Common immunological thinking can be insufficient
for attaining progress in reproductive immunology.
T. Hjort

Antisperm Antibodies and Sterility: Intractable Problem or Perspective Trend of Research?

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Conflicting opinions about the effects of antisperm antibodies on fertilization can be due to inadequacy of experimental approaches in evaluating the antisperm immunity. Detection of antisperm antibodies bound to the surface of live spermatozoa can be associated with aggregation of surface antigen-antibody complexes followed by metabolic activation of spermatozoa and acrosomal reaction, which impair cell resistance. New concept of antisperm immunity and its influence on reproduction can be formulated only after comprehensive studies of the mechanisms of spermatozoon response to binding of antisperm antibodies. Further improvement of quantitative assays of antisperm antibodies and evaluation of their effect on spermatozoon function should be aimed at the selection of experimental conditions preventing changes in spermatozoa coated with antisperm antibodies during *in vitro* manipulations.

Key Words: *sterility; antisperm antibodies; flow cytofluorometry*

Production of antisperm antibodies was first demonstrated by K. Landsteiner [41] and I. M. Mechnikov [50] at the close of the 19th century. Since first publications on detection of antisperm antibodies (ASAB) in the sera of infertile women by primitive nonspecific methods, which however were widely used in clinical practice for decades, the existence of immunological sterility (IS) determined by the presence of ASAB in sera and reproductive tract secretions of both men [66] and women [19] was postulated. The role of ASAB in the pathogenesis of sterility was intensely investigated, but the problem remains unsolved. Some authors revealed a correlation between the presence of antibodies and decreased incidence of pregnancy [10, 12,15,34], while others doubt the effect of ASAB on the probability of pregnancy. Studies of extracorporeal fertilization and introduction of the spermatozoon into the oocyte cytoplasm showed that the incidence of pregnancy was the same in patients with and without ASAB [17,40,46,72]. Moreover, implantation more often occurred in patients with ASAB [58,61]. The titles of reports "Immunological Sterility: Fact or Speculations?" [37], "Therapy and Antisperm Antibodies: Fraught or Victory?" [8], and "Antisperm Antibodies and Sterility: Insoluble Problem" [32] are sufficiently expressive; the rightfulness of the term "immunological sterility" is doubted [77].

It is now obvious that evaluation of the role of antisperm immunity directly depends on adequacy of the methods used for detecting ASAB. A discussion of the problem of antisperm immunity was unfolded in Human Reproduction journal in 1999. It was started with a report by F. M. Helmerhorst *et al.* who came to a depressing conclusion: "there is no universal standard method for detecting ASAB, no univer-

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sally acknowledged concept on the clinical significance of ASAB, and no proof of ASAB effect on reproduction" [29]. The authors claim that routine methods cannot be used for detection of ASAB for the diagnosis of sterility and there are no methods for evaluating treatment efficiency.

The absence of universal criteria for the presence of ASAB and notions on their clinical significance heighten skeptical attitude to the problem of IS and the necessity of immunological examinations of sterile patients. The number of studies of antisperm immunity decreased.

Mixed agglutination reaction (MAR test), based on cross-binding of Fc fragments of immunoglobulin adsorbed on latex beads and ASAB on spermatozoon membrane by antiserum to human immunoglobulin, is most often used for detecting ASAB [31,36]. Though MAR test is recommended by WHO for evaluation of antisperm immunity [82], its prognostic value is disputed. There is a report about pregnancy in patients with highly positive MAR test and ASAB of the IgG and IgA classes in the ejaculate [49]. Immunobead test (IBT) similarly to MAR test detected no difference between the development of pregnancy after *in vitro* fertilization with IBT-positive and IBT-negative semen [17,40,46,72].

Flow cytofluorometry (FCM) is one of the most up-to-date and promising methods allowing estimation of ASAB on live spermatozoa after their interactions with human IgG covalently bound to a fluorescent label. This method estimates the percentage of spermatozoa coated with antibodies and evaluates the number of various classes of ASAB on their surface [1-4,25,39,53-55,57,62-64,70]. This method allows to detect ASAB on immobile spermatozoa and to analyze up to 500 cells/sec. It makes possible to examine many samples within a short time.

The method is based on evaluation of IgG binding to the surface of live spermatozoa, which is regarded as an essential advantage excluding nonspecific reaction between immunoglobulins and dead cells occuring in enzyme immunoassay [5,81], radioimmunoassay [24], and immunofluorescence analysis [33,79].

However the sensitivity of FCM test is much lower than of MAR test. M. Rasanen *et al.* [62] obtained positive results in MAR test in 9 (82%) of 11 IBT-positive seminal samples, the percentage of MAR-positive spermatozoa was also high (71%), while FCM test was positive in only 5 (45%) samples and detected only 13% IgG-positive spermatozoa. MAR test detected 95% positive samples in testing of FCM-negative sera of infertile donors [59]. In our studies the percentage of FCM-positive spermatozoa in MAR-positive sera (MAR=100%) varied from 45 to 93% [57].

