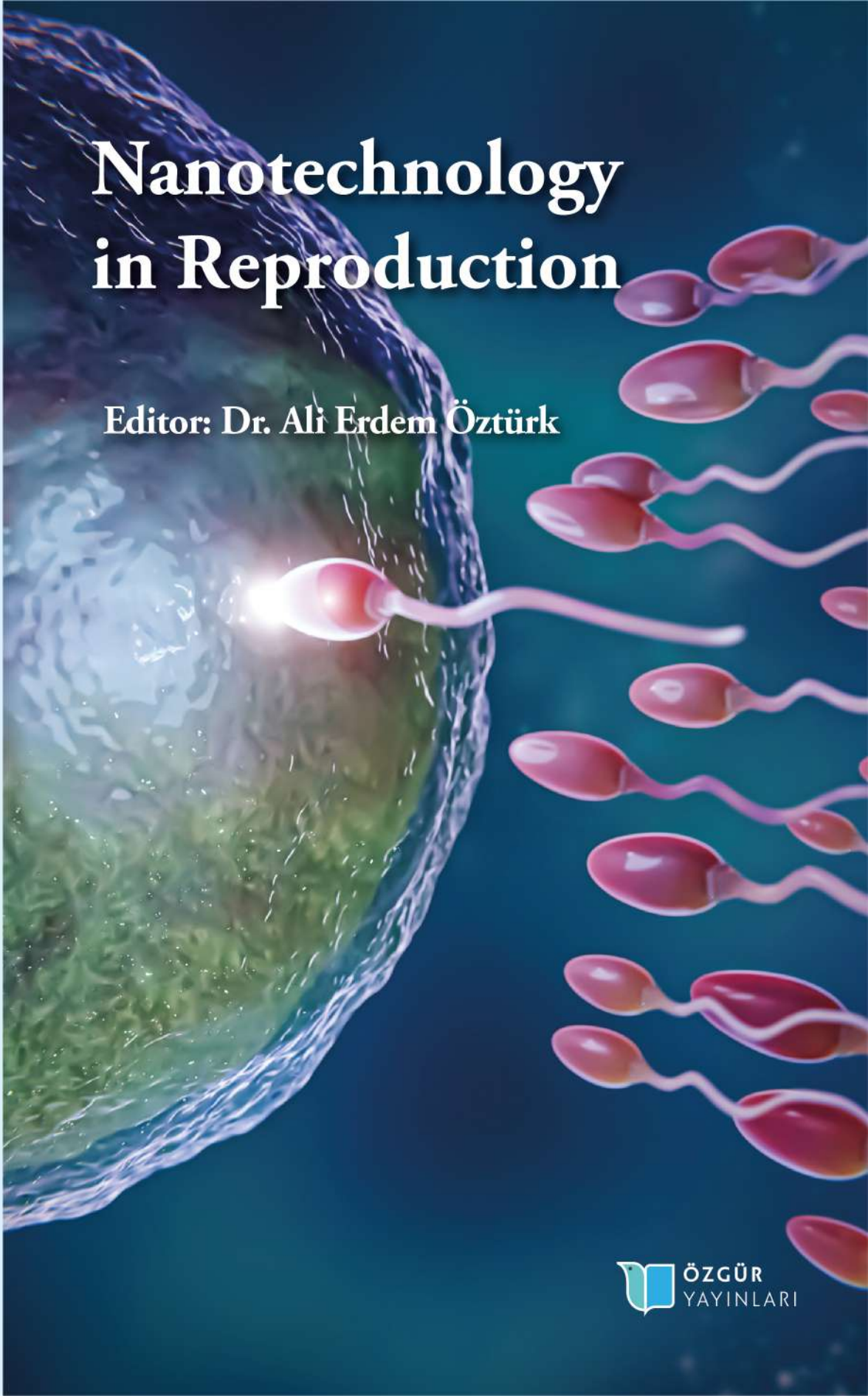
Nanotechnology in Reproduction

Editor: Dr. Ali Erdem Öztürk



ÖZGÜR YAYINLARI

Nanotechnology in Reproduction

Editor:
Dr. Ali Erdem Öztürk

ÖZGÜR
YAYINLARI

Published by
Özgür Yayın-Dağıtım Co. Ltd.
Certificate Number: 45503

15 Temmuz Mah. 148136. Sk. No: 9 Şehitkamil/Gaziantep
+90.850 260 09 97
+90.532 289 82 15
www.ozguryayınlari.com
info@ozguryayinlari.com

Nanotechnology in Reproduction

Editor: Dr. Ali Erdem Öztürk

Language: English
Publication Date: 2023
Cover design by Mehmet Çakır
Cover design and image licensed under CC BY-NC 4.0
Print and digital versions typeset by Çizgi Medya Co. Ltd.

ISBN (PDF): 978-975-447-692-7

DOI: https://doi.org/10.58830/ozgur.pub203

Suggested citation:
Öztürk, A. E. (ed) (2023). *Nanotechnology in Reproduction* Özgür Publications.
DOI: https://doi.org/10.58830/ozgur.pub203. License: CC-BY-NC 4.0

The full text of this book has been peer-reviewed to ensure high academic standards. For full review policies, see https://www.ozguryayinlari.com/

ÖZGÜR YAYINLARI

Contents

Chapter 1

The Positive Effects of In Vivo/In Vitro Suplementation of Nanoparticles on Semen 1

Recep Hakkı Koca

Chapter 2

Nanoparticles as Food Additives and their Possible Effects on Male Reproductive Systems 13

Serkan Ali Akarsu

Ali Doğan Ömür

Chapter 3

Effects of Fullerene Nanoparticles on Semen Quality 53

Tutku Can Acısu

Chapter 4

The Effects of Micro & Nano Pollution on Fish Reproduction 63

Ali Erdem Öztürk

Chapter 5

New Technology in Embryo Manipulations "Nano-Materials" 77

Aslıhan Turhan

Ahmet Bülent Turhan

Chapter 6

Genotoxic Effects of Nanoparticles on Gamete Cells and Their Potential Risks for Next Generations 101
Ayşe Sarı
Muhammet Mücahit Sarı
Emine Öksüz Durmaz

Chapter 7

Nanotechnology in the Purification of Semen 119
Mustafa Bodu
Mehmet Bozkurt Ataman
Mustafa Numan Bucak

Chapter 8

Nanotechnology in the Treatment of Infertility 139
Salih Narlıçay
Mehmet Buğra Kavrak

Chapter 1

The Positive Effects of In Vivo/In Vitro Suplementation of Nanoparticles on Semen

Recep Hakkı Koca[1]

Abstract

Nanoparticles (NPs) are small size molecules. It is divided into organic and inorganic. Organic NPs have many clinical uses, from inoculation to homeostasis, long-term storage and delivery to the system, and absorption of topically used drugs into the skin. Inorganic NPs can be used in intraoperative lymphatic scanning, tumors, and anemia treatments. NPs have a favorable effect on sperm motility, kinematic parameters or membrane integrity rates, they can also be used as antibacterial, antiviral and antifungal in genital organ diseases. There are many studies in which the addition of NPs can be beneficial or harmful to sperm. Researchers have stated that it may be beneficial or cytotoxic depending on in vivo (such as airway, skin or injection) or in vitro use. It has been reported that NPs have positive effects on motility, kinematic parameters, plasma membrane integrity, DNA damage and acrosomal integrity and can be used as an antioxidant. In addition, it has been reported that it can be used in sexsorting, bioimaging and nanopurification, and that more studies on NPs will increase in the coming years.

1. Introduction

Nanoparticles (NPs) are small size molecules (Falchi et al. 2018a) and with sizes in the range of 10-1000nm (Mohanraj and Chen 2006). Although it is not named as a nanoparticle, nanoparticle studies date back to the 1950s. And nowadays scientific studies are more focused on NPs (Kreuter 2007). Because NPs have many particle-based formulations and technologies, they are widely researched preclinically. They are proven methods approved by the Food and Drug Administration (FDA) for the distribute of NPs, based upon the desired application or targeted site, delivered as oral, local, topical

1 Corresponding author, Assist. Prof., Department of Reproduction and Artificial Insemination, Bingol University, Bingöl, Turkey, e-mail: rhkoca@bingol.edu.tr; https://orcid.org/0000-0002-1740-8016.

https://doi.org/10.58830/ozgur.pub203.c934

and systemic administration (Wagner et al. 2006; Torchilin 2014, Min et al. 2015). Treatment and identical NPs generally fall into two categories such as inorganic nanoparticles (e.g., gold, silica, iron oxide, etc.) and organic nanoparticles (e.g., polymeric, liposomes, micelles, etc.) (Anselmo and Mitragotri 2016). It has been stated that inorganic NPs can be used in intraoperative lymphatic scanning, tumors, and anemia treatments. (McCarthy and Weissleder 2008; Huang et al. 2011; Anselmo and Mitragotri 2015). In addition, organic NPs have many clinical uses, from inoculation to homeostasis, long-term storage and delivery to the system, and absorption of topically used drugs into the skin (Anselmo and Mitragotri 2016).

NPs have been highly used in the area of drug for treatment and they contain both lipophilic and some hydrophilic compounds. But hydrophobic drugs remains still a seriously trouble for clinical application (Falchi et al. 2018a).

There are in vivo studies showing that NPs cross the blood testicular barrier more easily than larger molecules of the same element. Researchers attributed the main reason for this to the fact that these particles received an inflammatory response when passing through Sertoli cells (Lan and Yang 2012).

There are many studies in which the addition of NPs can be beneficial or harmful to sperm. Researchers have stated that it may be beneficial or cytotoxic depending on in vivo (such as airway, skin or injection) or in vitro use (Park et al. 2010; Shi et al. 2010; Shi et al. 2011).

2. Positive Effect of Addition of Nanoparticles

Nowadays, there are nanoparticle technologies used for antioxidant, antiinflammatory and antimicrobial properties. (Stevanović et al. 2014; Lee et al. 2016; Kim et al. 2017). This case has enabled for most researchers in its in vivo and in vitro use for reproductive functions research (Barkalina et al. 2014).

NPs have a positive effect on sperm motility, kinematic parameters or sperm functionality, they can also be used as antibacterial, antiviral and antifungal in genital organ diseases (Samad et al. 2009; Mohamed et al. 2017; Raghunath and Perumal 2017; Gurunathan et al. 2018; Hassanein and El-Amir 2018; Siddiqi et al. 2018). Considering the antioxidative effects of NPSs, it can improve sperm functions and male fertility by eliminating the negative effects of reactive oxygen species (ROS) that occur in cooling or freezing reconstituted semen (Khalil et al. 2018). For example; Cerium oxide (CeO2) NPs have used as a ROS scavenger during cooling in ram

semen. There is a study reporting that the addition of CeO2 nanoparticle to ram semen extender has a positive effect on sperm velocity parameters, DNA integrity and plasma membrane integrity even after 96 hours at +5 °C, as well as a significant decrease in ROS level compared to the control group (Falchi et al. 2018b).

Gold NPs(nano-Au) with DNA conjugated and nanosensors have been used to research for hormonal assesment and for accumulating semen production. For imaging method, liposome which is called emulsions have been loaded with large payloads, such as doxorubicin and magnetic NPs to make possible application and reveal with actual imaging tools. Despite these NPs to increase male fertility through intratesticular application, their use in livestock is limited yet. NPs containing antioxidant enzymes to decrease the effect of oxidative stress have the potential to improve male fertility. The benefit of such NPs have showed in a cultured mouse Sertoli cell line, so there is potential for an affect on the fertility of males (Feugang et al. 2019). Although magnetic nanoparticles accumulate in Leydig cells in mice as a result of inhalation of magnetic nanoparticles (Kwon et al. 2008), there are studies showing that Fe3O4, one of the magnetic nanoparticles, can be put into semen extenders as an equivalent to antibiotics in boar (Tsakmakidis et al. 2020). It was also stated that magnetic nanoparticles used to separate X and Y chromosomes in rams before sperm freezing did not have any effect on sperm motility, viability rates, membrane integrity, abnormal sperm ratios and MDA levels (Moradi et al. 2022). It was also shown in a study conducted for fertilization in both unsorted and sexsorted semen that magnetic NPs used to remove DNA damage to the extender in bull semen had a positive effect on semen parameters and did not have a negative effect on the blastocyst cleavage rate (Farini et al. 2016). It was also stated that the addition of magnetic NPs to the semen extender in bulls did not adversely affect motility and other parameters undergo acrosome reaction (Makhluf et al. 2006). In addition, polivinil alcohol (PVA) iron oxide (Fe3O4), which are magnetic NPs, can get into sperm and the intracellular antibody is still active in binding the cellular antigen. The similarity among regular immunocytochemical analysis regarding the localization of proteins and the use of antibody-bound particles can be used as a very good tool for protein localization of antibody-bound particles in living cells (Makhluf et al. 2008). When using magnetic NPs for molecular-based selection of wild swine sperm, results have indicated that the nanoselected sperm cells have raised velocity characteristics with increased progressive motility (Durfey et al. 2017). Others studies have used NPs from FeO conjugated with annexin V in order to designate the apoptosis of boar and bull spermatozoon (Odhiambo et

al. 2014; Feugang et al. 2015). In a study conducted in rats to examine the time-dependent distribution of nano-Au in the testicles, it was stated that residues could be seen in the testicles even after 2 months. (Balasubramanian et al. 2010). Another studied rats that were injected intravenously with a single dose nano-Au showed that there may be residue in the testis even after one day. Even small doses of Nano-Au are given injectable, there may be a large amount of residue in the testis. (De Jong et al. 2008). Nano-Au added Tris-based extender have improved goat semen freezing by retaining the sperm membrane and acrosome integrity post-thawing. In addition, nano-Au has increased antioxidant level and ultimately have scavenged ROS in a goat semen extender (Ismail et al. 2020).

In a study examining the effects of nanoselenium(nano-Se) addition to rooster semen after thawing, it was stated that it increased total motility, progressive motility, sperm viability and total antioxidant capacity, and significantly decreased the abnormal rate of sperm and the level of malondialdehyde, which is end product of lipid peroxidation (Safa et al. 2016). Similarly, it was stated that the addition of nano-Se to the bull semen extender increased the progressive motility, viability rate, membrane integrity rate and TAC level after thawing, in addition to decreasing sperm chromatin damage, sperm apoptosis rate and abnormal sperm rate (Khalil et al. 2019). It has been stated that nano-Se added to the sperm extender in rams reduces lipid peroxidation and DNA damage after thawing, and positively increases sperm motility and membrane integrity (Hozyen et al. 2019; Nateq et al. 2020). In a study conducted in fish, it was stated that nano-Se added to the extender increased total and progressive motility, increased mitochondrial membrane integrity, plasma membrane integrity, and decreased ROS at 72 hours at +4^{o}C (Zhu et al. 2023). In another study given orally to male rats nano-Se, after its reproductive toxicity was induced by bisphenol, there is a study stating that it is beneficial as an antioxidative to the damage caused by toxicity, rearranges COX-2 and ER-2 genes and reduces DNA damage (Khalaf et al. 2019). In addition, orally administered nano-Se also preserved motility and DNA integrity and reduces oxidative stress (Rezvanfar et al. 2013). When given orally in goats, nano-Se increasing antioxidant levels and sperm quality when compared with other compound of selenium (Shi et al. 2010; Shi et al. 2011).

Addition of bull semen extender with nanoparticles of Zinc Oxide(nano-Zn) reduces MDA level and improved mitochondrial activity (Yazdanshenas et al. 2016). In addition, it was stated that it increased plasma membrane and acrosomal integrity and reduced DNA damage after thawing in frozen bull semen (Farhadi et al. 2022; Jahanbin et al. 2015). There is also the

opinion that it will be beneficial in reaching the blastocyst stage by developing the cumulus oocyte complex in the embryo obtained by using bull semen with nano-Zinc added (Jahanbin et al. 2021). In a study given orally to rams, nano-Zn has been shown to increase the level of superoxide dismutase and TAC, motility, viability, semen volume and density, as well as plasma membrane integrity in semen. They are also added it when there was a decrease in the rate of abnormal sperm (Abaspour Aporvari et al. 2018). In addition to the study supplementation of the diet with nano-Zn increase epididymal semen quality, seminal plasma antioxidant levels and superoxide dismutase (SOD) in rams (Zhang et al. 2015). In another study added to the diet of rams, researchers have stated that after 72 hours at +4°C, epididymal sperms increased total motility, viability, and plasma membrane and DNA integrity (Soltani et al. 2022). In diabetic rats, nano-Zinc have been expressed to raise the antioxidant activity in the testicular tissue, to increase spermatological parameters against harmful of oxidative stress (Afifi et al. 2015). In addition, there are studies showing that the addition of nano-Zn to sperm in humans protects the sperm chromatin structure and nano-Zn reduces oxidative stress in testicular tissue in mice (Snow Lisy et al. 2014; Sabanegh et al. 2014; Isaac et al. 2017).

A water-soluble nanotechnological method has been developed without using any solvent or stabilizer to produce C60 fullerene (C60HyFn). Chemically, C60HyFn water molecules are highly hydrophilic (Özer Kaya et al. 2021). In a study conducted to investigate whether hydrated C60 fullerene (C60HyFn) could prevent testicular dysfunction diabetic rats, researchers have stated that by preventing the negative effect of STZ, these NPs increase total motility and preserve sperm density (Bal et al. 2011). It has stated that C60HyFn added to ram semen extender was better for spermatological parameters between 100 nM and 40 μM doses at +5 °C at the end of the 144th hour (Özer Kaya et al. 2021). In addition, there are studies stating that C60HyFn added to semen extender in rams increases total and progressive motility after thawing, decreases abnormal sperm rate, decreases MDA levels, and increases vitamin A, K and amino acid levels (Türk et al. 2022).

It has been known that the progesterone hormone as a NPs increases the acrosome integrity, sperm capacitation and TAC level, as well as increases the expression of PTK PKA, SPACA1 and P38MAPK genes in the study conducted in asthenospermic humans (Baranizadeh et al. 2022).

In addition, there is a study stating that heparin-containing gold nanoparticle may be useful as a biomarker during sperm preparation in in vitro fertilization (Vidya and Saji 2018).

Plant extract nanoformulations such as mint, thyme, and curcumin increase progressive motility, vitality, and plasma membrane integrity ratios and antioxidative level, chromatin decondensation and also decreased apoptosis in goat semen. Curcumin NPs increase sperm motility and antioxidative level, while decreased apoptotic and necrotic spermatozoa in rabbit semen (Saadeldin et al. 2020).

Vitamin E NPs have preserved the acrosome integrity, decreased cell death ratio, and decreased ROS and LPO and hence improved sperm kinematic parameters in red deer epididymal sperm (Sanchez Rubio et al. 2020).

3. Conclusions

As a result, NPs not only have positive effects on sperm motility, membrane integrity, sperm morphology and antioxidant effects on sperm in vivo or in vitro addition, but also can be used in bioimaging, sexsorting and purification on sperm and that more studies on NPs will increase in the coming years.

References

Abaspour Aporvari, M., Mamoei, M., Tabatabaei Vakili, S., Zareei M. & Dadashpour Davachi N. (2018). The effect of oral administration of zinc oxide nanoparticles on quantitative and qualitative properties of arabic ram sperm and some antioxidant parameters of seminal plasma in the non-breeding season. Archives of Razi Institute 73(2): 121-129.

Afifi, M., Almaghrabi O. A. & Kadasa N. M. (2015). Ameliorative effect of zinc oxide nanoparticles on antioxidants and sperm characteristics in streptozotocin-induced diabetic rat testes. BioMed Research International 2015.

Anselmo, A. C. & Mitragotri S. (2015). A review of clinical translation of inorganic nanoparticles. The AAPS journal 17: 1041-1054.

Anselmo, A. C. & Mitragotri S. (2016). Nanoparticles in the clinic. Bioengineering & translational medicine 1(1): 10-29.

Bal, R., Türk, G., Tuzcu, M., Yilmaz, O., Ozercan, I., Kuloglu, T., Gür, S., Nedzvetsky, V. S., Tykhomyrov A. A. & Andrievsky G. V. (2011). Protective effects of nanostructures of hydrated C60 fullerene on reproductive function in streptozotocin-diabetic male rats. Toxicology 282(3): 69-81.

Balasubramanian, S., Jittiwat, K., J., Manikandan, J., Ong, C. N., Liya E. Y. & Ong W. Y. (2010). Biodistribution of gold nanoparticles and gene expression changes in the liver and spleen after intravenous administration in rats. Biomaterials 31(8): 2034-2042.

Baranizadeh, K., M. M. Mahboobian, I. Amiri, H. Tavilani & G. Shafiee (2022). Effects of progesterone nanoparticles on the sperm capacitation and acrosome reaction in asthenozoospermia men. Andrologia 54(1): e14258.

Barkalina, N., Jones, C., Kashir, J., Coote, S., Huang, X., Morrison, R., Townley H. & Coward K. (2014). Effects of mesoporous silica nanoparticles upon the function of mammalian sperm in vitro. Nanomedicine: Nanotechnology, Biology and Medicine 10(4): 859-870.

De Jong, W. H., Hagens, W. I., Krystek, P., Burger, M. C., Sips A. J. & Geertsma R. E. (2008). Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. Biomaterials 29(12): 1912-1919.

Durfey, C. L., Burnett, D. D., Liao, S. F., Steadman, C. S., Crenshaw, M. A., Clemente, H. J., Willard, S. T., Ryan P. L. & Feugang J. M. (2017). Nanotechnology-based selection of boar spermatozoa: growth development and health assessments of produced offspring. Livestock science 205: 137-142.

Falchi, L., Khalil, W. A., Hassan M. & Marei W. F. (2018a). Perspectives of nanotechnology in male fertility and sperm function. International Journal of Veterinary Science and Medicine 6(2): 265-269.

Falchi, L., Galleri, G., Dore, G. M., Zedda, M. T., Pau, S., Bogliolo, L., Ariu, F., Pinna, A., Nieddu, S., Innocenzi P. & Ledda S. (2018b). Effect of

exposure to CeO2 nanoparticles on ram spermatozoa during storage at 4 °C for 96 hours. Reproductive Biology and Endocrinology 16(1): 19.

Farhadi, F., Towhidi, A., Shakeri M. & Seifi-Jamadi A. (2022). Zinc Oxide Nanoparticles Have Beneficial Effect on Frozen-Thawed Spermatozoa of Holstein Bulls. Iranian Journal of Applied Animal Science 12(1): 49-55.

Farini, V. L., Camaño, C. V., Ybarra, G., Viale, D. L., Vichera, G., Yakisich J. S. & Radrizzani M. (2016). Improvement of bovine semen quality by removal of membrane-damaged sperm cells with DNA aptamers and magnetic nanoparticles. Journal of Biotechnology 229: 33-41.

Feugang, J. M., Rhoads, C. E., Mustapha, P. A., Tardif, S., Parrish, J. J., Willard S. T. & Ryan P. L. (2019). Treatment of boar sperm with nanoparticles for improved fertility. Theriogenology 137: 75-81.

Feugang, J. M., Youngblood, R. C., Greene, J. M., Willard S. T. & Ryan P. L. (2015). Self-illuminating quantum dots for non-invasive bioluminescence imaging of mammalian gametes. Journal of Nanobiotechnology 13: 1-16.

Gurunathan, S., Choi Y. J. & Kim J. H. (2018). Antibacterial efficacy of silver nanoparticles on endometritis caused by Prevotella melaninogenica and Arcanobacterum pyogenes in dairy cattle. International journal of molecular sciences 19(4): 1210.

Hassanein, K. M. & El-Amir Y. O. (2018). Ameliorative effects of thymoquinone on titanium dioxide nanoparticles induced acute toxicity in rats. International journal of veterinary science and medicine 6(1): 16-21.

Hozyen, H., El-Shamy A. & Farghali A. (2019). In vitro supplementation of nano selenium minimizes freeze-thaw induced damage to ram spermatozoa. International Journal of Veterinary Science 8(4): 249-254.

Huang, H. C., Barua, S., Sharma, G., Dey S. K. & Rege K. (2011). Inorganic nanoparticles for cancer imaging and therapy. Journal of controlled Release 155(3): 344-357.

Isaac, A. V., Kumari, S., Nair, R., Urs, D. R., Salian, S. R., Kalthur, G., Adiga, S. K., Manikkath, J., Mutalik, S., Sachdev D. & Pasricha R. (2017). Supplementing zinc oxide nanoparticles to cryopreservation medium minimizes the freeze-thaw-induced damage to spermatozoa. Biochemical and Biophysical Research Communications 494(3): 656-662.

Ismail, A. A., Abdel-Khalek, A., Khalil W. & El-Harairy M. (2020). Influence of adding green synthesized gold nanoparticles to tris-extender on sperm characteristics of cryopreserved goat semen. Journal of Animal and Poultry Production 11(2): 39-45.

Jahanbin, R., Yazdanshenas, P., Amin Afshar, M., Mohammadi Sangcheshmeh, A., Varnaseri, H., Chamani, M., Nazaran M. H. & Bakhtiyarizadeh M.

R. (2015). Effect of zinc nano-complex on bull semen quality after freeze-thawing process. Animal Production 17(2): 371-380.

Jahanbin, R., Yazdanshenas, P., Rahimi, M., Hajarizadeh, A., Tvrda, E., Nazari, S. A., Mohammadi-Sangcheshmeh A. & Ghanem N. (2021). In vivo and in vitro evaluation of bull semen processed with zinc (Zn) nanoparticles. Biological Trace Element Research 199: 126-135.

Khalaf, A., Ahmed, W., Moselhy, W., Abdel-Halim B. & Ibrahim M. (2019). Protective effects of selenium and nano-selenium on bisphenol-induced reproductive toxicity in male rats. Human & experimental toxicology 38(4): 398-408.

Khalil, W. A., El-Harairy, M. A., Zeidan, A. E., Hassan M. A. & Mohey-Elsaeed O. (2018). Evaluation of bull spermatozoa during and after cryopreservation: Structural and ultrastructural insights. International Journal of Veterinary Science and Medicine 6: S49-S56.

Khalil, W. A., El-Harairy, M. A., Zeidan A. E. B. & Hassan M. A. E. (2019). Impact of selenium nano-particles in semen extender on bull sperm quality after cryopreservation. Theriogenology 126: 121-127.

Kim, A., Ha, J. H. &. Park, S. N (2017). Selective release system for antioxidative and anti-inflammatory activities using H2O2-responsive therapeutic nanoparticles. Biomacromolecules 18(10): 3197-3206.

Kreuter, J. (2007). Nanoparticles—a historical perspective. International journal of pharmaceutics 331(1): 1-10.

Kwon, J.T., Hwang, S. K., Jin, H., Kim, D. S., Minai-Tehrani, A., Yoon, H. J., Choi, M., Yoon, T. J., Han D. Y. & Kang Y.W. (2008). Body distribution of inhaled fluorescent magnetic nanoparticles in the mice. Journal of occupational health 50(1): 1-6.

Lan, Z. & Yang W.X. (2012). "Nanoparticles and spermatogenesis: how do nanoparticles affect spermatogenesis and penetrate the blood–testis barrier." Nanomedicine 7(4): 579-596.

Lee, G. H., Lee, S. J., Jeong, S. W., Kim, H. C., Park, G. Y., Lee S. G., & Choi J. H. (2016). Antioxidative and antiinflammatory activities of quercetin-loaded silica nanoparticles. Colloids and Surfaces B: Biointerfaces 143: 511-517.

Makhluf, S. B. D., R. Qasem, S. Rubinstein, A. Gedanken & H. Breitbart (2006). "Loading magnetic nanoparticles into sperm cells does not affect their functionality." Langmuir 22(23): 9480-9482.

Makhluf, S. B. D., Abu-Mukh, R., Rubinstein, S., Breitbart H. & Gedanken A. (2008). Modified PVA–Fe3O4 nanoparticles as protein carriers into sperm cells. Small 4(9): 1453-1458.

McCarthy, J. R. & Weissleder R. (2008). Multifunctional magnetic nanoparticles for targeted imaging and therapy. Advanced drug delivery reviews 60(11): 1241-1251.

Min, Y., Caster, J. M., Eblan M. J. & Wang A. Z. (2015). Clinical translation of nanomedicine. Chemical reviews 115(19): 11147-11190.

Mohamed, M. M., Fouad, S. A., Elshoky, H. A., Mohammed G. M. & Salaheldin T. A. (2017). Antibacterial effect of gold nanoparticles against Corynebacterium pseudotuberculosis. International Journal of veterinary science and medicine 5(1): 23-29.

Mohanraj, V. & Chen Y. (2006). Nanoparticles-a review. Tropical journal of pharmaceutical research 5(1): 561-573.

Moradi, M., Hajarian, H., Karamishabankareh, H., Soltani L. & Soleymani B. (2022). Pre-treatment of ram semen extender with magnetic nanoparticles on freeze-thawed spermatozoa. Veterinary Medicine and Science 8(2): 792-798.

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

Odhiambo, J. F., DeJarnette, J., Geary, T. W., Kennedy, C. E., Suarez, S. S., Sutovsky M. & Sutovsky P. (2014). Increased conception rates in beef cattle inseminated with nanopurified bull semen. Biology of reproduction 91(4): 97, 91-10.

Özer Kaya Ş., Güngör. İ. H., Dayan Cinkara S., Acisu T.C., Koca R.H., Akarsu S.A., Can C., Çakir A., Yilmaz İ., Halici M.S., Gür S., Sönmez M. & Türk G. (2021). Effect of different doses of hydrated C60 fullerene nanoparticles on ram semen during cool - storage. Turk J Vet Anim Sci In Press.

Park, E. J., Bae, E., Yi, J., Kim, Y., Choi, K., Lee, S., Yoon, H. J., Lee B. C. & Park K. (2010). Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles. Environmental toxicology and pharmacology 30(2): 162-168.

Raghunath, A. & Perumal E. (2017). Metal oxide nanoparticles as antimicrobial agents: a promise for the future. International journal of antimicrobial agents 49(2): 137-152.

Rezvanfar, M. A., Rezvanfar, M. A., Shahverdi, A. R., Ahmadi, A., Baeeri, M., Mohammadirad A. & Abdollahi M. (2013). Protection of cisplatin-induced spermatotoxicity, DNA damage and chromatin abnormality by selenium nano-particles. Toxicology and Applied Pharmacology 266(3): 356-365.

Saadeldin, I. M., Khalil, W. A., Alharbi M. G. & Lee S. H. (2020). The current trends in using nanoparticles, liposomes, and exosomes for semen cryopreservation. Animals 10(12): 2281.

Safa, S., Moghaddam, G., Jozani, R. J., Kia H. D. & Janmohammadi H. (2016). Effect of vitamin E and selenium nanoparticles on post-thaw variables and oxidative status of rooster semen. Animal reproduction science 174: 100-106.

Samad, A., M. I. Alam and K. Saxena (2009). Dendrimers: a class of polymers in the nanotechnology for the delivery of active pharmaceuticals. Current pharmaceutical design 15(25): 2958-2969.

Sánchez-Rubio, F., Soria-Meneses, P. J., Jurado-Campos, A., Bartolomé-García, J., Gómez-Rubio, V., Soler, A. J., Arroyo-Jimenez, M. M., Santander-Ortega, M. J., Plaza-Oliver, M., Lozano, M. V., Garde J. J. & Fernández-Santos M. R. (2020). Nanotechnology in reproduction: Vitamin E nanoemulsions for reducing oxidative stress in sperm cells. Free Radical Biology and Medicine 160: 47-56.

Shi, L. G., Yang, R. J., Yue, W. B., Xun, W. J., Zhang, C. X., Ren, Y. S., Shi L. & Lei F. L. (2010). Effect of elemental nano-selenium on semen quality, glutathione peroxidase activity, and testis ultrastructure in male Boer goats. Animal reproduction science 118(2-4): 248-254.

Shi, L., Xun, W., Yue, W., Zhang, C., Ren, Y., Shi, L., Wang, Q., Yang R. & Lei F. (2011). Effect of sodium selenite, Se-yeast and nano-elemental selenium on growth performance, Se concentration and antioxidant status in growing male goats. Small Ruminant Research 96(1): 49-52.

Siddiqi, K. S., Husen A. & Rao R. A. (2018). A review on biosynthesis of silver nanoparticles and their biocidal properties. Journal of nanobiotechnology 16(1): 1-28.

Snow-Lisy, D. C., Sabanegh, E. S., Samplaski, M. K., Morris V. B. & Labhasetwar V. (2014). Superoxide dismutase-loaded biodegradable nanoparticles targeted with a follicle-stimulating hormone peptide protect Sertoli cells from oxidative stress. Fertility and Sterility 101(2): 560-567.e563.

Soltani, L., Samereh S. & Mohammadi T. (2022). Effects of different concentrations of zinc oxide nanoparticles on the quality of ram cauda epididymal spermatozoa during storage at 4° C. Reproduction in Domestic Animals 57(8): 864-875.

Stevanović, M., Bračko, I., Milenković, M., Filipović, N., Nunić, J., Filipič M. & Uskoković D. P. (2014). Multifunctional PLGA particles containing poly (l-glutamic acid)-capped silver nanoparticles and ascorbic acid with simultaneous antioxidative and prolonged antimicrobial activity. Acta biomaterialia 10(1): 151-162.

Torchilin, V. P. (2014). Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery. Nature reviews Drug discovery 13(11): 813-827.

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

Türk, G., Koca, R. H., Güngör, İ. H., Dayan Cinkara, S., Acısu, T. C., Erdem Erişir, F., Arkalı, G., Özer Kaya, Ş., Kızıl M., Sönmez M., Gür, S., Yılmaz, Ö., Yüce, A. & Karatepe, M. (2022). Effect of hydrated C60 fullerene on lipid, vitamin and amino acid composition in frozen-thawed ram semen. Animal Reproduction Science: 106939.

Vidya, R. & Saji A. (2018). Naked eye detection of infertility based on sperm protamine-induced aggregation of heparin gold nanoparticles. Analytical and bioanalytical chemistry 410: 3053-3058.

Wagner, V., Dullaart, A., Bock A. K. & Zweck A. (2006). The emerging nanomedicine landscape. Nature biotechnology 24(10): 1211-1217.

Yazdanshenas, P., Jahanbin, R., Mohammadi Sangcheshmeh, A., Aminafshar, M., Vaseghi Dodaran, H., Varnaseri, H., Chamani M. & Nazaran M. H. (2016). Effect of zinc nano-complex on bull semen quality and pregnancy outcome. Animal Production 18(1): 173-181.

Zhang, C., Qin, X., Guo, L., Zhang, G., Zhang J., & Ren Y. (2015). Effect of different Nano-zinc levels in dietary on semen quality, activities of antioxidant enzyme and expression of copper zinc superoxide in epididymis of ram lambs. Scientia Agricultura Sinica 48(1): 154-164.

Zhu, C., Li, L., Liu, Q., Li, J., Peng, G., Zhang, L., Qi, M., Yang, F., Ji H. & Dong W. (2023). Effect of selenium nanoparticles (SeNPs) supplementation on the sperm quality of fish after short-term storage. Aquaculture 562: 738876.

Chapter 2

Nanoparticles as Food Additives and their Possible Effects on Male Reproductive Systems

Serkan Ali Akarsu[1]

Ali Doğan Ömür[2]

Abstract

Nanoparticles (NPs) are substances that are used in many fields, especially in antimicrobial and food additives. Consumable nanoparticles, also known as food nanoparticles, are separated into organic and inorganic nanoparticles. Organic NPs can be classified as proteins, carbonates, phospholipids, and lipids, while inorganic NPs can be classified as silica (SiO_2, E571), zinc oxide (ZnO), titanium dioxide (TiO_2, E171), iron oxide (Fe_2O_3, E172), copper (Cu), gold (Au, E175) and silver (Ag, E174). Organic nanoparticles are not long lasting in the body. However, is it possible to make the same claim about inorganic nanoparticles? Inorganic nanoparticles are employed as food additives, vitamin supplements, and food packaging in the nutrition of both humans and animals. Food nanoparticles that make products brighter, tastier, more shelf-stable, and more antimicrobially resistant influence the liver, renal, digestive, respiratory, and genital systems once they enter the body. NPs can enter the male genital tract, adversely affect the testicles and sperm, and even affect the hypothalamo-pituitary axis, causing hormonal disorders. The effects of inorganic NPs on testes and spermatozoa vary depending on the diameter and composition of this NPS. Studies with some inorganic NPs show that low doses have positive effects on the antioxidant system and harmful effects occur when their concentrations are increased, while some have toxic effects even at very low concentrations. Given all of this information, might consumable nanoparticles be one of the causes of rising male infertility? The aim of this review is to explain how nanoparticles affect the male genital system and sperm quality and to provide insights into whether they might be one of the factors contributing to male infertility.

1 Corresponding author, Assist. Prof., Department of Reproduction and Artificial Insemination, Atatürk University, Erzurum, Turkey, e-mail: serkanaliakarsu@gmail.com; https://orcid.org/0000-0003-4450-6540.

