical mucus and penetrating the egg and its investments, determination of the percentage of motile sperm cells in the semen specimen is critical in the evaluation of semen. Sperm specimens which exhibit decreased motility may be competent to fertilize the ovum, but if the total motile sperm per ejaculate is decreased the number of sperm reaching the upper female reproductive tract may also be decreased, thereby lessening the chance of fertilization. The total number of motile sperm per ejaculate (total sperm count x % motility) may be more important than the % motility alone. Forward progression is a measure of the sperm cell's forward velocity.

Motility and forward progression is commonly estimated by technician on direct observation. More accurate and sophisticated motility assessment can be obtained with computer assisted semen analysis (CASA) (25). Videomicroscopic observations of a sperm sample is rapidly analyzed by such devices at a speed of 20-60 frames per second. The computer calculates motion characteristics. Typical reported parameters include straight line velocity, curvilinear velocity, average path velocity and amplitude of lateral head displacement.

4.1.3 Sperm morphology

Morphological analysis of sperm is an important aspect of the semen analysis. Semen samples with a low percentage of sperm exhibiting normal morphology have a greatly reduced prognosis for fertility, despite normal sperm numbers and motility. Although structural defects can be found in any region of the sperm cell, abnormalities of the sperm head are most common (19).

Morphological analysis of sperm is best done on fixed, air-dried smears carefully prepared and stained with one of several stain preparations (18). There are two major classifications of sperm morphology assessment. The WHO system has a normal value of >30% normal forms (18). The method employing strict criteria for normal shapes, which was developed by Kruger, has a normal range of >15% normally shaped sperm (19, 20). The Kruger system is more time-consuming, as detailed measurements of the sperm are taken by the technician viewing under the microscope, but the result of such an assessment correlates better with other methods of assessing fertility of sperm specimens, such as fertilization during IVF cycles (20).

Semen samples with reduced incidence of normal forms typically have reduced motility and a reduced capacity to penetrate the cervical mucus, to penetrate zona-free hamster eggs and to fertilize the human oocytes (26). Sperm classified as having round heads are acrosomeless and are incapable of penetrating the zona pellucida of eggs in IVF.

4.2 Sperm survival/longevity studies

Shortened longevity of sperm may play an important role in failed fertilization when semen is deposited either naturally in the vagina or by intrauterine insemination when the timing of sperm delivery is accomplished well before ovulation.

Measuring the ability of sperm cells to maintain motility in media *in vitro* is a relatively

simple assay. However, proper preparation and processing of the semen samples is essential for obtaining a motile fraction of sperm cells free from seminal plasma and other contaminating cell types. There are three primary means for achieving this separation: 1. Washing the sperm by centrifugation. This removes seminal plasma but not the contaminating cells but does not improve the motility of the recovered sperm; 2. Swim-up procedure. In this procedure motile sperm are allowed to migrate into sperm medium that overlays the semen. This technique improves the percent motility and removes seminal plasma and other cells but greatly reduces the sperm concentration; and 3. Percoll gradient centrifugation. This procedure yields a highly motile fraction of sperm relative to the starting sample, improves sperm number relative to the swim-up procedure, and removes seminal plasma and other cells (25). It was reported that subjecting processed sperm to a 4 hour temperature-stress (40°C) improved sensitivity of an assay in predicting the success of IVF (27).

Clinical studies inform us that some sperm may remain alive and fertile in the female reproductive tract for up to 5 days (28). Although the most information would be obtained by examining motility longevity directly in female tract, there is no non-surgical method of obtaining a sample for observation. In one study, normal sperm retained 66% of initial motility after 24 hours of incubation in standard media (29). Samples from men undergoing intrauterine insemination for male factor infertility and electroejaculation specimens from men with spinal cord injury have a significantly shortened life span. At 24 hours, these groups retain only 52% and 34% of initial motility, respectively (29). In cases of decreased motility longevity, accurate timing of ovulation is critical to successful insemination and fertility.

4.3 Hypo-osmotic swelling test

When intact sperm are subjected to a hypo-osmotic medium, the permeable cell membrane allows influx of water, with resultant swelling of the cell. The hypo-osmotic swelling (HOS) test is based on this principle (30). There is a correlation between the percentage of sperm in a semen sample that shows controlled swelling, indicated by coiling of the tails, and their ability to penetrate hamster eggs and to fertilize human oocytes *in vitro* (30). However, the results of this assay have not been uniformly consistent between laboratories, casting doubt on introducing this assay as a routine procedure (31). Even simple sperm morphology assessment has been demonstrated in one study to be a better predictor of sperm penetration assay (see below) results than the HOS assay (32). Despite these shortcomings, the HOS test remains a useful indicator of sperm viability and membrane fragility, if such information is deemed clinically relevant.

4.4 Cervical mucus migration assay

The ability of sperm to migrate through cervical mucus (CM) has been used as an assessment of both sperm and CM quality. The extent of the distance migrated and the motility and number of sperm penetrating the CM are routinely assessed in both the postcoital test and in *in vitro* assays (18). Male factors that affect the outcome of CM penetration test include swimming pattern of the sperm, presence of antisperm antibodies, initial sperm motility, sperm concentration, and sperm morphology. Female factors affecting sperm migration are stage of the menstrual cycle and hormonal state, cervical infections (pH changes and inflammatory factors) and female antisperm antibodies (33). To circumvent the need for human cervical mucus samples, *in vitro* assays have substituted bovine estrous cervical mucus and more recently hyaluronate. Results of these assays appear to be relatively comparable (25). Commercial kits are available for carrying out these tests.

Rectangular-shaped microslide capillary tubes (0.1 to 0.2 mm depth) filled with CM or hyaluronate and sealed at one end are placed open-end first in a 4 ml culture tube containing 0.2 ml semen or sperm sample. These are incubated at 37^{o}C for 30 min and then examined microscopically at 200x to assess the migration distance of sperm and to determine the relative sperm concentration. The latter can be done by counting the number of sperm in a set area of the microslide, and by use of an ocular micrometer, the volume is calculated. In our laboratory, a semen specimen is considered to have normal CM migration if a vanguard of $\geq$ 5 sperm achieve $\geq$ 30 mm distance in a column of CM or CM substitute. When an abnormal test is seen, *in vitro* cross-testing with female-partner CM/ substitute CM, and male-partner/donor sperm allows assaying the possible sources of problem (25).

