



## Review Article

# The impact of selenium nanoparticles on sperm quality

Dipan Rudra Paul, Dibyajyoti Talukdar, Sourabh Deori

### Abstract

The health of people, animals, and microbes depends on selenium, a trace micronutrient. Recently, several researchers have become interested in selenium nanoparticles (SeNPs) because of their biocompatibility, bioavailability, and low toxicity. Selenium nanoparticles are therefore widely used in a variety of biomedical applications due to their greater bioactivity. The antioxidant defence capacity of semen is reduced during processing and cryopreservation; nevertheless, the antioxidant inclusion in the freezing extender has a shield in opposition to the peroxidation of lipids, maintaining metabolic function and cellular function. Selenium acts as a crucial part of glutathione peroxidase (GSH-PX), an enzyme that defends cellular membrane lipids from free radical damage and protects interior cell structures. Semen from numerous animals, including sheep, dogs, man, goats, birds and cattle, has been found to have glutathione peroxidase activity. This review discusses the impact of selenium nanoparticles on the status of spermatozoa.

**Keywords** antioxidants, cryopreservation, fertility, selenium nanoparticles, semen

### Introduction

The growth of nanotechnology over the past thirty years has altered how people view the discovery and development of new drugs by revealing previously closed doors in disease pathophysiology and therapeutic alternatives. Nanotechnology is concerned with particles that are submicroscopic and have at least one dimension smaller than 100 nm. Jöns Jacob Berzelius discovered the selenium (Se) metal in 1817 [1]. The word "Se" is a translation of the Greek word "Selene," which signifies moon. Its atomic number is 34, and it is a member of Periodic Table Group 6. It was found to be a byproduct in the manufacture of sulfuric acid. Se is a colourless, non-toxic, physiologically inactive substance with a "zero" oxidation state. In general, physical, chemical, and biological techniques can be used to create selenium nanoparticles. The organically produced SeNPs, however, show improved compatibility with human organs and tissues. Numerous researchers have looked into how their manufacturing technique, size, and shape affect how they are used in biological systems.

The control of sperm motility and immunomodulatory activity are two crucial roles that selenoproteins play. The human genome has 25 selenoprotein genes. Several antioxidant enzymes, including glutathione peroxidase (GPX), thioredoxin reductase (TXNRD), and selenoprotein P (SELENOP) incorporates Se as selenocysteine (SEC). For all of these enzymes to function biochemically, Se, serves as the redox centre [2]. Reduced fertility results from severe detriments to the DNA of sperm, motility mechanism,

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**Authors:**  
D. R. Paul ✉, D. Talukdar  
College of Veterinary Sciences and Animal  
Husbandry, Central Agricultural University,  
Aizawl, Mizoram, India

