



Revolutionizing Animal Health: The Role of Nanoparticles in Veterinary Medicine

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Nanoparticles (NPs) have opened new doors in veterinary disease detection, therapy, and prevention. These particles have many functions due to their designed, inadvertent, and natural origins. NPs including quantum dots, iron oxide, gold, silver, dendrimers, liposomes, carbon-based, and polymeric NPs have unique functions and are better for biomedical applications. Characterization procedures include SEM, TEM, AFM, and Zeta Potential studies help understand

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NP characteristics. Veterinary medicine uses NPs in several ways. They aid disease diagnosis, including neurological disorders and mastitis prevention. Silver and copper NPs are effective against *Staphylococcus aureus* and *E. coli*. Nanoparticles also boost immune responses and target infections, including zoonotic diseases, improving vaccine development. *Neospora caninum* uses neo glycolipid-coated liposomes to induce neosporosis and high cellular and humoral immunity. Besides treatments, NPs improve the health status of animals by increasing reproductive, immunity, and growth performance. Pet care products and breeding methods use them for long-term gamete preservation, fertility control, and reproductive health assessment. Exploring transdisciplinary uses, standardizing characterization, and lowering toxicity are the future of veterinary nanomedicine. To employ NPs in veterinary medicine, standard methods and complete preclinical evaluations are needed.

Keywords: Nanoparticles; nanotechnology; antimicrobial; vaccine development; reproductive health; nanomedicine.

1. INTRODUCTION

Nanoparticles (NPs) are particles that are smaller than 100 nm but greater than 1 nm [1]. Richard Feynman, an American noble prize laureate first suggested the concept of nanotechnology in 1959 [2]. Iron oxides, silica, and titania are among the most used types of NPs. Currently NPs can exist in various forms, such as liposomes, polymeric NPs, nano shells, quantum dots, and magnetic iron oxide NPs. The reactivity of NPs grows in direct proportion to their surface area to volume ratio. NPs are encapsulated within a protective shell or capsule. NPs typically exhibit structural similarities to ligands, DNA, and proteins, enabling their interactions with cellular membranes and tissues across a range of biological circumstances. Initially, NPs were only used in nanotechnology but now they have been widely used in medical and veterinary sciences. Nanotechnology emerged in the 1980s but now it is developing rapidly. NPs are crucial in various applications, including electronics, catalysis, magnetism, biomedicine, pharmaceuticals, cosmetics, environmental protection, industry, and optoelectronics. Nanotechnology is used in therapeutic purposes. It facilitates the recognition and interpretation of environmental pollutants. In order to reduce emissions from mobile sources, catalysts are used. NPs function as UV (ultraviolet) filters. NPs modify the characteristics of cosmetic goods, such as their color, solubility, and chemical reactivity.

2. SOURCES AND CLASSIFICATION

2.1 Sources of Nanoparticles

The sources of nanoparticles can be divided into three categories which are discussed below.

2.1.1 Incidental nanomaterials

Human activities like charcoal burning and transportation and some natural processes like volcanic eruption, dust storms, radioactive decay, photochemical reactions, and forest fires lead to production of nanoparticles. Selenium and arsenic are derived from the process of coal combustion [3]. However, Vanadium and Nickel can be extracted from the process of oil combustion [4].

2.1.2 Engineered NPs

These nanoparticles are made by humans for desired applications and uses depending upon their dimension and characteristics [5]. Nanoparticles like carbon NPs, TiO₂ NPs and hydroxyapatites are present in sport goods, toothpaste, and some cosmetic products. Automobile exhaust is a main source of nanoparticles. Diesel engines produce 20–130 nm sized particles whereas gasoline engines release 20–60 nm sized particles. Cigarette smoke consists of 100,000 chemical compounds ranging from 10-700 nm particle size [6].

