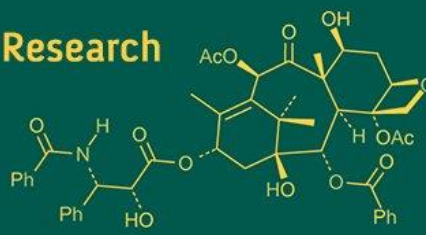
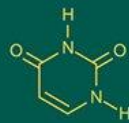


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Immune responses against spermatozoa: Role of Antisperm antibodies in infertility in bovines

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Abstract

Immunoinfertility caused by antisperm antibodies (ASAs) is an underrecognized factor contributing to reduced fertility in bovines despite advances in reproductive management. ASAs develop when immune tolerance toward sperm antigens is disrupted in bulls or cows due to infections, inflammation, trauma, blood-testis barrier damage, reproductive tract disorders, or repeated antigen exposure. These antibodies target sperm surface antigens and impair essential reproductive processes, including sperm motility, viability, capacitation, acrosome reaction, cervical mucus penetration, fertilization, and early embryonic development, leading to repeat breeding, reduced conception rates, prolonged calving intervals, and significant economic losses. This review summarizes the immunological mechanisms responsible for immune tolerance and ASA formation in both sexes, the major sperm antigens involved, and the pathological effects of ASAs on bovine reproduction. It also discusses current diagnostic methods, including direct and indirect immunological assays, and reviews available therapeutic and laboratory-based interventions. Improved understanding of ASAs will facilitate early diagnosis, targeted management, and enhanced reproductive efficiency in cattle herds.

Keywords: Antisperm antibodies, immunoinfertility, bovine fertility, reproductive immunology

Introduction

Infertility in bovines refers to a condition of reduced reproductive efficiency characterized by failure or delay in the production of a viable calf (Gautam *et al.*, 2025). Over the past several decades, a progressive decline in the fertility of lactating dairy cattle has been reported globally, despite substantial advancements in reproductive management practices (Lonergan *et al.*, 2016; Funeshima *et al.*, 2021). Infertility in dairy cattle may result from a wide range of physiological, anatomical, nutritional and management-related factors, many of which can be readily identified through routine clinical and reproductive evaluations. However, in a considerable proportion of cases, infertility persists despite the absence of overt abnormalities, indicating the involvement of less apparent mechanisms. Among these, immunologically mediated infertility remains poorly understood, frequently overlooked and often misdiagnosed, thereby contributing to unexplained or persistent reproductive failure (Parkinson, 2018). Immunoinfertility primarily involves immune sensitization against antigens associated with reproductive function. These antigens may originate from reproductive hormones, such as luteinizing hormone-releasing hormone (LHRH), gonadotropin-releasing hormone (GnRH), gonadal steroids, prostaglandin F₂-α and oxytocin, as well as from gamete- and semen-derived components, including spermatozoa, seminal plasma proteins and ova. (Cooper and Larsen, 2006). Immunoinfertility in cattle, predominantly mediated by antisperm antibodies (ASAs), represents a significant yet frequently underrecognized cause of reproductive failure, contributing to approximately 10-30% of infertility cases in bovines depending on breed, management practices and diagnostic sensitivity (Gupta *et al.*, 2023) [21, 31, 20, 37, 11, 23]. ASAs impair bovine fertility by targeting sperm surface antigens and disrupting essential processes including sperm motility, capacitation, acrosome reaction and sperm-oocyte interaction, ultimately compromising fertilization and early embryonic development. Clinically, ASAs associated immune responses are linked to repeat breeding, extended inter-estrus intervals, early embryonic loss, delayed uterine involution, prolonged calving intervals,

postpartum reproductive tract infections and reduced conception rates, cumulatively imposing substantial economic losses on cattle production systems (Srivastava *et al.*, 2017).

Research in cattle are limited and fragmented (Zodinsanga *et al.*, 2015; Hernández *et al.*, 2022) and because of that a comprehensive understanding of ASAs-mediated infertility in bovines is not revealed, despite its direct relevance to reproductive efficiency and herd productivity. In this context, the present review critically synthesizes existing evidence on antisperm antibodies in cattle, with emphasis on their etiology, mechanisms of action, diagnostic advancements and current strategies for treatment, control and prevention [53, 24].