We suggested that the use of live cells for detecting ASAB imposes serious limitations on the interpretation of experimental data. Binding of human spermatozoon surface antigens by polyvalent antibodies and binding of surface antigen-antibody complexes by second antibodies can lead to aggregation of immune complexes, formation of so-called patches, and aggregation of patches into caps [16, 26,33]. Immune complexes undergo endocytosis with subsequent degradation, which was demonstrated for many types of cells [14,47]. Endocytosis is hardly possible in mature spermatozoa, but there are reports on shedding of immune complexes from spermatozoon surface in rats [20], urchins [78], rabbits [69], and humans [16].

We demonstrated aggregation and shedding of antigen-antibody complexes during ASAB assay with polyvalent antibodies. That is why the content of ASAB bound to the surface and ASAB remaining on the surface of spermatozoa, detected by FCM, can differ by orders of magnitude.

In some cases intensive shedding resulting in complete removal of antigen-antibody complexes from the surface is responsible for false-negative results. The intensity of shedding is determined by the intensity of aggregation, which, in turn, depends on many factors: metabolism and functions of spermatozoa, plasma membrane fluidity, experimental conditions, concentrations of ASAB and second antibodies [56]. During FCM analysis shedding can be directly stimulated by hydrodynamic and mechanical factors (cell moving in a liquid flow under high pressure).

Plasma membrane of a spermatozoon consists of several domains with specific structure, functions, and biochemical features [9], *i. e.* there are mechanisms preventing lateral drift of surface receptors on the entire plasma membrane and ensuring their location in acrosomal or postacrosomal domains and in the tail. Presumably, aggregation of antigen-antibody complexes in these domains can be independent, which is confirmed by detection of caps in different domains by fluorescent microscopy. Different number of caps and different fluorescence intensity of ASAB-coated spermatozoa can reflect selective shedding of antigen-antibody complexes in different domains (Fig. 1).

The number of ASAB on spermatozoa estimated by FCM is an important parameter used in experimental studies and for the diagnosis of IS and evaluation of treatment efficiency. It depends on changes in spermatozoa in the course of experimental manipulations. These manipulations are minimal in MAR test, and therefore shedding of immune complexes is minor, which improves the sensitivity of this method. However, the results obtained by J. E. Gould *et al.* [22] and our findings (data not presented) suggest that antigen-antibody complexes in some seminal samples can be

shed during MAR test if the duration of incubation of ASAB-positive spermatozoa is increased.

Aggregation and shedding of antigen-antibody complexes limit interpretation of FCM results; it is obvious that the search for methods inhibiting these processes acquires special attention. Standard methods blocking aggregation (low temperature, sodium azide, and cytochalasine B) did not prevent aggregation and shedding of antigen-antibody complexes from the surface of spermatozoa in MAR-positive samples [56].

Facilitated shedding of immune complexes can be due to high content of polyenic fatty acids in spermatozoon plasma membrane determining high fluidity and high susceptibility to free radical (FR) damage [38,44]. Controlled free radical oxidation is very important for spermatozoon metabolism. It ensures high membrane fluidity necessary for normal capacitation and acrosomal reaction (AR), chromatin decondensation, and fertilization [6,18]. Though spermatozoa contains cytoplasmic antioxidant enzymes (glutathione peroxidase, catalase, and SOD) their small volume limits the potentialities of the intracellular antioxidant system. An important factor protecting the spermatozoon from damage is high concentration of antioxidant enzymes and FR scavengers in seminal plasma [67]. Generation of FR increases 20-450 times after isolation of spermatozoa from ejaculate and centrifugation [7,35].

Detection of ASAB by FCN involves repeated washouts and centrifugation for separation of spermatozoa and seminal plasma, serum, or fluorescein-labeled antibodies to IgG, which can stimulate FR generation. Therefore, generation of FR in spermatozoa is the most important factor affecting ASAB detection on cell surface by standard methods. It is obvious that uncontrollable stimulation of FR generation during ASAB assay can lead to damage or alteration of plasma membrane due to activation of lipid peroxidation [71], shedding of antigen-antibody complexes [30,60], and false-positive results in detection of ASAB.

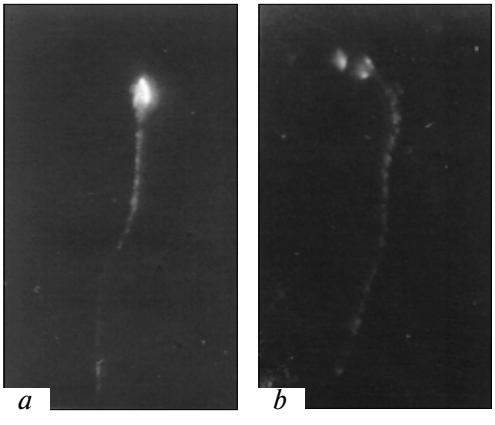

Fig. 1. Distribution of antigen-antibody complexes on the spermatozoon surface (fluorescent microscopy, *400): *a*) even distribution; *b*) fluorescent clusters of aggregated antigen-antibody complexes.

