Immunogenicity of human spermatozoa

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Abstract

Investigation and experimental design of the study was basically aimed at developing insight into the antigenicity of spermatozoa associated proteins. Apart from studying the natural antigenicity of washed whole spermatozoa their immunogenicity was also demonstrated in vitro. The whole live spermatozoa were immobilized and agglutinated in vitro by the antibodies they induced in the laboratory model- a female rabbit. A regular immunization routine induced a high titre of antisperm polyclonal antibodies.To prepare a spermatozoa specific antigen which will not produce a cross reacting antibody against other human tissues, only the motile and live spermatozoa were selected for antigen preparation.In investigation the laboratory bred female rabbits were used as the bioactive system of production of antisperm antibody.Agglutination of whole spermatozoa has been observed on slides. The technique though simple is highly eloquent; clumping of spermatozoa confirms the existence of antisperm antibodies in the serum under examination.The results show that nature and pattern of immobilization of active motile spermatozoa are different as observed in different graphs of immobilization.The variation in spermatozoal population in antigen distribution on individual spermatozoa is reflected in different patterns of agglutination.

Introduction

Human spermatozoa have antigenic potentials and can generate an immune response. The early reports of the antigenicity of spermatozoa by Landsteiner (1899) and Metchnikoff (1899) have led other investigators to study spermatozoa of various species, their specificity, antigenic properties and or their clinical relevance for reproduction (Wilson 1954, Rumke 1970). A number of spermatozoal antigens have been identified which are highly specific to spermatozoa. Identification and characterization of human spermtozoal antigens capable of eliciting the production of antisperm antibodies is important for understanding the mechanisms involved in antibody mediated impairment of reproduction (Shai and Naot 1992). There is considerable heterogeneity in antigenic distribution of spermatozoa of fertile and infertile subjects (Purakayastha et al. 1999). Evidences suggest that antigenic expression on the spermatozoal surface varies between individuals (Witkin et al. 1988).Several laboratories have identified spermatozoal antigens using polyclonal or monoclonal antibodies (Kamada et al. 1985), or antisperm antibodies (Kamada et al. 1998).

Spermatozoa are not present in men until puberty. Antigens specifically associated with spermatozoa are foreign to the immune systems of both adult men and women. Testis-specific autoantigens appear on the late pachytene spermatocytes in the seminiferous tubules and during their maturation within the epididymis additional antigens appear. As maturation progresses in the seminiferous tubules tight inter Sertoli junctions form behind developing gametes. Auto immunization to spermatozoal antigens is normally blocked because of sequestration in the testis by the blood testis barrier and in epididymis by tight junctions. Additionally these barriers retard the ingress of blood components. This has been demonstrated by the finding that fluid from the seminiferous tubules is normally deficient in immunoglobulins, macrophages and other leucocytes (Haas 1987).

Immunologic response to spermatozoa is polyclonal so that a population of antibodies directed against different epitopes exists between different individuals. (Clarke 1988).Antibodies can be generated experimentally for simulation studies. The antibodies may combine with high affinity and specificity for ligand antigen with innate effector elements. These functions are located in separate protein domains within the antibody correspond to the specific antigen structure. An understanding of the structural and functional properties of the antigenic domains

may indicate appropriate construct for novel molecules. These novel molecules can be produced in a range of expression systems. The monoclonal antibodies or affinity purified polyclonal antibodies may be raised against antigens indicated in the study playing a role in fertilization. It is possible to assess the effect of specific antibodies on motility of spermatozoa. Characterization of antisperm antibodies is also important for a greater understanding of the events that occur at fertilization.

Materials and Methods

The study was conducted in Life science and Biotechnology Department of Jadavpur University during my PhD program. The University Research Committee approved all studies involving human semen donors. All procedures involving the use of animals were conducted in accordance with the guide for care and use of laboratory animals.

1. Human semen collection and examination

Semen was obtained from individuals undergoing assessment at the laboratory. Liquefaction, viscosity and appearance of each sample were judged visually. Semen collected from individual donors was analyzed for primary physical characteristics. Semen analyses were performed following the WHO protocol (1999) to obtain volume, pH, spermatozoal concentration, motility, and normal forms.

