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Nanoparticles for preventing cryoinjuries during preservation of animal semen: A review

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ABSTRACT

Many biotechnological aided reproductive methods are now utilized to manage farm animal reproductive processes. Nowadays, smart artificial reproductive technology (ART) is being created that takes into account method efficiency, animal welfare, economic efficiency and environmental health. The nanotechnology based revolution has swept across all fields of science, including farm animal reproduction, allowing for considerable advances in this field. Nanotechnology has the potential to enhance and solve numerous technical challenges confronting many forms of ART. To deliver high-quality sperm doses, technologies for sperm preservation and purification have been developed using a variety of nanomaterials. Cryopreservation causes sperm damage through oxidative stress (OS) and disrupting the plasma membrane integrity. Free radicals and OS are regularly formed during the process of cryopreservation and nanoparticles (NPs) are routinely used to guard against them. We cover current nanoparticle-based approaches for preventing cryoinjuries during sperm cryopreservation in animals in this brief review.

1. Introduction

Artificial insemination (AI) with cryopreserved sperm leads to genetic improvement and promotes the conservation of endangered breeds, preserving biodiversity. Sperm freezing causes ultrastructural and functional alterations in the spermatozoa. Damage to chromatin integrity, increased sperm membrane permeability, free radical hyperoxidation and reactive oxygen species (ROS) generation are all detrimental to fertilization leading to early embryonic development (Ntemka *et al.* 2018). Membrane phospholipids concentrate due to van-der-Waals forces following sperm freezing, and the liquid crystal to gel phase transition occurs. During the thawing process, irregular voids form in the cell membrane, causing membrane damage as well as irregular water and ion leakage in and out (Patist and Zoerb, 2005). During semen cryopreservation, cold shock and ambient oxygen increases ROS generation, resulting in an imbalance between free radicals and antioxidant defense in the semen (Petruska *et al.* 2014). Increased ROS production can impair sperm function by inactivating glycolytic enzymes via acrosomal damage, resulting in lipid peroxidation (LPO), affecting sperm fertility (Sikka, 1996). The LPO mechanism is damaging to sperm viability because it is initiated by H_2O_2. Due to higher quantity of PUFA in the plasma membrane and a lack of antioxidant enzyme defence system, mammalian spermatozoa are prone to LPO-induced damage resulting in loss of sperm activity (Ziaullah *et al.* 2012). Increased ROS generation under OS results in increased permeability of sperm plasma membrane, diminished sperm cell cytoplasm, substantial loss in viability, sperm membrane integrity, fertilizing capacity and increased sperm DNA damage (Bucak *et al.* 2010). The majority of research in the previous few decades has focused on methods/approaches to improve sperm freezing efficiency. The methods utilized were primarily aimed at shielding spermatozoa from the damaging effects of freezing, such as the use of different extenders, cryoprotectant chemicals, antioxidants and nutritional components. Other investigations interrogated how injured spermatozoa were healed following freezing and thawing. . To guarantee that free radicals are scavenged and post-thaw sperm activity is enhanced, several tactics are employed, including the inclusion of antioxidants and cryoprotective chemicals (Lasso *et al.* 1994). Antioxidants, on the other hand, have several drawbacks, such as limited resistance to

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severe conditions and poor solubility and stability in aqueous environments (Martinelli *et al.* 2020). Antioxidants can be protected by encapsulation nanotechnology from deterioration caused by direct contact with external elements such as light, oxygen, chemicals, heat, and pressure. This method can also improve antioxidant solubility and stability in biological fluids, resulting in increased bioavailability (Khalil *et al.* 2019). Nanomaterials might be employed in farm animal reproduction in a number of ways, including transgenesis and targeted chemical delivery to sperm cells, antioxidants, as well as during sperm cryopreservation. The antioxidant properties of certain NPs are among the most propitious attributes for their usage in safeguarding sperm cell activity during cryopreservation (Hashem *et al.* 2020). The current review summarizes the different nanoparticles that are used as antioxidants during sperm cryopreservation for minimizing the deleterious effects of freezing.

How NPs confer spermatozoa better protection against cryoinjury

In ruminant species, the sperm membrane consists of higher concentration of phospholipids that are unsaturated and a lower concentration of cholesterol. This composition, notably the decreased cholesterol content, reduces sperm resilience to freezing–thawing (Darin- Bennet *et al.* 1977). Phospholipids redistribute across the membrane during freezing, and some of them transit from liquid to gel state faster than others owing to structural variations, resulting in lipid phase separation (Grotter *et al.* 2019). As a result, the lipid–protein interactions essential for appropriate membrane activity are disrupted, and certain sperm surface and membrane proteins are lost or translocated, resulting in the loss of function (Lemma, 2011).