Positive associations have been reported of the CM results with the data obtained from IVF cycles (34, 35).

4.5 Tests of capacitation: hyperactivation, acrosome reaction, acrosin assays

Capacitation requires separation of sperm from seminal plasma, followed by incubation for a defined period in the female tract or an appropriate laboratory medium. Laboratory methods to detect capacitation include; sperm penetration assay with zona-free hamster eggs (discussed separately because of its high use in clinical laboratories), detection of hyperactivated motility, determination of ability of

the sperm to undergo the acrosome reaction (AR) and acrosin content measurement. Results are based on the relative percentage of motile sperm cells with a positive result compared with a control sperm sample.

Analysis of hyperactivation (HA) requires use of a computer-assisted semen analyzer (CASA) system (11). There are commercially available systems that have programs to run the HA analysis. Although HA seems to be a prerequisite to sperm fertilizing capability, and some correlations to clinical fertility and infertility have been noted (11), the clinical value of this assay remains to be established.

Methods to assay the AR are dependent on detecting the presence of acrosomal membrane or contents by staining with dyes, fluorescent-labeled lectins or antibodies (25, 36, 37). Because of the low spontaneous rate of AR in human sperm specimens, induction of AR with reagents such as calcium ionophore A23187 produces a more consistent and controlled response in capacitated sperm cells (38). Once recombinant ZP3 becomes commercially available, an AR assay may be developed that more closely simulates the natural conditions.

Stimulation of AR is performed on the processed sperm suspension with either with a low concentration (1.25 or 2.5 µM) of A23187 for 3 hr (38) or a higher concentration (10 µM) for 30-60 min after a capacitation period of 3 hr (39). The cells are then washed once and resuspended in fresh protein-free medium or alcohol and smears are air dried on microslides. Multispot glass slides are convenient to use with multiple samples or experimental treatments. The medium suspended sperm need to be fixed in alcohol before staining. The use of the fluorescent-labeled lectins, peanut agglutinin (PNA), or Pisum sativum agglutinin (PSA) are most widely used and yield easy-to-read preparations under fluorescent microscopy (25). Test sperm samples are compared with control samples of positive reactive sperm, with tests performed simultaneously under the same conditions. Non-treated samples have a low rate of AR, with a substantial increase following ionophore treatment (39, 40). One study showed a spontaneous AR rate of 3.4, which increased to 23.3% with treatment (40). Sperm samples with high percentages of premature AR or semen which fail to respond to ionophore are unlikely to fertilize the ovum (16).

The degree of enzyme activity of acrosin in sperm correlates with the male fertility but clinical application of such an assay is not widely utilized (41). There are several approaches for assaying the activity: 1. use of a commercial kit (Accu-Sperm: OEM Concepts) that measures enzymatic conversion of substrate by some prescribed concentration of sperm (41), and 2. gelatin-film lysis by individual sperm cells (42). Individual halos are measured and averaged per sample. The clinical relevance of such tests is presently unknown.

The degree of correlation between the AR assay and acrosin assay and an analysis with fertility, *e.g. in vitro* fertilization, has not yet been established. A study on a small group of patients reported a strong correlation between acrosome morphology and acrosin activity with the results obtained from IVF (43).

4.6 Human sperm penetration assay (SPA) with zona-free hamster eggs

This assay assesses the ability of sperm to successfully undergo capacitation, the acrosome reaction, membrane fusion with oocytes and chromatin decondensation (44). The SPA has been used to classify men as likely to be fertile or infertile, to predict the success of ART procedures, and to assess certain male infertility therapy outcomes (45, 46). There are two basic methods for preparing the sperm cells for the assay: 1. processing and overnight incubation of the sperm in a sperm medium to effect capacitation (47); 2. mixing and storage of sperm, either fresh or processed, in TEST-yolk buffer (TES-tris buffer with egg yolk) for 24-48 hr, followed by thermal shock (48). The sperm concentration is adjusted, microdrops are made and the zona-free hamster eggs are added for 3 hr before determining the percentage of eggs penetrated and the number of sperm penetrations per egg. Penetrations are noted by detecting swollen sperm heads within the oocyte cytoplasm. These can be visualized in unstained eggs using phase-contrast or phase-interference microscopy or by staining the eggs with acridine orange, which allows easy identification of intact and swollen sperm heads under fluorescent microscopy (Fig 1).

Each SPA preparation method has advantages, but the TEST-yolk technique appears to be preferable, because it better correlates with fertility (48) and with the outcome of IVF (49). Johnson and colleagues have reported that this system allows a higher number of penetrations per oocyte (termed the sperm capacitation index), with a lower assay limit in fertile men of 5 sperm penetrations per egg (48).

Other SPA modifications have been suggested by different investigators to improve the SPA. This includes treatment of sperm with follicular fluid or the induction of the AR with an ionophore in an attempt to improve differentiation between fertile and nonfertile men (38). However, to date, no large scale prospective studies have been reported using these methods.

