Antisperm Antibodies: Invaluable Tools Toward the Identification of Sperm Proteins Involved in Fertilization

Mónica H. Vazquez-Levin[1], Clara I. Marín-Briggiler[1], Carolina Veaute[2]

[1]Instituto de Biología y Medicina Experimental (IBYME), National Research Council of Argentina (CONICET), Buenos Aires, Argentina; [2]Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. Ciudad Universitaria, Santa Fe, Argentina

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Correspondence
Mónica H. Vazquez-Levin, Instituto de Biología y Medicina Experimental, CONICET-UBA, Vuelta de Obligado 2490, Room B16 & B24, ZIP Code C1428ADN, Buenos Aires, Argentina.
E-mail: mhvazl@gmail.com

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The identification of sperm proteins involved in fertilization has been the subject of numerous investigations. Much interest has been dedicated to naturally occurring antisperm antibodies (ASA) and their impact in fertility. Their presence in men and women has been associated with 2–50% of infertility cases. ASA may impair pre- and post-fertilization steps. Experimental models have been developed using sperm proteins as immunogens to evaluate their involvement in sperm function. Our team has pursued investigations to assess ASA presence in biological fluids from patients consulting for infertility and their effect on fertilization. We found ASA in follicular fluids with ability of inducing the acrosome reaction and blocking sperm–*zona pellucida* interaction and used them to identify sperm entities involved in these events. We generated and utilized antibodies against proacrosin/acrosin to characterize the sperm protease system. We implemented an ELISA to detect proacrosin/acrosin antibodies in human sera and evaluated their impact upon fertility by developing *in vitro* assays and a gene immunization model. This review presents a summary of ASA history, etiology, current approaches for detection and effects upon fertility. ASA (naturally occurring, generated by animal immunization and/or of commercial origin) are invaluable tools to understand the molecular basis of fertilization, better diagnose/treat immunoinfertility and develop immunocontraceptive methods.

Introduction

Fertilization is an exceptional multistep process that involves two highly differentiated cells, the spermatozoon and the oocyte. Spermatozoa that complete spermatogenesis undergo several structural and functional modifications during epididymal maturation. At ejaculation, spermatozoa are deposited in the female reproductive tract, where they undergo several changes collectively known as capacitation, which are required for developing their full fertilizing competence. Once sperm cells arrive to the oocyte vicinity, they transiently interact with the cumulus oophorus cells and the *zona pellucida* (ZP) and finally bind and fuse to the oocyte plasma membrane (oolemma). Interaction with the oolemma is preceded by the sperm acrosome reaction (AR), a process wherein the sperm plasma and outer acrosomal membranes fuse and the acrosomal contents are released. The identification of sperm and oocyte proteins that participate in fertilization-related events has been the subject of numerous investigations aimed at unraveling the molecular basis of gamete interaction and improving diagnosis and treatment of infertility.[1–5]

Specifically regarding sperm proteins, much interest has been paid in the evaluation of antisperm antibodies (ASA), because their presence in the male and/or female partner has been related to impaired sperm function(s), diminishing the chance of

achieving a spontaneous pregnancy. For over a century, numerous reports published by experts in the reproductive immunology field have addressed in great extent findings on the etiology of ASA and methodologies for their detection, as well as their impact upon male and female infertility diagnosis and treatment (the reader may refer to a selection of contributions that summarize these topics[6–15]). Despite all these efforts, whether and/or to what extent these interfering effects occur in each individual patient has yet not been established. The identification of antigens of biological relevance involved in fertilization is essential to understand the mechanism by which ASA influence the sperm-fertilizing capacity and to develop reliable additional diagnostic methods for clinically relevant ASA.

This review report presents a brief summary of ASA history, etiology, detection methods and clinical impact, as well a revision of studies from our research team and others on the use of ASA as tools toward the identification of sperm proteins involved in fertilization-related events.