Cryopreservation also alters the amount of proteins that serve as ROS scavengers. Following cryopreservation, antioxidant enzymes such GPx, GR and SOD were reported to be redistributed on the surface of ram sperm (Marti *et al.* 2008). Sperm antioxidant defence is rather limited and is mostly dependent on the antioxidant capacity of SP (Martin-Hidalgo *et al.* 2019). Disruptions in the sperm antioxidant system during cryopreservation, as well as the activation of L-Amino acid oxidase in dead or faulty cryopreserved sperm, all contribute significantly to increased ROS generation.

Antioxidant supplementation in the freezing medium minimizes the deleterious effects caused by excessive ROS generation during cryopreservation, improving sperm cryosurvival. Antioxidants (both enzymatic and non-enzymatic) can be incorporated in the freezing medium, with varied consequences (Fernandez-Santos *et al.* 2007). Recent research studied the inclusion of several NPs in the freezing medium to address the primary limitations that traditional antioxidants may have, such as their limited durability under hard settings (Khalil *et al.* 2019). Advances in nanotechnology have aided in the development of new nano-compounds with antioxidant capabilities, such as Se, ZnO and apoferritin containing Ag-Au NPs.

Novel nano-compounds with antioxidant capabilities, such as selenium, zinc oxide, and apoferritin containing gold-silver nanoparticles, have been developed thanks to breakthroughs in nanotechnology. The addition of selenium nanoparticles to the semen extender improved the vitality, motility, and chromatin integrity of cryopreserved bull sperm, resulting in improved in vivo fertility (Khalil *et al.* 2019). When selenium particles were added to the freezing medium, similar results were reported in cryopreserved ram sperm (Hozyen *et al.* 2019). These findings explain so as why selenium nanoparticles reduce oxidative stress, lipid peroxidation, and apoptosis by increasing GPx activity. The post-thaw quality of dromedary camel sperm was also enhanced by supplementing the freezing media with selenium or zinc oxide nanoparticles (Shahin *et al.* 2020). Both nanoparticles have the capacity to boost antioxidant enzyme activity (GPx and SOD), as well as GSH and scavenge ROS. Cryopreserved sperm's viability, membrane integrity, and motility were all increased, while apoptosis was reduced. A silver–gold nanohybrid in an apoferritin cage was another nanoparticle recently created to imitate SOD, CAT, and GPx activities (Dashtestani *et al.* 2019). Because of the lowering of ROS levels and apoptosis during cryopreservation, enriching semen extender with apoferritin containing gold–silver nanoparticles resulted in increased sperm viability and motility after thawing. These findings confirm nanoparticles' ability to protect sperm from oxidative damage during freezing and thawing, but more research in various ruminant species is needed to rule out nanotoxicity in sperm cells.

The spermatozoa cytoplasmic membrane may be harmed by the cooling/freezing process because phospholipids are re-localized into a new pattern. In this regard, phospholipid-based NPs have been discovered to increase spermatozoa membrane integrity by compensating for the loss of free fatty acids and phospholipids during cryopreservation. When compared to lecithin-based and egg yolk-based extenders, Nadri et al. (2019) found that using a nano-lecithin-based extender (2% lecithin) for the dilution of goat semen improves sperm cryosurvival (in terms of lower apoptosis and higher motility, viability, and sperm membrane functionality).

Different types of Nanoparticles and their use in Sperm preservation

NPs, have a diameter of less than 100 nm, can be employed for a number of bioapplications, including reproductive biology, due to their unique physical and chemical characteristics (Shahin *et al.* 2020). Several atoms

and molecules have structural properties that differ from bulk materials in a variety of ways. The surface properties of NPs, such as their size, play an important role in their activity. Two significant parameters to consider are hydrophobicity and charge density. Manipulation of functional chemicals into nanoforms can increase absorption and bioavailability (Zhang *et al.* 2006).

Recent breakthroughs in nanoparticle technology have led in the creation of a number of NP compositions with powerful antioxidant, antibacterial and anti-inflammatory properties.

1. *Vitamin Nanoemulsions in Sperm cryopreservation*

Nanoemulsions (NEs) are vesicular systems that have an oily core and are stabilised by surfactants. They display remarkable adaptability by including oil from various sources and choosing the NE surface based on the characteristics of the administration method (Santander- Ortega *et al.* 2012). A new line of study focuses on nanotechnology as a breakthrough tool in spermatology, utilising NE to accommodate vitamin E within, protecting it from oxidation and stimulating its release into the medium. Nanosystems are nanoscale structures that can connect with or enclose active molecules. They've been used successfully to treat a number of disorders, with several formulations on the market and in clinical trials (Gil Guzman *et al.* 2001).