2. Collection of viable spermatozoa

Semen sample was mixed with Phosphate Buffered Saline (PBS) in 1:3 ratios and centrifuged for 10 minutes in 3000g. Supernatant was discarded and pellet was resuspended in PBS and centrifuged again for 10 minutes in 3000g. Again supernatant was discarded and pellet was resuspended in PBS. Then the centrifuge tube was kept undisturbed for 30 minutes in room temperature to allow the spermatozoa to swim up into medium in the top phase whereas non motile or vibrating spermatozoa remained in the bottom layer. The top layer was removed by pasteur pipette without disturbing the bottom layer. The motile spermatozoa were resuspended in PBS. Then spermatozoal count and percentage of motility was checked as of semen.

Preparation of antigen of spermatozoa

The motile spermatozoal fraction obtained from swim up procedure was used as antigen.

Raising of antisperm antibody

Female rabbits 1.5 to 2 kg taken from the department animal house from original New Zealand stock was maintained in controlled environment with minimum of noise. They were provided with food and water ad libitum. Young adult animals were used. Spermatozoal suspension collected after swim up was administered to female rabbit with the help of 1 ml syringe. Single exposure to antigen does not suffice to induce high level of immune response. Recurrent boosters are necessary to obtain a high titre of antibody. Rabbits were immunized 3 times in week up to 4 weeks for optimum level of immunization.

Preparation of antisperm antisera from rabbit

Rabbit was bled from marginal ear vein. Blood was collected drop wise into sterile glass tubes. Blood was allowed to clot for sufficient time at room temperature. Clot was allowed to retract and serum was withdrawn by pasteur pipette from the glass tube. Clear straw colored serum was stored at 4^0C in sterile plastic vials with airtight seals. Containers were properly labeled and Thiomersal (Merthiolate) 1:10000 was added for long term storage of sera.

Characterization of rabbit anti human sperm antisera

Fixed quantity of serially diluted serum was allowed to react with a calculated amount of antigen. Both were mixed thoroughly on the clean sterile glass slide, kept in moist chamber and incubated for 30 minutes at 37^0C. Both normal and antisera (sera containing antibody) were serially diluted and treated so that the difference was evident.

Agglutination

Spermatozoal agglutination assay was performed to evaluate the titre of antisera and study the characteristic patterns of antigen antibody interaction. The test was performed by taking two clean glass slides and on each one drop of antisera and one drop of control sera was placed respectively. Then one drop of spermatozoal suspension was added to each slide. The

preparation was incubated at 37^0 C for 1 hour and examined under magnification of 400x. The antisera was diluted in series of 1:5 to 1:625 to produce reactants of increasing dilution of antisperm antibody, that is a decreasing series of solutions containing antibody produced. The series of reactants were allowed to react with spermatozoa obtained from different semen samples. Nature of clumping, size of agglutination clumps; number of agglutination clumps, and number of free spermatozoa were analyzed to determine the nature of variation in spermatozoal agglutination.