S. Deori  
Division of Animal and Fisheries Sciences,  
ICAR Research Complex for NEH Region,  
Umiam, Meghalaya, India

✉ rudrapauldipan2013@gmail.com

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membrane integrity, and acrosomal integrity caused by the processing of sperm and cryopreservation. It is generally recognised that oxidative stress causes lipid peroxidation in bio-membranes, which in turn causes sperm abnormalities. To defend spermatozoa from reactive oxygen species, sperm contains a variety of antioxidants that shield against this reactive oxygen species (ROS) formation [3]. The antioxidant defence potential of semen is reduced during processing and cryopreservation; the antioxidant inclusion in the cooling diluents had a shield against lipid peroxidation, maintaining metabolic function and cellular function [4-5]. Infertility can have male, female, or both, as well as other unexplained causes. It is estimated that aberrant semen parameters connected with male factors account for about 50% of infertility [6]. These males may exhibit abnormalities such as oligospermia, asthenospermia, teratozoospermia (oligoasthenozoospermia), and azoospermia. A lot of man sperm is frozen for use in assisted reproduction, particularly before chemotherapy, radiotherapy, and some surgical treatments [7]. Reactive oxygen species (ROS) and oxidative stress, which can be created in semen, have been demonstrated to negatively impact spermatozoa [8]. Leukocytes and germinal cell precursors can create ROS in defective spermatozoa in semen [9]. On the other hand, spermatozoa's production of ROS is crucial for maintaining the ability to fertilise and for sperm capacitation, among other typical physiological functions [10]. Due to spermatozoa's high vulnerability to oxidative stress and ROS, many damages can be induced, including lipid peroxidation, DNA damage, apoptosis, and decreased sperm motility [11]. By scavenging released free radicals, antioxidants are molecules with the capacity to limit or diminish the oxidative process in other molecules [12]. The two categories of antioxidants are enzymatic and non-enzymatic. Superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase are examples of enzyme-based antioxidants, whereas vitamins A, C, and E, pyruvate, glutathione, and coenzyme Q are examples of non-enzymatic antioxidants. The antioxidant action of selenium (Se) is mediated by the enzyme glutathione peroxidase. Vitamins E and C, as well as Se and Zn, which are elements of antioxidant systems, are among the antioxidants in semen that are known to enhance sperm quality. The protective antioxidant systems in sperms originate from the cytoplasm, and sperms discard a significant portion of their cytoplasm as a residual body during the final stages of differentiation, so it is likely that these antioxidant agents are insufficient in preventing lipid peroxidation and sperm plasma membrane damage during the freezing-thawing process. Sperm motility and metabolic activity are significantly reduced during freezing and thawing operations, although vitamin E and selenium have been reported to alleviate freezing-induced damage and decreased sperm motility [13]. Semen is always exposed to cold shock and ambient oxygen during cryopreservation, which enhances its susceptibility to lipid peroxidation due to increased ROS generation. One of the crucial components changed by cryopreservation is the seminal plasma membrane, which might then have an impact on the function and fertility of the sperm cells [14]. Se is a vital metal-like trace element in animal diets, and its significance in humans has been well documented [15]. Selenium is mostly found naturally in foods including seafood, liver, grain, egg yolk, milk, water, and soil, with soil typically having the highest levels [16]. The average daily dietary consumption of selenium in the US is 80-120 mg, while the daily recommended requirement for men and women is 70 mg and 50 mg, respectively [17]. An essential antioxidant and indicator of oxidative stress, selenium is always a part of the glutathione peroxidase enzyme [18].

### ***Characterization of Se-NPs***

Scanning electron microscopy is used to examine the size, shape, and distribution of SeNPs. By using sonication, a diluted sample is prepared and evenly distributed. Following drying, samples are typed with a gold monolayer (sputter coating), and the Quanta 200 electron microscope is used to capture images. Additionally, SeNPs are identified using a UV-Vis absorption spectrophotometer (PerkinElmer, Lambda 25). The spectra are captured between the wavelengths of 200 and 500 nm [19].

### ***Se-NPs role in semen preservation***

It is generally recognised that the primary processes of cryopreservation and such as cooling, freeze-thawing, place sperm membranes under physical, chemical, and oxidative stress, impairing their



physiological features [20]. Numerous studies have looked into how adding antioxidant compounds to semen diluent affects the safety of sperm during the processing as well as cryopreservation of semen [21]. Several researchers found a strong favourable relationship between sperm quality and selenium levels in the plasma of semen. The totality of the possible anti-ROS enzymes, like GSH-PX, is represented by the total antioxidant capacity of the plasma of semen. The enzyme GSH-PX, which uses selenium as one of its components, guards against peroxidative damage to cellular membranes and organelles that contain lipids. Selenium in the form of selenite aids in the detoxification of the media in cell culture to shield the cells from oxidative damage [22-23]. Summary of SeNPs usage in semen extender along with its effect on sperm quality in Table 1.