Basic immunology of sperm in cattle

Sperm as an antigen in bulls

In bulls, spermatozoa are considered foreign to the immune system because they are produced only after puberty, once immune self-tolerance has already been established. As a result, sperm antigens are not recognized as self during early immune development. Under physiological conditions, these antigens remain sequestered; however, when immune privilege is compromised, sperm antigens may elicit immune responses, leading to the production of antisperm antibodies (Romrell and Michael, 1978)^[41].

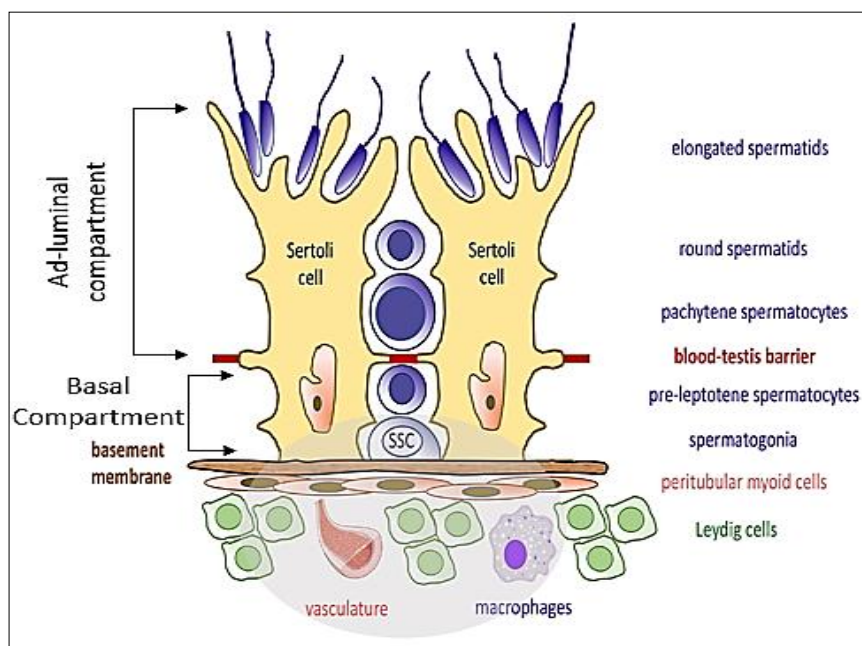


Fig 1: Blood testis barrier

Mechanism of immunological tolerance in bulls

The blood-testis barrier (BTB) is established at puberty through the development of highly specialized intercellular junction between adjacent Sertoli cells, presence of gap junctions, basement membrane of seminiferous tubules and surrounding peritubular myoid cells, collectively creating a highly selective and protective microenvironment for germ cell development. Functionally, the BTB divides the seminiferous epithelium into a basal compartment containing spermatogonia that remains accessible to systemic factors and immune cells and an ad-luminal compartment that encloses meiotic and post-meiotic germ cells and is completely secluded from immune surveillance. By restricting the movement of immune cells and circulating antibodies into the ad-luminal compartment, the BTB safeguards immunogenic germ cells from immune-mediated injury. Additionally, it prevents the leakage of germ cell and sperm antigens into the systemic circulation, thereby minimizing antigen presentation and subsequent activation of antisperm immune responses. Beyond its physical barrier function, the BTB contributes to immune privilege by maintaining a locally immunosuppressive environment, as Sertoli cells secrete immunomodulatory factors that suppress local immune activation, ensuring immune tolerance (Rode *et al.*, 2015)^[40].

Further, multiple cellular and molecular mechanisms act in concert in bull to maintain immune tolerance toward

developing germ cells. Sertoli cells play a central role by actively phagocytosing apoptotic and degenerating germ cells, thereby preventing the release of intracellular sperm antigens into the interstitial space and systemic circulation, which minimizes antigen leakage and immune sensitization (Fijak *et al.*, 2018).

