Antisperm antibodies and immune mediated infertility. Are we missing something?

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Abstract

Immune mediated infertility was first reported in stallions in the 1990s. Dysregulation of the tolerogenic testicular environment leads to development of autoimmune orchitis (AO), defined as an autoimmune reaction to germ cell and sperm antigens. AO is characterized by testicular interstitial infiltration with leukocytes, immunoglobulin binding to germ cells in seminiferous tubules, disruption of spermatogenesis, production of antisperm antibodies (ASAs) and infertility. Clinical diagnosis of AO is based on detection of ASAs. Furthermore, ASAs can cause infertility by preventing sperm binding to oviductal cells, undergoing capacitation and binding to the zona pellucida. However, ICSI circumvents these effects and enables pregnancies to be obtained from males with ASAs. Other reported treatments include glucocorticoid treatment for immunosuppression, or laboratory techniques to select ASA-free sperm. Although AO is poorly understood in stallions, presence of sperm-directed IgG and IgA has been described in stallions with poor semen quality and infertility. Immune mediated infertility may represent an overlooked cause of reduced reproductive efficiency in stallions.

Keywords: Autoimmune orchitis, equine, stallion, semen, sperm directed antibodies

First reports of antisperm antibodies in stallions

The presence of antisperm antibodies (ASAs) in stallions was first reported in the 1990s. Two case reports simultaneously linked the presence of sperm directed IgG in serum or seminal plasma to infertility.[1,2] Both infertile stallions had a history of scrotal trauma 6 months to 2 years prior to diagnosis of ASAs. On presentation, these stallions were oligospermic and asthenospermic. However, 1 week after treatment with glucocorticoids, sperm motility and fertility temporarily recovered. Concurrently, ASA titers decreased, providing evidence for an effect of ASAs on stallion fertility.[1,2] Autoantigenicity of stallion sperm was confirmed in 1994 when two stallions were immunized with autologous sperm.[3] Immunization induced an increase in serum IgG and seminal plasma IgA titers that peaked in 2 - 4 weeks, and returned to prevaccination levels by 20 weeks. Concomitant with increased ASA titers, both stallions developed oligospermia and teratospermia.[3]

Testicular immunology and the origin of ASAs

Initiation of spermatogenesis leads to expression of new proteins by developing germ cells that are recognized as foreign by the immune system (neoantigens) since immune self-tolerance is established in utero. However, these neoantigens are normally tolerated without inducing immune responses. Testicular immune privileged status is maintained by tissue physical structures, local immunosuppressive milieu and systemic immune tolerance working cooperatively.

The blood testes barrier (BTB) is formed during puberty by specialized junctions between adjacent Sertoli cells and is supported by the basement membrane of seminiferous tubules and peritubular myoid cells.[4,5] The BTB physically divides seminiferous tubules into basal and adluminal compartments. Spermatogenesis is initiated within the basal compartment, where Type A spermatogonia divide and start their differentiation. Type B spermatogonia progress to preleptotene spermatocytes, which then translocate to the adluminal compartment. The progression of meiosis, spermiogenesis and spermiation occurs within the adluminal compartment, where contact between neoantigens and the immune system is prevented by the BTB.[4] However, it must be noted that preleptotene spermatocytes, type B and type A spermatogonia within the basal compartment also express neoantigens capable of inducing an autoimmune response. Permeability of the BTB is regulated by FSH and testosterone.[6] In seasonal breeding animals, the absence of FSH results in increased permeability of the BTB during the non-breeding season.[6] In addition, sperm can interact with the immune system at the rete testis and throughout the excurrent duct system, as a blood tissue barrier is not present at these sites. Other factors are therefore also involved in preventing an autoimmune response against germ cell antigens.

Sertoli cells are key players in testicular immune tolerance. Apoptotic germ cells and their antigens are removed from the seminiferous tubules by phagocytosis by Sertoli cells. Prior to spermiation, redundant cytoplasm and plasma membrane of elongated spermatids is packaged into a large cytoplast, the residual body, retained in the seminiferous tubule and a small cytoplast, the cytoplasmic droplet, attached to the sperm. Residual bodies contain germ cell antigens that are captured by Sertoli cells in the adluminal compartment and are released basally and into the interstitial space through Sertoli cell basal projections, bypassing the BTB by intracellular transport.[7] The release occurs at spermiation and egressed neoantigens are crucial for maintenance of systemic tolerance mediated by regulatory T (Treg) cells.[7,8] Sertoli cells function as nonprofessional tolerogenic antigen presenting cells by inducing enrichment of Treg.[9] The testis and draining lymph nodes are enriched for sperm specific Tregs that normally suppress autoreactive T cells.[10]

