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Toxicological Impact of Nanoparticles on Reproductive System: A Review

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Abstract

The widespread utilization of Nanoparticles (NPs) in industrial, medical, and consumer goods prompted worries about their possible toxicity. Growing concerns towards individual well- being may however impede the widespread use of this intriguing invention. Although people are exposed to nanoparticles from an early age, but it has increased significantly in the past several years owing to anthropogenic sources of these nanoparticles. The consequences of nanoparticles on the male and female reproductive system are addressed in this review. NPs can infiltrate the epithelial barrier, placental barrier, and blood-testis-barrier and then amass in reproductive organs. NPs amassing impairs organs such as the testis, ovaries and uterus via obliterating Leydig cells, Sertoli cells and Germ Cells, exacerbating reproductive system impairment which unduly affects the quality, amount, morphology, and mobility of sperms or decreasing the amount of egg maturation and distress the folliculogenesis mainly primary and secondary follicular development. NPs may also alter secreted hormone levels and induce effects on sexual behavior. These detrimental effects correlate to nanoparticle composition, surface modification, dosage, mode of transmission and animal species. The current review, however, concentrates on nanoparticles induced toxicological issues and their potential toxicity mechanisms such as oxidative damage, apoptosis, inflammation as well as genotoxicity. NPs may enhance inflammatory response, and oxidative stress, and produce ROS damage, which leads to cytotoxicity at both molecular and genetic levels.

Keywords: Apoptosis, Nanoparticles, Oxidative Stress, Reproductive Toxicity

1. Introduction

"Nanoparticle" is a combination of the term "Nanos" (a dwarf) and "particulum" (a particle). The "nano" is specifically used to describe a particular magnitude of dimension 10^{-9} nm in size in the scientific context[1]. International Organization for Standardization (ISO) describes the word nanoparticle as a substance with one, two, or three external dimensions in the nanoscale domain, i.e., 1-100 nm in size. While contemporary technologies are responsible for manufacturing a significant portion of nanoparticles, natural processes, including volcanic ash, ocean spray, mineral composites, or forest fires, which are involved in creating nanoparticles[2]. Advanced studies and scientific breakthroughs have allowed the invention of different kinds of Nanoparticles (NPs), which have recently come to be used in many applications. These nanoparticles have been extensively used in medicine, biology, environment, manufacturing sectors, energy sectors, industries, and other fields of science. They exhibit exclusive physical (form, dimensions, charge, stability, and versatility), electrical, mechanical, magnetic,

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chemical, optical, and biological characteristics that vary widely from the characteristics of ordinary substances[3]. Engineered Nanoparticles (ENPs) include numerous different types of nanoparticles, including metals (e.g., silver, palladium, nickel, gold, aluminium), metal oxides (e.g., silicon dioxide, iron oxide, aluminium oxide, cerium oxide, copper oxide, molybdenum oxide, zinc oxide, titanium dioxide etc.), carbon-based nanoparticles [e.g., Carbon Nanotubes (CNTs), fullerenes, graphene oxide, carbon black] and polymer nanoparticles such as chitosan[4]. Nanoparticles provide a solution for early diagnosis of diseases, treating complex diseases, and metabolic disorders, used in drug delivery, antibacterial treatment, and cell repair processes[5]. More than 1814 commodities (including pharmaceuticals, food, textiles, sports equipment, and electronic materials) have been manufactured by 622 enterprises and commercialized within a short period and the number is still increasing firmly[6]. Due to this rise in production, usage, and discharge of these nanoparticles into the soil, air, and aqueous systems, it is essential to study nanoparticles and their impact upon humans and animals to their potential toxicities. Studies investigating some nanoparticles commonly utilized in our day-to-day lives have revealed many dangerous consequences on human well-being. Nanoparticle exposure occurs via the skin, inhalation of water vapours, potentially contaminated water, or a variety of other ways[7]. Due to the petite size of nanoparticles (< 100 nm), they can enter the cells, and their translocation can quickly occur across epithelial cells. Nanoparticles can also migrate to otherparts of the body. The size-dependent accumulation of nanoparticles has been observed in organs with substantial phagocytic activity, primarily the hepatic, renal, and spleen region[8]. Nanoparticles cause excess ROS generation and inflammatory cytokine production in human tissues and cell cultures[9]. Additionally, they may be absorbed by the nucleus and mitochondria, resulting in mitochondrial deformities, DNA abnormalities, and cell death[10]. Reproductive toxicity due to nanoparticles has become a significant concern in today's world. Developing embryos and gametes in mammals are at a high risk of toxicity as the nanoparticle exposure on gametes can cause significant development changes. Many research studies have discovered that nanoparticles are able to penetrate the protective biological barriers that isolate reproductive organs. According to recent research, nanoparticles are found to pierce the Blood-Testis Barrier (BTB) and then lodge in the testicular region affecting spermatogenesis[11]. A few studies have indicated that disorders in the development of male reproductive organs (including poor sperm production in maturity, undescended testes, genital deformities, and testicular cancer) are caused by inadvertent usage of nanoparticles[12]. Nanoparticles also can enter inside ovaries and accumulate in ovarian cells. Several different nanoparticles such as titanium dioxide, fullerene, gold, silicon, iron oxide, cerium dioxide, aluminium dioxide, quantum dots,silver carbon nanotubes have been found to cross the placenta in various experimental models[13]. Several research indicates that when adult female rats were exposed to nanoparticles, reproductive functions were severely impacted, harming uterine tissues and altering Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), and Oestradiol (E2) values, culminating in birth deformities[14]. Due to the widespread availability and usage of the nanoparticles, it is critical to recognize the potential risk to reproductive health. Therefore, in this review we will mainly focus on the harmful effects of the nanoparticles in different animal models via studying different literature available on both *in vitro* and *in vivo* models. This review aims to summarize the literature about the different nanoparticle classifications, their potential reproductive toxicities induced via different mechanisms at different levels (cellular, tissue, organ, and molecular levels).

2. Classification of Nanoparticles

Nanoparticles are primarily categorized into three types, namely carbon-based, organic, and inorganic[15] (Figure 1) and their wide applications are shown in Table (Table 1)

2.1 Carbon Based

These nanoparticles are completely carbon-based and can exist in various forms like carbon black, carbon nanotubes (single and multi-walled carbon nanotubes i.e., SWCNT and MWCNT), graphene, carbon nanofibers, fullerene (C_{60})[16].

2.2 Organic Nanoparticles

Non-covalent interactions are found in these nanoparticles, which means these may be modified into a wide range of forms, including dendrimers, liposomes, micelles, hydrogel and polymer nanoparticles[17]. Due to their unique features, they are an excellent alternative for delivering

drugs. Applications and efficiency are determined not just by typical features, such as size, composition, and surface shape, but also by the carrying capacity, stability, and delivery systems of the drug, whether it is in the form of entrapped or adsorbed medication[15].

Since organic nanoparticles are very beneficial for the biomedical industry by facilitating high levels of medication delivery, these nanoparticles may be injected into many regions of the body to assist with personalized treatment.

2.3 Inorganic Nanoparticles

It primarily contains metal and metal oxide nanoparticles. The fabrication techniques include either a constructive or destructive approach/top-down or bottom-down synthesis. Among others, metal elements like Aluminium (Al), lead (Pb), cadmium (Cd), silver (Ag), cobalt (Co), gold (Au), copper (Cu), zinc (Zn), iron (Fe) are all excellent for nanoparticle manufacturing[18]. Both crystalline and amorphous features may be seen in these formations. While metal oxide nanoparticles are constructed from several metals and also have unique properties distinct from metals. The commonly produced nanoparticles are Cerium oxide (CeO_2), Aluminium oxide (Al_2O_3), Magnetite (Fe_3O_4), Iron oxide (Fe_2O_3), Titanium oxide (TiO_2), Silicon dioxide (SiO_2), and Zinc oxide (Zn O)[15].