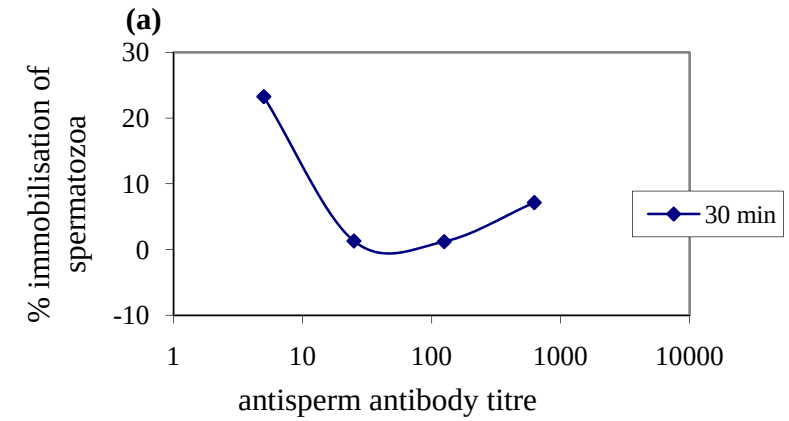
Immobilisation

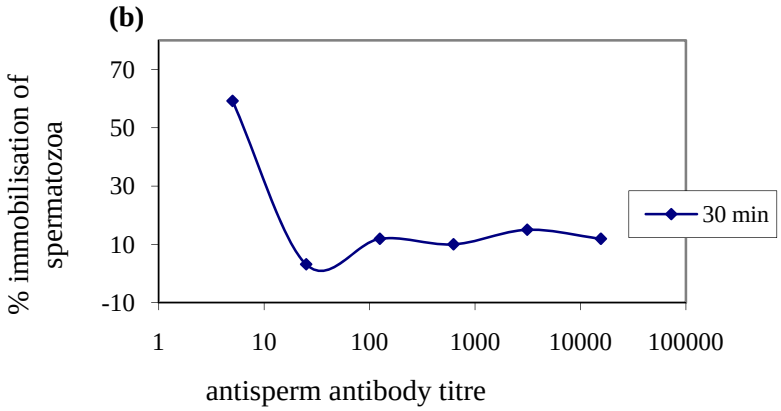
The test, introduced by Isojima et al (1968) was performed by placing one drop of serially diluted antisera on clean glass slides and for control one drop of normal sera was placed on a clean glass slide. Then one drop of spermatozoal suspension was placed on each of the slides containing serially diluted antisera and normal sera. The preparation was incubated at 37^0C for 30 minutes and then motility was checked under magnification of 400x. The antisera were diluted in series of 1:5 to 1:625 to produce reactants of increasing dilution of antisperm antibody. Nature of immobilization of active motile spermatozoa was analyzed to determine the nature of variation in spermatozoal immobilization.

Results

Exposure of viable human spermatozoa to rabbit antisera shows agglutinating and immobilizing effects. Table 1 shows the nature of agglutination after incubation with serially diluted antisera (5 times dilution) The majority of spermatozoal agglutinating and immobilizing antibodies in sera is directed against intrinsic spermatozoal antigens rather than at antigens adsorbed to spermatozoa from seminal plasma as the antigen is prepared with appropriate washing Agglutination as well as immobilization is dependent on the antibody concentration as indicated by increasing dilution the size of spermatozoal agglutinates and number of immobilized spermatozoa decreased and number of free spermatozoa increased. The numbers of agglutinated spermatozoa show different patterns of adherence to each other such as head to head, midpiece to midpiece, tail to tail, tail tip to tail tip, mixed and tangled positions. The shapes of immobilization curves (a-h) of active motile spermatozoa are found to be different when the values of spermatozoal immobilization are plotted against the dilutions of the antisperm antibody

in a logarithmic scale. The results show that nature and pattern of immobilization of active motile spermatozoa are different shown by different graphs of immobilization.





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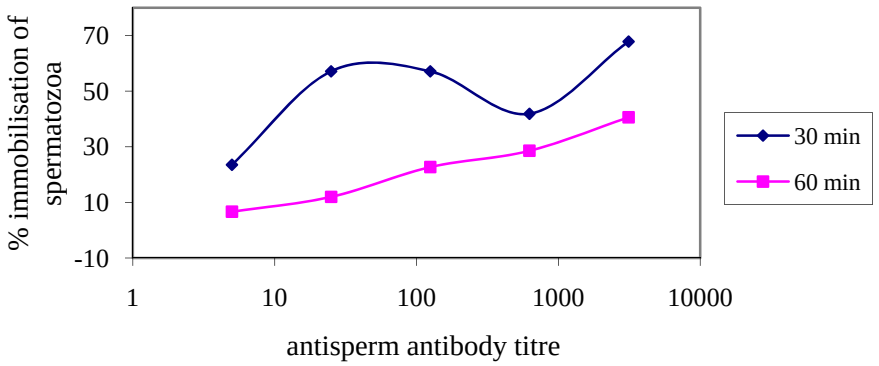