ASA brief history

Studies first demonstrating the antigenic properties of the spermatozoon were reported by the end of the 19th century by I. Metchnikoff[16] and K. Landsteiner.[17] Some of the experimental designs involved the injection of rodents with spermatozoa or testis homogenates from human beings, bulls or rodents and revealed the ability of serum from inoculated animals to agglutinate and immobilize spermatozoa from these species. Both scientists later received the Nobel Prize in recognition of their outstanding work on immunity. Since their pioneer work, numerous studies were published in the following 50 years, resulting in over 100 publications involving more than two hundred sets of experiments carried out with sperm or testis proteins, as elegantly summarized by A. Tyler.[18] A large number of these studies described sterility in animals subjected to active immunization as well as in those of passive immunization or sperm pre-treatment; in addition, other studies were also carried out with materials from the female reproductive tract.

In the 50s, the concept of 'antisperm antibodies' and their relationship with infertility was independently introduced in the reports by Rümke[19–21] and Wilson.[22,23] Numerous studies were later performed, some assessing the relationship between naturally occurring ASA, the impairment of male fertility and their impact in different infertility treatments (initially intrauterine insemination and later *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI)[24–26]), whereas others described either the relationship between ASA presence and vasectomy procedures,[27–29] or their use toward the identification of male contraceptives.[30–32]

With regard to female ASA, a report by S.R. Meaker in 1922 first documented their presence in women.[33] Based on these discoveries, the first recorded attempt at human immune contraception was made in 1929; the trial involved 20 women of proven fertility who became infertile after a year of follow-up after injection with their husband's semen.[34] A patent for a contraceptive 'spermatoxic' vaccine was issued in 1937 (US patent # 2103240); however, no product was developed worldwide from this project. Studies were later published demonstrating the ability to induce immunity to sperm antigens in female animal models,[35,36] ASA detection in sera from subfertile women and their impact in their fertility.[37–41] Altogether, these studies provided evidence on the presence of ASA in the male and female reproductive tract and their negative impact in fertility potential.

Etiology of male and female ASA

As the spermatogenic process is completed after puberty and the immune response against sperm antigens is delicately controlled during the adult life, it is expected that any imbalance or breach of the control mechanisms may lead to antisperm immunity. Under physiologic conditions, this control is accomplished by: (i) an anatomic barrier, mainly formed by the tight junctions between Sertoli cells that restrict the passage of molecules into the adluminal compartment of the testis; (ii) a physiologic barrier, composed of specific transporters located along the basolateral and apical membranes of the Sertoli cells that regulate the movement of molecules in/out of the lumen; and (iii) an immunological barrier, formed by immune cells and cytokines that induce a tolerogenic microenvironment in the testis (reviewed in[42] and[43]). On the other hand, the ability of the male gonad to produce an effective immune response against infectious agents demonstrates the existence of a defined highly regulated testis immunocompetence.

Development of ASA is typically observed in men who had suffered a tissue injury, that is, testicular

torsion, testicular surgery or trauma. Exposure of sperm antigens to the immune cells within an inflammatory context induces ASA in a high percentage of cases.[29,44–47] However, tolerance to sperm antigens was observed after vasectomy in mice in spite of persistent autoantigen stimulation in the presence of danger signals; this tolerance was dependent on the activity of T regulatory (Treg) cells and varied depending on the mouse strain.[48] Then, the development of autoimmunity to sperm is likely to be related to the ability of antigen-specific Tregs activated in the regional lymph nodes to control the immune response.

Infection is another condition that could alter the immune equilibrium in the testis. Several decades ago, it was proposed that a testicular damage after an infectious process would result from the host's immune response rather than a direct cytotoxic effect of the pathogen; the pathogen would induce an inflammatory response and change the testis tolerogenic milieu as well as the anatomic functionality of the blood-testis barrier.[49]

The presence of ASA has also been associated with obstruction of sperm transport in patients diagnosed with cystic fibrosis or congenital agenesis of vas deferens[50–52] and in those subjected to vasectomy procedures.[29,53,54] In association with these findings, serum-ASA have been reported as highly accurate in predicting obstructive azoospermia (IgG: sensitivity 85%, specificity 97%; IgA: sensitivity 99%, specificity 99%).[55] On the other side, no clear association has been found between varicocele,[56,57] cryptorchidism[58,59] and testicular sperm extraction procedures[60] with ASA occurrence.