2 Prof.Dr., Department of Reproduction and Artificial Insemination, Atatürk University, Erzurum, Turkey. https://orcid.org/0000-0002-2976-4368

https://doi.org/10.58830/ozgur.pub203.c935

1. Introduction

The term "nanotechnology" describes the interdisciplinary research and development activities that focus on the creation, design, and characterization of nanoscale materials and the systems that are made with these materials. Nanotechnology designs and synthesizes artificial structures which called nanoparticles (NPs) by processing known molecules with different atoms and molecules. Nanoparticles have a changeable surface structure and can be produced from a wide range of materials, including metals, proteins, polysaccharides, and lipids (Samrot, Sean et al. 2020). With nanotechnology, it has become possible to produce materials that are more functional, fast, take up less space, consume less energy, are more durable, cheap and have extraordinary new properties (Bayda, Adeel et al. 2019). Nanotechnology is used in many fields including manufacturing, electronics and computer technologies, the medical and health sector, aerospace researches, environment and energy, defense industry, biotechnology, agriculture and food technologies(Aithal and Aithal 2021) .

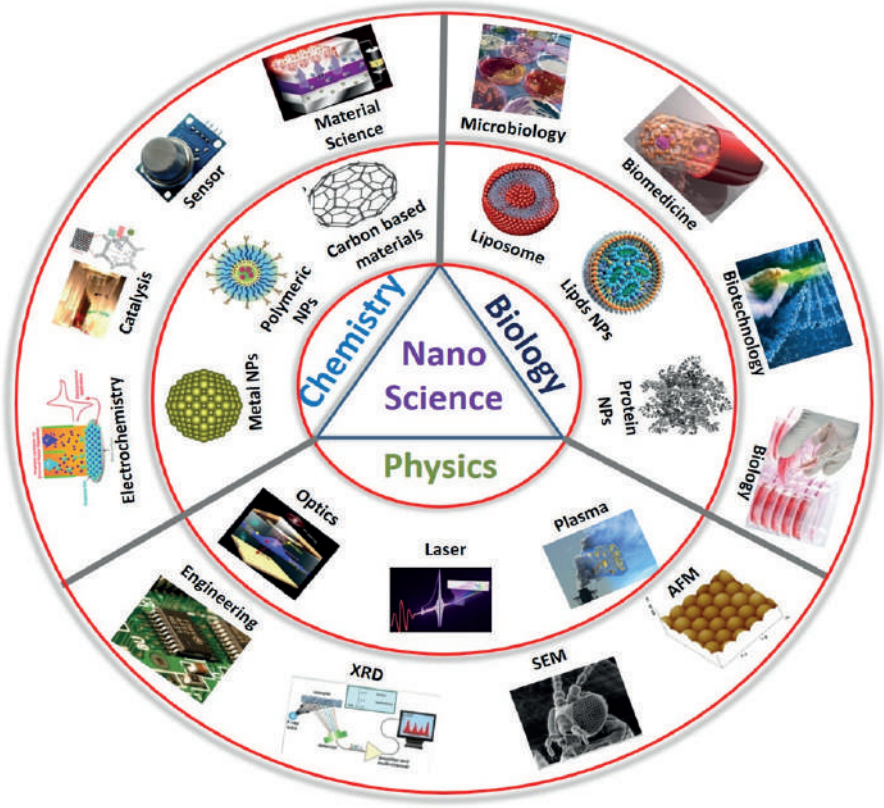


Figure 1. Nanoscience and related fields (Bayda, Adeel et al. 2019).

Nanoparticles (NPs) have various applications such as soil and groundwater remediation, air pollution control, drinking and wastewater treatment. Iron, silver, manganese, magnesium, aluminum and titanium nanomaterials are used in drinking water and wastewater treatment (Van Benschoten, Reed et al. 1994, Agrawal and Sahu 2006). Silver nanoparticles are mostly used for disinfection in the treatment of drinking water, and iron oxide nanoparticles are used to remove arsenic and other dangerous heavy metal pollutants from drinking water (Prathna, Sharma et al. 2018). Nanotechnology can be widely used in livestock and related enterprises. Foods not used for human consumption can be used as animal feed, and this includes important issues such as digestibility of feed, improvement of its quality and spread of diseases (Scott 2007). In the animal nutrition field of nanotechnology, it is mostly aimed at increasing the bioavailability of mineral nanoparticles. Nanoparticles are used to increase the rate of absorption in terms of specific surface area, surface activity, catalytic and efficiency. Thus, it makes it possible to increase the growth performance and utilization rate of the consumed feed in animals. Many nano-scale application systems such as micelles, liposomes, nano-emulsions, biopolymeric nanoparticles, protein-carbohydrate nano-scale complexes, solid nano-lipid particles have been developed in order to use nutrients effectively in the animal body (Chen, Weiss et al. 2006). From another point of view, nanobots that allow the study of the nervous system in animals have also been developed (Opara 2004).

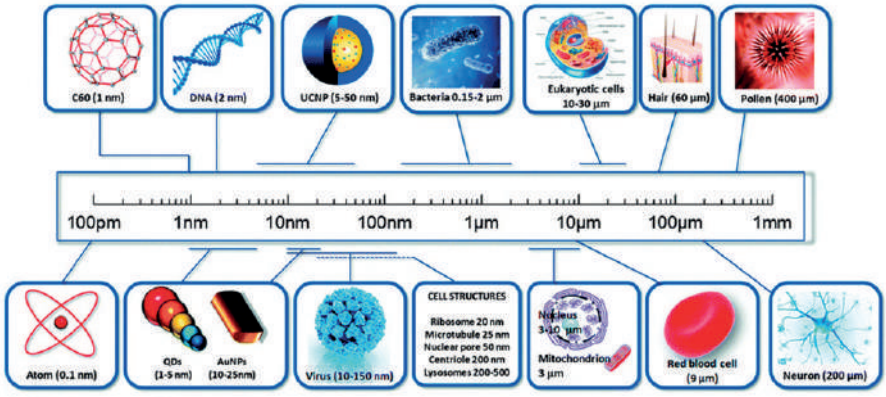


Figure 2. A basic comparison of nanomaterial sizes (Gnach, Lipinski et al. 2015).

Consumable nanoparticles can be classified as organic or inorganic nanoparticles (Moradi, Razavi et al. 2022). Organic NPs can be categorized as proteins, carbonates, phospholipids, and lipids. On the other hand,

inorganic NPs can be classified as silica (SiO_2, E571), zinc oxide (ZnO), titanium dioxide (TiO_2, E171), iron oxide (Fe_2O_3, E172), copper (Cu), gold (Au, E175), and silver (Ag, E174). Organic nanoparticles can be digestable in gastrointestinal tract and they are not bio- persistent. Thats why they are more less toxic than the inorganic NPs. However, when it comes to inorganic nanoparticles both society and the scientific community are concerned about the potential risk associated with oral consumption of inorganic NPs (Aisen, Medina et al. 2002, Moradi, Razavi et al. 2022). Inorganic nanoparticles are implemented in human nutrition as food additives, vitamin supplements, and food packaging (Chaudhry, Scotter et al. 2008, Go, Bae et al. 2017). Nanoparticles are utilized in food technology to improve health, safety, quality, and shelf life. Nanoparticles employed in food technology have beneficial effects on the color, smell and flavor of the products. For instance, TiO_2's naturally white color makes food appear brighter (Setyawati, Zhao et al. 2020) whereas Fe_2O_3 and ZnO nanoparticles are employed as necessary minerals in foods (Voss, Hsiao et al. 2020, Kim, Viswanathan et al. 2022). Furthermore, Fe2O3 nanoparticles are employed as food color pigments, whereas ZnO nanoparticles are used in sunscreens due to their UV radiation protective capabilities. While SiO_2 NPs are used to avoid sediment development in beverages like beer and wine(Antony, Sivalingam et al. 2015), Ag NPs are used to prevent microbial contamination of foods (Wang, Du et al. 2013).

2. Food Nanoparticles in Male Reproductive System

The use of nanoparticles in food and beverages is increasing day by day. This increase exposes living organisms to increasing numbers of nanoparticles through various factors. Nanotechnological developments expose the male reproductive system to nanoparticles (NPs). These NPs are reported to have negative consequences on male germ and somatic cells. (Lee 1998, Brohi, Wang et al. 2017). The male reproductive system is considered susceptible to oxidative stress and inflammation, and both can be used as hallmarks of nanoparticle exposure in other organs (Walczak–Jedrzejowska, Wolski et al. 2013, Azenabor, Ekun et al. 2015). Various nanoparticles induce reactive oxygen species (ROS) as one of the main mechanisms of cytotoxicity (Risom, Møller et al. 2005).

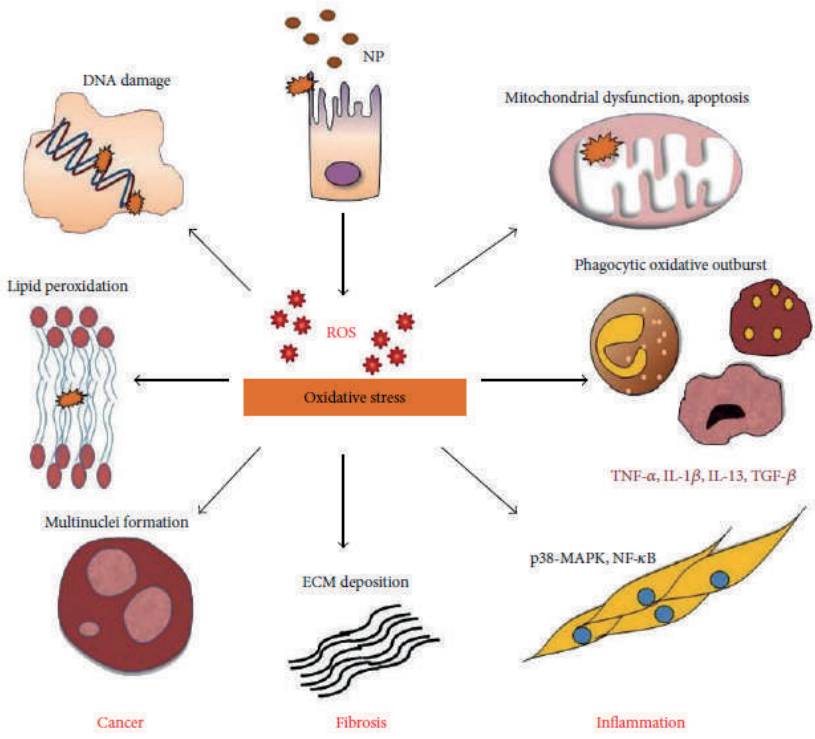


Figure 3. Prooxidant pathway for NP-induced toxicity (Manke, Wang et al. 2013).

It activates transcription factors by affecting intracellular calcium concentrations of nanoparticles and modulates cytokine production through free radical production. Cells exposed to nanoparticles respond to increased oxidative stress with antioxidant defense systems. Transcriptional activation of phase II antioxidant enzymes under mild oxidative stress conditions occurs via induction of erythroid-derived nuclear factor-like 2 (Nrf2). Moderately, the redox-sensitive mitogen-activated protein kinase (MAPK) and nuclear factor kappa-light chain enhancer (NF-κB) cascades of activated B cells produce a proinflammatory response. In addition, extremely toxic levels of oxidative stress lead to mitochondrial membrane damage and cell death (Huang, Aronstam et al. 2010). NP is oxidative in nature, inducing its pro-oxidant effects by reacting with cells and producing intracellular ROS, which includes activation of mitochondrial respiration and NADPH-like enzyme systems (Driscoll, Howard et al. 2001). The prooxidant effects of NP result in the activation of signaling pathways, transcription factors, and cytokine cascade contributing to a diverse range of cellular responses (Manke, Wang et al. 2013). NP-induced ROS induce changes in homeostatic redox state.

NPs activate nuclear factor kappa B (NF-kB) signaling by upregulating the transcription of various proinflammatory genes, including tumor necrosis factor-a and interleukins (IL)-1, IL-6 and IL-8, followed by severe DNA damage and apoptosis (Khanna, Ong et al. 2015).

Table 1. Effects of NPs on the male reproductive system.

Tissue, cell	Experimental Models	Nanoparticles	Authors
Sperm	*Fish*	TiO_2 and Silver NPs	**Carvalhais, Oliveira et al. (2022)**
Testicular tissue	Mouse	TiO_2 and Silver NPs	**Arslan, Keles et al. (2022)**
Sperm	Sea urchin	ZnO NPs	**Kukla, Chelomin et al. (2022)**
Epididymal sperm	Dog	ZnO NPs	**Fayez, El Sayed et al. (2022)**
Testicular tissue	Rat	ZnO NPs	**Hong, Shao et al. (2022)**
Epididymal sperm	Rat	Fe_2O_3 NPs	**Paskeh, Babaei et al. (2022)**
Testicular tissue	Mice	SiO_2 NPs	**Sun, Wang et al. (2022)**
Epididymal sperm	Mice	TiO_2	**Danafar, Khoradmehr et al. (2021)**
Sperm	Bull	Silver-Carbon NPs	**Yousef, Abdelhamid et al. (2021)**
Spermatocyte	Mouse	SiO_2 NPs	**Sang, Liu et al. (2021)**
Sperm	Rabbit	ZnO NPs	**Halo Jr, Bułka et al. (2021)**
Sperm	Bull	ZnO NPs	**Jahanbin, Yazdanshenas et al. (2021)**
Sperm	Human and Rat	CeO_2 NPs	**Cotena, Auffan et al. (2020)**
Sperm	*Fish*	TiO_2 NPs	**Özgür, Ulu et al. (2020)**
Sperm	Swine	Silver NPs	**Pérez-Duran, Acosta-Torres et al. (2020)**
Spermatogonia	Mouse	ZnO NPs	**Pinho, Martins et al. (2020)**
Sperm	*Fish*	SiO_2 NPs	**Özgür, Ulu et al. (2019)**
Sperm	Human	TiO_2 NPs	**Santonastaso, Mottola et al. (2019)**
Testicular tissue	Rat	Silver NPs	**Elsharkawy, Abd El-Nasser et al. (2019)**

Leydig cells	Mouse	ZnO NPs	**Shen, Yang et al. (2019)**
Sperm	Sea urchin	CuO NPs	**Gallo, Manfra et al. (2018)**
Spermatogenic ephitelium	Rat	TiO_2 NPs	**Sharafutdinova, Fedorova et al. (2018)**
Sperm	Buffalo bull	Silver NPs and Multiwalled carbon nanotubes	**Sanand, Kumar et al. (2018)**
Sperm	Bull	Fe_2O_3 NPs	**Caldeira, Paulini et al. (2018)**
Testicular tissue	Mice	CeO2 NPs	**Adebayo, Akinloye et al. (2018)**
Germ cell line		TiO_2 NPs	**Mao, Yao et al. (2017)**
Sperm	Human	Silver NPs	**Wang, Huang et al. (2017)**
Epididymal sperm	Mice	Silica NPs	**Ren, Zhang et al. (2016)**
Epididymal sperm	Mice	TiO_2 NPS	**Smith, Michael et al. (2015)**
Sperm	Rabbit	Silver NPs	**Castellini, Ruggeri et al. (2014)**
Sperm	Buffalo	TiO_2 NPs	**Pawar and Kaul (2014)**
Sperm	Mice	Mo NPs	**Zhai, Zhang et al. (2013)**
Sperm	Human	ZnO NPs	**Barkhordari, Hekmatimoghaddam et al. (2013)**
Sperm	Human	Gold and Silver NPs	**Moretti, Terzuoli et al. (2013)**

2.1. Titanium Dioxide Nanoparticles

Titanium Dioxide nanoparticles (TiO_2) occur naturally as anatase, rutile, and brookite. With its white, bright colors, anatase form is utilized as a preservative and coating in foods. (Irshad, Nawaz et al. 2021). Although the rutile form and the anatase form have similar characteristics, the rutile form is lighter and provides anti-corrosion capabilities and UV light protection. Brookite form, on the other hand, is a rare form and can transform into rutile form at high temperatures due to its unstable structure. (Vijayalakshmi and Rajendran 2012).

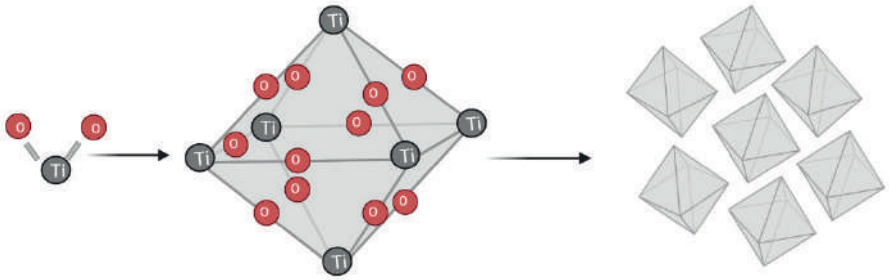


Figure 4. Structure of TiO_2 nanoparticles

On the other side, TiO_2 is used in infrastructure technologies including waterproof clothing, paint, glass coatings, and wallpapers. While it is used in agriculture to prevent pollution and pesticide residues, it is also used in electrical systems, solar panels, fluorescent lights, and refrigerators. It is used in the treatment of cancer and the manufacture of surgical instruments in medicine (Irshad, Nawaz et al. 2021). Although TiO_2 NPs, which helps to acquire a brighter color in meals and is commonly used in the cosmetics industry, is classified as a safe nanoparticle, the International Agency for Research on Cancer (Tsatsakis, Docea et al.) has warned that it can be carcinogenic when inhaled (Hong, Zhao et al. 2015).

TiO_2 nanoparticles are synthesized using a variety of techniques. According to production methods, it can be divided into two categories: physical/chemical method and biological method. The physical/chemical method can be classified as sol-gel method (Sharma, Sarkar et al. 2020), solvothermal method (Ramakrishnan, Natarajan et al. 2018) and hydrothermal method (Wang, Haidry et al. 2020). There are many different production techniques available when using the biological method. Various microorganisms, plants or plant wastes, fungi, and fruit extracts can all be used in this process, which is known as "green synthesis" (Singh, Kumar et al. 2019). While the chemical method is not preferred because it contains many toxic chemicals and can only produce a small number of TiO_2 nanoparticles, biological methods are far more preferred. As a result, TiO_2 nanoparticles, which are more easily and safely produced, are among the consumable nanoparticles. Many researchers have been studying the effects of TiO_2 nanoparticles on various tissues and organs, which can enter the body if foods such as fruit are eaten without washing. One example is research into the effects of TiO_2 nanoparticles on the male reproductive system. When the effects of these nanoparticles on the male reproductive system were investigated, it was discovered that motility decreased, DNA integrity was impaired,

DNA damages were observed, genomic stability of sperm decreased, and intracellular ROS formation increased in human semen exposed to TiO_2 nanoparticles in vivo (Santonastaso, Mottola et al. 2021). Considering these changes in human semen and the damages of TiO_2 nanoparticles in vitro, their effects in vivo have also been a matter of curiosity. In one of the studies based on this curiosity, Hong et al. Administered TiO_2 nanoparticles orally at doses of 1.25, 2.5 and 5 mg/kg in mice during a period of six months. The applications led to the discovery that nanoparticles aggregated in the testis and drastically decreased the quality of semen (Hong, Zhao et al. 2015). Again, in a study where TiO_2 nanoparticles were injected intravenously into rats, they were administered at doses of 5, 25, and 50 mg/kg and at a size of 21 nm. It has been observed that when TiO_2 nanoparticles are used in high doses, they accumulate in the testicles, activate the apoptotic enzyme caspase-3, and alter spermatological parameters (Meena and Kajal 2015). It is quite remarkable that TiO_2 nanoparticles taken in increasing doses have harmful effects on the male reproductive system. Therefore, Jia et al demonstrated that TiO_2 nanoparticles taken in the period from the postnatal 28th day to puberty inhibit the release and conversion of testosterone hormone, which is indispensable in the male reproductive system (Jia, Sun et al. 2014). The effects of consumable nanoparticles on the environment are equally as important as the effects of TiO_2 nanoparticles on humans, which have been the subject of studies with laboratory animals. It is stated that TiO_2 nanoparticles also damage bull semen, decrease sperm viability, membrane integrity and increase DNA damage fragmentation (Pawar and Kaul 2014). TiO_2 nanoparticles' effects on the male reproductive system are not limited to terrestrial creatures. TiO_2 nanoparticles were found to be harmful to both *Capoe trutta* and *Sparus aurata* sperm (Özgür, Ulu et al. 2018, Carvalhais, Oliveira et al. 2022). Application of 400 μg of subcutaneous TiO_2 NPs (<300 nm diameter) during pregnancy leads to a decrease in the number of Sertoli cells and changes in the testicular morphology and changes in the seminiferous tubules in male offspring (Takeda, Suzuki et al. 2009). TiO_2-NPs (21 nm in diameter) in testis cause a decrease in the level of antioxidant enzymes, an increase in the level of caspase 3, which is one of the apoptosis markers, and an increase in the rate of DNA damage (Meena and Kajal 2015). In addition, microarray analysis produces changes in spermatogenesis and gene expression associated with steroid hormones in testes exposed to TiO_2 NPs (Gao, Ze et al. 2013). It has been reported that TiO_2 NPs in mice also activate the MAPK signaling pathways (p38, c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK)) of testicular tissue of mice, leading to male reproductive dysfunction through this pathway (Lu,

Ling et al. 2021). TiO_2 NPs cause germ cell apoptosis by downregulation of Bcl-2, upregulation of Bax, Cleaved Caspase 3 and Cleaved Caspase 9 (Meng, Li et al. 2022).

2.2. Silver Nanoparticles

Three processes are used to create silver nanoparticles: physical, chemical, and biological synthesis. Although the physical method, which uses techniques like spark discharge and pyrolysis, is quick and doesn't require harmful chemicals, but it has several disadvantages, including energy consumption, the possibility of solvent contamination, and less uniform distribution. (Zhang, Liu et al. 2016). Another method, the chemical method, includes methods such as cryochemical synthesis (Sergeev, Kasaikin et al. 1999), lithography (Hulteen, Treichel et al. 1999) and chemical reduction (Zhang, Li et al. 2011). Chemicals used in the synthesis of nanoparticles in the chemical method are toxic and harmful. (Mallick, Witcomb et al. 2004). As a result, while they are simple and inexpensive to produce, they can be harmful to living organisms. (Gurunathan, Han et al. 2015). Despite the disadvantages of the physical and chemical method, the biological method is a good alternative as it is simple, inexpensive and harmless. Along with different biomolecules, bacteria, fungi, and plant extracts are also used in the biological synthesis method. As an example of bacterial production, Ag nanoparticles in spherical, triangular and hexagonal form can be produced from *Pseudomonas stutzeri* isolated from silver mines in Africa. (Klaus, Joerger et al. 1999). In another biological method, *Verticilim sp.* and *Fusarium oxysporum* can produce 25 nm Ag nanoparticles. (Mukherjee, Ahmad et al. 2001, Sastry, Ahmad et al. 2003).

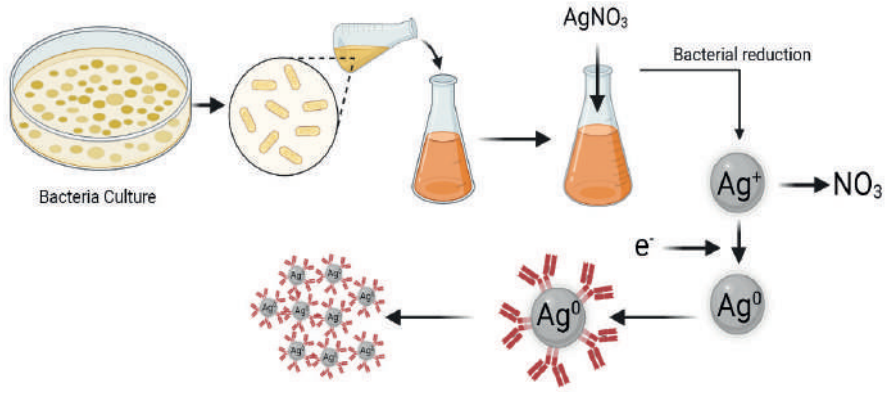


Figure 5. Biological production of silver nanoparticles.

Ag nanoparticles have found use in many areas such as toys, detergents, antibacterial chemicals, cosmetics, etc. Because of its antimicrobial properties, it is used in food packaging and as a food additive (Marambio-Jones and Hoek 2010, Abu-Taweel, Albetran et al. 2021). Antimicrobial effects of Ag nanoparticles are size dependent (Morones, Elechiguerra et al. 2005). When Ag nanoparticles come into contact with bacteria, they stick to the cell surface and alter it in various ways. It inhibits the enzymes that are part of the cellular respiratory chain, damages the cell membrane by forming pits and cracks, and alters the permeability of the cell as a result. (Morones, Elechiguerra et al. 2005, Pal, Dutta et al. 2007). In addition to these effects, metal ions form oxygen radicals and lead to oxidation of cellular structures. (Dastjerdi and Montazer 2010, Sweet and Singleton 2011). Silver nanoparticles are well known for their excellent antibacterial abilities and superior physical properties and are widely used in an increasing number of applications, from household disinfectants to medical devices and water purifiers. (Yu, Yin et al. 2013). The wide usage area of silver nanoparticles also causes increased exposure to it. For this reason, the effects of silver nanoparticle exposure on the reproductive systems of living things as a result of nano-pollution have also been a matter of interest. The effects of Ag nanoparticles, which we are exposed to at every stage of life, on the reproductive system are not different from TiO_2 nanoparticles. In a study, it was revealed that Ag nanoparticles administered intraperitoneally at a dose of 40 mg/kg decreased testicular weight in mice, reduced the antioxidative defense mechanism, and decreased motility in semen (Abu-Taweel, Albetran et al. 2021). While oral administration of 5.3 mg/kg and 13.4 mg/kg Ag nanoparticles to rats reduced testosterone levels, SOD levels, sperm viability, and DNA chromatin integrityi increase MDA levels, in electron density in the nucleus and cytoplasm of spermatogonia were observed (Elsharkawy, Abd El-Nasser et al. 2019). It was noted that 50 mg/kg Ag nanoparticles taken orally during the prepubertal period damaged the testicular tissue and slowed down reproductive development in addition to studies in adult rats (Sleiman, Romano et al. 2013, Mathias, Romano et al. 2015). The increase in ROS production by AgNPs stimulates insulin receptor substrate-1 (IRS-1), protein kinase B(AKT), mechanistic target of rapamycin (mTOR), p53, p21 and caspase 3 as well as SOD and CAT activity as defense mechanisms in the cell and preserves DNA integrity (Blanco, Tomás-Hernández et al. 2018). Furthermore, AgNPs significantly downregulated the hypothalamic–pituitary–gonadal axis (Arisha, Ahmed et al. 2019).

2.3. Zinc Nanoparticles

Along with silicon-based nanoparticles and titanium dioxide nanoparticles (TiO_2 NPs), zinc oxide nanoparticles (ZnO NPs) are thought to be among the three most produced nanoparticles. The production mechanisms of ZnO NPs are similar to other nanoparticles, and physical and chemical methods are widely used. This methods can be clasifies as chemical precipitation (Wang and Muhammed 1999), sol-gel method (Spanhel and Anderson 1991), solid-state pyrolytic method (Wang, Zhang et al. 2002) and solution free mechanochemical method (Shen, Bao et al. 2006). However, the environmental pollution problem that has emerged in recent years has led researchers to the biological production of ZnO NPs, which is much safer for the environment. For this reason, zinc oxide nanoparticles have been produced from many plant extracts and green synthesis studies are still continuing (Elumalai and Velmurugan 2015). Zinc oxide nanoparticles are used in industrial fields such as rubber, paints, coatings and cosmetics (Smijs and Pavel 2011). ZnO NPs were first used in the rubber industry (Kołodziejczak-Radzimska and Jesionowski 2014, Ruszkiewicz, Pinkas et al. 2017). Due to its UV absorption properties, ZnO is also used in personal care products such as cosmetics and sunscreen (Newman, Stotland et al. 2009). Zinc oxide nanoparticles are the form with low toxicity, which effectively overcomes cells and molecules in pathological conditions (Suri, Fenniri et al. 2007).

In general knowledge, zinc plays curicial roles in all body tissues such as bone, skin, brain and muscle. It is also the main component of various enzymatic systems and protein and nucleic acid synthesis mechanisms (Jiang, Pi et al. 2018). With so many important roles to play, ZnO NPs with very small particle sizes can be easily absorbed from the body. As a result, the effects of numerous consumable products and ZnO NPs entering the body on cell systems should be thoroughly explained.

ZnO NPs first bind to the cell membrane when they get to the target tissues and organs. The intracellular cytoplasmic structure leaks out of the cell as a result of ZnO NPs' damage to the cell membrane, which also compromises the integrity of the cell. In addition to the physical damage of the cell, ZnO NPs entering the cell damage the electron transport chain occurring in the mitochondrial membrane and inhibit respiratory dehydrogenase enzymes. ROS are produced when ATPase complexes are damaged and appear as H_2O_2, OH^- VE O_2^{-2}. These reactive oxygen species disrupt mitochondrial function, cause lipid peroxidation, and damage to plasmids and DNA. (Singh, Singh et al. 2018). For all nanomaterials, the mechanism shown below acts in a similar way (Figure 6).

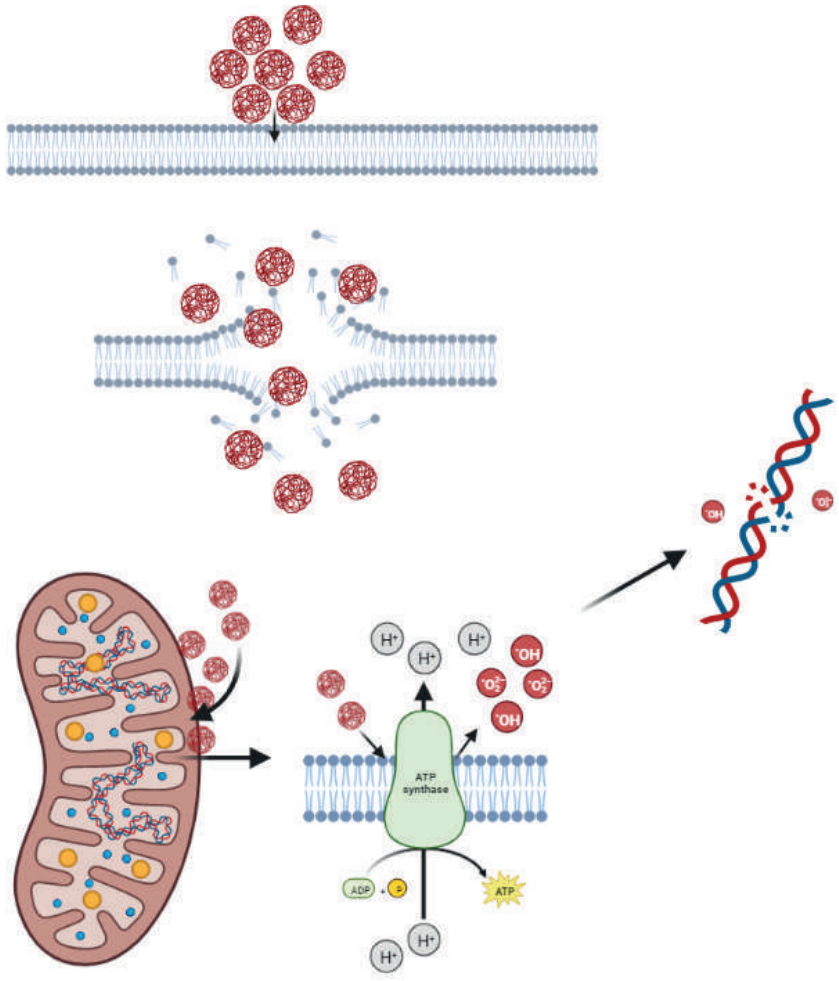


Figure 6. Cellular damage mechanism of ZnO nanoparticles.

This damage caused by high levels of ZnO NPs has drawn the attention of researchers, and numerous studies have been done to study the effects of ZnO NPs on various tissues and organs. ZnO NPs, which have low toxicity under normal conditions, increased sperm motility, antioxidant enzyme activity and mRNA expression level in diabetic rats (Afifi, Almaghrabi et al. 2015). However, when high-dose exposure occurs, ZnO NPs do not reveal such innocent results. YIn a study using high doses of ZnO NPs, different concentrations of ZnO NPs (10, 100, 500, and 1000 μg/mL) were reported to be cytotoxic at all time periods at the highest dose in human semen incubated (45, 90 and 180 min) (Barkhordari, Hekmatimoghaddam et al. 2013). In foods, ZnO serves as an antimicrobial agent as well as a mineral

component. This nanoparticle, which is crucial in preventing microbial contamination, endangers both aquatic and terrestrial life. Similar to earlier studies, it was found that 350 mg/kg of ZnO nanoparticles caused damage to rat testicular tissue, altered epididymal weight, sperm motility, and changed hormone levels in a study in which ZnO nanoparticles were given orally to adult rats (Hong, Shao et al. 2022). Studies conducted both in vivo and in vitro have shown that ZnO nanoparticles have negative effects, including harm to testicular tissue and induction of apoptosis and autophagy in mouse Leydig cells (Liu, Xu et al. 2016, Shen, Yang et al. 2019, Pinho, Martins et al. 2020). ZnO nanoparticles have been shown to be toxic not only in laboratory animals, but also in aquatic organisms. One of these studies was conducted by Oliviera et al. DNA damage was discovered in the sperm of *Paracentroutus lividus* after 30 minutes of exposure to ZnO nanoparticles (Oliviero, Schiavo et al. 2019). ZnO damages the seminiferous epithelium in the testis, reduces the semen density in the cauda epididymis and lowers the serum testosterone level. In addition, caspase 8, caspase 3, Bax, LC3-II, Atg 5, and Beclin 1 levels increase, while Bcl-2 levels decrease (Shen, Yang et al. 2019).

2.4. Iron Oxide Nanoparticles

Iron oxide nanoparticles are found in nature as magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3) and hematite (α-Fe_2O_3) (Ali, Zafar et al. 2016). The forms used in the food industry are maghemite, which has supraparamagnetic properties, and hematite, which can be used as a food dye. Maghemite nanoparticles, especially thanks to their high magnetic properties, bind to unwanted materials in foods and help them to precipitate in the magnetic environment and thus to purify foods (Dong, Chen et al. 2022). This process of purification allows foods to maintain their original flavor (Schwaminger, Fraga-García et al. 2019). In a study using this method, Mierczynska-Vasilev et al. succeeded in removing some proteins from wine (Mierczynska-Vasilev, Boyer et al. 2017). Maghemite nanoparticles (Fe_2O_3) are also widely used in clinical diagnosis for T2-weighted magnetic resonance imaging (Bansal and Bilaspuri) as a contrast agent (Yang, Wang et al. 2022). Fe_2O_3-NPs is also significant for its extensive uses, such as magnetic resonance imaging (Bansal and Bilaspuri) (Ha¨feli, Riffle et al. 2009). α-Fe_2O_3, which is known by the European Union with the code E172, is also used as a colorant in foods (Voss, Hsiao et al. 2020). The cytotoxic effect of iron oxide nanoparticles at high doses is similar to that of ZnO NPs. For this reason, there is a concern that they may have harmful effects on various tissues and organs when consumed in large amounts. In a study where this situation was sorely tested,

mice given 25 and 50 mg/kg Fe_2O_3 nanoparticles experienced an increase in caspase 3 activity and Bax levels, which led to apoptosis in the testicular tissue (Sundarraj, Raghunath et al. 2017). Similar findings were also seen in mouse semen, where 40 mg/kg Fe_2O_3 nanoparticles given intraperitoneally for two weeks reduced motility and density (Nasri, Rezai-Zarchi et al. 2015). Comparable to terrestrial life, aquatic life experienced similar effects, causing destruction to the gonads of *Poecilia reticulata* (Gonçalves, Dias et al. 2021). Oxidative stress has been implicated as a central mechanism for damage caused by Fe_2O_3-NPs. Fe_2O_3-NPs cause a decrease in body weight, gonodo somatic index, sperm motility and viability in mice. In addition, it causes an increase in the level of MDA, which is a lipid peroxidation marker, while it causes a decrease in the levels of CAT, SOD, GSH and GPx and the relative mRNA level (Ahmed, Hussein et al. 2022). Fe_2O_3 NPs cause significant changes in gene expression of mitochondrial transcription factor-A (mtTFA) and dissociation protein 2 (UCP 2) in testicles (Younus, Yousef et al. 2020).

2.5. Copper Nanoparticles

Copper Nanoparticles (Cu NPs) are used as antimicrobial and anticancer agents along with their use in the textile, electronics and chemical industrie. Fertilizers and herbicides containing CuNP are used in various agricultural applications (Cometa, Iatta et al. 2013). Cu NPs are used in animal feeds because of their good antibacterial and growth promoting effects that reduce the incidence of animal diseases (Tamilvanan, Balamurugan et al. 2014). CuNPs show their toxic effect by increasing reactive oxygen species (ROS) (Lin and Xing 2007). CuNPs decrease sperm quality parameters, male hormones, cause testicular damage, increase oxidative stress and apoptosis, decrease antioxidant enzymes and germ cell proliferation, and increase 8-oxoguanine DNA glycosylase-1 (OGG1) and apelin receptor (APJ) expression (Nicy, Das et al. 2022). Cu NPs cause testicular damage, decrease in sperm quality and fructose content, increase in oxidative stress and sperm malformations, and changes in Bax, Beclin, Bcl-2 and p52 expression in rats. Cu NPs cause testicular damage, decrease in sperm quality and fructose content, increase in oxidative stress and sperm malformations, and changes in Bax, Beclin, Bcl-2 and p52 expression in rats (Chen, Wang et al. 2022).