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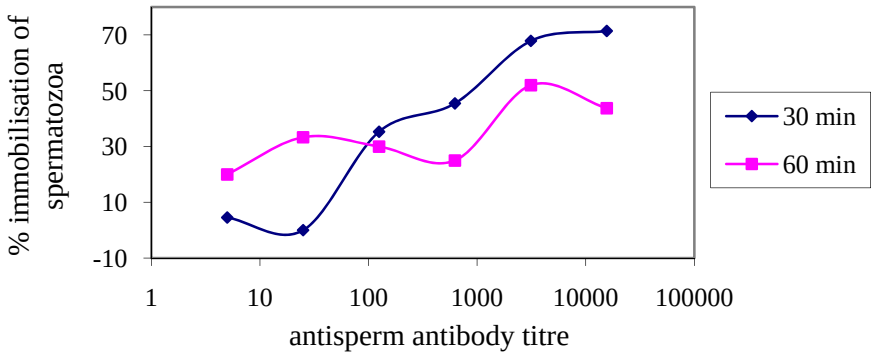

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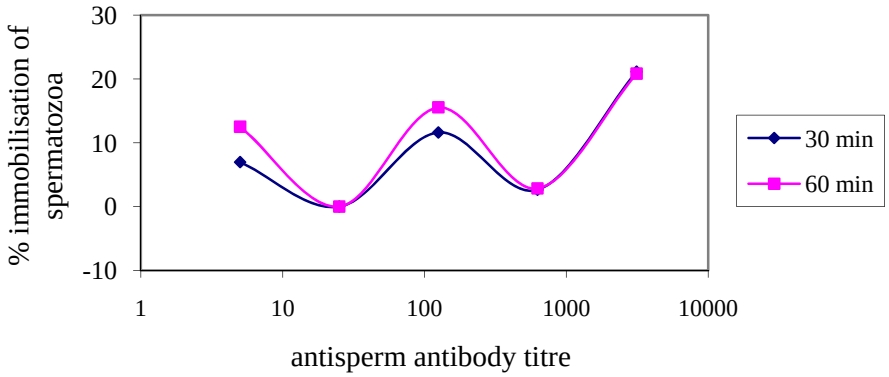

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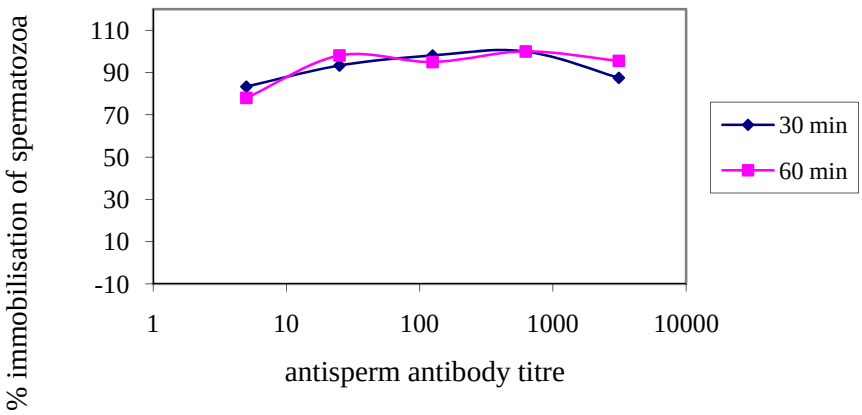

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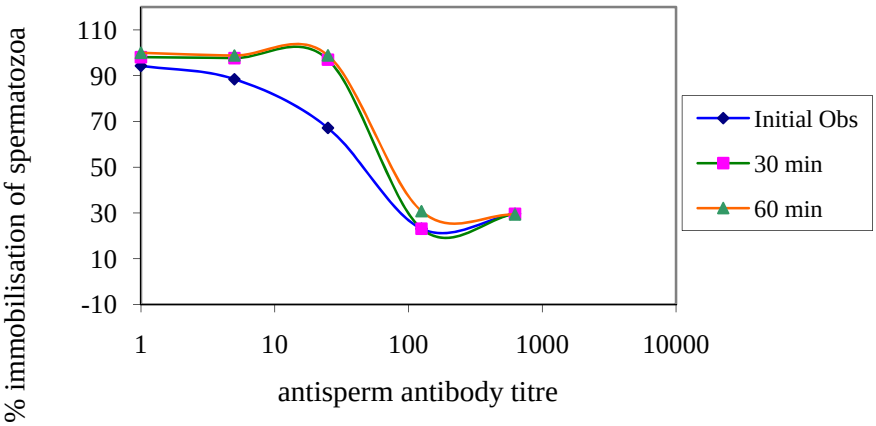