2.1.3 Naturally produced NPs

They are generally produced in bodies of organisms like humans, insects, and bacteria. In insects, nanoparticles are produced through evolutionary process which help them to survive in harsh conditions [7]. Plants also utilize nutrients from soil and water to grow. These nutrients accumulate in plants in the form of nanoparticles. Bones and some other structures are made by nanoparticles in the human body. Bone is made up of collagen which is an organic nanoparticle and hydroxyapatite which is an inorganic nanoparticle. Antibodies, enzymes and

other secretions are nanosized which are beneficial for the normal growth of humans [8]. Acid mine drainage site and radon gas decline are also sources of natural nanoparticles [9]. NPs are classified based on their origin. Their details description is as below.

2.2 Classification of Nanoparticles

2.2.1 Inorganic NPs

- **Quantum Dots:** They are semiconductors having unique fluorescent qualities, so they are being utilized in imaging. For instance, they serve as markers in in vivo imaging of cancers or sentinel lymph node mapping [10].
- **Iron Oxide NPs:** Iron oxide is the most studied material as FDA-approved nanomedicines. Used in magnetic resonance imaging (MRI) as contrast agents to enhance the visualization of organs, tissues, or abnormalities [11]. Ferumoxytol, commercially known as Feraheme, is employed as an MRI contrast agent specifically for the liver. Magnetic iron oxide nanoparticles comprise of magnetite Fe_3O_4 [12]. The dendritic- Fe_3O_4 nanoparticles have been established as successful delivery vehicles as well as diagnostic platforms in various studies.
- **Gold NPs:** They have been employed in photothermal therapy, a therapy which entails concentrating on cancer cells and heating them with infrared light. This destroys the cancer cells while protecting the surrounding healthy tissue. One example is AuroLase therapy, which uses gold nanoshells to target solid tumors specifically. Gold nanoparticles have great potential in gene therapy and enzyme immobilization [13].
- **Silver NPs:** These are being used to prevent infections as antibacterial agents in medical equipment coatings and wound dressings. Silver NPs are used in Acticoat, a wound dressing, to provide antibacterial effects [14,15].

2.2.2 Organic NPs

- **Dendrimers:** They are employed in gene delivery and as MRI contrast agents because of their well-defined and adjustable architectures, as well as their capacity to transport a payload [16]. SPL7013 Gel is a product that is based on

dendrimers and has strong antiviral and antibacterial effects [17].

- **Liposomes:** Liposomes are examples of complex structures that consist of amphiphilic elements like non-polar and polar lipids, which are scattered throughout a water solution. These Ludwig building blocks order themselves into circumstances with two layers of bilayers that are organized to create closed, hollow, and sphere-shaped vesicles. Due to a dual-layered-core design, these hollow shells can encase both hydrophilic and hydrophobic agents, mostly as a result of their watery interior [18]. Doxil is a drug that belongs to a liposomal colloidal suspension of doxorubicin. Liposomal doxorubicin has been known as an approved drug utilized in the treatment of various cancer types like ovarian cancer, acquired immunodeficiency syndrome (AIDS)-associated Kaposi's sarcoma, and multi-myeloma ADT, most notably due to its potential to minimize dose-related adverse responses in sufferers. This administration strategy also known as the target drug gave system-controlled and direct drug delivery, which reduces the side effects and enhances efficiency [19].
- **Carbon-based NPs:** In advance, carbon-based NPs have received much attention as conveyors of medications, proteins, and nucleic acids because of their distinctive features [20, 21], and on the other side, look for their use in tissue engineering and regenerative medicine [22].
- **Polymeric NPs:** Polymeric NPs are one of the most active components that are being employed in the modern medicine of these materials. The main priorities of almost all research facilities are rating the system, delivering the medicine, and minimizing the amount provided and its delivery [23]. The albumin-bound form of paclitaxel is Abraxane, which is especially utilized in the treatment of breast, lung, and pancreatic cancer [24].

2.2.3 Biological NPs

- **Protein NPs:** Albumin NPs are chemical compounds that utilize the inherent transport capabilities of albumin present in the body to facilitate medication delivery. This is a conventional depiction of Albumin-bound paclitaxel, known as

Abraxane, which is used for cancer treatment [25].

