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Reproductive Biology Insights

Immunobiology of Spermatozoa: A Review

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ABSTRACT: Immunobiological changes in spermatozoa within the male reproductive tract are vital for their viability, and hence for reproductive efficiency. The secretions of the seminal vesicles have immunosuppressive property that attenuates the antigenic effects of spermatozoa in the male reproductive tract. However, when spermatozoa find a way to outside of the rete testis or epididymis due to trauma or infection, they can cause significant inflammatory and immunological reactions in the surrounding tissues. This has also been observed in the female reproductive tract. However, such cases are rare and the reason for this is unknown. Antisperm antibodies are the main cause of infertility in humans. The purpose of this review is to analyze these immunobiological changes from the time of spermatozoa formation in rete testis to their role in fertilizing the oocytes in the female reproductive tract.

KEYWORDS: spermatozoa, immune, antibodies, testis, epididymis, seminal vesicle

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Introduction

Among many highly specialized cells in the body, the spermatozoa hold a very important role. They are the unique cells that contain unpaired chromosomes, perform the sole function of fertilizing an oocyte, and are responsible for the continuity of generations. After being produced in the testicle, the spermatozoa move along the epididymis over a period of 9–11 days before reaching the cauda epididymidis where they are stored until ejaculation. During this journey, they undergo a series of maturation changes required to convert them from infertile testicular spermatozoa to motile spermatozoa that are capable of fertilizing oocytes.[1]

The spermatozoon has an immune privileged status in the testis.[2–5] However, significant inflammatory and immunological reactions in the surrounding tissues have been observed when spermatozoa find a way to outside of the rete testis or epididymis due to trauma or infection.[6] Although less frequent, this has also been detected in the female reproductive tract. However, in most cases, there are no adverse immunological reactions in the female reproductive tract to ejaculated spermatozoa.[7] The reason for this could be the immuno-protective effect of seminal plasma,[8,9] which gets mixed with spermatozoa during ejaculation. In addition, factors like immune insult from bacterial infections[10,11] and female sex hormones[12] have been shown to influence the viability of spermatozoa and immune response against them in females.

It has been found that there is sequential developmental change in the immunogenicity of a spermatozoon as it moves from rete testis toward the caput epididymidis, corpus epididymidis, and finally the cauda epididymidis. Indeed, it has been shown that rete testis spermatozoa are most immunogenic and cauda epididymidis spermatozoa are the least. This suggests that the spermatozoon loses its immunogenicity as it moves from rete testis toward the cauda epididymidis, and the immunogenicity of ejaculated spermatozoa could be less than those from the cauda epididymidis.[13]

Researchers in past have observed the immunosuppressive property of the secretions of the seminal vesicles. These are mixed with spermatozoa when they move from the cauda epididymidis toward the exterior during ejaculation.[13] This supports the hypothesis that seminal fluid is responsible for the reduction of immunogenicity of spermatozoa. Indeed, the immunosuppressive components obtained from the seminal fluid have been found to reduce B lymphocyte activity to mitogens.[14] In addition, seminal protein coatings on sperms are essential for several processes in the female reproductive tract, such as formation of the oviductal sperm reservoir, sperm capacitation, oocyte recognition, and sperm binding to the oocyte.[15] Seminal plasma, containing cytokines and prostaglandins (PGs), is believed to provide the physiologically protective environment to the highly antigenic spermatozoa in the female reproductive tract.[9,14,16–19] It has been suggested that this immunosuppressive effect of seminal plasma may also compromise the immune system in females for viral and bacterial attack.[14,20–23]

After the initiation of studies in immunological infertility in 1954 by Rumke who was responsible for the discovery of antibody response to spermatozoa in two infertile men,[24] this field is still in its infancy stage and much work is required to determine the cause of infertility in both men and women. This literature review takes into account the possible causes and patterns of immunological responses to spermatozoa in males. The purpose of this review therefore is to discuss the immunobiology of spermatozoa from their formation in the rete testis to storage and maturation in the cauda epididymis followed by their ejaculation in males, and finally to their role in fertilizing the oocytes in the female reproductive tract.

Methods

Search and selection process. An electronic database search of PubMed and Google Scholar with the following key terms in various combinations and permutations was performed: sperm, spermatozoa, testis, epididymis, male reproductive tract, female reproductive tract, seminal vesicle, zona pellucida, spermatogenesis, immune, monoclonal antibodies, humoral immune, cellular immune, antigen, T cells, B cells, immunoglobulins, NK cells, immunogenicity, immunostimulatory, immunosuppressive, hormones, antisperm, systemic, infertility, and blood–testis barrier. At each stage of the search, titles and abstracts were scrutinized and the most appropriate were organized into separate folders using End Note X6.0.1 software. In addition, articles relevant to our discussion were retrieved from the reference list of other online articles on each subtopic. This in total yielded approximately 585 papers. After placing all inclusion and exclusion criteria into our search (shown in Fig. 1), 97 articles closely related