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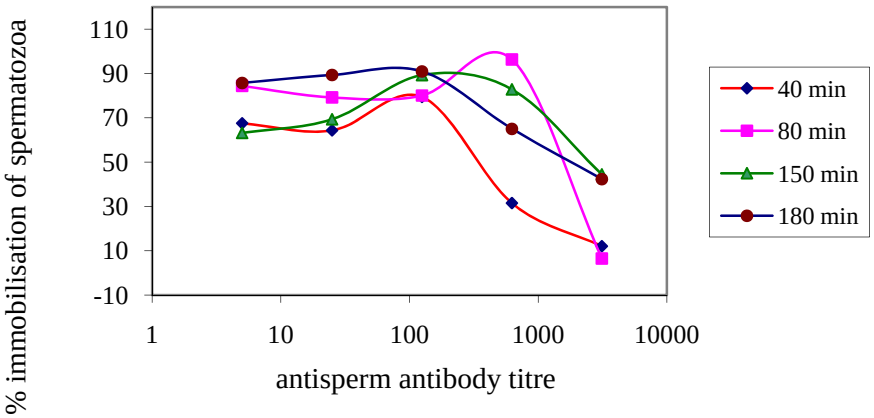

Table 5.Observation of behavior of spermatozoa after incubation with serially diluted antisera in a series of x5

Dilution grades	Serum content (in µl)	Agglutination or clumping	Immobilization
5	100	Heavy clumping of spermatozoa due to agglutination. Clumps are large in size and few in number	Few spermatozoa are free from clumps to be motile
5^2	20	Heavy clumping of spermatozoa due to agglutination, clumps are large in size and few in number	Few spermatozoa are free from clumps to be motile
5^3	4	Moderate clumping of spermatozoa due to agglutination. Clumps are large in size and few in number	Many free motile spermatozoa
5^4	0.8	Smaller clumps of agglutinated	Few free motile

		live spermatozoa. Number of clumps too many	spermatozoa
5^5	0.16	No visible clumping due to agglutination. Spermatozoa remain attached to each other at different points	Some spermatozoa still motile
5^6	0.032	No clumping due to agglutination of live spermatozoa. Spermatozoa remain attached to each other by their heads and tails	Almost all spermatozoa immotile
5^7	0.0064	No clumping is visible. Spermatozoa remain attached to each other by their heads and tails	Most spermatozoa immobile but few are mobile

Discussion

The generation of antibodies in heterologous systems helps to identify a host of molecules that are implicated in fertilization in man. Spermatozoal preparations from a donor are injected into a recipient that provokes generation of antibodies against the spermatozoal antigens. These antibodies are then isolated, purified and tested for their ability to inhibit fertilization in donor as well as in other organisms. Such antibodies become valuable tools to identify the actual molecules that mediate in fertilization. Assays for antisperm antibodies provide information on the antibody titre, percentage of spermatozoa bound by antibodies, the immunoglobulin (Ig) isotype and site (spermatozoal head, midpiece and tail) of antibody binding.Antisperm antibodies that interfere with fertility are heterogeneous and react against a multiplicity of epitopes.There is considerable heterogeneity in surface antigen distribution of human spermatozoa.

Investigation and experimental design of the study was basically aimed at developing insight into the antigenicity of spermatozoa associated proteins. Apart from studying the natural

antigenicity of washed whole spermatozoa their immunogenicity was also demonstrated in vitro. The whole live spermatozoa were immobilized and agglutinated in vitro by the antibodies they induced in the laboratory model- a female rabbit. A regular immunization routine induced a high titre of antisperm polyclonal antibodies.

In majority of studies the approach taken to isolate proteins from pooled spermatozoa is by application of several types of extraction methods. These have biological or physiological inconsistencies such as mixing up and exposing proteins of the inner surface of the membrane and presentation of the epitopes in a nonphysiological set up. So live human spermatozoa are taken as the ideal system of study.

