Chapter 7

Nanotechnology in the Purification of Semen

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Summary

Animals' reduced fertility poses a serious threat to animal reproduction. A number of factors have been demonstrated to reduce semen quality, including age, health, heredity, nutritional condition, seasonal variance, travel stress, artificial insemination (AI), and rising breeding demand. The properties and functioning of semen are altered by the in vitro environment and changes in semen after collection that occur during artificial insemination. Successful artificial insemination may also be achieved via the use of sperm selection and purification techniques. Invasive labeling and/or centrifugation processes are often used in current sperm manipulation approaches, which may be harmful to sperm and/or result in poor recovery rates. The biochemical properties and DNA status of the sperm are often disregarded in favor of selecting for physically normal and motile sperm. It is critical to develop alternative, noninvasive, label-free sperm selection methods to separate sperm based on biochemical features and DNA status. Magnetic nanoparticles provide intriguing new research opportunities for sperm selection. This book explains how sperm may be tagged and cleaned up with the use of nanotechnology, which has become more important in this area in recent years. This book analyzes the recent impact of nanotechnology on sperm labeling, selection, and purification techniques, both existing and planned.

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Introduction

Nanoparticles (NPs) are manufactured particles with a high surface ratio, flexible fabrication, and extremely small size - in the nanometer range. Metals, polysaccharides, and proteins are just some of the components that can be used to make nanoparticles. The potential applications of nanotechnology in many areas of science, including medicine, have increased dramatically in recent years. This is largely attributable to the engineering of nanoparticles with improved stability, solubility, and physiological efficacy over their naturally occurring counterparts. In addition, NPs are being employed more often in the pharmaceutical sector to provide therapeutic formulations for hydrophilic substances that are either lipophilic or unstable. Poor water solubility (hydrophobicity) of medications is still a serious issue in clinical pharmacology. Nanotechnology is particularly useful for the approximately 25% of essential medicines on the WHO (World Health Organization) that are thought to be poorly water soluble (Lindenberg et al., 2004).

Techniques to remove defective cells, impurities, and debris from an ejaculate are part of the process of selecting subpopulations of the best sperm (in terms of morphology and motility). These methods can be particularly useful for recovering sperm from suboptimal ejaculations from animals with high genetic value, but they also remove dead sperm, which is highly beneficial due to the detrimental effects that live sperm have on motility and membrane integrity (Brinsko et al., 2003; Öztürk and Ömür, 2022). The most common techniques for removing sperm from animals include density gradient centrifugation (Sieme et al., 2003), swim-up evaluation (Arias et al., 2017), column cleaning (Galarza et al., 2018), and single-layer centrifugation (Nongbua et al., 2017). Although these methods significantly enhance sperm motility and functional parameters in semen cans, particularly for post-thaw semen, they are still constrained by varying rates of recovery (resulting in low sperm concentration in semen cans), increased costs, long run times, and labor costs (Feugang, 2017).

It is necessary to do research in order to have a better comprehension of the cellular and tissue-level impacts of nanocompounds on the reproductive system, since little is currently known about their activity. Since nanocarriers can cross the hemato-testicular barrier (Lan and Yang, 2012), their systemic distribution and biocompatibility have been called into question, and it has been hypothesized that in vivo effects may be the concequence of both systemic changes and direct effects on the testes.

Recent studies on semen statistics show that anywhere from 16–33 percent of semen ejaculates from cattle and buffalo bulls are rejected due to

poor quality when they are still fresh (Manda et al., 2016; Tiwar et al., 2015; Gopinathan et al., 2016), and another 25–30 percent are rejected after being thawed (Bisla et al., 2020). This reduces their commercial viability since there is less high-quality germplasm available for breeding propagation. Increased oxidative stress because of the presence of dead and injured spermatozoa (25%-30%) producing ROS may be a primary cause of ejaculate rejection (Bisla et al., 2020; 2021; Kumar et al., 2018; Rautela et al., 2020).