Within the interstitial space, resident macrophages and dendritic cells also help maintain the tolerogenic environment. Resident macrophages express the M2 phenotype, characterized by production of antiinflammatory cytokines.[11] Testicular dendritic cells also have an immature and tolerogenic phenotype. Resident dendritic cells capture neoantigens and maintain immunologic tolerance by routinely migrating to draining lymph nodes and presenting self-antigens to lymphocytes in a tolerogenic manner.[12] Paracrine factors involved in testicular immune regulation include testosterone from Leydig cells, TGF β1 produced by Leydig and Sertoli cells, Activin A expressed by most testicular cells and IL10 from resident macrophages.[13]

Dysregulation of the tolerogenic environment leads to development of autoimmune orchitis (AO), which can be primary or secondary to inflammation, infection, trauma or toxins, among others. AO is defined as an autoimmune reaction to testicular antigens characterized clinically by presence of ASAs and infertility.[14] Several components of the innate immune system are present in the male genital tract. In the testis, Toll like receptors (TLRs) are expressed in all immune, somatic and germ cells.[15] In particular, activation of TLR 2 and 4 is critical for development of AO in mice models.[16] Microbial components and damaged germ cells induce pro-inflammatory cytokine production by Sertoli cells through TLR activation.[15] Heat shock proteins (Hsp60 and Hsp70), disulfide isomerase ER 60 and outer dense fiber protein 2 have been proposed as damage associated molecular patterns (DAMPs) released by germ cells in a rodent model of experimental auto immune orchitis (EAO).[17] In addition, activation of TLRs in Sertoli cells disassembles junctional complexes involved in the BTB and Sertoli cell germ cell interaction, resulting in sloughing of germ cells.[15] Furthermore, activation of TLRs in Leydig cells suppresses steroidogenesis, altering testicular function through decreased intratesticular testosterone concentration. Activation of TLRs in germ cells directly induces apoptosis.[15] These effects are mediated by inflammatory cytokines, which also induce recruitment of macrophages, neutrophils, mast cells and lymphocytes to the testis. Recruited leukocytes further contribute to production of proinflammatory cytokines and mast cells contribute to tissue fibrosis and thickening of the basement membrane. Under inflammatory conditions, immature testicular dendritic cells overcome immune tolerance. Proinflammatory cytokines induce maturation of testicular dendritic cells (DC) that capture germ cell antigens released to the interstitium during the course of the disease.[18,19] These inflammatory DC migrate to the draining lymph nodes and activate T lymphocytes. Sensitized lymphocytes in turn migrate to the testes where progressive amplification of the autoimmune response leads to chronic AO with continuous antigen presentation of DC to lymphocytes in lymph nodes and testis.[18,19]

Although pathophysiology of AO in stallions is not known, EAO has been induced by immunization with autologous sperm.[20] Stallions had increased serum IgG and seminal plasma IgA titers 1 week after immunization, concurrent with decreases in sperm numbers and morphologically normal sperm. Chronic inflammatory changes around the rete testis, hypospermatogenesis and presence of immature germ cells and low sperm numbers in the epididymis was described on histopathology.[20]

Diagnosis of autoimmune orchitis

Clinical diagnosis of AO is based on detection of ASAs. The current consensus in human andrology is that only sperm-bound IgG and IgA are relevant to fertility.[14] Therefore, assays considered most relevant are those that detect IgG or IgA directly bound to sperm (direct assays).

Indirect techniques detect ASAs in serum or seminal plasma. Serum or seminal plasma from the infertile animal are incubated with sperm from a "normal," ASA-free donor. Binding of serum- or seminal plasma derived antibodies to donor's sperm was detected in stallions using a sperm agglutination or immobilization test, indirect immunofluorescence or enzyme linked immunosorbent assay.[1-3,20,21] Other indirect tests used in human medicine include immunocytochemistry, indirect mixed antiglobulin reaction and indirect immunobead binding test.[14]

Direct techniques detect ASAs already bound to the infertile male's sperm at ejaculation. The direct mixed antiglobulin reaction and immunobead binding tests (dIBT) are most commonly used in human andrology.[14] In 2010, Ferguson et al. reported cross reaction between human and equine ASAs and used a dIBT to demonstrate sperm-bound ASAs in a stallion with unexplained infertility.[22] Although the dIBT has promise for clinical applications in the field, it has not been further validated in stallions. In addition, the dIBT is based on counting motile sperm bound to antibody-coated beads. Therefore, the estimation is subjective and requires good sperm motility in samples from infertile patients.[14,22] Instead, flow cytometry allows objective and quantitative estimation of ASAs on the surface of living or dead sperm and is a sensitive, specific and repeatable test. Flow cytometry also allows identification of antibody class, isotype and load.[23] A direct diagnostic test was standardized in veterinary medicine to detect sperm-bound ASAs using flow cytometry and was used to characterize IgG and IgA binding to bovine and equine sperm.[24-26]