The experimental work was carried out to cover the unexplored aspects of development of antisperm antibody. To prepare a spermatozoa specific antigen which will not produce a cross reacting antibody against other human tissues, only the motile and live spermatozoa were selected for antigen preparation. Spermatozoa of nonmotile and/or under motile nature were rejected to avoid leakage of intracellular components as prospective antigens. The swim-up technique was utilized for not only to isolate mobile spermatozoa but also to get spermatozoal surface washed free of any extraneous adsorbed antigens. The intravenous route of administration of spermatozoal suspension was adopted to expose the plasma membrane related antigens to the interacting immunocompetent cells. In investigation the laboratory bred female rabbits were used as the bioactive system of production of antisperm antibody.

Agglutination of whole spermatozoa has been observed on slides. The technique though simple is highly eloquent; clumping of spermatozoa confirms the existence of antisperm antibodies in the serum under examination. Absence of antibodies shall never cause spermatozoal clumping as is evident from the observation of preserum spermatozoa experiment. The spermatozoa remain as normal as those incubated without any serum. Incubation with preserum resulted in no clumping and confirms absence of antisperm antibodies on the experimental model prior to the immunization. With the dilution of antisera the number of free spermatozoa increases. These observations indicate the change in immunogenic behavior of spermatozoa on being exposed to antiserum. This can be accounted for by changes on the membrane components of whole spermatozoa.

The results show that nature and pattern of immobilization of active motile spermatozoa are different as observed in different graphs of immobilization. Immobilization is dependent on the antibody concentration as indicated by increasing dilution the number of immobilized spermatozoa decreased and number of free spermatozoa increased. The shapes of immobilization curves of active motile spermatozoa are found to be different when the values of spermatozoal immobilization are plotted against the dilutions of the antisperm antibody in a logarithmic scale. The results show that nature and pattern of immobilization of active motile spermatozoa are different shown by different graphs of immobilization. Different patterns of immobilization are due to difference in distribution of antigens on surface of spermatozoa of different semen samples.

Exposure of viable human spermatozoa to rabbit antisera shows agglutinating effects.The variation in spermatozoal population in antigen distribution on individual spermatozoa is reflected in different patterns of agglutination. The majority of spermatozoal agglutinating antibodies in sera is directed against intrinsic spermatozoal antigens rather than at antigens adsorbed to spermatozoa from seminal plasma as the antigen is prepared with appropriate washing Agglutination is dependent on the antibody concentration as indicated by increasing dilution the size of spermatozoal agglutinates decreased and number of free spermatozoa increased. The number of agglutinated spermatozoa show different patterns of adherence to each other such as head to head, midpiece to midpiece, tail to tail, tail tip to tail tip, mixed and tangled positions. Agglutination is dependent on the antisperm antibody concentration and distribution of antigenic determinants on the surface of spermatozoa. Spermatozoal agglutinating antibodies seemed to be directed against different surface antigens of the spermatozoa distributed at sites like acrosome, anterior and posterior segments, post acrosomal region, posterior ring, neck, midpiece and tip of the tail.

A polyclonal antiserum contains many different antibody specificities to the various epitopes of the structurally complex antigen. Complex antigens possess a mosaic of different antigenic determinants that may generate a multiplicity of antibodies in an antiserum as a result of polyclonal process of antibody production.An antiserum is heterogeneous at many levels; in the classes and subclasses (isotypes) of the antibody produced, their specificity, titre and affinity. In one antiserum there may be antibodies to many discrete antigens (multispecific or

polyspecific), to a few antigens (oligospecific) or to a single antigen (unispecific), but even in the latter case the reagent is not homogeneous as single antigens are of multi-determinant structures that generate a polyclonal, multi-determinant-specific response often in several isotypes. In addition the response to individual epitopes is clonally diverse and antibodies of different affinity may compete for the same epitope.The antisera due to their polyclonal, multispecific nature may be used to determine antigenic differences between molecules at the individual epitope level.

Precipitation of antigen, which is the basis of immunoassay, depends upon the multi-determinant specificity of antisera whose constituent antibodies bind to the mosaic of determinants on complex antigens, constructing a lattice of combined molecules, which, at appropriate relative concentrations of antigen and antibody, come out of solution.

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