In women, pelvic and genital infections have also been associated with ASA induction. Although the precise mechanisms by which females do not usually develop immunity against spermatozoa have not been fully elucidated, it is proposed that insemination induces a particular inflammatory reaction leading to an expansion of specific Treg cells that allows tolerance to male antigens.[61] Following this hypothesis, an inflammation caused by an infectious agent could potentially affect the female immune response, modifying the release of cytokines (IFN-γ, interleukin (IL)-10 and TNF-α, among others) and leading to immunopathological conditions related to infertility.[62,63]

Molecular mimicry between microorganisms and sperm antigens has been pointed as an alternative cause of antisperm immunity related to genital infection.[64,65] As an example, shared epitopes were demonstrated between *Chlamydia trachomatics* HSP70 and the human sperm chaperones HSPD1, HSPA2 and HSPA1L,[66] and between *Ureaplasma urealyticum* UreG and the nuclear autoantigenic sperm protein (NASP).[67] However, it still remains controversial whether an association between infection and ASA development exists, taking into account reports that show discrepancies found in several studies.[68–71] This divergence would result, at least in part, from differences in sensitivity of tests used for microbiological diagnosis and identification [molecular techniques, enzyme-linked immunosorbent assay, (ELISA)] and from the diagnosis of ASA (agglutination tests in most of the studies).

ASA in women have been reported to significantly correlate with ASA in the male partner in numerous cases.[72] The study by Witkin and Chaudhry involves an analysis carried out in over 600 couples and describes a 12.4% incidence of sperm-surface antibodies in men whose wives had antisperm antibodies in their sera. Based on these observations, two hypotheses have been proposed. The first one suggests that women would produce ASA through the idiotype/anti-idiotype mechanism; according to this hypothesis, women would produce antibodies against the paratope of the male antisperm antibody, generating an internal image that resembles the sperm antigen and thus is able to induce a humoral response.[73] The second is based on the ability of antibody-coated sperm to induce *in vitro* production of IFN-γ by lymphocytes from female donors, whereas antibody-free spermatozoa do not provoke such response. This cytokine, in turn, would activate macrophages and contribute to a pro-inflammatory cascade that would lead to an immune response against spermatozoa.[74] Notwithstanding the relevance, this topic has not been fully investigated and more studies are warranted.

Current approaches for ASA detection (direct, indirect): reach and limitations

Antisperm antibodies found associated with the sperm surface or free in the seminal fluid are mainly IgA and IgG classes; contrasting, IgM is rarely found in semen. Two tests have been worldwide used in the last 3 decades to test for the presence of ASA on the sperm surface of live spermatozoa: the mixed antiglobulin reaction (MAR) test[75] (or the commercially available SpermMAR test[76]) and the immunobead binding test (IBT).[77,78] Whereas the MAR test

is performed on a fresh semen sample, in the IBT, the seminal plasma is removed by centrifugation of the semen; beads bind to the sperm cells and the sperm/bead complexes are examined with a microscope. Beads bind to both motile and immotile spermatozoa that have surface-bound antibodies, but only motile spermatozoa with bound beads are recorded and the percentage of spermatozoa with bound beads is calculated. In addition to these methods, other authors have suggested the use of flow cytometry to quantify IgG and/or IgA levels in an objective fashion in each live sperm cell;[79,80] this assay may be coupled to an immunofluorescent test on the same sperm suspension, preferably using non-fixed spermatozoa, to assess immunolocalization of Igs in sperm regions, and preventing antigen denaturation associated with cell fixation.

In samples with a high percentage of immotile spermatozoa, an indirect test is run. In those cases, heat-inactivated seminal plasma is incubated with antibody-free washed donor spermatozoa, and ASA bound to donor spermatozoa are analyzed as described for the direct assay. Alternatively, the assay can be run using male or female sera, cervical mucus or follicular fluid from patients under evaluation. In addition to the indirect tests, serological tests to detect and quantitate isoantibodies and autoantibodies to sperm are usually performed for clinical purposes because high ASA titers in fluids have been related to ASA bound to the sperm membrane and may impair sperm performance. The use of ELISA with fixed spermatozoa or membrane extracts is not recommended, because sample processing may reduce antigen recognition by ASA.[81] On the other side, a radiolabeled antiglobulin assay[82] can be also used; this procedure is highly sensitive and specific in quantifying the antibody load but does not detect the sperm subcellular region where it is bound and cannot determine the proportion of ASA-positive spermatozoa. As recently summarized,[15] serological tests may be categorized based on the nature of the antigen source into three groups: (i) 'sperm extract' assays (immunodiffusion or immunoelectrophoresis), (ii) 'fixed spermatozoa' assays (immunofluorescence, mixed agglutination tests, ELISA and radioimmunoassay) and (iii) 'live sperm' assays (macroagglutination, microagglutination, cytotoxicity, immobilization or sperm/cervical mucus interaction).