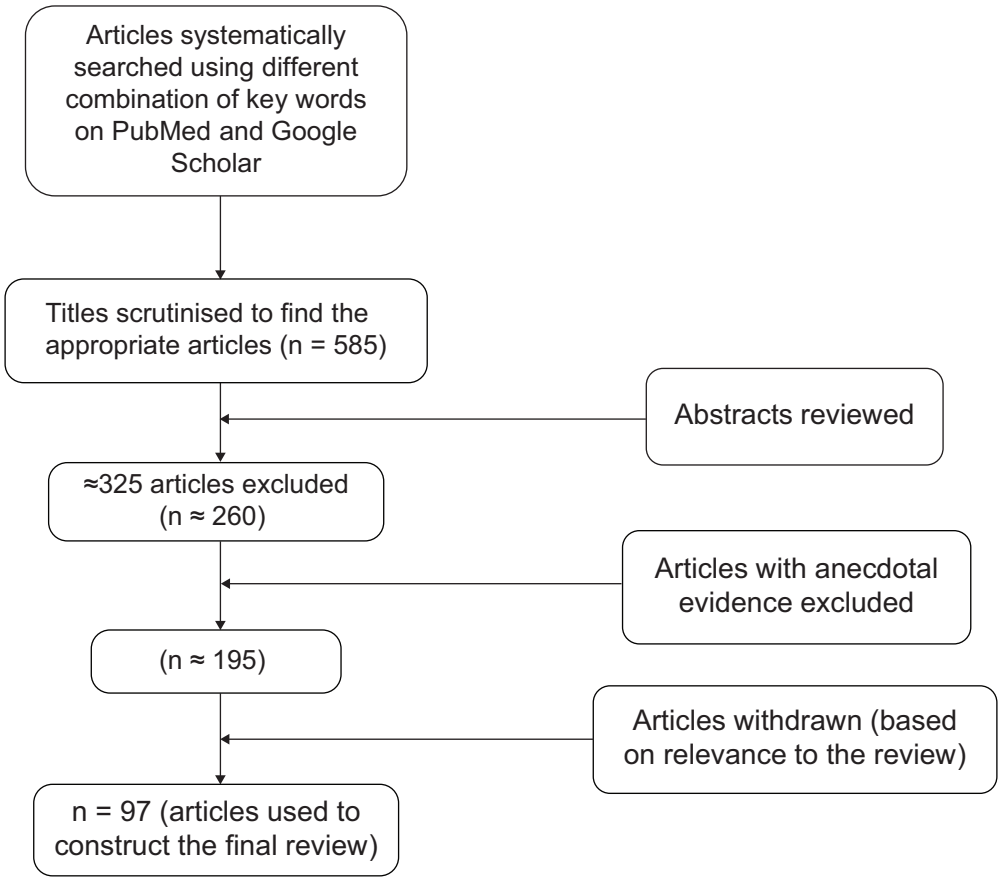

Figure 1. Study inclusion flowchart. The flowchart depicts the methodology for search and collection of relevant articles for this review.

to the aims set forth for this review were selected and hence utilized for writing the review.

Inclusion and exclusion criteria. Articles on immunobiological characteristics of spermatozoa in the male reproductive tract were mainly selected for detailed analysis. Articles that investigated the immunobiological effects of spermatozoa in the female reproductive tract were consulted in less depth and are cited wherever required for the convenience of readers. Similarly, structural and functional characteristics of spermatozoa, as well as physical and chemical properties of semen, as described by researchers in the past also find mention in this review. Articles with anecdotal evidence were excluded from the review. The articles included in this review have been published between 1978 and 2014.

General Immunobiology of Spermatozoa

Spermatozoon evolved to perform the vital function of fertilizing an oocyte required for producing offspring and hence in the continuity of generations. However, the survival of spermatozoa depends on the immune system whose main function is to differentiate between self and non-self and hence protecting the body against non-self. The spermatozoon has an immune privileged status in the testis despite the fact that cells of the immune system are present in the testis and drain the regional lymph nodes.[3] When spermatozoa move from rete testis toward the cauda epididymidis and finally toward the exterior at ejaculation, they become mixed with the secretions from various reproductive glands and hence their immunogenicity could vary at different locations of the tract. Once ejaculated in the female reproductive tract, spermatozoa act as the potential target for the female immune system due to their foreign nature. However, an immune recognition of spermatozoa and subsequent antibody production are not common.[7]

Contrary to the popular belief that the male reproductive system evolved to produce large numbers of elongated spermatozoa due to sperm competition, Gomendio and Roldan believed that this also counteracts the greater losses of spermatozoa occurring in the female reproductive tract that has continuously evolved to form a longer oviduct.[25] The authors also proposed that evolution in sperm size is nowhere related to the thickness of the zona pellucida. However, it is now known that longer spermatozoa move more rapidly in the female reproductive tract and hence have more chances of fertilizing the ovum.[26]

Spermatogenesis

Spermatogenesis begins during puberty long after the immune system has learned to distinguish between self-antigen expressed on the body's own tissues and non-self-extracorporeal antigens. Spermatogenesis takes months in lower vertebrates like chondrichthyes, and as such, the epididymis has taken over the testicular function of sperm storage.[27] The post-testicular storage is also accompanied by sperm maturation that occurs under the influence of proteins secreted by the duct system. Human beings produce fewer spermatozoa than other mammals. In addition, spermatozoa produced are poor in quality and less storage of spermatozoa occurs in the cauda epididymidis, which does not maintain sperm viability very well.[27]

The structural and functional entity of spermatozoa largely determines the fertilizing capacity of males, and as such, structural analysis of spermatozoa is essential in the infertile male.[28] The shape of the head varies greatly among species, which is determined during spermateliosis; the chromatin condenses and the nucleus of the spermatid gradually takes the shape characteristic of the species. The enzyme proteosome is present in the acrosome of the head, which removes abnormal and aged proteins and is essential for the process of fertilization.[29–31] While the head is responsible for carrying the genetic code to the oocyte, the middle piece helps in the production of energy required for the propulsion of sperm in the female reproductive tract.[28] The microscopic characteristics of spermatozoan tail could vary with the species. This is evident from the hook-shaped and long-tailed spermatozoa found in rodents belonging to the family Muroidea, a contrast to the other families of rodents. This represents the evolutionary development among rodents.[32,33]

Development of spermatozoa in the testis. Spermatogenesis can be divided into three main processes, ie spermatocytogenesis during which spermatogonia undergo multiple divisions to finally form primary spermatocytes, meiosis during which primary spermatocytes undergo reduction division to form haploid spermatids, and spermiogenesis during which spermatids are metamorphosed into spermatozoa.[34] Hormones including testosterone, estradiol, and inhibin directly or indirectly control the whole process of spermatogenesis.[35–37] It is important to note that testis-specific autoantigens appear on the membrane of spermatozoa in late pachytene spermatocytes and persist through spermiogenesis. Additional antigens appear after the mid spermatid stage of spermatogenesis and during epididymal transit.[34]

