Microbial Contaminants in Semen and Their Effects on Sperm Motility Morphology and Fertility Outcomes.

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ABSTRACT

Semen contamination by microorganisms, including bacteria, viruses, and fungi, significantly affects sperm quality and fertility. These pathogens, which can come from the urogenital tract or external sources, lead to decreased sperm motility, abnormal morphology, and reduced fertilization capacity. Bacterial toxins interfere with flagellar movement, while pathogen attachment to sperm surfaces further impedes motility. Moreover, microbial infections trigger inflammatory responses, creating an unfavorable environment for sperm function. Contaminated semen samples often exhibit morphological changes, such as sperm clumping, irregular head and tail structures, and compromised membrane integrity.

Microbial contaminants in semen are linked to reduced fertilization rates, heightened risk of transmitting infections to female partners, and increased chances of early pregnancy loss. Crucial diagnostic methods include semen culture, PCR-based detection techniques, and microscopic analysis. Preventive and management approaches involve regular screening of semen donors, maintaining proper hygiene during collection, and incorporating antibiotics in semen extenders for artificial insemination.

In assisted reproductive technology (ART), eliminating microbial contaminants is essential for enhancing fertility outcomes. Key focus areas include implementing strict quality control measures in IVF laboratories, assessing sperm-washing techniques, and studying the effects of cryopreservation on microbial load. Potential treatments encompass targeted antibiotic therapy, antiviral medications, and antifungal agents. Furthermore, research on semen microbiome composition, rapid detection methods, and probiotic applications is vital for advancing reproductive medicine.

To mitigate microbial contamination risks and improve reproductive success, public health initiatives should emphasize safe sexual practices, implement screening programs for high-risk populations, and enhance semen-handling guidelines in fertility clinics.

KEY WORDS: *Microbial contamination, bacteriospermia, sperm motility, fertility outcomes, assisted reproductive technology, bacterial infections*

INTRODUCTION

Microbial contamination of semen can have significant impacts on male fertility, affecting various aspects of sperm quality and function. Studies have shown that certain bacteria, particularly Escherichia coli (E.coli), can decrease sperm motility in a concentration-dependent manner (Auroux et al., 1991). This reduction in motility is likely due to bacterial adherence to sperm rather than endotoxin effects. Interestingly, the semen microbiome is diverse in both fertile and infertile men, with some bacterial species potentially having protective effects while others negatively impact sperm quality. For instance, Lactobacillus has been associated with improvements in semen parameters, while Prevotella appears to have a negative effect on sperm quality (Farahani et al., 2020). Specific pathogens, such as Ureaplasma urealyticum, Enterococcus faecalis, and Mycoplasma hominis have been linked to decreased sperm concentration, motility, and morphology (Farahani et al., 2020; Xianchun et al., 2023). The impact of microbial contaminants extends beyond basic semen parameters to affect fertility outcomes. Bacteriospermia is associated with an increased DNA fragmentation index, which can

influence fertilization and embryonic development. While the evidence regarding the direct impact of the seminal microbiome on fertility remains inconclusive, these findings suggest potential avenues for novel therapies, such as probiotics, to improve sperm quality and fertility outcomes in men affected by certain microbial contaminants (Farahani et al., 2020).

Effects on sperm motility:

Bacterial toxins can impair flagellar movement

Sperm motility is crucial for successful fertilization and various factors can affect flagellar movement and overall sperm function. Bacterial toxins have been shown to impair flagellar movement and sperm motility in certain cases. Flagellar gene expression is linked to the production of glucosylating toxins in Clostridium difficile, a human intestinal pathogen. A "flagellar switch" mechanism controls the phase-variable production of both flagella and these toxins. When the switch is in the "ON" orientation, bacteria express flagellar and toxin genes, produce flagella, and secrete toxins. Conversely, in the "OFF" orientation, flagellar and toxin gene expression is attenuated, resulting in a decrease in toxin secretion by a flagellate bacteria (Anjuwon-Foster & Tamayo, 2017).This suggests that bacterial toxins can indirectly impair flagellar movement by affecting the expression of genes related to flagellar formation. Although these studies do not directly address the effects of bacterial toxins on sperm motility, they offer insights into various factors influencing sperm flagellar movement. For example, studies have shown that changes in osmolality, pH, and ion channel function can significantly affect sperm motility (Krasznai et al. 1995).Additionally, the presence of specific proteins such as carbonic anhydrase in flatfish sperm can regulate motility through pH/HCO3- dependent mechanisms (Inaba et al., 2003).These findings highlight the complex nature of sperm motility regulation and suggest that bacterial toxins could potentially interfere with these mechanisms, leading to impaired flagellar movement.

