Journal of Nanoscience & Nanotechnology Research

Open Access Review Article

Silver Nanoparticles: Potential Uses and Significance as Semen Supplement

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ABSTRACT

Nanotechnology has emerged as a powerful tool in various scientific disciplines, including biology, reproduction and pharmaceuticals. This paper provides an overview of the history, types and applications of nanoparticles, with a particular focus on Silver Nanoparticles (AgNPs). AgNPs possess unique properties, such as high surface area-to-volume ratio, which make them suitable for diverse biomedical applications. This paper highlights the significant antibacterial, antifungal and antiviral properties of AgNPs and their potential to address the challenges of multidrug resistance. AgNPs interact with bacterial and fungal cells, causing damage to their membranes and nucleic acids, ultimately leading to cell death. Moreover, AgNPs exhibit strong antiviral activity by interfering with viral replication and limiting cell-to-cell spread. The antimicrobial, antifungal and antiviral efficacy of AgNPs is highly dependent on their size, shape, surface charge and coating. Furthermore, AgNPs have been utilized in various medical devices, food packaging materials and fabric coatings to prevent microbial infections. The unique properties and broad applications of AgNPs make them promising candidates for future advancements in nanomedicine and the fight against microbial diseases.

Keywords: Silver; Nano; Particle; Semen; Nanotechnology

INTRODUCTION

Advanced researches in the field of nanotechnology and their applications in the field of biology, reproduction and pharmaceuticals have revolutionized the twentieth century with new perspective and avenues of treatment. Nanotechnology is the production of substances at the molecular level. The prefix "nano" is a Greek word which means "dwarf". The word "nano" means very small. Nanotechnology is the treatment of individual atoms, molecules or compounds into structures to produce materials and devices with special properties. Nanotechnology includes materials within the size range of 0.1 nm to 100 nm and thus can be used for a broad range of applications and the creation of various types of nano materials and nano devices. In another words it is the science of materials and devices whose structures and properties demonstrate novelity and physical, chemical and biological properties considerably alter due to change in their nanoscale size and shape. Thus, nanotechnology is defined as the manipulation of matter on

Received:	13-July-2023	**Manuscript No:**	IPNNR-23-17583
Editor assigned:	18-July-2023	**PreQC No:**	IPNNR-23-17583 (PQ)
Reviewed:	01-August-2023	**QC No:**	IPNNR-23-17583
Revised:	18-August-2023	**Manuscript No:**	IPNNR-23-17583 (R)
Published:	15-September-2023	**DOI:**	10.12769/IPNNR.23.7.22

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Citation: Kanwar A, Virmani M, Chaudhary K, Sharma R, Saini P (2023) Silver Nanoparticles: Potential uses and Significance as Semen Supplement. J Nanosci Nanotechnol. 7:022.

an atomic, molecular and supramolecular scale involving the design, production, characterization and application of different nanoscale materials in different potential areas providing novel technological advances mainly in the field of medicine. Conventional drugs are characterized by poor biodistribution, limited effectiveness, undesirable side effects and lack of selectivity. Controlled drug delivery can be achieved by use of these metallic nanoparticles with release of the drug at particular place of action. It also enhances drug concentration in target tissues; therefore, lowering the dose of drug are required. Such type of therapy is required when there is a discrepancy between a dose or concentration of a drug and its therapeutic results or toxic effects. Nanomedicine is defined by European science foundation as 'the science and technology of diagnosing, treating and preventing disease and traumatic injury, of relieving pain and of preserving and improving human health, using molecular tools and molecular knowledge of the human body. This definition was revised by the US NIH as: 'Nanomedicine refers to highly specific medical intervention at the molecular scale for curing diseases or repairing damaged tissues, such as bone, muscle or nerve' [1].

LITERATURE REVIEW

History of Nanoparticle

The concept of nanotechnology came into existence in 1959 for the first time. Periodical development of Nanoparticle is given in the table below (**Table 1**).

Table 1: Periodical development of nanoparticle.