In parallel, the submucosa of the male reproductive tract is enriched with suppressor T cells, which actively downregulate immune activation, inhibit effector T-cell responses and prevent inappropriate immune reactions against sperm antigens, thereby reinforcing local immune suppression (Milovanovic *et al.*, 2005). Seminal plasma further contributes to immune protection by acting as an immunosuppressive milieu that masks sperm antigens and inhibits immune recognition, with high concentrations of polyamines such as spermine and spermidine suppressing lymphocyte proliferation through oxidative metabolites; their degradation products, including acrolein and putrescine, amplify local immunosuppression, while soluble FcγRIII (CD16) modulates antisperm immune responses, with reduced levels reported in ASAs-positive individuals (Cheema *et al.*, 2016)^[33, 6, 14, 6].

Testicular macrophages predominantly exhibit an alternatively activated M2 phenotype, which is characterized by potent anti-inflammatory and immunoregulatory functions essential for maintaining testicular immune privilege. These macrophages constitutively secrete high levels of

immunosuppressive cytokines, particularly interleukin-10 (IL-10) and transforming growth factor- β (TGF- β), which act synergistically to dampen local immune activation. Whereas, testicular dendritic cells are predominantly maintained in an immature and tolerogenic state, which is essential for preserving immune tolerance toward germ cell antigens. These cells efficiently capture sperm and germ cell antigens within the testicular microenvironment but exhibit low expression of MHC class II molecules and co-stimulatory signals such as CD80 and CD86, thereby limiting their capacity to activate effector T-cell responses (Ferrer, 2019). Additional protection is provided by complement-regulatory proteins expressed on spermatozoa and reproductive tissues, including decay-accelerating factor, membrane cofactor protein and CD59, which collectively inhibit complement activation and prevent membrane attack complex-mediated lysis of sperm cells (Cheema *et al.*, 2016). Moreover, paracrine factors such as testosterone, TGF- β 1, activin A and IL-10 synergistically sustain an immunosuppressive testicular niche, ensuring immune privilege and uninterrupted spermatogenesis (Ferrer, 2019) [6, 14].

Sperm as an antigen in cows

Spermatozoa from bulls are perceived by the female reproductive tract as allogeneic antigens because they originate from a genetically distinct individual and are therefore immunologically foreign to the female immune system. Upon exposure, these antigens can stimulate local innate and adaptive immune responses within the female reproductive tract, resulting in the recruitment and activation of immune cells such as neutrophils, macrophages and lymphocytes. In certain cases, this immune activation progresses to the generation of anti-sperm antibodies (Nagaria *et al.*, 2011) [35].

Mechanism of immunological tolerance in cows

The mucosal lining of the female reproductive tract constitutes a crucial first line of immunological regulation by establishing a physical and functional barrier that limits direct contact between spermatozoa and immune cells. This barrier is formed by epithelial cells interconnected through tight junctions, giving rise to an organized mucosal epithelium that operates as a transiently immunoprivileged site during sperm transport. It restricts antigen penetration, modulates immune cell access and ensures controlled exposure to paternal antigens, thereby facilitating immune tolerance (Vlasova and Saif, 2021). During natural mating, the female reproductive tract is routinely exposed to spermatozoa; however, this exposure does not normally elicit a deleterious immune response against sperm cells due to the presence of multiple immunoinhibitory factors. Immunosuppressive substances such as 19-hydroxy prostaglandin E, polyamines, transglutaminase and high-molecular-weight Fc receptor-binding proteins are present in seminal plasma as well as in follicular and uterine fluids and cervical mucus, actively modulate local immune activity. These factors suppress immune cell activation and inhibit antibody-mediated reactions (Lazarevic *et al.*, 2013) [49, 29]. Macrophages and dendritic cells residing in the bovine female reproductive tract predominantly exhibit a tolerogenic antigen-presenting phenotype that is essential for preventing immune reactions against spermatozoa. Instead of eliciting robust inflammatory or classical antigen-presenting responses, these cells limit antigen presentation, downregulate the production of pro-

inflammatory cytokines and preferentially activate regulatory immune pathways. Such tolerogenic programming ensures that sperm antigens are not perceived as pathogenic, thereby preventing immune-mediated sperm elimination (Vlasova and Saif, 2021).