3. Route of Entry of Nanoparticles in Animals

3.1 Ingestion

Humans can eat a wide variety of dietary components, additives, and supplements that contains nanoparticles. Although a considerable percentage of inhaled nanoparticles may pass through the airway and get to the stomach with help from mucociliary cells[29]. Nanoparticles may quickly diffuse into the bloodstream and therefore reach all organs after passing the epithelium (Figure 2). Nanoparticles potentially influence the gastrointestinal system's motility, the mucus layer of intestinal lumen, and the microbiome. Inside the intestinal cells, these nanoparticles are found to increase oxidative stress levels, DNA distortion, and even cause inflammation[30]. Since the year 2005, nanoparticle-based commodities usage in food industries has seen a tremendous surge-Silver Nanoparticles (Ag NPs), Titanium dioxide nanoparticles (TiO_2 NPs), Zinc oxide nanoparticles (ZnO NPs), Silicon dioxide nanoparticles (SiO_2 NPs) are among the

Table 1. Applications of various nanoparticles

Nanoparticles	Abbreviations / Chemical Formula	Applications
Titanium dioxide	TiO_2	Cosmetics, pharmaceutical, paint, paper, rubber, ink, plastics, toothpaste, E171(as food colorant), leather industry[19].
Aluminum	Al	Packaging materials, cutting tools, polishing materials, glass products, plastic, tape, and grinding belts, drug delivery, antimicrobial, immunotherapy[20].
Gold	Au	Electronics, cancer detection and treatment, biological sensors, medication delivery, and other applications rely on Catalyst[21].
Silver	Ag	Used in burns, wounds, and bacterial infections, food and textile industry, electronics, antibiotic resistance, cosmetics and paints[22].
Iron oxide	Fe_3O_4	Drug delivery, magnetic hyperthermia, chemotherapy, magnetic imaging, biosensors and imaging, waste water treatment, printed electronics, pollution sensor etc[23].
Zinc	ZnO	Rubber, paint, coating, cosmetics, biomedicine (anticancerous, antibacterial, antidiabetic), bioimaging, concrete production, photocatalysis, electronics, electrotechnology industries, wound healing etc[24,25].
Carbon nanotubes	CNT	Paper batteries, cable wire transistor, water filter, air filter, energy storage, supercapacitors, textiles, hydrogen sensors etc[26,27].
Quantum dots	QDs	Bioimaging, pH probe for study of enzyme kinetics, detection of cancerous biomarkers[28].

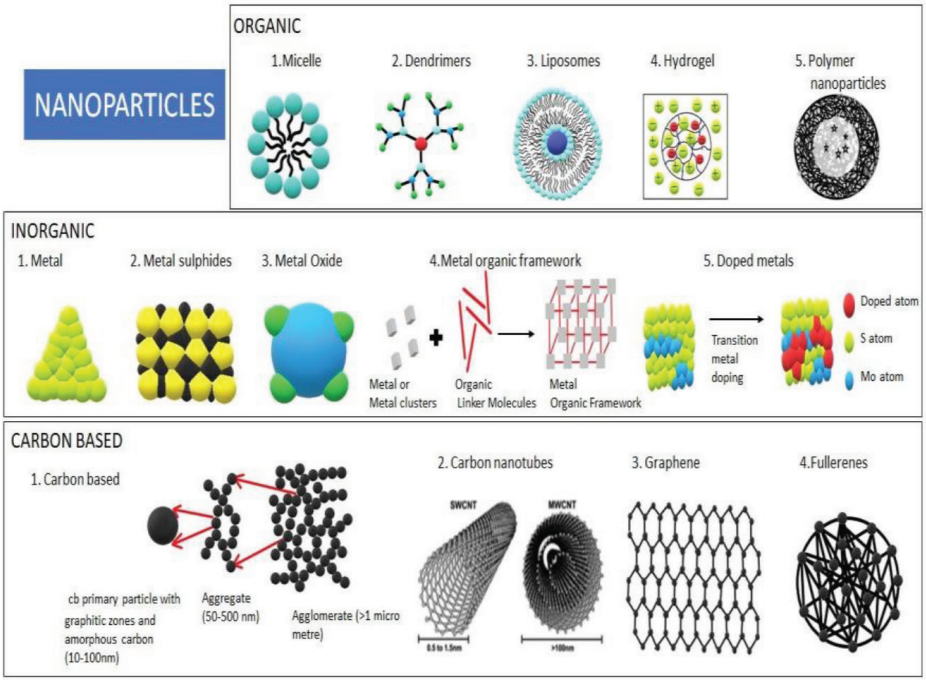


Figure 1. Classification of nanoparticles (NPs).

mostly used in food and other industries[31]. Sergent and co-workers, exposed SiO_2 NPs (25 and 100 nm) to HT 29 (Human epithelial intestinal cells) for approximately 24 hours and reported cytotoxicity[32]. The impact of the dimension, physical, and chemical characteristics of toxicity assessment in the gastrointestinal system is apparent in all published studies.

3.2 Inhalation/Nano Respiration

Nanoparticles can enter inside the body via various pathways like inhalation of mist, aerosol, powder, or artificial by instillation into the respiratory system via the trachea oropharynx, or pharynx[33]. The nanoparticle can be translocated to various pulmonary sites via blood flow in a size-dependent manner. Once the nanoparticles are present in the respiratory region, they interrelate with the cells for a prolonged duration and cause several toxic effects as the elimination mechanisms are not fast enough. (Figure 2). During the exposure of TiO_2 anatase nanoparticles in the male rats for approximately 6 hours via the inhalation route, nanoparticle deposition is found in the hepatic, renal, and spleen along with pulmonary region[34]. For 2–24 hours, Mc Carthy and co-workers studied the impacts of 50 g/mL SiO_2 NPs of varying dimensions of 10, 150, and 500 nm on human lung submucosal cells (Calu-3) *in vitro*, and nanoparticles resulted in enhanced malondialdehyde levels and genotoxicity[35]. All of this information suggests that the primary physicochemical characteristic affecting the airways is size. However, although it's true that smaller NPs are better at accumulating in the deepest areas, triggering inflammation and the production of ROS.

3.3 Skin Penetration

Skin serves various purposes, including protection from external chemicals, protection from ultraviolet radiation, and acts as a selectively permeable barrier. Additionally, the skin is critical for temperature control and immune response modulation in humans. With the growing field of nanotechnology, nanoparticles are amply used in the cosmetics industry. TiO_2 NPs (70%, 80%) are more commonly used in cosmetics along with ZnO NPs and Ag NPs[36]. Nanoparticlediffusion is found to be size-dependent and available data reported that 4 nm nanoparticles are found to penetrate intact skin while 45 nm can penetrate damaged skin[37]. ZnO NPs found to elicit mitochondria disruption and also cell membrane damage in HaCaT cells after exposure of around 24 hours at 0-80 µg/ml in addition to the ROS generation, lipid peroxidation, and apoptosis[37]. The published analysis indicates that NPs have a significant potential for skin penetration (Figure 2). However, this entrance path is very improbable to be

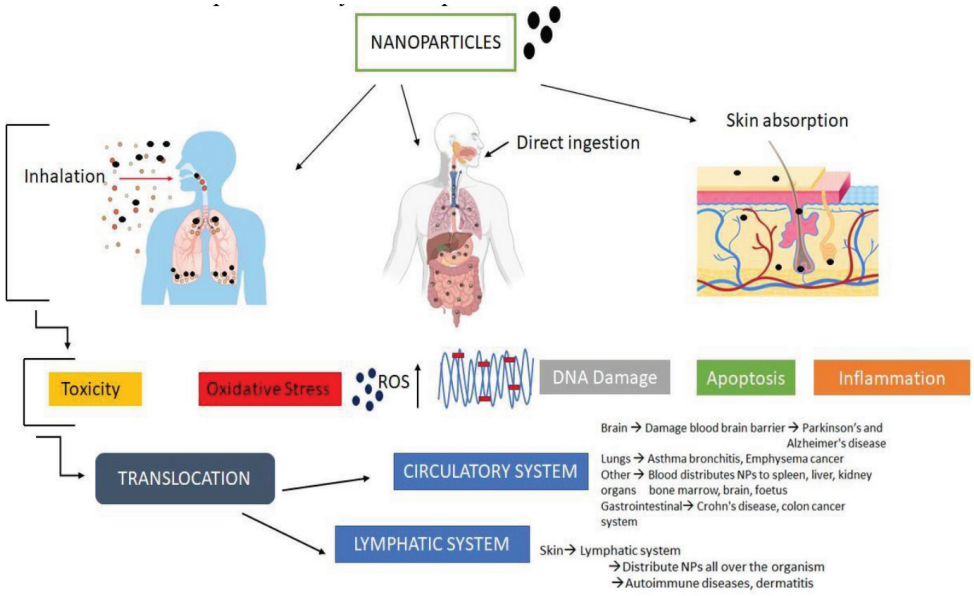


Figure 2. Various routes of nanoparticle exposure.

the preferred one since NPs enter the epidermis only via skin pores and abrasions.