Both the SpermMAR and IBT tests have been recommended by the World Health Organization, and detailed procedures and recommendations have been included in the last version of the Semen Manual;[83] a cutoff value of 50% of the motile spermatozoa carrying ASA has been considered clinically relevant. When an ASA test results positive, secondary tests such as sperm agglutination, sperm immobilization or blocking of fertilization have been recommended to determine the relevance of these antibodies upon sperm-fertilizing potential.[15] In any case, despite all the effort carried out to develop and to standardize tests for the assessment of ASA and the vast literature reporting the results obtained, it is still hard to establish in each individual patient, whether, or to what extent, these interfering effects occur. Part of these limitations have been attributed to (i) the interindividual high variability of semen parameters, not related to the presence of ASA, that make very difficult to obtain a study and a control population, homogeneous for semen quality, (ii) the low incidence of sperm autoimmunization in unselected infertile couples that require multicentric studies including a suitable number of patients and a suitable number of observed cycles. Antibodies may be directed to antigens that are not involved in any relevant functional event; also, the recommended tests assess the presence and localization but do not evaluate the concentration of antibodies present, which in some cases may result in impairment of a sperm function and in others not. The development of assays for the detection of ASA toward specific antigens relevant to fertilization may help improving diagnosis and eventually treatment of immunological infertility.

ASA effects upon fertility

The incidence of ASA in infertile couples is 9–55%, depending on the reporting center. Antisperm antibodies have been described in 8–21% of the infertile men (autoantibodies) and in 6–43% of infertile women (isoantibodies). However, the presence of ASA has also been detected in 1.2–19% of fertile couples, suggesting that not all ASA cause infertility. Moreover, ASA from infertile patients may be directed to dissimilar sperm antigens, and/or clusters of antigens, or possess different antigen-binding characteristics than those from fertile individuals.[84–86]

Antibodies directed against sperm components have shown to exert detrimental effects on different pre- and post-fertilization events. Antisperm antibodies can affect sperm transport, sperm motility and viability, gamete interaction and also early embryonic development, implantation and fetal

development.[11,87,88] In particular, agglutinating ASA can reduce sperm forward progressive motility and may affect sperm penetration through cervical mucus.[89] Moreover, ASA may alter sperm capacitation, AR, sperm binding and penetration of the ZP.[90–92] Other ASA may act as opsonins, facilitating the recognition and destruction of sperm by phagocytes or may evoke the complement cascade that leads to sperm lysis.[93,94] The substantial number of different sperm functions which may potentially be affected by ASA to reduce fertility indicates that several different sperm-surface entities might be involved in immunosubfertility.

The effect of female and male ASA on IVF, early embryonic development and pregnancy outcome has been extensively studied; however, results are still controversial. In a study reported in 1991, we described a deleterious effect of ASA in sera from women undergoing IVF; the study revealed significantly lower percentages of fertilization, inferior embryo quality and pregnancy rates in these cases when compared to ASA-negative patients, even though IVF procedures were carried out with bovine serum albumin in replacement of maternal serum.[95] Similar results were obtained in IVF cycles of men with sperm-bound ASA, in comparison with ASA-negative patients used as controls.[96] In agreement with these findings, some reports from the literature have shown a detrimental effect of ASA on fertilization rates after IVF;[97–101] however, others have found no ASA effect on fertilization outcome.[102–105] In a meta-analysis study combining more than 4000 cycles, no relationship was found between ASA levels detected by MAR/IBT in semen and pregnancy rates following IVF and ICSI and the reports suggested that both treatments are viable options to ASA-positive males.[106] However, conclusions from this type of studies may still be difficult to draw because of some limitations associated with the lack of standardized methods for ASA detection with appropriate cutoff levels and for the assessment of their effect upon sperm function, as well as to the difficulty in identifying the sperm epitopes responsible of ASA-related subfertility. Prospective controlled studies involving standardized methodologies for ASA assessment (method and cutoff levels), ART procedures (IVF- and ICSI-defined protocols) and endpoint determinations (i.e., fertilization rate, implantation and pregnancy rates) are essential to determine ASA impact upon fertility.