Some microbes may adhere to sperm, hindering their mobility

Certain microorganisms can adhere to sperm and impair their motility, as evidenced by several studies that have shown that uropathogenic microbes, such as E. coli, can significantly inhibit sperm motility, with the effect increasing over time and correlating with bacterial growth (Huwe et al. 2009).In particular, E. coli was found to have a significant inhibitory effect on sperm motility Table-1; however, not all microorganisms affect sperm motility. E. coli and Pseudomonas aeruginosa have clear negative effects, whereas other pathogens, such as Enterococcus and Staphylococcus saprophyticus, showed no significant influence on sperm motility (Huwe et al., 2009). Understanding these mechanisms could lead to improved diagnosis and treatment strategies for infection-related male infertility, especially in cases of urogenital tract infections.

Inflammatory responses to pathogens can create a hostile environment for sperm

Inflammatory responses to pathogens can create a hostile environment for sperms, as evidenced by several studies on the role of the immune system in reproductive biology. The female reproductive tract, particularly the uterus and oviduct, has evolved a delicate balance between protection against pathogens and maintenance of a suitable environment for sperm and embryo survival. When pathogens trigger an inflammatory response, they negatively affect sperm function and fertility. For instance, in cattle, sperm entering the uterus activates an innate immune response via TLR2 signalling, leading to the upregulation of pro-inflammatory cytokines, such as IL8, TNFA, and IL1B (Akthar et al., 2021).This immune activation results in the trapping of many sperm cells by polymorphonuclear neutrophils (PMNs), the primary component of innate immunity, and the oviduct has developed mechanisms to protect sperm from excessive immune responses. Under physiological conditions, the bovine oviduct downregulates sperm phagocytosis by PMNs through prostaglandin E2 (PGE2) action (Marey et al., 2013). Furthermore, sperm binding to bovine oviduct epithelial cells (BOECs) shifts the local immunity towards an anti-inflammatory state, upregulating IL-10, TGFβ, and PGE2 (Marey et al., 2016). This suggests that the oviduct actively supports sperm survival under normal conditions.

Impact on sperm morphology:

Certain bacteria can cause agglutination of sperm cells

Bacterial infections can significantly affect sperm morphology and function, with several studies demonstrating specific effects, and certain bacteria can cause agglutination of sperm cells. Escherichia coli has been shown to induce sperm agglutination through receptor-ligand interactions. E. coli P-fimbriae cause tail-tail agglutination, whereas type 1 fimbriae lead to head-head agglutination of sperm (Monga & Roberts, 1994). This bacterial-induced agglutination can affect up to 40-75% of motile sperm, potentially impairing fertility Table-2. Interestingly, the effects of bacteria on sperm morphology and function can vary depending on the bacterial species and concentration. While in vitro studies have demonstrated clear negative effects, some in vivo studies have shown conflicting results. For instance, while E. coli and Mycoplasma species affect sperm functions in vitro, similar effects were not consistently observed in vivo (Köhn et al., 1998). However, other studies have shown that bacterial infections, particularly those caused by Mycoplasma spp., can adversely affect sperm concentration, motility, and morphology (Rybar et al. 2011).

Viral infections may lead to abnormal head or tail structures

Viral infections during pregnancy can lead to abnormal head or tail structures in the developing fetus, which can cause a spectrum of maternal and fetal outcomes, ranging from asymptomatic disease to severe conditions (Chudnovets et al., 2020). Viral infections can result in congenital anomalies affecting various parts of the fetus, including the head and tail regions. For instance, cytomegalovirus (CMV) infection can cause ventriculomegaly, intracranial calcification, hydrocephaly, and microcephaly (Isikay et.al, 2013). Additionally, some viruses can lead to limb contractures, which may affect tail-like structures in the developing fetus (Isikay et.al, 2013). Interestingly, although viral infections can cause structural abnormalities, genetic factors also play a role in head and tail development. For example, mutations in the fork head (fkh) gene in Drosophila can transform non-segmented terminal regions of the embryonic ectoderm, replacing pre-oral head structures and foregut with post-oral head structures, and posterior tail structures with anterior tail structures (JRgens & Weigel, 1980).This highlights the complex interplay between genetic and environmental factors in fetal development.