4.7 Hemizona binding assay

Assessing sperm binding to the zona pellucida determines availability and functionality of the molecules on the sperm cell necessary for

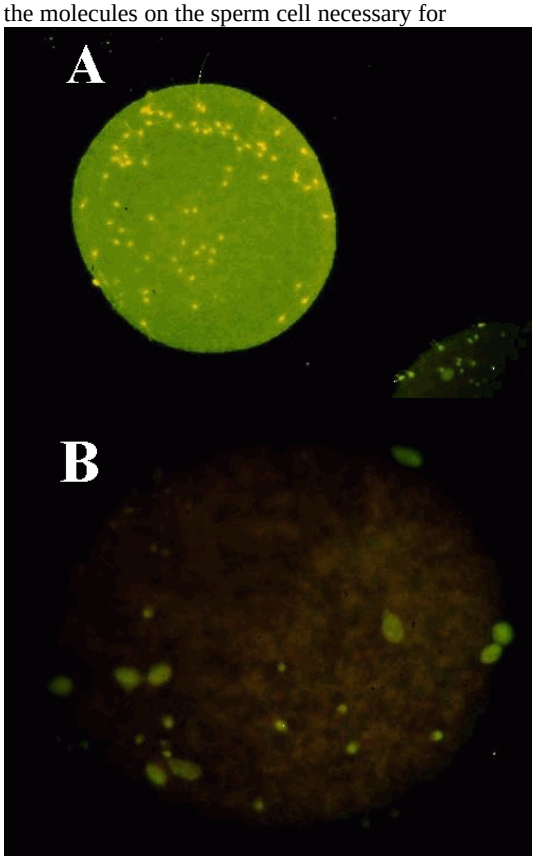

Figure 1: Fluorescent microscopy of acridine orange stained zona-free hamster oocytes, after exposure to capacitated human sperm. In A, sperm is bound to the oocyte but no penetration is seen. In B, sperm has penetrated the oocyte.

interacting with the egg. The SPA, while evaluating some aspects of the fertilizing process is unable to test zona binding, since the assays are performed on hamster oocytes devoid of zona pellucida. The hemizona assay uses a bisected zona pellucida from a human oocyte that was rendered nonfertilizable during a clinical IVF trial. The halves are placed with different sperm samples to assess sperm binding. Sperm are processed as for IVF and incubated with the ZP halves for 4 hr before counting the bound sperm cells. Known positive-binding sperm samples are used as controls (50). The assay has shown a high predictive value for IVF. Preparation for this assay requires skillful microdissection to halve the zonae.

Another procedure, known as competitive zona binding, labels the patient and donor sperm cells with different fluorochromes (*e.g.* fluorescein and rhodamine). Motile sperm from the two samples are mixed in equal numbers and added to intact zonae. After an appropriate incubation period, the bound sperm of the samples are counted under a fluorescent microscope. Using this method, the sperm binding results along with motility and morphology were predictive of the outcome of IVF (51).

Both methods require a supply of human oocytes which are not always readily obtainable and thus, general application of these assays appears limited. Oocytes have been successfully used when stored in salt solution or fresh/frozen.

4.8 Antisperm antibody assays

Successful detection and quantitation of antisperm antibodies is a necessary prerequisite in ART. Simple methods exist for direct analysis of antibodies bound to sperm cells using the SpermMar assay (Conception Technologies, LaJolla, CA) or Immunobead test (IBT) (Irvine Scientific, Santa Ana, CA) (18, 52). For preliminary screening, the SpermMar is rapid and allows the test to be performed on unprocessed semen, whereas, the IBT requires some sperm processing before assaying (53). Both assays permit semiquantitative assessment of the degree of antibody binding as well as the detection of IgA and IgG classes of antibodies. Samples should be considered positive if $\geq$20% of the sperm bind the beads (Fig 2).

Since mucosal immunity and systemic immunity may occur independently in the female partner, thorough antisperm antibody analysis requires assaying both the serum and the cervical mucus. An indirect IBT assay utilizing serum or CM-pretreated-donor sperm is an approach that identifies both the presence and class of antisperm antibodies. Other methods for detection of antibodies against sperm include the tray agglutination test and immobilization test routinely used for screening serum samples because of ease of assaying multiple samples in one run (54). Solubilized CM can also be assayed by these methods. Positive samples can be further evaluated, if necessary, by the IBT.

4.9 *In vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI)

The ability to fertilize oocytes is considered as the ultimate test of sperm function. Therefore, many studies have made comparisons of results of different assays with oocyte fertilization rates in IVF trials. However, a more appropriate parameter would be the percent of MII oocytes which fertilize and achieve the 8 cell stage by 3 days after insemination. Sperm factors such as morphology or antisperm antibodies can affect the quality of the embryo.

With the advent of ICSI, men classified as infertile due to the presence of a high titer of

antisperm antibodies in the semen or semen containing a low number of motile sperm or sperm with abnormal morphology now have a hope to become a parent. Even in the absence of sperm in the semen, aspiration of vas deferens, epididymis or

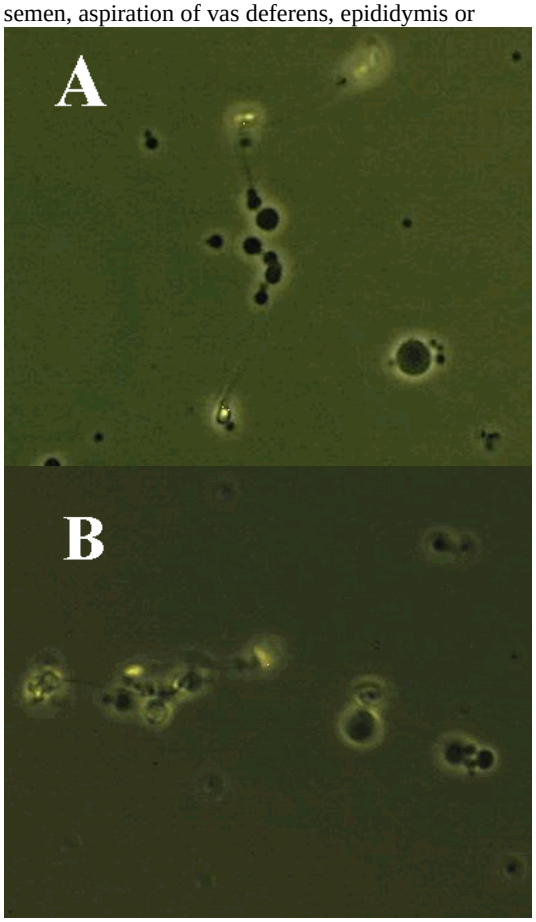

Figure 2: Immunobead test demonstrating the presence of antisperm antibodies. A. Immunobeads bound to the sperm tail. B. Diffuse binding of immunobeads, indicating presence of several locations of antibody binding.

testis often results in retrieval of an adequate number of viable sperm for the ICSI procedure (55). Embryo development and establishment of a pregnancy following ICSI suggests normal post-fertilization sperm function.