- **Virus-like Particles (VLPs):** VLPs are self-assembled nanostructures out of one or more structural viral proteins but do not have any genetic information. Because they have a native-like form, similar globular nanostructure to a real virus but do not have its harmful substances and infectivity; they have been an excellent nontoxic option to the inactivated and weakened live attenuated vaccines. Tissue targeting can be remarkably specific and whole cells can be penetrable [26]. For example, Gardasil vaccination contains VLPs that immunize people against the human papillomavirus; although the particles produce the coat proteins of the outer surface of the genuine virus, they cause a powerful immune reaction without the viral pathogens' genetic material [27].
- **Silica NPs:** Silica functions as a receptor for pharmaceutical drugs and aids the detection of different structures in medical imaging. The encapsulation of pharmaceuticals in mesoporous silica nanoparticles has been done in two manners: external and in-situ. The external approach was mainly utilized to alter drug delivery. Their exterior surface area is greater because the silica packs solid into a three-dimensional random structure and has more consistent pore breadth [28]. Consequently, SiO₂ has been utilized to monitor the performance and possibilities for targeting microenvironment of the universe especially within the cell [29].

- **Metal-Organic Frameworks (MOFs):** MOFs are newly developed material for the application in medicine as potential tool for effective drug delivery system [30]. Till now, it has been decided to employ the zeolitic imidazolate framework (ZIF-8) to sequester and release a potent combination of drug materials [31].

3. CHARACTERIZATION OF NPs

Even though NPs possess unique characteristics due to their small dimension and high surface to volume ratio, information such as size, shape, electrical charge, and structure are critical for their wide range of utilization in various fields. In medicine and materials science, the common methods of characterizing NPs are the following: Scanning Electron Microscopy, Transmission Electron Microscopy, Atomic Force Microscopy, and assessment of Zeta Potential [32].

3.1 Scanning Electron Microscopy (SEM)

SEM was used to obtain images with enough resolution, enabling the observation of NPs' exterior structures with great detail. The concentration of the electron when they hit the surface produces a variety of signals when atoms on the surface interact with the electrons. The SEM technique is appropriate for surface characterization of the features and condition of aggregate of NPs. According to a prior value estimation of the dispersion, it is appropriate for determining as well as monitoring the sizes and a series of external processes of matter [33].

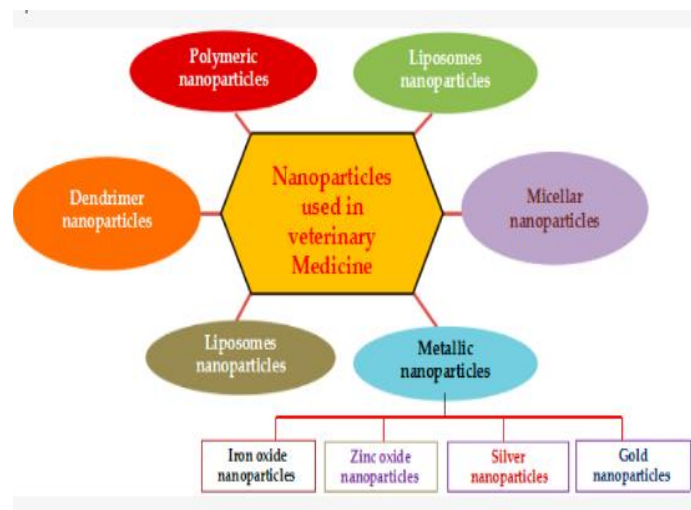


Fig. 1. Classification of NPs being used in Veterinary Medicine

3.2 Transmission Electron Microscopy (TEM)

TEM gives a better comprehension by shifting electrons through a very thinner specimen. Electrons that zigzag their paths across one another generate interactions that create precise pictures of specific aspects, allowing the evaluation of NPs crystalline character and the elemental composition. Confirm the internal structure of the NPs than SEM. TEM is also essential for research on NPs, which are less than 10 nm. It provides higher resolution and data on the internal structure than SEM [33,34].