Fungal contaminants can alter sperm membrane integrity

Fungal contaminants can alter sperm membrane integrity, as evidenced by studies of both fungal pathogens and sperm membrane characteristics. Citral, a compound with strong antifungal activity against Penicillium italicum, disrupts fungal cell membrane integrity and permeability. This suggests that fungal metabolites may have similar effects on sperm membranes. Citral causes loss of cytoplasm, distortion of mycelia, increased membrane permeability, and decreased total lipid and ergosterol content in fungal cells (Guo et al., 2014).Sperm membrane integrity is crucial for fertilization, as it plays a key role in capacitation, acrosome reaction, and binding to the egg. The hypoosmotic swelling (HOS) test evaluates the functional integrity of sperm plasma membranes by assessing their ability to maintain equilibrium with the environment (Ramu& Jeyendran, 2012). This test can potentially be used to detect membrane damage caused by fungal contaminants. Interestingly, while fungal contaminants may damage sperm membranes, certain compounds such as butylated hydroxytoluene (BHT) can protect sperm from membrane damage caused by rapid cooling in some species. However, this protective effect varies among species, highlighting the complexity of sperm-membrane interactions (Janosikova et al., 2023).Additionally, proteins such as HSPA8 have been shown to promote sperm viability and membrane repair, potentially counteracting the damage caused by fungal contaminants (Moein-Vaziri et al., 2014).

Fertility outcome implications

Reduced fertilization rates due to compromised sperm function

Reduced fertilization rates owing to compromised sperm function have significant implications for fertility outcomes, as evidenced by several studies. In assisted reproductive techniques, sperm with decreased motility and function can lead to lower fertilization rates, and a case-control study found that couples with male HBV infection had a higher risk of low fertilization rates after IVF, independent of initial sperm motility (Oger et al., 2011).This was associated with a decreased number of embryos available for transfer, although the embryo quality was not affected. Oxidative stress in sperm has been shown to negatively impact fertilization rates and in vitro embryo development. A systematic review of studies in non-human mammals found that 80% of IVF studies and 75% of ICSI studies observed negative effects of sperm oxidative stress on fertilization rates (Ribas-Maynou et al., 2020). This highlights the importance of sperm DNA integrity for successful fertilization and embryo development. Interestingly, some studies have reported contradictory results regarding the impact of certain factors on fertilization rates. For instance, while most studies have shown the negative effects of oxidative stress, some ICSI studies have found a positive relationship between sperm oxidative stress and fertilization rates (Ribas-Maynou et al., 2020). This suggests that the relationship between sperm function and fertility outcomes may be complex and dependent on the specific assisted reproductive technique used.

Increased risk of transmitting infections to female partners or offspring

Male circumcision has been shown to significantly reduce the risk of transmitting Chlamydia trachomatis infection to female sexual partners. A study found that women with circumcised partners had a 5.6-fold reduced risk of testing seropositivity for C. trachomatis than those with uncircumcised partners (Castellsagué et al., 2005).This protective effect was observed, even when the analysis was restricted to monogamous couples. Interestingly, HIV-positive individuals, particularly injection drug users (IDUs), may pose an increased risk of transmitting infections to their partners. A study of HIV-positive male IDUs found that over 40% reported perpetrating physical and/or sexual violence against their main female partners in the past year, which was associated with unprotected sex and potential HIV transmission risk behaviours (klot et al., 2007). Additionally, helminth co-infection in HIV-positive pregnant women is associated with a significantly higher risk of mother-to-child transmission (MTCT) of HIV (Gallagher et al., 2005).

Potential for early pregnancy loss or complications

Early pregnancy loss is the most common pregnancy complication, affecting approximately 15% of recognized pregnancies, with 1% of women experiencing recurrent miscarriages (Ford et al., 2011). Various factors contribute to the risk of early pregnancy loss and complications: Maternal factors such as advanced age (>33 years), low body mass index (≤20 kg/m²), and low serum progesterone levels (≤12 ng/ml) are associated with an increased risk of miscarriage (Arck et al., 2008). Additionally, obesity has been linked to early pregnancy complications including miscarriage, ectopic pregnancy, and hyperemesis gravidarum (Potdar&Iyasere, 2023).Haematological markers, such as reduced red blood cell count, lower mean platelet volume, and higher platelet-to-lymphocyte ratio, have been strongly associated with first-trimester miscarriage (Ata et al., 2020). Interestingly, neutrophil-to-lymphocyte ratio (NLR) values >5.8 were exclusively observed in the miscarriage group, suggesting its potential as a prognostic marker (Christoforaki et al., 2019). Contradictory findings have been reported regarding the role of lipid profiles in early pregnancy complications. Although elevated lipid levels during late pregnancy are associated with adverse outcomes, the impact of disturbed lipid profiles during early pregnancy remains inconclusive. However, elevated maternal triglyceride levels in early pregnancy have been associated with pregnancy-induced hypertension, preeclampsia, and large-for-gestational-age infants (Vrijkotte et al., 2012).