According to Stiavnická et al. (2017), sperm nano-purification is a process that does not include any invasive procedures and may be used to select high-quality sperm based on epigenetics. The process of nanopurification is used on sperm in order to differentiate unhealthy, intact sperm from sperm that contains moribund cells and sperm that has particular surface alterations. According to recent research (Degheidy et al., 2015; Romany et al., 2017), the technology known as magnetically activated cell sorting (MACS), which can differentiate between dead and living sperm, is the most efficient procedure for the nanopurification of sperm currently available. In this context, sperm nano-purification is a game-changing technique as a result of its innovative capacity to be more effective while also taking less time. It is possible for it to be utilized commercially on a significant scale. Iron oxide nanoparticles (Fe3O4 NPs/IONPs) are renowned for their magnetic, biocompatible, and biofunctional qualities, which constitute the foundation for sorting dead sperm from those with damaged membranes, as stated by Huang and Tang (2004). Binding iron oxide nanoparticles (IONPs) to antiubiquitin Abs (Abs against ubiquitin, a poor fertility indicator) or to other plant lectins like PNA/PSA, which may bind to sperm membrane glycans, might be employed for nanopurification (Bisla et al., 2020). This would allow the IONPs to be purified in a more targeted manner. It was proven that greater conception rates could be achieved using sull sperm nano-purified after being frozen and thawed using Fe3O4 plant lectin or anti-ubiquitin antibody-coated nanoparticles (Odhiambo et al., 2014). This was the case even when using just half of the needed dosage.

In the first nano-purification experiments, buffalo sperm was used. The results were promising in terms of improved spermatozoa motility, plasma membrane integrity, viability, reduction of DNA damage, acrosome integrity, oxidative stress with improved antioxidant properties, and in vitro fertilization rate (Bisla et al., 2021). So, using magnetic nanoparticles to remove dead, dying, and clumped sperm has been shown to improve seminal fluid properties and fertility in boars (Durfey et al., 2019), bulls (Zhang et al., 2018), buffaloes (Bisla et al., 2021), and stallions (Morris et al., 2018).

In both fresh and frozen ejaculations, a procedure known as sperm nano-purification is used to remove defective and moribund sperm. This is done with the intention of reducing the extent of damage to viable sperm. This approach offers several advantages over conventional methods used today. The use of traditional methods for similar purposes, such as Sephadex filtration, sperm swimming up and down, or gradient separation, is limited due to a number of factors. These factors include labor costs, variably low sperm yield (10% to 63%), and high time requirements (> 60 minutes) (Bisla et al., 2020).

The following are nano purification techniques in semen and research that are linked to them.

1. Magnetic nanoparticles' removing of apoptotic and dead spermatozoa

Apoptotic and dead spermatozoa both result in the production of reactive oxygen species (ROS), including the highly reactive superoxide anion (O_2^-), hydroxyl radical (OH^-), and hydrogen peroxide (H_2O_2). Durfey et al., (2019) state that ROS are free radicals that lead to oxidative damage. ROS are byproducts of oxidative phosphorylation, the primary energy pathway for animal spermatozoa. Antioxidants such as ergothioneine, catalase, and superoxide dismutase, are abundant in spermatozoa seminal plasma to protect against the destruction caused by ROS. In order to partially defend against oxidative damage from ROS, it is necessary to have both hyperactive antioxidant systems and endogenous ROS generation through oxidative phosphorylation. When ROS build up, they cause lipid peroxidation, which in turn creates toxic lipid dehydrates (Leemans et al., 2019). This leads to a dramatic decline in sperm mobility and an increase in midpiece abnormalities. These metabolic byproducts would cause rapid cell death and oxidative DNA damage as stated by Rappa et al., (2016). Limiting and preventing these oxidative stressors by eliminating the dead and apoptotic sperm might improve the viability, motility, and fertility of a semen sample (Durfey et al., 2019; Lone 2016).