4. General Mechanism of Nanoparticle Induced Toxicity

4.1 Nanoparticle Internalization

The tiny nanoparticles can penetrate several biological barriers, affecting and altering the basicbiological structure and mechanism of the reproductive system. Nanoparticles can cause severe proinflammatory response, which has the potential to affect the Biological Testis Barrier (BTB), and in addition, the genes which are essential for the generation of the BTB (tight junction genes in Sertoli cells) are also suppressed[27]. As a result, an increase in BTB gap size as well as nanoparticle accumulation results in nanoparticles crossing of BTB barrier and infiltrating spermatogenic cells. Several *in vitro* research also show that NPs could attach to the plasma membrane and infiltrate it along with the head region of the spermatozoa[38]. In someof the other studies, nanoparticles were also found to move to the ovaries along with accumulation in ovarian cells[39]. Nanoparticles detrimental effects on oocyte development (oogenesis) may be attributed to their ability to harm the local cellular milieu seen in developing follicles[40]. Thus, we conclude that nanoparticles impair the surrounding cellular milieu of growing follicles and cause oocyte development (oogenesis) to be disrupted.

4.2 Oxidative Stress

Oxidative stress is mainly generated due to the imbalance between the Reactive Oxygen Species (ROS) generation by the various cellular aerobic metabolism and the antioxidants produced by various defense mechanisms[41]. ROS can also be generated from different exogenous chemicals such as pesticides, industrial chemicals, and heavy metals as well as fromnanoparticles[42]. Some research indicates that oxidative stress is the primary mechanism for germ cell harm caused by nanoparticles[42]. Nanoparticles upregulate many different kinds of proinflammatory genes compromising the Tumor Necrosis Factor- Alpha (TNF-Alpha) and Interleukin- 1,6,8 (IL- 1,6,8), which results in increased oxidative stress, apoptosis, and DNA breaks down[43]. One well-known example of this type of toxicity is nano silver particles which enter cells by diffusing or being engulfed via endocytosis, where it directly affects mitochondria and induces the creation of ROS; the ROS causes mitochondrial dysfunction and destroys proteins and nucleic acid, finally inhibiting cell proliferation[44] (Figure 3). According to few studies, TiO_2 NPs enhanced ROS production and Malondialdehyde (MDA) concentrations and decrease antioxidant enzyme activity via reducing CAT (Catalase), GPx (Glutathione peroxidase), SOD (Superoxide Dismutase), and Glutathione Reductase (GR) levels or intracellular antioxidant concentration like GSH (Reduced glutathione) and ascorbic acid[45,46]. The most common

source of spermatozoa ROS is electron leakage through the electron transport chain and monovalent reduction in molecular oxygen[47]. Nanoparticles small size permits them to invade mitochondria and disrupt the structure of mitochondria which further leads to distortion in electron transport chain resulting in excess of ROS. In addition, research has shown that the nanoparticles interact with the oocytes in several ways: via direct contact with the oocytes *in vitro* or indirectly after emitted ions from the nanoparticles have penetrated the zona pellucida. Several *in vivo* and *in vitro* studies have shown that different types of nanoparticles can accumulate inside the female germ cells which increase ROS inside these cells[48-51].

4.3 DNA Damage

Nanoparticles increased exposure had led to ROS generation which causes the DNA damage and oxidative stress via a variety of direct and indirect processes finally leading to genotoxicity and reproductive toxicity (Figure 3). Nanomaterials that persist in the nucleus may damage the DNA by interacting with the DNA molecule or DNA-related proteins. Titanium dioxide nanoparticles can enter the nucleus[50], where they induce intracellular protein clumps, leading to inhibition of various processes such as replication, transcription, and cell proliferation[51]. Alternately, DNA damage may develop due to the nanomaterial interaction with other biological proteins involved in cell division. Moreover, studies show that they may lead to a cascade of reactions resulting in genotoxicity, which creates oxidative stress, inflammation, and abnormal signalling responses. Nanoparticle exposure has been associated with sperm DNA damage and male infertility[52]. Badouard and coworkers found that ROS may lead to the production of oxidized DNA adducts leading to the creation of abasic sites, which further leads to destabilization of DNA structure and single-strand breaks. Furthermore, it is essential for spermatozoa to have a proper balance of ROS with antioxidants for chromosome compaction. Incorrect chromatin compaction has also been linked with male infertility as loss of fertility is directly related to the incorrect chromatin compaction caused by nanoparticle- induced oxidative stress[53]. Additionally, nanoparticles may cause oxidative stress and DNA damage in follicular cells and oocytes *in vitro*. Courbiere and co-workers, found increased DNA damage in the follicular cells exposed to CeO_2 nanoparticles in a dose-dependent manner with the Olive Tail Moment (OTM)[48]. Plasmonic nanoparticles especially gold and silver exposure to zebrafish (*Danio rerio*) larvae and adults had reported DNA damage along with the production of ROS[54].

4.4 Cell Apoptosis

Cell self-destruction, known as apoptosis, is a regulated cell death that happens in multicellular organisms as a way to keep the inner environment (homeostasis) in balance[55]. Changes in cell shape, death of cells, and formation of apoptotic bodies are all characteristics of this process. When apoptotic bodies are phagocytosed, this phagocytosis protects the surrounding cells from being damaged by the discharged cellular contents[56]. Numerous experiments have shown that NPs are capable of causing apoptosis in cells. The mitochondrial pathway, which is triggered by the mitochondria implosion in membrane potential and higher ROS levels, causes apoptosis[14]. Another research found that Ag NPs reduced mitochondrial function, causing the cells to go through apoptosis and necrosis[57]. The literature reveals that certain NPs are able to modulate the BAX and BCL-2 genes expression, which is associated with apoptosis[58]. The graphene also activated Bim and Bax, however, the various outcomes may come from distinct materials, varying particle sizes, and differing surface treatments. MAPK (JNK, ERK, and p38) and TGF-β cascades have been found to be activated by pristine- graphene-treated cells[59]. Human kidney cells (HEK 293) when exposed to SiO_2 nanoparticles for 24 hours at a dose of 100 mg/l and 200 mg/l resulted in cell apoptosis via ROS mediated DNA damage along with activation of p53[60]. Human keratinocyte (HaCaT) cells and human lung carcinoma cells (A549) when exposed to Chromium (III) oxide (Cr_2O_3) led to activation of caspase 3 showing decrease in cell viability caused by the apoptosis[61].

5. Nanoparticle Induced Reproductive Toxicity

The existence of both female and male organisms, as well as their unborn babies, may be at risk from nanoparticle exposure. Blood, placenta, and epithelial barriers exist to protect reproductive tissues, yet nanoparticles may cross all three and accumulate in the reproductive region and damage reproductive organs (such as the testes, epididymis, ovary, and uterus), the accumulation of

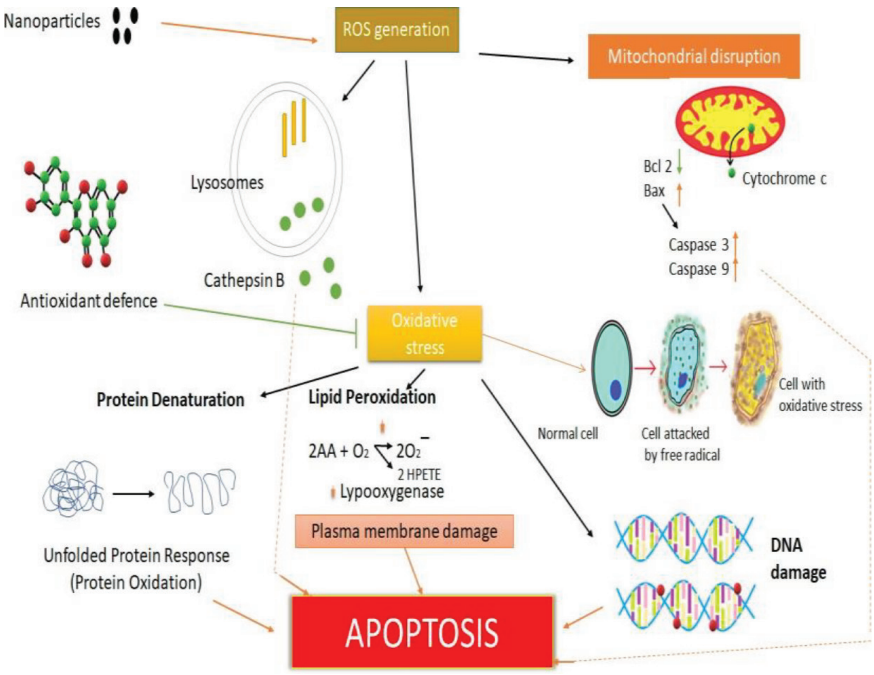


Figure 3. Different mechanisms of nanoparticle toxicity.

nanoparticles harms the cells such as Germline Stem Cells (GSCs), Sertoli Cells (SCs), Leydig Cells (LCs), and cause reproductive health complications, which negatively affects semen quality, number, morphology, and motility, as well as declines the number of mature oocytes and shown complications affecting primary and secondary folliculogenesis[62] (Table 2).