5. Selected clinical male infertility syndromes with impairment of sperm function

5.1 Antisperm antibodies

Antisperm antibodies may be detected in 8% -21% of infertile males (56-67). Due to initiation of spermatogenesis, sperm-specific antigens first appear at the time of puberty . Since such antigens are not present during development of immunological tolerance, these proteins are potential targets for an immune response and therefore generation of antisperm antibodies.

The specific antigens which are targets against which antisperm antibodies are generated are still being elucidated. Potentially relevant antigens include PH-20, LDH-C4, SP-10, HSA-63, FA-1, CS-1 and GA-1 (68). The roles of most of these antigens in sperm function are currently unknown. However, FA-1 (fertilization antigen-1) and CS-1 (cleavage signal protein) appear to be respectively important in the fertilization process (69) and in oocyte cleavage (70). Antibodies to sperm antigens may inactivate their functions and therefore lead to infertility. Specific antibodies against FA-1 have been demonstrated in a high percentage of clinical cases of human immunoinfertility (69).

Functional impairments due to antibodies can also be more generalized. Agglutination of sperm may lead to their inability to move through the female reproductive tract. Sperm cytotoxicity may result (71). Sperm with bound antibodies may be unable to penetrate through cervical mucus and there is some suggestion that the Fc region of IgA is responsible for this inhibition (72). Antisperm antibodies on the sperm head may impair development of the acrosome reaction (73). Decreased *in vitro* fertilization rates have been seen when the man harbors such antibodies (74).

Treatments devised to circumvent infertility related to antisperm antibodies have been disappointing to date (75). Generalized immuno-suppression with corticosteroids has had conflicting results, with some studies showing a mild improvement in pregnancy rate and others showing no improvement (76). Laboratory techniques to separate antibodies from sperm by physical means have had little effect (77). In most studies, intrauterine insemination afforded only a slight improvement in the pregnancy rate (78). *In vitro* fertilization may further improve the pregnancy rate but bypassing the block in fertilization may only reliably be achieved by intracytoplasmic sperm injection (ICSI) (79). Further experience with novel laboratory antibody removal techniques will hopefully yield better results in the future.

5.2 Immotile cilia syndrome

The development of somatic epithelial cilia and sperm flagella is under complex genetic control. When the construction of these structures is changed by a mutation of a crucial gene, the immotile cilia syndrome results (80). Electron microscopic examination of respiratory cilia and sperm tails (Fig 3), shows ultrastructural abnormalities of the microtubular structure.

The incidence of the immotile cilia syndrome is approximately 1/20,000 (81). Loss of microtubular function is most evident in the respiratory system. The inability to clear mucoid secretions from the lungs results in bronchiectasis and sinusitis (81). Association of these two

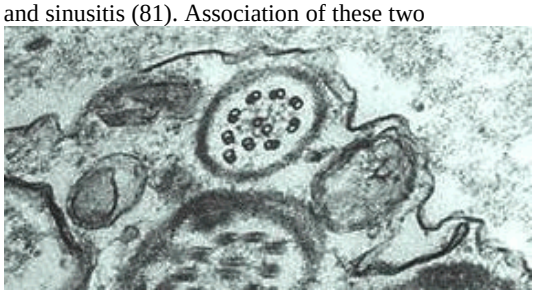

Figure 3: Transmission electron micrograph of a sperm tail lacking inner and outer dynein arms. This individual had immotile cilia syndrome and despite sperm viability of 60%, shows total absence of sperm motility

components of the syndrome with *situs inversus* (most likely to due lack of normal ciliary function during embryonic development, a 50/50 chance), is called Kartagener's syndrome (82*). Situs inversus* poses no health hazard and has no clinical implication.

The infertility of men with immotile-cilia syndrome is due to lack of sperm motility (83). There is no specific cure for this sperm defect. However, since the sperm from such individuals demonstrate normal viability, if they enter the oocyte, they should be able to function normally. In fact, intracytoplasmic sperm injection (ICSI) may be used to circumvent thismotility problem and to yield fertilized eggs that would undergo normal embryogenesis (84).

5.3 Spinal cord injury/anejaculatory infertility

Spinal cord injury (SCI) may lead to infertility due to erectile and ejaculatory dysfunction (85). There are reliable methods to induce release of semen in SCI men, including stimulation of a reflex ejaculation by penile vibration and electrical stimulation of seminal emission by rectal probe electroejaculation (EEJ). EEJ may also be used for men who have neurogenic anejaculation due to retroperitoneal surgery, diabetic neuropathy, multiple sclerosis and other conditions which affect this reflex. Sperm from induced ejaculation can be harvested and used for artificial insemination. However, the pregnancy rates remain disappointingly low from such endeavors (86).

The functional characteristics of sperm were examined in 32 anejaculatory men who underwent EEJ. Semen analysis showed high sperm counts and poor sperm motility. The average total antegrade sperm count was 448 x 10^6 and the average retrograde sperm count was 2.3 billion, but the motilities were 10.9% and 6.2%, respectively. Sperm viability very closely paralleled the sperm motility readings, indicating that the absence of motility was due to sperm cell death rather than an isolated

Table 2: Electroejaculation semen parameters in a population of anejaculatory men.

	Fraction:	
	Antegrade	**Retrograde**
Total sperm count	448 x 10^6	2304 x 10^6
Motility	10.9 %	6.2 %
Total motile sperm count	78 x 10^6	318 x 10^6
Normal morphology	43 %	46 %
Viability	10.4 %	5.3%

motility defect. Further functional testing showed that sperm survived more poorly after overnight incubation, again testifying to their poor longevity. In the cervical mucous penetration test, they performed poorly compared to normal donor sperm. Sperm penetration assay scores were also poor (87). The functional abnormalities in anejaculatory men are not due to the presence of antisperm antibodies (88).