**Table 1. Summary of SeNPs usage in semen extender along with its effect on sperm quality**

Species	Dosage	Effect on sperm quality parameters	References
Buffalo	1 and 2 µg/ml SeNPs	1. In frozen-thawed semen, diluents with 1 µg/ml and 2 µg/ml selenium dramatically increased sperm survival, motility, membrane integrity, and total antioxidant capability. Additionally, they caused a decline in sperms with DNA damage.	Dorostkar et al., [24].
Rooster	5 µg/ml VitE and 1% of SeNPs	1. Following the freeze-thaw process, sperm motility overall, sperm movement with time, live sperm percentage, and sperm membrane integrity were all improved.	Safa et al., [25].
Human	5 µg/ml SeNPs	1. Sperm movement that was higher than untreated samples. 2. percentage of DNA damage decreased.	Rezaeian et al., [26].
Bull	1 µg/ml SeNPs	1. Increased post-thaw sperm progressive motility, viability and membrane integrity. 2. Decreased TAC and MDA concentration.	Khalil et al., [27].
Ram	SeNPs @ 1 µg/ml	1. Increased total and progressive motility of sperm, plasma membrane integrity, and live sperm as a percentage. 2. Decreased acrosomal membrane damage and abnormal sperms.	Nateq et al., [28].
Camel	ZnONPs @50 µg/ml and SeNPs @ 1 µg/ml	1. Epididymal sperms held at 4 °C for up to 144 hours maintained their progressive motility, live spermatozoa, and membrane integrity while exhibiting reduced abnormalities and cytoplasmic droplet percentages.	Shahin et al., [30].

Selenium supplementation given in vitro has been shown to improve the status of buffalo bull fresh and frozen spermatozoa [24]. Five ejaculates from each of the five healthy buffalo bulls were used. One ejaculate at a time was extended at 37°C in a tris-based extender consisting of control, 0.5 µg/ml, 1 µg/ml, 2 µg/ml, 4 µg/ml, and 8 µg/ml sodium selenite. The motility and life per cent of the sperm were assessed at time points of 0 minutes (T0), 60 minutes (T1), and 120 minutes (T2) after the semen had been diluted. In the following stage, semen parameters (such as motility, live sperm percentage, the integrity of sperm membrane, and damaged sperm DNA) were calculated by dilution in tris-egg yolk-glycerol diluent consisting of the same concentrations of sodium selenite. The semen was next placed in 0.5 mL French straws and put into liquid nitrogen to freeze it. The results showed that, without changing other parameters, adding 1 µg/ml and 2 µg/ml selenium to the semen extender considerably enhanced the sperm movement of fresh and equilibrated sperm compared to the control. Extenders with 1 µg/ml and 2 µg/ml selenium, however, importantly increased spermatozoa survival, progressive motility, the integrity of sperm membrane, and sperm total antioxidant capability in frozen-thawed sperm. They also led to a decrease in sperms with DNA damage [24].



Safa et al., [25] have seen the effects of the Beltsville extender in conjunction with selenium nanoparticles (1 and 2%) and Vitamin E (5 and 10  $\mu\text{g/ml}$ ) for cryopreserving rooster sperm. It was discovered that, as compared to the control group, administering Vitamin E at a dosage of 5  $\mu\text{g/ml}$  and 1% selenium nanoparticles increased total sperm motility, progressive sperm movement, sperm live percentage, and sperm membrane integrity. Extenders treated with vitamin E at 5  $\mu\text{g/ml}$  or mixed with vitamin E at 5  $\mu\text{g/ml}$  and 1% selenium nanoparticle showed lower MDA contents as compared to control extenders [25].

The impact of selenium has been shown to improve the quality of human semen parameters [26]. Four groups were created out of each sample: two were given a wash, while the other two were left unwashed. One of the washed and unwashed groups also received 5  $\mu\text{g/ml}$  of selenium. According to the findings, unwashed selenium-treated samples had sperm motility that was higher than untreated samples that had not been washed. Comparing treated samples to untreated ones, the percentage of DNA damage decreased [26].