Lastly, testicular biopsies in men with testicular dysfunction of unknown origin often reveal inflammatory changes characterized by peritubular infiltration with lymphocytes, nonresident macrophages and mast cells, translocation of macrophages and mast cells from the interstitium to the seminiferous tubules, tubular atrophy, hyalinization of basement membrane with deposition of immunoglobulins and complement and interstitial fibrosis, representing varying stages of AO.[8]

Clinical manifestations and indications for testing

Immune mediated infertility does not have a pathognomonic clinical presentation. In men, testing for ASAs is recommended if sperm agglutination occurs in the absence of clinical infection, sperm motility is < 30%, there is poor mucus penetration, or unexplained infertility.[14]

In stallions, ASAs have been identified in the center of sperm clumps, indicating that they are involved in agglutination (Ferrer, unpublished); presence of this phenomenon warrants investigation of ASAs. Low sperm motility was also identified in IgA and IgG positive stallions and bulls,[1,2,25,26] and therefore ASA investigation may be indicated in cases of asthenospermia. In addition, presence of IgA was associated with poor sperm morphology in stallions and bulls.[25,26] Testing teratospermic animals may also be indicated.

Role of ASAs in unexplained infertility in normospermic stallions is less clear. In one study, 30% of unsatisfactory breeder stallions had significant IgA binding, whereas no satisfactory breeder stallions were IgA positive.[25] Therefore, IgA binding is less likely to be associated with idiopathic infertility. However, IgG binding was equally prevalent (30%) among unsatisfactory and satisfactory breeders.[25] A similar prevalence of IgG positive sera (34%) was described among subfertile, unsatisfactory breeder stallions, whereas only 5% satisfactory breeders were IgG positive.[20] Some of the IgG positive satisfactory breeder stallions developed low efficiency of spermatogenesis with time, suggesting they were in the early stages of AO.[20] Conversely, some of these stallions could have been in the recovery phase of testicular pathology. Persistence of ASAs has been implicated in long-term effects of genital infection on human fertility. Even when semen quality returns to normal values, ASAs are estimated to persist for an average of 5 years.[27] Persistence of ASAs for 1.5 - 2 years was described in stallions and bulls after traumatic or infectious orchitis.[1,28] Therefore, testing normospermic stallions with unexplained infertility for sperm-directed IgG is recommended.[25]

Additionally, presence of sperm bound IgA and IgG was associated with poor motility and membrane integrity of cooled stallion sperm.[29] Testing stallions with poor tolerance to cooling for ASAs may be recommended.

Effects on fertility

Initial reports linked presence of ASAs in serum or seminal plasma to infertility in stallions.[1,2,20,21] However, mechanisms by which ASAs affect equine fertility have not been described. Using a model of EAO in bulls, sperm bound ASAs reduced ability of bovine sperm to bind to oviductal explants in vitro.[30] This effect was mediated primarily by IgG. Since BI is correlated with non-return rates, it was proposed that sperm bound IgG may affect bovine fertility by reducing ability of sperm to form an oviductal reservoir.[30] Similarly, antiserum raised against proteins from the periacrosomal membrane reduced ability of equine sperm to attach to oviductal epithelial cells in vitro.[31] The antiserum recognized integral membrane components present on epididymal sperm and spermatids, as well as adsorbed seminal plasma proteins present in ejaculated sperm.[31]

Experimentally induced ASAs prevent sperm capacitation in vitro in bulls.[32] Membrane fluidity changes associated with sperm capacitation were prevented by sperm-bound IgA. Similarly, ASAs blocked sperm capacitation in humans by preventing removal of cholesterol from the plasma membrane and inhibiting membrane fluidity changes.[33,34] Furthermore, sperm-bound IgA reduced ability of bovine sperm to bind to the zona pellucida in vitro.[32] In men, ASAs block sperm zona binding by inhibiting capacitation-associated increases in surface expression of mannose receptors.[34] Binding to the zona pellucida or exposure to soluble factors in the vicinity of the cumulus oocyte complex stimulates the acrosome reaction, for which capacitation and loss of cholesterol is also a prerequisite. However, bovine and human ASAs did not affect the ability of sperm to undergo the acrosome reaction in response to calcium ionophore A23187.[32,34] Stimulation of the acrosome reaction with calcium ionophore A23187 is not physiological and bypasses the activation of calcium channels that occur during sperm capacitation. When a more physiologic stimulus was used (i.e. zona pellucida proteins), the acrosome reaction was inhibited by human ASAs.[34]