Reasons for poor survival and functional abnormalities in anejaculatory infertility are not known. However, since this heterogeneous clinical group exhibits similar deficiencies, a common neurological factor may be involved. There is experimental evidence to suggest that denervation of the testis can lead to changes in sperm motility in the rat model (89). Further work with this patient population will hopefully yield more clues into the neurological control of spermatogenesis and sperm function.

5.4 Nifedipine-associated infertility

The acrosome reaction is a complex calcium-dependent process (90). Premature spontaneous acrosome reaction prior to reaching the oocyte may lead to early sperm cell death. On the other hand, the inability of sperm to undergo stimulated acrosome reaction in response to the oocyte investments and/or follicular fluid may lead to failure of sperm to fertilize the ovum.

It has recently been demonstrated that nifedipine, a calcium channel blocker, has the capability of blocking the acrosome reaction (91).
A group of men taking nifedipine for hypertension were found to have reversible disordered expression of head-directed mannose-ligand receptors and low rates of acrosome reaction during capacitating conditions. The laboratory was also able to reproduce these findings *in vitro* by introducing nifedipine in the medium of sperm from normal donors (92). Clinical nifedipine-associated infertility has been reported. Following cessation of the drug, the acrosome reaction status has returned to normal and subsequent pregnancy has been achieved (5)

The fact that problems related to a very common treatment for hypertension went unrecognized for such a long time raises the question of how many other drug-related effects on sperm function may exist. Only through improved understanding of specific biochemical processes involved in human sperm function will enable us to identify and rectify such problems.

6. THERAPIES AND FUTURE DIRECTIONS

ICSI is one of the major advances in the treatment of male infertility and has overcome severe male factor problems. However, all cases of male factor do not require ICSI. Semen specimens with low motility or forward progression may respond to methylxanthine reagents stimulation of the cAMP system such as caffeine, 2-deoxyadenosine, and pentoxifylline. Evidence is mounting that these treatments may be effective in some cases of male infertility and in improving IVF results (93, 94). The pentoxifylline treatment appears to improve the acrosome reaction and *in vitro* fertilization rates in those cases in which the sperm had failed the ionophore A23187 challenge test (94, 95). Pentoxifylline also improves motility parameters and hyperactivation of sperm from both normal and asthenozoospermic semen (96) and increases sperm binding to the zona pellucida in the hemizona assay of some men (97).

The future availability of recombinant ZP3 protein should allow development of an AR assay which will be dependent on the natural inducer of the phenomenon and that would render specificity to the methodology (15). With the availability of specific sperm molecules for sperm-zona pellucida binding, it would be possible to determine the complete profile of the ability of sperm to interact with the egg.

Sperm coated with a high number of antisperm antibodies are inhibited from interacting with the egg. Attempts to remove such antibodies by washing, enzyme digestion and low pH media have largely failed. Identification of sperm antigens involved in immunologic infertility will allow molecular cloning of the genes and production of recombinant peptides/proteins that may facilitate removal of antibodies from sperm cells and allowing them to fertilize the egg.

Our understanding of the processes of sperm function and how these processes may become disordered is increasing rapidly. With each new discovery, we have a new potential opportunity to impact a great many couples suffering from male factor infertility.

7. REFERENCES

1. M. Sigman, L. I. Lipshultz & S. S. Howards: Evaluation of the subfertile male. In: *Infertility in the male*. Eds: Lipshultz L. I., Howards S. S., Mosby-Year Book Inc., St. Louis (1991)

2. R. A. Lobo: Unexplained infertility. *J Reprod Med* 38, 241-9 (1993)

3. A. Mackenna: Contribution of the male factor to unexplained infertility: a review. *Int J Androl* 1, 58-61 (1995)

4. C. B. Coulam, S. B. Moore & W. O'Fallon: Investigating unexplained infertility. *Am J Obstet Gynecol* 1374-81 (1988)

5. A. Hershlag, G. W. Cooper & S. Benoff: Pregnancy following discontinuation of a calcium channel blocker in the male partner. *Hum Reprod* 10, 599-606 (1995)

6. M. Busacca, F. Fusi, C. Brigante, N. Doldi, M. Smid & P. Vigano: Evaluation of antisperm antibodies in infertile couples with immunobead test: prevalence and prognostic value. *Acta Eur Fertil* 20, 77-82 (1989)

7. C. Huckins & D. Hellerstein: Spermatogenesis in the adult: characteristics, kinetics and control. In*: Infertility in the male.* Eds: Lipshultz L. I., Howards S. S., Mosby-Year Book Inc., St. Louis (1991)

8. D. E. Brooks: Metabolic activity in the epididymis and its regulation by androgens. *Physiol Rev* 61, 515-55 (1981)

9. D. S. Settlage, M. Motoshima & D. R. Tredway: Sperm transport from the external cervical os to the fallopian tubes in women: a time and quantitation study. *Fertil Steril* 24, 655-61 (1973)

10. M. C. Chang: The meaning of sperm capacitation. A historical perspective. *J Androl* 5, 45-50 (1984)

11. L. Burkman: The motility of human spermatozoa before and after capacitation. In: *Gametes-The Spermatozoon*. Eds: Grudzinskkas J., Yovich J., Cambridge University Press, Cambridge (1995)

12. J. M. Bedford: Sperm capacitation and fertilization in mammals. *Biol Reprod* 2, 128-58 (1970)

13. D. Y. Liu & H. W. Baker: Inhibition of acrosin activity with a trypsin inhibitor blocks human sperm penetration of the zona pellucida. *Biol Reprod* 48, 340-8 (1993)

14. U. A. Urch, N. J. Wardrip & J. L. Hedrick: Proteolysis of the zona pellucida by acrosin: the nature of the hydrolysis products. *J Exp Zool* 236, 239-43 (1985)

15. M. van Duin, J. E. Polman, I. T. De Breet, K. van Ginneken, H. Bunschoten, A. Grootenhuis, J. Brindle & R. J. Aitken: Recombinant human zona pellucida protein ZP3 produced by Chinese hamster ovary cells induces the human sperm acrosome reaction and promotes sperm-egg fusion. *Biol Reprod* 51, 607-17 (1994)