The various testicular characteristics determine the overall quality of spermatozoa and are easy to obtain. These are scrotal circumference, which is a measure of testicular size and is correlated to sperm production, and the testicular consistency, which is a measure of firmness of the testis and is correlated to seminal quality. The scrotal circumference and testicular consistency are easily measured, reproducible, and heritable, and they provide important information on the reproductive capacity of males. It has been found that animals with large testis reach puberty at a younger age and they provide better chances of fertilization of females. The other factors affecting the testicular efficiency of spermatozoa production are breed, age, body weight, and season of the year.[26,38,39]

Sperm competition in eutherian mammals, metatherian mammals, and monotremes, where female mates with many males has led to increase in the relative sizes of testes in order to accommodate more seminiferous tubular tissues to sustain an optimal level of spermatozoa production.[26]

Development of spermatozoa in the epididymis. During the course of evolution, extragonadal ducts have evolved in both males and females to allow internal fertilization to occur.[27,40,41] It has been an adaptation for better survival of species and more chances of production of viable offspring. The extragonadal duct system is thought to be very important in females for fertilization and embryonic development to occur in protected environment.[40] However, the biological significance of these extragonadal ducts is not clear in males as compared to females.

While external fertilization involves release of spermatozoa near the oocytes so that spermatozoa can directly enter, internal fertilization involves the release of an oocyte at a considerable distance to the site of fertilization and spermatozoa are required to propel themselves to reach the final site of fertilization and may have to wait for the oocyte to reach the site. As such, sperm motility is very important for internal fertilization and this is achieved in the epididymis.[27,40] The epididymis is responsible for the increased lifespan of spermatozoa and protecting the surface antigens from the female immune system. The epididymis also mixes spermatozoa of different ages and as such spermatozoa capable of fertilizing oocytes are present in almost all ejaculates, a major difference between internal and external fertilization. Because of the process of internal fertilization, sperm competition and the rivalry between males to achieve paternity is seen among reptiles, birds, and mammals.[26,27,40]

The epididymis also stores spermatozoa, which is advantageous for males to be used during periods when mating is frequent and the duration of spermatogenesis is too long. It also helps in continuous production of spermatozoa in testis and provides better control than the testis in releasing limited number of spermatozoa at each ejaculation. However, spermatozoa may lose some viability during storage and the numbers of spermatozoa are considerably reduced with successive ejaculations.[27]

Changes in the antigenic proteins on the surface of spermatozoa in the epididymis. There is strong evidence that testicular spermatozoa cannot fertilize oocytes when inseminated *in vitro* or *in vivo*, and it is essential for all spermatozoa to pass through the proximal region of epididymis where two important capacities are achieved.[27] First being most important for internal fertilization is the capacity to be motile, and the second is the capacity to bind to the zona pellucida and penetrate an oocyte. This maturation process in the proximal part of the epididymis commences in the initial segment of eutherian mammals and involves modification of the sperm surface antigens by epididymal proteins that are synthesized in epididymis under the influence of androgens. These modifications are a part of making the spermatozoon capable of controlling its metabolic rate in order to produce fewer reactive oxygen species that can damage spermatozoa.[27,42–44]

The metabolic rate of mammalian spermatozoa is reduced while stored in the epididymis and increases by three to five times upon dilution with secretions from accessory glands during ejaculation. The metabolic rate increases more when the spermatozoon capacitates and it further increases when it reaches the oocyte.[27]

Studies have found that spermatozoa isolated in the cauda epididymidis between ligatures are capable of fertilizing oocytes for three weeks and they maintain their motility for six weeks. It has also been demonstrated that there is direct relationship between the number of spermatozoa stored in epididymis and the body mass of vertebrates. However, some species differences to this are also possible.[27]

In a study conducted by Harkema et al, it was concluded that the binding sites on the epididymal sperm surface that are responsible for increased binding of zona proteins during incubation were not derived from seminal plasma. However, exposure to seminal plasma accelerated the cellular processes leading to binding with the zona pellucida.[45]

Function of the epididymis. Apart from its main function of serving as a transport channel for spermatozoa, the epididymis serves the function of achieving the paternity in the highly evolved and complex mating system.[27,41] The epididymis concentrates spermatozoa by absorbing fluid released from the testis. It has been established that spermatozoa produced in the testis are not capable of fertilizing ova until they pass through the epididymis. It is also known that the cauda epididymidis has a major role in sperm storage and hence in sperm competition. Apparently, this function of the cauda epididymidis is due to its lower temperature than the temperature of the testis.[27,41]

The epididymis plays many important roles for the purpose of sperm maturation. It modifies the pre-existing structures on the sperm surface directly or indirectly, provides new surface proteins for the membrane, and also helps in preserving active sites on the sperm membrane.[46]

Cooper reviewed the interaction between epididymal secretions and spermatozoa with respect to the nature of interactions, the maturity of spermatozoa, and the environment in which spermatozoa mature.[47] He observed two different kinds of interactions:

(1) Interactions that are dependent on the state of maturity of spermatozoa as in the case of peripheral proteins and some integral proteins. For this to occur, the charge on the sperm membrane must change in order to allow the recognition of peripheral proteins, and the insertion of secreted charged integral membrane proteins may precede the interaction with more distally secreted products.
(2) Interactions that are independent of the state of maturity of spermatozoa as in the case of most integral proteins for which partition into the membrane depend on the composition of plasma membrane.