Evaluation of active oxygen forms in the semen of fertile and infertile patients is a highly valuable prognostic test [73]. It is possible that different intensity of shedding of antigen-antibody complexes from the surface of spermatozoa of different donors can also indicate the intensity of free-radical reactions. Obviously inhibition of FR generation during ASAB assay can improve the sensitivity of FCM test.

A large body of evidence on the mechanisms of IS development was obtained from comparison of functional activities of ASAB-positive and ASAB-negative spermatozoa: ASAB decrease the motility of spermatozoa in cervical mucus [13,23], spermatozoon binding to oocyte [25], and AR [74]. However some reports are at variance with the common notion on ASAB as a factor negatively affecting fertilization. ASAB were shown to stimulate AR [28,42,68]. R. A. Bronson *et al.* [11] demonstrated that ASAB promote penetration of human spermatozoa into oocytes of golden hamsters. G. Palermo *et al.* [58] and S. V. Rajah *et al.* [61] showed that the percentage of pregnancies after *in vitro* fertilization and embryo implantation is higher in patients with ASAB-positive spermatozoa. A. I. Yudin *et al.* [80] regard binding of monoclonal antibodies to spermatozoon PH-20 antigen (surface hyaluronidase) in monkeys as the stage of AR preceding exocytosis. Hence, these data allow us to regard ASAB-spermatozoa interaction as a receptor dependent process which activates spermatozoon metabolism and probably regulates various stages of fertilization (capacitation, AR, and spermatozoon-oocyte interaction).

Contradictory notions on the functions of ASAB-positive spermatozoa can be due to (in)adequate methods of ASAB detection and high probability of *in vitro* changes in the membrane of ASAB-positive cells. It is important that in these experiments ASAB-positive spermatozoa are used after incubation of cells with ASAB-positive fluids, centrifugation, and washings, which can alter or damage plasma membrane and hence, modify the status of immune complexes on cell surface, stimulate aggregation, fusion of patches, and shedding of immune complexes.

In addition, aggregation of surface receptors drasticly activate metabolism of various cell types, in particular, increase of cell Ca^{2+} concentration [65], basophil degranulation [52], and activation of tyrosine-specific protein kinases [21]. Aggregation of surface antigen-antibody complexes can also be associated with spermatozoon activation [80]. Aggregation of spermatozoon surface antigens plays the key role in AR [43,76,80]. It was demonstrated that aggregation of surface antigen-antibody complexes affects AR in sheep [48] and mice [45]. The resistance of spermato-

zoa to experimental procedures can decrease after activation and AR. Hence, functional characteristics of ASAB-positive spermatozoa *in vivo* and *in vitro* can principally differ. The development of adequate methods for estimation of functional activity of ASAB-positive spermatozoa, excluding or reducing alterations of cell under experimental conditions is an important practical and theoretical task.

Contradictory data about the functions of ASAB-positive spermatozoa can be also due to subjectivity of methods used for evaluation of these functions. Acrosomal reaction (receptor-mediated activation and release of proteolytic enzymes) is the most important function of spermatozoa, playing the key role in the penetration of a spermatozoon through the oocyte membrane [51]. In 1996 Working Meeting on Modern Diagnostic Approaches of European Society of Human Reproduction and Embryology recommended evaluation of AR as an obligatory functional test for sterile men [75].

Fluorescent microscopy used for evaluation of the acrosomal status is a involved and subjective method. The data on the effect of ASAB on AR obtained by this method are contradictory.

In 1998 we proposed a new method for evaluating AR, based on the polymerase chain reaction (PCR) [54]. We found that the influence of ASAB on AR can depend on the status of the antigen-antibody complexes on the spermatozoon surface. AR never occurs when ASAB are equally distributed, but can be induced by their aggregation. Aggregation of surface complexes depends on specific features of spermatozoon plasma membrane, cell metabolism, antigen expression, and ASAB concentration [22]. Hence, ASAB concentration in seminal plasma or female reproductive tract (cervical mucus, peritoneal or follicular fluid) can be a factor determining the intensity of AR. Preterm induction of AR in the ejaculate can impair fertilization, at the same time, the presence of ASAB on the spermatozoon surface can promote gamete fusion.

Modern concepts on the role of antisperm immunity in reproductive disturbances in man can be developed only using adequate methods of ASAB detection in various biological fluids from sterile and fertile patients. Studies by standard immunological methods should take into account biological features of these cells. It is obvious that only parallel use of cell biological and immunological methods can lead to the development of adequate criteria for evaluation of local and systemic mechanisms of antisperm immunity in health and infertility.

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