Use of ASA in biological fluids toward the identification of proteins involved in fertilization

Patients with ASA can be considered naturally immunized subjects against sperm antigens involved in the fertilization process. Thus, ASA present in biological fluids (male and female sera, seminal plasma, follicular fluid and cervical mucus) from patients with immunological infertility have been widely used to identify fertilization-relevant sperm antigens. There are numerous studies aimed at identifying the sperm entities recognized by ASA, and using a variety of methods, several sperm antigens have been already characterized. Studies using immunoprecipitation, unidimensional and two-dimensional (2-D) electrophoresis of sperm extracts exposed to sera and/or seminal plasma from immunoinfertile men allowed the detection of specific sperm proteins.[107–109] Moreover, a thorough study using high-resolution 2-D electrophoresis [isoelectric focusing (IEF) or non-equilibrium pH gradient electrophoresis (NEPHGE) followed by polyacrylamide electrophoresis (PAGE)] led to the identification of 98 sperm auto- and iso-antigens, which were recognized by sera from infertile men and women but not from fertile subjects; after vectorial labeling at the sperm surface, 6 antigens were further characterized.[110] Other investigations led to the identification of specific sperm entities (see Table I; ref. 111–122). In most of these reports, it was assumed that if ASA were obtained from immunoinfertile patients, the cognate antigens would be relevant to sperm function, but the ability of these antibodies to block specific fertilization-related events was evaluated only in some cases.

In a study from our laboratory, we described that IgG recovered from the follicular fluid (hFFIgG) of patients participating in an IVF program had the ability of inducing the AR and impairing sperm–ZP interaction.[123] Our results show that 45% (18/40) of the hFFIgGs depicted a high AR-inducing power. Some of these antibodies also inhibited sperm–ZP binding in the hemizona assay. It is remarkable that when evaluated with the IBT, only 8% (3/40) had ASA, indicating that this assay was not able to detect some antibodies with a functional effect. With the aim of identifying sperm antigens recognized by the hFFIgGs, sperm extracts were subjected to SDS–PAGE followed by Western immunoblotting. The IgGs isolated from a hFF with the ability of evoking the AR and impairing sperm–ZP binding specifically

Table I Sperm Antigens Identified with Naturally Occurring ASA

Antigen	Antigen localization	Antigen function	Exclusive of sperm	Animal immunization trials	*In vitro* antibody blocking studies
Ac1[111]	Acrosome	–	No	–	–
Ac2[111]	Acrosome	–	Yes	–	–
FLJ32704[112]	–	–	–	–	–
80 kDa HSA[113]	Sperm head	–	Yes	Yes[114]	Yes[114]
HSP70 HSP70-2 Disulfide isomerase ER60 Inactive form of caspase-3 Component 2 and zeta chain (subunits of proteasome)[115]	Sperm membrane	–	No	–	–
NZ-2[116]	Sperm surface	ZP binding	No	–	–
P36[117]	Equatorial segment	Sperm–oolemma interaction	No	Yes[117]	Yes[117]
P18[117]	Acrosomal and post-acrosomal region	Sperm–oolemma interaction	–	Yes[117]	Yes[117]
Radial spoke protein 44 (human meichroacidin)[118]	Sperm axoneme	–	No	–	–
SLLP1[119] or SPRASA[120]	Acrosome	Sperm–oolemma interaction	Yes	Yes[121]	Yes[121]
SPAG2[122]	Outer dense fibers of the flagellum	–	No	–	–

The table lists some examples of studies that have used naturally occurring ASA to identify sperm antigens. Selected proteins are listed in alphabetic order.
–: not found.

recognized an entity of 78 ± 1 kDa. After MALDI-TOF analysis, we determined that the protein corresponded to glucose-regulated protein 78 (Grp78/BiP). Further studies led to describe that spermatozoa contain Grp78/BiP; additionally, it was found that this protein is secreted by the human oviductal epithelium in periovulatory period and can associate with the acrosomal region of human spermatozoa. The recombinant Grp78/BiP was also able to modulate sperm–ZP binding when present during the hemizona assay.[124] This is an example of the use of naturally occurring ASA present in a biological fluid of infertile patients as a tool for the identification of entities involved in the fertilization process.