Year	Development in nanotechnology
1965	Dr. Richard Feynman proposed "there's plenty of room at the bottom"
1974	Term "Nanotechnology" proposed by Norio Taniguchi
1981	IMB develops scanning tunnling microscope
1985	Curl, Kroto, Smalley discovered buckey balls
1986	First book on nanotechnology engines of creation published by k. eric drexler, atomic force microscope
1989	IMB Almaden research center wrote imb with 36 xenon atoms
1991	Discovery of carbon nanotubes by Sumin Ijima
1999	First US research lab devoted to nanoscience
2000	US launch National Nano Initiative (NNI)
2001	Charles M. Lieber was awarded feynman prize in nanotechnology was for developing theory of nanometer-scale electronic devices and for synthesis and characterization of carbon nanotubes and nano wires
2002	Chad Mirkin was awarded Feynman prize for using DNA to enable the self-assembly of new structures and for advancing our ability to model molecular machine systems
2003	Carlo Montemagno was awarded Feynman prize for modeling the molecular and electronic structures of new materials and for integrating single molecule biological motors with nano-scale silicon devices
2004	First policy conference on advanced nanotech held. First center for nano mechanical systems established, Feynman prize in nanotechnology awarded for designing stable protein structures and for constructing a novel enzyme with an altered function
2005-2010	3D nano structures: Robotics, 3D networking and other nano products were prepared that change their state during use
2011	Nano particles explored and a new era of nanotevhnology started

Types of Nanoparticles

Nanoparticles can be of many types and can be used medical sciences, electronics, and in development of technology (**Figure 1**).

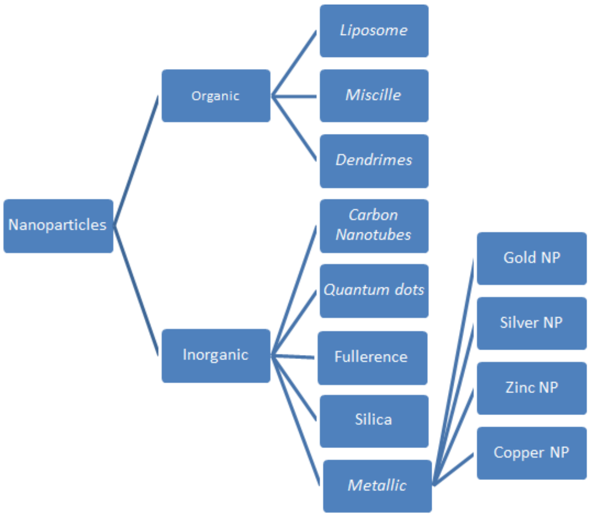

Figure 1: Medical sciences, electronics, and in development of technology.

Liposome: Size ranges from 50 nm-100 nm. It is characterized by presence of phospholipids vesicles. It is biocompatible, versatile and has good entrapment efficiency. It offers easy, passive and active delivery of gene, protein and peptide [2].

Metallic NP's: Size is usually smaller than 100 nm. Characterized by gold and silver colloids which are very small in size resulting in high surface area available for functionalization and are usually stable [3].

Nanocrystals quantum dots: Size ranges from 2 nm-9.5 nm. Characterized by semi conducting material synthesized with II-VI and III-V column element. These produce bright fluorescence, have narrow emission, broad UV excitation and high photo stability [4].

Polymeric micelles: Size ranges from 10 nm-100 nm. These block amphiphilic copolymer micelles, have high drug entrapment, payload and biostability. They are used for target specific active and passive drug delivery and have high diagnostic value [5].

Dendrimers: Size is less then 10 nm. These are highly branched, nearly monodisperse polymer system produced by controlled polymerization. They have three main parts-core, branch and surface. They are used in controlled drug delivery of bioactives, targeted delivery of bioactives to macrophages and liver targeting [6].

Carbon nanotubes: Size ranges from 0.5 nm-3 nm in diameter and 20 nm-1000 nm in length. These are allotropic crystalline form of carbon sheets either single layer (single walled nanotube, SWNT) or multiple layer (multi-walled nanotube, MWNT). These crystals have remarkable strength and unique electrical properties (conducting, semi conducting or insulating). These are used for penetration to cell cytoplasm and to nucleus, as carrier for gene and peptide delivery [7].