CuO nanoparticles are another type of nanoparticle that can affect an aquatic animal's reproductive system. Gallo et al. discovered that CuO nanoparticles increased ROS formation, resulting in a decrease in sperm viability and mitochondrial membrane potential in *Paracentrotus lividus* semen (Gallo, Manfra et al. 2018). In a different study, researchers found that Cu nanoparticles in *Onchorynchus mykiss* semen were more harmful than CuO

nanoparticles and emphasized that the toxicity or benefit of a nanoparticle depended on its particle diameter or its composition (Garncarek, Dziewulska et al. 2022). In a study conducted in laboratory animals, Cu nanoparticles applied at a dose of 40 mg/kg damaged the rat testicular tissue, causing a decrease in sperm viability and an increase in the amount of abnormal spermatozoa (Al-Bairuty, Taha et al. 2016).

2.6. Silicon Based Nanoparticles

Silicon is the second most abundant element in the earth's crust after oxygen and is used in agriculture because it is beneficial to plants (Epstein 1994). Silicon exists in nature as two forms, crystalline and amorphous, with the same molecular formula (Chen, Liu et al. 2018). SiO_2 nanoparticles are used in the fields of industry, biomedicine, food and environmental protection due to their properties such as good stability, excellent biocompatibility and easy modification (Xu, Wang et al. 2014).

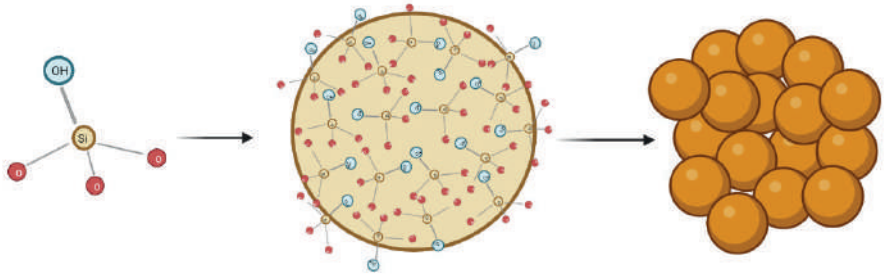


Figure 7. Nanostructure of Silica nanoparticles.

Silica used in foods is numbered with the code E551 and named as synthetic amorphous silica (Sastry, Ahmad et al.). The main usage of SAS are in noodles, soups, creams and coffee creamers as an anti caking agent (Dekkers, Krystek et al. 2011, Gubala, Giovannini et al. 2020). SiO_2 nanoparticles are used in pharmaceutical applications (Li, Barnes et al. 2012), medical diagnostics (Chen, Yin et al. 2012), in vivo imaging (Tu, Ma et al. 2010). When applied to semen, it reduced motility, viability, and DNA integrity; however, when applied to testicular tissue of lab animals, it induced apoptosis and oxidative stress-related cellular damage (Barkalina, Jones et al. 2015, Azouz, Korany et al. 2022, Sun, Wang et al. 2022). SiO_2 NPs cause histopathological changes in testis (Hassankhani, Esmaeillou et al. 2015). SiO_2 nanoparticles in water have a negative impact on marine organisms' reproductive systems, with exposure levels of 50 mg/L having

toxic effects on rainbow trout sperm (Özgür, Ulu et al. 2019). SiO_2 NPs increased lipid peroxidation in rat and decreased the activities of antioxidant enzymes. SiO_2 NPs induced apoptosis, demonstrated by upregulation of Bax and caspase 3 and downregulation of Bcl-2, as well as induction of DNA damage. SiO_2 NPs also caused upregulation of inflammation-related genes such as; IL-1β, TNF-α, NF-κB, cyclooxygenase 2 (COX2) (El-Sayed, El-Demerdash et al. 2021).

2.7. Molybdenum Nanoparticles

Molybdenum is mentioned as an essential trace element for humans and microorganisms. However, adverse effects of high molybdenum content in the diet on metabolism have been reported (Yang, Cui et al. 2011). Mo NPs are used in the electron industry, cutting tools, hard alloys, textiles, microelectronic films, coatings, plastics, nanowire and X-ray tubes. However, these industrial activities negatively affect the lives of people and animals (Chen, Yin et al. 2012, Siddiqui, Saquib et al. 2015). Mo NPs reduce the serum testosterone level in rats and cause histopathological changes in the testis (Asadi, Mohseni et al. 2017). High dose molybdenum administration in rats affected sperm parameters negatively and decreased SOD and GPx levels while increasing MDA levels (Zhai, Zhang et al. 2013). In the in vitro study, Mo NPs showed cytotoxic effect in mouse spermatogonial stem cell line (Braydich-Stolle, Hussain et al. 2005). Molybdenum trioxide (MoO_3) NPs with high toxicity are mainly used in industry, glass and the production of cracking catalysts, hydrogenation catalysts and refractory alloys, and they can significantly threaten public health (Gawande, Goswami et al. 2016). In a study, it was stated that MoO_3 changed the biochemical parameters in the blood and caused histological changes in the uterus (Fazelipour, Assadi et al. 2020). In a study, it was reported that the application of MoO_3 anoparticle caused the irregularity of spermatogenic cells in the seminiferous tubules and a decrease in the number of sperm and sertoli cells (Mirza Mohamadi and Sohrabi 2015).

2.8. Cerium Nanoparticles

Cerium is a member of the lanthanide group and exhibits antioxidant properties as well as catalytic properties (Dahle and Arai 2015, Dhall and Self 2018). CeO_2 NPs are used in various biomedical applications such as protection against radiation damage, retinal neurodegeneration, anti-inflammatory and antioxidant activities (Tarnuzzer, Colon et al. 2005). In one study, CeO NPs decreased blood hemoglobin, PCV and RBC count compared to controls. Additionally, luteinizing hormone (LH) and follicle

stimulating hormones (FSH), prolactin sperm quality parameters were significantly reduced in mice (Adebayo, Akinloye et al. 2018). In another study, it was stated that CeO_2 NPs improved testicular and sperm parameters in rats with diabetes (Artimani, Amiri et al. 2018). It was stated that the addition of CeO_2 NPs to the semen and the uptake of intracellular CeO_2 NPs did not affect the CASA parameters in the short-term storage of ram semen (Falchi, Bogliolo et al. 2016). It was stated that CeO_2 NPs improved sperm quality in rats treated with malathion (Moridi, Hosseini et al. 2018). CeO_2 NPs applied in the cryopreservation of human semen have been reported to improve sperm quality (Hosseinmardi, Siadat et al. 2022). A study in mice showed that CeO_2 NPs (20 mg/kg and 40 mg/kg) increased the Ce element content in the testis, testicular histopathological patterns and sperm DNA damage, while decreasing testicular weight, daily sperm production (DSP) and sperm motility. A remarkable reduction in testosterone levels and marker enzyme activities was noted, with downregulated mRNA expression levels of various steroidogenesis genes such as Star, P450scc, P450c17, 3β-Hsd and 17β-Hsd (Qin, Shen et al. 2019). Another study showed that the tubular diameter, epithelial height and spermiogenesis index were significantly reduced by CeO_2 NPs at 50 and 100 mg/kg doses. Sperm parameters were significantly reduced and also the percentages of immature sperm and sperm with DNA damage increased significantly compared to control. In addition, it was stated that in vitro fertilization and in vitro embryo development rates decreased (Hosseinalipour, Karimipour et al. 2021).

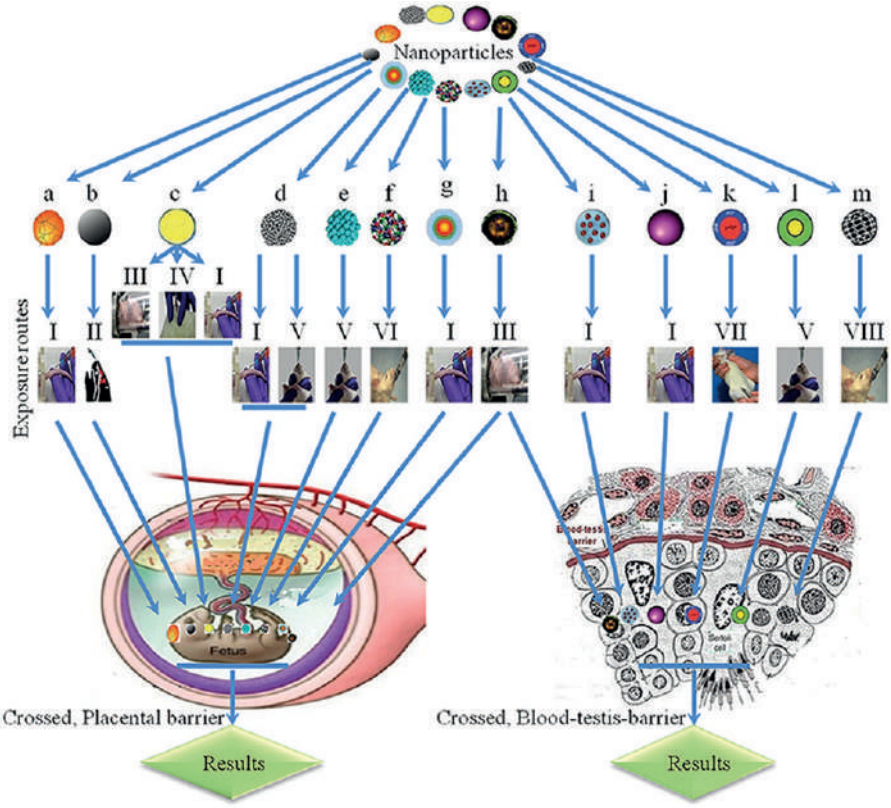


Figure 8. Effects of nanoparticles on male and female reproductive system (Brohi, Wang et al. 2017).

a,[198]Gold-nanoparticles(Semmler-Behnke, Fertsch et al. 2007); b, black carbons(Takahashi and Matsuoka 1981, Kubo-Irie, Oshio et al. 2011); c, titanium oxide(Wang, Zhou et al. 2007); d, single-walled carbon nanotubes(Sugamata, Ihara et al. 2006, Snyder, Fennell et al. 2015); e, platinum(Meng, Yang et al. 2010), f, multi-walled carbon nanotubes(Jackson, Vogel et al. 2011); g, cadmium telluride/cadmium sulfide quantum dots(Mattison, Plowchalk et al. 1990); h, diesel exhaust(Hamada, Suzaki et al. 2003, Hougaard, Jackson et al. 2010, Pietroiusti, Massimiani et al. 2011, Jackson, Halappanavar et al. 2013, Kyjovska, Boisen et al. 2013); i, sodium chloride-modified silica nanoparticles(Philbrook, Walker et al. 2011); j, silicon dioxide(Yoshida, Hiyoshi et al. 2009); k, silica-coated magnetite nanoparticles (rhodamine B isothiocyanate)(Bai, Zhang et al. 2010); l, metal-free polymethyl methacrylate(Kashiwada 2006); m, carbon(Kubo-Irie, Oshio et al. 2011); I, intravenous; II, intranasal; III, inhalation; IV,

subcutaneous; V, oral exposure; VI, by gavage; VII, intraperitoneal; VIII, intragastric.

3. Conclusion & Future Prospects

Nanoparticles show their effects in the biological process in various ways. Basically, the following situations can be mentioned;

- direct association with the cell membrane (Buchman, Hudson-Smith et al. 2019),

- physical effect by removing/destroying the lipid membrane (Tu, Lv et al. 2013, Mensch, Hernandez et al. 2017),

- interaction based on electrostatic attraction (Dickson and Koohmaraie 1989),

- inducing internal signaling pathways that damage the cell (Hussain, Garantziotis et al. 2014),

- releasing toxic ions by binding to proteins and enzymes (Bondarenko, Ivask et al. 2013),

- effect of metal ions on the phospholipid membrane and genetic material (Stohs and Bagchi 1995),

- inhibiting cellular functions (Barras and Fontecave 2011, Macomber and Hausinger 2011).

- the occurrence of oxidative stress conditions in which different key enzymes such as mononuclear iron proteins can be targeted (Sobota and Imlay 2011, Anjem and Imlay 2012) and mutation formation in the organism by the oxidation of DNA bases and deoxyribose by ROS (Galhardo, Almeida et al. 2000, Imlay 2008).

Successful fertilization depends on the healthy production and functioning of reproductive cells. The harmful effects of nanoparticles disrupt the fertilization process. This situation is summarized in the image below.

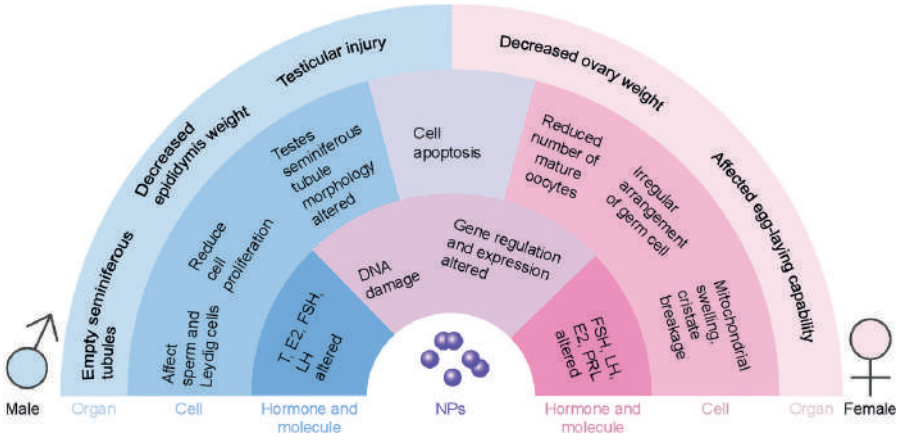


Figure 9. Negative effects of nanoparticles in the fertilization process (Wang, Song et al. 2018).

Consumable nanoparticles' benefits in the food industry have led to their widespread use, and various nanoparticles are involved in every stage of food processing. In vivo and in vitro studies show the effects of these nanoparticles on the reproductive system, which have passed many toxicity tests and have been approved for use. Toxicological studies on a wide range of animals, from laboratory animals to human experiments, aquatic creatures to farm animals, reveal that the reproductive toxicities of nanoparticles vary depending on the dose of use. The harmful effects of existing nanoparticles occur at high doses and frequent exposures. In the light of all this information, when taken in high quantities and on a continuous basis, consumable nanoparticles might be considered one of the major causes of male infertility.

Further advances in reproductive biotechnology may be possible with greater incorporation of nanoparticles into molecular biology techniques. Sperm-mediated gene transfer is an application in which nanoparticles can be loaded with nucleic acids and proteins (Barkalina, Jones et al. 2014). With continued research, it may be discovered that nanoparticles play a much more active role in the reproductive system. However, it should be kept in mind that some nanoparticles can lead to serious negative consequences due to their toxic effects.

References

Abu-Taweel, G. M., H. M. Albetran, M. G. Al-Mutary, M. Ahmad and I. M. Low (2021). "Alleviation of silver nanoparticle-induced sexual behavior and testicular parameters dysfunction in male mice by yttrium oxide nanoparticles." Toxicology Reports **8**: 1121-1130.

Adebayo, O., O. Akinloye and O. Adaramoye (2018). "Cerium oxide nanoparticle elicits oxidative stress, endocrine imbalance and lowers sperm characteristics in testes of balb/c mice." Andrologia **50**(3): e12920.

Afifi, M., O. A. Almaghrabi and N. M. Kadasa (2015). "Ameliorative effect of zinc oxide nanoparticles on antioxidants and sperm characteristics in streptozotocin-induced diabetic rat testes." BioMed Research International **2015**.

Agrawal, A. and K. Sahu (2006). "Kinetic and isotherm studies of cadmium adsorption on manganese nodule residue." Journal of hazardous materials **137**(2): 915-924.

Ahmed, M. M., M. M. Hussein, T. Saber and Y. M. Abd-Elhakim (2022). "Palliative Effect of Resveratrol against Nanosized Iron Oxide-Induced Oxidative Stress and Steroidogenesis-Related Genes Dysregulation in Testicular Tissue of Adult Male Rats." International Journal of Environmental Research and Public Health **19**(13): 8171.

Aisen, E., V. Medina and A. Venturino (2002). "Cryopreservation and post-thawed fertility of ram semen frozen in different trehalose concentrations." theriogenology **57**(7): 1801-1808.

Aithal, S. and P. Aithal (2021). "Green and eco-friendly Nanotechnology–concepts and industrial prospects." International Journal of Management, Technology, and Social Sciences (IJMTS) **6**(1): 1-31.

Al-Bairuty, G. A., M. N. Taha and M. Taha (2016). "Effects of copper nanoparticles on reproductive organs of male albino rats." International Journal for Sciences and Technology **11**(3): 17-24.

Ali, A., H. Zafar, M. Zia, I. ul Haq, A. R. Phull, J. S. Ali and A. Hussain (2016). "Synthesis, characterization, applications, and challenges of iron oxide nanoparticles." Nanotechnology, science and applications **9**: 49.

Anjem, A. and J. A. Imlay (2012). "Mononuclear iron enzymes are primary targets of hydrogen peroxide stress." Journal of Biological Chemistry **287**(19): 15544-15556.

Antony, J. J., P. Sivalingam and B. Chen (2015). "Toxicological effects of silver nanoparticles." Environmental toxicology and pharmacology **40**(3): 729-732.

Arisha, A. H., M. M. Ahmed, M. A. Kamel, Y. A. Attia and M. Hussein (2019). "Morin ameliorates the testicular apoptosis, oxidative stress, and impact on blood–testis barrier induced by photo-extracellularly synthesized

silver nanoparticles." Environmental Science and Pollution Research **26**(28): 28749-28762.

Arslan, N. P., O. N. Keles and N. Gonul-Baltaci (2022). "Effect of Titanium Dioxide and Silver Nanoparticles on Mitochondrial Dynamics in Mouse Testis Tissue." Biological Trace Element Research **200**(4): 1650-1658.

Artimani, T., I. Amiri, S. Soleimani Asl, M. Saidijam, D. Hasanvand and S. Afshar (2018). "Amelioration of diabetes-induced testicular and sperm damage in rats by cerium oxide nanoparticle treatment." Andrologia **50**(9): e13089.

Asadi, F., M. Mohseni, K. Dadashi Noshahr, F. H. Soleymani, A. Jalilvand and A. Heidari (2017). "Effect of molybdenum nanoparticles on blood cells, liver enzymes, and sexual hormones in male rats." Biological trace element research **175**(1): 50-56.

Azenabor, A., A. O. Ekun and O. Akinloye (2015). "Impact of inflammation on male reproductive tract." Journal of reproduction & infertility **16**(3): 123.

Azouz, R. A., R. Korany and P. A. Noshy (2022). "Silica Nanoparticle–Induced Reproductive Toxicity in Male Albino Rats via Testicular Apoptosis and Oxidative Stress." Biological Trace Element Research: 1-9.

Bai, Y., Y. Zhang, J. Zhang, Q. Mu, W. Zhang, E. R. Butch, S. E. Snyder and B. Yan (2010). "Repeated administrations of carbon nanotubes in male mice cause reversible testis damage without affecting fertility." Nature nanotechnology **5**(9): 683-689.

Bansal, A. K. and G. Bilaspuri (2011). "Impacts of oxidative stress and antioxidants on semen functions." Veterinary medicine international **2011**.

Barkalina, N., C. Jones, J. Kashir, S. Coote, X. Huang, R. Morrison, H. Townley and K. Coward (2014). "Effects of mesoporous silica nanoparticles upon the function of mammalian sperm in vitro." Nanomedicine: Nanotechnology, Biology and Medicine **10**(4): 859-870.

Barkalina, N., C. Jones, H. Townley and K. Coward (2015). "Functionalization of mesoporous silica nanoparticles with a cell-penetrating peptide to target mammalian sperm in vitro." Nanomedicine **10**(10): 1539-1553.

Barkhordari, A., S. Hekmatimoghaddam, A. Jebali, M. A. Khalili, A. Talebi and M. Noorani (2013). "Effect of zinc oxide nanoparticles on viability of human spermatozoa." Iranian journal of reproductive medicine **11**(9): 767.

Barras, F. and M. Fontecave (2011). "Cobalt stress in Escherichia coli and Salmonella enterica: molecular bases for toxicity and resistance." Metallomics **3**(11): 1130-1134.

Bayda, S., M. Adeel, T. Tuccinardi, M. Cordani and F. Rizzolio (2019). "The history of nanoscience and nanotechnology: from chemical–physical applications to nanomedicine." Molecules **25**(1): 112.

Blanco, J., S. Tomás-Hernández, T. García, M. Mulero, M. Gómez, J. L. Domingo and D. J. Sánchez (2018). "Oral exposure to silver nanoparticles increases oxidative stress markers in the liver of male rats and deregulates the insulin signalling pathway and p53 and cleaved caspase 3 protein expression." Food and Chemical Toxicology **115**: 398-404.

Bondarenko, O., A. Ivask, A. Käkinen, I. Kurvet and A. Kahru (2013). "Particle-cell contact enhances antibacterial activity of silver nanoparticles." PloS one **8**(5): e64060.

Braydich-Stolle, L., S. Hussain, J. J. Schlager and M.-C. Hofmann (2005). "In vitro cytotoxicity of nanoparticles in mammalian germline stem cells." Toxicological sciences **88**(2): 412-419.

Brohi, R. D., L. Wang, H. S. Talpur, D. Wu, F. A. Khan, D. Bhattarai, Z.-U. Rehman, F. Farmanullah and L.-J. Huo (2017). "Toxicity of nanoparticles on the reproductive system in animal models: a review." Frontiers in pharmacology **8**: 606.

Buchman, J. T., N. V. Hudson-Smith, K. M. Landy and C. L. Haynes (2019). "Understanding nanoparticle toxicity mechanisms to inform redesign strategies to reduce environmental impact." Accounts of chemical research **52**(6): 1632-1642.

Caldeira, D. F., F. Paulini, R. C. Silva, R. B. d. Azevedo and C. M. Lucci (2018). "In vitro exposure of bull sperm cells to DMSA-coated maghemite nanoparticles does not affect cell functionality or structure." International Journal of Hyperthermia **34**(4): 415-422.

Carvalhais, A., I. Oliveira, H. Oliveira, C. Oliveira, L. Ferrão, E. Cabrita, J. Asturiano, S. Guilherme, M. Pacheco and C. Mieiro (2022). "Ex vivo exposure to titanium dioxide and silver nanoparticles mildly affect sperm of gilthead seabream (Sparus aurata)-A multiparameter spermiotoxicity approach." Marine Pollution Bulletin **177**: 113487.

Castellini, C., S. Ruggeri, S. Mattioli, G. Bernardini, L. Macchioni, E. Moretti and G. Collodel (2014). "Long-term effects of silver nanoparticles on reproductive activity of rabbit buck." Systems biology in reproductive medicine **60**(3): 143-150.

Chaudhry, Q., M. Scotter, J. Blackburn, B. Ross, A. Boxall, L. Castle, R. Aitken and R. Watkins (2008). "Applications and implications of nanotechnologies for the food sector." Food additives and contaminants **25**(3): 241-258.

Chen, H., Y. Wang, J. Luo, M. Kang, J. Hou, R. Tang, L. Zhao, F. Shi, G. Ye and X. He (2022). "Autophagy and apoptosis mediated nano-copper-induced testicular damage." Ecotoxicology and Environmental Safety **229**: 113039.

Chen, H., J. Weiss and F. Shahidi (2006). "Nanotechnology in nutraceuticals and functional foods." Food technology (Chicago) **60**(3): 30-36.

Chen, L., J. Liu, Y. Zhang, G. Zhang, Y. Kang, A. Chen, X. Feng and L. Shao (2018). "The toxicity of silica nanoparticles to the immune system." Nanomedicine **13**(15): 1939-1962.

Chen, Y., Q. Yin, X. Ji, S. Zhang, H. Chen, Y. Zheng, Y. Sun, H. Qu, Z. Wang and Y. Li (2012). "Manganese oxide-based multifunctionalized mesoporous silica nanoparticles for pH-responsive MRI, ultrasonography and circumvention of MDR in cancer cells." Biomaterials **33**(29): 7126-7137.

Cometa, S., R. Iatta, M. A. Ricci, C. Ferretti and E. De Giglio (2013). "Analytical characterization and antimicrobial properties of novel copper nanoparticle–loaded electrosynthesized hydrogel coatings." Journal of bioactive and compatible polymers **28**(5): 508-522.

Cotena, M., M. Auffan, S. Robert, V. Tassistro, N. Resseguier, J. Rose and J. Perrin (2020). "CeO2 Nanomaterials from Diesel Engine Exhaust Induce DNA Damage and Oxidative Stress in Human and Rat Sperm In Vitro." Nanomaterials **10**(12): 2327.

Dahle, J. T. and Y. Arai (2015). "Environmental geochemistry of cerium: applications and toxicology of cerium oxide nanoparticles." International journal of environmental research and public health **12**(2): 1253-1278.

Danafar, A., A. Khoradmehr, M. H. Bondarabadi, F. Mazaheri, A. Tamadon, S. Pourmasoumi, L. Gholizadeh, M. Moshrefi, I. Halvaei and A. Hosseini (2021). "Impairment of sperm efficiency in mice following short-term nano-titanium dioxide exposure: An experimental study." International Journal of Reproductive BioMedicine **19**(12): 1045.

Dastjerdi, R. and M. Montazer (2010). "A review on the application of inorganic nano-structured materials in the modification of textiles: focus on anti-microbial properties." Colloids and surfaces B: Biointerfaces **79**(1): 5-18.

Dekkers, S., P. Krystek, R. J. Peters, D. P. Lankveld, B. G. Bokkers, P. H. van Hoeven-Arentzen, H. Bouwmeester and A. G. Oomen (2011). "Presence and risks of nanosilica in food products." Nanotoxicology **5**(3): 393-405.

Dhall, A. and W. Self (2018). "Cerium oxide nanoparticles: a brief review of their synthesis methods and biomedical applications." Antioxidants **7**(8): 97.

Dickson, J. S. and M. Koohmaraie (1989). "Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces." Applied and environmental microbiology **55**(4): 832-836.

Dong, L., G. Chen, G. Liu, X. Huang, X. Xu, L. Li, Y. Zhang, J. Wang, M. Jin and D. Xu (2022). "A review on recent advances in the applications of

composite Fe3O4 magnetic nanoparticles in the food industry." Critical Reviews in Food Science and Nutrition: 1-29.

Driscoll, K. E., B. W. Howard, J. M. Carter, Y. M. Janssen, B. T. Mossman and R. J. Isfort (2001). Mitochondrial-derived oxidants and quartz activation of chemokine gene expression. Biological Reactive Intermediates VI, Springer**:** 489-496.

El-Sayed, R. A., F. M. El-Demerdash and M. A. El-Magd (2021). "Ginseng ameliorates pulmonary toxicity induced by silicon dioxide nanoparticles in rats." Asian Pacific Journal of Tropical Biomedicine **11**(6): 254.

Elsharkawy, E. E., M. Abd El-Nasser and H. F. Kamaly (2019). "Silver nanoparticles testicular toxicity in rat." Environmental toxicology and pharmacology **70**: 103194.

Elumalai, K. and S. Velmurugan (2015). "Green synthesis, characterization and antimicrobial activities of zinc oxide nanoparticles from the leaf extract of Azadirachta indica (L.)." Applied Surface Science **345**: 329-336.

Epstein, E. (1994). "The anomaly of silicon in plant biology." Proceedings of the National Academy of Sciences **91**(1): 11-17.

Falchi, L., L. Bogliolo, G. Galleri, F. Ariu, M. T. Zedda, A. Pinna, L. Malfatti, P. Innocenzi and S. Ledda (2016). "Cerium dioxide nanoparticles did not alter the functional and morphologic characteristics of ram sperm during short-term exposure." Theriogenology **85**(7): 1274-1281. e1273.

Fayez, E., M. El Sayed, Z. Rawash and A. Salama (2022). "Influence of the addition of zinc oxide nanoparticles to cryopreservation medium for dog epididymal spermatozoa." Topics in Companion Animal Medicine: 100736.

Fazelipour, S., F. Assadi, Z. Tootian, M. T. Sheibani, M. Dahmardeh, O. Zehtabvar, S. Namdar and S. Farshidfar (2020). "Effect of molybdenum trioxide nanoparticles on histological changes of uterus and biochemical parameters of blood serum in rat." Comparative Clinical Pathology **29**(5): 991-999.

Galhardo, R. S., C. E. Almeida, A. C. Leita□o and J. r. B. Cabral-Neto (2000). "Repair of DNA lesions induced by hydrogen peroxide in the presence of iron chelators in Escherichia coli: participation of endonuclease IV and Fpg." Journal of bacteriology **182**(7): 1964-1968.

Gallo, A., L. Manfra, R. Boni, A. Rotini, L. Migliore and E. Tosti (2018). "Cytotoxicity and genotoxicity of CuO nanoparticles in sea urchin spermatozoa through oxidative stress." Environment international **118**: 325-333.

Gao, G., Y. Ze, X. Zhao, X. Sang, L. Zheng, X. Ze, S. Gui, L. Sheng, Q. Sun and J. Hong (2013). "Titanium dioxide nanoparticle-induced testicular damage, spermatogenesis suppression, and gene expression alterations in male mice." Journal of hazardous materials **258**: 133-143.

Garncarek, M., K. Dziewulska and M. Kowalska-Góralska (2022). "The Effect of Copper and Copper Oxide Nanoparticles on Rainbow Trout (Oncorhynchus mykiss W.) Spermatozoa Motility after Incubation with Contaminants." International Journal of Environmental Research and Public Health **19**(14): 8486.

Gawande, M. B., A. Goswami, F.-X. Felpin, T. Asefa, X. Huang, R. Silva, X. Zou, R. Zboril and R. S. Varma (2016). "Cu and Cu-based nanoparticles: synthesis and applications in catalysis." Chemical reviews **116**(6): 3722-3811.

Gnach, A., T. Lipinski, A. Bednarkiewicz, J. Rybka and J. A. Capobianco (2015). "Upconverting nanoparticles: assessing the toxicity." Chemical Society Reviews **44**(6): 1561-1584.

Go, M.-R., S.-H. Bae, H.-J. Kim, J. Yu and S.-J. Choi (2017). "Interactions between food additive silica nanoparticles and food matrices." Frontiers in microbiology **8**: 1013.

Gonçalves, B. B., F. C. Dias, N. S. de Souza Trigueiro, E. Marques, C. C. Rodrigues, I. B. Madureira, G. Qualhato, S. M. T. de Sabóia-Morais and T. L. Rocha (2021). "Chronic exposure to iron oxide nanoparticles (γ-Fe2O3) induces gonadal histopathology on male guppies (Poecilia reticulata)." Environmental Nanotechnology, Monitoring & Management **16**: 100522.

Gubala, V., G. Giovannini, F. Kunc, M. P. Monopoli and C. J. Moore (2020). "Dye-doped silica nanoparticles: synthesis, surface chemistry and bioapplications." Cancer Nanotechnology **11**(1): 1-43.

Gurunathan, S., J. W. Han, E. S. Kim, J. H. Park and J.-H. Kim (2015). "Reduction of graphene oxide by resveratrol: A novel and simple biological method for the synthesis of an effective anticancer nanotherapeutic molecule." International journal of nanomedicine **10**: 2951.

Ha¨feli, U. O., J. S. Riffle, L. Harris-Shekhawat, A. Carmichael-Baranauskas, F. Mark, J. P. Dailey and D. Bardenstein (2009). "Cell uptake and in vitro toxicity of magnetic nanoparticles suitable for drug delivery." Molecular pharmaceutics **6**(5): 1417-1428.

Halo Jr, M., K. Bułka, P. A. Antos, A. Greń, T. Slanina, Ľ. Ondruška, K. Tokárová, M. Massányi, G. Formicki and M. Halo (2021). "The effect of ZnO nanoparticles on rabbit spermatozoa motility and viability parameters in vitro." Saudi Journal of Biological Sciences **28**(12): 7450-7454.

Hamada, K., Y. Suzaki, A. Goldman, Y. Y. Ning, C. Goldsmith, A. Palecanda, B. Coull, C. Hubeau and L. Kobzik (2003). "Allergen-independent maternal transmission of asthma susceptibility." The Journal of Immunology **170**(4): 1683-1689.

Hassankhani, R., M. Esmaeillou, A. A. Tehrani, K. Nasirzadeh, F. Khadir and H. Maadi (2015). "In vivo toxicity of orally administrated silicon dioxide nanoparticles in healthy adult mice." Environmental Science and Pollution Research **22**(2): 1127-1132.

Hong, F., X. Zhao, W. Si, Y. Ze, L. Wang, Y. Zhou, J. Hong, X. Yu, L. Sheng and D. Liu (2015). "Decreased spermatogenesis led to alterations of testis-specific gene expression in male mice following nano-TiO2 exposure." Journal of hazardous materials **300**: 718-728.

Hong, X., N. Shao, L. Yin, C. Li, G. Tao, Y. Sun, K. Qian, J. Yang, P. Xiao and X. Yu (2022). "Exposure to zinc oxide nanoparticles affects testicular structure, reproductive development and spermatogenesis in parental and offspring male rats." Annals of translational medicine **10**(13).

Hosseinalipour, E., M. Karimipour and A. Ahmadi (2021). "Detrimental effects of cerium oxide nanoparticles on testis, sperm parameters quality, and in vitro fertilization in mice: An experimental study." International Journal of Reproductive BioMedicine **19**(9): 801.

Hosseinmardi, M., F. Siadat, M. Sharafi, N. H. Roodbari and M. Hezavehei (2022). "Protective Effect of Cerium Oxide Nanoparticles on Human Sperm Function During Cryopreservation." Biopreservation and Biobanking **20**(1): 24-30.

Hougaard, K. S., P. Jackson, K. A. Jensen, J. J. Sloth, K. Löschner, E. H. Larsen, R. K. Birkedal, A. Vibenholt, A.-M. Z. Boisen and H. Wallin (2010). "Effects of prenatal exposure to surface-coated nanosized titanium dioxide (UV-Titan). A study in mice." Particle and fibre toxicology **7**(1): 1-15.

Huang, C.-C., R. S. Aronstam, D.-R. Chen and Y.-W. Huang (2010). "Oxidative stress, calcium homeostasis, and altered gene expression in human lung epithelial cells exposed to ZnO nanoparticles." Toxicology in vitro **24**(1): 45-55.

Hulteen, J. C., D. A. Treichel, M. T. Smith, M. L. Duval, T. R. Jensen and R. P. Van Duyne (1999). "Nanosphere lithography: size-tunable silver nanoparticle and surface cluster arrays." The Journal of Physical Chemistry B **103**(19): 3854-3863.

Hussain, S., S. Garantziotis, F. Rodrigues-Lima, J.-M. Dupret, A. Baeza-Squiban and S. Boland (2014). "Intracellular signal modulation by nanomaterials." Nanomaterial: 111-134.

Imlay, J. A. (2008). "Cellular defenses against superoxide and hydrogen peroxide." Annu. Rev. Biochem. **77**: 755-776.

Irshad, M. A., R. Nawaz, M. Z. ur Rehman, M. Adrees, M. Rizwan, S. Ali, S. Ahmad and S. Tasleem (2021). "Synthesis, characterization and advanced sustainable applications of titanium dioxide nanoparticles: A review." Ecotoxicology and environmental safety **212**: 111978.

Jackson, P., S. Halappanavar, K. S. Hougaard, A. Williams, A. M. Madsen, J. S. Lamson, O. Andersen, C. Yauk, H. Wallin and U. Vogel (2013). "Maternal inhalation of surface-coated nanosized titanium dioxide (UV-Titan) in C57BL/6 mice: effects in prenatally exposed offspring on hepatic DNA damage and gene expression." Nanotoxicology **7**(1): 85-96.

Jackson, P., U. Vogel, H. Wallin and K. S. Hougaard (2011). "Prenatal exposure to carbon black (printex 90): effects on sexual development and neurofunction." Basic & clinical pharmacology & toxicology **109**(6): 434-437.

Jahanbin, R., P. Yazdanshenas, M. Rahimi, A. Hajarizadeh, E. Tvrda, S. A. Nazari, A. Mohammadi-Sangcheshmeh and N. Ghanem (2021). "In vivo and in vitro evaluation of bull semen processed with zinc (Zn) nanoparticles." Biological Trace Element Research **199**(1): 126-135.

Jia, F., Z. Sun, X. Yan, B. Zhou and J. Wang (2014). "Effect of pubertal nano-TiO2 exposure on testosterone synthesis and spermatogenesis in mice." Archives of toxicology **88**(3): 781-788.

Jiang, J., J. Pi and J. Cai (2018). "The advancing of zinc oxide nanoparticles for biomedical applications." Bioinorganic chemistry and applications **2018**.

Kashiwada, S. (2006). "Distribution of nanoparticles in the see-through medaka (Oryzias latipes)." Environmental health perspectives **114**(11): 1697-1702.

Khanna, P., C. Ong, B. H. Bay and G. H. Baeg (2015). "Nanotoxicity: an interplay of oxidative stress, inflammation and cell death." Nanomaterials **5**(3): 1163-1180.