5.1 Male Reproductive Toxicity

Environmental stressors such as toxins, chemicals, and NPs may be a concern to male reproductive health. Toxicity studies have investigated the particle distribution in different organs to trace the movement of NPs throughout the body. Because of their tiny size, nanoparticles are able to collect in the testes, prostate gland, and epididymis through the blood or via direct drug delivery to target organs, leading to organ toxicity[14]. Many investigations have shown that TiO_2 has negative effects on the reproductive system[63]. Certain NPs are excellent for both antibacterial and cancer treatment since they have the ability to generate Reactive Oxygen Species (ROS), DNA damage, and apoptosis, but there is considerable controversy about how easily they enter cells, and how much they accumulate in living creatures[64]. Research has shown that NP exposure may harm male reproductive health, which would lower fertility[65].

5.1.1 Potential Adverse Effect on Reproductive Organs Weight

Nanoparticles result in undue harmful damage to the reproductive organs, change in organ andbody weight is proof of the devastation inflicted on the body by chemical exposure. However,in a study where Nickel Nanoparticles (Ni NPs) in a dose dependent way (5, 15, 45 mg/kg B.W.) were exposed to fully grown adult rats, a decrease in body weight along with an increase in epididymis to body weight ratio was reported[66]. Recent research established that nanosized silver nanoparticles (Ag NPs) were hazardous to the male rat reproductive system. In male rats,subcutaneous exposure to Ag NPs at a dosage of 50 mg/kg B.W. over 4 weeks resulted in a reduction in body weight, while treatment to the same dose for 1 week resulted in a drop in therelative weight of the epididymis and testis[66]. As a result, it is clear that the effect of NPs on the body as well as organ weight depends upon dosage and time. Oral administration of ZnO NPs (50, 150, or 450 mg/kg/B.W.) to lab mice for 2 weeks substantially decreased body weight, and increase relative testicular weight in a dose-dependent manner, whereas relative epididymis weight increased more greatly in mice who received ZnO NPs at 50 and 450 mg/kg

B.W. than in mice who received ZnO NPs at 150 mg/kg/B.W[67]. Oral administration of carbon nanotubes

(SWCNTs and MWCNTs) at 10 mg/k.g./B.W. and 50mg/k.g./B.W. has also evoked a decrease in body weight and epididymal weight were also reported to be decreased upon exposure of high dose of SWCNTs[68].

5.1.2 Nanotoxicity on the Testes

Some *in vivo* experiments have indicated that NPs can accumulate in animal testes, and the particles display restricted dissemination in cells. In the experiment, Kunming albino mice were given a dose of silicon NPs coated in sodium chloride or sodium iodide and exposed to doses of 0-225 mg/kg/B.W. for 96 hours, the results showed some accumulation of NPs in the testes, however there were no adverse alterations in animal cells or in mortality[69]. Sliver nanoparticles (Ag-NPs) were found effect sexual behavior, distort the testicular basic histomorphology, altered testicular functions when exposed at the dose of 40mg/kg/B.W. for 35 days via injection[70]. Silver nanoparticles intravenously administered in male rats have altered luteinizing hormone concentration in plasma and sex hormone in testes[71]. Water soluble carbon nanotubes (MWCNTs) exposure to the male mice via intravenous route has resulted inincreased oxidative stress, decreased thickness of seminiferous tubules along with accumulation in testis[72]. Lead selenide nanoparticles (PbSe NPs) exposure to male rats via intraperitoneal route at 10mg/kg/B.W./ week for 60 days has resulted in the accumulation of nanoparticles in testis, increase in oxidative stress, Endoplasmic Reticulum (ER) and mitochondria mediated cell apoptosis alongside decreased fertility[73]. Testicular toxicity has been associated with immune compromise and excessive inflammation due to exposure to zincoxide nanoparticles (ZnO NPs), nickel nanoparticles (Ni NPs), titanium dioxide nanoparticles (TiO_2 NPs), manganese dioxide nanoparticles (MnO_2 NPs), multiwalled carbon nanotubes (MWCNTs), and other forms of pollutants[3,72,74].

5.1.3 Nanotoxicity on Sertoli and Leydig cells

Silver nanoparticles (Ag NPs) has reported mammalian reproductive toxicity on a primary culture on mouse Sertoli cells *in vitro* as they lead to increased oxidative stress, activation of p53, bcl-2 repression and DNA damage in Sertoli cells along with a reduced number of Sertoli cells[75]. Graphene oxide (size 20 and 100 nm) exposure on Leydig cells (TM3) and Sertoli cells (TM4) has caused oxidative stress, DNA damage, loss of mitochondrial membrane potential, and finally apoptosis due to EGFR/AKT pathway regulation[76]. Gold nanoparticles (Au NPs) intravenously administered in male mice BALB/c mice has accumulated in testis and shown an adverse effect on the male reproductive system including testicular histology changes. Au NPs were found to be internalized into lysosomes of TM3 Leydig cells, increase ROS, disrupt cell cycle S phase and further cause toxicity and DNA damage[77]. Copper nanoparticles (Cu NPs) administrated via intraperitoneal route with 0.5 ml of 20 and 40 mg/kg/B.W. for 3, 6 and 9 days has resulted in decreased body weight and increased in coefficient reproductive organ weights (Testis, epididymis, head epididymis tail, seminal vesicle and prostate gland) and significant weight of tunica albuginea was also found to be significantly increased[78]. Silver nanoparticles (Ag NPs) at dose of 10-40 µg/ml for 24 hours[27], titanium dioxide nanoparticles (TiO_2 NPs) dosage 5-15 g/ml for 6 to 24 hours[79] had accumulated in the cytoplasm and nuclei of Sertoli cells. Titanium dioxide exposure at 5-30 µg/ml for 12-48 hours has decreased cell viability and caused certain morphological changes such as cell and nucleus shrinkage, fragmented chromatin, swollen mitochondria, and endoplasmic reticulum expansion and microfilament network disorientation[79]. Ultrastructural changes, such as disturbance of cytoskeletal filaments and chromatin fragmentation, lead to Sertoli cell autophagy, a pro-survival method that gives the cell a possibility to self-repair while also promoting cell death in conjunction with apoptosis to expedite the removal of damaged cells[80]. Nanoparticles are also found to have toxic effects on the Leydig cells whose primary function is the production of testosterone and it must be properly regulated as insufficient or excessive production is found to be detrimental[81]. Carbon black nanoparticles, Diesel exhaust nanoparticles of size 14-70 nm at dosage of 10 or 100 µg /ml for 24 to 72 hours[82], Titanium dioxide nanoparticles of size 6-7 nm at a dosage 10-40 ug/ml for 24 hours[82] and Zinc oxide nanoparticles (70 nm for 5-20 µg/ml for 3-24 hours) has led to a change in the testosterone biosynthesis as the accumulation of these NMs have been observed in the Leydig cells cytoplasm as well as nuclei.

5.1.4 Nanotoxicity on Seminiferous Tubules and Spermatogenesis

Spermatogenesis occurs in the seminiferous tubules and ROS may be produced in this process, damaging sperm cell DNA. In experiments conducted by Takeda and coworkers (2009), pregnant mice who received subcutaneous

injections of titanium dioxide nanoparticles (TiO_2-400 mg dosage) measured less than 300 nm in diameter were shown to have reduced Sertoli cell count, distorted testicular morphology, distended seminiferous tubules, and significantly decreased sperm production[83]. Further research discovered that a single treatment of carbon NP (14nm) intratracheal injections (at a total dosage of 400 mg) in pregnant ICR mice at days 7 and 14 of gestation induced degeneration of germ cells in the seminiferous tubules, as well as a decrease sperm production in male offspring[84]. An *in vivo* mouse study found that after an intratracheal delivery of cobalt NPs at a dose of 0.1 mg once a week for 10 weeks, the seminiferous tubules showed changes in size. Exposure of silver nanoparticles at different doses (3000 mg/kg/B.W., 4000mg/kg/B.W., 5000mg/kg/B.W., 6000mg/kg/B.W.) for 5 consecutive days and observed for 30 days afterwards[85], and 20 µg/kg/B.W./day for 90 days[86] has resulted in adverse hypertrophic seminiferous tubules along with necrosis and degradation of spermatogenic cells and ultrastructural alterations. Animal studies have shown that when injecting mice with multi-walled carbon nanotubes, levels of ROS (Reactive Oxygen Species) rise and the thickness of the epithelium in the tubules increases, which may take up to 90 days to heal[87]. The 90-day test period didn't show any changes in the adult mice's sperm count, quality, or testosterone levels, making them viable and able to create healthy pups.

