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A review on applications and toxicities of metallic nanoparticles in mammalian semen biology

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Abstract

The advent of nanotechnology since the 1950s, when the well-known physicist Richard P. Feynman talked in his famous talk about "There's plenty of room at the bottom", has led to incredible contribution of nanotechnology in the fields of medical and veterinary therapeutics, diagnostics and other applications. Semen biology dealing with the study of spermatozoa and its related physiological and pathological aspects has not remained unscathed from the facets of nanotechnology. With each passing day investigators are revealing newer aspects of the nanoparticles, such as an antioxidants to relieve oxidative stress during semen cryopreservation, for the depletion of moribund spermatozoa from semen, gender selection of spermatozoa, bioimaging of gametes, sperm mediated gene transfer, as well as for male fertility evaluation. As, the uses of various magnetic nanoparticles in the industry have gained acceleration, the evaluation of their effects, either beneficial or otherwise on the mammalian spermatozoa becomes obligatory. Many toxicological studies have also been conducted in respect to the harmful effects of different metallic nanoparticles related to their applicability, and industry borne adverse effects on the male germ cells in human beings and the animals. This review has been designed to focus on the beneficial as well as toxicological effects of various metallic nanoparticles on the mammalian spermatozoa and the future prospects related to their applicability in the semen biology.

KEYWORDS
magnetic, nanoparticles, nanotoxicity, oxidative stress, spermatozoa

1 | INTRODUCTION

Any object with non-significant variation in longest and shortest axes and all dimensions falling within the size range of 1–100 nm are termed as nanoparticles (NPs) (ISO Technical Specification 80004). A well-known physicist Richard P. Feynman discussed the concept of particles of size ranging in nanoscale at first in 1959 his world famous talk to the undergraduate students "There's plenty of room at the bottom" (Feynman, 1960). Norio Taniguchi in 1974 first of all used the term "Nanotechnology" but it was not popularised. Later, in 1986, an American engineer Kim Eric Drexler, who was inspired by concepts of Feynman, wrote a book "Engines of Creations: An Era of Nanotechnology" and used the term "Nanotechnology". In 1986, Drexler co-founded "The Foresight Institute" which led to an era of start of nanotechnology with increasing knowledge about various concepts and applications of nanotechnology. Gerd Binning and Heinrich Rohrer in 1981 after the invention of scanning tunnelling microscope (Binning & Rohrer, 1986) as well as Harry Kroto, Richard Smalley and Robert Curl led discovery of fullerenes in 1985 (Kroto et al., 1985) ensured popularisation of the nanotechnology. Today, nanotechnology is one of the major spheres of science with multiple implications at industrial, biomedical, as well as allied fields. The utility of NPs can be found in successful diagnosis and treatment of certain forms of cancer or drug delivery tool as well as for delivery of genetic

constructs their destinations without any morphological or functional alterations.

NPs are classified into many classes on the basis of their properties, size and shape. NPs physical and chemical properties, their large surface area and nano size makes their use in many commercial and domestic applications (Khan et al., 2019). The bio-imaging applications of NPs are also possible due to their various colours characteristics and properties with variation in size and shape (Dreaden et al., 2012). NPs can be synthesised from different precursors and thereby classifying them into organic and inorganic types. NPs are conjugated with different ligands via ionic, hydrophobic, dative binding which is called as bio-conjugation and it forms a basis of their many bio-medical applications. Bio-conjugation of NPs with antibodies (Abs), surface biomarkers & drugs is used in cancer diagnosis, drug delivery and other diagnostic & therapeutic applications (Bisla, Rautela, et al., 2020). NPs have different applications in the field of medical and veterinary sciences. The emerging trends of using the NPs in the semen biology for various applications like depletion of dead/moribund and spermatozoa with various surface damages/alterations from the ejaculates (Nano-purification of semen) (Odhiambo et al., 2014; Feugang et al., 2015; Durfey, Swistek, et al., 2017; Bisla, Rautela, et al., 2020; Bisla, Rautela, et al., 2021), bio-imaging of gametes (Barchanski et al., 2015; Vasquez et al., 2016), sex-sorting of semen (Domínguez et al., 2018; Moradi et al., 2021) and evaluation of male infertility using different biomarkers (Vidya & Saji, 2018) have opened the tremendous opportunities in the reproductive biology.

NPs after bio-conjugation with various negative fertility biomarkers like Abs (e.g., Anti-ubiquitin Abs), various biomarkers for selective acrosome damage like glycans binding lectins like *Pisum satium* agglutinin (PSA), *Peanut* agglutinin (PNA) and *Lens cullinaris* have been used for nano-purification of semen and other procedures (Bisla, Rautela, et al., 2020; Bisla, Rautela, et al., 2021; Odhiambo et al., 2014). Several types of NPs have been used as an antioxidant, as well as for their antimicrobial properties thus paving the way for the spermatozoa quality and male fertility assessment and improvement. Cerium oxide NPs (CeO_2 NPs) had been shown to improve the plasma membrane- and nuclear-integrity of the ram spermatozoa stored for short-term, and long-term periods, particularly by their ability to decrease the oxidative damage by scavenging the reactive free radicals (Falchi et al., 2018). Others have shown improvement in the post-thaw quality in the rooster semen by addition of nano selenium and vitamin E (Safa et al., 2016).

Very limited studies in past have been employed to assess the effects of nano forms of metallic oxides and other metallic NPs (MNPs) on the spermatozoa either after directly adding into the semen or indirectly via either exposure to males or feeding. The studies on the beneficial as well as harmful effects of different MNPs is imperative because of their increasing applicability in the routine life of humans as well as their association with the livestock. Such studies are important, for the effect of MNPs on the spermatozoa could lead to the transmission of some genotypic and phenotypic changes into the next generation of animals as well as in humans. Therefore, the current review has been designed to comprehensively focus on the utilities of various MNPs into the semen biology for their direct applications and the toxicological effects of them on the mature male germ cells, that is, spermatozoa. The applications of important MNPs are summarised in Table 1.

2 | NANOPARTICLES AS ANTIOXIDANTS

The technique of semen cryopreservation and storage at −196°C in the liquid nitrogen is a routine process in the field of livestock semen biology (Alvarenga et al., 2016; Bisla, Rautela, et al., 2020; Bisla, Rautela, et al., 2021; Cheema et al., 2021; Kumar, Prasad, et al., 2018; Kumar, Prasad, et al., 2018; Lone et al., 2018; Mavi et al., 2020) and human (Santo et al., 2012). This process of freezing the spermatozoa causes several changes due to solution effect, formation of intracellular and extra cellular ice crystals, and oxidative damage arising from the reactive oxygen and nitrogen free radical, collectively known as cryoinjury to the spermatozoa. Oxidative damage is the crucial phenomenon which is required for physiological functions but the severity of that is one of the most important factors leading to poor post-thaw semen quality after sperm cryopreservation (Bisla, Rautela, et al., 2021; Bucak et al., 2010). Oxidative stress arises due to an imbalance between the production of reactive oxygen species (ROS) and a biological system's ability to detoxify it, that is; when concentration of the oxidants exceeds that of physiological tolerable limits. It is often associated with an increased rate of cellular damage induced by oxygen (O_2) and oxygen derived oxidants (Sikka, 1996; Bansal and Bilaspuri, 2011). In agreement, extensive reviews (Aitken & Baker, 2020; Alahmar, 2019; Bisht et al., 2017; Gharagozloo & Aitken, 2011; Kumar et al., 2019; Mannucci et al., 2021; Tremellen, 2008) show the various causes of male infertility associated with the increased oxidative stress in humans and animals.