Apoptosis is an example of a regulated cell death mechanism. The proportion of Sertoli to germ cells is regulated in part by apoptosis (Aitken and Baker, 2013). Fifty to sixty percent of germ cells during the initial meiotic division are apoptosized by Sertoli cells (Sakkas and Alvarez, 2010). The testis' Sertoli cells will phagocytose these cells after they acquire apoptotic markers (Valcarce et al., 2016). This is important for maintaining a healthy ratio between germ cells and the number of Sertoli cells available to nourish

them. Apoptosis kills damaged germ cells in the testicular epithelium in response to various physiological and environmental cues (Aitken and Baker, 2013). However, the elimination method has its limitations. Some of these injured germ cells may still participate in spermiogenesis and show up in the form of ejaculate, although the amount varies greatly. Therefore, it is not uncommon to find apoptotic cells among the spermatozoa of mammals that have just ejaculated (Valcarce et al., 2016). When this elimination process is unsuccessful, sperm morphology and genomic quality may differ, resulting in spermatozoa that appear normal but have apoptotic damage to their nuclei (Sakkas and Alvarez, 2010).

Apoptotic spermatozoa exhibit DNA fragmentation hastening, mitochondrial membrane potential shifts, and caspase activation. The two most important apoptotic events in the setting of nano-purification are caspase activation and the release of the phosphatidylserine phospholipid (Durfey et al., 2019; Gil et al., 2013). Proteases known as caspases have been linked to apoptosis in two distinct ways, as effectors (caspases 3, 6, and 7) and as initiators (caspases 8, 9, and 10), respectively (Paasch et al., 2003). By generating DNA strand breaks, blocking membrane activities, cleavage of various structural cell proteins, and caspase 3 is responsible for the ultimate phases of cell death (Said et al., 2008). Studies by Cortés-Gutiérrez et al., (2007) reveal that infertility is linked to genetic deficits. As part of the apoptotic process, DNA fragmentation occurs, although this by itself does not have a major impact on the effectiveness of assisted reproduction. Cells that have begun the dying process but have not yet reached the point of ultimate collapse may still contain intact DNA since DNA fragmentation is a late indicator of apoptosis (Rateb, 2021). Loss of membrane integrity, a prelude to apoptosis (Gil et al., 2013), is even more intriguing. When the plasma membrane of sperm is disrupted, phosphatidylserine is released from the inner leaflet and moves to the cell's surface. Ca^{2+}-dependent phospholipid-binding protein annexin V has a considerable affinity for exposed phosphatidylserine. For this reason, MNP conjugated to annexin V may be utilized to differentiate between normal and abnormal sperm (Gil et al., 2013; Said et al. 2008).

Healthy spermatozoa may be distinguished from unhealthy spermatozoa. Miltenyi Biotec (Bergisch Gladbach, Germany) created and trademarked the MACS® technique, which can identify various cell types. MACS® gets rid of sperm cells that have phosphatidylserine on their surface by using 50 nm colloidal superparamagnetic microbeads that are linked to annexin V. These beads identify and trap sperm in the magnetic field of the MACS® column as they enter apoptosis and death. Figure 2 shows how percent flow can be

used to isolate viable sperm. Healthy sperm are isolated from sperm that have died or undergone apoptosis. Because of this, coated microspheres with MNP-annexin V complexes can't bind to non-apoptotic cells with healthy membranes. If sperm are able to adhere to the microspheres, this indicates that the phosphatidylserine has been externalized and sperm membrane integrity is compromised (Daneshmandpour et al., 2019) (Figure 1).