Use of ASA toward specific proteins as tools for characterizing the molecular basis of fertilization

Experiments based on the use of ASA developed toward specific proteins present in sperm (induced ASA) have been highly relevant in the characterization of numerous structural proteins and in the evaluation of their involvement in sperm physiopathology. Immunological methods, including Western immunoblotting, immunoprecipitation, ELISA, immunohistochemistry, immunocytochemistry and immunoelectron microscopy procedures, have provided invaluable information about cellular and tissue localization of specific sperm proteins, regulation of their expression and transport in the male tract (i.e., spermatogenesis, sperm maturation, ejaculation), modifications during sperm transport in the female tract and their participation in signaling pathways, among others. Moreover, blocking ASA have been extensively used in protocols designed to assess the contribution of sperm proteins in fertilization, both *in vitro* and *in vivo*. Table II (ref. 125–156) summarizes information on human sperm antigens characterized using antibodies, evaluations that, in some cases, included induced ASA toward these proteins to block *in vitro* gamete interaction and *in vivo* fertility. Interestingly, detection of naturally occurring ASA toward some of these proteins on the sperm surface and fluids of patients consulting for infertility was reported in the literature (i.e., FA-1[157–160]; IZUMO1[161]; YLP-12[160]).

Table II Sperm Antigens Characterized with Induced ASA

Antigen	Antigen localization	Antigen function	Exclusive of sperm	Animal immunization trials	*In vitro* antibody blocking studies
CatSper[125]	Sperm membrane	Calcium channel	Yes	Yes[126]	Yes[127]
Classical cadherins					
Epithelial cadherin[128]	Acrosomal region (intact sperm) and acrosomal membrane (reacted sperm)	ZP binding Sperm–oolemma interaction	No	–	Yes[128]
Neural cadherin[129]	Acrosomal region (intact sperm) and equatorial segment (reacted sperm)	Sperm–oolemma interaction	No	–	Yes[129]
CRISP family members[130–133]					
CRISP-1	Acrosomal region (intact sperm) and equatorial segment (reacted sperm)	ZP binding Sperm–oolemma interaction	Yes	Yes[134]	Yes[135]
CRISP-2 or Tpx-1	Acrosomal region (intact sperm) and equatorial segment (reacted sperm)	Sperm–oolemma interaction Ion channel	Yes	Yes[136]	Yes[131]
CRISP-3	Seminal plasma	Innate immunity	–	–	–
FA-1[137]	Sperm membrane	ZP binding	Yes	Yes[138]	Yes[139]
Izumo1[140]	Acrosomal region (intact sperm) and equatorial segment (reacted sperm)	Sperm–oolemma fusion	Yes	Yes[141,142]	Yes[143]
P34H[144]	Sperm membrane	ZP binding	Yes	Yes[145]	Yes[144]
SAGA-1[146]	Entire sperm surface	ZP binding	Yes	–	Yes[147]
SP-10[148]	Acrosome	Sperm–oolemma interaction	Yes	Yes[149,150]	Yes[151]
YLP-12[152]	Acrosome	AR and ZP binding	Yes	Yes[153]	Yes[154]
Zonadhesin[155]	Acrosomal matrix	ZP binding	Yes	–	Yes[156]

The table lists some examples of studies that have used induced ASA to characterize structural and/or functional aspects of sperm antigens. Selected proteins are listed in alphabetic order.
–: not found.