The major detrimental changes due to oxidative stress mainly occur at the plasma membrane and nucleus of the spermatozoa (Bisla, Rautela, et al., 2020; Bisla, Rautela, et al., 2021). These changes in the seminal attributes arising due to oxidative stress could be assessed by various staining procedures (Rautela et al., 2020) and computer assisted semen analysis (Singh et al., 2021). Many attempts have been done in past for the reduction of oxidative damage in the semen, namely, neutralising ROS through enzymatic, non-enzymatic, plant-based antioxidants or reductants (reviewed by Kumar et al., 2019; Silvestre et al., 2021; Kumar et al., 2021, 2022); or by minimising the sources like the semen radiation exposure, leucocytes and dead and defective spermatozoa to reduce the level of free radical generation (Arzondo et al., 2012; Bisla, Ramamoorthy, et al., 2020; Bisla, Rautela, et al., 2020; Bisla, Rautela, et al., 2021; Durfey et al., 2019; Durfey, Burnett, et al., 2017; Durfey, Swistek, et al., 2017; Feugang, 2017; Feugang et al., 2015; Odhiambo et al., 2014); or by using oxygen scavenger like *E. coli* derived enzyme oxyrase (Dalal et al., 2020; Darr et al., 2016; Dong et al., 2010; London et al., 2017; Ngou et al., 2020; Shore, 2019); or by using nitrogen gassing to reduce the oxygen tension for partially deoxygenating the extender, a source of generation of free

TABLE 1 Applications of various metallic nanoparticles (MNPs) in semen biology.

Sl. No.	Applications	Metallic nanoparticles	Species	Mechanism of action/results	References
1.	Antioxidants	• Zinc oxide NPs (Oral in diet @0–150 mg/Kg)	• Ram	• Improved semen quality, seminal plasma anti-oxidase activity and expression of copper-zinc superoxide dismutase (SOD) with optimal concentration 50–100 mg/Kg diet.	• Zhang et al., 2015
		• Zinc oxide NPs (in semen @0.001 M)	• Bull	• Decreased malondialdehyde (MDA) production and improved mitochondrial activity.	• Yazdanshenas et al., 2016
		• Zinc oxide NPs (in semen @10^{-6} M)	• Bull	• No adverse effect on semen quality with increase blastocyst rate without affecting embryo development and pregnancy rates.	• Jahanbin et al., 2015, 2021
		• Zinc oxide NPs (in semen @ 100 μg/ml)	• Human	• Diminished freeze-thaw induced damage to the spermatozoa with formation of protective layer to prevent lipid peroxidation and preserving functionality.	• Issac et al., 2017
		• Zinc oxide NPs (intraperitoneal @ 10 mg/Kg)	• Rats	• Ameliorated the oxidative stress in diabetic rats with increased activity and expression of enzymatic antioxidants and reduced MDA.	• Afifi et al., 2015
		• Cerium oxide NPs (in semen @ 220 μg/ml)	• Ram	• Protective effect on the plasma membrane integrity with ability to store and release oxygen, deliberating their scavenger activity against oxidative damage.	• Falchi et al., 2016, 2018
		• Cerium oxide NPs (intraperitoneal@ 30 mg/Kg)	• Rats	• Reduced malathion induced oxidative damage with improved sperm counts, motility and viability, reduced lipid peroxidation and improved total antioxidant capacity.	• Moridi et al., 2018
		• Cerium oxide NPs (in semen @0.1 μg/ml)	• Human	• Improved progressive and total motility, viability, membrane functionality, DNA integrity and protamination with reduced lipid peroxidation.	• Hosseinmardi et al., 2022
2.	Nano-purification of semen	• Iron oxide nanoparticles	• Beef cattle	• Fe_3O_4 NPs coated with the plant lectins or anti-ubiquitin antibodies removed dead/damaged spermatozoa from frozen-thawed bull semen and showed more conception rates even in the half dose.	• Odhiambo et al., 2014
			• Buffalo	• Improved sperm plasma membrane integrity, motility, viability, acrosome integrity and reduction in DNA damage, oxidative stress with improved antioxidant characteristics and in vitro fertilisation rate.	• Bisla, Rautela, et al., 2020; Bisla, Rautela, et al., 2021
			• Swine	• Removal of dead, moribund and aggregated spermatozoa showed increase in the seminal attributes and fertility in boar.	• Feugang et al., 2015

(Continues)

TABLE 1 (Continued)

Sl. No.	Applications	Metallic nanoparticles	Species	Mechanism of action/results	References
			• Stallion	• Good structural and functional attributes in the purified semen without any impairments of fertility, and no adverse effect on the development and health status of future progeny.	• Durfey, Swistek, et al., 2017; Durfey, Burnett, et al., 2017; Durfey et al., 2019
				• Magnetic nanoparticles coated with the PNA lectins and gender X specific factors in the stallion semen had no negative effect on the sperm quality parameters with less DNA damage and more pregnancy rate.	• Morris et al., 2018
3.	Sex-sorting of spermatozoa	• Iron oxide nanoparticles	• Ram	• Polymerase chain reaction (PCR) confirmed that the treatment of semen with 50 μg/ml magnetic NPs had the highest effects on the recovery of X sperm with no resultant toxic effects on spermatozoa, sperm viability and DNA integrity.	• Moradi et al., 2021, 2022
			• Donkey	• A success rate of around 90% for separation of the X-containing spermatozoa without any adverse effect on the spermatozoa.	• Dominguez et al., 2018
			• Stallion	• Pregnancy outcomes using magnetic sex-sorted stallion semen was nearly 80%, with 95% of conceptuses confirmed female by ultrasonography.	• Ramírez Castex et al., 2017
4.	Bio-imaging of spermatozoa	Iron oxide nanoparticles	• Boar	• IONPs and a natural enzyme luciferase in the form of nanocomposite has potential for a lower toxicity than quantum dots, as well as the ability to magnetically manipulate cells and track them in vivo.	• Vasquez et al., 2016
5.	Fertility evaluation	Gold nanoparticles	• Human	• GNPs based biosensor termed as fructose blue for detection of fructose in human semen, a marker of seminal vesicle function and fertility.	• Vidya et al., 2014
			• Human	• Assessment of level of protamine as a probable marker of fertility in human males using heparin GNPs.	• Vidya & Saji, 2018
			• Cattle	• ReproTest a lateral flow device based on the colloidal GNPs design to evaluate the bull fertility based on fertility associated antigen HBP-30 (Heparin binding protein-30).	• Sutovsky and Kennedey, 2013

radicals in the microenvironment of spermatozoa (Amin et al., 2018; Balamurugan et al., 2018, 2020; Bhutia et al., 2021; Katiyar et al., 2020; Kumar, Prasad, et al., 2018; Kumar, Prasad, et al., 2018; Mustapha et al., 2021; Pande et al., 2015).

The metallic oxides of NPs have been shown to possess antioxidant properties which could be more effective in prevention of oxidative stress induced damages to the spermatozoa, primarily owing to their nanosize with more surface area and thus greater efficacies in

the reduction of oxidative stress. In concurrence, dietary supplementation of zinc oxide NPs (ZnO NPs) improved the semen quality, seminal plasma anti-oxidase activity and expression of copper-zinc superoxide dismutase (SOD) enzyme of ram epididymal spermatozoa (Zhang et al., 2015). Similar results were also observed when the bull semen was supplemented with the ZnO NPs, resulting in the decreased malondialdehyde (MDA) production and improved mitochondrial activity (Yazdanshenas et al., 2016). Moreover, Zinc-nano complex improved the spermatozoa plasma membrane functionality in a dose dependent manner without any harmful effects (Jahanbin et al., 2015). In a recent study, Jahanbin et al. (2021) depicted that in vitro supplementation of ZnO NPs in bovine semen at concentration of 10^{-6} M increased blastocyst rate without any adverse effect on semen quality, embryo development and pregnancy rates.