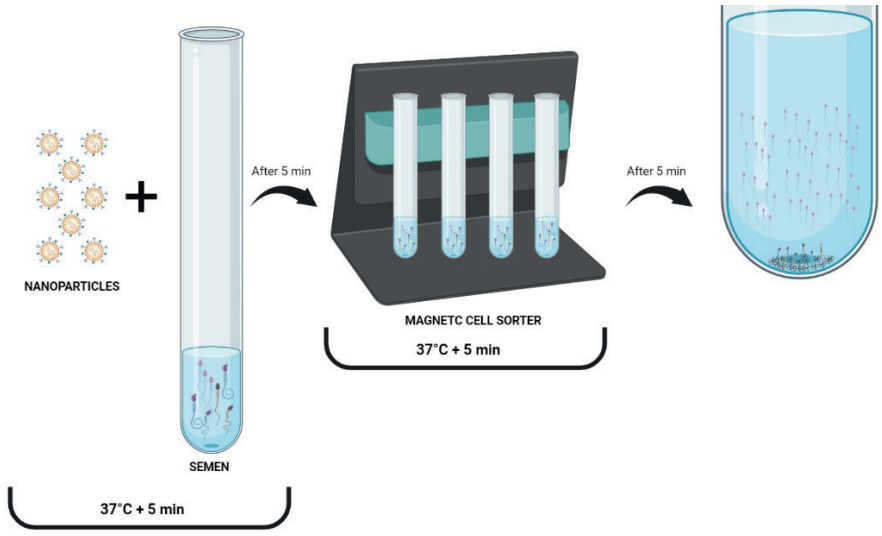

Figure 1. Schematic Diagram of Nanopurification of Semen

Performance of the MACS® system is restricted to less than 10^9 sperm for one factor in sperm viability, like apoptosis. The use of MACS® by the swine industry is thus hindered as a result (Durfey et al., 2019). The amount of boar semen used in postcervical artificial insemination is often increased to 50 milliliters and contains between one and one and a half million sperm. Extended amounts of 80-100 mL of swine semen containing $1.5\text{-}3.0\text{x}10^9$ spermatozoa are considered to be typical for conventional artificial insemination, as stated by Schulze et al., (2019). Due to the fact that the dosage necessary to enhance fertility after AI is only $500\text{x}10^6$, the aforementioned constraint presents less of a concern in horse reproduction (Samper, 2009).

Human sperm with a significantly higher motility and better cryosurvival rate after freezing and thawing are the result of MACS® separation. Oocytes that have been sorted have a better chance of being penetrated (Said et al., 2008). Paasch et al., (2003) found that both the magnetic field and the

separation columns had no appreciable impact on sperm viability in their experiments. The sperm count did not decrease, and neither did their motility. When compared to annexin-positive sperm, annexin-negative sperm had significantly higher increasing motile speeds after separation.

In a human study, Said et al., (2008) found that MACS® screening for non-apoptotic sperm subpopulations resulted in better sperm morphology. Lower sperm deformity index scores were observed, and fewer sperm had midpiece abnormalities, acrosomal defects, tail defects, or persisting cytoplasmic droplets. MACS® has several benefits when used to sort sperm, including cheap cost, convenience of use, high sensitivity, high specificity, quick response kinetics, and the production of stable suspensions. Annexin V, a cell protein, reacts immunospecifically with the phospholipid phosphatidylserine, resulting in a highly sensitive and specific assay. One of the few limitations of MACS is that the microbeads are so small. Miltenyi Biotec GmbH's MACS® Microbeads and Columns (MiniMACS® column) can produce this powerful magnetic field without compromising cell viability or performance. The tiny magnet needs a magnetic field of around 1 Tesla to preserve the tagged cells. Using MACS® to isolate a non-apoptotic fraction improves the overall quality and fertilization potential of the recovered sperm. MACS® can sort a large number of cells by surface marker expression. Apoptotic sperm aren't the only thing in an ejaculate that has to be filtered out if you want better outcomes. Leukocytes, debris, and plasma are all examples of these components. Thus, MACS® can enhance current methods of preparation (Said et al., 2008). For instance, double density gradient centrifugation paired with MACS® has been shown to be the most effective procedure for sperm selection in human fertility trials (Gil et al., 2013).