5.1.5 Nanotoxicity in Germline

Reduction in testicular weight is because of the demise of germ cells and owing to complications with spermatogenesis. Changes may be because of accumulated NPs, which are hazardous to sperm. In a recent experiment, fresh semen was exposed to Au NPs (9 nm) at the dose 44 mg/ml; some spermatozoa (approximately 25 percent) lost motility, as the gold particles settled with in sperm tails and heads[88]. An experiment recently found that a 20 nm sized Mn_3O_4 NP treatment for a whole year decreased rat sperm quality, lowering the male's fertility[89]. Spermatogonial Stem Cells (SSCs), also considered a critical aspect of sperm development and male fertility, have been used in animal research to evaluate the toxicity of various NPs on the male reproductive system[90]. Nanoparticle accumulation has also resulted in morphological changes such as cell shrinkage, chromatin condensation and necrosis[91]. Silver nanoparticles are also found to inhibit SSCs function like their ability to self-renew stem cell population and generation of self-progenitor cells that proceed through spermatogenesis to form sperm[92,93]. Further exposure of SSCs in a cell line of TM3 and TM4 to silver nanoparticles of size 10-20 nm at 10-40 µg/ml for 24 hours resulted in downregulation of various genes involved in cell proliferation such as (Bcl-6, Etv 5), meiosis (Stra8, Dazl, Sycp3) and differentiation (c-Kit)[27]. Although NPs have been widely used in many sectors and professions, humans living near industrial regions who are exposed to NPs in contaminants in the environment have a desperate need for additional studies for improved health assessments.

5.2 Female Reproductive Toxicity

Several research have been done to investigate the toxicity of NPs within the female reproductive system, which

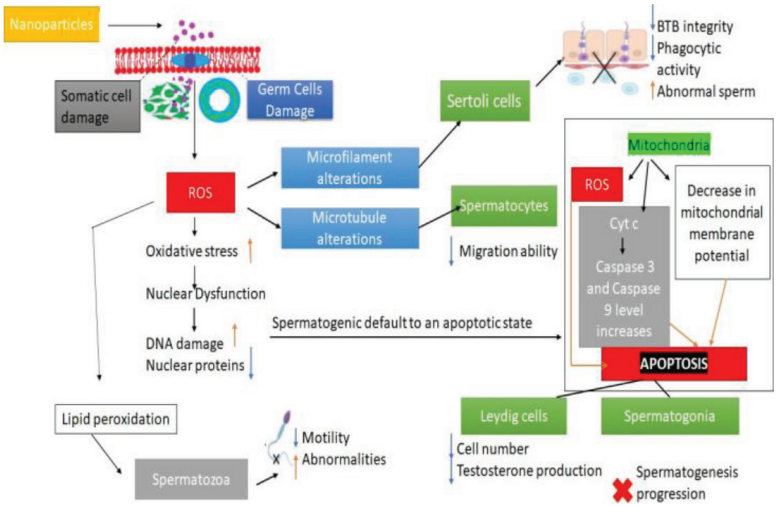


Figure 4a. Nanoparticle-induced male reproductive toxicity.

generally focuses on fertility, teratogenic effects on embryos, and disastrous effects on infants after birth. In mammals, the women's reproductive system consists of organs, such as the oviducts, uterus, vagina, and external genitalia, which are all in close proximity to the hypothalamus-pituitary-ovarian axis[40]. Positive and negative interaction between several elements of the axis is necessary for proper reproductive system function. Xenobiotics may affect normal reproductive system functions like as oogenesis, ovulation, and granulosa cell hormone synthesis, as well as accessory reproductive structures. A study evaluating the effects of nanoparticles in non-pregnant female mice showed that exposure of the animals to TiO_2 nanoparticles (5-6 nm) at a dosage of 10 mg/kg/B.W. causes ovarian dysfunction and changed gene expression profiles and changes were also detected in the expression of immunological and inflammatory reactions genes, oxidative stress, ion transport, proliferation of the cell, transcription and activity of ovary oxidoreductase[39]. These mice had nanoparticles of TiO_2 in their ovarian cells, which caused the cells to be damaged, resulting in a sex hormone imbalance and infertility. Silver nanoparticles (34.9 ± 14.8 nm) were shown to move from mother to baby via the placental and breastfeeding when inserted in pregnant female rats intragastrically via a gavage needle[94]. Research shows that pregnant CD-1 mice exposed to Cadmium Oxide (CdO) nanoparticles (230 μg/m^3) in daily inhalation had their uterine and placental weights changed[94]. And furthermore, changes in expression levels of estrogen receptors α and β (ERα and ERβ) in the uterus and lower amounts of 17β-estradiol resulted in lowered implantation levels[94]. Cd ions produced from CdO nanoparticles may interfere with implantation and blastocyst implantation by affecting the hormonal environment of the womb. Research indicates that hazardous chemicals, such as nano-sized particles, are capable of negatively affecting the female reproductive system and fertility, as has been seen for other dangerous substances.

5.2.1 *Potential Adverse effect on Uterus and Ovaries*

NPs negatively impacted the uterus and ovaries due to secondary deposition which occurred as a result of blood circulation, smaller NPs were discovered to be more suited for accumulation in the uterus[95]. Exposure to ZnO NPs may be hazardous to pregnant women and their babies whether it occurs before, during, or after pregnancy[96]. Additionally, there are suggestions that NPs deposits influence reproductive organs by lingering in the ovaries. Decreased ovarian weight ratios were seen in one experiment, and the scientists attributed these to nickel nanoparticles (Ni NPs). The weight of ovary and uterine body was found to be decreased significantly in the Molybdenum trioxide nanoparticles (MoO_3 NPs) group in comparison with the control group[97]. When Drosophila were exposed to Ag NPs for a short or long period of time throughout adulthood, they were unable to produce eggs and their ovaries were stunted[98]. Silica nanoparticles (20 nm) at dose of 0.2 mM, 0.4 mM and 0.8 mM were found to accumulate inside the cytoplasm of uterine muscle cells, causing ROS generation and apoptosis finally impairing the uterine contractile activity[99]. Fullerenol nanoparticles ($C_{60}OH_{24}$) were found to decrease the relaxant effect of Dimethyl Sulfoxide (DMSO) on rat uterus spontaneous contraction[100]. Copper nanoparticles (Cu NPs) dosage via intraperitoneal injection for 14 days at dose of 3.12, 6.25 and 12.5 mg/kg/d had resulted in uterus damage, and a decrease in relative uterine weight[101]. Liu and coworkers have studied the amplified effect of silica nanoparticles (Si NPs) on the cadmium chloride ($CdCl_2$) induced reproductive toxicity on ovaries when adult female zebrafish were exposed to dose of 1 μmol/L $CdCl_2$ and/or 25 μg/mL Si NPs for 30 days. The results observed reduced ovarian quality, less count of mature oocytes and malformed offsprings[96]. Exposure of CdSe/ZnS QDs in female mice has caused severe adverse effects on ovarian functions, oocyte and fertility[103]. Titanium dioxide nanoparticles (TiO_2 NPs) administration in female NMRI mice at a dosage of 100mg/kg/bw via oral route had caused histological alterations in ovaries including degeneration and low count of the ovarian follicles[102].