Kim, I., K. Viswanathan, G. Kasi, S. Thanakkasaranee, K. Sadeghi and J. Seo (2022). "ZnO nanostructures in active antibacterial food packaging: preparation methods, antimicrobial mechanisms, safety issues, future prospects, and challenges." Food Reviews International **38**(4): 537-565.

Klaus, T., R. Joerger, E. Olsson and C.-G. Granqvist (1999). "Silver-based crystalline nanoparticles, microbially fabricated." Proceedings of the National Academy of Sciences **96**(24): 13611-13614.

Kołodziejczak-Radzimska, A. and T. Jesionowski (2014). "Zinc oxide—from synthesis to application: a review." Materials **7**(4): 2833-2881.

Kubo-Irie, M., S. Oshio, Y. Niwata, A. Ishihara, I. Sugawara and K. Takeda (2011). "Pre-and postnatal exposure to low-dose diesel exhaust impairs murine spermatogenesis." Inhalation toxicology **23**(13): 805-813.

Kukla, S. P., V. P. Chelomin, A. A. Mazur and V. V. Slobodskova (2022). "Zinc Oxide Nanoparticles Induce DNA Damage in Sand Dollar Scaphechinus mirabilis Sperm." Toxics **10**(7): 348.

Kyjovska, Z. O., A. M. Z. Boisen, P. Jackson, H. Wallin, U. Vogel and K. S. Hougaard (2013). "Daily sperm production: application in studies of

prenatal exposure to nanoparticles in mice." Reproductive Toxicology **36**: 88-97.

Lee, P. C. (1998). "Disruption of male reproductive tract development by administration of the xenoestrogen, nonylphenol, to male newborn rats." Endocrine **9**(1): 105-111.

Li, Z., J. C. Barnes, A. Bosoy, J. F. Stoddart and J. I. Zink (2012). "Mesoporous silica nanoparticles in biomedical applications." Chemical Society Reviews **41**(7): 2590-2605.

Lin, D. and B. Xing (2007). "Phytotoxicity of nanoparticles: inhibition of seed germination and root growth." Environmental pollution **150**(2): 243-250.

Liu, Q., C. Xu, G. Ji, H. Liu, Y. Mo, D. J. Tollerud, A. Gu and Q. Zhang (2016). "Sublethal effects of zinc oxide nanoparticles on male reproductive cells." Toxicology in Vitro **35**: 131-138.

Lu, T., C. Ling, M. Hu, X. Meng, Y. Deng, H. An, L. Li, Y. Hu, H. Wang and G. Song (2021). "Effect of nano-titanium dioxide on blood-testis barrier and MAPK signaling pathway in male mice." Biological Trace Element Research **199**(8): 2961-2971.

Macomber, L. and R. P. Hausinger (2011). "Mechanisms of nickel toxicity in microorganisms." Metallomics **3**(11): 1153-1162.

Mallick, K., M. Witcomb and M. Scurrell (2004). "Polymer stabilized silver nanoparticles: a photochemical synthesis route." Journal of materials science **39**(14): 4459-4463.

Manke, A., L. Wang and Y. Rojanasakul (2013). "Mechanisms of nanoparticle-induced oxidative stress and toxicity." BioMed research international **2013**.

Mao, Z., M. Yao, B. Xu, X. Ji, H. Jiang, X. Han, Q. Tang, Z. Zhou, R. Chen and X. Li (2017). "Cytoskeletons of two reproductive germ cell lines response differently to titanium dioxide nanoparticles mediating vary reproductive toxicity." Journal of Biomedical Nanotechnology **13**(4): 409-416.

Marambio-Jones, C. and E. Hoek (2010). "A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment." Journal of nanoparticle research **12**(5): 1531-1551.

Mathias, F. T., R. M. Romano, M. M. Kizys, T. Kasamatsu, G. Giannocco, M. I. Chiamolera, M. R. Dias-da-Silva and M. A. Romano (2015). "Daily exposure to silver nanoparticles during prepubertal development decreases adult sperm and reproductive parameters." Nanotoxicology **9**(1): 64-70.

Mattison, D. R., D. R. Plowchalk, M. J. Meadows, A. Z. Al-Juburi, J. Gandy and A. Malek (1990). "Reproductive toxicity: male and female repro-

ductive systems as targets for chemical injury." Medical Clinics of North America **74**(2): 391-411.

Meena, R. and K. Kajal (2015). "Cytotoxic and genotoxic effects of titanium dioxide nanoparticles in testicular cells of male wistar rat." Applied Biochemistry and Biotechnology **175**(2): 825-840.

Meng, J., M. Yang, F. Jia, H. Kong, W. Zhang, C. Wang, J. Xing, S. Xie and H. Xu (2010). "Subcutaneous injection of water-soluble multi-walled carbon nanotubes in tumor-bearing mice boosts the host immune activity." Nanotechnology **21**(14): 145104.

Meng, X., L. Li, H. An, Y. Deng, C. Ling, T. Lu, G. Song and Y. Wang (2022). "Lycopene Alleviates Titanium Dioxide Nanoparticle-Induced Testicular Toxicity by Inhibiting Oxidative Stress and Apoptosis in Mice." Biological Trace Element Research **200**(6): 2825-2837.

Mensch, A. C., R. T. Hernandez, J. E. Kuether, M. D. Torelli, Z. V. Feng, R. J. Hamers and J. A. Pedersen (2017). "Natural organic matter concentration impacts the interaction of functionalized diamond nanoparticles with model and actual bacterial membranes." Environmental science & technology **51**(19): 11075-11084.

Mierczynska-Vasilev, A., P. Boyer, K. Vasilev and P. A. Smith (2017). "A novel technology for the rapid, selective, magnetic removal of pathogenesis-related proteins from wines." Food chemistry **232**: 508-514.

Mirza Mohamadi, M. and D. Sohrabi (2015). "The effect of the molybdenum trioxide (MoO3) nanoparticles on histological changes of testis and spermatogenesis process in adult male Wistar rats." Journal of arak University of Medical Sciences **17**(12): 64-74.

Moradi, M., R. Razavi, A. K. Omer, A. Farhangfar and D. J. McClements (2022). "Interactions between nanoparticle-based food additives and other food ingredients: A review of current knowledge." Trends in Food Science & Technology.

Moretti, E., G. Terzuoli, T. Renieri, F. Iacoponi, C. Castellini, C. Giordano and G. Collodel (2013). "In vitro effect of gold and silver nanoparticles on human spermatozoa." Andrologia **45**(6): 392-396.

Moridi, H., S. A. Hosseini, H. Shateri, N. Kheiripour, A. Kaki, M. Hatami and A. Ranjbar (2018). "Protective effect of cerium oxide nanoparticle on sperm quality and oxidative damage in malathion-induced testicular toxicity in rats: An experimental study." International journal of reproductive biomedicine **16**(4): 261.

Morones, J. R., J. L. Elechiguerra, A. Camacho, K. Holt, J. B. Kouri, J. T. Ramírez and M. J. Yacaman (2005). "The bactericidal effect of silver nanoparticles." Nanotechnology **16**(10): 2346.

Mukherjee, P., A. Ahmad, D. Mandal, S. Senapati, S. R. Sainkar, M. I. Khan, R. Parishcha, P. Ajaykumar, M. Alam and R. Kumar (2001). "Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis." Nano letters **1**(10): 515-519.

Nasri, S., S. Rezai-Zarchi, P. Kerishchi and S. Sadeghi (2015). "The effect of iron oxide nanoparticles on sperm numbers and mobility in male mice." Zahedan Journal of Research in Medical Sciences **17**(10).

Newman, M. D., M. Stotland and J. I. Ellis (2009). "The safety of nanosized particles in titanium dioxide–and zinc oxide–based sunscreens." Journal of the American Academy of Dermatology **61**(4): 685-692.

Nicy, V., M. Das, G. Gurusubramanian, P. Mondal and V. K. Roy (2022). "Treatment of copper nanoparticles (CuNPs) for two spermatogenic cycles impairs testicular activity via down-regulating steroid receptors and inhibition of germ cell proliferation in a mice model." Nanotoxicology **16**(5): 658-678.

Oliviero, M., S. Schiavo, S. Dumontet and S. Manzo (2019). "DNA damages and offspring quality in sea urchin Paracentrotus lividus sperms exposed to ZnO nanoparticles." Science of the Total Environment **651**: 756-765.

Opara, L. (2004). "Emerging technological innovation triad for smart agriculture in the 21st century. Part I. Prospects and impacts of nanotechnology in agriculture."

Özgür, M. E., A. Ulu, S. Balcıoğlu, I. Özcan, S. Köytepe and B. Ateş (2018). "The toxicity assessment of iron oxide (Fe3O4) nanoparticles on physical and biochemical quality of rainbow trout spermatozoon." Toxics **6**(4): 62.

Özgür, M. E., A. Ulu, S. A. A. Noma, İ. Özcan, S. Balcıoğlu, B. Ateş and S. Köytepe (2020). "Melatonin protects sperm cells of Capoeta trutta from toxicity of titanium dioxide nanoparticles." Environmental Science and Pollution Research **27**(15): 17843-17853.

Özgür, M. E., A. Ulu, İ. Özcan, S. Balcioglu, B. Ateş and S. Köytepe (2019). "Investigation of toxic effects of amorphous SiO2 nanoparticles on motility and oxidative stress markers in rainbow trout sperm cells." Environmental Science and Pollution Research **26**(15): 15641-15652.

Pal, G., A. Dutta, K. Mitra, M. S. Grace, A. Amat, T. B. Romanczyk, X. Wu, K. Chakrabarti, J. Anders and E. Gorman (2007). "Effect of low intensity laser interaction with human skin fibroblast cells using fiber-optic nano-probes." Journal of Photochemistry and Photobiology B: Biology **86**(3): 252-261.

Paskeh, M. D. A., N. Babaei, M. Entezari, M. Hashemi and A. Doosti (2022). "Protective Effects of Coenzyme Q10 Along with Fe2O3 Nanoparticles

On Sperm Parameters in Rats with Scrotal Hyperthermia." Galen Medical Journal **11**: e2046-e2046.

Pawar, K. and G. Kaul (2014). "Toxicity of titanium oxide nanoparticles causes functionality and DNA damage in buffalo (Bubalus bubalis) sperm in vitro." Toxicology and industrial health **30**(6): 520-533.

Pérez-Duran, F., L. S. Acosta-Torres, P. N. Serrano-Díaz, I. A. Toscano-Torres, I. B. Olivo-Zepeda, E. García-Caxin and R. E. Nuñez-Anita (2020). "Toxicity and antimicrobial effect of silver nanoparticles in swine sperms." Systems biology in reproductive medicine **66**(4): 281-289.

Philbrook, N. A., V. K. Walker, A. N. Afrooz, N. B. Saleh and L. M. Winn (2011). "Investigating the effects of functionalized carbon nanotubes on reproduction and development in Drosophila melanogaster and CD-1 mice." Reproductive Toxicology **32**(4): 442-448.

Pietroiusti, A., M. Massimiani, I. Fenoglio, M. Colonna, F. Valentini, G. Palleschi, A. Camaioni, A. Magrini, G. Siracusa and A. Bergamaschi (2011). "Low doses of pristine and oxidized single-wall carbon nanotubes affect mammalian embryonic development." Acs Nano **5**(6): 4624-4633.

Pinho, A. R., F. Martins, M. E. V. Costa, A. M. Senos, O. A. da Cruz e Silva, M. d. L. Pereira and S. Rebelo (2020). "In vitro cytotoxicity effects of zinc oxide nanoparticles on spermatogonia cells." Cells **9**(5): 1081.

Prathna, T., S. K. Sharma and M. Kennedy (2018). "Nanoparticles in household level water treatment: an overview." Separation and Purification Technology **199**: 260-270.

Qin, F., T. Shen, J. Li, J. Qian, J. Zhang, G. Zhou and J. Tong (2019). "SF-1 mediates reproductive toxicity induced by Cerium oxide nanoparticles in male mice." Journal of nanobiotechnology **17**(1): 1-13.

Ramakrishnan, V. M., M. Natarajan, A. Santhanam, V. Asokan and D. Velauthapillai (2018). "Size controlled synthesis of TiO2 nanoparticles by modified solvothermal method towards effective photo catalytic and photovoltaic applications." Materials Research Bulletin **97**: 351-360.

Ren, L., J. Zhang, Y. Zou, L. Zhang, J. Wei, Z. Shi, Y. Li, C. Guo, Z. Sun and X. Zhou (2016). "Silica nanoparticles induce reversible damage of spermatogenic cells via RIPK1 signal pathways in C57 mice." International Journal of Nanomedicine **11**: 2251.

Risom, L., P. Møller and S. Loft (2005). "Oxidative stress-induced DNA damage by particulate air pollution." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **592**(1-2): 119-137.

Ruszkiewicz, J. A., A. Pinkas, B. Ferrer, T. V. Peres, A. Tsatsakis and M. Aschner (2017). "Neurotoxic effect of active ingredients in sunscreen products, a contemporary review." Toxicology Reports **4**: 245-259.

Samrot, A. V., T. C. Sean, T. Kudaiyappan, U. Bisyarah, A. Mirarmandi, E. Faradjeva, A. Abubakar, H. H. Ali, J. L. A. Angalene and S. S. Kumar (2020). "Production, characterization and application of nanocarriers made of polysaccharides, proteins, bio-polyesters and other biopolymers: A review." International journal of biological macromolecules **165**: 3088-3105.

Sanand, S., S. Kumar, N. Bara and G. Kaul (2018). "Comparative evaluation of half-maximum inhibitory concentration and cytotoxicity of silver nanoparticles and multiwalled carbon nanotubes using buffalo bull spermatozoa as a cell model." Toxicology and Industrial Health **34**(9): 640-652.

Sang, Y., J. Liu, X. Li, G. Zhou, Y. Zhang, L. Gao, Y. Zhao and X. Zhou (2021). "The effect of SiNPs on DNA methylation of genome in mouse spermatocytes." Environmental Science and Pollution Research **28**(32): 43684-43697.

Santonastaso, M., F. Mottola, N. Colacurci, C. Iovine, S. Pacifico, M. Cammarota, F. Cesaroni and L. Rocco (2019). "In vitro genotoxic effects of titanium dioxide nanoparticles (n-TiO2) in human sperm cells." Molecular reproduction and development **86**(10): 1369-1377.

Santonastaso, M., F. Mottola, C. Iovine, F. Cesaroni, N. Colacurci and L. Rocco (2021). "In Vitro Effects of Titanium Dioxide Nanoparticles (TiO Genotoxicity 2NPs) on in Cadmium Human Sperm Chloride Cells." Nanotechnology for Environmental and Biomedical Research: 123.

Sastry, M., A. Ahmad, M. I. Khan and R. Kumar (2003). "Biosynthesis of metal nanoparticles using fungi and actinomycete." Current science: 162-170.

Schwaminger, S. P., P. Fraga-García, M. Eigenfeld, T. M. Becker and S. Berensmeier (2019). "Magnetic separation in bioprocessing beyond the analytical scale: from biotechnology to the food industry." Frontiers in bioengineering and biotechnology **7**: 233.

Scott, N. (2007). "Nanoscience in veterinary medicine." Veterinary research communications **31**(1): 139-144.

Semmler-Behnke, M., S. Fertsch, G. Schmid, A. Wenk and W. G. Kreyling (2007). "Uptake of 1.4 nm versus 18 nm gold nanoparticles in secondary target organs is size dependent in control and pregnant rats after intratracheal or intravenous application." Proceedings of the EuroNanoForum 2007: 19-21 June 2007; Düsseldorf: 102-104.

Sergeev, B. M., V. A. Kasaikin, E. A. Litmanovich, G. B. Sergeev and A. N. Prusov (1999). "Cryochemical synthesis and properties of silver nanoparticle dispersions stabilised by poly (2-dimethylaminoethyl methacrylate)." Mendeleev communications **9**(4): 130-132.

Setyawati, M. I., Z. Zhao and K. W. Ng (2020). "Transformation of nanomaterials and its implications in gut nanotoxicology." Small **16**(36): 2001246.

Sharafutdinova, L., A. Fedorova, S. Bashkatov, K. Sinel'nikov and V. Valiullin (2018). "Structural and functional analysis of the spermatogenic epithelium in rats exposed to titanium dioxide nanoparticles." Bulletin of experimental biology and medicine **166**(2): 279-282.

Sharma, R., A. Sarkar, R. Jha, A. Kumar Sharma and D. Sharma (2020). "Sol-gel–mediated synthesis of TiO2 nanocrystals: Structural, optical, and electrochemical properties." International Journal of Applied Ceramic Technology **17**(3): 1400-1409.

Shen, J., D. Yang, X. Zhou, Y. Wang, S. Tang, H. Yin, J. Wang, R. Chen and J. Chen (2019). "Role of autophagy in zinc oxide nanoparticles-induced apoptosis of mouse LEYDIG cells." International journal of molecular sciences **20**(16): 4042.

Shen, L., N. Bao, K. Yanagisawa, K. Domen, A. Gupta and C. A. Grimes (2006). "Direct synthesis of ZnO nanoparticles by a solution-free mechanochemical reaction." Nanotechnology **17**(20): 5117.

Siddiqui, M. A., Q. Saquib, M. Ahamed, N. N. Farshori, J. Ahmad, R. Wahab, S. T. Khan, H. A. Alhadlaq, J. Musarrat and A. A. Al-Khedhairy (2015). "Molybdenum nanoparticles-induced cytotoxicity, oxidative stress, G2/M arrest, and DNA damage in mouse skin fibroblast cells (L929)." Colloids and Surfaces B: Biointerfaces **125**: 73-81.

Singh, A., N. á. Singh, S. Afzal, T. Singh and I. Hussain (2018). "Zinc oxide nanoparticles: a review of their biological synthesis, antimicrobial activity, uptake, translocation and biotransformation in plants." Journal of materials science **53**(1): 185-201.

Singh, J., S. Kumar, A. Alok, S. K. Upadhyay, M. Rawat, D. C. Tsang, N. Bolan and K.-H. Kim (2019). "The potential of green synthesized zinc oxide nanoparticles as nutrient source for plant growth." Journal of Cleaner Production **214**: 1061-1070.

Sleiman, H. K., R. M. Romano, C. A. d. Oliveira and M. A. Romano (2013). "Effects of prepubertal exposure to silver nanoparticles on reproductive parameters in adult male Wistar rats." Journal of Toxicology and Environmental Health, Part A **76**(17): 1023-1032.

Smijs, T. G. and S. Pavel (2011). "Titanium dioxide and zinc oxide nanoparticles in sunscreens: focus on their safety and effectiveness." Nanotechnology, science and applications **4**: 95.

Smith, M. A., R. Michael, R. G. Aravindan, S. Dash, S. I. Shah, D. S. Galileo and P. A. Martin-DeLeon (2015). "Anatase titanium dioxide nanoparticles in mice: evidence for induced structural and functional sperm defects after short-, but not long-, term exposure." Asian Journal of Andrology **17**(2): 261.

Snyder, R. W., T. R. Fennell, C. J. Wingard, N. P. Mortensen, N. A. Holland, J. H. Shannahan, W. Pathmasiri, A. H. Lewin and S. C. Sumner (2015). "Distribution and biomarker of carbon-14 labeled fullerene C60 ([14C (U)] C60) in pregnant and lactating rats and their offspring after maternal intravenous exposure." Journal of Applied Toxicology **35**(12): 1438-1451.

Sobota, J. M. and J. A. Imlay (2011). "Iron enzyme ribulose-5-phosphate 3-epimerase in Escherichia coli is rapidly damaged by hydrogen peroxide but can be protected by manganese." Proceedings of the National Academy of Sciences **108**(13): 5402-5407.

Spanhel, L. and M. A. Anderson (1991). "Semiconductor clusters in the sol-gel process: quantized aggregation, gelation, and crystal growth in concentrated zinc oxide colloids." Journal of the American Chemical Society **113**(8): 2826-2833.

Stohs, S. J. and D. Bagchi (1995). "Oxidative mechanisms in the toxicity of metal ions." Free radical biology and medicine **18**(2): 321-336.

Sugamata, M., T. Ihara, H. Takano, S. Oshio and K. Takeda (2006). "Maternal diesel exhaust exposure damages newborn murine brains." Journal of health science **52**(1): 82-84.

Sun, F., X. Wang, P. Zhang, Z. Chen, Z. Guo and X. Shang (2022). "Reproductive toxicity investigation of silica nanoparticles in male pubertal mice." Environmental Science and Pollution Research **29**(24): 36640-36654.

Sundarraj, K., A. Raghunath, L. Panneerselvam and E. Perumal (2017). "Iron oxide nanoparticles modulate heat shock proteins and organ specific markers expression in mice male accessory organs." Toxicology and applied pharmacology **317**: 12-24.

Suri, S. S., H. Fenniri and B. Singh (2007). "Nanotechnology-based drug delivery systems." Journal of occupational medicine and toxicology **2**(1): 1-6.

Sweet, M. and I. Singleton (2011). "Silver nanoparticles: a microbial perspective." Advances in applied microbiology **77**: 115-133.

Takahashi, S. and O. Matsuoka (1981). "Cross placental transfer of 198Au-colloid in near term rats." Journal of radiation research **22**(2): 242-249.

Takeda, K., K.-i. Suzuki, A. Ishihara, M. Kubo-Irie, R. Fujimoto, M. Tabata, S. Oshio, Y. Nihei, T. Ihara and M. Sugamata (2009). "Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems." Journal of Health science **55**(1): 95-102.

Tamilvanan, A., K. Balamurugan, K. Ponappa and B. M. Kumar (2014). "Copper nanoparticles: synthetic strategies, properties and multifunctional application." International Journal of Nanoscience **13**(02): 1430001.

Tarnuzzer, R. W., J. Colon, S. Patil and S. Seal (2005). "Vacancy engineered ceria nanostructures for protection from radiation-induced cellular damage." Nano letters **5**(12): 2573-2577.

Tsatsakis, A., A. O. Docea, C. Constantin, D. Calina, O. Zlatian, T. K. Nikolouzakis, P. D. Stivaktakis, A. Kalogeraki, J. Liesivuori and G. Tzanakakis (2019). "Genotoxic, cytotoxic, and cytopathological effects in rats exposed for 18 months to a mixture of 13 chemicals in doses below NOAEL levels." Toxicology Letters **316**: 154-170.

Tu, C., X. Ma, P. Pantazis, S. M. Kauzlarich and A. Y. Louie (2010). "Paramagnetic, silicon quantum dots for magnetic resonance and two-photon imaging of macrophages." Journal of the American Chemical Society **132**(6): 2016-2023.

Tu, Y., M. Lv, P. Xiu, T. Huynh, M. Zhang, M. Castelli, Z. Liu, Q. Huang, C. Fan and H. Fang (2013). "Destructive extraction of phospholipids from Escherichia coli membranes by graphene nanosheets." Nature nanotechnology **8**(8): 594-601.

Van Benschoten, J. E., B. E. Reed, M. R. Matsumoto and P. McGarvey (1994). "Metal removal by soil washing for an iron oxide coated sandy soil." Water Environment Research **66**(2): 168-174.

Vijayalakshmi, R. and V. Rajendran (2012). "Synthesis and characterization of nano-TiO2 via different methods." Arch. Appl. Sci. Res **4**(2): 1183-1190.

Voss, L., I.-L. Hsiao, M. Ebisch, J. Vidmar, N. Dreiack, L. Böhmert, V. Stock, A. Braeuning, K. Loeschner and P. Laux (2020). "The presence of iron oxide nanoparticles in the food pigment E172." Food Chemistry **327**: 127000.

Walczak–Jedrzejowska, R., J. K. Wolski and J. Slowikowska–Hilczer (2013). "The role of oxidative stress and antioxidants in male fertility." Central European journal of urology **66**(1): 60.

Wang, E., Y. Huang, Q. Du and Y. Sun (2017). "Silver nanoparticle induced toxicity to human sperm by increasing ROS (reactive oxygen species) production and DNA damage." Environmental Toxicology and Pharmacology **52**: 193-199.

Wang, H., L.-J. Du, Z.-M. Song and X.-X. Chen (2013). "Progress in the characterization and safety evaluation of engineered inorganic nanomaterials in food." Nanomedicine **8**(12): 2007-2025.

Wang, J., G. Zhou, C. Chen, H. Yu, T. Wang, Y. Ma, G. Jia, Y. Gao, B. Li and J. Sun (2007). "Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration." Toxicology letters **168**(2): 176-185.

Wang, L. and M. Muhammed (1999). "Synthesis of zinc oxide nanoparticles with controlled morphology." Journal of Materials Chemistry **9**(11): 2871-2878.

Wang, R., B. Song, J. Wu, Y. Zhang, A. Chen and L. Shao (2018). "Potential adverse effects of nanoparticles on the reproductive system." International journal of nanomedicine **13**: 8487.

Wang, Z., A. A. Haidry, L. Xie, A. Zavabeti, Z. Li, W. Yin, R. L. Fomekong and B. Saruhan (2020). "Acetone sensing applications of Ag modified TiO2 porous nanoparticles synthesized via facile hydrothermal method." Applied Surface Science **533**: 147383.

Wang, Z., H. Zhang, L. Zhang, J. Yuan, S. Yan and C. Wang (2002). "Low-temperature synthesis of ZnO nanoparticles by solid-state pyrolytic reaction." Nanotechnology **14**(1): 11.

Xu, Y., N. Wang, Y. Yu, Y. Li, Y.-B. Li, Y.-B. Yu, X.-Q. Zhou and Z.-W. Sun (2014). "Exposure to silica nanoparticles causes reversible damage of the spermatogenic process in mice." PloS one **9**(7): e101572.

Yang, F., H. Cui, J. Xiao, X. Peng, J. Deng and Z. Zuo (2011). "Increased apoptotic lymphocyte population in the spleen of young chickens fed on diets high in molybdenum." Biological trace element research **140**(3): 308-316.

Yang, H., H. Wang, C. Wen, S. Bai, P. Wei, B. Xu, Y. Xu, C. Liang, Y. Zhang and G. Zhang (2022). "Effects of iron oxide nanoparticles as T2-MRI contrast agents on reproductive system in male mice." Journal of nanobiotechnology **20**(1): 1-18.

Yoshida, S., K. Hiyoshi, T. Ichinose, H. Takano, S. Oshio, I. Sugawara, K. Takeda and T. Shibamoto (2009). "Effect of nanoparticles on the male reproductive system of mice." International journal of andrology **32**(4): 337-342.

Younus, A. I., M. I. Yousef, K. M. Abdel-Nabi, M. I. Younus and J. M. Abdulrahman (2020). "Reproductive toxicity of iron oxide nanoparticles, silver nanoparticles and their mixture in male rats: Effects on testicular gene expression." World Journal of Advanced Research and Reviews **7**(2): 075-081.

Yousef, M., H. N. Abdelhamid, M. Hidalgo, R. Fathy, L. Gómez-Gascón and J. Dorado (2021). "Antimicrobial activity of silver-carbon nanoparticles on the bacterial flora of bull semen." Theriogenology **161**: 219-227.

Yu, S.-j., Y.-g. Yin and J.-f. Liu (2013). "Silver nanoparticles in the environment." Environmental Science: Processes & Impacts **15**(1): 78-92.

Zhai, X.-W., Y.-L. Zhang, Q. Qi, Y. Bai, X.-L. Chen, L.-J. Jin, X.-G. Ma, R.-Z. Shu, Z.-J. Yang and F.-J. Liu (2013). "Effects of molybdenum on sperm

quality and testis oxidative stress." Systems biology in reproductive medicine **59**(5): 251-255.

Zhang, Q., N. Li, J. Goebl, Z. Lu and Y. Yin (2011). "A systematic study of the synthesis of silver nanoplates: is citrate a "magic" reagent?" Journal of the American Chemical Society **133**(46): 18931-18939.

Zhang, X.-F., Z.-G. Liu, W. Shen and S. Gurunathan (2016). "Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches." International journal of molecular sciences **17**(9): 1534.

Chapter 3

Effects of Fullerene Nanoparticles on Semen Quality

Tutku Can Acısu[1]

Abstract

Fullerene or Buckminsterfullerene (also known as "Buckyballs"), which is composed entirely of 60 carbon (C) atoms, is a kind of molecule that is considered to be the most prominent member of the nano-materials family. Fullerene has been an attractive research topic in the field of nano-materials since 1985, the date when it is first discovered, due to the unique physicochemical properties and biological activities. Carbon 60 (C_{60}) Fullerene (FUL) nanoparticles, prepared through various dissolution and modification methods, exhibit different and distinct biological effects. Carbon 60 (C_{60}) Fullerene (FUL) and some of its derivatives are known to provide effective protection in vivo and in vitro against oxidative stress, without causing acute or subacute toxicity. Carbon 60 (C_{60}) Fullerene (FUL), which has been the subject of numerous studies particularly in recent years, has been proven to have beneficial biological effects as neuro-protectant, anti-cancer, anti-inflammatory, anti-atherogenic and radioprotective agents on the grounds of its unique bio-antioxidant properties that can manifest unexpectedly even at small concentrations and doses.

1. Introduction

Galaxies and stars formed approximately 300.000 years after the Big Bang, which occurred 13,7 billion years ago. There are a high percentage of hydrogen (H), some helium (He), and lesser amounts of other heavy elements in the first ring of stellar development. The phenomenon that keeps the stars in equilibrium against the gravitational pull of the stellar core is the release of heat as the H present in the structure fuses into He. The decrease in the amount of H over time results in the weakening of this heat source. In a supernova explosion, stars consequently scatter elements such as

1 Corresponding author, Dr., Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Firat University, Elazig, Turkey, e-mail: tcacisu@firat.edu.tr; http://orcid.org/0000-0002-0882-937X.

https://doi.org/10.58830/ozgur.pub203.c936

C, oxygen (O_2) and iron (Fe) into space. Hence, C became one of the basic elements of the life on Earth as a consequence of such a supernova explosion (Hanaoka et al., 2002).

C, which is one of the most important elements, ranks the fourth after H, He and O_2 in terms of the amount existing in the universe. It exists both in pure form and as a compound. C, as one of the basic building blocks of all living creatures, constitutes 18.5% of the human body. C atom, with 6 electrons, bears nonmetallic properties and is the first element in Group IV of the Periodic Table. Furthermore, C has the highest hybridization tendency among all Group IV-A elements (Saito, 1998).

C initially played a minor role for chemists working on creating and shaping a new substance. However, this fact changed significantly when the classical graphite and diamond structures were replaced by the C-allotrope family enriched by Fullerene (FUL). Unlike graphite and diamond, FULs have spherical molecules consisting of a combination of pentagons and hexagons and they can be dissolved in some organic solvents. The most known FULs containing the least C is Buckminsterfullerene C60, which is shaped like a soccer ball (Figure 1). The principle that creates FULs is a consequence of Euler's Theorem, which states that 12 pentagons are needed to fulfill each spherical mesh of n hexagons (Demirbakan, 2015). FULs with high numbers of C atoms are accompanied by multiple isomers and point group symmetry (Godly and Taylor, 1997).

The structure of these three-dimensional systems has attracted the attention of many scientists, as they have a more aesthetic appearance compared to smaller two-dimensional molecules. For this reason, Buckminsterfullerene C60 has become one of the molecules that has been widely researched in a very short time (Demirbakan, 2015).

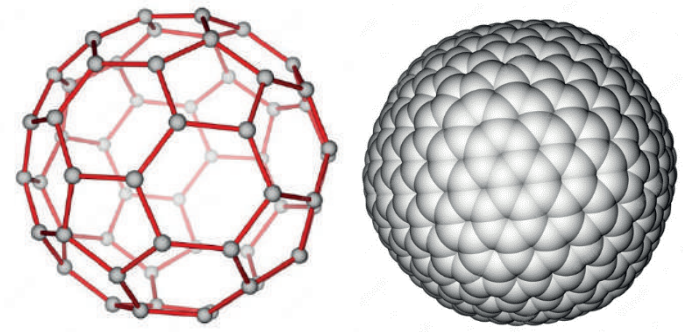


Figure 1. Schematic presentation of the Buckminsterfullerene C60 (left) Balls and Sticks model, (right) Space Filling model.

2. History of Carbon 60 (C_{60}) Fullerene

For the first time in 1966, Deadalus also known as D. E. H Jones suggested that it would be possible to generate C-cages (so called as FULs); however, this idea was not supported by the scientific community. In 1970, exactly 4 years after this idea was put forward, the synthesis of the bowl-shaped molecule called 'Corannulene' was found to be similar to the idea put forward before. Eiji Osawa was the first who hypothesized the spherical icosahedral (I_h) symmetric football structure for the C_{60} molecule (Barth and Lawton, 1966). The first article on FULs was also published in 1970 by the Japanese chemist Osawa who suggested that FULs could theoretically be stable (Yoshida and Osawa, 1971). However, these publications were not well known around the world as they were published in Japanese.

It was observed in 1984 that large, carbon-only clusters (C_n, n=30–190) can be produced upon laser vaporization of graphite. FULs were experimentally investigated in 1985, when Kroto visited Rice University in Houston (Kroto et al., 1985). Furthermore, Smalley et al. from the same University developed a laser ablation technique on a solid, such as graphite, to study refractory clusters with mass spectrometry (Dietz et al., 1981). These studies helped to observe C_{60} FUL with an intense mass spectrum peak. In the light of the results derived by this experimental study; Kroto and Smalley discovered the stable, 32-face truncated icosahedral (I_h) shaped C_{60} (with 12 regular pentagonal faces and 20 regular hexagonal faces), generated on similar principles and named also as Buckminsterfullerene after the architect Buckminster Fuller known for its geodesic dome and announced it to the world of science with their article published in Journal of Nature dated November 14, 1985 (Figure 2) (Kroto et al., 1985).

Figure 2. The geodesic dome built in 1967 by R. B. Fuller at the Montreal Fair.

3. The Structure of Carbon 60 (C_{60}) Fullerene

Truncated icosahedral (I_h) shaped C_{60} [60-I_h] FUL, which looks like a football and approximately 1 nm in diameter, is the smallest and most stable FUL. The pentagonal structures contain single bonds (–) and the hexagonal structures contain double bonds (=). The structure of [60-I_h] FUL has been revealed by theoretical and experimental studies. It has a number of features like all 12 pentagons being isolated by hexagons and the bonds (bonds [5,6]) between a single hexagon and a single pentagon being longer than the bonds (bonds [6,6]) connecting two hexagons (Demirbakan, 2015; Schulman et al., 1987).

While the hexagonal patterns in the FUL structure is only planar, the bonding structure consisting of pentagons provide multi-dimension (Schmalz et al., 1986; Bendikov et al., 2004).

Due to the structural properties, FULs are compounds suitable for chemical modifications. Therefore, it is possible to transform FULs into structures with various physical and chemical properties by using different modifications. For instance, Hydroxyl (-OH), Carboxyl (-COOH) and Amine (-NH2) groups can be introduced onto the surfaces of FUL nano-

particles in order to impart hydrophilic properties or it is possible to obtain endohedral metallofullerenes, which are used as radiotracers in X-ray imaging, by placing metal atoms in the FUL cage (Bosi et al., 2003).

Contrary to the methods for the chemical modification of FUL, a method has been developed to produce water-soluble and chemically unmodified C_{60} FUL without using any solvents or stabilizers and obtained preparation was named as "Hydrated C_{60} Fullerene" (C60HyFn). Stable Hydrated C60HyFn solutions in water contains single hydrated C60 FUL molecules as well as their variable clusters with a size of 3-36 nm (Andrievsky et al., 1995; Andrievsky et al., 1999; Andrievsky et al., 2002; Avdeev et al., 2004). C60HyFn has been the subject of numerous studies over the last 20 years and has been found to have beneficial effects as a neuro-protectant, anticancer, anti-inflammatory, anti-atherogenic and radioprotective agent. Unexpected manifestation of the beneficial effects of C60HyFn, even at very small concentrations and doses, is attributed to its unique bio-antioxidative properties (Andrievsky et al., 2009; Andrievsky et al., 2005; Podolski et al., 2007; Tykhomyrov et al., 2008; Andrievsky et al., 2010).

Consequently, FULs have a significant potential power in many areas at the molecular level due to their unique electron structure and chemical properties (Diederich, 1997). Various synthesized FUL derivatives have been observed to serve promising developments particularly in medical applications or nano-technology.

4. On the Health Effects of Carbon 60 (C_{60}) Fullerene

C60 FUL, which is the third allotrope of C and is a kind of molecule composed entirely of 60 C-atoms, is considered to be the most prominent member of the nano-materials family. Due to its unique physico-chemical properties and significant biological activities, C60HyFn derivative in water-soluble form has been widely researched in many areas by emphasizing the strong antioxidant activity caused by FUL particularly at very low doses. Recent studies revealed that FUL and some of its derivatives have beneficial effects and exhibit effective protection in the treatment of diseases related to oxidative stress (Andrievsky et al., 2009; Markovic and Trajkovic, 2008; Yin, 2009).

FUL is assumed to have the ability to initiate and catalyze reactions with reactive oxygen species (ROS) besides reacting directly with free radicals. This assumption explains how FUL can be effective even at very low doses (Andrievsky et al., 2009).

Studies have revealed that pure C_{60} FUL does not cause acute or subacute toxicity on humans, mice, rats and guinea pigs. Furthermore, it is known that chemically modified FULs are connected to nano-drug carrier systems when used alone or in the form of cylindrical C Nanotubes (CNTs) (Kolosnjaj et al., 2012; Aydın, 2016).