Separation of motile and high-quality spermatozoa and an improved conception rate were also reported by Feugang et al., (2015) using Fe3O4 NPs coated with PNA/PSA lectins for nanopurification of swine semen. Improved semen quality with more viable cells was seen by Farini et al., (2016) when synthetic DNA (deoxyribonucleic acid) aptamers were associated with spermatozoa with damaged membranes using avidin-coated superparamagnetic Fe3O4 NPs. Researchers Durfey et al., (2017, 2019) found that using the nanoselection technology to produce purified semen led to sperm with excellent structural and functional features without negatively impacting fertility. In addition, it did not compromise the well-being or growth of future generations.

Successful removal of apoptotic spermatozoa from ejaculate by MACS® has been reported in stallions by da Silva et al., (2010). Caspase activation, sperm motility, and membrane integrity were all negatively affected in spermatozoa that had connected to annexin V-conjugated microbeads, as predicted. In addition, MACS® did not alter the sperm's natural form in any way. In this investigation, only 46.3% of the sperm were retrieved using MACS®. More study is needed to determine if MACS® can strengthen sperm quality in infertile stallions and freeze-thawing of sperm (da Silva et al., 2010).

2. Magnetic nanoparticles removal early acrosome-reacted spermatozoa

Normal sperm undergo an acrosome response when they encounter the zona pellucida of an egg during fertilization in the fallopian tube. Yousef et al., (2020) and Leemans et al., (2019) found that sperm with premature acrosome reactions are unable to fertilize an egg because their reactions occur too early or in the wrong places. Acrosome injuries caused by osmotic, mechanical, or cryogenic pressure may cause premature acrosome reactions, and these injuries can be inherited. The inner acrosome membrane, combined with the remaining unfused component of the plasma membrane, creates the new outer sperm membrane in sperm that have responded prematurely with their acrosomes or in sperm that have damaged acrosomes. This happens in sperm that are either healthy or injured (Leemans et al., 2019).

Specific carbohydrates are released from the inner acrosomal membrane after premature capacitation of spermatozoa, an acrosomal damage, or acrosomal reaction reaction (Lone, 2016). Head-to-head agglutination of spermatozoa may be triggered by the interaction of these particular carbohydrates with lectins. The rate of conception drops when this happens because fewer sperm are able to reach the egg. Carbohydrates on the epithelial membrane of the fallopian tube and the zona pellucida of the egg may interact with lectins, glycoproteins on the surface of sperm. Lectin-carbohydrate interplays are used by the oviduct to choose sperm with optimal shape and performance. Pisum sativum agglutinin (PSA) and Peanut agglutinin from Arachis hypogaea (PNA) are two examples of lectins that have a high affinity for carbohydrates found exclusively in the inner acrosomal membrane (Feugang et al., 2015). Accurate indications of acrosomal damage in mammalian sperm include the lectins PSA and PNA. Yousef et al., (2020) and Odhiambo et al., (2014) propose that PNA lectins or MNP coated with PSA may be used to exclude sperm with a damaged acrosome.

3. Magnetic nanoparticle elimination of ubiquitinated spermatozoa

In MNP-assisted sperm selection, ubiquitin, a regulatory chaperone molecule, may be used as a marker, as revealed by Štiavnická et al., (2017). The reproductive organs of both sexes are common sites of ubiquitination (Lone, 2016). When it comes to X chromosome silencing, cell signaling, and gene transcription during gametogenesis, fertilization, and early embryonic development, mono-ubiquitination plays a key role (Odhiambo et al., 2014). Polyubiquitination, in which several ubiquitin chains are attached to the internal lysine residues of a substrate protein, is a stable covalent post-translational modification. Štiavnická et al., (2017) shown that poly-ubiquitination is crucial to the ubiquitin-proteasome system's role in protein turnover. According to the findings of study conducted by Odhiambo et al., (2014), ubiquitin in mammalian epididymal fluid helps in the turnover and aggregation of epididymal proteins that are secretory. This, in turn, assists in the maturation of sperm by encasing damaged or dead sperm, which helps the maturation process.