Additionally, upon exposure to sperm antigens, regulatory T cells undergo controlled activation and limited expansion. They actively suppress the activation and proliferation of potentially harmful effector T lymphocytes through tolerogenic signalling pathways and the release of regulatory cytokines. This finely coordinated immune response prevents immune-mediated damage to spermatozoa. The interaction of spermatozoa with oviductal epithelial cells induces a significant upregulation of transforming growth factor- β 1 (TGF- β 1) expression. Together with the prominent localization of TGF- β 1 and interleukin-10 (IL-10) within the oviductal epithelium and the surrounding vascular endothelium, this response establishes a strong anti-inflammatory and immunoregulatory microenvironment. These cytokines directly modulate local antigen-presenting cells, particularly macrophages and dendritic cells, promoting their differentiation toward a tolerogenic phenotype. As a result, sperm antigen presentation occurs in a non-inflammatory context, preferentially driving the induction and expansion of regulatory T cells rather than effector immune responses, thereby maintaining immune tolerance to spermatozoa. Besides, estrogen plays a pivotal role in modulating immune activity within the female reproductive tract by regulating cytokine expression and immune cell function, thereby establishing a dynamic and tightly controlled immune zone. In contrast, progesterone exerts potent immunosuppressive effects by attenuating inflammatory responses and limiting excessive activation of immune effector cells. Acting synergistically, these ovarian hormones orchestrate an immunotolerant microenvironment that restrains immune recognition of sperm antigens, protects spermatozoa from immune-mediated damage (Yousef *et al.*, 2016) [49, 29, 51].

Formation of antisperm antibodies in bulls

Blood-testis barrier may disrupt due to mechanical injury, infectious, inflammatory or degenerative insults, leads to the abnormal exposure of immunogenic sperm antigens to the systemic immune system. Such antigenic leakage initiates an immune response that may progress from an initial regulatory or anti-inflammatory phase to the activation of humoral immunity, culminating in the production of antisperm antibodies (Ferrer *et al.*, 2015).

Structural similarities between microbial antigens and sperm surface proteins can result in molecular mimicry, whereby the immune system fails to discriminate between pathogenic and self-antigens. Consequently, immune responses directed against infectious agents may inadvertently target spermatozoa, leading to the generation of cross-reactive antibodies (Vickram *et al.*, 2019) [16, 3]. Frequent semen collection or repeated inflammatory episodes lead to prolonged and cumulative exposure of sperm antigens to the immune system. This sustained antigenic stimulation enhances immune surveillance and increases the likelihood of antigen recognition and presentation leads to elevated antisperm antibody levels. In some bulls, antisperm antibodies develop in the absence of any clearly identifiable pathological or environmental trigger. This phenomenon is thought to be associated with inherent genetic variations

affecting immune regulation, which may predispose certain individuals to heightened immune reactivity. Hormonal influences can further modulate immune responses, altering the balance between tolerance and activation. Additionally, defects in normal immune tolerance mechanisms may allow sperm antigens to be recognized as non-self, ultimately leading to spontaneous ASAs production (Gupta *et al.*, 2023) [23].

Formation of antisperm antibodies in cows

Inflammatory conditions of the genital organs, including vaginitis, cervicitis and metritis, as well as trauma or bleeding during natural mating or artificial insemination, facilitate antigenic exposure. Any mechanical or chemical disruption of the mucosal barrier allows sperm antigens to interact with the mucosal immune system, triggering local immune activation and the subsequent formation of ASAs (Risvanli *et al.*, 2003). Certain infectious agents like *Campylobacter fetus*, *Trueperella pyogenes* and *Histophilus somni* cause vaginitis, cervicitis and endometritis, leading to mucosal damage and increased exposure of sperm antigens to the female immune system. Bovine herpesvirus type 1 (BoHV-1) and *Chlamydia* spp. produce ulcerative or chronic inflammatory lesions in the vagina, cervix and uterus, disrupting immune tolerance to sperm (Sardoy *et al.*, 2013). Egg yolk is a key component of many semen extenders; however, its protein constituents can act as foreign antigens within the female reproductive tract. This antigenicity has been demonstrated by the detection of antibodies directed against egg yolk proteins in inseminated heifers during the early postpartum period, as well as in the vaginal and uterine secretions of inseminated cows, indicating that extender-derived antigens can elicit local immune responses following artificial insemination (Bratanov *et al.*, 1965; Griffin *et al.*, 1971) [38, 42, 5, 22].