Lastly, experimentally induced ASAs can affect fertilization rates in vitro and in vivo.[35-37] Bovine ASAs in sera of immunized bulls and heifers had a negative effect on in vitro fertilization (IVF).[35,36] Mating of female wallabies to males with sperm-bound ASAs resulted in 20% in vivo fertilization rate compared to 95% in females mated to control, nonimmune males.[37] Mechanisms proposed for these effects were alterations in sperm motility, or inhibition of sperm capacitation, zona pellucida binding and oocyte penetration.[35,37]

Immunolocalization of experimentally induced ASAs was evaluated in bulls using immunofluorescence. Both IgG and IgA bound mainly to the acrosomal, equatorial and post-acrosomal areas, and to cytoplasmic droplets.[24] However, antigen specificity is not known. In stallions, 62 and 70 kD proteins were identified as autoantigens.[3] In humans, antibodies against fertilization antigen 1 (FA 1) inhibited sperm capacitation, acrosome reaction and zona pellucida binding. Addition of anti FA 1 antibodies to the IVF medium also decreased fertilization rate in cattle.[38] YLP12 is another protein involved in binding to the zona pellucida protein ZP3. Anti YLP12 antibodies inhibited sperm zona binding in humans and mice.[39] Antibodies against other antigens, e.g. epithelial cadherin, CRISP 1, zonadhesin, P34H and SAGA 1, blocked sperm–zona binding in humans and rodents.[40] Most of these antigens are located on the sperm membrane around the acrosomal area.

Treatment

To date, there are no critically tested treatments for male immune mediated infertility. Immunosuppressive compounds have been used to decrease ASA production. Treatment of stallions with dexamethasone (20 mg IV SID) or prednisolone (0.5 g IV BID) for 1 - 3 weeks decreased ASA titers, improved semen quality and increased pregnancy rates.[1,2] The effect was transient and semen quality deteriorated after treatment was discontinued.[1] Whereas glucocorticoids have been used in humans, fertility was transiently increased in only 20% of men during prednisolone treatment.[41] Other studies have failed to identify an increase in pregnancy rate or semen quality, in spite of decreased IgG binding.[42] Benefits of glucocorticoid treatment are controversial and their side effects include potential endocrine disruption with a detrimental effect on semen quality.[43]

Laboratory techniques aim at removing ASA bound sperm from the ejaculate, or removing ASAs from the sperm surface. In stallions, washing by centrifugation did not remove ASAs. However, single layer centrifugation enriched a population of ASA-free sperm and improved semen quality and tolerance to cooling (Ferrer, unpublished). Similar findings were reported in men.[44,45] Immunomagnetic separation was also able to reduce ASA bound sperm in humans, although with a low efficiency.[46] Proteases have been used to destroy ASAs on the sperm surface. Although these treatments were successful at reducing ASA binding, they also caused damage to the sperm.[45]

Lastly, IVF circumvents the problem of the reduced sperm reservoir. In consequence, IVF has helped achieve pregnancies in some, but not all, reports in human medicine.[45] Controversial results with IVF are likely due to the heterogeneity in antibody class and antigen specificity. Because intracytoplasmic sperm injection (ICSI) circumvents most of the known effects of ASAs on sperm function, pregnancy rates are similar between ASA positive and ASA negative men after ICSI. Therefore, ICSI is currently the most commonly used treatment for ASA mediated male infertility in humans.[45]

Antisperm antibodies in mares

Females can also mount an immune response against sperm. Indeed, due to the lack of tolerogenic sperm antigen presentation, females mount a stronger immunological response to sperm antigens than males.[7] When mares were immunized with sonicated sperm, serum IgG was detected using ELISA from 1 to 5 weeks after immunization, with a detectable serum IgA response present on week 5.[47] Immunized mares were then inseminated over 5 consecutive estrous cycles the following breeding season. While ASA titers had returned to prevaccination levels, serum IgG rapidly increased by 1 week post-ovulation in the first insemination cycle and remained elevated throughout the study. Similarly, uterine IgA increased after the first insemination in 1 mare and after the fourth insemination in the other mare.[48] Serum IgA and uterine IgG titers did not differ from levels observed in control mares. In spite of increased ASA titers, pregnancy rates were not different between immunized and control mares.[48] However, definitive conclusions on the effect of mare ASAs on fertility cannot be made due to limited numbers of mares in each group (n = 2).

Conclusion

Presence of ASAs represent a relevant entity in male infertility. Advances have been made in diagnostic methods and understanding how ASAs affect sperm function. However, pathophysiology of AO is poorly understood in domestic animals. Without this knowledge, designing effective treatments will remain challenging. Nevertheless, recommendations in this review regarding indications for testing and semen processing techniques may improve sample quality.

Conflict of interest

There are no conflicts of interest to declare.

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