Safa *et al.* (2016) investigated the effects of Vitamin E (5 and 10 g/mL) and Nano-Se (1 and 2%), and their combination in Beltsville extender for cryopreservation of rooster sperm. They observed that, as compared to the control group, supplementing Vitamin E @ 5 g/mL and 1% Nano-Se improved total sperm motility, progressive sperm motility, sperm vitality and sperm membrane integrity after the freeze–thaw technique. Furthermore, extenders treated with vitamin E @5 g/mL only or in combination with Vitamin E @ 5 g/mLand 1% Nano-Se had lower MDA contents than control extenders.

Vitamin E NE conferred protection against sperm oxidative damage. The beneficial effects of these NE appear to be mediated by motility parameters improvement, as progressivity and sperm velocity, maintaining and protecting mitochondrial activity. In addition, vitamin E NE protect the integrity of the acrosome as well as prevent cell death (Sanchez-Rubio *et al.*, 2020).

2. *Herbal Extract NPs in sperm preservation*

In a number of recent investigations, several herbal extracts have been explored as lipid peroxidation suppressor and natural antioxidants in farm animal sperm preservation.

When cryopreserved bovine and rabbit sperm were treated with curcumin extract, the post-thaw quality of the spermatozoa improved. Curcumin NPs added to the extender can increase post-thawed rabbit sperm motility, membrane integrity, vitality, and sperm ultrastructure. These benefits might be attributed to their ability to reduce lipid and protein oxidation, thereby reducing apoptosis (Tvrda *et al.* 2018). When 1.5 g/mL Cucurmin NPs were employed, the largest increase in post-thawed rabbit semen quality was found (Tvrda *et al.* 2016). Cucurmin (50 mol/L) has significant ROS-scavenging properties, hinting that it may protect cryopreserved bovine spermatozoa from OS and hence boost male gamete post-thaw functional activity (Tvrda *et al.* 2018). Alnusincana bark extract (Abadjieva *et al.* 2020) and Albiziaharveyi leaf extract (Sobeh *et al.* 2017) displayed protective antioxidative effects when supplemented in cryopreserved ram and bovine semen. Echinacea and ginger extracts improved spermatozoa quality and fertility potential when added in the freezing medium for cryopreserved ram sperm (Merati and Farshad, 2020). Thyme, mint and Curcumin nanoformulations improved post-thawed buck sperm characteristics and redox status while decreasing sperm death and chromatin decondensation (Pagl *et al.* 2006).

3. *Metal Nanoparticles in sperm preservation*

Increased ROS levels are known to cause apoptosis, reduced cellular metabolism and acrosome response impairment (Nizanski *et al.* 2016). Durfey *et al.* (2017) used magnetic NPs in conjugated form for molecular selection of spermatozoa, and the nanoselected spermatozoa showed improved motion characteristics, such as a higher proportion of advancing spermatozoa and straightness in boars.

Tsakmakidis *et al.* (2020) investigated the effect of Fe_3O_4 NPs (Fe-0.192 mg/mL semen) on boar semen incubated for 0.5 h at the standard storage temperature (17º C) and reported that the use of Fe_3O_4 NPs during semen processing and preservation provided slight anti-microbial effect with no deleterious effect on sperm characteristics. Basioura *et al.* (2020) studied the kinetic sperm motion characteristics of Fe_3O_4 NPs (0.192 mg/mL semen) and Ag/Fe NPs consisting of Ag and a 5% of zero-valent Fe (0.128 mg/mL semen) in boar semen incubated at 17ºC for 30 minutes following magnetic removal of the said NPs. In conclusion, they reported that Ag/Fe NPs were toxic to boar spermatozoa, however the tested Fe_3O_4 NP concentration had no influence on CASA motility characteristics in boar sperm.

ZnO NPs minimizes ROS generation and boosts sperm viability (Heidari *et al.* 2018). When spermatozoa are damaged by ROS, they lose PUFAs from the plasma membrane, limiting their survival and fertilizing potential (Bucak *et al.* 2010). Zn can remove free radicals from a number of sources, including ionising radiation and reduce lipid peroxidation, giving it the title "high-protection antioxidant" (Patist and Zoerb, 2005). Another study revealed that ZnO NPs may protect the cell membrane from oxidative damage by increasing the number of antioxidant enzymes,

decreasing the quantity of MDA, thereby enhancing antioxidant activity by decreasing the amount of free radicals. The use of ZnO NPs (50 g/mL) and Se NPs (1 g/mL) in the SHOTOR extender enhanced sperm ultrastructure and morphology of camel epididymal spermatozoa during cryopreservation by reducing apoptosis and MDA levels (Shahin *et al.* 2020).