3.3 Atomic Force Microscopy (AFM)

AFM works by moving a small tip very precisely across the particle's surface. A cantilever is deflected due to the interaction of NPs with surfaces. These deflections are quantified, making it possible to generate highly comprehensive and well-resolved surface topographical maps with nanoscale resolution. Unlike most other approaches, it gives very precise topography, roughness, and mechanical characteristics of NPs that may be applied to both gaseous as well as liquid settings [35,36].

3.4 Zeta Potential Measurements

The zeta potential is established by measuring the movement of the NPs in an applied electric field. It gives information on the intensity of electrostatic interactions between the suspended particles in a liquid. Zeta potential is critical in predicting the stability of the nanoparticle suspension. High zeta potential values indicate strong repulsive forces, thus reducing the likelihood of aggregation and improving suspension stability [36].

4. APPLICATIONS OF NPs IN VETERINARY MEDICINE RESEARCH

4.1 Therapeutic Applications

Veterinary Sciences utilize NPs in a number of significant ways. NPs are also used for disease diagnosis and treatment in animals and human beings. NPs increase the activity of drugs and the immune system against foreign bodies [37]. They also counter drug resistance in human beings and animals [38]. NPs are also used for diagnosis and treatment in animal and human neurodegenerative diseases such as Alzheimer's, Parkinson's, and amyotrophic lateral

sclerosis among others. This is due to the fact that the majority of medications are unable to cross the blood-brain barrier and enter the central nervous system. NPs can cross the blood-brain barrier. In this way NPs help in the delivery of drugs to central nervous system. NPs transport drugs and therapeutic agents to target portions by one of the two methods. Firstly, drugs could be attached on the surface of nanoparticle. Secondly, drug get trapped inside the NPs. Some metal NPs and quantum dots are used to cure neurodegenerative diseases [39,40,41].

4.2 Disease Treatment

Some diseases like tuberculosis, foot and mouth diseases are very common in animals. The use of nano-drugs greatly reduces the unwanted side effects. NPs are very effective in diagnosis and treatment of such diseases. The first investigated nanoparticles in treatment of diseases were liposomes. Liposomes are spherical vesicles mainly consisting of phospholipids and steroids which maintain their shape. Chemotherapeutic drugs are encapsulated in liposomes to treat cancer. Liposomes release drugs depending upon the structure of liposomes, pH and pressure in surrounding area [42].

4.3 Diagnostic Applications

4.3.1 Disease diagnosis

NPs are also used as administrative agents to diagnose mastitis. Mastitis is the inflammation of mammary glands that get worsened by trauma and microbial action. It is being diagnosed by increased somatic cell count, California mastitis test, blood in milk, udder edema, and increase udder temperature.

4.3.2 Imaging techniques

The activity of microorganisms such as bacteria, viruses and fungi can be inhibited by photodynamic therapy (PDT). This therapy involves interaction between a photosensitizer (PS) compound, molecular oxygen, and light with adequate wavelength. PS compounds are activated by light and convert into an excited state (3PS*) which interact with molecular oxygen and convert it into singlet oxygen. This singlet oxygen cell is toxic for the cells and can kill diseased cells and microorganisms. Safranine-O drug is used as synthetic photosensitizers in inactivation of bacteria and fungi by photodynamic inactivation of

microorganisms (PDIM). Micelles and some polymeric nanoparticles play an important role in inactivation of microorganisms [43].

4.4 Applications in Drug Delivery System

NPs are autonomous decision-making particles that operate in a highly specialized manner. NPs react to variations in temperature, pH, and the presence of specific compounds. Self-regulatory methods can be used to construct smart delivery systems so they can make decisions on their own [44]. *Pseudomonas aeruginosa* is a common bacterium causing many skin and urinary tract infections. Gentamicin is the antibiotic used against *Pseudomonas aeruginosa*. Gentamicin is attached to a nanoparticle, hydrogel by a peptide linker. Gentamicin remains medically inactive as long as it remains attached by peptide linker. This peptide linker is broken by a protease enzyme which is produced by *Pseudomonas aeruginosa* [45,46].