Dead or membrane-compromised sperm lose membrane integrity, leading to the release and surface exposure of intracellular antigens that are normally concealed from immune recognition. Once exposed, these antigens are readily detected by immune cells within the female reproductive tract. This triggers antigen presentation and local inflammatory or humoral immune responses. Consequently, such sperm become highly immunogenic and may contribute to antisperm antibody formation (Hunter, 1989) [25].

Estrus cycle dependent hormonal fluctuations markedly regulate mucosal immune responsiveness in the bovine reproductive tract. During estrus, estrogen dominance enhances immune cell recruitment, antigen presentation and

local antibody production, increasing immune vigilance. In contrast, during diestrus, progesterone predominance exerts immunosuppressive effects by dampening inflammatory responses and reducing immune cell activation, thereby favouring sperm survival and potential embryo implantation. Despite extensive research, a significant proportion of antisperm antibody (ASAs) cases in cows lack a clearly identifiable cause. Approximately 15-20% of cows develop ASAs in the absence of recognized predisposing factors, suggesting the involvement of complex interactions among genetic predisposition, individual immune regulatory differences and subtle environmental or management-related influences (Gupta *et al.*, 2023) [23].

Sperm antigens, antisperm antibody classes and their localization on spermatozoa

During the early phase of the primary immune response following exposure to an antigenic stimulus, IgM is the first immunoglobulin class to be synthesized. Upon subsequent or repeated exposure to the same antigen, class switching occurs, leading to a secondary immune response characterized predominantly by the production of IgG and IgA antibodies (Batard *et al.*, 1993). IgM, IgG and IgA have been implicated in immunologically mediated infertility. Among these, IgG is the principal antibody detected in blood serum, whereas IgA predominates in genital tract secretions. Both antibody classes are present in the reproductive fluids of male and female bovines, where they may interact with sperm antigens and impair sperm function, transport or fertilizing capacity (Kim *et al.*, 1999).

Antisperm antibodies exert their deleterious effects on spermatozoa in a site-specific manner, depending on their binding location along the sperm surface. Antibody binding to the sperm head region interferes with critical fertilization events by blocking sperm-zona pellucida interaction, preventing oocyte binding, inhibiting the acrosome reaction and impairing sperm-olemma fusion. When ASAs localize to the midpiece, they disrupt mitochondrial function, leading to reduced ATP production and compromised energy metabolism, ultimately shortening sperm longevity. In contrast, antibody attachment to the tail region predominantly affects sperm motility by restricting forward progressive movement, reducing migration efficiency within the female reproductive tract, and impairing penetration of cervical mucus. Collectively, these region-specific actions of ASAs significantly compromise sperm functional competence and contribute to immunologically mediated infertility (Kuntareddi *et al.*, 2024) [4, 27, 28].

Table 1: Sperm antigens and their location

Antigens on bull spermatozoa	Location on spermatozoa	References
Hyaluronidase	Acrosomal matrix and inner acrosomal membrane of the sperm head	(Robertson and Sharkey, 2001) [39]
Acrosin	Acrosomal vesicle, predominantly bound to the inner acrosomal membrane	(Robertson and Sharkey, 2001) [39]
Neuraminidase	Outer acrosomal membrane and acrosomal contents	(Robertson and Sharkey, 2001) [39]
Corona-dispersing enzyme	Acrosomal cap of the sperm head (acrosomal contents)	(Robertson and Sharkey, 2001) [39]
ADAM1, ADAM2 and ADAM3	Sperm head	(Evans, 2002) [13]
PH-20, PH-30, FA-1	Plasma membrane of sperm head	(Shibahara <i>et al.</i> , 2005) [43]
HBP (heparin binding proteins)	Posterior head region	(Cheema <i>et al.</i> , 2015; Dawson, 2005) [7, 12]
SP-10 (seminal plasma protein-10)	Intra acrosomal region	(Coonrod <i>et al.</i> , 1996) [9]
BS-17 (calpastatin)	Over the acrosomal region and to a low extent in tail.	(Yudin <i>et al.</i> , 2000) [52]
TIMP-2	Posterior head region	(Cheema <i>et al.</i> , 2015; Dawson, 2005) [7, 12]
IZUMO 1 protein	Integral membrane protein	(Clark and Naz, 2013; Sutovsky, 2009) [8, 44]
ACTL7a	Acrosome and tail of mature spermatozoa	(Fu <i>et al.</i> , 2016) [19]