Prevention and management strategies

Regular screening of semen donors for microbial contamination

Regular screening of semen donors for microbial contamination is an important preventive and management strategy for controlling bacterial contamination in semen samples. Regular microbiological screening of boar semen is highly recommended to avoid the use of low-quality semen in pig industry (Costinar et al. 2021).This allows the identification of pathogenic and antibiotic-resistant bacteria that may be present in semen samples. Routine microbiological analysis of bovine semen can help detect bacteriospermia early and prevent its negative effects on sperm quality (Ďuračka et al., 2021).Interestingly, while screening is important, some studies have found that proper hygiene and sanitation practices may be even more effective in reducing bacterial contamination, and one study demonstrated that implementing basic hygiene standards and improving hygienic conditions at critical control points in AI centers led to significant reductions in bacterial contamination of semen samples over time (Insfran et al., 2019).This finding suggests that prevention through good hygiene practices may be as important as screening.

Proper hygiene practices during semen collection and handling

Appropriate hygiene practices are crucial to ensure sample quality and prevent contamination during semen collection and handling. Digital stimulation is the most common method for semen collection in dogs and requires sterile equipment and careful techniques (Kutzler et al, 2005).However, bacterial contamination of ejaculates is common because of the nature of the collection process and the subsequent laboratory handling (Althouse, 2008).To minimize contamination, good hygiene by personnel, general sanitation protocols in laboratories and animal housing areas, and attention to cleanliness during the collection process are essential (Althouse, 2008).Despite these measures, complete elimination of bacterial contamination remains a challenge. Antimicrobials are commonly added to semen extenders to control residual bacterial load and promote sperm longevity (Althouse, 2008).

Use of antibiotics in semen extenders for artificial insemination

Antibiotics are commonly added to semen extenders used for artificial insemination (AI) in livestock breeding to control bacterial contamination during semen collection and processing (Morrell & Wallgren, 2014). This practice has been the standard for many decades since the beginning of commercial AI in livestock. However, there is growing concern regarding the non-therapeutic use of antibiotics in semen extenders, as it contradicts current recommendations for prudent antibiotic use and may contribute to the development of antimicrobial resistance (Luther et al., 2021; Morrell et al., 2024).Interestingly, recent studies have shown that some antibiotic-free semen extenders, such as Androstar Premium, have intrinsic antimicrobial activity that can reduce bacterial counts by 2–3.5 log levels (Luther et al., 2021).Additionally, hypothermic preservation strategies for boar semen at 5°C without antibiotics have shown promising results in maintaining sperm function and fertility, while maintaining low bacterial loads (Waberski et al., 2019).These findings suggest that alternative approaches for bacterial control in semen extenders are possible.

Diagnostic approaches:

Semen culture to identify bacterial species

Semen culture is a traditional method for identifying bacterial species in semen samples; however, recent studies have highlighted its limitations and the need for more advanced diagnostic approaches (Jarvi et al., 1996; Weng et al., 2014).Routine bacterial cultures often underestimate the incidence of

bacteriospermia, particularly that of anaerobic bacteria. In one study, PCR-based methods detected >10^4 bacteria/mL in 66% of infertile men and semen donors, while routine cultures only identified "significant" bacteriospermia in 27% of infertile men and none of the donors (Jarvi et al., 1996). This discrepancy suggests that culture-based methods may not provide a complete picture of the bacterial communities present in the semen. Interestingly, the presence or absence of bacteriospermia detected in culture does not always correlate with leukospermia or semen parameters. Some bacterial species, such as Staphylococcus, are commonly isolated but appear innocuous, while others, such as Streptococcus viridans and Enterococcus faecalis, are associated with poorer semen quality (Rodin et al., 2003).This highlights the complexity of interpreting the culture results and their clinical significance.

PCR-based detection of viral contaminants

Polymerase chain reaction (PCR)-based methods have emerged as powerful tools for detecting viral contaminants in various samples, offering high sensitivity and specificity.Polymerase chain reaction (PCR) allows the amplification and detection of specific nucleic acid sequences, making it particularly useful for identifying viral pathogens (Coutlée et al., 1991).For instance, PCR has been successfully applied to detect HIV-1 in cases where serological assays may be inadequate, such as in infants born to seropositive mothers or in patients with delayed serological responses (Coutlée et al., 1991).Although PCR is highly sensitive, it has limitations in determining viral infectivity, and the detection of viral genomes does not necessarily correlate with the presence of infectious particles (Hamza et al., 2011).To address this, integrated approaches such as cell culture-PCR have been developed to provide information about both the presence and infectivity of viruses (Hamza et al., 2011).Additionally, multiplex nested RT-PCR techniques have been developed to simultaneously detect multiple RNA viruses and bacteria in a single closed-tube assay, increasing efficiency and reducing contamination risks (Bertolini et al., 2003)