4.5 Antimicrobial Activity

Silver NPs have potential effects on some bacteria like *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas*. *E. coli* may cause diarrhea in animals. *S.aureus* is a major cause of mastitis in animals. *Pseudomonas* may cause mastitis in dairy cows and urinary tract infections in dogs. Silver NPs affect the cell division and respiratory chain which may cause the death of bacterial cell [45]. Nanoparticles affect bacteria by three mechanisms.

4.5.1 Interaction with membrane

Silver nanoparticles act on the outer membrane of bacteria and penetrate it. They used to accumulate and adhere on inner membrane causing destabilization, damage, increasing membrane permeability which may lead to the leaking of cellular contents which results in death of bacterial cell [47].

4.5.2 Interaction with proteins, sulfur, thiol, and phosphorous groups

After breaking and crossing the cell membrane, silver NPs can affect the permeability and structure of bacterial cells. Due to its properties, Ag nanoparticles have affinity with sulfur and phosphorous groups present in intracellular contents like DNA. They also interact with proteins and alter their function and structure. They also interact with thiol groups in enzymes

inducing reactive oxygen species and free radicals which alter respiratory chain in inner membrane. This can result in damage to intracellular machinery and activation of apoptotic pathways [48].

4.5.3 Interaction with cellular components

Silver NPs interact with cellular components due to their size and charge. This can alter many metabolic pathways, membranes, and even genetic makeup [49].

Some nanoparticles have a higher ability to control bacterial growth than silver nanoparticles like copper nanoparticles. In contrast, Copper nanoparticles have toxic effect toward the cell. They penetrate the bacterial membrane and transfer electrons by photo-catalytic processes. Copper nanoparticles are mostly unstable, and they usually oxidize to copper oxide nanoparticles.

Gold nanoparticles are also important for antimicrobial activity, but they are less effective than silver nanoparticles. Their mechanism of action only involves the adherence on bacterial membrane by electrostatic forces. Their mechanism of action depends upon the size of nanoparticles. Smaller the size of nanoparticle, lesser the minimum inhibition concentration (MIC) [50]. MIC is the lower concentration of antimicrobial agent expressed in mg/L which completely prevents the growth of microorganism under strict vitro conditions [51].

4.6 Vaccines and Nanoadjuvants

Nanocarriers are very beneficial in therapeutics as they have following benefits:

- Nanocarriers accumulate and target therapeutic agents at the site of action.
- Nanocarriers often mask the unpleasant odor and taste of drugs.
- Nanocarriers also influence the release profile of therapeutics.
- Nanocarriers modify the pharmacokinetic parameters of therapeutics by increasing their bioavailability.
- Nanocarriers protect the therapeutics usually by encapsulation and increase their stability.

NPs are used in the production of veterinary vaccines. NPs are used to potentiate immune responses. Nanoparticles are necessary for the stimulation of humoral and cell-mediated immune

response to combat pathogens and prevent the spread of infection [52]. They play a very important role in the activation of antigen presenting cells. NPs are used as nanoadjuvants which slow the release of antigens due to which the efficiency of vaccine increases [45]. NPs loaded with antigens are directed towards lymph nodes to boost efficiency of vaccine. Poly lactic-co-glycolic acid (PLGA) is a nanoparticle which is used in making vaccines against bovine parainfluenza type 3 and tetanus which increases IgA and IgG immune responses. One glucosamine polymer is chitosan that is extracted from the shells of shellfish. It is a nanoparticle used in vaccines against tuberculosis. Gold NPs are used in vaccines against foot and mouth diseases. Nano-vaccines are used in combating zoonotic diseases which are transmitted between species [53].