Isaac et al. (2017) supplemented the normozoospermic semen of men with ZnO NPs in the cryopreservation medium and observed diminished freeze-thaw induced damage to the spermatozoa without any adverse effects. On the plus side, ZnO NPs neither penetrated into the spermatozoa and nor it altered the functionality of the spermatozoa. The protective effect of ZnO NPs on the chromatin of the spermatozoa was assumed to be due to formation of protective layer on the spermatozoa and also preventing the peroxidative damage to the lipid bilayer in the plasma membrane (Isaac et al., 2017). ZnO NPs also ameliorated the oxidative stress in diabetic rats either alone or in combination with insulin with increase in the sperm count and motility (Afifi et al., 2015). The activity and mRNA expression of different antioxidant enzymes like SOD, catalase (CAT), glutathione peroxidase, glutathione reductase and Glutathion-S-Transferase were significantly increased with reduction in MDA in the testis of diabetic rats.

CeO_2 NPs exposure to Ram spermatozoa found to have protective effect on the plasma membrane integrity with ability to store and release oxygen, deliberating their scavenger activity against oxidative damage, however, the ROS levels were not altered (Falchi et al., 2016, 2018). Moridi et al. (2018) had found that CeO_2 NPs supplementation in the wistar rats had restorative effects on the adversely affected testicular parenchyma due to malathion induced oxidative damage with improved sperm counts, motility and viability, reduced lipid peroxidation (LPO) and improved total antioxidant capacity (TAC) indicative of antioxidant property of CeO_2 NPs. In another study in human, the results showed that supplementation of CeO_2 NPs at the concentration of 0.1 μg/ml in semen significantly improved the progressive and total motility, viability, membrane functionality, DNA integrity and protamination compared with the frozen control group with reduction in LPO along with no effect on sperm normal morphology and mitochondria activity (Hosseinmardi et al., 2022).

3 | NANO-PURIFICATION OF THE SEMEN

The available literature on the semen statistics shows that about 16%–33% of the semen ejaculates were found non-freezable in cattle and buffalo bull due to poor quality and were discarded at the fresh stage (Gopinathan et al., 2016; Manda et al., 2016; Singh et al., 2013; Tiwari et al., 2012, 2015; Zafar et al., 1988) and further at post-thaw the discard rate was found to be around 25%–30% (Bisla, Ramamoorthy, et al., 2020; Bisla, Rautela, et al., 2020). This causes the shortage of the quality germplasm for the breeding propagation and limits their economic exploitation. One of the important causes in the rejection of ejaculates could be greater oxidative stress arising from the accompanied dead and damaged spermatozoa (25%–30%) releasing ROS (Bisla, Ramamoorthy, et al., 2020; Bisla, Rautela, et al., 2020; Bisla, Rautela, et al., 2021; Kumar, Prasad, et al., 2018; Maurya & Tuli, 2003; Rautela et al., 2020). Since, the harmful effect of the dead and damaged spermatozoa on the contemporary live spermatozoa has been proved categorically, for obvious reasons improvement in viability, motility and functionality of the contemporary live spermatozoa can be achieved by depletion of such unwanted cells from the fresh ejaculates.

Nano-purification of semen is an epigenetic-based non-invasive approach selection of good quality spermatozoa (Štiavnická et al., 2017). Nano-purification of semen is done for separation of moribund cells and spermatozoa with some surface alterations from undamaged, healthy spermatozoa. The most successful method till date for nano-purification of semen is magnetic-activated cell sorting (MACS), which separates dead and live spermatozoa (Degheidy et al., 2015; Romany et al., 2017). The nano-purification of the semen is a novel technique in this regard with potential applicability at the large commercial scales due to its property of less time consuming as well as better efficiency. Ferric oxide NPs (Fe_3O_4 NPs/IONPs) are known for their magnetic property, biocompatibility and bio-functionalisation property (Huang & Tang, 2004) which makes their basis for the separation of dead spermatozoa with the damaged membranes. Iron oxide NPs (IONPs) could be used for nano-purification by either binding them to the various plant derived lectins like PNA/PSA which have ability to bind to spermatozoa membrane glycans, or anti-ubiquitin Abs (Abs against ubiquitin which is a negative fertility biomarker) (Bisla, Rautela, et al., 2020; Bisla, Rautela, et al., 2021). Fe_3O_4 NPs coated with the plant lectins or anti-ubiquitin antibodies were used for the nano-purification of frozen-thawed bull semen and showed more conception rates even in the half dose than recommended (Odhiambo et al., 2014).

Fe_3O_4 NPs coated with the PNA/PSA lectins for the nano-purification of the boar semen also showed promising results in the separation of motile and good quality spermatozoa and improved conception rate as well (Feugang et al., 2015). Farini et al. (2016) used the avidin coated superparamagnetic Fe_3O_4 NPs to bind with the synthetic DNA (Deoxyribo nucleic acid) aptamers associated with the spermatozoa with damaged membranes and observed the improved semen quality with more viable cells without affecting the in vitro embryo development. The nano-selection process showed spermatozoa of good structural and functional attributes in the purified semen without any impairments of fertility, as well as no adverse effect on the development and health status of future progeny (Durfey, Burnett, et al., 2017; Durfey, Swistek, et al., 2017).

Morris et al. (2018) used magnetic nanoparticles coated with the PNA lectins and gender X specific factors in the stallion semen and

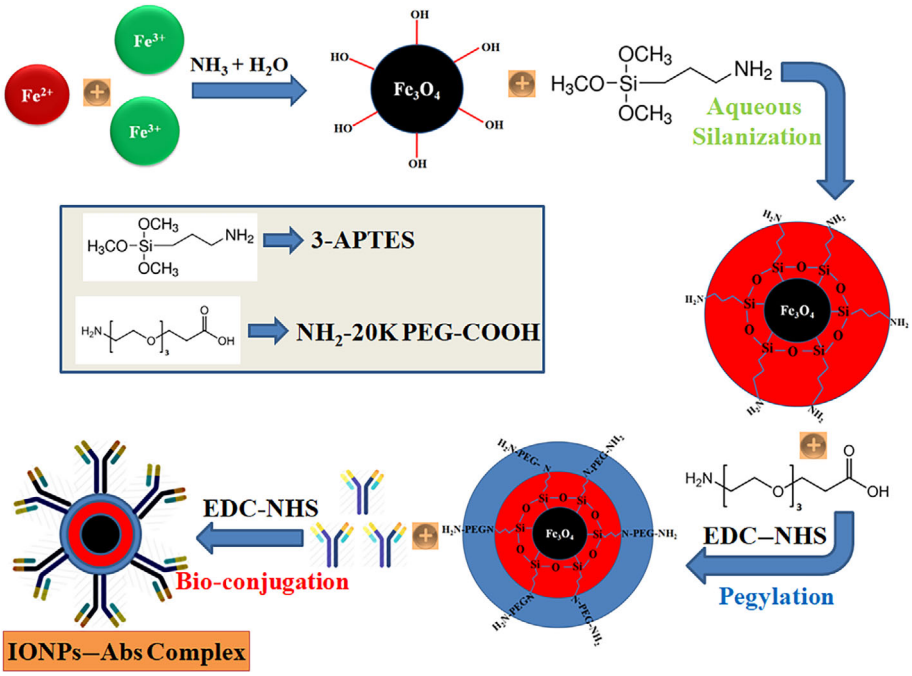

FIGURE 1 Schematic representation of the synthesis, serial functionalisation (silanisation followed by pegylation) and bioconjugation of IONPs with monoclonal antiubiquitin antibodies. 3-APTES, 3-aminopropyl (triethoxy) silane; Abs, antibodies; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; IONPs, iron oxide nanoparticles; NHS, N-hydroxysuccinimide; PEG, polyethylene glycol. (Taken from Bisla, Rautela, et al., 2021)

observed that nanoparticles treatments does not have any negative effect on the spermatozoa quality parameters. The NPs treated spermatozoa had less DNA damage due to reduced oxidative stress and the 80% pregnancy rate (4/5). Zhang et al. (2018) used magnetic beads coated with the anti-ubiquitin antibodies in the bull semen and found that degree of ubiquitination was found to be greater association with structural surface damages as well as impaired nuclear integrity of the bull spermatozoa. The use of modified MACS techniques with NPs conjugated with anti-ubiquitin antibody efficiently removed the spermatozoa with morphological damages from bull semen by migrating ubiquitinated spermatozoa attached with NPs towards the bottom of the magnetic field.