In a study, it was determined that the administration of doxorubicin caused a significant increase in lipid peroxidation (LPO) levels and changed antioxidant enzyme activities in male rats. It was further reported that FUL treatment is quite effective in preventing oxidative stress (Srdjenovic et al., 2010).

The administration of FUL together with cyclophosphamide on rats was found to result in improvement in oxidative stress and antioxidant system parameters (Elshater et al., 2018).

In another study conducted on rats with diabetes, C60HyFn administration was reported to provide a protective effect against the damage in the seminiferous tubules of the testicles due to oxidative stress and prevents the adverse effects on epididymal sperm concentration and motility in addition to reproductive organ weights at varying levels (Bal et al., 2011).

Some in-vitro studies conducted on rams have revealed that FUL added to the semen extender has a positive effect on spermatozoon motility and membrane integrity for the purpose of both short-term and long-term semen storage (Özer Kaya et al., 2021; Güngör et al., 2022).

Similar studies performed on pigs have revealed that FUL added to the semen extender protects spermatozoa against oxidative stress in short-term storage at 4°C (Xinhong et al., 2019).

The number of studies on C60 FUL have recently been increasing and kept up to date. However, the number of studies on male reproductive characteristics and male fertility are still very few.

References

Andrievsky, G., Shakhnin, D., Tronza, A., Zhernosekov, D., & Tykhomyrov, A. (2010). The acceleration of blood plasma clot lysis in the presence of hydrated C60 fullerene nanostructures in super-small concentration. Fullerenes, Nanotubes, Carbon Nanostructures, 18, 303–311.

Andrievsky, G.V., Bruskov, V.I., Tykhomyrov, A.A., & Gudkov, S.V. (2009). Peculiarities of the antioxidant and radioprotective effects of hydrated C60 fullerene nanostuctures in vitro and in vivo. Free Radical Biology and Medicine, 47, 786–793.

Andrievsky, G.V., Klochkov, V.K., & Derevyanchenko, L.I. (2005). Is C60 fullerene molecule toxic? Fullerenes, Nanotubes, Carbon Nanostructures, 13, 363–376.

Andrievsky, G.V., Klochkov, V.K., Bordyuh, A., & Dovbeshko, G.I. (2002). Comparative analysis of two aqueous-colloidal solutions of C60 fullerene with help of FT-IR reflectance and UV–vis spectroscopy. Chemical Physics Letters, 364, 8–17.

Andrievsky, G.V., Klochkov, V.K., Karyakina, E.L., & Mchedlov-Petrossyan, N.O. (1999). Studies of aqueous colloidal solution of fullerene C60 by electron microscopy. Chemical Physics Letters, 300, 392–396.

Andrievsky, G.V., Kosevich, M.V., Vovk, O.M., Shelkovsky, V.S., & Vashchenko, L.A. (1995) On the production of an aqueous colloidal solution of fullerenes. Journal of the Chemical Society, Chemical Communications, 12, 1281–1282.

Avdeev, M.V, Khokhryakov, A.A., Tropin, T.V., Andrievsky, G.V., Klochkov, V.K., Derevyanchenko, L.I., Rosta, L., Garamus, V.M., Priezzhev, V.B., Korobov, M.V., & Aksenov, V.L. (2004). Structural features of molecular-colloidal solutions of C60 fullerenes in water by small-angle neutron scattering. Langmuir, 20, 4363– 4368.

Aydın, E. (2016). Tek Duvarlı Kiral Karbon Nanotüplerde Fonon Dağınım Bağıntıları. Doktora Tezi, Ankara, Ankara Üniversitesi, Fen Bilimleri Enstitüsü.

Bal, R., Turk, G., Tuzcu, M., Yilmaz, O., Ozercan, I., Kuloglu, T., Gur, S., Nedzvetsky, V.S., Tykhomyrov, A.A., Andrievsky, G.V., Baydas, G., & Naziroglu, M. (2011). Protective effects of nanostructures of hydrated C(60) fullerene on reproductive function in streptozotocin-diabetic male rats. Toxicology, 282(3), 69-81.

Barth, W.E., & Lawton, R.G. (1966). Dibenzo [ghi, mno] fluoranthene. Journal of the American Chemical Society, 88, 380.

Bendikov, M., Wudl, F., & Perepichka, D.F. (2004). Tetrathiafulvalenes, oligoacenenes, and their Buckminsterfullerene derivatives: The brick and mortar of organic electronics. Chemical Reviews, 104(11), 4891–4946.

Bosi, S., Da Ros, T., Spalluto, G., & Prato, G. (2003). Invited Review: Fullerene derivatives: an attractive tool for biological applications. European Journal of Medicinal Chemistry, 38: 1 – 3.

Demirbakan, B. (2015). HSP70'in Hassas ve Ekonomik Analizi İçin Fulleren C_{60} Temelli Bir Biyosensör. Yüksek Lisans Tezi, Tekirdağ, Namık Kemal Üniversitesi, Fen Bilimleri Enstitüsü.

Diederich, F. (1997). Covalent fullerene chemistry. Pure and Applied Chemistry, 69(3), 395-400.

Dietz, T.G., Duncan, M.A., Powers, D.E., & Smalley, R.E. (1981). Laser production of supersonic metal cluster beams. The Journal of Chemical Physics, 74, 6511.

Elshater, A.A., Haridy, M.A.M., Salman, M.M.A., Fayyad, A.S., & Hammad, S. (2018). Fullerene C60 nanoparticles ameliorated cyclophosphamide-induced acute hepatotoxicity in rats. Biomedicine and Pharmacotherapy, 97, 53–59.

Godly, E.W., & Taylor, R. (1997). Nomenclature and terminology of fullerenes: A preliminary survey. Pure and Applied Chemistry, 69(7), 1411-1434.

Gungor, İ.H., Dayan Cinkara, S., Acisu, T.C., Arkali, G., Koca, R.H., Akarsu, S.A., Can, C., Ozer Kaya, Ş., Kizil, M., Cakir, A., Firat, F., Halici, M.S., Yilmaz, I., Badilli, N., Yuce, A., Gur, S., Sonmez, M., & Turk, G. (2022). Effect of hydrated carbon 60 fullerene on frozen ram semen quality. Biopreservation and Biobanking, 20(4), 340-347.

Hanaoka, T., Kawamura, N., Hara, K., & Tsugane, S. (2002). Urinary bisphenol A and plasma hormone concentrations in male workers exposed to bisphenol A diglycidylether and mixed organic solvents. Occupational and Environmental Medicine, 59(9), 625-628.

Kolosnjaj, T.J., Baati, T., Szwarc, H., & Moussa, F. (2012). Toxicity studies of [60] fullerene and carbon nanotubes: State of the art. In: D'Souza, F., Kadish, K.M. (Editors). Handbook of Carbon Nano Materials 1st Edition, Singapore, World Scientific: 49-75.

Kroto, H.W., Heath J.R., O'Brien, S.C., Curl, R.F., & Smalley, R.E. (1985). C_{60}: Buckminsterfullerene. Nature, 318, 162-163.

Markovic, Z., & Trajkovic, V. (2008). Biomedical potential of the reactive oxygen species generation and quenching by fullerenes (C60). Biomaterials, 29(26), 3561-3573.

Ozer Kaya, Ş., Gungor, İ.H., Dayan Cinkara, S., Acisu, T.C., Koca, R.H., Akarsu, S.A., Can, C., Çakir, A., Yilmaz, İ., Halici, M.S., Gur, S., Sonmez, M., & Turk, G. (2021). Effect of different doses of hydrated C60 fullerene nanoparticles on ram semen during cool-storage. Turkish Journal of Veterinary and Animal Sciences, 45(1), 139-147.

Podolski, I.Y., Podlubnaya, Z.A., Kosenko, E.A., Mugantseva, E.A., Makarova, E.G., Marsagishvili, L.G., Shpagina, M.D., Kaminsky, Y.G., Andrievsky, G.V., & Klochkov, V.K. (2007). Effects of hydrated forms of C60 fullerene on amyloid-peptide fibrillization in vitro and performance of the cognitive task. Journal of Nanoscience and Nanotechnology, 7, 1479–1485.

Saito, R., Dresselhaus, G., & Dresselhaus M.S. (1998). Physical Properties of Carbon Nanotubes. 1st Edition, London, Imperial College Press.

Schmalz, T.G., Seitz W.A., Klein, D.J., & Hite, G.E. (1986). C60 carbon cages. Chemical Physics Letters, 130(3), 203-207.

Schulman, J.M., Disch, R.L., Miller, M.A., & Peck, R.C. (1987). Symmetrical clusters of carbon atoms: The C_{24} and C_{60} molecules. Chemical Physics Letters, 141(1–2), 45-48.

Srdjenovic, B., Torres, V.M., Grujic, N., Stankov, K., Djordjevic, A., & Vasovic, V. (2010). Antioxidant properties of fullerenol $C_{60}(OH)_{24}$ in rat kidneys, testes, and lungs treated with doxorubicin. Toxicology Mechanisms and Methods, 20(6), 298-305.

Tykhomyrov, A.A., Nedzvetsky, V.S., Klochkov, V.K., & Andrievsky, G.V. (2008). Nanostructures of hydrated C60 fullerene (C60HyFn) protect rat brain against alcohol impact and attenuate behavioral impairments of alcoholized animals. Toxicology, 246, 158–165.

Xinhong, L., Lirui, W., Huan, L., Fu, J., Zhen, L., Li, Y., Zhang, Y., & Zhang, Y. (2019). C_{60} Fullerenes Suppress Reactive Oxygen Species Toxicity Damage in Boar Sperm. Nano-Micro Letters, 11(104), 1-17.

Yin, J. J., Lao, F., Fu, P. P., Wamer, W. G., Zhao, Y., Wang, P. C., Qiu, Y., Sun, B., Xing, G., Dong, J., Liang, X. J., & Chen, C. (2009). The scavenging of reactive oxygen species and the potential for cell protection by functionalized fullerene materials. Biomaterials, 30(4), 611-622.

Yoshida, Z., & Osawa, E. (1971). Aromaticity. Chemical Monograph Series, 22, 174–178.

Chapter 4

The Effects of Micro & Nano Pollution on Fish Reproduction

Ali Erdem Öztürk[1]

Abstract

The changing world and the increasing use of nanomaterials in industry have caused industrial nano wastes to enter the water and oceans in recent years. By a similar mechanism, plastic wastes that enter the waters erode over time and turn into microplastics. Fish, which encounter micro & nano pollutants (MNP) in their habitats, ingest these materials through the food chain, respiration and direct contact. Although water pollution attracts attention in the media and society as a result of mass fish deaths, disruptions in the reproductive cycles of fish exposed to MNPs and anomalies in juvenile development stages threaten the future of fish population in aquatic ecosystems. Fish that experience limitations in their ability to reproduce or produce offspring with anomalies are at risk of facing extinction within a short timeframe. In this chapter, the effects of various industrial nanomaterials and microplastics on fish reproductive systems are discussed and an awareness is tried to be created.

1. Sources of Microplastics and Nanoparticles in Aquatic Ecosystems

Nanomaterials are one of a kind structural properties due to their dimensions, which typically range from 1 to 100 nanometers (Biswas and Wu 2005). By reducing materials to nanoscale dimensions compared to their normal size, they exhibit distinct properties. Nanomaterials possess a significantly higher surface area due to their size, enhancing their effectiveness compared to their bulk counterparts. Incorporating structures like nanotubes or nanosheets can impart desirable properties such as enhanced strength or flexibility to the final product. Furthermore, nanomaterials display

1 Assist. Prof., Department of Reproduction and Artificial Insemination,Faculty of Veterinary Medicine,Erciyes University, Kayseri, Türkiye. aeozturk@erciyes.edu.tr https://orcid.org/0000-0002-3053-4615

https://doi.org/10.58830/ozgur.pub203.c937

varying electrical and thermal conductivity properties based on their size and structure, making them suitable for applications in electronic devices and energy storage systems. With their expansive surface areas and reactive groups, nanomaterials find utility in diverse fields such as catalysts for chemical reactions, sensors, and medical applications (Neuberger, Schöpf et al. 2005, Mathew and Juang 2007, Ghazanfari, Kashefi et al. 2016, Koo, Ismail et al. 2019).

Nanomaterials find applications in a wide range of industries, including electronics, drug development, materials science, and energy storage. As research in nanotechnology and nanoscience continues to advance, new nanomaterials will be developed and utilized. Industrial nanomaterials are specifically designed using nanotechnology for use in industrial applications, and they exhibit diverse properties. Carbon nanotubes, for instance, have numerous uses in areas such as electronics, energy storage, and material reinforcement. On the other hand, nanocomposites enhance the mechanical, thermal, or electrical properties of polymers, resulting in more durable and functional materials. The continued development and utilization of nanomaterials contribute to advancements and innovations in various industries. (Darwish, Mostafa et al. 2022, Khan, Mubarak et al. 2022, Mishra, Devi et al. 2022, Öztürk and Ömür 2022). Nanoparticles like gold, silver, and platinum possess valuable catalytic properties and a large surface area, enabling their application in a wide range of fields. These tiny particles find utility in sensors, electronic and optical devices, as well as health and agricultural applications (Rastogi, Kumari et al. 2022, Husain, Nandi et al. 2023). TiO2 nanoparticles are semiconducting materials known for their remarkable photocatalytic activity. As a result, they are extensively employed in a variety of applications such as solar energy units, sunscreens, coatings, and air and water pollution control (Jiang, Zhou et al. 2022, Nandiyanto, Salsabila et al. 2023). Quantum dots, a type of semiconductor nanomaterial, possess the unique ability to absorb and emit light at precise wavelengths. This distinctive property makes them valuable for a range of applications including LED displays, solar panels, and biomedical imaging (Abdellatif, Younis et al. 2022, Xu, Niu et al. 2022, Jang and Jang 2023). Microplastics refer to tiny particles composed of different types of plastic materials, typically measuring less than 5 mm in size. These particles may originate from the degradation of plastic waste or be generated during the production, usage, or processing of specific products (Zhang, Xu et al. 2022). The degradation of large plastic waste in marine and natural environments can occur over time through various factors such as exposure to sunlight, wave movements, and mechanical abrasion. As a result of this degradation process, microplastics are

formed and released into the environment (Fan, Zou et al. 2022). Garments made from synthetic fibers, such as polyester or acrylic, are widely used and are known for their plastic-based composition. When these garments are washed, microplastic particles can be released from the fiber materials and enter the water system. Eventually, these microplastics may find their way into natural environments through the sewage system (Chen, Chen et al. 2022). Over time, plastic-based packaging products like plastic bottles, bags, and packaging materials can undergo degradation and transform into microplastics. These microplastics can originate from various sources, including landfills, leaks from garbage, or plastics that have been accidentally discarded in natural environments (Chen, Xu et al. 2023). Certain cosmetic products and cleaning agents, such as exfoliating peels and toothpastes, may include particles that contain microplastics. When these products are used, the microplastics can enter the water system and subsequently disperse into the environment (Zhou, Ashokkumar et al. 2023) (Fig 1).

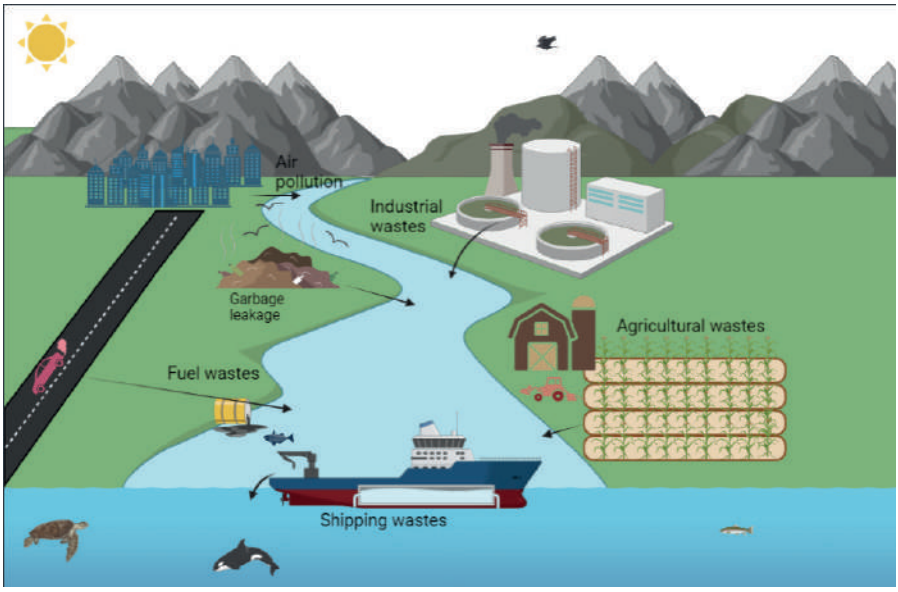


Figure 1. The sources of MNPs in water and oceans.

The presence and buildup of microplastics in natural environments and water resources can pose a potential threat to ecosystems and living organisms. Hence, it is crucial to prioritize initiatives that aim to raise awareness about microplastic pollution and promote the reduction of plastic consumption. It's worth noting that microplastics and nanoparticles have the potential to contaminate various consumable items, including food

and beverages, underlining the significance of addressing this issue for the well-being of both humans and the environment (Vitali, Peters et al. 2023). The escalating issue of nanoparticle and microplastic pollution in water, as discussed earlier, raises concerns about the potential impact on aquatic organisms. Research indicates that nanomaterials and microplastics primarily pose risks to the intestinal system and gills of these organisms (Alak, Uçar et al. 2022, Nabi, Ahmad et al. 2022). While studies on the effects of micro-nano pollutants (MNPs) on fish reproductive systems are still ongoing, there is growing evidence indicating potential impacts. Recent research compiled in this section aims to shed light on this issue, highlighting the alarming problem that these pollutants pose to the survival and potential extinction of various living organisms.

2. Effects of Nano Pollutants on Fish Reproductive System

For a considerable period, water pollution resulting from industrial growth and inadequate disposal of industrial waste into rivers and oceans was chiefly associated with visible waste materials. Nonetheless, recent studies has shed light on the presence of MNPs in water pollution, prompting scientists to investigate the potential ramifications of these pollutants. Remarkable findings have emerged from the diligent efforts of scientists studying fish reproduction and its relation to MNPs in aquatic environments.

Deepa, Mamta et al. (2022) conducted a study highlighting the detrimental effects of carbon nanotubes on testicular tissue in *Common carp*. Their findings revealed an increase in reactive oxygen species levels within the testicular cells, leading to cellular apoptotic changes. Another study by Deepa, Murugananthkumar et al. (2019) reported that ZnO nanoparticles resulted in the downregulation of steroid enzymes in testicular tissue. Additionally, they observed that increasing doses of ZnO nanoparticles led to oxidative stress and damage in the testicular tissue of *Cyprinus carpio* fish.

When Asian striped catfish (*Mystus vittatus*), which is known for their resilient structure, were exposed to ZnS nanomaterials, abnormalities were observed in testicular cell development. Additionally, damage to the spermatozoa membrane was detected. (Bhattacharjee and Chatterjee 2016). Cu nanoparticles were introduced to *Clarias bathrachus*, a catfish species, resulting in damage to the basal lamina of the testicular tissue. This damage led to the presence of different types of spermatogonia and enlargement of spermatocytes (Murugananthkumar, Rajesh et al. 2016).

Zebrafish (*Danio rerio*) is widely recognized as the primary experimental fish in aquatic studies. This versatile fish species is commonly employed for modeling human conditions and is also extensively utilized in studies examining the potential toxicity of nanoparticles. In zebrafish studies conducted by (Kotil, Akbulut et al. 2017), it was observed that TiO2 nanoparticles led to mitochondrial degeneration and necrosis specifically in Sertoli cells. Similarly, Ma, Lu et al. (2018) discovered that silver nanoparticles induced the formation of reactive oxygen species (ROS) in the testes and ovaries after 5 weeks or longer exposure, subsequently triggering mitochondria-dependent apoptosis in the cells. In a study conducted by Sumi and Chitra (2020), it was found that exposure to C60 fullerene nanoparticles resulted in decreased testosterone levels in male fish and estrogen levels in female fish, specifically in their research with *Anabas testudineus*. They concluded that such exposure to C60 fullerene nanoparticles caused reproductive toxicity in *Anabas testudineus* fish.

Nanotoxicity studies focusing on females are equally crucial in understanding the effects on reproduction. While males primarily experience damage to testicular tissue and spermatozoa, females face additional risks, including damage to the ovary, oocyte, and even the potential harm to larvae in live-bearing species. Notably, since a male can fertilize multiple females, a decrease in the male population may be "partially" tolerable. However, a decline in the number of females can lead to irreversible damage, significantly endangering the species' continuity. This highlights the significance of considering the broader impact on females in nanotoxicity studies for the preservation of species.

Studies investigating nano-toxicity in various species of female fish have indicated that silver (Ag) nanoparticles can inhibit the release of steroid hormones from ovarian cells of *Oryzias melastigma* (Degger, Anna et al. 2015). Ag nanoparticles were also found to affect meiosis and cause oxidative stress in Zebrafish gonads (Szudrowicz, Kamaszewski et al. 2022). In a study conducted on zebrafish, Dayal, Thakur et al. (2016) stated that gold nanoparticles cause damage to the ovarian structure. Similarly, TiO2 nanoparticles cause 29.5% egg loss in females at 13 weeks of exposure (Wang, Zhu et al. 2011).

Nanomaterials can be produced in two forms: chemical and biological (green synthesis). The general opinion is that green synthesis nanoparticles are less toxic to biological systems(Nadaroglu, Güngör et al. 2017). In a study proving this information, Sarkar, Netam et al. (2014)stated that plant derived silver nanoparticles have no toxic effect on Zebrafish. Therefore, it is necessary

to leave an open door for the toxicity of these industrial nanomaterials to those of biological origin. Numerous studies have demonstrated the toxicity of silver nanoparticles, revealing that they not only accumulate in the mother fish but can also be transferred to the fry during larval development, as highlighted in the study by Yan and Wang (2022). This maternal transfer of nanoparticles poses a significant risk, impacting not only the current generation but also potentially impacting future generations. Similarly, in a related study conducted by Shi, Zhang et al. (2018), it was observed that selenium nanoparticles induced malformations in the offspring.

3. Effects of Microplastics on Fish Reproductive System

Fish can come into contact with microplastics through various routes, including inhalation, ingestion through water, or direct consumption as food. The potential impacts of microplastics on fish can be categorized as; i. digestive problems by the accumulation in the intestinal tract, ii. toxic effects iii. food chain bioaccumulation iv. behavioral changes such as feeding, swimming behavior, and reproduction. (Andrady 2011, Wang, Tan et al. 2016, Pinheiro, Oliveira et al. 2017, Bessa, Barría et al. 2018).

When investigating the reproductive system in fish, one frequently mentioned type of microplastic is polystyrene microplastics. These microplastics consist of small particles made from a polymer known as polystyrene and are utilized in various industries. One prominent use of polystyrene foam is found in packaging materials due to its lightweight and insulating properties (Abidin, Sanny et al. 2022). Additionally, polystyrene-based materials are commonly employed for thermal and sound insulation in buildings, including walls, ceilings, and roofs (Ramli Sulong, Mustapa et al. 2019). They have also been utilized in medical devices and laboratory equipment (Loos, Syrovets et al. 2014), as well as electronic devices, computer cases, and cell phone casings (Sen, Zhao et al. 2004).

The utilization of polystyrene microplastics in various applications has faced criticism due to their detrimental environmental impacts. These tiny particles can persist in the environment for thousands of years and are prone to ingestion by marine organisms in aquatic ecosystems. This ingestion poses a significant threat to marine organisms, as the effects of polystyrene microplastics can propagate through the food chain. The environmental impact of microplastics, including their potential to exacerbate pollution in marine and aquatic ecosystems, has raised significant concerns. Consequently, numerous countries and companies have taken measures to reduce or completely eliminate the use of polystyrene microplastics in response to these environmental concerns (Wagner 2020).

Microplastics remain in aquatic ecosystems for a long time. Therefore, studies have shown that long-term exposure to polystyrene microplastics introduced into aquatic ecosystems causes ROS formation in both the ovaries and testes of zebrafish. In addition, they emphasized that increasing doses of polystyrene microplastics activate the p53 mediated apoptotic pathway in gonads. (Qiang and Cheng 2021). Another study conducted on zebrafish highlighted the impact of polystyrene microplastics, emphasizing their role in increasing the bioaccumulation of microcystin-LR and causing endocrinological disorders through gonadal damage. (Lin, Luo et al. 2023). In a separate endocrinological study involving male *Oryzias melastigma* fish, the presence of polystyrene microplastics resulted in a significant estrogenic effect (Wang, Li et al. 2022). Although chronic studies have revealed that microplastics cause endocrinological disorders and gonadal damage, the low transgenerational effects are promising for now (Qiang, Lo et al. 2020). Despite the transgenerational effect being generally limited, the reproductive development of growing *Oryzias latipes* juveniles demonstrates a different outcome. The exposure of larvae to polyethylene microplastics resulted in a noteworthy decrease in the gene expression of 11-beta-dehydrogenase isozyme 2 (HSO11β2), which is directly linked to sperm quality during the developmental stage. This decrease led to poor sperm quality in the affected individuals (DiBona, Haley et al. 2022). Although it is encouraging that microplastics are not transmitted to the next generation, the increasing concentration of microplastics in freshwater and oceans is posing limitations on fish reproduction.

4. Conclusion

The emergence of various industrial products has been driven by increasing population and consumption needs. This industrial development has also led to the rise of new technologies, including nanotechnology, which has found applications in numerous sectors such as healthcare, transportation, technology, construction, food, and agriculture. Nanotechnology has played a transformative role, providing easy and cost-effective solutions in technological products, acting as a preservative or enhancer in agriculture, enhancing strength in mechanical and mechatronic fields, serving as colorants or coatings in textiles, and acting as targeted carriers in the field of healthcare. However, these widespread applications have also resulted in a significant amount of nano waste being generated in developed and developing countries. Sadly, the ultimate destination for much of this nano waste, which combines with soil, air, and water, has been the seas and oceans. On the other hand, the emergence of microplastics presents a slightly different challenge. MNPs can have a direct effect on the reproductive systems of fish. The physical

degeneration of the gonads causes a decrease in the quality of sperm and ovum and a decrease in the number of offspring. On the other hand, MNPs, which have also been found to cause estrogenic effects in male fish, also bring about changes in gender characteristics. Although not in microplastics, nano-pollutants also affect the next generation, causing offspring born with anomalies or developmental problems. Exposure to MNPs (magnetic nanoparticles) during the developmental stages of naturally-born offspring has been found to have detrimental effects on their development. Studies in this field indicate that the extent of damage is dependent on the dosage of MNPs. Fish, being among the last organisms affected by nano-pollution in the oceans, will likely experience harm only when the concentration of MNPs required to cause damage is already present. By that point, algae, plankton, and other marine creatures may have already perished. Even if MNPs do not directly impact fish, they can still disrupt aquatic ecosystems, leading to disturbances in the marine ecosystem and ultimately resulting in secondary factors contributing to fish mortality. In his review, (Dedman 2022) showed a few of the studies examining the effects of nano-pollution on marine ecosystems. The results from this section are simply shown in Figure 2.

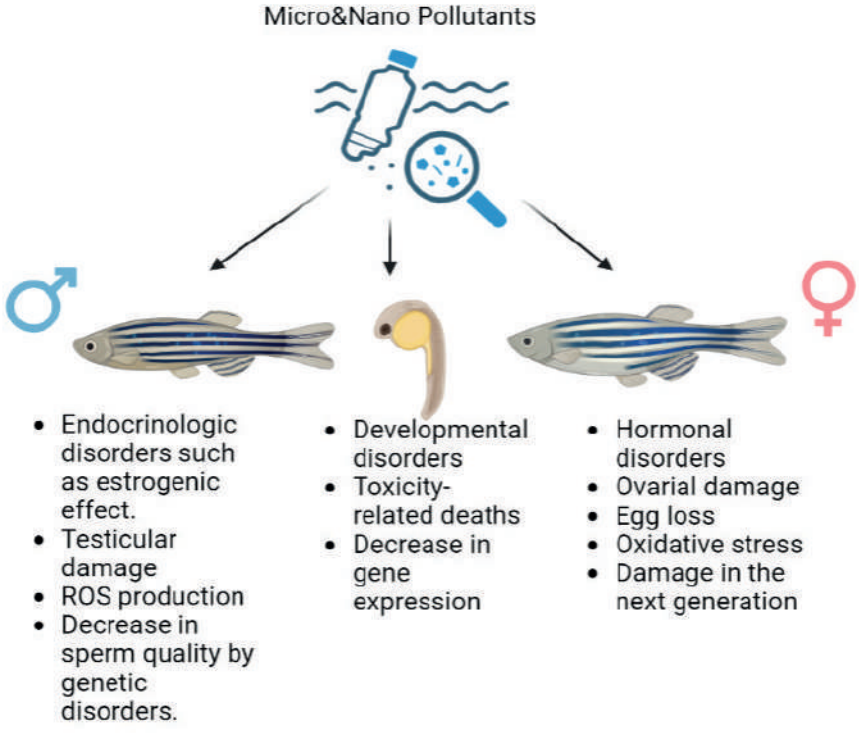


Figure 2. Effect of MNPs on males, females and larvae

In conclusion, while MNPs may not currently pose as significant of a threat as other factors such as global warming or chemical wastes, the ongoing industrial development raises concerns about their potential to become a major source of pollution in the coming decades. Without proper measures and precautions, MNPs could emerge as a significant contributor to environmental pollution. This, in turn, could lead to detrimental consequences such as fish mortality, reproductive impairments, and even species extinctions. It is crucial to address and mitigate the potential risks associated with MNPs to prevent such outcomes from occurring in the future.

References

Abdellatif, A. A., M. A. Younis, M. Alsharidah, O. Al Rugaie and H. M. Tawfeek (2022). "Biomedical applications of quantum dots: overview, challenges, and clinical potential." International journal of nanomedicine: 1951-1970.

Abidin, U. F. U. Z., M. Sanny and N. H. Z. Abedin (2022). Knowledge, attitude, and practice (KAP) of polystyrene food packaging usage among food operators. Food Safety Practices in the Restaurant Industry, IGI Global**:** 100-122.

Alak, G., A. Uçar, V. Parlak and M. Atamanalp (2022). "Identification, characterisation of microplastic and their effects on aquatic organisms." Chemistry and Ecology **38**(10): 967-987.

Andrady, A. L. (2011). "Microplastics in the marine environment." Marine pollution bulletin **62**(8): 1596-1605.

Bessa, F., P. Barría, J. M. Neto, J. P. Frias, V. Otero, P. Sobral and J. Marques (2018). "Occurrence of microplastics in commercial fish from a natural estuarine environment." Marine pollution bulletin **128**: 575-584.

Bhattacharjee, B. and N. Chatterjee (2016). "Perilous effect of ZnS nanoparticles on testicular cell development and sperm morphology in the Asian striped catfish Mystus vittatus (Bloch, 1794)." Advanced Science Letters **22**(1): 64-70.

Biswas, P. and C.-Y. Wu (2005). "Nanoparticles and the environment." Journal of the air & waste management association **55**(6): 708-746.

Chen, Y., Q. Chen, Q. Zhang, C. Zuo and H. Shi (2022). "An Overview of Chemical Additives on (Micro) Plastic Fibers: Occurrence, Release, and Health Risks." Reviews of Environmental Contamination and Toxicology **260**(1): 22.

Chen, Y., H. Xu, Y. Luo, Y. Ding, J. Huang, H. Wu, J. Han, L. Du, A. Kang and M. Jia (2023). "Plastic Bottles for Chilled Carbonated Beverages as a Source of Microplastics and Nanoplastics." Water Research: 120243.

Darwish, M. S., M. H. Mostafa and L. M. Al-Harbi (2022). "Polymeric nanocomposites for environmental and industrial applications." International Journal of Molecular Sciences **23**(3): 1023.

Dayal, N., M. Thakur, P. Patil, D. Singh, G. Vanage and D. Joshi (2016). "Histological and genotoxic evaluation of gold nanoparticles in ovarian cells of zebrafish (Danio rerio)." Journal of Nanoparticle Research **18**: 1-12.

Dedman, C. J. (2022). "Nano-ecotoxicology in a changing ocean." SN Applied Sciences **4**(10): 264.

Deepa, S., S.-K. Mamta, A. Anitha and B. Senthilkumaran (2022). "Exposure of carbon nanotubes affects testis and brain of common carp." Environmental Toxicology and Pharmacology **95**: 103957.

Deepa, S., R. Murugananthkumar, Y. Raj Gupta, M. Gowda KS and B. Senthilkumaran (2019). "Effects of zinc oxide nanoparticles and zinc sulfate on the testis of common carp, Cyprinus carpio." Nanotoxicology **13**(2): 240-257.

Degger, N., C. Anna and R. S. Wu (2015). "Silver nanoparticles disrupt regulation of steroidogenesis in fish ovarian cells." Aquatic Toxicology **169**: 143-151.

DiBona, E., C. Haley, S. Geist and F. Seemann (2022). "Developmental Polyethylene Microplastic Fiber Exposure Entails Subtle Reproductive Impacts in Juvenile Japanese Medaka (Oryzias latipes)." Environmental Toxicology and Chemistry **41**(11): 2848-2858.

Fan, J., L. Zou, T. Duan, L. Qin, Z. Qi and J. Sun (2022). "Occurrence and distribution of microplastics in surface water and sediments in China's inland water systems: a critical review." Journal of Cleaner Production **331**: 129968.

Ghazanfari, M. R., M. Kashefi, S. F. Shams and M. R. Jaafari (2016). "Perspective of Fe 3 O 4 nanoparticles role in biomedical applications." Biochemistry research international **2016**.

Husain, S., A. Nandi, F. Z. Simnani, U. Saha, A. Ghosh, A. Sinha, A. Sahay, S. K. Samal, P. K. Panda and S. K. Verma (2023). "Emerging trends in advanced translational applications of silver nanoparticles: a progressing dawn of nanotechnology." Journal of Functional Biomaterials **14**(1): 47.

Jang, E. and H. Jang (2023). "Quantum Dot Light-Emitting Diodes." Chemical Reviews **123**(8): 4663-4692.

Jiang, L., S. Zhou, J. Yang, H. Wang, H. Yu, H. Chen, Y. Zhao, X. Yuan, W. Chu and H. Li (2022). "Near-infrared light responsive TiO2 for efficient solar energy utilization." Advanced Functional Materials **32**(12): 2108977.

Khan, F. S. A., N. Mubarak, M. Khalid, M. M. Khan, Y. H. Tan, R. Walvekar, E. Abdullah, R. R. Karri and M. E. Rahman (2022). "Comprehensive review on carbon nanotubes embedded in different metal and polymer matrix: fabrications and applications." Critical Reviews in Solid State and Materials Sciences **47**(6): 837-864.

Koo, K. N., A. F. Ismail, M. H. D. Othman, N. Bidin and M. A. Rahman (2019). "Preparation and characterization of superparamagnetic magnetite (Fe3O4) nanoparticles: A short review." Malaysian Journal of Fundamental and Applied Sciences **15**(1): 23-31.

Kotil, T., C. Akbulut and N. D. Yön (2017). "The effects of titanium dioxide nanoparticles on ultrastructure of zebrafish testis (Danio rerio)." Micron **100**: 38-44.

Lin, W., H. Luo, J. Wu, X. Liu, B. Cao, Y. Liu, P. Yang and J. Yang (2023). "Polystyrene microplastics enhance the microcystin-LR-induced gonadal damage and reproductive endocrine disruption in zebrafish." Science of The Total Environment **876**: 162664.

Loos, C., T. Syrovets, A. Musyanovych, V. Mailänder, K. Landfester, G. U. Nienhaus and T. Simmet (2014). "Functionalized polystyrene nanoparticles as a platform for studying bio–nano interactions." Beilstein journal of nanotechnology **5**(1): 2403-2412.

Ma, Y.-B., C.-J. Lu, M. Junaid, P.-P. Jia, L. Yang, J.-H. Zhang and D.-S. Pei (2018). "Potential adverse outcome pathway (AOP) of silver nanoparticles mediated reproductive toxicity in zebrafish." Chemosphere **207**: 320-328.

Mathew, D. S. and R.-S. Juang (2007). "An overview of the structure and magnetism of spinel ferrite nanoparticles and their synthesis in microemulsions." Chemical engineering journal **129**(1-3): 51-65.

Mishra, K., N. Devi, S. S. Siwal, Q. Zhang, W. F. Alsanie, F. Scarpa and V. K. Thakur (2022). "Ionic Liquid-Based Polymer Nanocomposites for Sensors, Energy, Biomedicine, and Environmental Applications: Roadmap to the Future." Advanced Science **9**(26): 2202187.

Murugananthkumar, R., D. Rajesh and B. Senthilkumaran (2016). "Copper nanoparticles differentially target testis of the catfish, Clarias batrachus: In vivo and in vitro study." Frontiers in Environmental Science **4**: 67.

Nabi, G., S. Ahmad, S. Ullah, S. Zada, M. Sarfraz, X. Guo, M. Ismail and K. Wanghe (2022). "The adverse health effects of increasing microplastic pollution on aquatic mammals." Journal of King Saud University-Science **34**(4): 102006.