Metallic nanoparticles being of utmost importance in medicine are used in drug delivery, treatment of cancer and development of biosensors. Amongst various metallic NP, silver and gold nano particles are of prime importance in biomedicine. Various properties of silver and gold NP depend on its size (surface area), shape, surface charge and coating, agglomeration and dissolution rate. The shape of silver and gold nanostructures dramatically affect the physical and chemical properties. As the shape and size of these NP's can be controlled, these can be efficiently used in biomedicine. Gold NP that we use are mainly in Colloidal gold solution or colloidal suspension of gold nanoparticles in a fluid, usually water. Silver nanoparticle falls in the size range between 1 nm-100 nm. Silver NP have unique properties due to their large ratio of surface to bulk silver atoms. Silver NP can be of various shapes depending upon the method followed for its preparation. It has been proved that nanoparticles with different size and shape have different efficacy. Silver nanoparticles have a size-dependent interaction with HIV-1, with particles in the range of 1 nm-10 nm getting attached to the virus and having highest efficacy [8].

Recent advancement in nanoparticle technologies is the engineering of nanostructure materials with potent antioxidant, antiinflammatory and antimicrobial properties. The Nanoparticles (NPs) include particles with extremely small size, at the nanometer scale, with higher surface-area ratio. Nanoparticles can be made from a variety of materials including metals, polysaccharides and proteins. Since they are more stable, soluble and more biologically effective compared to their corresponding unengineered homologues, thus can be used for a broad range of applications. Among metallic nanoparticles, Silver Nanoparticles (AgNPs) are widely employed in catalysis, optics and biosensing; however, their most common use is in medicine and hygiene as biocides due to the notable activity against bacteria, fungi and viruses. Silver is more toxic to microorganisms than other metals; at the same time, it exhibits a relatively low toxicity and a low propensity to induce microbial resistance. Undoubtedly, AgNPs represent a new generation of antimicrobials, exerting more efficient antimicrobial activity than silver ions and other silver salts. Silver nanoparticles are highly commercialized due to their antimicrobial properties and are being used in many daily life products as well as in several medical devices like catheters, bone and dental cements, anticonceptional foams to prevent microbial infections [9].

Properties and Applications of Silver NP

Silver NP as an antibacterial agent, silver nanoparticles have significant antibacterial activity and are being used in different ways to benefit animal and human life. Nanoparticles are now used as a workable alternative to antibiotics and prove to be a game changer in solving the problem of multidrug resistance. Silver NPs interact with bacterial cell membrane, damage it, make it permeable to silver nanoparticles ultimately leading to its death by binding to the protein and nucleic acid causing structural changes and deformations in the wall, in the membranes and in the nucleic acids of the bacterial cell. Silver ions can enter the body of microbe and cause damage to ribosomes leading to inhibition of protein synthesis. Translation and transcription are also blocked when silver ions bind with the genetic material of the bacteria. Silver NPs also promote the release of Reactive

Oxygen Species (ROS), forming free radicals that lead to the powerful bactericidal action. The activity of AgNP greatly depends on the size of the particle. Smaller nanoparticles have a greater ability to penetrate the bacteria. Electrostatic forces develop when nanoparticles with a positive zeta potential come across a bacterium with negative surface charge and promotes attraction or interaction between the two units and promote penetration into bacterial membrane. Therefore, positive zeta potential with a reduced size of the nanoparticles is the fundamental parameter that control the antimicrobial activity and efficacy of nanoparticles. In addition, silver nanoparticles have been reported to enhance the antibacterial properties of silk fabrics by attaching it upon the fabrics. Nanomaterials are being used to develop new, intelligent, better and more durable and efficient food packaging material. Nanoparticle-based devices and materials are being used like nanofilter and nanosensors for standardising food saftey and traceability. Silver Nanoparticle/Poly (DL-Lactic-co-Glycolic Acid) (PLGA)-coated Stainless Steel Alloy (SNPSA) prove to be a potential antimicrobial implant material. Also, SNPSA showed promising antibacterial activity *in vitro* and *ex vivo* and promoted osteogenesis while suppressing bacterial survival in contaminated rat femoral canals [10].