Epididymal epithelial cells use an apocrine secretory pathway to release ubiquitin as a quality control marker when sperm move through the epididymis. This happens as the sperm go through the epididymis. Epididymal epithelial cells are responsible for the release of unconjugated ubiquitin as well as the enzymes that are necessary for its covalent attachment to certain proteins found on the surface of damaged sperm. Ubiquitination of defective sperm triggers their phagocytosis and elimination. The ejaculate still contains ubiquitinated defective sperm (Štiavnická et al., 2017). That's why ubiquitination may cause morphological abnormalities, DNA fragmentation, acrosomal damage, dysregulated and ectopic accumulation of fertility-associated proteins, or all three in bull sperm. Odhiambo et al., (2014) found a negative correlation between ubiquitination of sperm and male fertility in various mammalian species. These data support the use of ubiquitin as a reliable spermatozoa marker. To eliminate incorrectly ubiquitinated spermatozoa, we used MNP coated with an anti-ubiquitin monoclonal antibody (Štiavnická et al., 2017).

Measurements of sperm quality were unaffected by the application of magnetic nanoparticles coated with PNA lectins and sex-specific components, as reported by Morris et al., (2018). Sperm treated with NPs demonstrated less DNA damage and had a higher conception rate (80%). Bull sperm nuclear integrity and structural surface damage were shown to be correlated with increasing ubiquitination (Zhang et al., 2018). Updated MACS techniques utilizing NPs coated with anti-ubiquitin antibodies

effectively removed morphologically damaged sperm from bull sperm by pushing sperm that were ubiquitinated and connected to NPs downward into the magnetic field.

4. A two-step procedure for removing spermatozoa that have undergone apoptosis and acrosome reaction

In two separate studies using the same methodology, researchers treated porcine sperm with magnetic nanoparticles (MNP) conjugated to the lectins PNA/PSA and MNP conjugated to annexin V (Durfey et al.,2019). By altering the acrosome of the sperm, lectin-MNP was used to remove the sperm. The nuclear diameter of these lectin-MNP conjugates is 14±0.4 nm, as determined by transmission electron microscopy (TEM). Annexin V-MNP was used to remove the apoptotic and dead spermatozoa. The results of this Annexin V-MNP conjugates TEM show that the nuclear diameter is about 7.1±0.2 nm. Freshly collected porcine semen was combined with lectin MNPs or Annexin V-MNP to produce 3-4x10^9 spermatozoa/80 mL per insemination dose. Gentle rotation was performed during the 10-15 min incubation period at 37°C. The sperm sample was exposed to a 12,000 gauss neodymium magnet for 10 min at room temperature after being exposed for 15 to 20 min to capture all unbound and sperm-bound MNP. Following Durfey et al. (2019), the unbound sperm were eluted into fresh tubes.

In a two-step process, the spermatozoa that were still alive and had their acrosomes intact were separated. In the first phase, 87.5 g of Annexin V-MNP was added to the enlarged semen sample, and the mixture was incubated at 37 degrees Celsius for 30 minutes to kill off any apoptotic or dead spermatozoa. There were between 1.6 and 2.0x10^9 spermatozoa in 40 mL of the concentrated semen sample. The second step involves combining the non-apoptotic, nano-selected spermatozoa with 87.5 gr of lectin-MNP and incubating the mixture for the optimal incubation time of 30 minutes to remove any spermatozoon that have undergone a change in their sperm acrosome (Durfey et al., 2019). To extract viable, apoptosis-free spermatozoa, one must use a new tube.