The addition of the Se-NP (1.0 µg/mL) in the semen expander improved the quality of post-thaw sperm in Holstein bulls by lowering apoptosis, LPO and sperm damage (Khalil *et al.* 2019). When compared to sodium selenite, the use of Se at extremely low quantities in the form of NPs produces better results in terms of sperm quality. Furthermore, Hozyen *et al.* (2019) and Nateq *et al.* (2020) used SeNPs (1 µg/mL) in rams and discovered that they increased motility, viability index and membrane integrity while decreasing acrosome defects, DNA fragmentation and MDA levels.

Falchi *et al.* (2018) reported that supplementing ram spermatozoa with CeO_2 NPs enhanced motility metrics even after 48 h to 96 h of incubation.

Summary of NP usage in animal species along with its effect on sperm quality

Animal Spp.	Nanoparticle	Dosage	Effect on sperm quality parameters	References
Rabbit	ZnO	6–391 mg/mL	1.Significant increase on MOT, viability and cell integrity in vitro at lowest concentration. 2. Decreased spermatozoa parameters generated by higher concentrations of ZnO NPs.	Halo Jr *et al.* (2021)
Rabbit	Curcumin	1.5 µg/mL	1. Improved the post-thawed quality of rabbit sperm via redox signaling and reduce the apoptosis process.	Abdelnour *et al.* (2020)
Ram	Se	1 µg/ml	1.Increased the percentage of viability, total and progressive motility, plasma membrane integrity. 2. Decreased acrosome membrane damaged and abnormal sperms. 3. Decreased LPO levels.	Nateq *et al.* (2020)
Rabbit	Curcumin	1.5 µg/	1.Positive influence on post-thawing sperm progressive motility, viability and membrane integrity. 2. Reduced percentages of dead sperm, abnormalities, early apoptotic, apoptotic and necrotic sperm cells. 3. Improved TAC and GPx. 4. Decreased MDA and protein oxidation (POC).	Abdelnour *et al.* (2020)
Red deer	Vit E NE	12 mM	1.Improved sperm kinematic variable and preserved sperm viability in samples subjected to OS. 2. Preserved the acrosomal integrity, maintained and protected mitochondrial activity, prevented sperm lipoperoxidation and reduced ROS production in samples subjected to OS.	Sanchez Rubio *et al.* (2020)
Camel	Se-NP & Zn-NP	SeNPs @ 1 µg/mL ZnONPs @50 µg/mL	1.Maintained the progressive motility, livability and membrane integrity and decreased abnormalities and cytoplasmic droplet percentages of epididymal spermatozoa stored at 4 °C up to 144 h.	Shahin *et al.* (2020)
Boar	Fe_3O_4	Fe_3O_4 (Fe; 0.192 mg/mL semen)	1.Slight anti-microbiological effect with no adverse effects on sperm characteristics.	Tsakmakidis *et al.* (2020)
Boar	Fe_3O_4	Fe_3O_4 (Fe; 0.192 mg/mL semen)	1.No effect on sperm CASA motility parameters	Basioura *et al.* (2020)

Boar	Ag/Fe NPs of diameter 30 nm, consisted of Ag and a 5% of zero-valent Fe (0.128 mg/mL semen)	0.128 mg/mL semen	1. Ag/Fe NPs demonstrated a harmful effect on boar spermatozoa.	Basioura *et al.* (2020)
Rat	Se	1 µg/ml	1. Positive effect on post-thawing sperm progressive motility, livability and membrane integrity. 2. Increased percentages of viable sperm. 3. Decreased percentages of early apoptotic, apoptotic and necrotic sperm cells. 4. Increased TAC and decreased MDA concentration in the SP.	Khalil *et al.* (2019)
Bull	Se	1 µg/ml	1.Increased post-thaw sperm progressive motility, livability and membrane integrity. 2. Decreased TAC and MDA concentration.	Khalil *et al.* (2019)
Ram	Se NP	0.5 µg/ml in extender	1.During freezing process potentially protected spermatozoa from lipid peroxidation and maintained motilityand sperm membrane integrity.	Hozyen *et al.* (2019)
Ram	CeO_2	220 µg/mL	1.Beneficial effects on morphologic and kinematic parameters of ram semen such as motility and plasma membrane integrity after 96 h of exposure.	Falchi *et al.* (2018)
Rooster	Vit E and Nano Se	5 µg/mL VitE 1% of Nano-Se	1.Improved total sperm motility, progressive sperm motility, sperm viability and integrity of the sperm membrane after the freeze–thawing process.	Safa *et al.* (2016)

Harmful effects of NPs on sperm quality parameters

Toxicological screening is a frequent method used to confirm the possible clinical usage of novel medications and substances. The toxicity of a drug is determined by a variety of parameters including gender, age and species and is mediated by the organism's capacity to absorb and metabolize the chemical. The varying sensitivity of animal species to various harmful chemicals has been recognized for many years, and the scientific community fully accepts this reality. The analysis of a new drug's potential hazardous qualities in different animal models and the testing of different dosages is a standard scientific strategy that frequently yields diverse results and assures the safety and potential wide usage of that agent.