Neospora caninum is an intracellular protozoan parasite causing neosporosis in dogs. Neosporosis is transmitted by trans-placental transmission of pathogens from mother to her fetus or by ingestion of oocysts. Pathogenic effects of neosporosis are reduced by the stimulation of humoral and cellular immunity. Mainly cellular immunity is involved in producing responses against neosporosis by type 1 T helper or Th 1 cell activation, Interleukin (IL)-12 and gamma interferon cytokines production [54]. Activation of some antigen presenting cells like macrophages and dendritic cells also provide immunity against neosporosis. Parasite specific CD-4 and CD-8 cells eliminate *N. caninum* infected cells. The most common vector used in the vaccine development against neosporosis is liposomes coated with neoglycolipids that contain oligomannose residues (OMLs) which play role as adjuvants to induce Th 1 cells and T lymphocytes immune responses. OML takes up phagocytic cells from peripheral tissues and migrates to lymphoid tissues. Phagocytic cells respond to this increase by selectively releasing IL-12 and enhancing the production of certain costimulatory-molecules, which indicates its adjuvant action. OMLs transport enclosed antigens to the major histocompatibility complex (MHC) class I and MHC class II pathways, resulting in the generation of antigen-specific cytotoxic T lymphocytes (CTLs) and T helper 1 (Th1) cells, respectively. OMLs carry lipid antigen to CD1d to activate natural killer T cells. OMLs encapsulated with dense granule protein 7 (NcGRA7) and apical membrane antigen 1 (NcAMA1) antigen induces the *N. caninum* specific cellular and humoral immune responses.

This also prevent transmission of parasites from mother to fetus [55].

4.7 Other Applications

4.7.1 Animal health and nutrition

NPs are cheaper and required in low concentrations for improving the reproductive, immune and growth status of animals. NPs are used as liquid vitamins for feeding purposes in poultry. The nanosized minerals pass through GIT and move in bloodstream and increase their bioavailability [45]. Nanoparticles containing minerals like selenium can pass through stomach wall and into body cells faster than inorganic salts with large particle size like sodium selenite [56]. NPs in food are used to control pathogens and regulate the rumen fermentation process. Nanoparticle zinc is used to increase milk production. Nano-sensors in small concentrations are useful to detect any chemical or biological contamination [57,58].

NPs provide better packing materials which provides anti-microbial effect, protection from external environment, UV radiation [59]. Mycotoxicosis is a very common disease in animals and humans. It is due to the intake of forage containing many toxic metabolites produced by certain fungi. It is present about 25 percent in food. Silica and magnesium oxide are used as strong anti-mycotoxins that bind successfully to aflatoxins and inactivate them [60]. Sodium selenite NPs having coating of methacrylate polymer improves the absorption of selenium when supplemented with ruminants. Nanosized selenium absorbed in blood and tissues enhance ruminal fermentation and feed utilization in sheeps [61].

Nanoparticles are supplemented to animal diet not only to increase milk, egg and meat production but also to enhance the quality of animal derived products [62]. Oxidative stress is also decreased by the supplementation of NPs. This is due to the catalase and glutathione peroxidase like activity of NPs which are responsible for elimination of reactive oxygen species. There is decrease in malondialdehyde (MDA) which can be caused due to Selenium NPs, zinc NPs [58] and silver nanoparticles [63]. MDA represents the result of oxidative stress.

4.7.2 Pets care

NPs are used in the production of different industrial products for the care of pets. Silver