Impact of antisperm antibodies on cattle reproduction

ASAs negatively influence key sperm functions, including motility, viability, capacitation and the acrosome reaction, as discussed in the following sections.

Impact of ASAs on sperm motility: Antisperm antibodies directed against the sperm head and principal piece markedly impair the ability of spermatozoa to penetrate cervical mucus (Coddington *et al.*, 1997). IgG and IgA isotypes of ASAs present in cervical mucus have been shown to induce a characteristic “shaking” phenomenon, wherein progressive sperm movement is converted into a non-progressive, immobilized state upon contact with mucus. Evidence suggests that ASAs binding to the sperm head or main tail segment effectively inhibit cervical mucus penetration, whereas antibodies targeting only the tail tip do not exert a similar effect (Wang *et al.*, 1985). Sperm immobilization further promotes agglutination, leading to clumping of spermatozoa and restricted motility within cervical mucus (Lazarević *et al.*, 2013). Elevated concentrations of IgG and IgA ASAs in the cervical mucus of crossbred cows have also been associated with antigen-antibody interactions on the sperm surface, resulting in head-to-head, head-to-tail or tail-to-tail agglutination (Vikrant *et al.*, 2016). The lower ASAs levels have more improved mucus penetration capacity, higher mitochondrial membrane potential, and superior sperm kinematic parameters, including straightness, linearity, curvilinear velocity, straight-line velocity and average path velocity (Archana *et al.*, 2021) [9, 50, 29, 48, 2].

Impact of ASAs on sperm viability: Antisperm antibodies are known to alter the permeability and fluidity of the sperm plasma membrane, thereby compromising sperm viability. The circulating ASAs have negative effect on sperm viability. The number of membrane intact spermatozoa are more with lower level of ASAs (Ahuja *et al.*, 2016) [1].

Impact of ASAs on sperm capacitation

Antisperm antibodies bound to the sperm surface markedly impair the capacity of bovine spermatozoa to undergo normal membrane fluidity changes during capacitation. This disruption adversely affects sperm-oocyte interactions and limits the ability of spermatozoa to penetrate the zona pellucida, ultimately resulting in fertilization failure (Ferrer *et al.*, 2017) [1].

Impact of ASAs on sperm acrosome reaction

Antisperm antibodies in the uterine fluid of cows can cause the induction of a premature acrosome reaction (Ahuja *et al.*, 2016). The zona pellucida-triggered acrosome reaction can be suppressed by the majority of ASA-positive sera (Francavilla *et al.*, 1997). The findings indicate that antisperm antibodies exert a variable influence on sperm capacitation and the acrosome reaction; certain ASAs impair the ability of spermatozoa to undergo these processes, whereas others appear to have no significant effect [1, 18].

Broader reproductive impact beyond sperm functions

Beyond their direct effects on sperm function, antisperm antibodies exert broader adverse consequences on reproductive performance at the herd level. The presence of ASAs has been associated with fertilization failure and early embryonic death, ultimately contributing to reduced conception rates and the development of repeat breeding syndrome. These reproductive inefficiencies lead to extended calving intervals and a marked decline in the success of artificial insemination programs. Collectively, such outcomes translate into substantial economic losses for livestock production systems. Thus, ASAs represent not only a biological impediment to fertilization but also a significant factor compromising overall reproductive efficiency and productivity in cattle (Gupta *et al.*, 2023) [23].