Nano-purification attempted in the form of preliminary studies in buffalo semen also had promising results with improved spermatozoa plasma membrane integrity, motility, viability, acrosome integrity and reduction in DNA damage, oxidative stress with improved antioxidant characteristics and in vitro fertilisation rate (Bisla, 2019; Bisla, Rautela, et al., 2020; Bisla, Rautela, et al., 2021; Rautela, 2020). Therefore, in several studies, it has been established that application of magnetic NPs for the removal of dead, moribund and aggregated spermatozoa has been shown to increase the seminal attributes and fertility in boar (Chung & Son, 2016; Durfey et al., 2019; Durfey, Burnett, et al., 2017; Durfey, Swistek, et al., 2017; Feugang et al., 2015), bull (Odhiambo et al., 2014; Zhang et al., 2018), buffalo (Bisla, 2019; Bisla, Rautela, et al., 2020; Bisla, Rautela, et al., 2021; Rautela, 2020) and the stallion (Morris et al., 2018).

Nano-purification of the semen is a newly emerging and promising technique with many advantages over the contemporary traditional techniques used for the separation of moribund and defective spermatozoa from the fresh as well as frozen-thawed semen ejaculates so that their adverse effect on the contemporary lives spermatozoa can be minimised. The traditional techniques, such as Sephadex filtration, sperm swim-up or down, or gradient separation used for the similar objectives have various limitations like labour, cost, varying low recovery of spermatozoa (10% to 63%) and are time consuming (>60 min) which limits their use in the large and commercial applications (Bisla, Ramamoorthy, et al., 2020; Feugang et al., 2015). The process of bio-conjugation of IONPs with anti-ubiquitin antibodies for nano-purification and outlay of process of nano-purification are depicted in Figures 1 and 2.

4 | NANO-PARTICLE ENABLED SEMEN SEXING

Semen sexing is an assisted reproductive technique with an increasing demand in the livestock industry nowadays. The semen sexing for the production of female calves is of prime importance in country like India where the cattle slaughter is banned and the number of surplus stray animals creates havoc on daily basis. Also, the prior sex-sorting of spermatozoa to get desired calf sex is requisite in frame of increasing livestock population causing global warming due to production of methane gas after rumination. Semen sexing requires specific target and cell penetration which is currently an unsatisfactory attempt due to absence of good sex-chromosome linked biomarkers (Campos et al., 2011; O'Brien & Robeck, 2006; Rath et al., 2015; Vazquez et al., 2009).

Currently, only flow cytometric based sex-selection of spermatozoa is shown to be the best suited technique used practically in the livestock sector. This technique involves exposure of cells to the UV (ultraviolet) light spectrum, combination of fluorescent dye bisbenzimide (Hoechst 33342) for cell labelling (Cran et al., 1993),

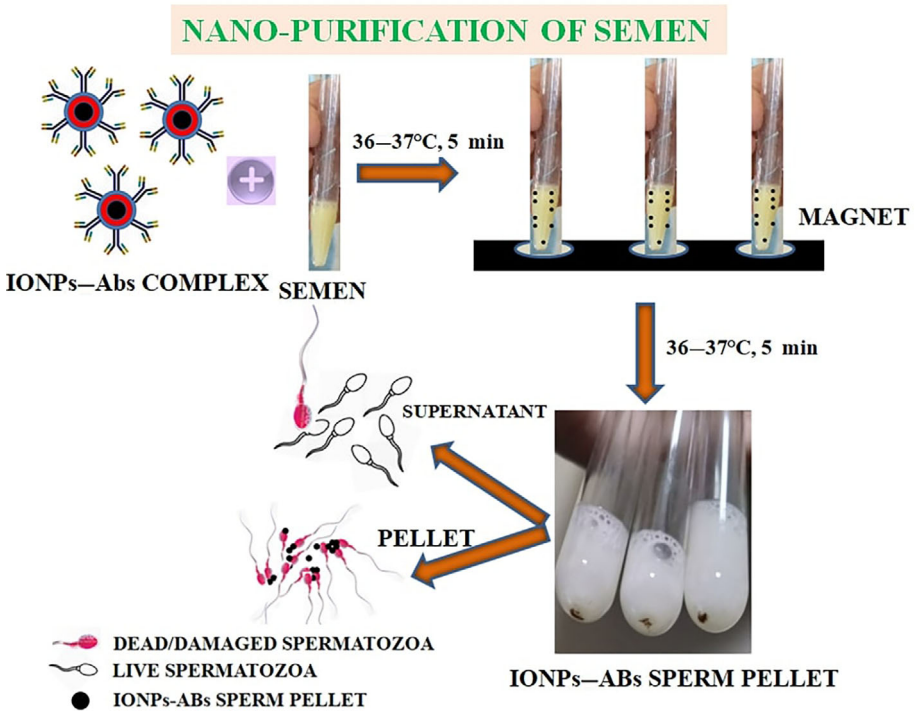

FIGURE 2 Pictorial representation of nano-purification process. (Taken from Bisla, Rautela, et al., 2021)

therefore, adversely affecting both sperm motility, morphology, membrane integrity as well as nuclear integrity (Balao da Silva et al., 2016; Domingues et al., 2017; Quan et al., 2015). Also, the number of dead or damaged sperm cells is increased by almost 20% due to the mechanical stresses induced on sperm cells during sorting and centrifugation (Carvalho et al., 2010). Over the last many years, innumerable efforts have been made in developing the methods for prior sex-sorting of spermatozoa to obtain desired sex of offspring especially in farm animals, for getting the reduced number of male claves in country like India where cow slaughter is banned so that number of stray cattle is reduced, for commercial purposes or for human medical reasons (e.g., genetic sex-linked disorders), for reducing the overall unproductive livestock due to increasing threats of global warming due to large methane gas production by ruminants. But, the international animal breeding industry is presently having a challenge with an unmet market demand of an inexpensive and efficient pre sperm sex-selection technology. Thereby, the development of alternative laboratory techniques for sperm sex-sorting has become a need of the hour for scientists in this field.

The sex sorting of the semen with MNPs have advantage over the technique of flow cytometry as it does not exposes the spermatozoa to the high speed, fluorescent dyes, UV light and other factors which can deplete the semen quality (Gaur et al., 2020). Whereas, with short duration of time required and ease to perform the procedure using MNPs for spermatozoa sorting makes better promise (Domínguez et al., 2018). In the initial efforts, a Y-chromosome-specific sequence containing oligonucleotide probe was conjugated to gold NPs (GNPs) for selective targeting of Y-chromosome bearing spermatozoa in bull (Gamrad et al., 2017). This methodology resulted in the decrease in the sperm motion attributes but no effect was observed on the morphology and viability without any penetration of GNPs into the spermatozoa. Morris et al. (2018) used gender X specific factors coated on the magnetic beads and achieved 80% (4/5) pregnancy rate in the mares.

Magnetic NPs were used for the sex sorting of the semen of donkey and the efficiency of the procedure was found much promising with short duration of time required and easiness to perform the procedure. The magnetic NPs bound to the spermatozoa containing the Y-chromosome were separated from the X bearing population (Domínguez et al., 2018). This technique was based on the fact that the zeta electrical potential exists between the sperm plasmalemma and the surrounding environment (Chan et al., 2006; Kheirollahi-Kouhestani et al., 2009) and thereby, this property was used to separate the X and Y spermatozoa according to their differential capability to align along an electrophoretic field. Thus, most Y spermatozoa have a zeta potential of −16 mV whereas the X spermatozoa have zeta potential of −20 mV (Ishijima et al., 1991). This methodology had a success rate of around 90% for separation of the X-containing spermatozoa without any adverse effect on the spermatozoa (Domínguez et al., 2018). The preliminary experiments with MNPs carried out in mares showed that the pregnancy outcomes when using magnetic sex-sorted stallion semen was nearly 80%, with 95% of conceptuses were confirmed female by ultrasonography (Ramírez Castex et al., 2017).