16. J. Tesarik: Appropriate timing of the acrosome reaction is a major requirement for the fertilizing spermatozoon. *Hum Reprod* 4, 957-61 (1989)

17. K. Ahmad & R. K. Naz: Effects of human antisperm antibodies on development of preimplantation embryos. *Arch Androl* 29, 9-20 (1992)

18. World Health Organization*: Laboratory manual for the examination of human semen and semen-cervical mucus interaction*, Cambridge University Press, Cambridge (1992)

19. T. Kruger, R. Menkveld, F. Stander, C. Lombard, J. Van der Merwe, J. Van Zyl & K. Smith: Sperm morphologic features as a prognostic factor in *in vitro* fertilization. *Fertil Steril* 46, 1118-23 (1986)

20. T. Kruger, A. Acosta, K. Simmons, R. Swanson, J. Matta & S. Oehninger: Predictive value of abnormal sperm morphology in *in vitro* fertilization. *Fertil Steril* 49, 112-7 (1988)

21. S. Sikka, M. Rajasekaran & W. Hellstrom: Role of oxidative stress and antioxidants in male infertility. *J Androl* 16, 464-8 (1995)

22. R. Schoysman & J. Gerris: Twelve-year follow-up study of pregnancy rates in 1291 couples with idiopathically impathically impaired male fertility. *Acta Eur Fertil* 14, 51-6 (1983)

23. E. Carlsen, A. Giwercman, N. Keiding & N. E. Skakkebaek: Evidence for decreasing quality of semen during past 50 years. *BMJ* 305, 609-13 (1992)

24. H. Fisch, E. T. Goluboff, J. H. Olson, J. Feldshuh, S. J. Broder & D. H. Barad: Semen analyses in 1,283 men from the United States over a 25-year period: no decline in quality. *Fertil Steril* 65, 1009-14 (1996)

25. D. Mortimer: *Practical Laboratory Andrology*, Oxford University Press, New York (1994)

26. D. Liu & H. Baker: Tests of human sperm function and fertilization *in vitro*. *Fertil Steril* 58, 465-83 (1992)

27. J. Alvarez, D. Minaretzis, C. Barrett, J. Mortola & I. Thompson: The sperm stress test: a novel test that predicts pregnancy in assisted reproductive technologies. *Fertil Steril* 65, 400-5 (1996)

28. A. J. Wilcox, C. R. Weinberg & D. D. Baird: Timing of sexual intercourse in relation to ovulation. Effects on the probability of conception, survival of the pregnancy, and sex of the baby. *N Engl J Med* 333, 1517-21 (1995)

29. J. Denil, D. A. Ohl, W. W. Hurd, A. C. Menge & M. R. Hiner: Motility longevity of sperm samples processed for intrauterine insemination. *Fertil Steril* 58, 436-8 (1992)

30. R. S. Jeyendran, H. H. Van der Ven, M. Perez-Pelaez, B. G. Crabo & L. J. Zaneveld: Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J Reprod Fertil* 70, 219-28 (1984)

31. D. Liu, Y. DuPlessis, P. Nayudu, W. Johnston & H. Baker: The use of *in vitro* fertilization to evaluate putative tests of human sperm function. *Fertil Steril* 49, 272-7 (1988)

32. A. Menge, L. Keller & M. Korte: Associations of sperm parameters, hypo-osmotic swelling test and hamster egg-sperm penetration assay of semen from men of infertile couples. *Assist Reprod Tech Androl* 2, 293-8 (1991)

33. S. P. Boyers: Evaluation and treatment of disorders of the cervix. In: *Infertility--evaluation and treatment*. Eds: Keye W. R., Chang R. J., Rebar R. W., Soules M. R., W. B. Saunders, Philadelphia (1995)

34. P. Berberoglugil, Y. Englert, Van, den, Bergh, M, C. Rodesch, E. Bertrand & J. Biramane: Abnormal sperm-mucus penetration test predicts low *in vitro* fertilization ability of apparently normal semen. *Fertil Steril* 59, 1228-32 (1993)

35. F. I. Sharara, E. H. Illions, C. C. Coddington & R. T. Scott: Evaluation of the Tru-Trax cervical mucus penetration test in predicting fertilization and pregnancy rates in *in vitro* fertilization. *Hum Reprod* 10, 1481-5 (1995)

36. M. Lee, G. Trucco, K. Bechtol, G. Kopf, L. Blasco & B. Storey: Capacitation and acrosome reactions in human spermatozoa monitored by a chlortetracycline fluorescence assay. *Fertil Steril* 48, 649-58 (1987)

37. N. Cross & J. Overstreet: Glycoconjugates of the human sperm surface: Distribution and alterations that accompany capacitation *in vitro*. *Gamete Res* 16, 23-35 (1987)

38. J. Aitken: Cell biology of human spermatozoa. In: *Gametes-The Spermatozoon*. Eds: Grudzinskkas J., Yovich J., Cambridge University Press, Cambridge (1995)

39. J. Cummins, S. Pember, A. Jequier, J. Yovich & P. Hartman: A test of the human sperm acrosome reaction following ionophore challenge (ARIC): relationship to fertility and other seminal parameters. *J Androl* 12, 98-103 (1991)

40. J. Parinaud, G. Vieitez, H. Moutaffian, G. Richoilley & B. Labal: Variations in spontaneous and induced acrosome reaction: correlations with semen parameters and *in vitro* fertilization results. *Hum Reprod* 10, 2085-9 (1995)

41. W. Kennedy, J. Kaminski, H. Van der Van, D. Jayendran, D. Reid, J. Blackwell & L. Zaneveld: A simple clinical assay to evaluate the acrosin activity of human spermatozoa. *J Androl* 10, 221-31 (1989)

42. G. Fiscor, L. Ginsberg, G. Oldford & R. Becker: Gelatin substrate film technique for detection of acrosin in single mammalian sperm. *Fertil Steril* 39, 548-52 (1983)

43. R. Menkveld, J. Rhemrev, D. Franken, J. Vermeiden & T. Kruger: Acrosomal morphology as a novel criterion for male fertility diagnosis: relation with acrosin activity, morphology (strict criteria) and fertilization *in vitro*. *Fertil Steril* 65, 637-44 (1996)