Bahamonde *et al.* (2018) recently demonstrated that there are significant species-specific changes in the biodistribution, excretion and toxicity potential of Au NPs. Furthermore, the impacts of NPs on cells are constantly being studied, and according to the literature, these effects on spermatozoa might vary depending on the kind of NPs, as well as their *in vitro* or *in vivo* delivery (Falchi *et al.* 2018). Although time and dose are important factors in NP toxicity, many other factors such as shape, size, stability, surface, magnetic activity, physicochemical properties and thermal and electrical conductivity of NPs, are thought to affect the dynamic of toxicity potential (Budama- Kilinc *et al.* 2018). Even after 48 hours of storage, no harmful impact of Fe_3O_4 NPs on swine spermatozoa was reported by Tsakmakidis *et al.* (2020). Falchi *et al.* (2016) reiterated that attention should be paid to the unique effects of NPs on male gametes, where cytotoxicity occurs in a time/dose dependent way, with species susceptibility acting as a possible diversification factor. Furthermore, Ag NPs has been documented to have a time and dose dependent negative effect on rat epididymal spermatozoa (Gromadzka-Ostrowska *et al.* 2012). Ag NP supplementation enhanced toxicity in rat and mouse sperm but not in human sperm (Yoisungnen *et al.* 2015). Through an equilibrium of dissolved Fe ions and the formation of free radicals via Fenton-like reactions, magnetite NPs can create ROS thus damaging the cell (Arakha et al. 2015). Low concentrations of CeO_2 NPs had a substantial influence on in vitro fertilization in mice and were genotoxic to both male and female gametes (Preaubert *et al.* 2015). In the mouse,

CeO_2 NPs caused DNA damage in spermatozoa and oocytes, affecting in vitro fertilizing potential (Preaubert *et al.* 2015), whereas in ovine species, supplementing maturation media with CeO_2 NPs (44g/mL) enhanced fertilization and blastocyst rate, and no adverse effects in chromatin configuration of oocytes exposed to NPs were observed (Ariu *et al.* 2017). In contrary, chromatin damage in mice (Asare *et al.* 2012) and bull spermatozoa (Zakhidov *et al.* 2013) exposed to Ag NPs has been documented.

2. Conclusion

NPs and natural or synthetic nanovesicles are increasingly being employed to improve sperm cryopreservation. NPs, when compared to equivalent metals or plant extracts, primarily perform as antioxidants with significant advantages. Metal or natural herb NPs work largely as antioxidants, protecting sperm against cryoinjury caused by free radicals and by-products of LPO cascade. More study is required to understand the ultrastructural dynamics and how NP supplementation impacts fertilization and the early phases of embryonic development.

Abbreviations

Ag- Gold; AI- Artificial Insemination; Al- Aluminium; Al_2O_3- Aluminium oxide; ALH- amplitude of lateral head displacement; ART- Artificial Reproductive Technology; Au- Silver; CASA- Computer assisted semen analysis; Cd- Cadmium; CeO_2- Cerium oxide; Co- Cobalt; Cu- Copper; DNA- Deoxyribonucleic acid; Fe- Iron; Fe_2O_3- Iron(III) oxide ; Fe_3O_4- Iron(II,III) oxide; GPx- Glutathione peroxidase; GR- Glutathione reductase; GSH- Reduced glutathione; H_2O_2- Hydrogen peroxide; LIN- linearity; LPO- Lipid peroxidation; MDA- Malondialdehyde; NE(s)- Nanoemulsion(s); nm- nanometer; NP(s)- Nanoparticle(s); Nrf2- Nuclear factor erythroid 2–related factor 2; OS- Oxidative Stress; Pb- Lead; PUFA- Polyunsaturated fatty acids ; ROS- Reactive Oxygen Species; Se- Selenium; Sio2- Silica; SOD- Superoxide dismutase; SP- Seminal plasma; STR- straightness (VSL/VAP); TiO2- Titanium dioxide; VAP- average path velocity; VCL- curvilinear velocity; VSL- straight-line (rectilinear) velocity; Zn- Zinc; ZnO- Zinc oxide

3. References

Abadjieva D, Yotov S, Mladenova V, Lauberte L, Kalvanov I, Krasilnikova J, Telesheva G and E Kistanova (2020). Positive effect of natural antioxidant oregonin from Alnus incana bark on ram semen quality stored at 5 ºC for 48 h. *Research in Veterinary Science* 131: 153–158.