As a result of the above mentioned interactions, membrane changes occur in spermatozoa, which allow other distal

epididymal secretions to interact with the membrane and provide specific components for fertilization. This also provides stability to the potentially unstable membrane.[47]

Semen

Importance of the male over the female in reproductive capacities. The male produces a large number of gametes, whereas the female produces relatively few. In an animal production system such as dairy cattle, spermatozoa from a single male are frequently used to fertilize oocytes in as many females as possible.[25] Interestingly, production of large number of spermatozoa also increases the sperm competition to fertilize an oocyte; however, this is also determined by the sperm morphology, copulatory behavior, and female influences.[26,48]

Nevertheless, with the development of superovulation and embryo transfer techniques and their increasing use in females, the role of the female's fertility is slowly gaining importance especially in order to increase reproductive rates in species such as cattle and horses, which have characteristically low reproductive rates in terms of number of offspring per year.

Evaluation of seminal quality. The ejaculate or the semen is made from the secretions of testes, excurrent ducts, and accessory sex glands. As such, it reflects the function and properties of each contributing portion of the reproductive tract and their interactions with the rest. Because the secretion of accessory gland fluid is reasonably concurrent with ejaculation, the contributions of the accessory gland are a fairly accurate reflection of current functional status, and the epididymal and testicular contributions, on the other hand, reflect past events in these parts of the tract.[15,20]

Physical and chemical properties of semen. In their research, Jonakova et al suggested that semen is highly variable in its composition, and mainly depends on the species, individuals within the same species, and ejaculates from the same individual.[15] Seminal quality may be modified by disease, frequency of ejaculation, nutrition, and other management factors such as season, age, amount of sexual preparation, method of collection, and magnitude of retrograde flow of semen into urinary bladder. In addition, procedures for handling the ejaculate during and after collection, analytical techniques, pharmacological agents, and normal physiological variations are all sources of variation that affect seminal quality.[15] As such, each potential source of variation should be recognized and accounted for in the interpretation of analysis of seminal quality. Many of these sources of variation can be controlled. Collection, handling, and analytical techniques for semen evaluation must be standardized.

No single measurement of seminal quality has been found to be a reliable criterion for predicting fertility of a given male. It is nearly impossible to obtain reliable coefficients of correlation that apply to mating single females which are naturally inseminated during mating by a single ejaculate containing large yet variable numbers of spermatozoa. Furthermore, there is no way to separate the contributing secretions from the female reproductive tract to accurately measure the seminal characteristic of that ejaculate.[15]

The evaluation of seminal quality should incorporate as many useful measurements of seminal characteristics as possible within the limits of practicality. The procedure used should be based on the purpose of re-evaluation. Routine seminal analysis at artificial insemination centers usually includes volume, concentration of spermatozoa, and total number of spermatozoa per ejaculate, and the percentage and rate of motility as minimal procedures. Examination of seminal quality for research purposes usually includes these, as well as a variety of additional measures of physical, chemical, and metabolic characteristics. Since seminal plasma is formed from the secretions of the testis, the epididymis and the male accessory sex glands including seminal vesicles, ampulla, prostrate, and the bulbourethral gland, the characteristics of seminal fluid could vary among different mammalian species.[15,20]

Immunosuppressive properties of semen. The immunosuppressive components obtained from the seminal fluid have been found to reduce B lymphocyte activity to mitogens. As such, seminal plasma is believed to provide the physiologic protective environment to the highly antigenic spermatozoa in the female reproductive tract.[9,14,16–18] The immunosuppressive proteins in the seminal plasma can be separated with different chromatographic separation techniques such as affinity chromatography, size exclusion chromatography, and high-performance liquid chromatography with reversed phase. Similarly, techniques to study the isolation and binding interaction of seminal proteins have also been developed.[15] Cytokines and PGs produced in the seminal vesicle serve as a potent signal for conception and pregnancy in the female reproductive tract.[9] As such, they help in increasing the conception and fertilization rates.

The immunosuppressive activity of human seminal plasma has been known for the last two decades, though the exact reason behind it is still unknown. But, the very high content of PGs of the "E" series in seminal plasma of man and higher primates and the known suppressive activity of PGE_2 on both lymphocytic proliferation and the activation by an interleukin-2-dependent mechanism support this fact.[49,50] Natural killer cells are also sensitive to PGE_2.[51] Concentrations of 10^{-8} M of PGE can suppress cytotoxicity, and the mean concentration of about 5000 times higher is present in human seminal plasma. Apart from these, seminal plasma is also responsible for class switching of antibodies, activation of suppressor cells in peripheral mononuclear cell preparations, and the positive selection of CD8 (suppressor) lymphocytes from precursors.[52] The major PG in human seminal plasma is 19-hydroxy PGE, but whether this class of PGs is present in domestic animals is still to be determined. The suppression activity of PGE is due to the rise in intracellular cAMP.[3,52]

Complement inhibitory agents are incorporated in seminal plasma during the early stages of spermatogenesis. The role

of complement inhibition prior to ejaculation is still unknown. However, since spermatozoa are susceptible to be attacked by various complements, the inhibition of the complement system post-ejaculation is important for successful conception.[3] Four inhibitors of complement are present in semen, the clusterin being the most important.[3]

Immunosuppressive activity of seminal plasma in mouse has been reported by Anderson and Tarter both *in vivo* and *in vitro*.[53] Murine seminal plasma is a mixture of secretions from the prostate glands, seminal vesicle, and epididymis, and it suppresses both humoral and cell-mediated immune response. This is essential to protect sperm from immunological damage and to prevent sensitization of females to sperm antigens after insemination. Peluso et al demonstrated that epididymal spermatozoa pre-treated with SV–IV, one of the major secretory proteins produced by the epithelium of adult rat seminal vesicles, has less ability to induce *in vivo* peritoneal macrophage activation, measured as class II major histocompatibility complex surface antigen expression, superoxide anion production, phagocytic activity, and antigen presentation.[13]

Dostal et al isolated the immunosuppressive components of boar seminal plasma and injected them into the rectum of female mice.[20] They observed that this reduced the number of white blood cells and decreased the activity of plaque-forming cells. This could be important to prevent anti-sperm action in the female reproductive tract. However, the immunosuppressive effect of seminal plasma could also compromise the immune system in females during viral and bacterial attack.[14,20–23]