Moradi et al. (2021) in a recent study conducted in rams used magnetic NPs for the pre-conceptual separation of X and Y containing spermatozoa with varying concentrations (0–200 μg/ml) of NPs as well as to assess the nanotoxicity using methyl thiazole tetrazolium assay. The polymerase chain reaction (PCR) was used to conclude that the treatment of semen with 50 μg/ml magnetic NPs had the highest effects on the recovery of X sperm with no resultant toxic effects on

spermatozoa, sperm viability and DNA integrity. Only high concentration (200 μg/ml) of magnetic NPs resulted in the reduced DNA integrity of spermatozoa. In another study, Moradi et al. (2022) depicted the detailed effect of magnetic NPs on the sperm characteristics and inferred that it did not significantly affect the semen parameters such as viability, membrane functionality, abnormality, as well as LPO levels and DNA integrity in comparison with the control group.

5 | BIO-IMAGING OF SPERMATOZOA

The majority of bio-imaging technologies related to spermatozoa have been done with use of quantam dots NPs but their applicability is questionable due to cadmium content which is toxic for the spermatozoa (Gao et al., 2004). An alternative non-invasive bio-imaging system can have possibility of naturally occurring luciferase enzymes which have inherent light emission characteristics and could be suitable for bioluminescence imaging in whole animal and cellular systems. Luciferase obtained from *Renilla reniformis* (So et al., 2006) and *Photinus pyralis* (Alam et al., 2012) were coupled with quantam dots nanoparticles for dual imaging purposes. But, the content of cadmium in the qunatam dots predisposed the limitation of their applicability in the semen biology due to the toxic effects of cadmium on male germ cells.

Therefore, the advent of magnetic NPs for bio-imaging applications could be promising. In one study, Vasquez et al. (2016) used naturally found luciferase enzyme obtained from *Photinus pyralis* and conjugated it with magnetite NPs. It was concluded that Food and drug administration approved IONPs and a natural enzyme luciferase in the form of nanocomposite has potential for a lower toxicity than quantum dots, as well as the ability to magnetically manipulate cells and track them in vivo and depicted the promising applicability of these for imaging and detection of spermatozoa.

In another study, Barchanski et al. (2015) demonstrated that GNPs conjugated with cell-penetrating markers are suitable candidates for in vitro and in vivo imaging of bull spermatozoa. Although, only few studies have been conducted to assess the bio-imaging properties of MNPs so, more emphasis is required for assessment of their characteristics for live tracking and imaging of spermatozoa in the female reproductive tract.

6 | MALE FERTILITY/INFERTILITY EVALUATION

The MNPs also have promising roles in development of tests for evaluation of fertility/infertility in human as well as male animals which otherwise, is very tedious to done. The ReproTest (Midland Bioproducts Corporation, Boone, IA) is a test based on lateral flow device which is similar to over-the counter human pregnancy tests (i.e., a dipstick fertility test), which is based on the colloidal GNPs design, had been developed and marketed to evaluate the bull fertility (Sutovsky & Kennedy, 2013). Heparin-binding proteins (HBPs) secreted by the accessory sex glands formed the basis of this test which are found in bull spermatozoa as well as seminal plasma. HBP-30 is one of the most abundant proteins of HBPs in semen of bulls and found to be associated with high fertility (Bellin et al., 1994, 1996, 1998; Sprott et al., 2000) and commonly known as fertility-associated antigen (FAA).

The bovine seminal plasma proteins (BSPs) represent the major portion of bovine seminal plasma with three major heparin binding acidic proteins secreted by seminal vesicles which are BSP-A1/A2 (also known as PDC-109), BSP-A3 and BSP-30-kDa amongst which PDC-109 was found to mainly associated with cholesterol efflux from sperm membrane, binding with oviductal membrane as well as a role in induction of sperm capacitation and acrosome reaction (reviewed by Srivastava et al., 2013). In another studies, Pande et al. (2018; 2019) concluded that presence of FAA on the sperm membrane was related to greater protection against oxidative stress as well as better freezability ensuing improved acrosome intact live-sperm cells with functional mitochondria which could have a direct implication on the fertility. Therefore, these types of fertility markers could be used in prior selection of male animals before their induction in the breeding programmes.

In a study for detection of human infertility with naked eyes, a GNPs based biosensor was synthesised by conjugating GNPs with 3-aminophenyl boronic acid and L-glutamic acid-(2,2,2)-trichloroethyl ester which was termed as fructose blue. This test was based on the principle of the detection of fructose in human semen, a marker of seminal vesicle function as well as the plasmon resonance properties of GNPs (Vidya et al., 2014). Protamine is the most abundant nuclear protein in the human sperm nucleus with rich basic arginine residues (Balhorn, 2007) with very important role in the protection as well as in affecting the morphology of sperm and hence male infertility (Oliva & Dixon, 1991). Therefore, the assessment of level of protamine has been viewed as a probable marker of fertility in human males. One assay has recently been developed consisting of heparin GNPs as a biomarker for human fertility based on the protamine (antidote of heparin), the most abundant nuclear protein in the sperm. Similar way, the surface plasmon resonance properties of GNPs was basis of this assay. The electrostatic interactions between polycationic protamine and polyanionic heparin depicted a colour change that can be detected by naked eye (Vidya & Saji, 2018). There is a much need for the development of similar other "lab-free" tests can be used in the future as animal-side tests for prior selection of the animals based on the genetic merits for breeding purposes.

7 | TOXICITIES OF DIFFERENT METALLIC NPS TO SPERMATOZOA

The increasing applicability of MNPs in the routine life as well as directly in the semen biology warrants their toxicological studies. The harmful effects arising due to any MNPs could lead to the transgenerational effects which could be dangerous for future generations of animals as well as human beings. Several toxicity trials have been made to study the adverse effects of NPs on the spermatozoa of

various livestock species and human. The toxicities of various NPs on the reproductive system in the animal models have been reviewed by Brohi et al. (2017). The first reports on studies of toxicities of MNPs directly into the semen started in 2009 where Wiwanitkit and co-workers tested the effect of GNPs supplementation on human spermatozoa. However, the direct in vitro or in vivo effects of supplementation of various NPs to the spermatozoa have not been reviewed yet. The toxicities of important MNPs are summarised in Table 2.

7.1 | Gold NPs (GNPs)

The main purpose of spermatozoa is to safely deliver male genome to the oocyte after passing through the potentially harsh environment. The nucleus of spermatozoa is consisting of chromatin material which is responsible for the transmission of paternal information in the future developing zygote. Therefore, the study of effects of different NPs on the sperm chromatin material is very essential. GNPs were the first NPs to be studied in relation to negative effects on the DNA of the spermatozoa. Initially, Wiwanitkit et al. (2009) observed that motility of human spermatozoa was affected by presence of GNPs, since these can penetrate spermatozoa, resulting in fragmentation of chromatin material. Zakhidov et al. (2013) found that bovine spermatozoa chromatin was not protected from the adverse effects of the GNPs and the changes observed were irreversible. Such changes include poor ability to undergo nuclear decondensation resulting in the fragmented and destroyed nuclei. GNPs can have dose dependent deleterious effects on mouse spermatozoa parameters and chromatin structure (Nazar et al., 2016; Zakhidov et al., 2010).