Abdelnour SA, Hassan MA, Mohammed AK, Alhimaidi AR, Al-Gabri N, Al-Khaldi K O and AA Swelum (2020). The effect of adding different levels of curcumin and its nanoparticles to extender on post-thaw quality of cryopreserved rabbit sperm. *Animals* 10(9): 1508.

Aitken RJ (1995). Free radicals, lipid peroxidation and sperm function. *Reproduction Fertility and Development* 7(4): 659–668.

Arakha M, Pal S, Samantarrai D, Panigrahi T K, Mallick BC, Pramanik K, Mallick B and S Jha (2015). Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface. *Scientific Reports* 5(1): 1-12.

Ariu F, Bogliolo L, Pinna A, Malfatti L, Innocenzi P, Falchi L, Bebbere D and S Ledda (2017). Cerium oxide nanoparticles (CeO_2 NPs) improve the developmental competence of in vitro-matured prepubertal ovine oocytes. *Reproduction, Fertility and Development* 29(5):1046–1056.

Asare N, Instanes C, Sandberg WJ, Refsnes M, Schwarze P, Kruszewski M and G Brunborg (2012). Cytotoxic and genotoxic effects of silver nanoparticles in testicular cells. *Toxicology* 291(1-3):65–72.

Bahamonde J, Brenseke B, Chan M Y, Kent R D, Vikesland P J and MR Prater (2018). Gold nanoparticle toxicity in mice and rats: Species differences. *Toxicologic Pathology* 46(4): 431–443.

Bailey JL, Bilodeau JF and N Cormier N (2000). Semen cryopreservation in domestic animals: A damaging and capacitating phenomenon. *Journal of Andrology* 21(1): 1–7.

Basioura A, Michos I, Ntemka A, Karagiannis I and CM Boscos (2020). Effect of iron oxide and silver nanoparticles on boar semen CASA motility and kinetics. *Journal of the Hellenic Veterinary Medical Society* 71(3): 2331-2338.

Bucak MN, Tuncer PB, Sariozkan S, Ba¸spınar N, Taspınar M, Çoyan K, Bilgili A, Akalin PP, Buyukleblebici S and S Aydos (2010). Effects of antioxidants on post-thawed bovine sperm and oxidative stress parameters: Antioxidants protect DNA integrity against cryodamage. *Cryobiology* 61(3): 248–253.

Budama-Kilinc Y, Cakir-Koc R, Zorlu T, Ozdemir B, Karavelioglu Z, Egil A C and S Kecel-Gunduz (2018). Assessment of nanotoxicity and safety profiles of silver nanoparticles. In Silver Nanoparticles—Fabrication, Characterization and Applications; Khan, M., Ed.; Intech Open: London, UK.

Chatterjee S, De Lamirande E and C Gagnon (2001). Cryopreservation alters membrane sulfhydryl status of bull spermatozoa: Protection by oxidized glutathione. *Molecular Reproduction and Development* 60(4): 498–506.

Darin-Bennett A and IG White (1977). Influence of the cholesterol content of mammalian spermatozoa on susceptibility to cold-shock. *Cryobiology* 14(4): 466–470.

Dashtestani F, Ghourchian H and A Najafi (2019). Silver-gold-apoferritin nanozyme for suppressing oxidative stress during cryopreservation. *Materials Science and Engineering: C* 94: 831-840.

Durfey CL, Burnett DD, Liao SF, Steadman CS, Crenshaw MA, Clemente HJ, Willard ST, Ryan PL and JM Feugang (2017). Nanotechnology-based selection of boar spermatozoa: growth development and health assessments of produced offspring. *Livestock Science* 205: 137-142.

Falchi L, Galleri G, Dore GM, Zedda MT, Pau S, Bogliolo L, Ariu F, Pinna A, Nieddu S, Innocenzi P and S Ledda (2018). Effect of exposure to CeO_2 nanoparticles on ram spermatozoa during storage at 4ºC for 96 hours. *Reproductive Biology and Endocrinology* 16(1):1-10.

Fernández-Santos MR, Martínez-Pastor F, García-Macías V, Esteso MC, Soler AJ, Paz P, Anel L and JJ Garde (2007). Sperm characteristics and DNA integrity of Iberian red deer (Cervus elaphus hispanicus) epididymal spermatozoa frozen in the presence of enzymatic and nonenzymatic antioxidants. *Journal of Andrology* 28(2): 294–305.