Prostasomes are multilamellar submicron organelles present in human semen in large numbers and act as the reservoirs of complement inhibiting activity and also inhibit the lymphocyte proliferation assay.[3] This prostasome activity is responsible for very high amount of immunosuppressive activity in human seminal plasma. These prostasomes adhere themselves to spermatozoa and are carried up to the female tract with them. These prostasomes have also been associated with immunosuppressive activity in bull semen.[3]

One of the other important functions of seminal plasma is to provide protection to spermatozoa from phagocytic ingestion immediately after ejaculation.[54] Tomlinson et al demonstrated that seminal leukocytes are responsible for the phagocytosis of morphologically abnormal spermatozoa in the semen.[55] This has also been confirmed by the work of Troedsson et al.[19]

Use of monoclonal antibodies in establishing immunosuppressive effect of seminal plasma. Since several antibodies are present in seminal plasma, this polyclonal nature makes it impossible to study the effect of one antibody on infertility, and hence, the use of monoclonal antibody is important in studying the individual effect of antibodies on infertility.[56,57] Metteler et al investigated the potential of spermatozoal antigens as targets of immunological contraception by using the monoclonal antibodies directed against human spermatozoa and found that 91% of all cell lines produced antibodies reacted with both sperm and seminal plasma.[57] Likewise, Vaselky et al used monoclonal antibodies for their research and concluded that there is no effect of the antibodies to the immunosuppressive molecule on the ability of boar spermatozoa to penetrate the porcine zona pellucida.[58]

The monoclonal antibody D7G3, which was directed to human spermatozoa and cross-reactive with mouse and rat spermatozoa, agglutinated spermatozoa by reacting with antigens present on the post-acrosomal region of human spermatozoa and acrosomal region of mouse spermatozoa. This antigen was found to be localized in the mouse testicular germ cells, Sertoli cells, epithelium of spermatozoa of the caput, corpus, and cauda epididymidis and also in the epithelium cells and secretions of prostrate and seminal vesicles.[59]

Immunostimulatory properties of semen. In contrast to the above fact, semen containing human seminal plasma seems to contain negligible amount of immuno-stimulatory compounds such as C3, C9, IL-1β, and IL-2. Cytokines like IL-8 and IL-6 are present in seminal plasma but they are not related to immuno-stimulation. Instead, IL-8 has been shown to cause leukospermia.[3]

Blood–Testis Barrier

The lymphatic and blood vessels runs through the interstitial spaces in testis and the blood–testis barrier plays an important role in preventing autoimmunogenicity to antigens present over the spermatozoal membrane. This barrier in the rete testis is less efficient than that in the seminiferous tubules, which is believed to be due to the presence of serum proteins in the rete testis.[60] However, it is not yet confirmed if the immunoglobulins observed in rete testis entered through peritubular capillaries in the rete testis. The peritubular capillaries around the efferent duct, which are permeable to water and some inorganic ions are more permeable than testicular blood capillaries. In addition to this, it has also been found that the tight junctions between adjacent epithelial cells in the ductus epididymis and seminiferous tubules are more highly developed than elsewhere in the rat and does not allow proteins and compounds like pyroentimote, lanthanum, and peroxidase to pass through them.[60] However, the blood–testis barrier can break due to chemical or physical trauma or infection and can become a cause for the production of antisperm antibodies in males.[34]

Role of sertoli cells in the regulation of blood–testis barrier dynamics and maintenance of immunological equilibrium in testis. Sertoli cells are somatic nurse-like cells in the seminiferous epithelium responsible for providing nutrition and structural support to the developing germ cells.[61,62] The close contact between the sertoli cells and developing germ cells is essential for the maintenance of spermatogenesis and fertility.[63] However, the recent discovery of their role in the formation of blood–testis barrier[63,64] suggests that sertoli cells also have a role to play in providing protection to the

developing spermatozoa from antibodies and autoimmune cells present in the systemic circulation, as well as from both interstitial and ascending invading pathogens. Any insult to the blood–testis barrier integrity could provide a stimulus for the production of antisperm antibodies and their movement within testis, thus leading to the death of spermatozoa and infertility.

Blood–testis barrier is composed of coexisting tight junctions, ectoplasmic specializations, desmosome-like junctions, and gap junctions, which collectively function to maintain the blood–testis barrier integrity.[61] Desmosomes-like junctions and ectoplasmic specializations are present in the seminiferous epithelium. Sertoli cells coexist with blood–testis barrier and the developing germ cells using these junctions and provide a protective microenvironment within testis for the development of spermatozoa. Like other somatic cells, sertoli cells have also been shown to express toll-like receptors on their membranes that potentially respond to a wide variety of bacteria and augment secretion of chemokine monocyte chemoattractant protein-1 and expression of intracellular adhesion molecules (ICAM), such as ICAM-1 on sertoli cells.[65] This attracts leukocytes within the testis and enhances binding of lymphocytes to the sertoli cells, as well as secretion of cytokine interleukin-6.[66] This suggests the potential role of sertoli cells in promoting inflammation in the testis and hence in the modulation of local immune activity within the testicular environment.

Immunogenicity of Spermatozoa

Spermatozoa rarely show autoimmunity in males; but despite being foreign in nature, they do not generate an immunogenic reaction in females in most of the cases. This is contrary to what might be expected.