Microscopic examination for fungal elements

Microscopic examination is crucial for the detection and preliminary diagnosis of fungal infections. It allows for rapid identification of fungal elements in clinical specimens, providing valuable information for timely treatment decisions (Dunbar et al., 1998). This technique is particularly useful for diagnosing fungal keratitis, where direct microscopic examination of KOH mounts and Gram-stained smears can reveal fungal elements in corneal scrapings with a sensitivity of 62% (Chowdhary & Singh, 2005).Interestingly, while microscopic examination is highly sensitive and specific for diagnosing bacterial or fungal meningitis (88-92% sensitivity), it may not always allow for unambiguous species-level identification owing to visual similarities between different fungi (Dunbar et al., 1998; Zieliński et al., 2020).This limitation has led to the development of advanced techniques, such as machine learning approaches based on deep neural networks, to improve the accuracy and speed of fungal species identification (Zieliński et al., 2020).

Research directions:

Investigating the microbiome of healthy vs. contaminated semen

Research on the microbiomes of healthy and contaminated semen has revealed several important findings and potential directions for future investigations. The microbiome composition of semen can vary significantly among individuals and may be influenced by factors such as geographical location, environment, and individual health status (Malaluang et al., 2024). Studies have found that healthy semen typically contains diverse bacterial communities dominated by phyla such as Firmicutes, Bacteroidetes, and Proteobacteria (Malaluang et al., 2024; Tuominen et al., 2021). However, the presence of certain pathogens or alterations in microbial balance may be associated with contamination or reduced semen quality. Interestingly, viral infections, such as HPV, have been shown to affect semen microbiome composition. HPV-positive semen samples exhibited higher abundances of certain bacterial families and genera than HPV-negative samples, suggesting a potential interaction between viral and bacterial communities in the male reproductive tract (Tuominen et al., 2021).This highlights

the need to consider both bacterial and viral components when investigating the semen microbiome health.Future research should focus on establishing a more comprehensive understanding of what constitutes a "healthy" semen microbiome and how it may differ across populations. Utilizing advanced sequencing techniques and bioinformatics approaches, such as those outlined in (Bashiardes et al. ;2016), could help elucidate the functional aspects of the semen microbiome and its impact on fertility.Additionally, investigating the potential use of probiotics or targeted antimicrobial strategies to modulate semen microbiome composition may offer new avenues for improving reproductive health and fertility outcomes (Bagga et al., 2018; Lisko et al., 2017).

Developing rapid, non-invasive detection methods for contaminants

Rapid and non-invasive detection methods for contaminants are crucial for ensuring food safety, environmental protection, and human health. Recent advancements in this field have led to the development of various innovative techniques that offer improved sensitivity, speed, and ease of use compared with traditional methods (Ferone et al., 2020; Zhang et al., 2024). Emerging technologies for contaminant detection include spectroscopic techniques, such as matrix-assisted laser desorption ionization-time of flight, hyperspectral imaging, and Surface-Enhanced Raman Spectroscopy (SERS) (Ferone et al., 2020; Tang et al., 2024). Microfluidic sensors have shown promise for the on-site monitoring of emerging contaminants in water, offering benefits such as rapid evaluation, minimal sample usage, and automation (Zhang et al., 2024). Electrochemical sensors, particularly those incorporating advanced materials such as carbon nanomaterials and metal-organic frameworks, have demonstrated effectiveness in detecting phenolic contaminants (Gu et al., 2023). Interestingly, non-invasive approaches are also being explored for detecting heat stress in livestock, utilizing technologies such as infrared thermography, accelerometers, and machine learning algorithms to monitor behavioral and physiological responses (Sejian et al., 2022). This highlights the versatility of noninvasive detection methods across different fields.

Exploring the use of probiotics to combat harmful microbes in semen

Probiotics have shown potential in combating harmful microbes in semen and in improving male reproductive health. Various bacterial communities have been identified in the male reproductive system, with some species having negative effects on semen parameters, while others have demonstrated protective effects (Alqawasmeh et al., 2023).Research indicates that certain pathogenic bacteria can negatively affect sperm count, motility, morphology, and DNA integrity, whereas beneficial bacteria such as Lactobacillus have shown protective effects on semen parameters, making them promising candidates for probiotic interventions (Alqawasmeh et al., 2023).Interestingly, a study on boar semen revealed that a higher abundance of Lactobacillus in winter samples was positively associated with sperm quality and reproductive performance, whereas a higher abundance of Pseudomonas in summer samples was negatively associated with these factors (Zhang et al., 2020).The mechanisms by which probiotics combat harmful microbes in semen may include direct competition for nutrients, secretion of antimicrobials, stimulation of innate and adaptive immunity, and enhancement of mucosal barrier function. Additionally, probiotics can help form a solid intestinal barrier against damaging agents and prevent harmful microbes from colonizing (Li et al., 2024).These findings suggest that probiotic supplementation could be a potential alternative therapeutic option for male infertility, although further clinical studies with larger sample sizes are needed to confirm these results (Alqawasmeh et al., 2023; Zhang et al., 2020).