Gil-Guzman E, Ollero M, Lopez M, Sharma R, Alvarez J, Thomas A Jr and A Agarwal A (2001). Differential production of reactive oxygen species by subsets of human spermatozoa at different stages of maturation. *Human Reproduction* 16(9): 1922–1930.

Gromadzka-Ostrowska J, Dziendzikowska K, Lankoff A, Dobrzy´nska M, Instanes C, Brunborg G, Gajowik A, Radzikowska J, Wojewódzka M and M Kruszewski (2012). Silver nanoparticles effects on epididymal sperm in rats. *Toxicology Letters* 214(3): 251–258.

Grötter LG, Cattaneo L, Estela P, Kjelland ME and LB Ferre (2019). Recent advances in bovine sperm cryopreservation techniques with a focus on sperm post—Thaw quality optimization. *Reproduction in Domestic Animals* 54(4): 655–665.

Halo Jr M, Bučka K, Antos PA, Gren A, Slanina T, Ondruska L, Tokarova K, Massanyi M, Formicki G, Halo M and P Massanyi (2021). The effect of ZnO nanoparticles on rabbit spermatozoa motility and viability parameters in vitro. *Saudi Journal of Biological Sciences*.

Hashem NM and A Gonzalez-Bulnes (2020). State-of-the-Art and Prospective of Nanotechnologies for Smart Reproductive Management of Farm Animals. *Animals* 10(5): 840.

Heidari J, Seifdavati J, Mohebodini H, Sharifi RS and HA Benemar (2018). Effect of nano zinc oxide on post-thaw variables and oxidative status of Moghani ram semen. *Kafkas Universitesi Veteriner Fakultesi Dergisi* 25(1): 71–76.

Hozyen HF, Shamy AAE and AA Farghali (2019). In vitro Supplementation of nano selenium minimizes freeze-thaw induced damage to ram spermatozoa. *International Journal of Veterinary Sciences* 8(4): 249–254.

Khalil WA, El-Harairy MA, Zeidan AEB and MAE Hassan (2019). Impact of selenium nano-particles in semen extender on bull sperm quality after cryopreservation. *Theriogenology* 126: 121–127.

Lasso JL, Noiles EE, Alvarez JG and BT Storey (1994). Mechanism of superoxide dismutase loss from human sperm cells during cryopreservation. *Journal of Andrology* 15(3): 255-265.

Lemma A (2011). Effect of Cryopreservation on sperm quality and fertility. *Artificial Insemination in Farm Animals* 12:191–216.

Marti E, Marti JI, Muino-Blanco T and JA Cebrian-Perez (2008). Effect of the cryopreservation process on the activity and immunolocalization of antioxidant enzymes in ram spermatozoa. *Journal of Andrology* 29(4): 459–467.

Martinelli C, Pucci C, Battaglini M, Marino A and G Ciofani (2020). Antioxidants and nanotechnology: promises and limits of potentially disruptive approaches in the treatment of central nervous system diseases. *Advanced Healthcare Materials* 9(3): 1901589.

Martin-Hidalgo D, Bragado MJ, Batista AR, Oliveira PF and MG Alves (2019). Antioxidants and male fertility:From molecular studies to clinical evidence. *Antioxidants* 8(4): 89.

Merati Z and A Farshad (2020). Ginger and echinacea extracts improve the quality and fertility potential of frozen-thawed ram epididymal spermatozoa. *Cryobiology* 92: 138–145.

Nateq S, Moghaddam G, Alijani S and M Behnam (2020). The effects of different levels of Nano selenium on the quality of frozen-thawed sperm in ram. *Journal of Applied Animal Research* 48(1): 434–439.

Niżanski W, Partyka A and S Prochowska (2016). Evaluation of spermatozoal function—useful tools or just science. *Reproduction in Domestic Animals* 51: 37-45.

Ntemka A, Tsakmakidis IA, Kiossis E, Milovanovic A and CM Boscos (2018). Current status and advances in ram semen cryopreservation. *Journal of the Hellenic Veterinary Medical Society* 69(2): 911-924.

Pagl R, Aurich JE, Muller-Schlosser F, Kankofer M and C Aurich (2006). Comparison of an extender containing defined milk protein fractions with a skim milk-based extender for storage of equine semen at 5 ºC. *Theriogenology* 66(5): 1115–1122.

Patist A and H Zoerb (2005). Preservation mechanisms of trehalose in food and biosystems. *Colloids and Surfaces B: Biointerfaces* 40(2): 107–113.