The spermatozoon acts as an allogenic cell in the female body and contains human leucocyte antigen (HLA) on their surface. These HLA are recognized by the T lymphocytes in the female body. The epididymal spermatozoa have not been found to possess these HLA antigens, but the presence of HLA-G and HLA-B has been detected in ejaculated spermatozoa using reverse transcriptase and polymerase chain reaction. This is believed to be due to the possible contamination of spermatozoa with RNA of somatic cells.[3]

In some women, genital secretions and serum showed the presence of sperm antibodies and this raises the question as to whether these sperm antibodies are produced in response to the immunogenicity of spermatozoa in reproductive tissues or it is a transudate from the serum. However, the reason for this autoimmunization is still unknown.[56] Nevertheless, the titer of the antibodies to spermatozoa is generally lower in serum than in genital secretions that support the hypothesis that these antibodies are produced in response to spermatozoa in the genital tract and not in the serum.[24]

Spermatozoa in the male reproductive tract. Although spermatozoa bear developmental antigens to which immune system is not tolerant, autoimmune responses against spermatozoa in males are uncommon. The reason for this lack of immunologic responsiveness in males is still unknown but the available evidence suggests that it resides within the immune system itself. There is evidence that immunosuppressive factors exist within the seminal fluid and that the population of lymphocytes with T suppressor markers detected in seminal fluid are also found in the interstitium of the testis and in the submucosal regions of the epididymis.[54,67] Activated macrophages capable of acting as antigen-processing cells have access to spermatogonia in the basal compartment of the seminiferous tubules. These spermatogonia have been found to express unique antigens even though they are not protected by the sequestration offered by the blood–testis barrier. These findings suggest that active immunosuppression at the level of testis is present.[54,67] Conversely, both the male and the female reproductive tracts have been found to be capable of producing a local immune response and to participate in generalized mucosal immunity. The female reproductive tract can initiate and participate in an immune response.[24]

Spermatozoa in the female reproductive tract. Spermatozoa also have an unusual relationship with the immune system of females. Following their deposition in the vagina, spermatozoa pass through various compartments of the female reproductive tract, which are capable of mounting an immune response. However, despite the established fact that single physiological exposure to semen by natural insemination initiates an immune response involving the lymph nodes that drain the uterus, a significant immune reaction rarely occurs in females even with frequent coital activity.[68]

Spermatozoa must travel the length of the female reproductive tract in order to reach and fertilize the oocytes. This tract forms part of the greater common mucosal immune system, which is sometimes responsible for antisperm immunity causing infertility in some females.[14] Unfortunately, relatively little is known about the regulation of immunoglobulin production and secretion within the genital tracts of male and female, either under normal conditions or in response to infection. Further studies are needed to determine how the local immune system in the female reproductive tract can be manipulated to elicit safe and effective antisperm immunity and to examine the interrelationship of the mucosal immune system of reproductive tracts with the other immune systems of body.[14]

Spermatozoa survive in the female genital tract for few days or until the time of ovulation in almost all domestic animals. Removal of ejaculated spermatozoa from the female tract is done after mating by mechanical evacuation through the vulva and by neutrophils and macrophages as part of the process of post-insemination leukocytosis.[69] The female reproductive tract has to meet the challenges of transporting and clearing a large number of spermatozoa without producing an immune response that otherwise will impair fertility. On the other hand, spermatozoa have to migrate and remain

alive in the female host long enough for fertilization to occur. Depending on the species, the interval between coitus and fertilization can vary from minutes to months. Therefore, spermatozoa must have the ability to evade the immune defenses of the female or the female must have the ability to recognize spermatozoa as distinct from other foreign cells, and either not mount an immune response or respond in a protective non-destructive manner, which may result in infertility.[70]

Although spermatozoa bear antigens that are foreign to the female reproductive tract, repeated exposure to these cells at coitus does not elicit an immunological response with an adverse effect on conception. This suggests two possibilities: (1) spermatozoa in the female genital tract do not evoke an immunological response or (2) the immune response to spermatozoa is of a suppressive or protective type.[14,71] Although the first possibility does not seem likely as the female genital tract is not an immunologically privileged site as in the case of males, the existence of competent local immune system has been demonstrated unequivocally and the seminal fluid secretions are supposed to be basically involved in suppressing the primary antibody response in the female reproductive tract. This they do by reducing the lymphocytic proliferation and inhibiting the antibody response.[14,71]

In some cases, females produce a local immune response to spermatozoa but only in cervical mucus and not in the serum. As such, the local immune response is more common in females than males, and systemic immune response is seen in females in both serum and mucus. The cervical mucus forms the major fraction of the female secretions that can be studied for antisperm antibodies by using serological procedures. Gentle mechanical agitation followed by addition of a small amount of bromelin and low-speed centrifugation is done to extract the fluid content of cervical mucus without damaging the immunoglobulins. The presence of antibodies in infertile women was found to be more than in those who were fertile.[24,56,72] Migration of spermatozoa in cervical mucus is impeded by antisperm antibodies. The motile spermatozoa become stationary or have shaky motility and their forward progression is halted.[56,73]

The female immune system has to be competent enough to accept spermatozoa for fertility, but on the other hand, it should kill the harmful invading organisms that cause sexually transmitted diseases. However, the rejection of spermatozoa and prevalence of sexually transmitted infectious organisms have increased during last 40 years probably due to non-barrier contraception, multiple sexual partners, and greater mobility.[3] The increased prevalence of sexually transmitted organisms could be due to the immunosuppression caused by seminal plasma. In addition, semen can also contain bacteria and viruses in a highly buffered medium, which are capable of neutralizing the low pH of the vagina and together with the immunosuppression caused by seminal plasma proteins, they can establish themselves in the female body.[3] However, semen also possesses antibacterial activity due to the presence of prostatic secretions containing zinc and spermine. Zinc has been found to bind to proteins and peptides in seminal secretions, in turn providing protection. Likewise, the main function of spermine is to protect against DNA damage.[3]

Taken together, it is clear that seminal plasma is responsible for immunosuppression and can prevent cell-mediated and humoral response to spermatozoa in females but may also be responsible for the spread of various sexually transmitted diseases due to its immunosuppressive effect. However, it has to be noted that females exposed to spermatozoa have an increase in the weight of the lymph nodes that drain the reproductive tract even though there is an immunosuppressive effect of seminal plasma.[34]