Nadaroglu, H., A. A. Güngör and İ. Selvi (2017). "Synthesis of nanoparticles by green synthesis method." International Journal of Innovative Research and Reviews **1**(1): 6-9.

Nandiyanto, A. B., D. Salsabila, F. Aulia, F. R. Hafidza, P. S. Ashfiya and S. Salsabila (2023). "Literature Review: Measuring Titanium Dioxide Particles Size in Sunscreen and Its Effectiveness as UV Radiation Absorber." Fullerene Journal of Chemistry **7**(1): 1-7.

Neuberger, T., B. Schöpf, H. Hofmann, M. Hofmann and B. Von Rechenberg (2005). "Superparamagnetic nanoparticles for biomedical applications: Possibilities and limitations of a new drug delivery system." Journal of Magnetism and Magnetic materials **293**(1): 483-496.

Öztürk, A. E. and A. D. Ömür (2022). "Current Approaches to the Use of Nanoparticles in Reproductive Biotechnologies: Spermatological Researches." The Trends In Nano Materials Synthesis And Applications: 29.

Pinheiro, C., U. Oliveira and M. Vieira (2017). "Occurrence and impacts of microplastics in freshwater fish." J. Aquac. Mar. Biol **5**(6): 00138.

Qiang, L. and J. Cheng (2021). "Exposure to polystyrene microplastics impairs gonads of zebrafish (Danio rerio)." Chemosphere **263**: 128161.

Qiang, L., L. S. H. Lo, Y. Gao and J. Cheng (2020). "Parental exposure to polystyrene microplastics at environmentally relevant concentrations has negligible transgenerational effects on zebrafish (Danio rerio)." Ecotoxicology and Environmental Safety **206**: 111382.

Ramli Sulong, N. H., S. A. S. Mustapa and M. K. Abdul Rashid (2019). "Application of expanded polystyrene (EPS) in buildings and constructions: A review." Journal of Applied Polymer Science **136**(20): 47529.

Rastogi, S., V. Kumari, V. Sharma and F. Ahmad (2022). "Gold Nanoparticle-based sensors in food safety applications." Food Analytical Methods: 1-17.

Sarkar, B., S. P. Netam, A. Mahanty, A. Saha, R. Bosu and K. Krishnani (2014). "Toxicity evaluation of chemically and plant derived silver nanoparticles on zebrafish (Danio rerio)." Proceedings of the National Academy of Sciences, India Section B: Biological Sciences **84**: 885-892.

Sen, R., B. Zhao, D. Perea, M. E. Itkis, H. Hu, J. Love, E. Bekyarova and R. C. Haddon (2004). "Preparation of single-walled carbon nanotube reinforced polystyrene and polyurethane nanofibers and membranes by electrospinning." Nano letters **4**(3): 459-464.

Shi, M., C. Zhang, I. F. Xia, S. T. Cheung, K. S. Wong, K.-H. Wong, D. W. Au, D. E. Hinton and K. W. Kwok (2018). "Maternal dietary exposure to selenium nanoparticle led to malformation in offspring." Ecotoxicology and environmental safety **156**: 34-40.

Sumi, N. and K. C. Chitra (2020). "Possible role of C 60 fullerene in the induction of reproductive toxicity in the freshwater fish, Anabas testudineus (Bloch, 1792)." Environmental Science and Pollution Research **27**: 19603-19615.

Szudrowicz, H., M. Kamaszewski, A. Adamski, M. Skrobisz, J. Frankowska-Łukawska, M. Wójcik, J. Bochenek, K. Kawalski, J. Martynow and P. Bujarski (2022). "The Effects of Seven-Day Exposure to Silver Nanoparticles on Fertility and Homeostasis of Zebrafish (Danio rerio)." International Journal of Molecular Sciences **23**(19): 11239.

Vitali, C., R. J. Peters, H.-G. Janssen and M. W. Nielen (2023). "Microplastics and nanoplastics in food, water, and beverages; part I. Occurrence." TrAC Trends in Analytical Chemistry **159**: 116670.

Wagner, T. P. (2020). "Policy instruments to reduce consumption of expanded polystyrene food service ware in the USA." Detritus **9**(March): 11-26.

Wang, J., X. Li, M. Gao, X. Li, L. Zhao and S. Ru (2022). "Polystyrene microplastics increase estrogenic effects of 17α-ethynylestradiol on male marine medaka (Oryzias melastigma)." Chemosphere **287**: 132312.

Wang, J., Z. Tan, J. Peng, Q. Qiu and M. Li (2016). "The behaviors of microplastics in the marine environment." Marine Environmental Research **113**: 7-17.

Wang, J., X. Zhu, X. Zhang, Z. Zhao, H. Liu, R. George, J. Wilson-Rawls, Y. Chang and Y. Chen (2011). "Disruption of zebrafish (Danio rerio) reproduction upon chronic exposure to TiO2 nanoparticles." Chemosphere **83**(4): 461-467.

Xu, Q., Y. Niu, J. Li, Z. Yang, J. Gao, L. Ding, H. Ni, P. Zhu, Y. Liu and Y. Tang (2022). "Recent progress of quantum dots for energy storage applications." Carbon Neutrality **1**(1): 13.

Yan, N. and W.-X. Wang (2022). "Maternal transfer and biodistribution of citrate and luminogens coated silver nanoparticles in medaka fish." Journal of Hazardous Materials **433**: 128862.

Zhang, K., S. Xu, Y. Zhang, Y. Lo, M. Liu, Y. Ma, H. S. Chau, Y. Cao, X. Xu and R. Wu (2022). "A systematic study of microplastic occurrence in urban water networks of a metropolis." Water Research **223**: 118992.

Zhou, Y., V. Ashokkumar, A. Amobonye, G. Bhattacharjee, R. Sirohi, V. Singh, G. Flora, V. Kumar, S. Pillai and Z. Zhang (2023). "Current research trends on cosmetic microplastic pollution and its impacts on the ecosystem: A review." Environmental Pollution: 121106.

Chapter 5

New Technology in Embryo Manipulations "Nano-Materials"

Aslıhan Turhan[1]

Ahmet Bülent Turhan[2]

Summary

Embryo manipulations have experienced significant increases in recent years. This has led to the development of new methods for the creation of reproductive technologies. When it comes to changing the genetic material of embryonic beings in multiple species– humans especially– there's a particular term involved. It's called nanotechnology and it's quickly becoming an ultra-important area of study. The medical, electricity and energy industries all stand to benefit from its broad range of applications. That's because this revolutionary process involves manipulating materials in a super small size. It involves altering materials at the nanoscale. Nanotechnology offers a range of uses in numerous sectors like medical electronics and energy. In medicine, nanotechnology has led to significant advances in the delivery of drug imaging and diagnosis. Besides, it has facilitated the manipulation of embryos in a new manner. The utilization of nanomaterials in embryo manipulations has the benefit of increasing embryo implantation rates through assisted reproductive technology (ART) and the implantation rates of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Another advantage of using nanomaterials in embryo modification is that genetic abnormalities are reduced. Other than that the relevance of nanoparticles has led to new potential in the field of artificial insemination. This book explains the most recent breakthroughs in the modification of human and animal embryos as well as the numerous types of nanoparticles and the benefits of their use in the process. Additionally, the book discusses the applications of nanoparticles in IVF and gene editing. This book also discusses ethical concerns issues and limitations of the new technology it also highlights the potential impact on the field of reproductive medicine.

1 International Center for Livestock Research and Training, Ankara, Turkey, turhannaslihan@gmail.com

2 Aksaray University, Faculty of Medicine, Department of Medical Biology, Aksaray, Turkey, bulentturhan@aksaray.edu.tr

https://doi.org/10.58830/ozgur.pub203.c938

1. Introduction

Positive outcomes in reproductive medicine can be difficult to guarantee with the widespread use of artificial insemination, which is commonly utilized for preventing genetic abnormalities and increasing fertility. Innovative developments are necessary to improve the success rate of these procedures. Manipulation of embryos has already demonstrated encouraging results, but the potential use of nanotechnology has opened up even more possibilities in this field.

Novel opportunities to increase the success rates of artificial insemination have emerged through the use of embryo manipulation technologies. Genetic diseases are reduced while increasing the chances of a successful pregnancy through the use of various techniques, like in vitro fertilization (IVF), Preimplantation Genetic Diagnosis (PGD), and Intracytoplasmic sperm injection (ICSI), that have been developed. By selecting healthy embryos, these methods achieve their goa

With the passing of time, innovative techniques have surfaced to modify embryos with greater precision, including the impressive CRISPR-Cas9, which has allowed genome editing to finally become achievable. The intriguing possibility of using this to eliminate all genetic abnormalities is a promising development. Another breakthrough is mitochondrial replacement therapy (MRT), which involves the transfer of defective mitochondria from an affected individual's embryo to a healthy donor's embryo. These reproductive advancements are undoubtedly transformative, but they also raise significant moral questions that warrant deep consideration.

2. Embryo manipulation technologies in humans

The transformation of human reproductive medicine has been massive in recent times due to embryo manipulation technologies. Fertilization of an egg by a sperm in a laboratory dish, commonly known as in vitro fertilization (IVF), is the most widely used technique. After the developed embryo is transferred to the uterus, the procedure is complete (Takashi Kohda, 2013). IVF necessitates the manipulation of an embryo in vitro and its growth in a strictly controlled environment (Juncà et al., 2015). Advancements in technology have simplified the supply of IVF, and social and demographic changes have increased their demand in recent times (Kushnir et al., 2022). In vivo-like microenvironments have been created using microfluidic systems to manage gametes, mature oocytes, culture embryos, and carry out fundamental procedures (Wheeler et al., 2017).

Preimplantation Genetic Diagnosis (PGD) is a sophisticated embryo manipulation process, most often seen in tandem with IVF. The main purpose is to minimize the risk of passing hereditary diseases to offspring. This is achieved by testing embryos for potential complications and only selecting those that are completely normal to be implanted. Various research studies have proven PGD an effective method for combating severe hereditary issues. Moreover, doctors can utilize pre-implantation genetic testing to weed out abnormal embryos before they're implanted. PGT is a kind of prenatal diagnosis and only allows normal genetic embryos to be put inside the womb (Knoppers et al., 2006).

Somatic cell nuclear transfer– otherwise known as SCNT– is a method that's been used in both animals and humans. Basically, it involves moving the nucleus of a somatic cell into an egg that has had its nucleus removed. The result? A cloned embryo (Tian et al., 2003). This technique has been used to make genetically engineered critters by using somatic cells with mutations (Lee et al., 2013). But due to ethical issues, reproductive cloning in humans isn't allowed in most countries (David Stocum, 2023). Although, SCNT does come in handy for reprogramming somatic cells into pluripotent stem cells which hold immense promise for medical applications (Tachibana et al., 2013).

3. Embryo manipulation technologies in cows

Advancement in embryo manipulation techniques is an absolute game-changer for animal breeding, especially among cows. Artificial Insemination (AI) is a much-used method where semen is directly inserted into the female reproductive system (Yuksel Agca & John K. Critser, 2006). This is less invasive and more cost-effective than natural breeding as it makes use of superior males without having to bring them around females.

IVF is a popular embryo manipulation technique when it comes to cows. Mainly, the process consists of fertilizing oocytes outside of the cow's body and then transferring the resulting embryos to other cows (Juncà et al., 2015). People have employed this method to cultivate better genes and even protect endangered species. ICSI is another player in this game, which involves injecting only one sperm directly into an oocyte (Takashi Kohda & Fumitoshi Ishino, 2013). Scientists have successfully created transgenic cows with superior disease immunity and increased milk production through the implementation of these methods.

Microfluidic systems have become a trusted option for working with embryos in recent years. Moreover, using embryo manipulation techniques it's possible to clone elite cows and quickly spread superior genetics around

the world (Juncà et al., 2015). All in all, these manipulation technologies have had a substantial impact on the quality of dairy and beef products, as well as on cattle genetics.

4. Embryo manipulation technologies in pigs

Embryo manipulation technologies have been utilized in pigs to improve breeding and genetic selection. Superovulation and embryo recovery are commonly used methods for the rapid multiplication of animals (Moore et al., 2017). The embryos are then collected and transferred to recipient female pigs, resulting in the birth of multiple offspring with desired traits. Recipient female pigs receive collected embryos resulting in multiple offspring with desired traits. Pig production in the industry benefits from these techniques that enhance both quality and quantity (Takashi Kohda & Fumitoshi Ishino, 2013).

Pig breeding also employs in vitro fertilization (IVF) (Juncà et al., 2015). Oocytes are collected from female pigs and fertilized with sperm in a laboratory setting. The resulting embryos are then transferred to recipient female pigs for gestation and birth. This technique allows for greater control over the genetic makeup of the offspring and can be used to produce pigs with specific traits, such as disease resistance or improved meat quality (Church et al., 1985).

Sperm and embryo cryopreservation are also important techniques in pig breeding. Cryopreservation has also been used to preserve endangered pig breeds and maintain genetic diversity (Ibeas et al., 2019). As technology advances, new techniques such as microfluidic systems are being developed to handle gametes, mature oocytes, culture embryos, and perform other basic procedures in a microenvironment that more closely mimics the in vivo environment (Wheeler et al., 2017).

5. Embryo manipulation technologies in sheeps

One of the most widely used techniques in sheep is embryo transfer (ET) (Church et al., 1985). ET involves the transfer of embryos from a genetically superior female to a recipient female that will carry the pregnancy to term. This technique enables the production of multiple offspring from a single female, which is particularly useful when dealing with valuable or rare genetics. ET has been used successfully in sheep for many years and is a valuable tool for improving the genetic potential of flocks (Dominiguez et al., 2020).

In vitro fertilization (IVF) is another commonly used embryo manipulation technique in sheep. IVF involves the fertilization of oocytes (eggs) outside of the female's body, followed by the transfer of resulting embryos to recipient females (Takashi Kohda, 2013). This technique has been used to produce genetically superior offspring, preserve rare genetics, and improve reproductive efficiency in flocks. IVF has also been used to produce transgenic animals, which can be useful for biomedical research (Kushnir et al., 2022).

Cloning is another embryo manipulation technique that has been used in sheep. Cloning has several advantages, including the preservation of endangered species and the improvement of animal production through valuable genetics. The continuous research performed in this area of cloning may pave the way to new discoveries and innovative applications in animal genetics and breeding.

6. Embryo manipulation technologies in horses

Embryo manipulation technologies have been widely used in horses to improve breeding efficiency and genetic selection. Juncà et al. (2015) highlight a technique called in vitro fertilization (IVF), which takes the fertilization process outside the mare's body and into a laboratory setting. The oocytes are fertilized with sperm in this setting. After fertilization, the embryos are left to develop to a specific stage under specific conditions. IVF has been used successfully in horses to produce offspring from mares with fertility issues or to breed mares with stallions that are not physically able to mate (Kushnir et al., 2022).

Embryo transfer allows for the production of multiple offspring from a single mare in a single breeding season (Moisyadi et al., 2009). Additionally, ET can be used to preserve the genetics of valuable mares and stallions by producing multiple offspring from them (Church et al., 1985).

Intracytoplasmic sperm injection (ICSI) is a technique that involves injecting a single sperm directly into an oocyte to fertilize it (Takashi Kohda & Fumitoshi Ishino, 2013). ICSI has been used in horses to overcome fertility issues in stallions or to produce offspring from mares with a history of failed fertilization attempts (Takashi Kohda, 2013). Recent studies have also explored the use of ICSI in horses for genome editing purposes (Mizushima et al., 2023). These embryo manipulation technologies have significantly impacted the horse breeding industry, allowing for improved breeding outcomes and genetic selection.

7. Embryo manipulation technologies in dogs

In dogs, embryo manipulation has become a trendy way of breeding. It's particularly helpful for dogs with sperm that moves poorly or has low counts. IVF is another method of fertilizing in vitro that has been applied to dogs. Following fertilization outside the body, an embryo is returned to the uterus (Takashi Kohda, 2013).

Advanced technology has ushered in a new era of precise and efficient embryo manipulation techniques for dogs. One innovative method is the use of microfluidic systems that create a microenvironment that closely resembles the reproductive tract making it easier to manipulate gametes and embryos (Wheeler et al., 2017). For dogs suffering from male infertility, ICSI has also been used. The dog breeding population can now have better results and improved genetic diversity thanks to high-tech techniques that involve injecting a single sperm directly into the egg resulting in successful fertilization (Church et al., 1985; Takashi Kohda & Fumitoshi Ishino, 2013).

The implementation of embryo manipulation technologies has brought about significant advancements in breeding efficiency and genetic diversity for dogs. But the ethical considerations surrounding these practices cannot be ignored. Modifying the natural reproductive process comes with potential risks and must consider the welfare of the animals in question. Manipulating early embryos can also have lasting effects on the growth and development of offspring. Careful consideration of both benefits and risks is crucial for breeders and researchers before implementing these techniques.

8. Nanomaterials in Embryo Manipulations

In various fields, such as medicine and biotechnology, nanomaterials are materials with at least one dimension less than 100 nanometers (Fraser et al., 2021) that offer unique properties. Sperm-mediated gene transfer could be made more efficient through the use of nanomaterials in embryo manipulations, which could result in the production of genetically modified embryos (Remiào et al., 2018). Nanomaterials are also being studied for their potential in stem-cell-based therapy for brain diseases, anti-tumor treatments, and gene delivery (Dong et al., 2021). With the integration of nanomaterials in embryo manipulations, the reproductive medicine field has the potential for significant advancement and new discoveries (Barhoum et al., 2022).

Various techniques can be improved in efficiency and precision by nanomaterials, making them an important factor in embryo manipulations. One way this can be achieved is through the development of smart materials

with novel functionalities that are biocompatible. These materials can lead to significant advancements in sub-cellular monitoring, creating a necessary non-incremental step forward according to M.J. Lopez-Martinez & E.M. Campo in 2011. Nanotechnology has been considered a tool for reproductive medicine to help overcome some impairments (Remiào et al., 2018). Recent research has investigated the applicability of nanomaterials to improve sperm selection and deliver antioxidants and hormones to preantral follicles (Silva et al., 2021). Using nanotechnologies to measure, understand and manipulate stem cells is a developing field (Ferreira et al., 2008). Incorporating nanomaterials may boost the accuracy and effectiveness of embryo manipulations, ultimately increasing success rates.

Technologies for manipulating embryos are rapidly evolving, with a plethora of options available to enhance precision and efficiency. These methods often involve micro and nanofabrication techniques (M.J. Lopez-Martinez & E.M. Campo, 2011). One innovative approach, which utilizes electrowetting-on-dielectric technology, is the creation of microfluidic chips that are capable of manipulating bovine embryos in vitro (Karcz et al., 2023). By integrating nanotechnology with various other fields, researchers have developed unique nanomaterials that can revolutionize diagnostic and therapeutic applications (Barhoum et al., 2022). It's exciting to imagine the possibilities as more advanced nanomaterials are developed, leading to even more groundbreaking embryo manipulation techniques.

9. Types of nanomaterials used in embryo manipulations

The use of metal nanoparticles in embryo manipulations has become ubiquitous owing to their small size-to-volume ratio and remarkable thermal stability (Tiwari et al., 2011). A bio-barcode assay using anti-PSA antibodies functionalized gold nanoparticles has been shown to be ultra-sensitive (Remiào et al., 2018). In embryo manipulations, gold nanoparticles have been utilized in an assortment of methods such as gene delivery, drug delivery, and imaging due to their biocompatibility and lack of toxicity. This discovery makes gold nanoparticles an attractive option for biomedical applications (Adawale et al., 2019; Carnovale et al., 2019).

Reportedly, silver nanoparticles synthesized from natural sources are a viable replacement for antibiotics in porcine sperm as they serve as effective antimicrobial agents (Silva et al., 2021; Lee et al., 2018). Meanwhile, iron oxide nanoparticles have been utilized in embryo manipulations for cell therapy and tissue engineering (Friedrich et al., 2021). In effecting embryo manipulation, metal nanoparticles' unique features serve them amply in their purpose. Silver nanoparticles, highlighted for their optical, electrical,

and antibacterial capabilities, are highly valuable in gene and medication transport, as well as in imaging (Silva et al., 2021; Lee et al., 2018).

Iron oxide nanoparticles, particularly magnetic iron oxide nanoparticles, have been implemented in the process of sexing semen in swine and bovine species (Silva et al., 2021). They have additionally been employed in fostering the development of zebrafish embryos to assess the toxicity of metal nanoparticles (Martha Sharisha Johnson, 2019; Magro et al., 2018). Embryo manipulations have been intensively studied for potential application with carbon-based nanomaterials.

One of the top materials used nowadays is carbon nanotubes or CNTs. There are two types of CNTs, namely single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs), as identified by Al Moustafa et al. in 2016. Thanks to their exceptional mechanical, electrical, and thermal features, they serve different purposes, one of which is embryo manipulation. Meanwhile, another carbon-based nanomaterial that holds potential is graphene oxide or GO, obtained from graphene by chemically oxidizing graphite flakes with strong oxidizing agents as noted by Liao et al. in 2018. Studies suggest that GO can improve in vitro fertilization in mice without impacting embryo development (Bernabo et al., 2020). As such, carbon-based nanomaterials have displayed promising outcomes in enhancing embryo manipulation techniques (Gaur et al., 2021).

A promising prospect for embryo manipulation is graphene oxide (GO), which boasts unusual properties. In vitro fertilization has demonstrated increased success rates due to alterations in cell behavior facilitated by the application of a graphene oxide nanofilm to embryo surfaces (Zielinska-Gorska et al., 2020). Lopez et al.'s examination of graphene oxides' biocompatibility for in vitro use involved gauging various well-defined examples (Lopez et al., 2022), where they discovered that in general, their use remained safe.

Embryo manipulations frequently utilize lipid-based nanomaterials due to their exceptional properties and versatility. One commonly used type of lipid-based nanoparticle in reproductive research is liposomes, which are spherical nanovesicles consisting of a single lipid bilayer (Saadeldin et al., 2020). Liposomes can transport various materials, including drugs and genetic material, and their size can range from 50 to 1000 nanometers. Their effectiveness in delivering genes to embryos has been proven through research (Pritchard et al., 2021). Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are two other lipid-based nanoparticles that have been utilized in embryo manipulations. These nanoparticles can

improve the bioavailability of therapeutic agents and drugs in embryos (Naseri et al., 2015).

One alternative form of lipid-based nanoparticle employed in embryo manipulations is nanoemulsions. These emulsions are stable, consisting of oil droplets dispersed in water, and can measure between 20 to 200 nanometers. Their efficacy has been established in various studies, proving their competence in delivering nutrients and medications to embryos (Silva et al., 2021). Another alternative is cubosomes, consisting of lipids arranged in a bicontinuous bilayer cubic phase. In experiments, cubosomes have been effective in delivering genes and other therapeutic agents to embryos (Tenchov et al., 2021).

With boundless possibilities, polymer-based nanomaterials possess immense potential in embryo manipulation. Dendrimers, a particular type of polymeric nanoparticle, have garnered significant interest for their applicability in a multitude of arenas, namely drug delivery, biomedical diagnostics, and protein emulation (Noriega-Luna et al., 2014). Extensively studied, these nanomaterials confirm their amenability to biological systems and possess the ability to permeate cell membranes, which renders them an optimal selection for employment in embryo manipulations. Furthermore, polymeric nanoparticles have been utilized as theragnostic tools, combining diagnosis and treatment in a single application (Cheng et al., 2021). With this in mind, polymer-based nanomaterials present a hopeful and promising opportunity for enhancing the field of reproductive medicine (Remiao et al., 2018; Silva et al., 2021).

Magnetoliposomes are nanoparticles that are magnetic in nature. Comprised of an iron oxide core and a bilayer of lipids, they have been utilized in targeted drug delivery and imaging for embryo manipulations, as demonstrated by Remiao et al., (2018). As reported in 2023 by Pacheco et al., clusters of magnetic nanoparticles composed of multiple magnetic nanoparticles connected by a biocompatible polymer have contributed to targeted drug delivery and hyperthermia during embryo manipulations. Magnetic nanoparticle chains, consisting of long chains of magnetic nanoparticles, have been employed to manipulate cells and tissues, as shown in Materon et al., (2021) research. Finally, Bongaerts et al., (2020) demonstrated the use of these magnetic nanoparticles in embryo manipulations for cell migration and neurite outgrowth.

Nanomaterials known as quantum dots possess distinct photophysical characteristics and have been employed in embryo manipulation. Among these, semiconductor quantum dots, which exhibit optical properties

contingent upon the size, have been used to label and track embryos during their developmental stages (Shao et al., 2011). Metal chalcogenide quantum dots, another class of quantum dots, have gained significant traction due to their quantum confinement effects and potential applications in biosensing and bioimaging (Mal et al., 2016). Furthermore, magnetic quantum dots have been utilized to treat cervical cancer in both human cell cultures and xenograft mice (Remiao et al., 2018). Owing to their exceptional properties, quantum dots have become a valuable instrument in the study of embryonic development and manipulation for research purposes.

In vitro, mesoporous silica nanoparticles (MSNs) have been proven to be effective vehicles for therapeutic drugs by Sharif et al., (2020). As a result, MSNs, a type of silica-based nanomaterial, have become a popular option for manipulating embryos due to their exceptional properties. Remiao et al. (2018) proved that MSNs have also been utilized successfully in the transfer of genes through sperm-mediated gene transfer, resulting in higher efficiency rates of genetically modified embryos. Additionally, MSNs have been researched for their potential use in cancer treatment due to their ability to specifically target tumors, as reported by Violetta Carolina Niculescu (2019). As a result, MSNs show great potential in a variety of embryo manipulation applications.

The unique properties and potential applications of titanium dioxide nanoparticles (TiO2 NPs) make them a popular choice in embryo manipulations. Synthesis methods for TiO2 NPs are diverse, including eco-friendly green synthesis with plant extracts, as demonstrated by Rajeshkumar et al., (2021). These nanoparticles offer a range of functions in embryo manipulations, such as serving as a gene delivery tool and a contrast agent for imaging techniques, as shown by Waghmade et al., (2019). However, the pervasiveness of TiO2 NPs across industries poses a potential hazard in terms of their toxicity, as highlighted by Lyashenko et al., (2023). It is therefore critical to comprehend the synthesis, applications, and toxicity of TiO2 NPs in embryo manipulations to ensure their safe and secure utilization.

10. Benefits of using nanomaterials in embryo manipulations

Reducing toxicity to embryos has been made possible with the promising use of nanomaterials in embryo manipulations. In vitro culture, gamete and embryo manipulations often harm embryo viability and result in oxidative stress (Remiào et al., 2018). By delivering antioxidants and hormones to preantral follicles through nanomaterials, sperm selection and pregnancy rates become enhanced. Furthermore, studies have shown that toxicity to

embryos can be lowered with the use of nanomaterials since aggregation formation was observed to have occurred due to the highest concentration of nanoparticles (Silva et al., 2021). In manipulating embryos, incorporating nanomaterials has the potential to enhance embryo development and improve viability.

An exploration by Zhang et al., (2022) studied the outcomes of particle size and surface charge in, in vitro mutagenic response and in vivo embryonic toxicity, discovering that the implementation of nanomaterials can heighten both. The efficacy of gene transfer through sperm-mediated gene transfer is escalated when utilizing nanomaterials, which ultimately allows for the creation of genetically modified embryos. Nanoparticles can also increase diagnostic testing and imaging quality so as to better supervise and trace embryo development. Mouse embryos, held in an electrowetting-on-dielectric (EWOD) chip, experienced a dynamic environment that led to improved reproductive outcomes (Huang et al., 2015). Thanks to all these benefits, implementing nanomaterials in embryo manipulation can advance embryo development.

Nanomaterials have shown great potential in enhancing gene delivery during embryo manipulations. The use of nanomaterials has shown promise in enhancing the efficiency of sperm-mediated gene transfer, a widely employed technique for producing genetically modified embryos (Remiào et al., 2018). Additionally, they may aid in the delivery of hormones and antioxidants to preantral follicles, thereby bolstering sperm selection and fertilization success rates (Silva et al., 2021). Overall, introducing nanomaterials into embryo manipulation protocols has the potential to elevate gene transfer performance and increase the likelihood of generating genetically modified embryos.

Enhanced drug delivery resulting from the use of nanomaterials in embryo manipulations has shown a remarkable potential to improve the efficacy of treatments. With the ability to design nanoparticles that specifically target cells or tissues, drug delivery has become more precise (Joshi et al., 2023). Moreover, the increased solubility of hydrophobic compounds using nanoparticles can improve the bioavailability of drugs for better results (Silva et al., 2021). With the potential to target specific cells and improve drug solubility, the use of nanomaterials can significantly enhance treatments and advance patient outcomes (Mitchell et al., 2021)

Nanomaterials can also reduce drug toxicity, which is a significant concern in embryo manipulations. The use of nanoparticles can minimize the impact of patient heterogeneity, allowing for more accurate patient stratification and

reducing the risk of adverse reactions (Mitchell et al., 2021). Improvement in toxicity testing is crucial for ensuring treatments are safe and effective, and nanomaterials can aid in achieving this. Nanoparticles can be used to deliver antioxidants and hormones to preantral follicles, which can improve the viability of embryos and reduce oxidative stress (Celà et al., 2014).

The use of nanomaterials in toxicity testing can lead to more reliable results and improved safety in embryo manipulations. Nanotechnology provides unique advantages for stem cell research, including precise stem cell manipulation. The development of nanomaterials and tools has made it possible to manipulate endogenous stem cells from their neurogenic niche, triggering neurogenesis and enhancing the regenerative potential of the brain (Masoudi Asil et al., 2020). The small size of nanomaterials makes them of interest in bioengineering and stem cell research, and their interaction with proneurogenic factors within the stem cell niche can promote self-renewal, proliferation, and differentiation (Masoudi Asil et al., 2020). The delivery of signaling molecules with spatial-temporal precision can guide stem cell behavior and improve therapeutic outcomes (Chueng et al., 2016). These findings suggest that the use of nanomaterials in stem cell research can lead to more precise and effective outcomes.

11. Applications of nanomaterials in in vitro fertilization (IVF)

Studies have displayed optimistic results for the utilization of nanomaterials in improving sperm motility during in vitro fertilization (IVF). Nano-zinc, for instance, has displayed the capacity to enhance semen quality in young rams (Falchi et al., 2018). Another possible application for nanomaterials is the enhancement of gene transfer efficacy via sperm-mediated transfer, which could be particularly valuable for the production of genetically modified embryos (Remiào et al., 2018). With the help of nanomaterials, sperm motility can be amplified, and enhancements can be made to the selection, morphology, and DNA fragmentation levels- ultimately leading to higher fertilization rates and increased IVF success (Fraser et al., 2021; Silva et al., 2021).

Nanomaterials could be the answer to bettering the quality of oocytes, which is essential for IVF success.To achieve this, on-chip manipulation of culture medium microdroplets and electrical stimulation of embryos and gametes have displayed encouraging results in a study conducted by Karcz et al. (2023). Additionally, Morimoto et al. (2023) found that mitochondrial transfer to human oocytes leads to acceptable clinical outcomes, enhancing embryo quality. Moreover, Palay et al. (2022) have experimentally employed

engineering-based models to estimate oocyte quality. Thus, implementing nanomaterials in IVF treatment has the potential to boost the oocyte quality and unlock better success rates.

Nano-bio technology has shown potential in helping with embryo selection during IVF. This includes improving sperm motility and oocyte quality. The future use of chick embryo chorioallantoic membrane (CAM) in nano-bio is also promising. All of these techniques can lead to higher success rates for IVF.

12. Applications of nanomaterials in gene editing

The manipulation and modification of embryos' genetic material has been revolutionized by nanomaterials. This has given rise to numerous tools for gene editing. One gene editing technique gaining popularity is CRISPR-Cas9, which employs a guide RNA for targeting DNA sequences slated for modifications. The research world has created a novel dual-targeted polymer nano-system. This nano-system delivers the CRISPR/Cas9 plasmid with pinpoint precision straight to the nucleus of tumor cells, resulting in the elimination of genome CDK11 (Duan et al., 2021).CRISPR-Cas9 has shown great potential in oncological research, allowing for precise targeting of cancer cells and genetic mutations (David Cyranoski and Sara Reardon, 2015; Kazemian et al., 2022; Wang et al., 2022).

Another gene editing technique that has gained attention is Zinc Finger Nucleases (ZFNs), which are artificial restriction enzymes designed for custom site-specific genome editing. ZFNs are created by fusing a non-sequence-specific cleavage domain to a site-specific DNA-binding domain loaded on the zinc finger (Li et al., 2020). Engineered endonucleases, such as ZFNs, provide genome-editing approaches that allow for specific targeting and manipulation of disease-causing genes (Ahmad M. Khalil,2020; Aguado et al., 2020; Chou et al., 2012; Kaneko et al., 2014). These techniques have been used in conjunction with nanomaterials to increase the efficiency of gene transfer in embryos (David Cyranoski and Sara Reardon, 2015; Remiàò et al., 2018).

The use of nanomaterials in embryo manipulation has allowed for precise delivery of substances into cells and has opened up new avenues for research in early embryo development (Butler et al., 2022; Zhao et al., 2018; Maria Luz Garcia, 2018). By adopting microscopic observation and micro-nano handling, scientists can carry out a more precise examination of how gene editing affects embryo development and investigate prospective remedies for genetic diseases. The application of nanomaterials in gene editing and

embryo manipulation is still nascent, but it offers considerable potential in pushing forward our comprehension of genetics and creating fresh treatments for an array of ailments.

13. Ethical considerations in using nanomaterials in embryo manipulations

Safety concerns arising from the usage of nanomaterials in embryo manipulation can not be overlooked, especially in regards to the toxicity potential of these substances. Pregnant organisms and developing embryos are particularly at risk from the harmful effects of certain nanomaterials (as noted by Remiào et al., in 2018). Therefore, it is crucial to assess the potential risks and toxicity levels of newly developed nanomaterials before utilizing them in embryo manipulations (as recommended by Lebre et al., in 2022). The cautious utilization of nanomaterials is of utmost importance, as although they may provide advantages like enhancing gene transfer efficiency (Remiào et al., 2018; Silva et al., 2021), the risks involved must be thoroughly analyzed.

Across generations, phenotypic alterations ignited by embryo manipulations are believed to persist, according to previous research (Dominiquez et al., 2020; 2021). Nanomaterials used in manipulations during embryonic development may pose future health risks and safety concerns that demand attention. These manipulations could potentially alter epigenetic markers, leading to unforeseen health outcomes. Thus, it is crucial to conduct a comprehensive evaluation of long-term health hazards pertaining to nanomaterial use during embryo manipulations.

Embryo manipulations utilizing nanomaterials pose significant societal implications that should not be overlooked. The ability to edit genes in human embryos is particularly concerning as it raises ethical issues regarding inheritable changes (Morrison and de Saille, 2019). An open and genuine dialogue must be had about the potential benefits and dangers of utilizing nanomaterials in embryo manipulation and the ethical concerns surrounding it (Brezia and Zhao, 2012). It is critical to consider these ethical implications thoroughly.

14. Current research on nanomaterials in embryo manipulations

In manipulating embryos, nanomaterials have been found to be a valuable resource. They have been utilized in delivering target molecules within the intra-gamete and embryo, thus improving assisted reproductive techniques (Lucas et al., 2019). As evidenced in animal studies, nanomaterials can

aid in sperm selection, provide antioxidants and hormones to preantral follicles, and even manipulate bovine embryos in vitro (Karcz et al., 2023; Silva et al., 2021). Remarkably, Eto et al. in 2021 were able to produce genetically modified animals using fully automated nuclear injection (Eto et al., 2021). Clearly these findings highlight the potential nanomaterials have to revolutionize animal breeding techniques.

The use of nanomaterials in enhancing human embryo manipulations has been evaluated through clinical trials. Reproductive medicine has found a potential ally in nanotechnology, aiding in overcoming obstacles related to assisted reproductive techniques (Remiào et al., 2018). Furthermore, scientific research has found that DNA methylation patterns in the F3 generation are modified by embryo manipulation, indicating a connection between epigenetic variability and this procedure (Dominiguez et al., 2021). These discoveries point to the possibility that nanomaterials may prove to be instrumental in improving human embryo manipulations.

As the future looms ahead, novel advancements are sprouting up for the progression of assisted reproductive techniques and stem-cell-based therapy. Among them are the creation of microrobotic tools that can smoothly shift gametes or embryos to the fallopian tube without being invasive (Nauber et al., 2023).

15. Challenges and limitations of using nanomaterials in embryo manipulations

Improving outcomes in reproductive medicine and embryo manipulations through nanotechnology is hindered by challenges and limitations. Cost and accessibility are among the main obstacles, with nanomaterials being not only expensive to produce but also requiring specialized equipment and expertise. As a result, many clinicians and researchers are unable to obtain them, which restricts their wide-scale use and hinders progress towards better patient outcomes. (Remiào et al., 2018).

Nanomaterials present a limitation for embryo manipulations due to safety concerns. Specifically during pregnancy and embryo development, organisms have shown toxicity to certain types of nanomaterials (Remiào et al., 2018). This has raised red flags concerning the potential dangers and undesired effects of incorporating these materials into reproductive medicine. Furthermore, a comprehensive evaluation of the potential risks and toxicity of new nanomaterials should occur at the same pace as their rapid expansion (Lebre et al., 2022). Therefore, it is necessary to take sufficient measures in

researching and ensuring the safety of nanomaterials prior to use in embryo manipulations.