5.2.2 *Effects on Oocytes and Granulosa cells*

The three layers comprising granulosa, thecal, and zona pellucida acts as a protective barrier and serve to keep extraneous chemicals from accessing the developing oocyte during follicular development[90,115]. Premature oogenesis is caused by several nanoparticles accumulating in the ovaries. The results of both *in vitro* and *in vivo* studies showed that when nanoparticles of various sizes are introduced into female germ cells, they accumulate inside them and initiate a variety of responses, such as apoptosis[104], inflammation, and the disruption of signal transduction. Several recent investigations have demonstrated that mammalian oocytes are stimulated by gold, silver, and gold-silver alloy nanoparticles, all of which have been identified in various phases of oocyte maturation and results show that silver

and gold NPs both accumulate in cumulus cell layers and oocytes[105]. Research studies found that QDs TF and QDs are both reproductive toxicants because of their effect on antral development of oocytes, disruption of the somatic and germ cell signaling pathways, and the dysfunction of cumulus cells, respectively[106]. In other studies, researchers have found that, as dosage goes up, the distribution of Cerium oxide (CeO_2) nanoparticles in follicular cells by endocytosis and the accumulation of the nanoparticles around the *Zona pellucida* are observed in oocytes in a dose- dependent manner along with DNA damage and oxidative stress[48]. Mouse oocytes that were exposed to CeO_2 nanoparticles at very low concentrations (0.01mg/l) had a decreased fertilization rate when they were grown in a medium containing nanoparticle, as compared to control oocytes[49]. Female rats were exposed to 15 and 45 mg/kg/B.W. of nickel nanoparticles (approx. 90 nm) in *in vivo* research, and toxicity in the ovaries was seen, as the mitochondria swells and cristae disappeared, with ER enlargement as well[104]. Ovaries collected from the pups of ZnO nanoparticle-exposed female mother mice at a dose of 16 mg/kg/BW resulted in DNA damage of primordial follicles leading to reduced follicle assembly[107]. When treated to TiO_2 nanoparticles (1.25, 2.5 and 5 mg/kg/BW for 60 days), female mice lost more primordial, antral follicle, and corpora lutea than controlled mice, and further showed increase in the atretic follicle. TGF-β1, TGF-βR1, PTEN, and FOXO3A proteins levels are increased, whereas PI3K, AKT, p-mTOR, p70S6K, p-p70S6K1, rpS6, TSC1 and TSC2 protein levels are found to be decreased.[108] All of the above-mentioned elements are engaged in signaling pathways, such as TGF-/PI3K/AKT/mTOR signaling, which play critical roles in the growth, maturation, and ovulation of follicular structures[108]. A time-dependent reduction in the number of follicles was seen in mice after intravenous exposure to Ag NPs (1 mg/kg BW i.v.) for 1.5 weeks, as well as an increase in ovarian cell death. 15 days following treatment, the scientists found that Figla, Nobox, Lhx8, and Foxo3A mRNA levels were reduced in comparison to the control group, and immuno localization of Lhx 8 was similarly reduced in Ag NP-treated ovaries. The deletion of FOXO3A and the failure of primordial follicle formation in Figla null mice show the importance of these components in follicular development. Carbon nanotubes (MWCNTs) exposure to *Xenopus tropicalis* at dosage of 0.5 and 2.5 mg/L for 56 days had resulted in oocyte formation alteration, and decreased fertilization and embryos survival rate. Administration of nickel nanoparticles has shown decreased activity of Superoxide Dismutase (SOD), Catalase (CAT) and increased Reactive Oxygen Species, MDA as well as increased expression of pro apoptotic proteins such as Fas, AIF, Cyt C, Bax, Bid[104]. However, accumulation of nanoparticles is mainly observed in cytoplasm and nuclei of theca as well as granulosa cells which resulted in ovarian cell apoptosis and antrum formation.

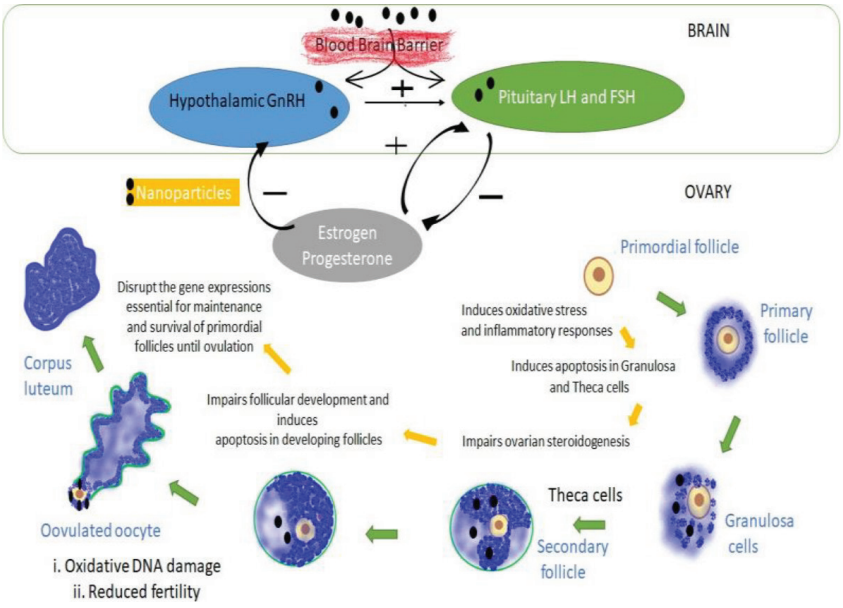


Figure 4b. Nanoparticles induced female reproductive toxicity.

Table 2. Different nanoparticle exposure and their toxicity

Male

Nanoparticles (NPs)	Concentrations	*In vitro/ in vivo* studies	Durations	Model Organisms/ Tissue	Parameters	Results	References
TiO_2 NPs anatase, 5.5 nm size	5-30 µg/ml	*In vitro*	6-24 hours	Sertoli cells	Cell viability, morphological changes, Sertoli cell apoptosis, Immunocytochemistry analysis.	Decrease in viability, increase in morphological changes, increase in apoptosis, Increase in Wnt signaling pathways, Decrease in GSK-3 β levels	[109]
TiO_2 NPs 5-6 nm size	5-30 µg/ml	*In vitro*	24 hours	Sertoli cells	Morphology, cell viability, cytokine expression assay, immunocytochemistry analysis	Decrease in cell viability, Increase in morphological changes, Increase in inflammatory cytokines (IL-lβ, IL-6, TNF-α) Increase in TLR 3 signaling channel.	[110]
TiO_2 NPs 30-90 nm size	1-100 µg/ml	*In vitro*	3-6 hours	Buffalo sperm	Sperm viability, Assessment of membrane integrity, Enzyme assay for damaged membrane detection, DNA fragmentation, Assessment of capacitation	Decrease in viability, Decrease in membrane integrity, Increase in DNA strand breaks, Decrease in acrosomal reaction, Increase in cell and mitochondria distortion	[38]
TiO_2 NPs 20-60 nm size	1 µg/ml 10 µg/ml	*In vitro*	0-72 hours	Human sperm cells	Comet assay, TUNEL assay, DCF assay, Polymorphic DNA profile	Increase in Motility, Decrease in DNA integrity, increase in variation in the polymorphic profiles, decrease in genomic stability, Increase in reactive oxygen species.	[111]
TiO_2 NPs Aeroxide P25	0.1 – 10 mg/ kg/B. W.	*In vivo*	Once a week for 4 consecu-tive weeks	C57BL/ CJ	Sperm motility, head numbers Measurement of ALT, AST activities, Plasma sex hormone levels	Decrease in sperm motility, No significant changes in sperm number of testis or epididymis.	[112]
TiO_2 NPs 25nm Anatase	10- 250mg/ k g/B.W.	*In vivo*	42 consecutive days	Kunmi ng mice	Serum testosterone levels, Epididymal sperm analysis, Histology, Real- time quantitative PCR analysis, Western blotting	Decrease in testosterone level, Increase in histopathological changes, Increase in sperm malformation, Alteration in steroidogenesis-related genes.	[113]
ZnO NPs	10, 100, 500, and 1000 µg/mL	*In vitro*	45 min, 90 min, 180 min.	Human spermatozoa	Viability	Increase in apoptosis at all concentrations.	[114]

Table 2. *Continued...*

Nanoparticles (NPs)	Concentrations	*In vitro/ in vivo* studies	Durations	Model Organisms/ Tissue	Parameters	Results	References
ZnO NPs	0.04 – 16 µg/ml	*In vitro*	24 hours	Mouse Sertoli cell line (TM-4)	Viability assay, Oxidative stress status	Decline in GSH and MDA levels, Disorganization of cell membrane of Sertoli cells, Decline in BTB junction protein, ROS level surge	115
Al_2O_3 NPs 50nm	70 mg/kg B.W.	*In vivo*	75 consecutive days	Wistar male albino rats	Quantitative analysis of testicular gene expression, Oxidative stress, DNA fragmentation, Reproductive and thyroid hormones, Histology	Suppression of the testicular gene expression, Increase in the level of p53, TNF-α, and IL-6, Reduction in sperm motility and sperm number, Increase in 17β-HSD activity and decrease in 17-ketosteroid reductase (17-KSR).	116
Al_2O_3 NPs	70mg/kg/ B.W.	*In vivo*	28 successive days	Wistar rats male	Sperm analysis, Comet assay, Histopathologic al studies, testosterone level and oxidative stress analysis	DNA damage is observed in testicular cells, The histological alteration was marked, Decline in GSH and CAT activities, Decline in serum testosterone levels, ROS generated in excess	23
Ag NPs 60 nm	30mg/kg/B.W.-1000mg/kg/B.W.	*In vivo*	28 days	Spragu e-Dawley Rats	Organ weight, Histopathology	Dose dependent deposition is found in testes	117
Ag NPs	0, 1, and 5 mg/ kg/B.W. 10-40µg/ml	*In vivo/ i n vitro*	Five times postnatal day [PND] 8–21 atintervalof 3 days	Neonatal malemice TM3 lines	Histological examination, RNA isolation, Sperm analysis,MTT and LDH assays	Sperm abnormalitiesincreased, The diameter of theconvoluted tubule shrank, Decrease in cell viability andcell proliferation, Decrease in steroidogenesis-related proteins, theexpression membrane integrity, Increase in ROS,increase autophagosomeformation, Increase in apoptosis-related genes	27
Ag NPs	0, 1, and 5 mg/ kg/B. W. 10-40µg/ml	*In vivo/ in vitro*	Five times postnatal day [PND] 8–21 at interval of 3 days	Neonatal male mice TM3 lines	Histological examination, RNA isolation, Sperm analysis, MTT and LDH assays	Sperm abnormalities increased, The diameter of the convoluted tubule shrank, Decrease in cell viability and cell proliferation, Decrease in steroidogenesis- related proteins, the expression membrane integrity, Increase in ROS, increase autophagosome formation, Increase in apoptosis-related genes	27