When it is compared with the somatic cells in relation to the permeability of NPs, spermatozoa are quite impermeable to incorporate them as it have been proved in the studies conducted by Taylor et al. (2014, 2015) and Tiedemann et al. (2014) using ligand-free and oligonucleotide conjugated, bovine serum albumin (BSA) coated GNPs, Silver NPs (SNPs) and gold silver alloy NPs on bovine and porcine spermatozoa. A controversial result reported by Taylor et al. (2014) showed that GNPs interfere with spermatozoa functionality by membrane adsorption without penetration. Atei et al. (2015) also found that GNPs could decreases the motility of human spermatozoa and can penetrate into the spermatozoa causing the DNA fragmentation but Moretti et al. (2013) found no adverse effects of GNPs on the human spermatozoa up to a concentration of 250–500 mM. Moretti et al. (2013) observed a significant dose-dependent decrease of motility and viability after incubation of human spermatozoa with high-concentration of SNPs and GNPs and also described the internalisation of GNPs in sperm cells (Moretti et al., 2013).

It is evident that toxicity trials of GNPs on the spermatozoa is yet to be validated with further extensive in vitro and in vivo studies as varying studies have demonstrated that GNPs could either penetrate the spermatozoa (Atei et al., 2015; Moretti et al., 2013; Nazar et al., 2016; Wiwanitkit et al., 2009; Zakhidov et al., 2010) or not (Taylor et al., 2014, 2015; Tiedemann et al., 2014). It is established that NPs enter into the somatic cells after membrane wrapping and endocytosis (Chithrani et al., 2006; Gao et al., 2005; Shukla et al., 2005) the cellular phenomenon which are not present in spermatozoa due to rather rigid and tensely stretched plasma membrane. The size of NPs and their coating also matters in regard to the entry of the NPs into the spermatozoa and the resultant DNA damage (Taylor et al., 2015). Barchanski et al. (2015) found that bioconjugated GNPs penetrate into the spermatozoa depending on plasma membrane status indicating that cell membrane of bovine spermatozoa with intact acrosome was impermeable to either ligand free or conjugated GNPs whilst, after acrosome reaction due to changes in the membrane fluidity the penetration of GNPs did occur.

7.2 | Silver NPs (SNPs)

SNPs are amongst the top five NPs used in the pharmaceutical products, building materials, and so forth, therefore, very important is the study of the toxicological effects of SNPs on the male germ cells of animals as well as humans. It was observed that there was increase in production of ROS as well as increased apoptosis in stem cell line C18-4 of mouse spermatogonia incubated in vitro with SNPs (Braydich-Stolle et al., 2005). Vinita et al. (2017) observed that the motility of the bull spermatozoa was affected by presence of SNPs with a toxicity potential causing immediate precipitation/coagulation of spermatozoa in the semen sample. Also, SNPs penetrated into the spermatozoa head causing its nuclear fragmentation, and with the increasing concentration of SNPs the acrosomal damage, tail damage and coagulation of spermatozoa increased. However, Tiedemann et al. (2014) observed that though GNPs did not have any effect on oocytes–cumulus complex and spermatozoa but the detrimental effects of SNPs on oocytes–cumulus complex were evident but no adverse effect on the spermatozoa.

Wang et al. (2017) reported that the toxic effect of SNPs on the human spermatozoa and its DNA was due to increased ROS production. On the other hand, SNPs shows a slightly higher toxicity than GNPs for both motility and viability to human spermatozoa (Moretti et al., 2013). Yoisungnern et al. (2015) showed that internalisation of SNPs into mouse sperm in vitro altered fertilisation and compromised embryo development. Even small amounts of 20 nm sized SNPs caused decrease in the total sperm production as well as the chromatin damage in rats (Gromadzka-Ostrowska et al., 2012). Baki et al. (2014) found that SNPs caused interruption in functions of sex hormones along with changes in the sperm indices of rats in a dose dependent manner. Castellini et al. (2014) in another study in rabbits revealed that SNPs compromises sperm motility, speed, acrosome and mitochondria shape and function. Layali et al. (2016) reported LPO in the spermatozoa following SNPs administration in rats, thus leading to injurious changes in the seminal attributes. It is evident from these studies that SNPs causes penetration of the spermatozoa and causes chromatin damage which could be harmful, and trans-generational harmful effects could even occur. Therefore, the exposure of SNPs to either animals or human could be detrimental to the male germ cells.

TABLE 2 Toxicities of various metallic nanoparticles (MNPs) to mammalian spermatozoa.

Sl. No.	Type of MNPs	Dosage and duration	Species	Size of MNPs	Toxic effects	References
1.	Gold (GNPs)	• 500 µl (15 min)	• Human	• 2–3 nm	• GNPs can penetrate spermatozoa, resulting in fragmentation of chromatin material and lowered motility.	• Wiwanitkit et al., 2009
		• 1×10^{15} particles/ml (30 min)	• Mice	• 3 nm	• GNPs can have dose dependent deleterious effects on mouse spermatozoa parameters and chromatin structure.	• Zakhidov et al., 2010
		• 1×10^{15} particles/ml (20 min)	• Bull	• 50 nm	• Irreversible nuclear decondensation resulting in the fragmented and destroyed nuclei of spermatozoa.	• Zakhidov et al., 2013
		• 30–500 µM (60–120 min)	• Human	• 100–125 nm	• No adverse effects of GNPs on the human spermatozoa up to a concentration of 250–500 µM but internalisation occur.	• Moretti et al., 2013
		• 01–10 µg/ml (2 h)	• Bull & Boar	• 50 nm	• GNPs interfere with spermatozoa functionality by membrane adsorption without penetration.	• Taylor et al., 2014
		• 0.89 µg/ml (10–20 min)	• Human	• <10 nm	• GNPs decreased motility of spermatozoa and penetrated into the spermatozoa causing the DNA fragmentation.	• Atei et al., 2015
		• 10 µg/ml (10 min)	• Bull	• 10–30 nm	• GNPs penetrate into the spermatozoa depending on plasma membrane status with no penetration in intact acrosome.	• Barchanski et al., 2015
		• 40–200 µg/kg/day (7–35 days, IP injection)	• Mice		• GNPs can have dose dependent deleterious effects on mouse spermatozoa parameters and chromatin structure.	• Nazar et al., 2016
2.	Silver (SNPs)	• 5–100 µg/ml (48 h)	• Mice	• 15 nm	• Increase in production of ROS and increased apoptosis in stem cell line of mouse spermatogonia.	• Braydich-Stolle et al., 2005
		• 5–10 mg/Kg (24 h–28 days IV)	• Rats	• 20–200 nm	• SNPs caused decrease in the total sperm production as well as the chromatin damage.	• Gromadzka-Ostrowska et al., 2012
		• 30–500 µM (60–120 min)	• Human	• 65 nm	• SNPs shows a slightly higher toxicity than GNPs for both motility and viability to human spermatozoa.	• Moretti et al., 2013
		• 10 µg/ml (10 min)	• Boar	• 11 nm	• SNPs had no adverse effect on the spermatozoa.	• Tiedemann et al., 2014
		• 25–100 mg/kg/day (oral 45 days)	• Rats	• 60 nm	• SNPs caused interruption in functions of sex hormones with changes in the sperm indices of rats.	• Baki et al., 2014
			• Rabbit	• 45 nm	• SNPs compromises sperm motility, speed, acrosome	• Castellini et al., 2014

TABLE 2 (Continued)