Incorporating nanomaterials into embryo manipulations poses significant ethical challenges. Inherent ethical, social, and policy issues have surrounded the creation and destruction of embryos for some time (Tatay et al., 2017). Furthermore, manipulating germline cells raises concerns regarding human trait enhancement and unintentional mutations (David Cyranoski and Sara Reardon, 2015; Mette Ebbesen and Thomas G. Jensen, 2006). As a result, it is crucial to weigh the ethical implications of utilizing nanomaterials in embryo manipulations and to ensure that research is conducted in an ethical and responsible manner (David Cyranoski and Sara Reardon, 2015).

16. Conclusion and future outlook for nanomaterials in embryo manipulations

The use of nanomaterials in embryo manipulations has the potential to revolutionize reproductive medicine. Nanotechnology can help overcome some of the impairments that have previously limited the success of reproductive interventions (Remiàо et al., 2018). Artificial gametes and embryos generated through manipulation of progenitor cells or stem cells can offer new treatment options for infertility and genetic disorders (Zhang et al., 2020). The investigation of gamete cells using nanotechnology has also revealed new possibilities for improving animal reproduction (L.P Silva, 2014). Due to their potential use in regenerative medicine and tissue engineering, mesenchymal stem cells have caught the interest of medical professionals.

Nanomaterials may have the ability to better the field of reproductive medicine. Clinical trials continue to explore their practicality (Pittenger et al., 2019). An in-depth investigation into the advantages and drawbacks of implementing nanomaterials in reproductive medicine would expand our knowledgebase.

Efficient processes like gene transfer and editing can be improved by utilizing nanomaterials in embryo manipulations, but ethical considerations must be taken into account alongside scientific progress. The CRISPR gene editing technology and related methods present potential unintended consequences that have sparked ethical concerns. Thus, it is essential to thoughtfully evaluate the ethical implications of implementing nanomaterials in embryo manipulations. Responsible use of these materials in reproductive medicine can ultimately bring about notable advancements while simultaneously upholding ethical standards according to Mara Almedia and Robert Ranisch (2022).

References

Adewale, O.B., Davis, H., Cairncross, L., & Roux, S. (2019). Toxicological Behavior of Gold Nanoparticles on Various Models: Influence of Physicochemical Properties and Other Factors. International Journal of Toxicology.38(5):357-384.

Agca, Y., & Critser, J. K. (2006). Assisted Reproductive Technologies and Genetic Modifications in Rats. In Elsevier eBooks (pp. 165–189).

Al Moustafa, A.E., Mfoumou, E., Roman, D.E., Nerguizian, V., Alazzam, A., Stiharu, I., & Yasmeen, A. (2016). Impact of single-walled carbon nanotubes on the embryo: a brief review, International Journal of Nanomedicine,11, 349-355.

Almeida, M., & Ranisch, R. (2022). Beyond safety: mapping the ethical debate on heritable genome editing interventions. Humanities & Social Sciences Communications, 9(1).

Barhoum, A., García-Betancourt, M. L., Jeevanandam, J., Hussien, E. A., Mekkawy, S. A., Mostafa, M., Omran, M. M., S. Abdalla, M., & Bechelany, M. (2022). Review on Natural, Incidental, Bioinspired, and Engineered Nanomaterials: History, Definitions, Classifications, Synthesis, Properties, Market, Toxicities, Risks, and Regulations. Nanomaterials, 12(2), 177.

Bernabo, N., Valbonetti, L., Raspa, M., Fontana, A., Palestini, P., Botto, L., …& Barboni, B. (2020). Graphene Oxide improves in vitro fertilization in mice with no impact on embryo development and preserves the membrane microdomains architecture, Frontiers in Bioengineering and Biotechnology, 8.

Bongaerts, M., Aizel, K., Secret, E., Jan, A., Nahar, T., Raudzus, F., … & Coppey, M. (2020). Parallelized Manipulation of Adherent Living Cells by Magnetic Nanoparticles-Mediated Forces. International Journal of Molecular Sciences, 21(18), 6560.

Brezina, P. R., & Zhao, Y. (2012). The Ethical, Legal, and Social Issues Impacted by Modern Assisted Reproductive Technologies. Obstetrics and Gynecology International, 2012, 1–7.

Butler, K. S., Brinker, C. J., & Leong, H. S. (2022). Bridging the In Vitro to In Vivo gap: Using the Chick Embryo Model to Accelerate Nanoparticle Validation and Qualification for In Vivo studies. ACS Nano, 16(12).

Carnovale, C., Bryant, G., Shukla, R., & Bansal, V. (2019). Identyfying Trends in Gold Nanoparticle Toxicity and Uptake: Size, Shape, Capping, Ligand, and Biological Corona, ACS Omega,4(1), 242-256.

Celá, P., Veselá, B., Matalová, E., Večeřa, Z., & Buchtová, M. (2014b). Embryonic Toxicity of Nanoparticles. Cells Tissues Organs, 199(1), 1–23.

Cheng, Z., Li, M., Dey, R., & Chen, Y. (2021). Nanomaterials for cancer therapy: current progress and perspectives. Journal of Hematology and Oncology, 14(1).

Chou, S. K., Leng, Q., & Mixson, A. J. (2012). Zinc finger nucleases: Tailor-made for gene therapy. Drugs of the Future, 37(3), 183.

Chueng, S. D., Yang, L., Zhang, Y., & Lee, K. (2016). Multidimensional nanomaterials for the control of stem cell fate. Nano Convergence, 3(1).

Church, R.B., Schaufele, F.J., & Meckling, K. (1985). Embryo manipulation and gene transfer in livestock. Animal Science, 65, 527-537.

Cyranoski, D., & Reardon, S. (2015). Chinese scientists genetically modify human embryos. Nature.

Dong, Y., Wu, X., Chen, X., Zhou, P., Xu, F., & Liang, W. (2021). Nanotechnology shaping stem cell therapy: recent advances, application, challenges, and future outlook, Biomedicine & Pharmacotherapy,137.

Ebbesen, M., & Jensen, T. (2006). Nanomedicine: Techniques, Potentials, and Ethical Implications. Journal of Biomedicine and Biotechnology, 2006, 1–11.

Eto, T., Ueda, H. R., Ito, R., Takahashi, T., Watanabe, T., Goto, M., Sotomaru, Y., Tanaka, N., & Takahashi, R. (2021). Establishment of an integrated automated embryonic manipulation system for producing genetically modified mice. Scientific Reports, 11(1).

Falchi, L., Khalil, W. A., Hassan, M., & Marei, W. F. (2018). Perspectives of nanotechnology in male fertility and sperm function. International Journal of Veterinary Science and Medicine, 6(2), 265–269.

Ferreira, L., Karp, J.M., Nobre, L., & Langer, R. (2008). New opportunities: the use of nanotechnologies to manipulate and track stem cells, Cell Stem Cell, 3(2), 136-146.

Fraser, B., Peters, A., Sutherland, J. M., Liang, M., Rebourcet, D., Nixon, B., & Aitken, R. J. (2021). Biocompatible Nanomaterials as an Emerging Technology in Reproductive Health; a Focus on the Male. Frontiers in Physiology, 12.

Friedrich, R.P., Cicha, I., & Alexiou, C. (2021). Iron Oxide Nanoparticles in Regenerative Medicine and Tissue Engineering, Nanomaterials, 11(9),2337.

García-Domínguez, X., Diretto, G., Peñaranda, D. S., Frusciante, S., García-Carpintero, V., Cañizares, J., … & Marco-Jiménez, F. (2021). Early Embryo Exposure to Assisted Reproductive Manipulation Induced Subtle Changes in Liver Epigenetics with No Apparent Negative Health Consequences in Rabbit. International Journal of Molecular Sciences, 22(18), 9716.

Garcia-Dominguez, X., Marco-Jiménez, F., Peñaranda, D. S., Diretto, G., Garcia-Carpintero, V., Cañizares, J., & Vicente, J. (2020). Long-term and

transgenerational phenotypic, transcriptional and metabolic effects in rabbit males born following vitrified embryo transfer. Scientific Reports, 10(1).

Garcia-Dominiguez, X., Vicente, J.S., Viudes-de-Castro, M.P., & Marco-Jimenez, F. (2020). Long-Term Effects Following Fresh/Vitrified Embryo Transfer Are Transmitted by Paternal Germline in a Large Size Rabbit Cohort, Animals,10(8), 1272.

García, M. L. S. (2018). Embryo Manipulation Techniques in the Rabbit. In IntechOpen eBooks.

Gaur, M., Misra, C., Yadav, A. B., Swaroop, S., Maolmhuaidh, F. Ó., Bechelany, M., & Barhoum, A. (2021). Biomedical Applications of Carbon Nanomaterials: Fullerenes, Quantum Dots, Nanotubes, Nanofibers, and Graphene. Materials, 14(20), 5978.

Gómez-Aguado, I., Rodríguez-Castejón, J., Vicente-Pascual, M., Rodríguez-Gascón, A., Solinís, M. Á., & del Pozo-Rodríguez, A. (2020). Nanomedicines to Deliver mRNA: State of the Art and Future Perspectives. Nanomaterials, 10(2), 364.

Gómez-Tatay, L., Hernández-Andreu, J., & Aznar, J. (2017). Mitochondrial Modification Techniques and Ethical Issues. Journal of Clinical Medicine, 6(3), 25.

https://www.britannica.com/science/somatic-cell-nuclear-transfer

Huang, H., Shen, H., Tien, C., Li, C., Fan, S., Liu, C., Hsu, W., & Yao, D. (2015). Digital Microfluidic Dynamic Culture of Mammalian Embryos on an Electrowetting on Dielectric (EWOD) Chip. PLOS ONE, 10(5), e0124196.

Ibeas, P.R., Heras, S., Gomez-Renondo, I., Planells, B., Fernandez-Gonzales, R., Pericuesta, E., …& Gutierrez-Adàn, A. (2019). Embryo responses to stress induced by assisted reproductive technologies. Molecular Reproduction and Development, 86(10), 1292-1306.

Johnson, Martha S. (2019). Study of the Effects of Silver Ions and Silver Nanoparticles on Embryonic Development, Chemistry & Biochemistry, Old Dominion University.

Joshi, S., Allabun, S., Ojo, S., Alqahtani, M. S., Shukla, P. K., Abbas, M., …& Almohiy, H. M. (2023). Enhanced Drug Delivery System Using Mesenchymal Stem Cells and Membrane-Coated Nanoparticles. Molecules, 28(5), 2130.

Kaneko, T., Sakuma, T., Yamamoto, T., & Mashimo, T. (2014). Simple knockout by electroporation of engineered endonucleases into intact rat embryos. Scientific Reports, 4(1).

Karcz, A., Van Soom, A., Smits, K., Van Vlierberghe, S., Verplancke, R., Pascottini, O. B., Van den Abbeel, E., & Vanfleteren, J. (2023). Development

of a Microfluidic Chip Powered by EWOD for In Vitro Manipulation of Bovine Embryos. Biosensors, 13(4), 419.

Karcz, A., Van Soom, A., Smits, K., Verplancke, R., Van Vlierberghe, S., & Vanfleteren, J. (2022). Electrically-driven handling of gametes and embryos: taking a step towards the future of ARTs. Lab on a Chip, 22(10), 1852–1875.

Kazemian, P., Yu, S., Thomson, S., Birkenshaw, A., Leavitt, B. R., & Ross, C. J. D. (2022). Lipid-Nanoparticle-Based Delivery of CRISPR/Cas9 Genome-Editing Components. Molecular Pharmaceutics, 19(6), 1669–1686.

Khalil, A. S. (2020). The genome editing revolution: review. Journal of Genetic Engineering and Biotechnology, 18(1).

Knoppers, B. M., Bordet, S., & Isasi, R. (2006). Preimplantation Genetic Diagnosis: An Overview of Socio-Ethical and Legal Considerations. Annual Review of Genomics and Human Genetics, 7(1), 201–221.

Kohda, T. (2013). Effects of embryonic manipulation and epigenetics. Journal of Human Genetics, 58(7), 416–420.

Kohda, T. (2013). Effects of embryonic manipulation and epigenetics. Journal of Human Genetics, 58, 416-420.

Kohda, T., & Ishino, F. (2013). Embryo manipulation via assisted reproductive technology and epigenetic asymmetry in mammalian early development. Philosophical Transactions of the Royal Society B, 368(1609), 20120353.

Kushnir, V. A., Smith, G. A., & Adashi, E. Y. (2022). The Future of IVF: The New Normal in Human Reproduction. Reproductive Sciences, 29(3), 849–856.

Kushnir, V. A., Smith, G. A., & Adashi, E. Y. (2022). The Future of IVF: The New Normal in Human Reproduction. Reproductive Sciences, 29(3), 849–856.

Lebre, F., Chatterjee, N., Costa, S., Fernández-de-Gortari, E., Lopes, C., Meneses, J., … & Alfaro-Moreno, E. (2022). Nanosafety: An Evolving Concept to Bring the Safest Possible Nanomaterials to Society and Environment. Nanomaterials, 12(11), 1810.

Lebre, F., Chatterjee, N., Costa, S., Fernández-de-Gortari, E., Lopes, C., Meneses, J., … & Alfaro-Moreno, E. (2022). Nanosafety: An Evolving Concept to Bring the Safest Possible Nanomaterials to Society and Environment. Nanomaterials, 12(11), 1810.

Lee, K., & Prather, R. S. (2013). Advancements in somatic cell nuclear transfer and future perspectives. Animal Frontiers, 3(4), 56–61.

Lee, W., Kim, E., Cho, H.-J., Kang, T., Kim, B., Kim, M., Kim, Y., Song, N., Lee, J.-S., & Jeong, J. (2018). The Relationship between Dissolution

Behavior and the Toxicity of Silver Nanoparticles on Zebrafish Embryos in Different Ionic Environments. Nanomaterials, 8(9), 652.

Li, H., Yang, Y., Hong, W., Huang, M., Wu, M., & Zhao, X. (2020). Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. Signal Transduction and Targeted Therapy, 5(1).

Liao, C., Li, Y., & Tjong, S.C. (2018). Graphene Nanomaterials: Synthesis, Biocompatibility, and Cytotoxicity, International Journal of Molecular Sciences, 19(11), 3564.

Lopez-Martinez, M. J., & Campo, E. (2011). Micro-Nano Technologies for Cell Manipulation and Subcellular Monitoring. In InTech eBooks.

Lopez, R. M., White, J. A., Truong, L., & Tanguay, R. L. (2022). Size- and Oxidation-Dependent Toxicity of Graphene Oxide Nanomaterials in Embryonic Zebrafish. Nanomaterials, 12(7).

Lucas, C., Chen, P. R., Seixas, F. K., Prather, R. S., & Collares, T. (2019). Applications of omics and nanotechnology to improve pig embryo production in vitro. Molecular Reproduction and Development, 86(11), 1531–1547.

Lyashenko, E. N., Uzbekova, L. D., Polovinkina, V. V., Dorofeeva, A. K., Ibragimov, S.-U. S., Tatamov, A.,… & Maslova, A. Y. (2023). Study of the Embryonic Toxicity of TiO2 and ZrO2 Nanoparticles. Micromachines, 14(2), 363.

Magro, M., De Liguoro, M., Franzago, E., Baratella, D., & Vianello, F. (2018). The surface reactivity of iron oxide nanoparticles as a potential hazard for aquatic environments: A study on Daphnia magna adults and embryos, Scientific Reports, 8, 13017.

Mal, J., Lens, P. N., Van Hullebusch, E. D., & Lens, P. N. (2016). Metal chalcogenide quantum dots: biotechnological synthesis and applications. RSC Advances, 6(47), 41477–41495.

Masoudi Asil, S., Ahlawat, J., Guillama Barroso, G., & Narayan, M. (2020). Application of Nanotechnology in Stem-Cell-Based Therapy of Neurodegenerative Diseases. Applied Sciences, 10(14), 4852.

Materon, E. M., Ferreira, M., Carr, O., Joshi, N., Picciani, P. H. S., Dalmaschio, C. J., Davis, F. W., & Shimizu, F. M. (2021). Magnetic nanoparticles in biomedical applications: A review. Applied Surface Science Advances 6, 6, 100163.

Mitchell, M. E., Billingsley, M. M., Haley, R. M., Wechsler, M. E., Peppas, N. A., & Langer, R. (2020). Engineering precision nanoparticles for drug delivery. Nature Reviews Drug Discovery, 20(2), 101–124.

Mizushima, S., Sasanami, T., Ono, T., & Kuroiwa, A. (2023). Current Approaches to and the Application of Intracytoplasmic Sperm Injection (ICSI) for Avian Genome Editing. Genes,14(3),757.

Moisyadi, S., Kaminski, J.M., & Yanagimachi, R. (2009). Use of intracytoplasmic sperm injection (ICSI) to generate transgenic animals. Comperative Immunology, Microbiology and Infections Diseases, 32(2), 47-60.

Moore, S. S., & Hasler, J. (2017). A 100-Year Review: Reproductive technologies in dairy science. Journal of Dairy Science, 100(12), 10314–10331.

Morimoto, Y., Gamage, U. S. K., Yamochi, T., Saeki, N., Morimoto, N., Yamanaka, M., … & Yanagimachi, R. (2023). Mitochondrial Transfer into Human Oocytes Improved Embryo Quality and Clinical Outcomes in Recurrent Pregnancy Failure Cases. International Journal of Molecular Sciences, 24(3), 2738.

Morrison, M. L., & De Saille, S. (2019). CRISPR in context: towards a socially responsible debate on embryo editing. Palgrave Communications, 5(1).

Naseri, N., Valizadeh, H., & Zakeri-Milani, P. (2015). Solid Lipid Nanoparticles and Nanostructured Lipid Carriers: Structure, Preparation and Application. Advanced Pharmaceutical Bulletin, 5(3), 305–313.

Nauber, R., Goudu, S. R., Goeckenjan, M., Bornhäuser, M., Ribeiro, C., & Medina-Sánchez, M. (2023). Medical microrobots in reproductive medicine from the bench to the clinic. Nature Communications, 14(1).

Niculescu, V. (2020). Mesoporous Silica Nanoparticles for Bio-Applications. Frontiers in Materials, 7.

Noriega-Luna, B., Godínez, L. A., Rodríguez, F., Rodríguez, A., De Larrea, G. Z. L., Sosa-Ferreyra, C. F., Mercado-Curiel, R. F., Manríquez, J., & Bustos, E. (2014). Applications of Dendrimers in Drug Delivery Agents, Diagnosis, Therapy, and Detection. Journal of Nanomaterials, 2014, 1–19.

Pacheco, A. R. F., Cardoso, B. D., Pires, A., Pereira, A. M., Araújo, J. P., Carvalho, V. M., …& Castanheira, E. M. S. (2023). Development of pH-Sensitive Magnetoliposomes Containing Shape Anisotropic Nanoparticles for Potential Application in Combined Cancer Therapy. Nanomaterials, 13(6), 1051.

Palay, P., Fathi, D., & Fathi, R. (2022). Oocyte quality evaluation: a review of engineering approaches toward clinical challenges. Biology of Reproduction, 108(3), 393–407.

Pittenger, M. F., Discher, D. E., Péault, B., Phinney, D. G., Hare, J. M., & Caplan, A. I. (2019b). Mesenchymal stem cell perspective: cell biology to clinical progress. Npj Regenerative Medicine, 4(1).

Pritchard, N., Kaitu'u-Lino, T. J., Harris, L. K., Tong, S., & Hannan, N. J. (2021). Nanoparticles in pregnancy: the next frontier in reproductive therapeutics. Human Reproduction Update, 27(2), 280–304.

Rajeshkumar, S., Santhoshkumar, J., Jule, L. T., & Krishnaraj, R. (2021). Phytosynthesis of Titanium Dioxide Nanoparticles Using King of Bitter Andrographis paniculata and Its Embryonic Toxicology Evaluation and

Biomedical Potential. Bioinorganic Chemistry and Applications, 2021, 1–11.

Remião, M. H., Segatto, N. V., Lehr, C., Guterres, S. S., Seixas, F. K., & Collares, T. (2018). The Potential of Nanotechnology in Medically Assisted Reproduction. Frontiers in Pharmacology, 8.

Saadeldin, I. M., Khalil, W. A., Alharbi, M. G., & Lee, S. (2020). The Current Trends in Using Nanoparticles, Liposomes, and Exosomes for Semen Cryopreservation. Animals, 10(12), 2281.

Shao, L., Gao, Y., & Yan, F. (2011). Semiconductor Quantum Dots for Biomedicial Applications. Sensors, 11(12), 11736–11751.

Sharif, F., Porta, F., Meijer, A. H., Kros, A., & Richardson, M. K. (2012). Mesoporous silica nanoparticles as a compound delivery system in zebrafish embryos. International Journal of Nanomedicine, 1875.

Silva, J. R. V., Barroso, P. A. A., Nascimento, D. R., Figueira, C. S., Azevedo, V. A. N., Silva, B. R., & Santos, R. P. D. (2021). Benefits and challenges of nanomaterials in assisted reproductive technologies. Molecular Reproduction and Development, 88, 707– 717.

Silva, L.P. (2014). Potential practical implications of nanotechnology in animal reproductive biotechnologies, Brazilian College of Animal Reproduction, 11(3), 278-280.

Stocum, D. (2023). Somatic cell nuclear transfer. Encyclopedia Britannica.

Tachibana, M., Amato, P., Sparman, M., Gutierrez, N.M., Tippner-Hedges,R., Ma,H., … Mitalipov,S. (2013). Human Embriyonic Stem Cells Derived by Somatic Cell Nuclear Transfer. Cell,153(6), 1228-1238.

Tenchov, R., Bird, R., Curtze, A. E., & Zhou, Q. (2021). Lipid Nanoparticles–From liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. ACS Nano, 15(11), 16982-17015.

Tian, X. C., Kubota, C., Enright, B. P., & Yang, X. (2003). Cloning animals by somatic cell nuclear transfer--biological factors. Reproductive Biology and Endocrinology, 1(1), 98.

Tiwari, P., Vig, K., Dennis, V., & Singh, S. (2011). Functionalized Gold Nanoparticles and Their Biomedical Applications. Nanomaterials, 1(1), 31–63.

Ventura-Juncá, P., Irarrázaval, I., Rolle, A., Gutiérrez, J. a. T., Moreno, R. A., & Santos, M. F. (2015). In vitro fertilization (IVF) in mammals: epigenetic and developmental alterations. Scientific and bioethical implications for IVF in humans. Biological Research, 48(1).

Ventura-Juncá, P., Irarrázaval, I., Rolle, A., Gutiérrez, J. a. T., Moreno, R. A., & Santos, M. F. (2015b). In vitro fertilization (IVF) in mammals: epigenetic and developmental alterations. Scientific and bioethical implications for IVF in humans. Biological Research, 48(1).

Waghmode, M. S., Gunjal, A., Mulla, J., Patil, N., & Nawani, N. (2019). Studies on the titanium dioxide nanoparticles: biosynthesis, applications and remediation. SN Applied Sciences, 1(4).

Wang, S., Gao, C., Zheng, Y., Lee, K., Lu, J., Huang, X., Cai, J., Zhang, P., Cui, Y., & Ke, A. (2022). Current applications and future perspective of CRISPR/Cas9 gene editing in cancer. Molecular Cancer, 21(1).

Wheeler, M.B., & Rubessa, M. (2017). Integration of microfluidics in animal in vitro embryo production, Molecular Human Reproduction, 23, 248–256.

Zhang, P., Fan, Y., Tan, T., & Yu, Y. (2020). Generation of Artificial Gamete and Embryo From Stem Cells in Reproductive Medicine. Frontiers in Bioengineering and Biotechnology, 8.

Zhang, X., Zhang, J., Wang, A., Ghimire, S., Mei, L., & Wu, C. (2022). Effects of Particle Size and Surface Charge on Mutagenicity and Chicken Embryonic Toxicity of New Silver Nanoclusters. ACS Omega, 7(21), 17703–17712.

Zhao, Y., Sun, H., Sha, X., Gu, L., Zhan, Z., & Li, W. J. (2018). A Review of Automated Microinjection of Zebrafish Embryos. Micromachines, 10(1), 7.

Zielińska-Górska, M., Hotowy, A., Wierzbicki, M., Bałaban, J., Sosnowska, M., Jaworski, S., Strojny, B., Chwalibog, A., & Sawosz, E. (2020). Graphene oxide nanofilm and the addition of L-glutamine can promote development of embryonic muscle cells. Journal of Nanobiotechnology, 18(76).

Chapter 6

Genotoxic Effects of Nanoparticles on Gamete Cells and Their Potential Risks for Next Generations

Ayşe Sarı[1]

Muhammet Mücahit Sarı[2]

Emine Öksüz Durmaz[3]

Summary

Nanoparticles occur in our natural habitats due to biological, physical and chemical processes. Nanoparticles and nanomaterials are increasingly used in food packaging, textiles, electronics, biomedicine, cosmetics (lipstick, sunscreen, etc.), and many aspects of daily life. Therefore, the potential risk of exposure of humans and surrounding organisms to nanoparticles should not be ignored. Nanomaterials are materials with small dimensions and large surface area, as well as other physical and chemical properties, such as polluting metals and charged surfaces, and genotoxic properties. Because of these properties, they can cause mutations and damage to chromosomes. It is required to consider the influence of nanoparticles not only on humans but also on the genetic components of other species in the environment. Recently, adverse effects from exposure of the reproductive system to nanoparticles have emerged, creating the risk of reproductive toxicity. Reproductive toxicity refers to effects that affect the development of healthy embryos, the reproductive cycle, and any stage of pregnancy. The studies about reproductive toxicity of NP is increasing, but research is ongoing. This section focuses on the potential genotoxic efficacy of nanoparticles on germ cells and reproductive systems, and potential risks these effects may cause in the next generations.

1 Necmettin Erbakan University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Konya, Türkiye

2 Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Veterinary Biology and Genetics, Afyonkarahisar, Türkiye

3 Selcuk University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Konya, Türkiye

https://doi.org/10.58830/ozgur.pub203.c939

1. Introduction

Nanomaterials are descriptive of materials that have a physicochemical construction of more small than 100 nm (nanoconstruction) and possess biological, chemical or physical properties connected with a nanostructure (Oberdörster et al., 2005).

According to the International Standards Organization (ISO), nanomaterials are divided into two categories: Nanoobjects and nanostructured materials. Nanoparticles (NPs) in nanoobjects are generally classified into 3 groups based on their chemical structures. These 3 groups include carbon based structures like nanotubes and C60-class corridors, nanoparticles like metals or metal oxides, and semiconductor nanocrystals such as CdSe and PbSe known as quantum dots (Krug & Wick, 2011).

Nanoparticles are formed in the nature owing to physical, biological and chemical processes. (Buzea et al., 2007). Nanoparticles and nanomaterials are increasingly used in food packaging, textiles, electronics, biomedicine, cosmetics (lipstick, sunscreen, etc.), and many aspects of daily life (Cotena et al., 2020). The potential risk of mankind and ecological exposure to nanoparticles should not be ignored.

Nanotoxicology is a branch of toxicology that to strive the disadvantageous health effects of nanoparticles. (Donaldson et al., 2004). Factors that may negatively affect human health include NPs' characteristics such as form, inorganic or organic coatings, size, and structure. At the same time, special factors like genetics and diseases also affect the extent of the negative efficacy on human health (Hoet et al., 2004; Nohynek & Dufour, 2012).

Owing to their little particle dimensions and comparatively large surface domain, nanoparticles can join the bloodstream via inhalation, ingestion, dermal uptake, especially outside of applications (Das et al., 2016; Hou & Zhu, 2017). Studies indicated that NPs can enter the cell and cause intracellular oxidative damage by enhance the formation of reactive oxygen radicals (ROS). This can disrupt intracellular biological structures and the normal functions of these structures (Nel et al., 2006). These toxic effects caused by NP lead the cell to apoptosis and can cause conditions like tissue inflammation (Foldbjerg et al., 2009; Li et al., 2010). Moreover, some NPs can cross natural barriers for example the blood-testis barrier, blood-brain barrier (BBB), the placenta and cumulates in various cells (Lan & Yang, 2012; Muoth et al., 2016)

Inhaled NPs may accumulate in the respiratory tract and lungs, because of their high surface reactivity properties, can cause inflammation at the site of

accumulation and cause the produc of reactive oxygen radicals (Hougaard et al., 2010; Møller et al., 2010). Generally, reactive oxygen radicals formation is assumed a major agent in toxic efficacy of nanoparticles (Brohi et al., 2017). ROS molecules are unstable and are characterised by the fact that they do not extend far beyond the region in which they occur (Wells et al., 2005). But, when antioxidant mechanisms fail to keep up with the level of oxidants, oxidative stress rises and triggers or intensifies inflammation which stem from Nps. In this case, a situation occurs in which the area where the mediators or nanoparticles associated with the inflammation occur cannot be limited (Brohi et al., 2017).

In other words, the NPs gain the ability to migrate and inflammatory mediators can also enter the systemic circulation. Through the systemic circulation, NPs can reach organs associated with pregnancy and fetal development and cross barriers such as the placenta, causing oxidative damage and sensitivity in this area. This has indirect negative effects on growing up of fetus (Brohi et al., 2017; Erdely et al., 2011). Particularly in the cells of the placenta, there are receptors called Toll-like receptors (TLR2 or TLR4) are to be included in the inflammatory reagent triggered by respiratory exposure NP (Koga & Mor, 2010; Zhao et al., 2012).

In conclusion, there is evidence that the increase in inflammation and ROS as a result of NP -induced toxicity may have adverse effects on reproduction and development, but these mechanisms cannot be fully explained, and it is reported that further studies are needed to elucidate them (Brohi et al., 2017).

2. Genotoxic Effect of Nanoparticles

Nanomaterials are materials with small dimensions and large surface areas, as well as other physical and chemical properties, such as polluting metals and charged surfaces, and genotoxic properties (Singh et al., 2009). Due to these properties, they can cause mutations and damage to chromosomes (Aloisi et al., 2022).

Genotoxins that cause only genetic changes without cell death can be called classical genotoxins. Carcinogenesis of these genotoxins results in DNA injury. As a consequence of DNA injury, not only the development of cancer is observed, but also significant problems for fertility and health of future generations may occur, as they affect gamete cells (Singh et al., 2009).

DNA injury by NPs can occur directly or indirectly in two ways. Direct interaction of NPs with genetic material results in direct DNA injury. The raise of oxidative damage in cell and the creation of chemical composition

such as ROS leads to indirect DNA injury (de Jesus & Kapila, 2013; Vales et al., 2016).

If the NPs are small enough, they can penetrate the cell membrane and arrive the nucleus, where they bring about damage by interacting with DNA. Even if they accumulate in the cell at a size where they cannot reach the nucleus, they can cause damage by direct contact with DNA if the integrity of the membrane is disrupted during mitosis. In this regard, silica and titanium dioxide nanoparticles have been found to penetrate the nucleus, and have been reported to cause intranuclear protein cluster that can obstruct processes such as cell differentiation, replication, transcription (Singh et al., 2009).

Indirectly, DNA damage can occur when nanoparticles interact with other cellular proteins during cell division. In addition, they cause damage when they trigger cellular responses that cause oxidative damage, intracellular unnatural signalling, inflammation and, causing genotoxicity (Singh et al., 2009).

2.1. Oxidadative Damage and Nanogenotoxicity

Oxidative damage is considered the main reason why nanoparticles cause genotoxic effects. Oxidative damage is the conclusion of an unbalance among the ROS present in the cell and the cell's antioxidant capacity. ROS react negatively with cellular macromolecules like proteins, DNA and lipids compounds in the cell, disrupting cell homeostasis. Examples of adverse reactions of ROS with DNA include strand breaks of DNA, base changes, and DNA cross-links. If these adverse conditions are not corrected, there is a high potential for the onset and progression of carcinogenesis (Singh et al., 2009; Toyokuni, 1998).

Reactive oxygen species are also divided into two type of ROS. One of them primary ROS, could be produced during metabolic duration or by oxygen activation leading to the formation of superoxide anions. Primary ROS do not react directly with polypeptides or DNA. Secondary ROS involves the formation of hydroxyl radicals in the cell as a result of catalysis of hydrogen peroxide with iron or other active transition metals, usually after the Fenton reaction. These hydroxyl radicals are considered to be the primary mediators of DNA damage (Valko et al., 2006).

The formation of hydroxyl radicals may lead to cross-links in chromatin and to changes in free iron ions and purine and pyrimidine bases (Valko et al., 2006; Zastawny et al., 1995). Accordingly, iron-containing nanoparticles be able to cause raised formation of highly reactive hydroxyl radicals by acting

as an additional source of iron in the cell by means of the Fenton reaction (Singh et al., 2009).

In addition to metal catalysts, the surface of nanoparticles brings with it the ability to boost the creation of ROS. Smaller nanoparticles are, have ability to cause higher oxidative stress (Brown et al., 2001; Knaapen et al., 2004). In large quantities studies have indicate that exposure to nanoparticles induces the production of ROS and has genotoxic effects by causing oxidative DNA injury (Gurr et al., 2005; Karlsson et al., 2008; Papageorgiou et al., 2007).

DNA damage following oxidative stress by nanoparticles can trigger several important cellular reply such as DNA repair, cell cycle stoppage or apoptosis. When cellular mechanisms such as DNA repair mechanisms, which prevent permanent mutations that can be caused by genetic damage, are compromised, mutations can occur if the damaged DNA is not repaired and replication occurs. This negatively affects the genetic integrity and life of the cell (Singh et al., 2009).

NP-induced ROS adversely affects the balance of homeostasis of the NP-affected organism. As a result, interleukins like IL-1,6,8 and tumor necrosis factor-a increase the transcription of pre-inflammatory genes. By triggering the nuclear factor kappa B (NF-kB) signal, it creates oxidative stress, and thus events such as DNA injury and apoptosis occur (Brohi et al., 2017; Khanna et al., 2015).

2.2. Nanogenotoxicity Due to Transcription Repression

The most important factor that comes into play in DNA damage is the molecule p53. P53 is a tumor suppressor gene. It shows its action by stopping the cell cycle, triggering the transcription of genes responsible for DNA reparation, or triggering apoptosis to destroy the cell for the utility of the organism if the DNA damage present is too great. This prevents the damage from turning into a mutation (Lane, 1992; Singh et al., 2009).

Nanoparticles are reported to repress transcription of other DNA repair genes (such as BRCA1, Hus1) involved in maintaining genome integrity. This suggests that nanoparticles can potentially lead to more serious genetic problems (Li et al., 2008).

It is essential to take into account the impact of nanoparticles not only on humans but also on the genetic components of other species in the environment. Pollution of water resources in the vicinity of production facilities increases the possibility of exposure to nanomaterials for other

creatures in the environment. Therefore, monitoring endangered species in native wildlife areas for DNA damage from nanotoxicity is essential to prevent adverse efficacy. DNA damage, in particular, is of concern because it may cause hereditary abnormalities and negative efficacy on harmony within the ecosystem (Baun et al., 2008).

3. Genotoxic Effects of Nanoparticles on Male and Female Gamete Cells

3.1. Nanogenotoxicity in the Male Reproductive System

The antenatal term of germ cell growth symbolizes an important viewpoint for epigenetic programming in males. Germ cells and testis have diverse methylation models that may be suitable for sustaining the matchless chromosome construction in male germ cells. Epigenetic changes could beimpacted by ecological determinants that are inherited thanks to the paternal germline and crossed on to subsequent generations. In addition, recent evidence suggests that the antenatal environment can also affect DNA integrity in offspring (Håkonsen et al., 2012; Poma et al., 2014).

Spermatogenesis is a complicated process of germ cell multiplication and differentiation providing to the generation and deliver of spermatozoa from the testis, and it's depends on hormonal interplays among Sertoli cells and germ cells. (Boekelheide et al., 2000). Thight junctions among contigous Sertoli cells form two distinct sections inside of the seminiferous epithelium, an upper and a basal adluminal section. Sertoli cells excrete hormonal and nutritional elements inside the adluminal section, which forms a private microenvironment for germ cell growth and viability.

Exposure of testicular tubules to nanoparticles affects spermatogenesis and the male reproductive system from where it begins in the testicular tubules. The complex cellular arrangement and cellular coactions in the testis create an environment in which spermatogenesis can be affected by nanoscale toxic substances. Numerous in vitro and in vivo researches indicate that many nanoparticles have counter effects on male germ cells (Braydich-Stolle et al., 2005; Braydich-Stolle et al., 2010), and the effect of NP exposure multifarious from species to species, and reduces sperm production (Boisen et al., 2013; Brohi et al., 2017). The reason for the diminished sperm production is owing to the molecular changes that happen as a consequence of the alteration in the expression grades of the genes included in spermatogenesis. In addition, some researches have represented that application of NP to mice caused residue in varied tissues, bearing the

testis and brain. This proposes that some NPs with ease cross the blood testis and blood brain barriers. (Hong, Wang, et al., 2016; Lan & Yang, 2012).

Proposed causes of cellular damage from exposure to nanoparticles include the formation of reactive oxygen radicals (ROS), as mentioned above, and the potential for DNA damage from engineered nanomaterials. Such injury to somatic cells be able to cause inflammation and even malignant cell proliferation, but in the case of germline cells, both types of damage can occur and lead to loss of fertility or inborn fault in the offspring (Poma et al., 2014; Singh et al., 2009).