Petruska P, Capcarova M and P Sutovsky (2014). Antioxidant supplementation and purification of semen for improved artificial insemination in livestock species. *Turkish Journal of Veterinary and Animal Science* 38(6): 643–652.

Pini T, Leahy T and SP de Graaf (2018). Sublethal sperm freezing damage: Manifestations and solutions. *Theriogenology* 118: 172–181.

Pons-Rejraji H, Bailey JL and P Leclerc (2009). Cryopreservation affects bovine sperm intracellular parameters associated with capacitation and acrosome exocytosis. *Reproduction Fertility and Development* 21(4): 525–537.

Preaubert L, Courbiere B, Achard V, Tassistro V, Greco F, Orsiere T, Bottero JY, Rose J, Auffan M and J Perrin (2015). Cerium dioxide nanoparticles affect in vitro fertilization in mice. *Nanotoxicology* 10(1): 111–117.

Safa S, Moghaddam G, Jozani RJ, Kia HD and H Janmohammadi (2016). Effect of vitamin E and selenium nanoparticles on post-thaw variables and oxidative status of rooster semen. *Animal Reproduction Science* 174: 100-106.

Sanchez-Rubio F, Soria-Meneses PJ, Jurado-Campos A, Bartolome-Garcia J, Gomez-Rubio V, Soler AJ, Arroyo-Jimenez MM, Santander-Ortega MJ, Plaza-Oliver M, Lozano MV and JJ Garde, (2020). Nanotechnology in reproduction: Vitamin E nanoemulsions for reducing oxidative stress in sperm cells. *Free Radical Biology and Medicine* 160: 47-56.

Santander-Ortega MJ, De La Fuente M, Lozano MV, Bekheet ME, Progatzky F, Elouzi A, Uchegbu IF and AG Schätzlein (2012). Hydration forces as a tool for the optimization of core–shell nanoparticle vectors for cancer gene therapy. *Soft Matter* 8(48): 12080-12092.

Shahin MA, Khalil WA, Saadeldin IM, Swelum AAA and MA El-Harairy (2020). Comparison between the effects of adding vitamins, trace elements, and nanoparticles to SHOTOR extender on the cryopreservation of dromedary camel epididymal spermatozoa. *Animals* 10(1): 78.

Sikka SC (1996). Oxidative stress and role of antioxidants in normal and abnormal sperm function. *Frontiers in Bioscience* 1: 78–86.

Sobeh M, Hassan S, El Raey M, Khalil W, Hassan M and M Wink M (2017). Polyphenolics from Albizia harveyi exhibit antioxidant activities and counteract oxidative damage and ultra-structural changes of cryopreserved bull semen. *Molecules* 22(11): 1993.

Tsakmakidis IA, Samaras T, Anastasiadou S, Basioura A, Ntemka A, Michos I, Simeonidis K, Karagiannis I, Tsousis G, Angelakeris, M and Boscos CM (2020). Iron oxide nanoparticles as an alternative to antibiotics additive on extended boar semen. *Nanomaterials* 10(8): 1568.

Tvrda E, Greifova H, Mackovich A, Hashim F and N Lukac (2018). Curcumin offers antioxidant protection to cryopreserved bovine semen. *Czech Journal of Animal Science* 63(7): 247-255.

Tvrda E, Kovacik A, Tusimova E, Paal D, Mackovich A, Alimov J and N Lukac (2016). Antioxidant efficiency of lycopene on oxidative stress-induced damage in bovine spermatozoa. *Journal of Animal Science and Biotechnology* **7**(1): 1-13.

Yoisungnern T, Choi YJ, Woong Han J, Kang MH, Das J, Gurunathan S, Kwon DN, Cho AG, Park C, Kyung Chang WK and BS Chang (2015). Internalization of silver nanoparticles into mouse spermatozoa results in poor fertilization and compromised embryo development. *Scientific Reports* 5: 11170.

Zakhidov ST, Pavlyuchenkova SM, Samoylov AV, Mudzhiri NM, Marshak TL, Rudoy VM, Dement'eva OV, Zelenina IA, Skuridin SG and YM Yevdokimov (2013). Bovine sperm chromatin is not protected from the effects of ultrasmall gold nanoparticles. *The Biological Bulletin* 40(6):493–499.

Zhang N, Ping Q, Huang G, Xu W, Cheng Y and X Han (2006). Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin. *International Journal of Pharmaceutics* 327(1-2): 153–159.

Ziaullah M, Ijaz A, Aleem M, Mahmood A, Rehman H, Bhatti S, Farooq U and M Sohail (2012). Optimal inclusion level of butylated hydroxytoluene in semen extender improves the quality of post-thawed canine sperm. *Czech Journal of Animal Sciences* 57(8): 377–381.