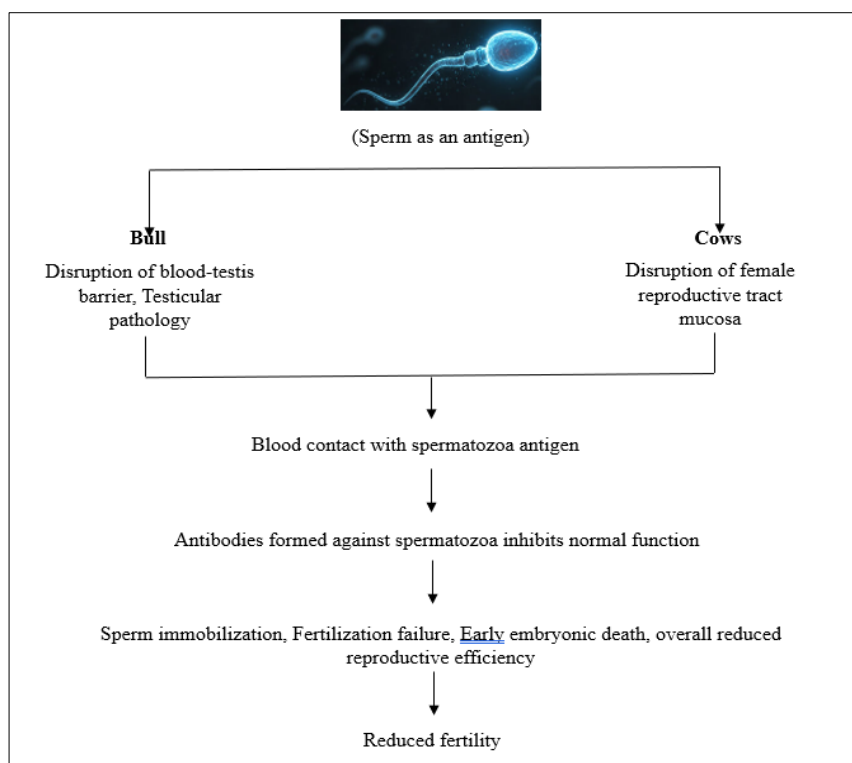


Fig 2: Immunoinfertility caused by antisperm antibodies

Diagnosis of antisperm antibodies

Diagnosis of antisperm antibodies in cattle involves the detection of specific immunoglobulin classes, predominantly IgG, IgA and IgM directed against sperm antigens. A range of biological samples are utilized to achieve a comprehensive assessment of ASAs presence and distribution. Semen samples are examined to identify antibodies bound directly to the sperm surface, providing insight into sperm-associated immune responses. In addition, blood serum and cervico-

vaginal mucus are analysed to detect circulating and locally produced antibodies targeting sperm antigens within the female reproductive tract. Uterine and oviductal fluids further contribute to diagnostic evaluation by revealing antibodies present at the site of fertilization. Collectively, analysis of these sample types enables accurate diagnosis of ASAs and improves understanding of their role in impaired fertility in cattle (Gupta *et al.*, 2023)^[23].

Table 2: Summary of direct and indirect diagnostic assays for the detection of antisperm antibodies Direct tests

Test	Principle	Advantage(s)	Disadvantage(s)
Immunobead test (IBT)	Beads coated with anti-Ig bind to antibody-covered sperm	Class-specific, detects binding site	Semi quantitative, low throughput
Flow cytometry	Fluorescent anti-Ig binds sperm antibodies and is quantified by laser detection.	Class-specific, quantitative, high throughput	Not field-suitable, expensive and needs skilled labour
Direct Immuno-fluorescence Test (DIFT)	Fluorescent anti-Ig attaches directly to sperm-bound antibodies and emits fluorescence.	Class-specific, detects binding site	Not quantitative, needs fluorescence microscope
Mixed Antiglobulin Reaction (MAR)	Anti-Ig coated particles agglutinate with antibody-coated sperm.	Class-specific, Simple and rapid	Not quantitative