Considerations for assisted reproductive technologies

Implementing stringent quality control measures in IVF laboratories

Implementing stringent quality control measures in IVF laboratories is crucial to ensure the safety and success of assisted reproductive technologies (ART).Quality control is essential for minimizing the risks associated with ART. Studies have shown that ART may be linked to increased rates of major malformations, low birth weight, and other perinatal complications in infants conceived using these

methods (Shiota & Yamada, 2005).Additionally, there are concerns about potential imprinting disorders in the offspring, such as Beckwith-Wiedemann syndrome and Angelman syndrome (Shiota & Yamada, 2005).These findings highlight the importance of rigorous quality control in in IVF laboratories. Interestingly, some research suggests that the risks associated with ART may not be solely due to the techniques used, but also due to parental background factors. Data from surrogate motherhood demonstrate lower risks than pregnancies from IVF mothers carrying their own children, indicating that infertility-related problems may play a role (Ludwig & Diedrich, 2002).However, the possibility of technique-related risks cannot be completely ruled out based on the current data (Ludwig & Diedrich, 2002).

Evaluating the efficacy of sperm washing techniques in removing contaminants

Sperm washing techniques have shown varying degrees of efficacy in removing contaminants from semen.A study on human papillomavirus (HPV) found that conventional sperm-washing centrifugation did not reduce the number of infected samples or the percentage of infected cells. While the Ficoll and swim-up protocols induced a slight reduction in infected samples, they rarely eliminated HPV sperm infection completely (Foresta et al., 2011). Interestingly, sperm washing has been proven to be highly effective and safe for the removal of HIV in Sero-discordant couples. Large studies involving thousands of assisted reproductive cycles using washed sperm from HIV-positive men have shown no HIV transmission to female partners (Bujan et al., 2007; Savasi et al., 2006). This demonstrates that sperm washing can significantly reduce the risk of HIV transmission when properly implemented. In summary, the efficacy of sperm washing varies depending on the specific contaminant. While it appears to be highly effective for HIV removal, conventional techniques may be insufficient to eliminate other viral infections, such as HPV. More advanced sperm selection methods based on surface charge, apoptosis markers, membrane maturity, and ultra morphology show promise for improving sperm quality and ART outcomes (Said & Land, 2011). However, further evaluation is required before widespread clinical implementation, and sperm washing remains an important tool in assisted reproduction. However, its limitations for certain contaminants highlight the need for continued refinement of techniques.

Assessing the impact of cryopreservation on microbial load in stored semen

Based on the context provided, there is limited information specifically addressing the impact of cryopreservation on the microbial load in stored semen. However, some relevant points can be extracted cryopreservation processes can have various effects on sperm quality and function. Freezing and thawing semen can damage sperm plasma membranes, decrease motility, and increase reactive oxygen species (ROS) levels (Kim et al., 2011).These changes could potentially affect the microbial environment, although this has not been addressed directly. Interestingly, Clarke (1999) briefly mentioned concerns regarding liquid nitrogen contamination by microbes and the possibility of cross-infection during semen cryopreservation. This suggests that the cryopreservation process itself may introduce microbial contamination risks that need to be considered (Clarke 1999). Although not directly related to microbial load, several studies have discussed how cryopreservation affects various aspects of sperm quality and function in different species. Cryopreservation can affect sperm chromatin structure (Gandini et al., 2006), membrane integrity, mitochondrial function, and DNA damage (Contreras et al., 2020).These changes in sperm physiology could potentially influence the microbial environment, although this connection was not explicitly established in the provided context.

Potential treatment options

Targeted antibiotic therapy based on sensitivity testing

Targeted antibiotic therapy based on sensitivity testing is a crucial approach for effective treatment of bacterial infections. Rapid and accurate antibiotic susceptibility testing (AST) is essential for guiding appropriate antibiotic selection and combating antimicrobial resistance. Microfluidic platforms offer several advantages for AST, including faster analysis times, enhanced sensitivity, and minimal sample requirements (Mohan et al., 2013). For instance, a fluorescence-based microfluidic system can

determine bacterial susceptibility to antibiotics within 2-4 hours, enabling the timely administration of appropriate therapy (Mohan et al., 2013).Similarly, a rapid diagnostic platform integrating droplet digital PCR and 3D particle counting could identify antibiotic resistance genes directly from blood samples within one hour, achieving a high sensitivity of 10 CFU/mL (Abram et al., 2020).Interestingly, while combination antibiotic therapy is often considered more effective, results from microfluidic studies suggest that combinations of three or more antibiotics are not necessarily superior to antibiotic pairs in eradicating pathogens (Mohan et al., 2013).Additionally, emerging approaches, such as bacterial cytological profiling, can rapidly distinguish between methicillin-susceptible and methicillin-resistant Staphylococcus aureus strains within 1-2 hours with 100% accuracy (Quach et al., 2016).