44. N. Bar-Chama & D. J. Lamb: Evaluation of sperm function--what is available in the modern andrology laboratory? *Urol Clin N Amer* 21, 433-46 (1994)

45. Y. Soffer, A. Golan, A. Herman, M. Pansky, E. Caspi & R. Ron-El: Prediction of *in vitro* fertilization outcome by sperm penetration assay with TEST-yolk buffer preincubation. *Fertil Steril* 58, 556-62 (1992)

46. E. J. Margalioth, M. Feinmesser, D. Navot, N. Mordel & R. A. Bronson: The long-term predictive value of the zona-free hamster ova sperm penetration assay. *Fertil Steril* 52, 490-4 (1989)

47. R. Yanagamachi, H. Yanagamachi & B. Rogers: The use of zona free animal ova as a test system for the assessment of the fertilizing capacity of human spermatozoa. *Biol Reprod* 15, 471-6 (1976)

48. A. Johnson, B. Bassham, L. Lipshultz & D. Lamb: Methodology for the optimized sperm penetration assay. In: *Handbook of the Laboratory Diagnosis and Treatment of Infertility*. Eds: Keel B., Webster B., CRC Press, Boca Raton, FL (1990)

49. Y. Soffer, A. Golan, A. Herman, M. Pansky, E. Caspi & R.-E. Raphael: Prediction of *in vitro* fertilization outcome by sperm penetration assay. *Fertil Steril* 58, 547-50 (1992)

50. S. Oehninger: Diagnostic significance of sperm-zona pellucida interaction. In: *Reproductive Medicine Review*. Eds: Smith S., Hodden and Stoughton, Kent, UK (1992)

51. D. Liu, G. Clarke, A. Lopata, W. Johnston & H. Baker: A sperm-zona pellucida binding test and *in vitro* fertilization. *Fertil Steril* 52, 281-7 (1989)

52. G. Clarke: Detection of antisperm antibodies using immunobeads. In: *CRC Handbook of the Laboratory Diagnosis and Treatment of Infertility*. Eds: Keel B., Webster B., CRC Press, Boca Raton (1990)

53. W. J. Hellstrom, S. J. Samuels, A. B. Waits & J. W. Overstreet: A comparison of the usefulness of SpermMar and immunobead tests for the detection of antisperm antibodies. *Fertil Steril* 52, 1027-31 (1989)

54. M. Korte & A. Menge: Detection of agglutinating and immobilizing antisperm antibodies. In*: CRC Handbook of the Laboratory Diagnosis and Treatment of Infertility*. Eds: Keel B., Webster B., CRC Press, Boca Raton (1990)

55. M. J. Tucker, P. C. Morton, M. A. Witt & G. Wright: Intracytoplasmic injection of testicular and epididymal spermatozoa for treatment of obstructive azoospermia. *Hum Reprod* 10, 486-9 (1995)

56. A. C. Menge, N. E. Medley, C. M. Mangione & J. W. Dietrich: The incidence and influence of antisperm antibodies in infertile human couples on sperm-cervical mucus interactions and subsequent fertility. *Fertil Steril* 38, 439-46 (1982)

57. A. C. Menge & O. Beitner: Interrelationships among semen characteristics, antisperm antibodies, and cervical mucus penetration assays in infertile human couples. *Fertil Steril* 51, 486-92 (1989)

58. J. A. Collins, E. A. Burrows, J. Yeo & E. V. YoungLai: Frequency and predictive value of antisperm antibodies among infertile couples. *Hum Reprod* 8, 592-8 (1993)

59. J. P. Jarow & J. J. Sanzone: Risk factors for male partner antisperm antibodies. *J Urol* 148, 1805-7 (1992)

60. A. A. Sinisi, B. Di Finizio, D. Pasquali, C. Scurini, A. D'Apuzzo & A. Bellastella: Prevalence of antisperm antibodies by SpermMARtest in subjects undergoing a routine sperm analysis for infertility. *Int J Androl* 16, 311-4 (1993)

61. H. Mohan, S. Yadav, U. Singh, A. Kadian & P. Mohan: Circulating iso- and auto-antibodies to human spermatozoa in infertility. *Indian J Pathol Microbiol* 33, 161-5 (1990)

62. A. Heidenreich, R. Bonfig, D. M. Wilbert, W. L. Strohmaier & U. H. Engelmann: Risk factors for antisperm antibodies in infertile men. *Am J Reprod Immunol* 31, 69-76 (1994)

63. P. Devine, B. J. Sedensky, H. S. Jordan, A. J. Friedman & B. M. Berger: Detecting semen antisperm antibodies in the clinical laboratory. *Arch Pathol Lab Med* 117, 784-8 (1993)

64. M. Bujas, B. Beric & A. Kapamadzija: Incidence of infertility of immune origin in a group of marriages with unexplained infertility. *Hum Reprod* 3, 301-2 (1988)

65. M. Busacca, F. Fusi, C. Brigante, N. Doldi, M. Smid & P. Vigano: Evaluation of antisperm antibodies in infertile couples with immunobead test: prevalence and prognostic value. *Acta Eur Fertil* 20, 77-82 (1989)

66. G. N. Clarke, P. J. Elliott & C. Smaila: Detection of sperm antibodies in semen using the immunobead test: a survey of 813 consecutive patients. *Am J Reprod Immunol Microbiol* 7, 118-23 (1985)

67. G. N. Clarke: Sperm antibodies in normal men: association with a history of nongonococcal urethritis (NGU). *Am J Reprod Immunol Microbiol* 12, 31-2 (1986)

68. R. K. Naz & A. C. Menge: Antisperm antibodies: origin, regulation, and sperm reactivity in human infertility. *Fertil Steril* 61, 1001-13 (1994)

69. R. K. Naz: Involvement of fertilization antigen (FA-1) in involuntary immunoinfertility in humans. *J Clin Invest* 80, 1375-83 (1987)