Indirect tests

Test	Principle	Advantage(s)	Disadvantage(s)
Sperm Immobilization Test (SIT)	Detects ASAs by observing antibody-mediated immobilization of motile sperm.	Class-specific	Not quantitative
Sperm Agglutination Test (SAT)	Identifies antibodies that cause visible clumping of motile sperm.	Used as a primary screening test	Not quantitative, Not class-specific
Enzyme-Linked Immunosorbent Assay (ELISA)	ASAs binds sperm antigen; enzyme-linked antibody produces color proportional to antibody level.	Class-specific and quantitative, high throughput	No site localization, required well-equipped lab
Immunodiffusion Test	ASAs and sperm antigen diffuse in agar; a precipitation line forms where they meet at optimal concentration.	Useful for screening in resource-limited laboratories	Not class-specific and qualitative only

Treatment of antisperm antibodies

Research on the treatment of antisperm antibodies remains at an early and evolving stage. Existing clinical and experimental efforts are largely centered on three complementary strategies.

By reducing ASAs production

One of the principal strategies for managing antisperm antibodies involves reducing their production by addressing underlying pathological and immunological triggers. Prompt diagnosis and treatment of genital infections or inflammatory conditions using appropriate antibiotics or anti-inflammatory agents can help control local inflammation and thereby limit the induction of ASAs. In addition, immunosuppressive interventions, corticosteroids can substantially reduce ASAs levels. However, such approaches must be applied with caution, as corticosteroid therapy is associated with potential adverse effects, including endocrine disturbances and negative impacts on semen quality (Turek and Lipshultz, 1994)^[46]. Consequently, while suppression of immune-mediated ASAs production remains a viable strategy, careful consideration of risk-benefit balance is essential in clinical and herd fertility management.

By laboratory techniques for ASAs removal

Several laboratory-based approaches have been explored to minimize the impact of antisperm antibodies on sperm

function. Sperm washing combined with density-gradient centrifugation is a routinely employed method that helps eliminate ASAs-associated components while selectively enriching highly motile spermatozoa for use in artificial insemination. Immunomagnetic separation represents a more targeted approach, wherein magnetic microbeads coated with anti-immunoglobulins bind ASA-positive sperm, allowing their selective removal through magnetic cell sorting and thereby improving the quality of the processed semen sample (Vigano *et al.*, 1991)^[47]. In addition, experimental enzymatic or protease-based surface treatments, using agents such as chymotrypsin or IgA-protease, have been investigated to detach antibodies bound to the sperm membrane (Mazumdar and Levine, 1998)^[32]. Collectively, these techniques aim to reduce ASA burden and enhance the functional competence of spermatozoa, although their practical applicability and efficacy require further validation.

Assisted Reproductive Technology

The application of preparative procedures in assisted reproductive technologies such as intrauterine and intracervical insemination, *in vitro* fertilization, gamete intrafallopian tube transfer, subzonal sperm injection and intracytoplasmic sperm injection minimizes the exposure of gametes to antisperm antibodies, thereby enhancing gamete functionality and improving fertilization potential (Naz, 2004)^[36].

8. Conclusions

Antisperm antibodies represent a significant yet frequently underestimated cause of reproductive failure in bovine species. Despite spermatozoa being immunologically non-self, both bulls and cows normally maintain immune tolerance through specialized regulatory mechanisms that prevent pathological immune recognition. Disruption of this balance most commonly due to compromise of the blood-testis barrier, genital tract infections, mucosal injury, underlying reproductive pathologies or repeated exposure to sperm antigens which can lead to the production of ASAs in both sexes. Functionally, different immunoglobulin classes exert region-specific effects on spermatozoa; IgG and IgA preferentially bind to distinct sperm domains, thereby impairing motility, capacitation, acrosome reaction and sperm-oocyte interaction. The presence of ASAs can be identified using direct assays that detect antibodies bound to sperm surfaces or indirect methods that measure circulating antibodies in seminal plasma, serum or reproductive tract secretions. However, despite advances in diagnostic approaches, therapeutic strategies for managing ASA-associated infertility remain limited and are still in an early stage of development, underscoring the need for further targeted research in bovine reproductive immunology.

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