Table 2. *Continued...*

Nanopar ticles (NPs)	Concentrations	*In vitro/ in vivo* studies	Durations	Model Organi sms/ Tissue	Parameters	Results	Refe rences
Ag NPs	0, 1, and 5 mg/ kg/B. W. 10-40µg/ml	*In vivo/ in vitro*	Five times postnat al day [PND] 8–21 at interval of 3 days	Neonat al male mice TM3 lines	Histological examination, RNA isolation, Sperm analysis, MTT and LDH assays	Sperm abnormalities increased, The diameter of the convoluted tubule shrank, Decrease in cell viability and cell proliferation, Decrease in steroidogenesis- related proteins, the expression membrane integrity, Increase in ROS, increase autophagosome formation, Increase in apoptosis-related genes	27
Ag NPs coated with PVP	50-400 µg/ml	*In vitro*	15, 30 and 60 min.	Human semen sample	Sperm motility, Viability and morphological abnormalities,	Decrease in cell viability and sperm motility, Increase in morphological deformities, Increase in ROS, Increase DNA damage.	118
Diesel exhaust NPs (DE NPs)	41.73 µg/m^3-152.01 µg/m^3	*In vivo*	5h per day for 8 weeks (5 day per week)	C57BL/ Jcl mice	Sperm count Radioimmunoas say Histology quantitative real-time PCR	No such difference in the body and organ weights, Loss of germ cells, Enlargement of both tubule structures and cells, Decrease in P450scc level.	57
Au NPs 5nm	12.5 µg/ml	*In vitro*	24 h, 48 h, 72 h and 96 h	TM3 L eydig cells mice	Comet assay, *in vitro* cytotoxicity assay,ROS measurement, Testosterone production in TM3 cell lines	Exhibited time and dose- dependent cytotoxicity, DNA damage was detected, Inhibition of testosterone in TM3 cells, Increased ROS were observed	119
Carbon nanotubes	10mg/kg/ B.W. 50mg/kg/ B.W. Both MWCNT and SWCNT used in this	*In vivo*	5 weeks	male BALB/ c mice	Sperm quality evaluation, Oxidative stress parameters, sperm mitochondria indices, testicular histopathology	Epidydimal sperm quality decreased, Increase in ROS level, TBARS level, and GSSG level in higher dosages were reported, Decrement in dehydrogenase activity and ATP content, seminiferous tubules desquamation increased at higher dosages, Higher doses had low litter size	68
TiO_2 NPs anatase 6nm	2.5, 5, and 10 mg/kg of body weight	*In vivo*	90 consecu tive days	CD-1 (ICR) female mice	Histopathology analysis, ROS and DNA oxidation assay, Sex hormone assay, Quantitative real-time PCR	Accumulation seen in ovaries, Decrease in mating rate, pregnancy weight, and number of fetuses, Increase in E2 and FSH while the reduction in P4, LH, and T levels	39

Table 2. *Continued...*

Female

Nanoparticles (NPs)	Concentrations	*In vitro/ in vivo* studies	Durations	Model Organisms/ Tissue	Parameters	Results	References
						Histological alternations like ovary atrophy, impaired primary and secondary follicles, irregularly shaped antrum, increased ROS production causing DNA peroxidation, and finally apoptosis, several gene alterations were seen.	
Zn O NPs 35 nm	500mg/k g/B.W.	*In vivo*	2 weeks before mating to day 4 of lactatio n	Female SD rats	Biodistribution of zinc nanoparticle, Histopathology observation	Increase in zinc concentration in mammary tissue of adult rats, Uterus showed pathological lesions, local necrosis, and histocyte infiltration observed	[96]
Zn O NPs 80.145 nm	10, 20 and 30 µg/ml	*In vitro*	1 day/ 7 days	Mouse ovarian germ cells	Intracellular ROS species generation, Cytotoxicity assessment, Gene expression analysis	Cell membrane integrity was lost, nuclear membrane disruption was also observed, the presence of autophagosomes, damaged organelles are found, cell viability significantly decreased, increased ROS level, Expression level of Nobox and Sycp3 were decreased	[120]
Al_2O_3 NPs	0.5 µg/ml-100 µg/ml	*In vitro*	24hours	CHO- K1 cells	MTT assay, Neutral Red uptake assay. SCE test, CBMN	Cell viability decreased, Formation of vesicles within the cells, loss of lysosomal activity, Reduction in MTT dye reduction in treated cells	[121]
Ag NPs	0.001–100 µg/ml	*In vitro*	24, 48and 72 hours	Ovarian granulo sa cells	Hormonal analysis via RIA and ELISA	Inhibitory effect on basal and FSH-induced P4 secretion by GCs in a dose- dependent manner Decline in E 2 synthesis.	[122]
Iron oxide NPs 28, 30 nm Positively(PEINPs/ negative charged (PAA NPs)	10 and 100 mg/ kg/B. W.	*In vivo*	GD 8,9,10	CD1 mice	Behavioral alteration in the fetus, histology, Mating and second- generation changes	Morphological alteration in uterus in dose-dependent manner, PEI NPs caused both short- and long-term loss, short- term fetal loss, long-term alterations, and loss in F2 generation while PAA NPs show more fetal death earlier rather and less long- term losses	[13]

Table 2. *Continued...*

Nanopar ticles (NPs)	Concentrations	*In vitro/ in vivo* studies	Durations	Model Organi sms/ Tissue	Parameters	Results	Refe rences
UV- Titan 21nm	42mg/m^3	*In vivo*	GD 8–18 for 1 h/day by whole body exposure	C57BL/ 6BomT ac	Gene Expression	Disturb gene expression in offspring	[123]
Calcium phosphat e NPs	10 and 100 µM	*In vitro*	2,4,24,48 and 72 hours	Human granulo sa cells	Apoptosis, cell cycle distribution, Radioimmuno assay	Apoptosis induced, cells in S/ (G2/M) phase increased perceptibly, Nanoparticles accumulated in lysosome, mitochondria, and thecal organelles	[124]
Silica NPs	100 ml, 0.8 mg per mouse	*In vivo*	Two consecu tive days, at GD16 and GD17	Mice	Fetotoxicity and placental dysfunction	Maternal body weight decreased along with uterus body weight, low fetus weight, and structural abnormalities in placenta found	[125]
Ag NPs 50 nm	0, 35, or 66 mg Ag/mous e	*In vivo*	GD 7,8,9	CD1 mice	Histopathologic al analysis, TEM and EDS	NPs accumulation was observed, Morphological changes in embryos at low frequencies, Yolk sac tissue contains silver nanoparticles	[126]
Ni NPs 90 nm	5 mg/kg/B. W 15 mg/ kg/B. W.45mg/ kg/B.W	*In vivo*	10 weeks before mating	Spragu e-Dawley rats	SOD, MDA, GSH, CAT, NO, ROS, RNA isolation, Western blot analysis.	Slight swelling, Crest order of mitochondria observed at high dosage, Ovary tissue ROS was increased in dose- dependent manner, Level of SOD is decreased while MDA level is increased, CAT level raised while NO levels were declined	[66]