Sl. No.	Type of MNPs	Dosage and duration	Species	Size of MNPs	Toxic effects	References
					and mitochondria shape and function.	
		• 0.6 mg/Kg IV	• Mice	• 34–46 nm	• Internalisation of SNPs into mouse sperm in vitro altered fertilisation and compromised embryo development.	• Yoisungnern et al., 2015
		• 0.1–50 μg/ml (3 h)	• Rats	• <50 nm	• Increased lipid peroxidation in the spermatozoa following SNPs administration.	• Layali et al., 2016
		• 0.07–0.28 mg/Kg oral (5 week)	• Human	• 10–100 nm	• Toxic effect of SNPs on human spermatozoa and its DNA due to increased ROS production.	• Wang et al., 2017
		• 0–400 μg/ml (15–60 min) • 100–500 μl (immediately)	• Bull	• 20.5–40 nm	• Immediate precipitation/ coagulation of spermatozoa with nuclear fragmentation, and acrosomal and tail damage.	• Vinita et al., 2017
3.	Cerium oxide	• 0–220 μg/ml (96 h)	• Ram	• 8.5 ± 1.3 nm	• No intracellular uptake and no adverse impairment on the functional and morphological characteristics of sperm.	• Falchi et al., 2016, 2018
		• 0.01 mg/ml (1–5 h)	• Mice	• 350 nm	• Reduced fertilisation rates in mice.	• Preaubert et al., 2016
		• 100–300 μg/Kg (3/week for 5 weeks)	• Mice	• <10 nm	• Decreased sperm motility and count with increased total sperm abnormality.	• Adebayo et al., 2018
		• 0.01–10 mg/L	• Human	• <10 nm	• Significant DNA damage without affecting the vitality in human spermatozoa.	• Préaubert et al., 2018
		• 20–40 mg/Kg (32 days)	• Mice	• 27.62 ± 3.01 nm	• Reduction in total sperm production, sperm motility with greater susceptibility to the sperm DNA damage.	• Qin et al., 2019
		• 20–40 mg/Kg (35 days)	• Mice	• 30 nm	• Reducing seminal attributes with diminished in vitro fertilisation rate and embryonic development.	• Hosseinalipour et al., 2021
4.	Iron oxide	• 1 ng/ml (4 h)	• Bull	• 10–50 nm	• NPs entered into the spermatozoa and attached to mitochondria in the tail and in the acrosome region no apparent impact on the acrosome reaction and motility.	• Makhluf et al., 2006
		• 5–20 mg/Kg IP (2 weeks)	• Mice	• 30 nm	• Reduction in sperm number along with increased ROS production.	• Nasri et al., 2015
		• 0.015–0.06 mg/ml (4 h)	• Bull	• 5.3 nm	• Did not affect cell functionality or structure.	• Caldeira et al., 2018
		• 5–20 mg/Kg IP (5 weeks)	• Mice	• 50 nm	• Reduction in sperm number along with increased ROS production.	• Varzeghani et al., 2018
		• 0.5–2.0 μg/ml (5 min)	• Buffalo bull	• 12.09 ± 0.91 nm • 40 nm	• No adverse effect on plasma membrane integrity,	• Bisla, Rautela, et al., 2020; Bisla, Rautela, et al., 2021

(Continues)

TABLE 2 (Continued)

Sl. No.	Type of MNPs	Dosage and duration	Species	Size of MNPs	Toxic effects	References
					motility, DNA integrity and acrosome integrity.	
		• 0.192 mg/ml (30 min)	• Boar		• No adverse effect on plasma membrane integrity, motility, DNA integrity and acrosome integrity.	• Basioura et al., 2020
5.	Zinc oxide	• 10–1000 µg/ml (45–180 min)	• Human	• 30–70 nm	• Higher concentrations and higher exposure periods induce higher toxicity to spermatozoa.	• Barkhordari et al., 2013
		• 5–300 mg/Kg (35 days)	• Mice	• 10–30 nm	• Increased sperm abnormalities.	• Talebi et al., 2013
		• 50–200 mg/Kg (10 days)	• Rats	• <100 nm • 50–90 nm	• Poor semen quality with reduction in sperm viability, motility and total antioxidant capacity.	• Abbasalipourkabir et al., 2015
		• 6–391 mg/ ml (0–3 h)	• Rabbit		• Negative effect of ZnO NPs on the viability and motility of rabbit spermatozoa.	• Halo et al., 2018; 2021
		• 75–300 mg/Kg (28 days)	• Mice		• Adverse effect on epididymal sperm parameters.	• Iqbal et al., 2021
6.	Titanium oxide	• 1–100 µg/ml (6 h)	• Buffalo bull	• <100 nm	• Reduction in viability, plasma membrane integrity, acrosome integrity, and increase in capacitation, acrosome reaction and DNA fragmentation with uptake of NPs mainly in the sperm head.	• Pawar & Kaul, 2014
		• 0–100 mg/Kg (28 days)	• Mice	• 5–10 nm	• Reduced sperm count and function with induced germ cell apoptosis.	• Song et al., 2017
		• 300 mg/Kg (28 days)	• Rats	• <50 nm	• Enhanced oxidative stress, with reduction in antioxidants.	• Hussein et al., 2019
		• 1–10 µg/L (0–72 h)	• Human	• 400 ± 50 nm	• Sperm viability was not affected with increase in progressive and non-progressive sperm but the DNA damage and ROS production was increased.	• Santonastaso et al., 2019, 2020

7.3 | Iron oxide NPs (IONPs)

There are three different forms of existence of Iron oxide in the nature, which are magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3) and haematite (α-Fe_2O_3) (Cornell & Schwertmann, 2003). Haematite also known as ferric oxide is the oldest known of the iron oxides. Magnetite is also known as black iron oxide, exhibits the strongest magnetism of any transition metal oxide (Cornell & Schwertmann, 2003; Majewski & Thierry, 2007). Maghemite occurs in soils mainly as a weathering product of magnetite, or produced as a product of heating of other iron oxides. IONPs could be synthesised for various biomedical applications (reviewed by Ali et al., 2016; Samrot et al., 2021) from various sources where chemical co-precipitation method using various iron precursors is common method which requires ultrasonication or other methods like peptisation for increasing their colloidal stability (Bisla, Srivastava, et al., 2020).

It has been shown that IONPs did not affect the spermatozoa motility and ability to fertilise the oocytes (Makhluf et al., 2006). Makhluf et al. (2006) found that when mixing of polyvinyl alcohol coated IONPs was done with the bovine spermatozoa, NPs entered into the spermatozoa and attached to mitochondria in the tail and in the acrosome region of the spermatozoa head but no apparent impact on the spermatozoa acrosome reaction and motility was seen. Caldeira et al. (2017) demonstrated that in vitro exposure of bull spermatozoa cells to dimercaptosuccinic acid coated maghemite NPs did not affect cell functionality or structure. Controversial to these findings it

was observed in some studies that chronic exposure of mice to Fe_2O_3 NPs led to reduction in sperm number along with increased ROS production (Nasri et al., 2015; Varzeghani et al., 2018). However, the results of majority of studies conducted till date indicate IONPs does not have any detrimental effect on the spermatozoa in large animals like cattle bull, boar and buffaloes (Basioura et al., 2020; Bisla, Rautela, et al., 2020; Bisla, Rautela, et al., 2021; Makhluf et al., 2006, 2008; Odhiambo et al., 2014).

7.4 | Cerium oxide NPs (CeO_2 NPs)

CeO_2 NPs have wider applications in the medical as well as industrial fields. Their effects on the human and animal spermatozoa have been studied. The ability of crossing of blood–testis barrier and accumulation into the testis has been observed in rats after in vivo exposure of CeO_2 NPs (Geraets et al., 2012) indicating a possible interaction with spermatozoa. The oral supplementation of 2–5 nm CeO_2 NPs coated with citrate had improvement in sperm concentration, motility and morphology due to their antioxidant properties in aged rats in vivo (Kobyliak et al., 2015) with improved seminal attributes. Similarly, in another study by Falchi et al. (2016) reported no intracellular uptake with no adverse impairment on the functional and morphological characteristics of ram sperm after in vitro exposure to high concentrations of CeO_2 NPs. Also, the DNA of ram spermatozoa was not affected by CeO_2 NPs even in the increasing concentration for 24 h at a low temperature (4°C) and seemed to well tolerate these NPs in particular ROS production and mitochondrial activity (Falchi et al., 2016).