Silver NP as Antifungal Agent

Silver nanoparticles can be effectively used as an antifungal drug against various species of fungus replacing the conventional antifungal drugs thereby combating the problem of drug resistance. He studied that AgNP suspensions made from aqueous raspberry extract have effective antifungal activity as growth inhibition factors against two resistant fungal strains. Nanosilver suspension of 50 ppm concentration inhibited the growth of *Cladosporium cladosporoides* by 90%and *Aspergillus niger* by 70%. The greener-synthesized silver nanoparticles synthesized using turnip leaf extract against wood rotting pathogens showed promising result as an antifungal agent against wood-degrading fungal pathogens. AgNPs synthesized by green method using ribose sugars as reducing agents and Sodium Dodecyl Sulfate (SDS) as the capping agent used against *C. albicans* and *C. tropicalis* reported visible inhibitory effect on the growth of *Tricosporan T. asahii.* The minimum inhibitory concentration of silver nanoparticles against *T. asahii* was 0.5 mg/mL, which was lower than amphotericin B, 5-flucytosine, caspofungin, terbinafine, fluconazole and itraconazole and higher than voriconazole [11].

Silver Nanoparticle as Antiviral Agent

Virus is the leading cause of death all over the world leading to huge economic losses to the livestock industry. Indiscriminate use of antiviral drugs is developing antiviral resistance among humans and animal specially in immune compromised patients. Studied that use of silver nanoparticle in size dependent manner showed steady decrease in replication efficiency of HSV-1 and HPIV-3 and a little effect on the replication of HSV-2. Replication of the viruses is inhibited when the virus and nanoparticles are present on the cell together during post-attachment step or when the virus has already entered the cells. This effect can be due to interference with the replication machinery inside the cells or by limiting cell-to-cell spread of viruses after the first replication cycle (Table 2) [12].

Table 2: Summary of action of metal nanoparticle against viruses.

Virus	Family	Metal nanoparticle composition (size)	Mechanism of action
Human Immunodeficiency Virus type 1 (HIV-1)	*Retroviridae*	PVP-coated silver nanoparticles (1 nm-10 nm)	Interaction with gp120
Herpes Simplex Virus type 1 (HSV-1)	*Herpesviridae*	MES-coated silver and gold nanoparticles (4 nm)	Competition for the binding of the virus to the cell
Respiratory syncytial virus	*Paramyxoviridae*	PVP-coated silver nanoparticles (69 nm ± 3 nm)	Interference with viral attachment
Monkeypox virus	*Poxviridae*	Silver nanoparticles and polysaccharide-coated silver nanoparticles (10 nm-80 nm)	Block of virus-host cell binding and penetration
Influenza virus	*Orthomyxoviridae*	Sialic-acid functionalized gold nanoparticles (14 nm)	Inhibition of virus binding to the plasma membrane
Tacaribe Virus (TCRV)	*Arenaviridae*	Silver nanoparticles and polysaccharide-coated Silver nanoparticles (10 nm)	Inactivation of virus particles prior to entry
Hepatitis B Virus (HBV)	*Hepadnaviridae*	Silver nanoparticles (10 nm-50 nm)	Interaction with double-stranded DNA and/or binding with viral particles

Silver nanoparticle in wound healing: Jeong and coworkers studied effect of silk fibroin nanofibrous containing SSD on rats *in vivo* and studied wound healing effect of SF nanofibers as compared to commercially available wound dressing Acticoat™ and concluded that SF matrix with 1.0 weight% SSD produced faster wound healing than Acticoat. Studied effect of silver dressings in normal and diabetic wounds in mice. Artificially created wounds were treated with silver gauze dressing and a reduction in bioburden and improved wound healing in diabetic mice was observed in silver-treated wounds. Tested wound healing material made from chitosan, Polyvinylpyrrolidone (PVP) and silver oxide nanoparticles (Ag_2O) (CPS films) and studied their effect *in vitro* and *in vivo*. It was found that wounds treated with CPS healed faster than chitosan treated groups and gauze dressing. Anisha and coworkers developed antimicrobial sponge containing chitosan, Hyaluronic Acid (HA) and nanosilver (nAg) as a wound dressing material for Diabetic Foot Ulcers (DFU) infected with drug resistant bacteria and its antibacterial property was checked against *E. coli, S. aureus,* Methicillin-Resistant S. aureus (MRSA), *P. aeruginosa* and *K. pneumonia*. They concluded that sponge could be used as a potent material for wound dressing for DFU infected with antibiotic-resistant bacteria. Velazquez-Velazquez and coworkers studied the antibiofilm activities of AgNP impregnated in commercial dressings against *P. aeruginosa*. Results suggest that the use of dressings material with AgNP can either prevent or reduce microbial growth in the wound. Also, this reducing wound bioburden which may improve wound healing [13].