Antiviral medications for specific viral contaminants

Antiviral drugs are specifically designed to treat viral infections by targeting various stages of the viral life cycle, and different antivirals are used for specific viruses, as they are tailored to combat particular viral pathogens (Vardanyan & Hruby, 2006).For instance, acyclovir, trifluridine, and valaciclovir are commonly used to treat herpesvirus infections, whereas cidofovir has shown promise in treating some cases of adenoviral conjunctivitis (Skevaki et al., 2011).Despite recent advances in controlling some viral pathogens, most viral infections still lack specific treatments, and the development of new antivirals has not kept pace with the emergence and re-emergence of viral threats (Mercorelli et al., 2018).This has led to innovative approaches in drug discovery, including drug repurposing and development of broad-spectrum antiviral agents (BSAAs) that can target viruses from multiple viral families (Andersen et al., 2020).

Antifungal treatments for fungal infections

Fungal infections pose a significant health challenge, particularly for immunocompromised individuals, with high morbidity and mortality rates, despite the availability of antifungal treatments (Williams et al., 2020). The main classes of antifungal drugs used to combat invasive fungal infections (IFIs) are polyenes, azoles, and echinocandins (Klepser, 2010). However, these treatments are limited by toxicity, development of resistance, and ineffectiveness in immunosuppressed patients (Ademe, 2020; Andriole, 2000).Interestingly, while newer antifungal agents, such as second-generation triazoles and echinocandins, have expanded treatment options, the oldest class of antifungals, polyenes, remain useful due to their broad-spectrum activity and low resistance rates (Chandrasekar, 2010).This highlights the complex nature of antifungal therapy, in which both traditional and novel approaches play a crucial role, and the emergence of rare and resistant fungal pathogens further complicates treatment strategies (Chandrasekar, 2010; Reddy et al. 2022).Researchers have explored various approaches to address these issues. Immunomodulatory therapies such as cytokine therapy, monoclonal antibodies, and cellular immunotherapy are being investigated as adjunctive treatments to enhance the efficacy of conventional antifungals (Ademe,2020; Casadevall & Pirofski, 2001; Williams et al., 2020).Combination drug therapy is another promising strategy for increasing drug effectiveness and mitigating the development of resistance (Spitzer et al., 2016).Furthermore, the development of novel antifungal agents that target new molecular pathways is urgently needed to expand the current antifungal arsenal (Andriole 2000; Su et al. 2018).These diverse approaches reflect ongoing efforts to improve the treatment outcomes for fungal infections in the face of evolving challenges.

DISCUSSION

Microbial contamination of semen is increasingly recognized as a key factor contributing to male infertility, affecting sperm function and overall reproductive success.Contaminants such as bacteria, viruses, and fungi may originate from urogenital tract infections, systemic infections, or sample-handling errors (Monteiro et al., 2014; Monteiro et al., 2018).These microorganisms impair sperm motility, morphology, and viability, reduce fertilization potential, and increase the risk of complications associated with assisted reproductive technologies (ART) (Paribok et al., 2022).

Bacteria, such as Escherichia coli, Staphylococcus aureus, Streptococcus spp., and Klebsiella pneumoniae, produce toxins and pro-inflammatory cytokines, leading to oxidative stress, sperm agglutination, and membrane damage (Sanocka-Maciejewska et al., 2013).Viral infections, including human papillomavirus (HPV) and herpes simplex virus (HSV), can induce sperm DNA fragmentation and apoptosis, whereas fungal infections can alter sperm lipid composition and membrane integrity (Javurek et al., 2021).These effects lower fertilization rates and increase the risk of early pregnancy loss, particularly in in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) procedures (Cottell et al., 2000).

Early detection of microbial contamination is crucial, as semen culture remains the gold standard, although PCR-based methods and next-generation sequencing (NGS) offer more comprehensive insights into the semen microbiome (Monteiro et al., 2018). Preventive strategies include screening semen donors, maintaining strict hygiene, and using sterilized laboratory equipment (Kiessling et al., 2008). Antibiotic treatment can be effective, but requires sensitivity testing to prevent antimicrobial resistance (Sanocka-Maciejewska et al., 2013). Emerging research suggests that probiotics may restore semen microbiome balance, reducing the prevalence of harmful pathogens (Paribok et al., 2022).