The synergistic and supplementary benefits of the two-step nanopurification method are evident. At each nanoselection stage, the fraction of total motile, forward, and fast spermatozoa is statistically significantly higher compared to the control sample, while the fraction of static spermatozoa is statistically significantly lower. MNP conjugates may be able to reduce the number of faulty spermatozoa since there are fewer of them. There is a significant relationship between the first phase of the

two-step nanopurification process and the resulting fast sperm motility, total sperm motility, curvilinear velocity (VCL), average path velocity (VAP), and straight-line velocity (VSL), The directional parameters improve to varying degrees after each elimination stage. The second stage significantly reduced the lateral head amplitude of the spermatozoa, while simultaneously increasing their beating frequency, straightness (STR), and linearity (LIN), in comparison to the control specimen and the specimens in which only the first step was conducted. Evidence like this highlights the need for a sequential removal technique to effectively filter out defective spermatozoon from sperm specimen (Durfey et al., 2019).

Sperm kinematic qualities are indicators of how well sperm will swim through the uterus and oviduct. Both male fertility and the rate of fertilization are positively correlated with these factors. However, the viability of the enriched samples was not improved compared to the controls after being enriched with better nano-selected sperm. To survive freezing conditions and be fertilized, the nano-selected sperm maintain a more stable plasma membrane, a greater mitochondrial membrane potential and a lower ROS level. These measures of sperm vitality do not vary from those of control sperm. Nano-selected semen samples did not increase the reproductive rate of gilts in field experiments. Concerns about potential danger or toxicity may be disregarded since there was no difference between the control and nano-selected groups in terms of litter size, weight, developmental pace, or offspring health. No significant associations between sperm treatment with MNP and offspring were found by Durfey et al., (2019).

5. Current studies with sperm nano purification

Metallic nanoparticles	*Species*	*Mechanism of action/results*	*References*
Fe_3O_4 (Iron Oxide) Nanoparticles	Buck	Sperm selection on Angora buck semen using nanoparticles at three distinct temperatures (37°C, 21°C, and 4°C). In comparison to the control group, total motility, LIN, VCL, VSL, and acrosome integrity parameters were all higher at 37oC. On the other hand, at 21oC, the control group had higher values for LIN, STR, VCL, VSL, VAP, wobble, and plasma membrane-acrosome integrity (PMAI). There were changes, although they were insignificant, between the group that was exposed to 4oC and the control group.	Alemdar and Tirpan, 2022
	Bull	Antibodies against ubiquitin-coated (Abs) IONPs for reducing oxidative damage to living spermatozoa obtained from unprocessed semen. In Groups II, III, and IV, IONPs-Abs complex was added in the following ratios: 1:1 (0.5 g/ml), 1:2 (1.0 g/ml), and 1:4 (2.0 g/ml), respectively. In order to reduce oxidative stress, IONPs conjugated with anti-ubiquitin Abs at a concentration of 2.0 g/ml may be an efficient way to remove damaged or dead spermatozoa from buffalo ejaculates.	Bisla et al., 2020
	Boar	Samples were classified into 4 groups based on their levels of motility: >90% (1), 80–90% (2), 70–80% (3), and <70% (4).Although there was a similar pattern in the sperm motility character measured by VCL, VSL, VAP, and LIN. The motility enhancement was more obvious in the group of sperm with less than 70% motility.	Chung and Son, 2016
	Buffalo	The efficacy of the swim-up approach and nano-purification in removing dead or damaged spermatozoa was compared in different concentrations of semen. The current study's findings showed that nano-purification utilizing anti-ubiquitin particles eliminated defective or dead spermatozoa at the lag stage considerably more successfully than swim up procedure.	Din et al., 2018