Antigenic structure on the surface of spermatozoa. Koyama et al identified epididymal sperm surface antigens using monoclonal antibodies against hamster spermatozoa, and in this process, seven hybridomas (GHS-1, -2, -3, -4, -5, -6, and -7) that produced monoclonal antibodies binding to epididymal spermatozoa were established.[74] Out of the seven, three of them were IgM and other four were IgG1. The authors found that GHS-1 to -4 and GHS-6 reacted to hamster spermatozoa from the cauda epididymidis, whereas GHS-5 and GHS-7 reacted with the acrosome region of the spermatozoa in both testis and epididymis. Veselsky et al detected antigens on the acrosome of boar spermatozoa using an anti-complement immunofluorescence test and antisera against spermatozoa, seminal fluid, and epididymis fluid. The weak fluorescence observed on the tail suggested presence of less number of antigens on the tail region, while no fluorescence on the post-nuclear segments indicated the absence of any antigens.[23]

Antibodies against epididymal spermatozoa have been found in infertile men. The antigens present on epididymal spermatozoa could have a predominant role in the etiopathogenesis of immunological infertility.[75] It is possible that the antibodies that are responsible for immunological infertility are produced in response to the surface antigens acquired or expressed on spermatozoa before they are mixed with accessory gland secretions during ejaculation, and not to the seminal plasma sperm-coating antigens. Riera et al also observed conventional and anaphylactic antibodies to epididymal and seminal spermatozoa in sera of rabbits.[76]

Antisperm antibodies as a cause of infertility. While the main cause for infertility in males is the impairment of the sperm quality (generally measured in terms of count, motility, and morphology), the infertility in females could be due to several reasons, including the problems in ovulation, obstruction of the Fallopian tubes, presence of endometriosis, and the reduced quality of cervical mucus.[77] However, a common factor reported for infertility in both men and women is the presence of antibodies to spermatozoa, and this has been observed in large number of infertile males and females.[77] Indeed formation of anti-sperm antibodies has been established as an important cause of both male and female

infertility, especially in humans.[78,79] Interestingly, it has been reported that the infertility due to antisperm antibodies can become reversed and such people can subsequently succeed in producing children.[24,56] This shows that immunological infertility depends on many factors, the presence or absence of which may lead to production of antisperm antibodies. These factors are still to be determined, although several of them have been proposed[24] including:

(1) The length of the time for which a couple, one of which contains antisperm antibodies, try to achieve pregnancy. However, some cases of couples who actually conceived after trying for few years have been reported. This suggests that the antisperm antibodies delays pregnancy but does not make the person infertile.
(2) The individuals with the blocked sperm passage, hence suffering from azoospermia, showed presence of spermatozoa, although some with antibodies, once their sperm passage was cleared surgically. The wives of such individuals did conceive but the wives of individuals with antibodies formed the lesser part of this percentage.
(3) The type of antibody as for example IgG, IgA, or IgM present in the genital secretions, seminal plasma, on spermatozoa, and in cervical mucus altered the fertilization rate in different proportions.

Cytological specificity of the antibody also seems to contribute to the antisperm antibody response and hence in infertility. As such, it seems essential to take into account that the antibodies are directed to the head or tail of spermatozoa and also upon how much of the surface of spermatozoa are covered by antibodies.

The role of antisperm antibodies in enhancing the phagocytic activity of leukocytes for spermatozoa in females and reducing the capacity of spermatozoa to migrate and enter the oocyte could be the reason for this infertility.[80–82] The oocyte is surrounded by two significant layers, the outer layer of cumulus cells and the inner one is species-specific extracellular glycol–protein matrix, the zona pellucida. The spermatozoon must pass through these two layers to fertilize the oocyte, and this process is facilitated by certain enzymes. Presence of antisperm antibodies reduces this ability of sperm penetration into oocytes, the reason for which is still unclear.[80–82] There is also evidence of antisperm antibodies being associated with disordered spermatogenesis causing oligospermia and azooseprmia.[67]

Presence of antisperm antibodies in males after vasectomy and testicular/epididymal injury. The disruption of the vas deferens during sterilization or due to trauma of the genital tract can lead to autoantigenicity and hence production of antisperm antibodies.[24,73] Conversely, it has been found that necrozoospermia in infertile males decreases with improvement in sperm transport through the epididymis.[83] Hence, the obstruction of sperm transport through the epididymis due to epididymal lesions or epididymal injury can cause male infertility by increasing the production of antisperm antibodies.

The production of spermatozoa continues even after vasectomy, but they degenerate with time and leak soluble products through the distended rete testis, epididymis, and efferent ductules into interstitial spaces, which could then be responsible for immunological reactions and the production of antisperm antibodies.[34] The immunological reaction may lead to the formation of sperm granulomas that are often seen when the testicular or epididymal tissue is degraded physically or chemically. However, it has also been reported that fertility is not reduced in cases of sperm granulomata in men with sperm antibodies.[84,85] Similar observations regarding the formation of sperm granulomas have been made in vasectomized rams.[86]

Antisperm antibodies from infertile women and men with vasectomies have been shown to impede the penetration of human spermatozoa in hamster oocytes and thus prevented fertilization.[56] Vasectomy blocks the excurrent ducts and is a type of autoimmunization and results in antibody production.