DISCUSSION

Silver Nanoparticle as Supplement in Semen

Semen quality is the ability of semen to successfully accomplish fertilization, thus estimating its fertilizing potential and decrease in sperm quality leads to reduced fertility. Although the advances in the cryobiology have made way to new methods that allow the conservation of gametes at low-temperature, but a loss of fertility potential with repeated manipulation and preservation has been observed in both human and animals. Routine semen assessment is usually done by microscopic evaluation of semen parameters such as total sperm count, sperm concentration, percent motile sperm and percent normal sperm morphology. Some of these parameters may be correlated with fertility even though their capacity of predicting male fertility is limited. Bacterial contamination in semen affects the quality and longevity of sperm and consequently fertility is reduced. Some pathogenic microorganisms, such as *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella spp., Pseudomona aeruginosa,* etc. are common contaminants of semen. Antibiotics have been used to prevent bacterial growth, but the frequency of bacterial resistance to various antibiotics is increasing [14].

In the current era of extensive antibiotic usage, including the widespread application of antimicrobials in livestock for disease control, treatment and growth promotion, the detrimental consequences of emerging Antimicrobial Resistance (AMR) are already evident worldwide. While antimicrobial-resistant infections currently pose a significant threat to human lives globally, the true extent of the burden on livestock and the potential transmission through the food chain to humans remains uncertain. It is crucial to develop alternative treatment strategies utilizing innovative approaches to combat antimicrobial resistance. One such approach involves the utilization of nanoparticles for the treatment of bacterial infections. Pothuraju conducted a study on the impact of silver nanoparticles on the functionality of buffalo spermatozoa and observed an increase in spermatozoa motility with higher nanoparticle concentrations. Bisla et al., synthesized iron oxide nanoparticles-antiubiquitin antibody conjugates to eliminate dead/damaged spermatozoa from buffalo semen. The research demonstrated that the in-house synthesized IONPs-Abs complex effectively removed dead/damaged spermatozoa from buffalo semen, leading to an improvement in quality. Morones et al. examined the effect of silver nanoparticles on bacterial growth at various concentrations and concluded that the concentration required to inhibit bacterial growth varied among different types, with *P. aeruginosa* and *V. cholera* exhibiting greater resistance than *E. coli* and *S. typhus*. Rezvanfar et al., reported that oral supplementation of SeNPs in rats protected spermatozoa quality (motility, DNA integrity) and spermatogenesis against oxidative damage induced by cisplatin, an anticancer agent.

Nano-Se was found to be effective in preventing CIS-induced gonadotoxicity through its antioxidant properties. Castilini C, et al. investigated the long-term effects of intravenous administration of silver nanoparticles on the reproductive activity of rabbit bucks and found no morphological damage in the testes. Although the libido, sperm concentration, semen volume and serum testosterone were not affected by NPs, NP-treated rabbits exhibited higher seminal Reactive Oxygen Species (ROS), less motile sperm and lower curvilinear velocity and oxygen consumption compared to control animals. Barkalina et al. suggested that NPs could serve as potential carriers for intragamete delivery. In boar spermatozoa, Mesoporous Silica Nanoparticles (MSNPs) loaded with fluorescent nucleic acid (lamin siRNA) or a fluorescent protein (mCherry) interacted with cell membranes without compromising sperm function. Safa et al. reported that the addition of Nano-Se to semen extender improved the post-thawing quality of rooster semen. Yazdanshenas et al. found that supplementation of bull semen extender with zinc nano-complex, Copper-Zinc Superoxide Dismutase (Cu-Zn SOD), reduced the levels of Malondialdehyde (MDA) and enhanced mitochondrial activity [15]. Basioura et al. investigated the effects of iron oxide and silver-iron nanoparticles on boar semen, specifically studying its motility and kinetic parameters using CASA at 0, 24 and 48 hours after treatment. The comparison within and between groups and storage time points revealed that the values of total motility, progressive motility of spermatozoa, VCL (Curvilinear velocity), ALH (Amplitude of Lateral Head displacement), VAP