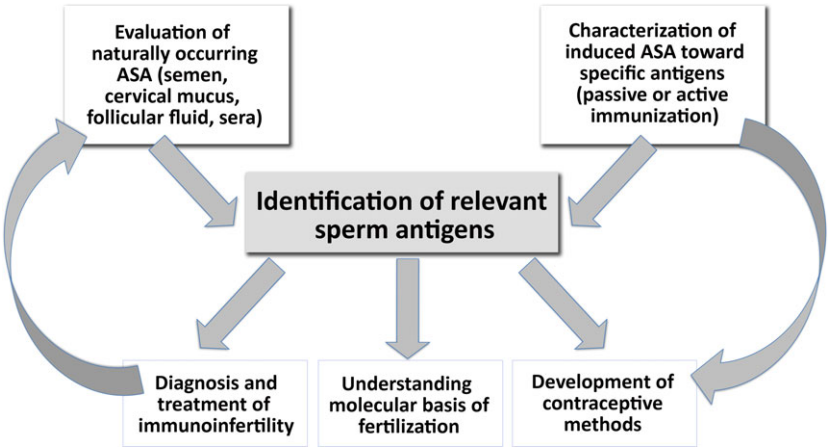

Fig. 1 Antisperm antibodies and sperm antigens relevant to fertilization. Naturally occurring (semen, cervical mucus, follicular fluid, sera) and induced (passive or active immunization) antisperm antibodies toward specific proteins have constituted invaluable tools toward the identification of relevant sperm proteins involved in fertilization. The identification to these proteins has contributed to the understanding of the molecular basis of fertilization, as well as to an improvement in the diagnosis and treatment of immunoinfertility and the development of contraceptive methods. An improvement in the diagnosis and treatment of immunoinfertility has had a direct impact upon the detection of specific naturally occurring ASA. Moreover, characterization of induced ASA has provided new insights toward the development of novel contraceptives.

Studies using these immunological strategies, in conjunction with molecular approaches involving the production of recombinant proteins and gene manipulation (transgenesis and gene knockout), had contributed to our current knowledge on sperm physiology and the fertilization process (reviewed

in[1–5]). Moreover, the inhibition of fertilization by antibodies had encouraged the assessment of immunization schemes with the aim of developing immunocontraceptive strategies.[32]

Using several immunological tools, our research team has completed a set of studies in the major sperm acrosomal proteinase acrosin (EC 3.4.21.10).[162] Localization of the proteinase in human spermatozoa was characterized by immunocytochemistry with a commercial monoclonal antibody, and identification of the human proenzyme, intermediate and mature active protein forms was carried out by Western immunoblotting of sperm protein extracts.[163] Using these tools, abnormalities in the proenzyme activation were identified in a subset of male patients, which may be related to their infertility.[164] In addition, an ELISA was designed to detect antiproacrosin/acrosin antibodies in sera of women consulting for infertility; interestingly, this assay identified antibodies in patient sera that were negative when tested with the IBT.[165] The recombinant acrosin was used to develop an ELISA test to assess the involvement of proacrosin/acrosin binding to ZP glycoproteins.[166–168] Moreover, a monoclonal antibody toward proacrosin was used to demonstrate the participation of acrosin in the mechanism of ZP-induced AR of human spermatozoa.[169] The relevance of this proteinase system in sperm function as well as the potential impact of specific anti-acrosin antibodies upon fertility regulation was further evaluated using mouse gene immunization. These studies revealed a reduced fertility in immunized female[170] and male mice[171] with high anti-acrosin antibody titers. Altogether, our studies using naturally occurring and induced (both commercially available and laboratory-produced by protein and DNA-immunization protocols) antiproacrosin/acrosin antibodies, in combination with biochemical and biological strategies, have contributed to a better understanding of the involvement of the sperm proacrosin/acrosin system in sperm function(s) and its role(s) in the physiopathology of male fertility.

Conclusion

Naturally occurring and induced antisperm antibodies have become invaluable tools in the identification of sperm proteins relevant to fertilization. The identification of these entities has been essential to understand the molecular basis of fertilization and the mechanism by which ASA may influence the sperm-fertilizing potential, as well as for designing new diagnostic methods for clinically relevant ASA and eventually for developing novel strategies for fertility control. A scheme is presented in Fig. 1 that relates the findings with ASA from both sources toward the identification of sperm proteins relevant to fertilization and the understanding of immunological infertility.

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