In a study conducted on IgG and Fab fractions from vasectomized guinea pig serum, the inhibition of sperm–sperm adhesion, the acrosome reaction, sperm binding to ova, and sperm fusion to ova were observed.[3] These antisperm antibodies affect sperm motility, acrosome reaction, sperm penetration of cervical mucus, binding to zona pellucida, and sperm–egg fusion.[73] The immunofluorescence study conducted on the vasectomized individuals found that the antisperm antibodies bind to the acrosomal region, midpiece, and tail of spermatozoa. However, this binding was more on the midpiece, and spermatozoa with antisperm antibodies both on the acrosome and the mid-piece were not observed. The binding of antisperm antibodies to the acrosomal region of spermatozoa has been shown to alter the acrosomal reaction but binding over the midpiece and tail did not have any significant effect on the properties of spermatozoa.[73]

Men with high titers of antisperm antibodies are less likely to produce a pregnancy than those with low titers or no antibodies. The precise mechanism for this is not known, but the immotility and agglutination of spermatozoa are the main effects of antisperm antibodies. Some antibodies may interfere with spermatozoa capacitation and their oocyte penetrating ability. Some antibodies also act specifically on sperm enzymes or receptors for the zona pellucida and ovum.[56]

Experimental injection of spermatozoa into tissues. Mice injected intragastrointestinally with human sperm antigens developed a humoral immune response to them and the antigenicity of the sperm membrane extract was higher than the whole sperm. Antisperm antibodies secreted by lymphocytes of the epididymis and the uterus were also detectable.[87] Similarly, the immunological response of mice was examined after intrauterine, intraperitoneal, and anal immunization with human sperm. The authors observed

that intrauterine immunization was capable of inducing a higher antibody response to sperm antigens compared to intraperitoneal and anal immunization. Antibody response was least after anal immunization.[82]

Characterization of Specific Antisperm Immunity in the Male Reproductive Tract Secretions Especially Seminal Fluid

The secretions from the accessory glands are not essential for fertility but are important for the viability of spermatozoa in the female reproductive tract. These secretions help in coagulation and liquefaction of the semen and in immunosuppression, which help spermatozoa to survive any immunological attack in the female reproductive tract.[3]

The immunosuppressive components extracted from the boar seminal fluid when injected intravenously in mice lead to the suppression of primary and secondary immune response to boar epididymal spermatozoa and to bacterial antigens.[88] The production of IgG and IgM antibodies to spermatozoa was delayed in mice treated with immunosuppressive components. Importantly, this immunosuppression was most prominent in female mice treated with immunosuppressive components three days before immunization with antigen than in those treated with immunosuppressive components 3 days after immunization with antigen.

Approximately 5–10% of men from unselected infertile couples manifest autoimmunity to spermatozoa. About 85% of those men with circulating humoral antisperm antibodies could pass antisperm antibodies in their ejaculates.[89] While spermatozoa with antibodies bound to them move well within the seminal fluid, their ability to penetrate and survive within the human cervical mucus could be impaired. Hence, the more spermatozoa in the ejaculate coated with antibodies, the fewer pass through the cervical mucus and populate the female reproductive tract. In addition, the oxidative stress and changes in the integrity of nuclear and mitochondrial DNA can also contribute to male infertility.[89]

Antisperm humoral immunity. The ejaculates of men with autoimmunity to spermatozoa commonly contain a mixture of IgA and IgG antisperm antibodies.[90,91] Most of the free seminal plasma antibodies are in the form of IgA and small amounts as IgG. These are present as a result of transudation from the serum but in a low concentration of approximately 1% of total. Antibodies coated on spermatozoa in semen form a major fraction of the total antisperm antibodies.[24] Antisperm IgG presence in the ejaculate is therefore expected to correlate with titer of circulating antisperm antibodies. The local production of antisperm IgA in the male genital tract has been suggested since the presence of IgA has been reported in semen but not always in serum.[92]

Both the intrarectal inoculation of rabbits and gastric inoculation of rats with spermatozoa have been shown to enhance the production of antisperm antibodies. These antisperm antibodies could in turn inhibit the acrosomal reaction by the zona pellucida leading to infertility.[73,93,94] Although the presence of antisperm antibodies to the sperm head is highly correlated with the antibody concentration, it is not a predictor of the fertilization rate.[95] While these antibodies can bind to the spermatozoon head, middle piece, or tail, their binding is most prominent to the middle piece.[73]

Antisperm cell-mediated immunity. Antisperm cell-mediated immunity may not be an exclusive response but could be an essential part of the overall immune response to spermatozoa following systemic or local immunization. It has been demonstrated that while immunization with sperm-specific antigens induce an antibody response that reduces or blocks fertility, these antigens also activate pre-sensitized lymphocytes that have cytotoxic effects on spermatozoa and embryos.[96] More research on antisperm cell-mediated immunity could potentially yield significant results that can be utilized to understand and develop new, as well as optimize the current, infertility treatments.

Infertility in mice injected with spermatozoa. Mice injected with murine spermatozoa become infertile and the level of this infertility directly correlates with the number of spermatozoa injected. The reduction in the percentage of expanded blastocysts that formed was directly correlated with the extent of immunization with spermatozoa.[97] The antisperm antibodies can affect gametes, the process of fertilization, the pre-implantation development, implantation of the blastocyst, and the early post-implantation development. However, no effect was observed on late developmental stages and no abortion occurred. The research was done both *in vitro* and *in vivo* and the results obtained were the same.[97]

In addition, antisperm antibodies also caused a reduction in the number of oocytes that reached the two-cell stage and also interfered with the normal development of fertilized oocytes. However, the antisperm antibodies did not impair the viability of oocytes. The dilution of antisperm antibodies in blood resulting in reduced immunological reaction has been suspected. This is supported by the fact that few mice immunized with antisperm antibodies still possessed fertilizable oocytes.[97]

Conclusion

Taken together, it is evident that antisperm antibodies are produced in some human females due to unclear reasons and are the main reason for female infertility. On the contrary, the reasons are clearer in males and antisperm antibodies have been reported after testicle or epididymal injury resulting in male infertility. Moreover, vasectomy can also stimulate production of antisperm antibodies. It is known that the antigenicity of a spermatozoon reduces as it moves from testis to outside while ejaculation and the epididymis is believed to play an important role in this process. The understanding of the process however is very limited and as such the role of epididymis in modifying the immunogenicity of spermatozoa requires more attention in future research.

Author Contributions

Conceived the concepts: GS. Analyzed the data: GS. Wrote the first draft of the manuscript: GS. Made critical revisions: GS. The author reviewed and approved of the final manuscript.

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