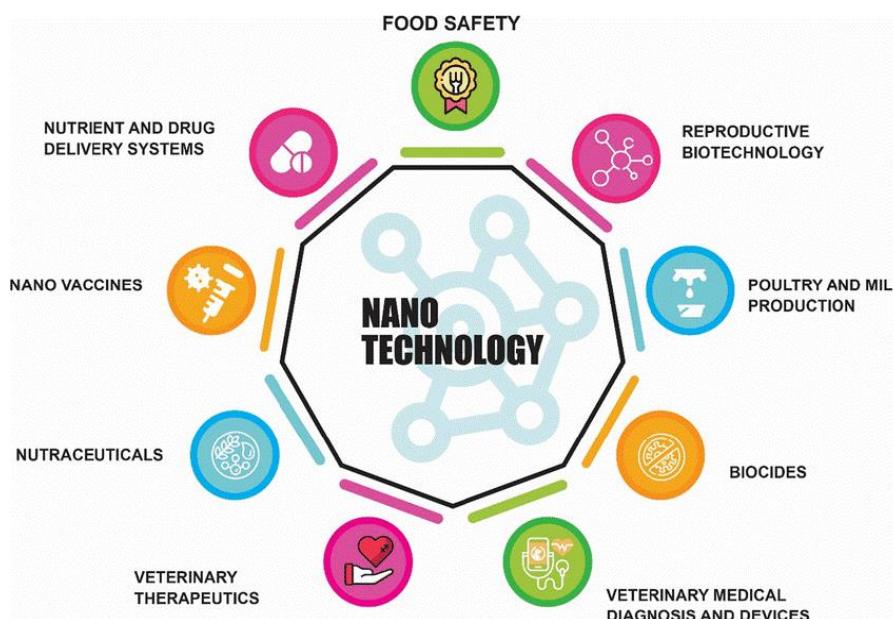


Fig. 2. Applications of Nanotechnology in veterinary medicine and food safety [69]

NPs are used in shampoos for pets. NPs also act as surface disinfectants depending upon their physical and chemical properties [45].

4.7.3 Reproduction and breeding

NPs have many applications in breeding and reproduction like long term preservation of gametes and embryos, fertility control and sustained release of molecules which may include some hormones, vitamins, antibiotics, antioxidants, and nucleic acids [64]. NPs are used for diagnosis and treatment of reproductive problems like retained placenta [45]. In sperm nano purification, the desired healthy sperm is collected, and a library is formed for the fertilization of more females with a single collection. NPs enhance the fertilization efficiency of sperm. In vitro fertilization and development of embryos are assisted by nanoparticles [65]. NPs are used as a cell probe to diagnose the genital tract illness, hormonal or metabolic issues. NPs play an important role in the protection and sustaining the release of hormones for reproduction like steroid hormones [66]. NPs also help in detection of estrous by inserting nanotubes under the skin as these nanotubes detect and bind with estradiol antibody [67]. Oxidative stress due to lesser amounts of antioxidants can cause production of reactive oxygen species (ROS). Reactive chemicals known as reactive oxygen species adversely impact sperm function and fertility. So,

antioxidants reduce level of reactive oxygen species and inhibits the ROS toxicity in sperms [68].

5. FUTURE PERSPECTIVES AND CONCLUSION

NPs are significantly contributing to the presentation of new and more peculiar and precise methods of diagnosis and cure of diseases in animals. NPs are very essential for animal hygiene, vaccination, reproduction, growth, and the security of animals. Owing to the unique biological and chemical characteristics, NPs hold significant potential for application in biomedical. They may be used to convey and send drugs, in the medicine and cure of a disease, detection, as well as its cure. As a result, it becomes important to gain a full broad understanding of the biological impacts and toxicity after exposure to nanoparticles in the execution of laboratory tests and living forms. Although substantial physiological work has been performed on different NPs, the possible toxicity concerns in vivo and fresh remedies remain significant topics of debate. The principal reason for these concerns is the variety of size, surface charge, and coatings of NPs observed in various publications. The number of cell lines, tissue, exposure dosage, and timing of interaction are the remaining parameters. This diversity significantly influences

biological interaction within the body of an individual [70].

The scientific community's focus on the practical and consumer use of nanotechnology has generated an extensive database. We need to figure out how different nanoparticles interact with specific biological barriers and compartments to enhance the process integration and targeted medication release [71]. Subsequent research would then need to address these issues to ensure safe and effective use. It will now be important to comprehend the methods of creation of nanoparticles, what can be done to lessen the risk, and how they may be produced in a factory for utilization in the biological application area. It also necessitates experiments with many animal models of cancerous growth to ensure consistent findings, rather than using a single model that is very efficient. One must remember that the model employed only partially resembles humans physiology [72]. Therefore, it is crucial to establish standardized methodologies for studying the biological effects of NPs of differing physical characteristics. Furthermore, prior to embarking on clinical and preclinical investigations, additional laboratory work may be required to assess fresh potential applications for human and animal use.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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