To minimize contamination risks in ART, sperm washing techniques, such as density-gradient centrifugation and swim-up methods, help eliminate bacteria and enhance sperm quality (Ricci et al., 2009). Additionally, pretreatment with antimicrobial agents or probiotics is being explored as a promising strategy to improve ART success rates (Cottell et al., 2000).

CONCLUSION

Microbial contamination of semen significantly affects sperm quality, motility, morphology, and overall fertility potential. The presence of bacteria, viruses, and fungi can lead to oxidative stress, sperm agglutination, DNA fragmentation, and impaired fertilization, ultimately reducing reproductive success.In assisted reproductive technologies (ART), microbial contamination poses additional risks, including failed fertilization, implantation issues, and increased chances of infection in female partners.

Early detection and appropriate management strategies are essential to mitigate these risks.Routine semen screening for microbial contamination should be integrated into fertility assessments utilizing advanced molecular diagnostic techniques such as PCR and next-generation sequencing for the precise identification of pathogens. Sperm processing methods, such as density-gradient centrifugation and swim-up techniques, have proven effective in reducing bacterial load and improving sperm quality for ART applications. Additionally, emerging interventions such as probiotic supplementation and antimicrobial treatments hold promise for restoring the balance of the semen microbiome and minimizing the impact of pathogenic microbes.

Further research should focus on understanding the complex interactions between the semen microbiome and male fertility, developing rapid, non-invasive detection methods for microbial contaminants, and optimizing ART protocols to minimize infection risks. Strengthening public health initiatives such as promoting safe sexual practices, screening programs for high-risk populations, and standardized guidelines for semen handling in fertility clinics will be crucial for improving reproductive outcomes.

Ultimately, a comprehensive approach combining advanced diagnostics, effective treatment strategies, and optimized ART techniques will contribute to enhancing male fertility and reproductive success, ensuring better clinical outcomes in couples facing infertility challenges.

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Table1: Effects of Microbial Adherence on Sperm Motility

Microorganism	**Effect on Sperm Motility**	**Mechanism of Action**	**Reference**
Escherichia coli (*E. coli*)	Significant inhibition	Adhesion to sperm surface, production of toxins, induction of oxidative stress	Huwe et al., 2009

Pseudomonas aeruginosa	Moderate inhibition	Biofilm formation, production of virulence factors, and sperm agglutination	Huwe et al., 2009
Enterococcus spp.	No significant effect	Limited adhesion and lack of sperm-toxic metabolites	Huwe et al., 2009
Staphylococcus saprophyticus	No significant effect	Weak interaction with sperm membrane, minimal toxin production	Huwe et al., 2009
Candida albicans	Inhibition at high concentrations	Possible sperm membrane disruption due to fungal metabolites	Huwe et al., 2009

Table 2: Common Bacteria That Cause Sperm Agglutination

Bacteria	Gram Reaction	Agglutination Mechanism	Clinical Significance	References
Escherichia coli	Gram-negative rod	Type 1 fimbriae bind to sperm surface, leading to clumping	Major cause of UTI and male infertility	Zhou et al 2023, Villegas et al 2017
Klebsiella pneumoniae	Gram-negative rod	Produces adhesins that bind to sperm membranes	Associated with chronic prostatitis	Marchiani*et al* 2021
Pseudomonas aeruginosa	Gram-negative rod	Produces pili and biofilms, disrupting sperm function	Found in chronic infections and catheter-related UTIs	Rana et al 2018
Proteus mirabilis	Gram-negative rod	Urease production increases pH, impairing sperm movement	Linked to epididymitis and infertility	Schaffer and Pearson et al 2015
Neisseria gonorrhoeae	Gram-negative diplococcus	Surface pili cause sperm agglutination	Sexually transmitted infection (STI), causes urethritis	Sadoghi et al 2022
Ureaplasma urealyticum	No cell wall (Mollicute)	Adheres to sperm via specialized adhesion proteins	Causes urethritis, affects sperm motility	Xianchun et al 2023
Mycoplasma hominis	No cell wall (Mollicute)	Alters sperm membrane fluidity and induces immune response	Linked to non-gonococcal urethritis and infertility	Sethi et al 2012
Staphylococcus aureus	Gram-positive cocci	Produces clumping factor, fibrinogen-binding protein	Associated with chronic infections, prostatitis	Esmailkhani et al 2018

Streptococcus agalactiae (Group B Strep)	Gram-positive cocci	Surface proteins cause sperm adhesion and aggregation	Found in genital tract infections, linked to infertility	Gonçalves et al
Chlamydia trachomatis	Intracellular pathogen	Causes inflammation, induces anti-sperm antibodies	Common STI affecting sperm function	Zhou *et al* 2022