Boar	Studied magnetic nanoparticle conjugates to eliminate non-viable spermatozoa and evaluate their motility and vitality. According to research, it is possible to increase male fertility by successfully removing moribund (static) spermatozoa without harming their viability.	Durfey et al., 2017
Boar	According to research, moribund sperm, also known as static sperm, may be successfully eliminated without negatively impacting viability, resulting in an increase in male fertility. The findings demonstrate that magnetic nanoselection is beneficial for high-throughput targeted removal of damaged sperm as well as simple and rapid enrichment of sperm dosages with highly mobile, viable, and fertile sperm. Therefore, magnetic nanoselection, which involves removing abnormal sperm from seminal fluid, has the potential to improve male fertility. This is especially true during times of heat stress in the summer.	Durfey et al., 2019
Bull	Using Cell-SELEX, it was possible to identify sperm cells that had suffered heat damage by using single-stranded DNA aptamers with the ideal combination of affinity and specificity. First, aptamers that bind to the membrane of heat-damaged spermatozoa were isolated; these aptamers contain two conserved motifs with a total length of 6 nucleotides. Then, synthetic biotin-labeled aptamers with the conserved motif were used to find membrane-damaged cells and separate them from healthy cells by using superparamagnetic iron oxide nanoparticles (SPION) coated with avidin. By dramatically increasing the proportion of viable sperm, this method improved sperm quality without decreasing the rate of blastocyst separation. Both asexual and sex-selected sperm suspensions performed well when treated with this method.	Farini et al., 2016
Boar	Improved Reproductive Performance with Lectin-Functionalized Magnetic Iron Oxide Nanoparticles. Sperm motility was considerably enhanced by nanopurification. The viability of the piglets and the ability of sperm to fertilize them were not adversely affected by the magnetic nanoparticles utilized in this early investigation. Semen fertility may have positive improvements, with potential for application in gender selection.	Feugang et al., 2015

Bull	The goal of this study was to see how well a nanoparticle-based magnetic purification method improved the viability and fertilization ability of sperm samples in vitro and in vivo by getting rid of the 30% of the sample that was made up of bad spermatozoa. Therefore, this study describes the successful implementation of a new nanotechnology to improve artificial insemination in cattle through field trials. The offspring of males whose spermatozoa were nanopurified to remove all traces of PNA and ubiquitin looked perfectly healthy. In the first year of this artificial intelligence field study, heifers conceived with both the non-purified control semen and the artificial intelligence showed normal fertility.	Odhiambo et al., 2014
Camel	This study set out to determine whether a magnetic nanoparticle-based sperm purification approach was adequate for removing damaged and apoptotic camel spermatozoa from dosages of cryopreserved semen liquefied using a protease. These results suggest that protease-based liquefaction of sperm before cryopreservation, followed by magnetic nanopurification after thawing, is promising to decrease the percentage of damaged and dead sperm and increase the fertilization efficiency of camelid sperm.	Rateb, 2021
Buffalo	A negative fertility marker is employed in this test to exclude any damaged or dead sperm that may have been present. Assays using hypoosmotic (HOS) and fluorescein-conjugated Pisum sativum agglutinin (FITC-PSA) demonstrate the effects of this substance. The success of this treatment is supported by evidence such as Ca2+-regulatory mechanisms, depolarization of the sperm membrane, a reduction in the quantity of free radicals, and an in vitro fertility test. An antibody concentration of 1.0 g/ml that was biotinylated with IONPs proved to be the most effective method for increasing the number of viable zona-bound sperm that were present in the ejaculate.	Rautela et al., 2022

Conclusion

Nano-based methods for mammalian sperm purification using MNPs coated with biomarkers are promising and offer new opportunities for developing easy, effective, and noninvasive methods, as stated by Odhiambo et al. (2014), and Yousef et al. (2020). Research by Durfey et al. (2019) suggests that MNPs may be utilized to selectively filter out damaged sperm from a semen sample in a high-throughput setting. The method is easily incorporated into semen cryopreservation protocols, is low-cost, does not call for extensive manipulation of semen, employs commercially available components, is highly sensitive and specific, requires little to no labor, does not impede fertilization or the establishment of a healthy pregnancy, and does not increase the risk of infections in the reproductive tract. MNP's use in spermatology has grown during the last several years. There have been several scholarly articles written on the subject, however not all of them can be considered scientifically sound. Manufacturers of MNPs state that various strains of their product have several potential uses. However, these initiatives frequently rely on research that has not been reviewed by experts in the field. Future research is required to separate fact from fiction.

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