(Average Path Velocity) and BCF (Beat Cross Frequency) decreased after 24 hours of storage in the Ag-Fe NPs group but not in the control and Fe NPs group (**Table 3**) [16].

Table 3: Summary of nanoparticles supplemented in semen and their effect.

Nanoparticle used	Effect
Complex of antiubiquitin Antibodies (Abs) with Iron Oxide Nanoparticles (IONPs)	Accomplishing the effective removal of deceased or impaired spermatozoa from buffalo (*Bubalus bubalis*) semen
Sexing in donkeys using Magnetic Nanoparticles (MNPs)	Capable of efficiently choosing X spermatozoa while leaving various physiological sperm parameters unaffected
Nanoparticles of iron oxide with Magnetic Properties (MNPs)	The utilization of magnetic tools, specifically Magnetic Nanoparticles (MNP), enhances fertility in magnetic-assisted cell sorting, commonly known as MACS, proving to be a highly efficient technique
Cryopreservation using zinc oxide nanoparticles	Increased proportion of total and progressive motility in the frozen-thawed samples when compared to the control group. Furthermore, there was a notable decrease in chromatin damage and the level of Malondialdehyde (MDA) was significantly lower as well
Silver nanoparticles	Substituted conventional antibiotics with a potent antimicrobial agent for enhanced effectiveness in porcine sperm
Zinc nanoparticles	Enhanced mitochondrial function, promote membrane integrity, decrease lipid peroxidation and enhance overall antioxidant capacity in bovine sperm cells
Cerium oxide (CeO_2)	After 48 hours of incubation, the motility characteristics of semen showed improvement. Additionally, the integrity of spermatozoa's plasma membranes remained intact
Selenium Nano-Particles (Se-NPs)	The use of a concentration of 1.0 μg/ml resulted in enhanced sperm quality in Holstein bulls after thawing, leading to a decrease in apoptosis, lipid peroxidation and cryopreservation-induced sperm damage
Ag@C nanoparticles, also known as silver-carbon NPs	After 48 hours of cold storage, the antimicrobial activity of the substance showed high efficiency (MIC: 3.125-12.5 μg/mL) against *E. Coli*, *S. aureus* and *P. aeruginosa*. It also exhibited a strong bactericidal effect on *S. aureus* and *P. aeruginosa* (MBC: 3.125 μg/mL). Importantly, at concentrations of 15 and 30 μg/mL, it did not adversely affect the percentage of sperm motility, plasma membrane integrity, acrosome integrity or normal sperm morphology
Gold nanoparticles synthesized using green methods, known as GSGNPs, were prepared at a concentration of 5 to 10 parts per million per milliliter (ppm/mL)	At a concentration of 10 ppm GSGPNs, tris-extended samples exhibited enhanced motility, livability and membrane integrity after the equilibration process. Post-equilibrated and post-thawed semen showed an increase in viable sperm count, while the percentages of early apoptotic, apoptotic and necrotic sperm decreased

CONCLUSION

Nanomaterials have emerged as a promising tool for advancing healthcare by serving as effective alternatives to antibiotics, acting as potent antimicrobial agents. In addition to silver, metallic nanoparticles have demonstrated remarkable antibacterial properties, making them valuable supplements that can replace conventional antibiotics in semen. This innovative approach not only helps prevent antimicrobial resistance but also mitigates the negative consequences associated with the use of antibiotics. As research in this field continues to expand our understanding, the potential applications of metallic nanoparticles in benefiting both humans and animals appear boundless. However, further exploration is required to investigate the *in-vivo* development of nanoparticles, including their capacitation ability, potential toxicity and other effects, to ensure their safe and effective utilization.

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