Abbreviations- µg- microgram, ml- millilitre, 17 β-HSD-17 Hydroxysteroid Dehydrogenase, 17-KSR-17 Ketosteroid Reductase, Ag NPs- Silver Nanoparticles, Al_2O_3- Aluminum Oxide nanoparticles, ALT-Alanine Transaminase, AST- Aspartate Transaminase, ATP-Adenosine Triphosphate, Au NPs-Gold Nanoparticles, BTB- Blood Testis Barrier, CAT-Catalase, CBMN- Cytokinesis-Block Micronucleus assay, Cd Te QDs- Cadmium Telluride Quantum Dots, COCs- Cumulus-Oocyte Cell Complexes, DCF- Dichlorofluorescein assay, DE NPs- Diesel Exhaust Nanoparticles, E2- Estradiol, EDS- Energy Dispersive X ray spectroscopy, ELISA- Enzyme Linked Immune Sorbent Assay, FSH- Follicle Stimulating Hormone, GCs- Germ Cells, GSH- Reduced Glutathione, GSK-3 Glycogen Synthase Kinase 3, GSSG- Glutathione disulphide, IL-6-Interleukin-6, IL-lβ - Interleukin-lβ, JC-1 - Tetraethylbenimidazolycarbocyanine iodide, JNK- cJun-N-terminal kinase, kg- Kilogram, LDH- Lactate Dehydrogenase, MDA- Malondialdehyde, mg- milligram, MHC II- Major Histocompatibility Complex, MnO_2- Manganese Dioxide Nanoparticles, MTT- 3-(4,5- Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MWCNTs- Multiwalled Carbon Nanotubes, Ni NPs- Nickel Nanoparticles, NO- Nitric Oxide, NPs – Nanoparticles, NR DE- Nanoparticle Rich Diesel Exhaust, P4- Progesterone, LH-Luteinizing Hormone, p53- Tumor Protein, PAA- Polyacrylic Acid Coated, PCR-Polymerase Chain Reaction, PEI- Polyethyleneimine, PND- Postnatal Days, QDs- Quantum Dots, RIA- Radioimmunoassay, ROS- Reactive Oxygen Species, SCD- Sperm Chromatin Dispersion, SCE – Sister Chromatid Exchange, SOD- Superoxide Dismutase, SWCNTs- Single Walled Carbon Nanotubes, Sycp3- Synaptonemal Complex Protein 3, T-Testosterone, TBARS- Thiobarbituric Acid Reactive Substances, TEM- Transmission Electron Microscopy, TiO_2 – Titanium dioxide Nanoparticles, TLR3-Toll-Like Receptor 3, TNF-α Tumor Necrosis Factor, TUNEL-Terminal deoxynucleotidyl Transferase dUTP Nick End Labelling, Wnt- Wingless-Related Integration site, ZnO- Zinc oxide Nanoparticles

6. Endocrine Disrupting Nature of Nanoparticles

Hormones are required for the maintenance and development of the reproductive system, as well as for the subsequent regulation of its functions once established. Numerous *in vitro* and *in vivo* experiments have shown that Endocrine Disrupting Chemicals (EDCs) are able to cause a variety of adverse effects, including testicular cancer, damaged reproductive tissue, prostate cancer, inadequate sperm production (declined sperm counts, low ejaculate volume, large number of abnormal sperm, low number of mobile sperm), and certain other irregularities in male reproductive system. Different nanoparticles exhibit varying effects on male hormonal levels. Male mice's testosterone levels were raised when they received a modest dose (1 mg/kg/dose) of Ag NPs via intravenous injection[27]. In an *in vitro* study, NPs had a detrimental impact on the mitochondria and membrane functions of Spermatogonial Stem Cells (SSCs), thereby allowing Leydig cells to increase the synthesis of steroid hormones. The research discovered that the exposure of male rats to Ag NPs caused substantial down-regulation of a variety of genes associated with sex hormone concentrations, steroidogenesis, and steroid metabolism, including Cyp11a1, Star, Hsd17b3, Hsd3b1 and Srd5a1[71]. Furthermore, Ag NPs impair the activities of sex hormones, lowering sperm counts and diminishing Leydig cells.[75] Male adult rats treated to Manganese Oxide Nanoparticles (Mn_2O_3 NPs) induced decreases in LH, FSH, and testosterone levels[127]. The impact on the development of spermatozoa is particularly significant, since NPs may influence levels of spermatogenesis and sex hormones, affecting fertility. Future research should address the likelihood of epigenetically mediated effects resulting from exposure to NPs that play a crucial role in EDCs. The female reproductive process and conception are generally controlled by balanced hormonal levels. The hypothalamic-pituitary gonadal axis controls reproductive function, fertility, and oogenesis. The hypothalamus, via the secretion of GnRH (Gonadotropin-Releasing Hormone), stimulates the pituitary gland, regulating the amount of estrogen and progesterone, helping to keep menstruation and reproduction functioning normally[128]. When female Wistar rats were injected with Polyethylene Glycol-Black-Poly (Lactic Acid) (PEG-B-PLA) nanoparticles (20mg/kg) from potential day 4 to day 7, the findings revealed disrupted Luteinizing Hormone (LH) release from the pituitary gland, which resulted in reproductive issues throughout maturity[124]. 50 or 100 mg/kg of zinc nanoparticles were shown to regulate the number of neuroendocrine cells in the ovaries by altering gene and protein expression of neuronal components via ingestion in hen's ovarian granulosa cells[67]. The 36-nm nanoparticles were especially found in the cerebrum and cerebellum, and particles smaller than 90 nm were shown to affect GnRH, FSH, and LH balance, including Ni, PEG-B-PLA[104]. Studies have shown that Qf bioconjugates concentrate in the theca and granulosa cells cytoplasm, disturbing mitochondrial inner membrane SSC activity and therefore disrupting the steroid biosynthesis process[103,] Inhaling the nanoparticle-rich Diesel exhaust in pregnant rats lowers levels of P4, elevates LH, and raises corticosterone and estradiol 17 β levels, according to research. Nanoparticles may indirectly influence or create PCOS by changing the amount of GnRH, which would alter the ratio of LH and FSH, producing hormonal imbalances. Finally, we can conclude that nanoparticles can have effects on the hypothalamic-pituitary-gonadal axis in the very two ways-

- The nanoparticles enter the hypothalamus (via penetrating the brain barrier) and pituitary secretory cells, influencing GnRH, LH, and FSH levels and causing a variation in ovarian estrogen and progesterone secretions.
- The theca and granulosa cells, which are cells that are part of the ovary, may collect nanoparticles via blood circulation, which can influence steroidogenesis.

7. Conclusion and Future Perspective

The number of research on the toxicity of NPs in animal reproductive systems is rising although the discipline still needs further active development to reveal the mechanisms of toxicity, dose- dependent effects, safe exposure levels, and other toxicological effects. Although several NPs have been shown to penetrate male and female reproductive organs both in utero as well as in adult individuals, the experiments were conducted with extremely diverse dosages and delivery methods, rendering direct comparisons and decisive conclusions challenging. Nanoparticle induced toxicity has been related to the production of oxidative stress, inflammation, and DNA damage finally leading to apoptosis. In males, there are several studies indicating

the amassing of NPs in the testes, and severely impacting seminiferous tubules, sperm morphology, and motility. In addition, these nanoparticle accumulations resulted in reduced testicular weight and changes in histomorphology of the seminiferous tubules along with lowering testosterone levels, and DNA damage via infiltrating nucleus in spermatozoa and spermatogenic cells. A number of nanoparticles including gold, cadmium, palladium, and titanium dioxide are also found to target the uterus and ovaries in the female animals, however, the findings of investigations in terms of indication of morphological impacts differ extensively. Preclinical studies with precisely articulated parameters have been needed immediately to resolve the situation with reverence to fetal vulnerability to distinct NPs forms, dimensions, surface charges, treatment options, dosage, and pathways of delivery, and the various hazardous effects. The challenge is to conduct better pre-clinical investigations and long-term studies using animal models that are phylogenetic similar to humans having the same exposure conditions mimicking actual scenarios. Researchers, nano toxicologists and scientists should develop protocols to analyze research with the appropriate study designs, addressing critical issues, such as hypothesis and understanding dose-dependent effects on NPs concentrations in the body. Another gap to overcome is the potential to evaluate studies in terms of exposure regimens and outcomes. Nanoparticle translocation across biological barriers is influenced by a variety of factors including the frequency of nanoparticle administration and several other underlying mechanisms. In addition, similar studies for indicator species should be done in vulnerable ecosystems to quantify the consequences on nanoparticles following their intentional and unintentional release into the environment. As a result, all of these considerations should be taken into account when planning future experiments, whether it be in order to ascertain ways to counteract nanoparticle-caused reproductive toxicity or to figure out how nanoparticles are harmful to reproductive organs.

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