70. R. K. Naz: Effects of antisperm antibodies on early cleavage of fertilized ova. *Biol Reprod* 46, 130-9 (1992)

71. S. Mathur, C. Rosenlund, M. Carlton, J. Caldwell, M. Barber, P. F. Rust & H. O. Williamson: Studies on sperm survival and motility in the presence of cytotoxic sperm antibodies. *Am J Reprod Immunol Microbiol* 17, 41-7 (1988)

72. R. A. Bronson, G. W. Cooper, D. L. Rosenfeld, J. V. Gilbert & A. G. Plaut: The effect of an IgA1 protease on immunoglobulins bound to the sperm surface and sperm cervical mucus penetrating ability. *Fertil Steril* 47, 985-91 (1987)

73. R. Bandoh, S. Yamano, M. Kamada, T. Daitoh & T. Aono: Effect of sperm-immobilizing antibodies on the acrosome reaction of human spermatozoa. *Fertil Steril* 57, 387-92 (1992)

74. R. Zouari, M. de Almeida, D. Rodrigues & P. Jouannet: Localization of antibodies on spermatozoa and sperm movement characteristics are good predictors of *in vitro* fertilization success in cases of male autoimmune infertility. *Fertil Steril* 59, 606-12 (1993)

75. D. A. Ohl & R. K. Naz: Infertility due to antisperm antibodies. *Urology* 46, 591-602 (1995)

76. P. J. Turek & L. I. Lipshultz: Immunologic infertility. *Urol Clin North Am* 21, 447-68 (1994)

77. G. G. Haas, O. J. D'Cruz & B. M. Denum: Effect of repeated washing on sperm-bound immunoglobulin G. *J Androl* 9, 190-6 (1988)

78. E. J. Margalioth, E. Sauter, R. A. Bronson, D. L. Rosenfeld, G. M. Scholl & G. W. Cooper: Intrauterine insemination as treatment for antisperm antibodies in the female. *Fertil Steril* 50, 441-6 (1988)

79. S. V. Rajah, J. M. Parslow, R. J. Howell & W. F. Hendry: The effects on *in vitro* fertilization of

autoantibodies to spermatozoa in subfertile men. *Hum Reprod* 8, 1079-82 (1993)

80. Y. Watanabe & M. Okuda: Ultrastructural study of immotile cilia syndrome. *Rhinology* 22, 193-9 (1984)

81. J. Palmblad, B. Mossberg & B. A. Afzelius: Ultrastructural, cellular, and clinical features of the immotile- cilia syndrome. *Annu Rev Med* 35, 481-92 (1984)

82. B. A. Afzelius: Genetical and ultrastructural aspects of the immotile-cilia syndrome. *Am J Hum Genet* 33, 852-64 (1981)

83. B. A. Afzelius & R. Eliasson: Male and female infertility problems in the immotile-cilia syndrome. *Eur J Respir Dis Suppl* 127, 144-7 (1983)

84. H. Tournaye, J. Liu, Z. Nagy, G. Verheyen, A. Van Steirteghem & P. Devroey: The use of testicular sperm for intracytoplasmic sperm injection in patients with necrospermia. *Fertil Steril* 66, 331-4 (1996)

85. J. Sønksen & F. Biering-Sørensen: Fertility in men with spinal cord or cauda equina lesions. *Semin Neurol* 12, 106-14 (1992)

86. A. Nehra, M. A. Werner, M. Bastuba, C. Title & R. D. Oates: Vibratory stimulation and rectal probe electroejaculation as therapy for patients with spinal cord injury: semen parameters and pregnancy rates. *J Urol* 155, 554-9 (1996)

87. J. Denil, D. A. Ohl, A. C. Menge, L. M. Keller & M. McCabe: Functional characteristics of sperm obtained by electroejaculation. *J Urol* 147, 69-72 (1992)

88. A. C. Menge, D. A. Ohl, J. Denil, M. K. Korte, L. Keller & M. McCabe: Absence of antisperm antibodies in anejaculatory men. *J Androl* 11, 396-8 (1990)

89. K. L. Billups, S. L. Tillman & T. S. Chang: Reduction of epididymal sperm motility after ablation of the inferior mesenteric plexus in the rat. *Fertil Steril* 53, 1076-82 (1990)

90. L. R. Fraser: Calcium channels play a pivotal role in the sequence of ionic changes involved in initiation of mouse sperm acrosomal exocytosis. *Mol Reprod Dev* 36, 368-76 (1993)

91. L. R. Fraser & K. McIntyre: Calcium channel antagonists modulate the acrosome reaction but not capacitation in mouse spermatozoa. *J Reprod Fertil* 86, 223-33 (1989)

92. S. Benoff, G. W. Cooper, I. Hurley, F. S. Mandel, D. L. Rosenfeld, G. M. Scholl, B. R. Gilbert & A. Hershlag: The effect of calcium ion channel blockers on sperm fertilization potential. *Fertil Steril* 62, 606-17 (1994)

93. B. Rizk, S. Fountain, S. Avery, M. Palmer, M. Blayney, M. MacNamee, C. Mills & P. Brindsen: Successful use of pentoxifylline in male-factor infertility and previous failure of *in vitro* fertilization: a prospective study. *J Assist Reprod Genet* 12, 710-713 (1995)
.
94. J. Yovich, W. Edirisinghe & J. Yovich: Use of the acrosome reaction to ionophore challenge test in managing patients in an assisted reproduction program. *Fertil Steril* 61, 902-910 (1994)

95. J. Tesarik & C. Mendoza: Sperm treatment with pentoxifylline improves the fertilizing ability in patients with acrosome reaction insufficiency. *Fertil Steril* 60, 141-148 (1993)

96. J. Tesarik, A. Thebault & J. Testart: Effect of pentoxifylline on sperm movement characteristics in normospermic and asthenozoospermic specimens. *Hum Reprod* 7, 1257-1263 (1992)

97. L. Yogev, R. Gamzu, A. Botchan, A. Amit, J. Lessing & H. Yavetz: Pentoxifylline improves sperm binding to the zona pellucida in the hemizona assay. *Fertil Steril* 64, 146-149 (1995)