Khalil et al., [27] have studied the effect of varying selenium nanoparticles (SeNPs) concentrations in the semen extender on the cryopreservation of bull sperm @ 0 (T1, control), 0.5 (T2), 1.0 (T3), and 1.5 (T4)  $\mu\text{g/ml}$  and 1.5  $\mu\text{g/ml}$ , respectively, in a tris-yolk fructose (TYF) extender. Semen that had been diluted was packaged in 0.25 ml straws and kept in liquid nitrogen (196 °C) for a month. Following thawing, the sperm quality indicators of sperm progressive motility, viability, morphological abnormalities, plasma membrane integrity, and chromatin integrity were assessed in the semen of each treatment. In each treatment, the total antioxidant capacity and indicators of peroxidation of lipid membrane were assessed in the seminal plasma of the semen. Finally, n = 81 cows were used to test in vivo the impact of Se-NPs on fertilisation capacity. Results demonstrated that when compared to the control, T2 and T3 had a positive impact on post-thawing sperm progressive motility, livability, and membrane integrity. Comparing T3 to T1, the percentage of viable sperm increased while the percentages of early, apoptotic, and necrotic sperm cells dropped. Malondialdehyde (MDA) content was reduced and the total antioxidant capacity (TAC) in seminal plasma rose in T3 compared to T1, but not in T4 [27]. On ram sperm, the effects of selenium nanoparticles acting as an antioxidant were investigated [28]. Over two months, samples were taken from 4 rams twice a week. Selenium nanoparticle (1 and 2  $\mu\text{g/ml}$ ) was added to the extenders, but no antioxidants (control group). The sperm samples were frozen and kept for 30 days. On days 0, 15, and 30 of storage, as well as the level of malondialdehyde on day 30, measurements of sperm viability, total motility, progressive motility, plasma membrane integrity, aberrant sperms, and acrosome membrane damage were made. The findings demonstrated that, in comparison to 2  $\mu\text{g/ml}$  SeNPs, 1  $\mu\text{g/ml}$  SeNPs considerably increased the percentage of viability, total and progressive motility, plasma membrane integrity, and decreased sperms with damaged or aberrant acrosome membranes [28].

The antioxidant activity of various doses of selenium nanoparticles (SeNPs) (0.1, 0.5, 1.5, and 4.5 mg/L) was investigated during storage at 4 °C for 72 hours in the investigation of the effect of selenium nanoparticles supplementation on the sperm quality of fish following short-term storage [29]. By stabilising the number of reactive oxygen species (ROS) within the sperm after 24 hours, 0.5 mg/L SeNPs was more successful at enhancing the quality of *Onychostoma macrolepis* sperm. SeNPs in *Schizothorax prenanti* sperm was likewise shown to have a similar function. The effect of SeNPs on the sperm quality of *Onychostoma macrolepis* was considerably reduced by RAS-selective lethal 3 (RSL3). Additionally, RSL3 reversed the action of SeNPs, which prevented *Onychostoma macrolepis* sperm from degrading the protein glutathione peroxidase 4 (GPX4). The midpiece, where mitochondria were more abundant in *Onychostoma macrolepis* sperm, is where the GPX4 protein is primarily found. These findings showed that GPX4 was crucial for sperm preservation in vitro and that SeNPs primarily controlled GPX4 in *Onychostoma macrolepis* sperm to stabilise ROS levels. The midpiece of the activated sperm also showed distortion and had more ATP than the midpiece of the non-active sperm [29].

## Conclusion

Selenium is an important trace element that the mammalian system needs continuously. Selenium plays a role in male reproduction in addition to other essential roles. However, studies have shown that semen



preserved with selenium nanoparticles reduces spermatic membrane lipid peroxidation, oxidative damage, and decreases sperm acrosome membrane damage. As a result, the quality of the sperm improves, increasing its ability to fertilize and increasing the likelihood of conception.

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