However, in one study, Préaubert et al. observed that in vitro exposure to very low concentrations of CeO_2 NPs can induce significant DNA damage without affecting the vitality in human spermatozoa (Préaubert et al., 2018), whereas reduced fertilisation rates in mice was observed (Preaubert et al., 2016). All the studies related to CeO_2 NPs have indicated that their cellular internalisation does not occur in the spermatozoa but variability in the effects is evident. Intraperitoneal supplementation of CeO_2 NPs in mice resulted in the decreased sperm motility and count with increased total sperm abnormality (Adebayo et al., 2017). Qin et al. (2019) also observed that chronic oral exposure of CeO_2 NPs to mice led to reduction in total sperm production, reduction in sperm motility with greater susceptibility to the sperm DNA damage. In another recent study conducted in mice, it was concluded that the oral administration of CeO_2 NPs resulted in testicular tissue alterations, reducing seminal attributes, and also diminishing the in vitro fertilisation rate and embryonic development (Hosseinalipour et al., 2021).

Nemati et al. (2020) had demonstrated that intraperitoneal injections of CeO_2 NPs to pregnant mice could have a dose dependent adverse effect on development of testicular tissue and blood biochemical parameters in neonates. However, in a study conducted on human spermatozoa (Hosseinmardi et al., 2022), it had been concluded that 0.1 μg/ml CeO_2 NPs significantly ($p < 0.05$) improved the progressive motility, total motility, viability, membrane functionality, DNA integrity and protamination of frozen-thawed spermatozoa from normozoospermic men. It was also observed that there was reduced concentration of malondialdehyde without any adverse effect on morphology and mitochondrial function after supplementation of human sperm with CeO_2 NPs (Hosseinmardi et al., 2022). Certainly, the antioxidant as well a pro-oxidant effect of these NPs has been found a controversy in different species with induction of protection of DNA as well as sperm quality and also, the DNA damage with reduction in seminal attributes. Therefore, these variability in the effects of CeO_2 NPs in different species indicates a species specific either harmful or beneficial response and may need further validation to establish their applicability.

7.5 | Zinc oxide NPs (ZnO NPs)

The wider application of ZnO NPs in various fields poses the importance of study of their toxicological effects on human and animal male germ cells. Kumar et al. (2006) in their earlier attempts found that qualitative and quantitative attributes of bull semen were improved after zinc supplementation in their diet either in the inorganic or organic form. It has been observed that the toxicity of ZnO NPs on human spermatozoa is dependent on concentration and time of exposure where, higher concentrations and higher exposure periods induce higher toxicity (Barkhordari et al., 2013). Talebi et al. (2013) in one study observed that exposure of ZnO NPs causes increased sperm abnormalities in mice. Abbasalipourkabir et al. (2015) in their study on wistar rats found that ZnO NPs caused poor semen quality with reduction in sperm viability, motility and TAC. However, Aporvari et al. (2018) in the study on ram semen found beneficial effects of ZnO NPs with improvement in the qualitative and quantitative properties of sperm and some antioxidant parameters of seminal plasma with neutralisation of the ROS effects.

Halo et al. (2018; 2021) found the dose dependent negative effect of ZnO NPs on the viability and motility of rabbit spermatozoa. In contrary, Ashtari et al. (2021) observed that ZnO NPs at a concentration of 100 ppm can maintain pH, motility and survival of men sperm in the freezing process. Barkhordari et al. (2013) observed that ZnO NPs affected the human spermatozoa in dose and time dependent manner but its effect was lesser than titanium oxide and silver NPs. Iqbal et al. (2021) also observed ZnO NPs had adverse effect on epididymal sperm parameters and testicular tissues of male albino mice. In another study conducted by Ogunsuyi et al. (2020) on Swiss mice, the evidence of toxicological effect of ZnO NPs alone and in combination with titanium oxide NPs (TiO_2 NPs) were studied and it was concluded that both NPs and their mixture altered sperm motility, reduced sperm numbers and increased abnormalities adversely with reduction in anti-oxidant enzymes and increased byproduct of lipid per-oxidation. It was also observed that the mixture of ZnO and TiO_2 NPs induced more sperm abnormalities than either of them (Ogunsuyi et al., 2020). In a recent study on ram epididymal spermatozoa, it was observed that there were some beneficial effects (increased sperm total motility, viability, DNA and membrane integrity, TAC and SOD with reduced lipid per-oxidation) of ZnO NPs supplementation in

sperm extender during oxidative stress conditions and cooled storage (Soltani et al., 2022). It is evident that the studies on effects of ZnO NPs on male germ cells are still scarce and need further validation at in vitro and in vivo levels.

7.6 | Titanium oxide NPs (TiO_2 NPs)

The wider applicability of TiO_2 NPs in pharmaceuticals, cosmetics, paints, medicine and engineering stresses that their toxicological studies in the semen of animals and human beings is necessary. However, certain concerns about the possible biological effects associated with their use have been raised. Pawar and Kaul (2014) reported detrimental effect of TiO_2 NPs on buffalo spermatozoa after in vitro incubation with reduction in viability, plasma membrane integrity, acrosome integrity, and increase in capacitation, acrosome reaction and DNA fragmentation with uptake of NPs mainly in the sperm head. Song et al. (2017) also reported the detrimental effects of TiO_2 NPs in mice with reduced sperm count and function with induced germ cell apoptosis. Hussein et al. (2019) in a study conducted on wistar rats observed that TiO_2 NPs enhanced oxidative stress, with reduction in antioxidants such as SOD, CAT, and glutathione in testicular tissues, and increased levels of the LPO. However, Santonastaso et al. (2019, 2020) found that sperm viability was not affected after TiO_2 NPs exposure to human sperm along with increase in progressive and non-progressive sperm but the DNA damage and ROS production was increased.

8 | FUTURE PERSPECTIVES

The increasing trend in utility of nanotechnology in the bio-medical and veterinary field has gained much height now a day. The promising applicability of MNPs in semen biology related to nano-purification of semen to improve post-thaw seminal attributes, to reduce the discard percentage of elite male germplasm and to treat poor quality ejaculates need to be well established. Sperm sex-sorting using magnetic NPs could ensure a novel technology with a new light in the arena of livestock industry with easier to perform, safer and cheaper alternative to flow cytometry. NPs enabled bio-imaging of gametes using safer magnetic NPs needs further elucidation and studies on the larger scale for the establishment of facts. Studies could be conducted relating to identification of potential negative or positive fertility biomarkers and the establishment of tests for the prior evaluation of fertility in male animals before selection. Along with the promising applicability, the toxicity studies on these MNPs on spermatozoa of animals as well as human need further more in vitro as well as in vivo studies. Also, till date the majority of nanotoxicity studies of various metal oxide NPs on the spermatozoa has been conducted on mainly laboratory animal models like mice and rats which need to be implicated in large animal models as well as in human beings for the more elucidation of direct toxicological effects and associated underlying mechanisms.

9 | CONCLUSION

It could be concluded that the magnetic NPs have many favourable applications in the field of semen biology like nano-purification of semen to improve post-thaw seminal attributes, reduction in discard of elite male germplasm due to poor quality, sex-sorting of spermatozoa, evaluation of male infertility and bio-imaging of spermatozoa but the acute and chronic toxicological effects of these NPs on mammalian spermatozoa cannot be ignored.

AUTHOR CONTRIBUTIONS

Amarjeet Bisla: Conceptualisation, drafting and typesetting of manuscript, preparation of figures, Mrigank Honparkhe: Conceptualisation, manuscript drafting, typesetting and grammatical corrections, Neeraj Srivastava: Conceptualisation, manuscript drafting and proof-reading. All authors have equal contribution in collection of information, drafting and proof-reading of manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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