It was noticed that 25% of the sperm were non-motile when gold nanoparticles were added directly to the sperm, while normal motility was 95% in the group without nanoparticles. When the researchers examined the sperm, they found that the gold nanoparticles penetrated the heads and tails of the sperm and the sperm were fragmented (Ema et al., 2010; Wiwanitkit et al., 2009).

When the impact of another nanoparticle applied directly to the spermatozoa was examined, it was found that the NPs penetrated the spermatozoa, bound to the tail, mitochondria and the acrosome, but had no significant effect on the acrosome response and motility (Ben-David Makhluf et al., 2006).

In male mice were also found to have decreased fertility in response to exposure to NP, increased apoptosis or necrosis of both spermatogenic cells and Sertoli cells, and increased inflammatory reactions (Ritz et al., 2011).

3.2. Nanogenotoxicity in the Female Reproductive System

Oocyte growth and maturation are increasingly vulnerable to differences in the microenvironment, especially to extracellular chemical compounds (Hou & Zhu, 2017). There are some arguments that various NPs may modify the expression levels of genes codifying proteins included in steroidogenesis and genes included in estrogen or progesterone synthesis. (Brohi et al., 2017).

Besides, it is indicated to cause alters in the expression of genes such as cytochrome P450 17A1 (Cyp17a1) and aldoketoreductase family 1 member C18 (Akr1c18), which are to be included in the synthesis and metabolism of estrogen and progesterone. In addition, changes in apoptosis-related genes, increase in inflammatory and immune responses, cell proliferation, increase in oxidative damage and alteratşon in the expression levels of genes to be included in ion transport can be listed as a consequence of longtime

and high-dose be exposed to nanoparticles (Gao et al., 2012). Considering that all these damages and impairments may be interrelated, it is noted that as a consequence of long-term be exposed to nanoparticles, there may be changes in sex steroid hormone levels, a decrease in fertility, and a decrease in pregnancy rates (Brohi et al., 2017).

In different study investigating the efficacy of exposure of some Nps on oocytes, it is reported that the presence of zona pellucida (ZP) can protect the oocyte from oxidative damage and DNA damage at low concentrations, but exposure of np at high concentrations induces oxidative stress and DNA damage is viewed in oocytes with or without zona pellucida. It is found that genotoxicity and aggregation of NPs depend on physicochemical properties of the cell environment, which determine redox modifications and factors such as surface adsorption (Browning et al., 2009; Courbiere et al., 2013).

Studies about toxicity of NP in the female reproductive system mostly involve examining the effects on fertility, embryonic development and perinatal offspring. In addition, the number of studies on reproductive toxicity in in vitro germ cell lines or in vivo animal models is increasing day by day. (Hou & Zhu, 2017).

Some studies report that NPs can enter the cell by endocytosis of granulosa cells, which can lead to changes in hormone levels that result in oocyte dysplasia or abortion of oocyte development in vivo (Hou & Zhu, 2017). In addition, NPs can spread over theca cells and granulosa cells. Hence, affect their normal function and most important one relation to their crucial role in hormone production process (Stelzer & Hutz, 2009).

In vivo studies in female mice showed that be exposed to long time Nps caused an imbalance in the levels of sex hormones and distribution of mineral elements, resulting in decreased pregnancy rate and expression of ovarian genes, as well as increased oxidative stress. Consequently, NPs of a certain size can directly influence hormone excretion in the ovaries, as they can pile up in secretory cells. (Gao et al., 2012; Hou & Zhu, 2017; Melnik et al., 2013).

It has been indicated that NPs be able to pass the blood brain barrier and pile up in the central nervous system. Another potentially harmful effect of NPs causing hormone imbalance is disruption of hormone regulation as a result of induced of the nervous system by NPs. It is stated that NPs could impress the oogenesis process and ovarian health implicitly by damaging the balance of these hormones in addition to the direct effect mentioned in the previous paragraph (Oberdörster et al., 2004).

The efficacy of NPs on hormone excretion occur in two different pathway: 1. NPs pass the BBB and alter the secretion of reproductive system hormones. This affects normal feedback mechanisms. 2. Pathological phenomena can be observed in oocytes and ovaries by NPs entering the ovaries through the circulation and accumulating in cells that play an important role in steroidogenesis. (Hou & Zhu, 2017).

It is also reported that NPs of certain size can enter and pile up in various female germ cells. As a result of these effects of nanoparticles, undesirable changes in the process of oogenesis may occur. These can be listed as the observation of different cell responses in female germ cells like oxidative stress, dysfunction of cumulus cells, apoptosis, disordering of antral formation in oocytes, DNA damage or inhibition of signal carrying among germ cells and somatic cells (Hou & Zhu, 2017). In addition, another study reported that the genotoxic and cytotoxic effects of NPs may be dose-dependent (Di Virgilio et al., 2010).

In studying the influence of np on mouse oocytes in in vitro fertilization (IVF) studies, it was found that np added to the culture medium decreased the fertilization ratio even at quite low concentrations. It was suggested that the reason for this could be genotoxicity or oxidative damage in germ cells because of nps. At high concentrations of NP exposure, it was observed that NPs diffused the cumulus cell layers through out the oocytes' zona pellucida and accumulated in it (Preaubert et al., 2016). Some CeO2 engineered NPs (ENPs) with biomedical properties that are effective in treating endometriosis and protecting the adverse effects of endometriosis on oocytes should be used for limited medical applications because of toxicity, given the results of the above-mentioned in vitro studies (Chaudhury et al., 2013; Hou & Zhu, 2017).

It is also claimed that the accumulation of nanoparticles on the ovaries causes the early onset of oogenesis. Such abnormal processes may lead to the formation of potentially malformed oocytes and dysfunction of the reproductive system. In other words, the accumulation of NP may trigger apoptosis because of the prompt of modifying BCL2 factor (BMF) and mitochondria-related apoptotic pathway (Gao et al., 2012). As mentioned above, most follicles in the ovary undergo a hormonally controlled process of apoptosis during their development, which is regulated by several factors (Hou & Zhu, 2017). As a result of long-term exposure to NPs, it was observed that the expression grades of 288 genes participate in cytokine and hormone pathways were changed in mouse ovaries (Zhao et al., 2013).

4. Developmental Toxicity of Nanoparticles

Adverse effects from exposure of the reproductive system to nanoparticles have recently emerged, posing a risk of reproductive toxicity. Reproductive toxicity refers to effects that interfere with the development of healthy embryos, the reproductive cycle, and any stage of pregnancy. Effects on offspring at any phase of life due to parental exposure are considered developmental toxicity (Brohi et al., 2017).

The quality of gamete cells influences the developmental process. Therefore, a negative effect of nanoparticles on gamete cells or gametogenesis can lead to significant developmental differences (Das et al., 2016). Gametogenesis is a complicated biological process that is sensible to environmental factors. Problems in gamete cells and during gamete cell maturation may affect fertility, induce cancer, and impair embryo development. For example, mutagens resulting from cell impairment can cause inherited gene mutations in germ cells by causing structural and numerical chromosomal damage. Germ cell mutations can result in genetic phenotypic changes, reduced fertility, embryonic death, or congenital malformations and genetic diseases of varying severity, even if the disease does not manifest in subsequent generations (Poma et al., 2014).

In addition, epigenetic changes that happen during gamete cell growth and early embryo growth play a very crucial role in embryo development and successful pregnancy (Khoureiry et al., 2008; Market-Velker et al., 2010). Numerous in vitro studies in animals and humans have demonstrated that diseases of the reproductive system are particularly associated with epigenetic alterations. For this reason, the mechanism of epigenetic reprogramming is important for germ cell development and early embryogenesis. Any problem that may arise from situations such as exposure to nanoparticles in epigenetic mechanisms in the embryonic period may lead to alteration in the expression of related genes, resulting either in the death of the embryo or in permanent diseases that may be transmitted to the next generations.

The placenta is a structure that regulates exchanges between mother and offspring, ensures the continuation of gravidity and embryonic development, and protects the fetus from detrimental situations. While the placental barrier allows the passage of nutrients, hormones, and antibodies, it cannot entirely prevent the passage of all toxic substances. Therefore, developmental toxicity may occur due to transplacental transfer from mother to offspring. Owing to the small size of nanoparticles, they can easily penetrate into the reproductive organs and thus cross the placental barrier easily. Some studies

also show that NP like Au, TiO2, SiO2, carbon (C) may easily cross the placenta (Brohi et al., 2017).

The placenta differentiates after implantation in the uterus wall during pregnancy. Accordingly, as noted in the studies, the effect of NPs may alter be attached on the exposure time of the placenta and fetus, which may alter the embryo's ability to defend against exogenous toxic substances. There are also studies showing that mice in the early stages of pregnancy have higher fetal susceptibility. Nanoparticles were observed in the brain structure of the mouse pups that had received subcutaneous injection of nanoparticles on days 3, 7, 10, and 14 of their pregnancy. Constriction of blood vessels was observed in the hippocampus and cerebral cortex of mouse offspring. These studies suggest that prior to the creation of a functional blood brain barrier, the fetal brain may have little defense against the toxicity of diverse types of NPs (Brohi et al., 2017). Besides, it has been indicated to cause changes in the expression of genes connected with cell death, oxidative damage response, mitochondria, and neurotransmitters, and to affect brain development in the prenatal period (Ema et al., 2010; Fedulov et al., 2008).

It has been reported that the release of metal ions belonging to nanoparticles exposed by inhalation caused to a decline in 17b estradiol levels and an augmentation in mRNA expression level of uterine estrogen receptors, disruption of endocrine mechanism. However, the mechanism of action is not certainly explained. In summary, the possible toxic influence of NP affect both the reproductive function of the offspring and the mothers, and pose a risk to the next generations of offspring exposed directly in utero (Blum et al., 2012; Brohi et al., 2017).

Nanoparticle administration has toxic effects on offspring development, bearing the fetal reproductive system, and results in loss of fertility. In addition to female offspring, NPs were detected by electron microscopy in spermatids, Leydig cells, and Sertoli cells in the testes of 4-day-to 42-day-old male offspring of mothers exposed to subcutaneous nanoparticles at 3 weeks of age. As a result, a reduce in the amount of Sertoli cells, loss of disorganization and integrity in testicular tubules, changes in testicular morphology, and a decrease in daily sperm production were observed. In addition, epididymal sperm motility was found to significantly lower in 42-day-old male offspring (Takeda et al., 2009). It has also been explained that diesel-derived exhaust particles (Diesel Exhaust (DE)) and nanoparticles such as TiO2 transiently suppress Leydig cell proliferation (Hong, Zhao, et

al., 2016). It has also been found to augmentation mutations in the male germline whilst offspring grow up (Boisen et al., 2013).

5. Conclusion

In summary, nanoparticles thanks to their unique properties allow them to provide significant therapeutic benefits in commercial products for example clinical applications, drug delivery systems, cosmetics such as sunscreen lotions or lipstick and textiles they are effectively used in. However, because NPs are non-degradable, in vivo and in vitro researches have indicated the possible for numerous disadventageous health effects from their use or contact (Hou & Zhu, 2017).

The number of studies on reproductive toxicity of NP is increasing, but research is ongoing. While there is evidence that some NPs enter reproductive tissues and organs directly in adult animals and their uteri, it is difficult to make comparisons and definitive conclusions as studies have used various doses and routes of administration.

There is a need to determine minimum doses and exposure pathways for environmental, occupational, therapeutic, and cosmetic uses through human and animal studies of various nanoparticles. Determination of "safe" concentrations of nanoparticles for human and animal health (Brohi et al., 2017).

References

Aloisi, M., Rossi, G., Colafarina, S., Guido, M., Cecconi, S., & Poma, A. M. (2022). The Impact of Metal Nanoparticles on Female Reproductive System: Risks and Opportunities. *International Journal of Environmental Research and Public Health*, *19*(21), 13748.

Baun, A., Hartmann, N. B., Grieger, K., & Kusk, K. O. (2008). Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. *Ecotoxicology*, *17*, 387-395.

Ben-David Makhluf, S., Qasem, R., Rubinstein, S., Gedanken, A., & Breitbart, H. (2006). Loading magnetic nanoparticles into sperm cells does not affect their functionality. *Langmuir*, *22*(23), 9480-9482.

Blum, J. L., Xiong, J. Q., Hoffman, C., & Zelikoff, J. T. (2012). Cadmium associated with inhaled cadmium oxide nanoparticles impacts fetal and neonatal development and growth. *Toxicological sciences*, *126*(2), 478-486.

Boekelheide, K., Fleming, S. L., Johnson, K. J., Patel, S. R., & Schoenfeld, H. A. (2000). Role of Sertoli cells in injury-associated testicular germ cell apoptosis. *Proceedings of the Society for Experimental Biology and Medicine: Minireview*, *225*(2), 105-115.

Boisen, A. M. Z., Shipley, T., Jackson, P., Wallin, H., Nellemann, C., Vogel, U., . . . Hougaard, K. S. (2013). In utero exposure to nanosized carbon black (Printex90) does not induce tandem repeat mutations in female murine germ cells. *Reproductive Toxicology*, *41*, 45-48.

Braydich-Stolle, L., Hussain, S., Schlager, J. J., & Hofmann, M.-C. (2005). In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. *Toxicological sciences*, *88*(2), 412-419.

Braydich-Stolle, L. K., Lucas, B., Schrand, A., Murdock, R. C., Lee, T., Schlager, J. J., . . . Hofmann, M.-C. (2010). Silver nanoparticles disrupt GDNF/Fyn kinase signaling in spermatogonial stem cells. *Toxicological sciences*, *116*(2), 577-589.

Brohi, R. D., Wang, L., Talpur, H. S., Wu, D., Khan, F. A., Bhattarai, D., . . . Huo, L.-J. (2017). Toxicity of nanoparticles on the reproductive system in animal models: a review. *Frontiers in pharmacology*, 606.

Brown, D. M., Wilson, M. R., MacNee, W., Stone, V., & Donaldson, K. (2001). Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. *Toxicology and applied pharmacology*, *175*(3), 191-199.

Browning, L. M., Lee, K. J., Huang, T., Nallathamby, P. D., Lowman, J. E., & Xu, X.-H. N. (2009). Random walk of single gold nanoparticles in zebrafish embryos leading to stochastic toxic effects on embryonic developments. *Nanoscale*, *1*(1), 138-152.

Chaudhury, K., Babu, N., Singh, A. K., Das, S., Kumar, A., & Seal, S. (2013). Mitigation of endometriosis using regenerative cerium oxide nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, *9*(3), 439-448.

Courbiere, B., Auffan, M., Rollais, R., Tassistro, V., Bonnefoy, A., Botta, A., . . . Perrin, J. (2013). Ultrastructural interactions and genotoxicity assay of cerium dioxide nanoparticles on mouse oocytes. *International journal of molecular sciences*, *14*(11), 21613-21628.

Das, J., Choi, Y.-J., Song, H., & Kim, J.-H. (2016). Potential toxicity of engineered nanoparticles in mammalian germ cells and developing embryos: treatment strategies and anticipated applications of nanoparticles in gene delivery. *Human reproduction update*, *22*(5), 588-619.

de Jesus, M. B., & Kapila, Y. L. (2013). Cellular mechanisms in nanomaterial internalization, intracellular trafficking, and toxicity. In *Nanotoxicology: Materials, Methodologies, and Assessments* (pp. 201-227). Springer.

Di Virgilio, A. L., Reigosa, M., Arnal, P. M., & De Mele, M. F. L. (2010). Comparative study of the cytotoxic and genotoxic effects of titanium oxide and aluminium oxide nanoparticles in Chinese hamster ovary (CHO-K1) cells. *Journal of hazardous materials*, *177*(1-3), 711-718.

Donaldson, K., Stone, V., Tran, C., Kreyling, W., & Borm, P. J. (2004). Nanotoxicology. In (Vol. 61, pp. 727-728): BMJ Publishing Group Ltd.

Ema, M., Kobayashi, N., Naya, M., Hanai, S., & Nakanishi, J. (2010). Reproductive and developmental toxicity studies of manufactured nanomaterials. *Reproductive Toxicology*, *30*(3), 343-352.

Erdely, A., Liston, A., Salmen-Muniz, R., Hulderman, T., Young, S.-H., Zeidler-Erdely, P. C., . . . Simeonova, P. P. (2011). Identification of systemic markers from a pulmonary carbon nanotube exposure. *Journal of occupational and environmental medicine*, S80-S86.

Fedulov, A. V., Leme, A., Yang, Z., Dahl, M., Lim, R., Mariani, T. J., & Kobzik, L. (2008). Pulmonary exposure to particles during pregnancy causes increased neonatal asthma susceptibility. *American journal of respiratory cell and molecular biology*, *38*(1), 57-67.

Foldbjerg, R., Olesen, P., Hougaard, M., Dang, D. A., Hoffmann, H. J., & Autrup, H. (2009). PVP-coated silver nanoparticles and silver ions induce reactive oxygen species, apoptosis and necrosis in THP-1 monocytes. *Toxicology letters*, *190*(2), 156-162.

Gao, G., Ze, Y., Li, B., Zhao, X., Zhang, T., Sheng, L., . . . Sun, Q. (2012). Ovarian dysfunction and gene-expressed characteristics of female mice caused by long-term exposure to titanium dioxide nanoparticles. *Journal of hazardous materials*, *243*, 19-27.

Gurr, J.-R., Wang, A. S., Chen, C.-H., & Jan, K.-Y. (2005). Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. *Toxicology*, *213*(1-2), 66-73.

Håkonsen, L., Spano, M., Bonde, J., Olsen, J., Thulstrup, A., Ernst, E., & Ramlau-Hansen, C. (2012). Exposures that may affect sperm DNA integrity: two decades of follow-up in a pregnancy cohort. *Reproductive Toxicology*, *33*(3), 316-321.

Hoet, P. H., Brüske-Hohlfeld, I., & Salata, O. V. (2004). Nanoparticles–known and unknown health risks. *Journal of nanobiotechnology*, *2*, 1-15.

Hong, F., Wang, Y., Zhou, Y., Zhang, Q., Ge, Y., Chen, M., . . . Wang, L. (2016). Exposure to TiO2 nanoparticles induces immunological dysfunction in mouse testitis. *Journal of agricultural and food chemistry*, *64*(1), 346-355.

Hong, F., Zhao, X., Chen, M., Zhou, Y., Ze, Y., Wang, L., . . . Ye, L. (2016). T i O 2 nanoparticles-induced apoptosis of primary cultured Sertoli cells of mice. *Journal of Biomedical Materials Research Part A*, *104*(1), 124-135.

Hou, C.-C., & Zhu, J.-Q. (2017). Nanoparticles and female reproductive system: how do nanoparticles affect oogenesis and embryonic development. *Oncotarget*, *8*(65), 109799.

Hougaard, K. S., Jackson, P., Jensen, K. A., Sloth, J. J., Löschner, K., Larsen, E. H., . . . Wallin, H. (2010). Effects of prenatal exposure to surface-coated nanosized titanium dioxide (UV-Titan). A study in mice. *Particle and fibre toxicology*, *7*(1), 1-15.

Karlsson, H. L., Cronholm, P., Gustafsson, J., & Moller, L. (2008). Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. *Chemical research in toxicology*, *21*(9), 1726-1732.

Khanna, P., Ong, C., Bay, B. H., & Baeg, G. H. (2015). Nanotoxicity: an interplay of oxidative stress, inflammation and cell death. *Nanomaterials*, *5*(3), 1163-1180.

Khoureiry, R., Ibala-Rhomdane, S., Mery, L., Blachere, T., Guerin, J., Lornage, J., & Lefevre, A. (2008). Dynamic CpG methylation of the KCNQ1OT1 gene during maturation of human oocytes. *Journal of medical genetics*, *45*(9), 583-588.

Knaapen, A. M., Borm, P. J., Albrecht, C., & Schins, R. P. (2004). Inhaled particles and lung cancer. Part A: Mechanisms. *International journal of cancer*, *109*(6), 799-809.

Koga, K., & Mor, G. (2010). Toll-like receptors at the maternal–fetal interface in normal pregnancy and pregnancy disorders. *American journal of reproductive immunology*, *63*(6), 587-600.

Lan, Z., & Yang, W.-X. (2012). Nanoparticles and spermatogenesis: how do nanoparticles affect spermatogenesis and penetrate the blood–testis barrier. *Nanomedicine*, *7*(4), 579-596.

Lane, D. P. (1992). p53, guardian of the genome. *Nature*, *358*(6381), 15-16.

Li, J. J., Zou, L., Hartono, D., Ong, C. N., Bay, B. H., & Lanry Yung, L. Y. (2008). Gold nanoparticles induce oxidative damage in lung fibroblasts in vitro. *Advanced Materials*, *20*(1), 138-142.

Li, P.-W., Kuo, T.-H., Chang, J.-H., Yeh, J.-M., & Chan, W.-H. (2010). Induction of cytotoxicity and apoptosis in mouse blastocysts by silver nanoparticles. *Toxicology letters*, *197*(2), 82-87.

Market-Velker, B. A., Zhang, L., Magri, L. S., Bonvissuto, A. C., & Mann, M. R. (2010). Dual effects of superovulation: loss of maternal and paternal imprinted methylation in a dose-dependent manner. *Human molecular genetics*, *19*(1), 36-51.

Melnik, E., Demin, V., Demin, V., Gmoshinski, I., Tyshko, N., & Tutelyan, V. (2013). Transfer of silver nanoparticles through the placenta and breast milk during in vivo experiments on rats. *Acta Naturae (англоязычная версия)*, *5*(3 (18)), 107-115.

Møller, P., Jacobsen, N. R., Folkmann, J. K., Danielsen, P. H., Mikkelsen, L., Hemmingsen, J. G., . . . Loft, S. (2010). Role of oxidative damage in toxicity of particulates. *Free radical research*, *44*(1), 1-46.

Muoth, C., Aengenheister, L., Kucki, M., Wick, P., & Buerki-Thurnherr, T. (2016). Nanoparticle transport across the placental barrier: pushing the field forward! *Nanomedicine*, *11*(8), 941-957.

Nel, A., Xia, T., Madler, L., & Li, N. (2006). Toxic potential of materials at the nanolevel. *Science*, *311*(5761), 622-627.

Nohynek, G. J., & Dufour, E. K. (2012). Nano-sized cosmetic formulations or solid nanoparticles in sunscreens: a risk to human health? *Archives of toxicology*, *86*(7), 1063-1075.

Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., & Cox, C. (2004). Translocation of inhaled ultrafine particles to the brain. *Inhalation toxicology*, *16*(6-7), 437-445.

Papageorgiou, I., Brown, C., Schins, R., Singh, S., Newson, R., Davis, S., . . . Case, C. (2007). The effect of nano-and micron-sized particles of cobalt–chromium alloy on human fibroblasts in vitro. *Biomaterials*, *28*(19), 2946-2958.

Poma, A., Colafarina, S., Fontecchio, G., & Chichiriccò, G. (2014). Transgenerational effects of NMs. *Nanomaterial: impacts on cell biology and medicine*, 235-254.

Preaubert, L., Courbiere, B., Achard, V., Tassistro, V., Greco, F., Orsiere, T., . . . Perrin, J. (2016). Cerium dioxide nanoparticles affect in vitro fertilization in mice. *Nanotoxicology*, *10*(1), 111-117.

Ritz, C., Ruminski, W., Hougaard, K. S., Wallin, H., Vogel, U., & Yauk, C. L. (2011). Germline mutation rates in mice following in utero exposure to diesel exhaust particles by maternal inhalation. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *712*(1-2), 55-58.

Singh, N., Manshian, B., Jenkins, G. J., Griffiths, S. M., Williams, P. M., Maffeis, T. G., . . . Doak, S. H. (2009). NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials. *Biomaterials*, *30*(23-24), 3891-3914.

Stelzer, R., & Hutz, R. J. (2009). Gold nanoparticles enter rat ovarian granulosa cells and subcellular organelles, and alter in-vitro estrogen accumulation. *Journal of Reproduction and Development*, *55*(6), 685-690.

Takeda, K., Suzuki, K.-i., Ishihara, A., Kubo-Irie, M., Fujimoto, R., Tabata, M., . . . Sugamata, M. (2009). Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems. *Journal of Health science*, *55*(1), 95-102.

Toyokuni, S. (1998). Oxidative stress and cancer: the role of redox regulation. *Biotherapy*, *11*, 147-154.

Vales, G., Rubio, L., & Marcos, R. (2016). Genotoxic and cell-transformation effects of multi-walled carbon nanotubes (MWCNT) following in vitro sub-chronic exposures. *Journal of hazardous materials*, *306*, 193-202.

Valko, M., Rhodes, C., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-biological interactions*, *160*(1), 1-40.

Wells, P. G., Bhuller, Y., Chen, C. S., Jeng, W., Kasapinovic, S., Kennedy, J. C., . . . Nicol, C. J. (2005). Molecular and biochemical mechanisms in teratogenesis involving reactive oxygen species. *Toxicology and applied pharmacology*, *207*(2), 354-366.

Wiwanitkit, V., Sereemaspun, A., & Rojanathanes, R. (2009). Effect of gold nanoparticles on spermatozoa: the first world report. *Fertility and Sterility*, *91*(1), e7-e8.

Zastawny, T. H., Altman, S. A., Randers-Eichhorn, L., Madurawe, R., Lumpkin, J. A., Dizdaroglu, M., & Rao, G. (1995). DNA base modifications and membrane damage in cultured mammalian cells treated with iron ions. *Free Radical Biology and Medicine*, *18*(6), 1013-1022.

Zhao, C., Liao, J., Chu, W., Wang, S., Yang, T., Tao, Y., & Wang, G. (2012). Involvement of TLR2 and TLR4 and Th1/Th2 shift in inflammatory responses induced by fine ambient particulate matter in mice. *Inhalation toxicology*, *24*(13), 918-927.

Zhao, X., Ze, Y., Gao, G., Sang, X., Li, B., Gui, S., . . . Cheng, Z. (2013). Nano-sized TiO2-induced reproductive system dysfunction and its mechanism in female mice. *PloS one*, *8*(4), e59378.

Chapter 7

Nanotechnology in the Purification of Semen

Mustafa Bodu[1]

Mehmet Bozkurt Ataman[2]

Mustafa Numan Bucak[3]

Summary

Animals' reduced fertility poses a serious threat to animal reproduction. A number of factors have been demonstrated to reduce semen quality, including age, health, heredity, nutritional condition, seasonal variance, travel stress, artificial insemination (AI), and rising breeding demand. The properties and functioning of semen are altered by the in vitro environment and changes in semen after collection that occur during artificial insemination. Successful artificial insemination may also be achieved via the use of sperm selection and purification techniques. Invasive labeling and/or centrifugation processes are often used in current sperm manipulation approaches, which may be harmful to sperm and/or result in poor recovery rates. The biochemical properties and DNA status of the sperm are often disregarded in favor of selecting for physically normal and motile sperm. It is critical to develop alternative, noninvasive, label-free sperm selection methods to separate sperm based on biochemical features and DNA status. Magnetic nanoparticles provide intriguing new research opportunities for sperm selection. This book explains how sperm may be tagged and cleaned up with the use of nanotechnology, which has become more important in this area in recent years. This book analyzes the recent impact of nanotechnology on sperm labeling, selection, and purification techniques, both existing and planned.

1 Selcuk University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Konya, Turkey

2 Selcuk University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Konya, Turkey

3 Selcuk University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Konya, Turkey

https://doi.org/10.58830/ozgur.pub203.c940

Introduction

Nanoparticles (NPs) are manufactured particles with a high surface ratio, flexible fabrication, and extremely small size - in the nanometer range. Metals, polysaccharides, and proteins are just some of the components that can be used to make nanoparticles. The potential applications of nanotechnology in many areas of science, including medicine, have increased dramatically in recent years. This is largely attributable to the engineering of nanoparticles with improved stability, solubility, and physiological efficacy over their naturally occurring counterparts. In addition, NPs are being employed more often in the pharmaceutical sector to provide therapeutic formulations for hydrophilic substances that are either lipophilic or unstable. Poor water solubility (hydrophobicity) of medications is still a serious issue in clinical pharmacology. Nanotechnology is particularly useful for the approximately 25% of essential medicines on the WHO (World Health Organization) that are thought to be poorly water soluble (Lindenberg et al., 2004).

Techniques to remove defective cells, impurities, and debris from an ejaculate are part of the process of selecting subpopulations of the best sperm (in terms of morphology and motility). These methods can be particularly useful for recovering sperm from suboptimal ejaculations from animals with high genetic value, but they also remove dead sperm, which is highly beneficial due to the detrimental effects that live sperm have on motility and membrane integrity (Brinsko et al., 2003; Öztürk and Ömür, 2022). The most common techniques for removing sperm from animals include density gradient centrifugation (Sieme et al., 2003), swim-up evaluation (Arias et al., 2017), column cleaning (Galarza et al., 2018), and single-layer centrifugation (Nongbua et al., 2017). Although these methods significantly enhance sperm motility and functional parameters in semen cans, particularly for post-thaw semen, they are still constrained by varying rates of recovery (resulting in low sperm concentration in semen cans), increased costs, long run times, and labor costs (Feugang, 2017).

It is necessary to do research in order to have a better comprehension of the cellular and tissue-level impacts of nanocompounds on the reproductive system, since little is currently known about their activity. Since nanocarriers can cross the hemato-testicular barrier (Lan and Yang, 2012), their systemic distribution and biocompatibility have been called into question, and it has been hypothesized that in vivo effects may be the concequence of both systemic changes and direct effects on the testes.

Recent studies on semen statistics show that anywhere from 16–33 percent of semen ejaculates from cattle and buffalo bulls are rejected due to

poor quality when they are still fresh (Manda et al., 2016; Tiwar et al., 2015; Gopinathan et al., 2016), and another 25–30 percent are rejected after being thawed (Bisla et al., 2020). This reduces their commercial viability since there is less high-quality germplasm available for breeding propagation. Increased oxidative stress because of the presence of dead and injured spermatozoa (25%-30%) producing ROS may be a primary cause of ejaculate rejection (Bisla et al., 2020; 2021; Kumar et al., 2018; Rautela et al., 2020).

According to Stiavnická et al. (2017), sperm nano-purification is a process that does not include any invasive procedures and may be used to select high-quality sperm based on epigenetics. The process of nanopurification is used on sperm in order to differentiate unhealthy, intact sperm from sperm that contains moribund cells and sperm that has particular surface alterations. According to recent research (Degheidy et al., 2015; Romany et al., 2017), the technology known as magnetically activated cell sorting (MACS), which can differentiate between dead and living sperm, is the most efficient procedure for the nanopurification of sperm currently available. In this context, sperm nano-purification is a game-changing technique as a result of its innovative capacity to be more effective while also taking less time. It is possible for it to be utilized commercially on a significant scale. Iron oxide nanoparticles (Fe3O4 NPs/IONPs) are renowned for their magnetic, biocompatible, and biofunctional qualities, which constitute the foundation for sorting dead sperm from those with damaged membranes, as stated by Huang and Tang (2004). Binding iron oxide nanoparticles (IONPs) to antiubiquitin Abs (Abs against ubiquitin, a poor fertility indicator) or to other plant lectins like PNA/PSA, which may bind to sperm membrane glycans, might be employed for nanopurification (Bisla et al., 2020). This would allow the IONPs to be purified in a more targeted manner. It was proven that greater conception rates could be achieved using sull sperm nano-purified after being frozen and thawed using Fe3O4 plant lectin or anti-ubiquitin antibody-coated nanoparticles (Odhiambo et al., 2014). This was the case even when using just half of the needed dosage.

In the first nano-purification experiments, buffalo sperm was used. The results were promising in terms of improved spermatozoa motility, plasma membrane integrity, viability, reduction of DNA damage, acrosome integrity, oxidative stress with improved antioxidant properties, and in vitro fertilization rate (Bisla et al., 2021). So, using magnetic nanoparticles to remove dead, dying, and clumped sperm has been shown to improve seminal fluid properties and fertility in boars (Durfey et al., 2019), bulls (Zhang et al., 2018), buffaloes (Bisla et al., 2021), and stallions (Morris et al., 2018).

In both fresh and frozen ejaculations, a procedure known as sperm nano-purification is used to remove defective and moribund sperm. This is done with the intention of reducing the extent of damage to viable sperm. This approach offers several advantages over conventional methods used today. The use of traditional methods for similar purposes, such as Sephadex filtration, sperm swimming up and down, or gradient separation, is limited due to a number of factors. These factors include labor costs, variably low sperm yield (10% to 63%), and high time requirements (> 60 minutes) (Bisla et al., 2020).

The following are nano purification techniques in semen and research that are linked to them.

1. Magnetic nanoparticles' removing of apoptotic and dead spermatozoa

Apoptotic and dead spermatozoa both result in the production of reactive oxygen species (ROS), including the highly reactive superoxide anion (O_2^-), hydroxyl radical (OH^-), and hydrogen peroxide (H_2O_2). Durfey et al., (2019) state that ROS are free radicals that lead to oxidative damage. ROS are byproducts of oxidative phosphorylation, the primary energy pathway for animal spermatozoa. Antioxidants such as ergothioneine, catalase, and superoxide dismutase, are abundant in spermatozoa seminal plasma to protect against the destruction caused by ROS. In order to partially defend against oxidative damage from ROS, it is necessary to have both hyperactive antioxidant systems and endogenous ROS generation through oxidative phosphorylation. When ROS build up, they cause lipid peroxidation, which in turn creates toxic lipid dehydrates (Leemans et al., 2019). This leads to a dramatic decline in sperm mobility and an increase in midpiece abnormalities. These metabolic byproducts would cause rapid cell death and oxidative DNA damage as stated by Rappa et al., (2016). Limiting and preventing these oxidative stressors by eliminating the dead and apoptotic sperm might improve the viability, motility, and fertility of a semen sample (Durfey et al., 2019; Lone 2016).

Apoptosis is an example of a regulated cell death mechanism. The proportion of Sertoli to germ cells is regulated in part by apoptosis (Aitken and Baker, 2013). Fifty to sixty percent of germ cells during the initial meiotic division are apoptosized by Sertoli cells (Sakkas and Alvarez, 2010). The testis' Sertoli cells will phagocytose these cells after they acquire apoptotic markers (Valcarce et al., 2016). This is important for maintaining a healthy ratio between germ cells and the number of Sertoli cells available to nourish

them. Apoptosis kills damaged germ cells in the testicular epithelium in response to various physiological and environmental cues (Aitken and Baker, 2013). However, the elimination method has its limitations. Some of these injured germ cells may still participate in spermiogenesis and show up in the form of ejaculate, although the amount varies greatly. Therefore, it is not uncommon to find apoptotic cells among the spermatozoa of mammals that have just ejaculated (Valcarce et al., 2016). When this elimination process is unsuccessful, sperm morphology and genomic quality may differ, resulting in spermatozoa that appear normal but have apoptotic damage to their nuclei (Sakkas and Alvarez, 2010).

Apoptotic spermatozoa exhibit DNA fragmentation hastening, mitochondrial membrane potential shifts, and caspase activation. The two most important apoptotic events in the setting of nano-purification are caspase activation and the release of the phosphatidylserine phospholipid (Durfey et al., 2019; Gil et al., 2013). Proteases known as caspases have been linked to apoptosis in two distinct ways, as effectors (caspases 3, 6, and 7) and as initiators (caspases 8, 9, and 10), respectively (Paasch et al., 2003). By generating DNA strand breaks, blocking membrane activities, cleavage of various structural cell proteins, and caspase 3 is responsible for the ultimate phases of cell death (Said et al., 2008). Studies by Cortés-Gutiérrez et al., (2007) reveal that infertility is linked to genetic deficits. As part of the apoptotic process, DNA fragmentation occurs, although this by itself does not have a major impact on the effectiveness of assisted reproduction. Cells that have begun the dying process but have not yet reached the point of ultimate collapse may still contain intact DNA since DNA fragmentation is a late indicator of apoptosis (Rateb, 2021). Loss of membrane integrity, a prelude to apoptosis (Gil et al., 2013), is even more intriguing. When the plasma membrane of sperm is disrupted, phosphatidylserine is released from the inner leaflet and moves to the cell's surface. Ca^{2+}-dependent phospholipid-binding protein annexin V has a considerable affinity for exposed phosphatidylserine. For this reason, MNP conjugated to annexin V may be utilized to differentiate between normal and abnormal sperm (Gil et al., 2013; Said et al. 2008).

Healthy spermatozoa may be distinguished from unhealthy spermatozoa. Miltenyi Biotec (Bergisch Gladbach, Germany) created and trademarked the MACS® technique, which can identify various cell types. MACS® gets rid of sperm cells that have phosphatidylserine on their surface by using 50 nm colloidal superparamagnetic microbeads that are linked to annexin V. These beads identify and trap sperm in the magnetic field of the MACS® column as they enter apoptosis and death. Figure 2 shows how percent flow can be

used to isolate viable sperm. Healthy sperm are isolated from sperm that have died or undergone apoptosis. Because of this, coated microspheres with MNP-annexin V complexes can't bind to non-apoptotic cells with healthy membranes. If sperm are able to adhere to the microspheres, this indicates that the phosphatidylserine has been externalized and sperm membrane integrity is compromised (Daneshmandpour et al., 2019) (Figure 1).

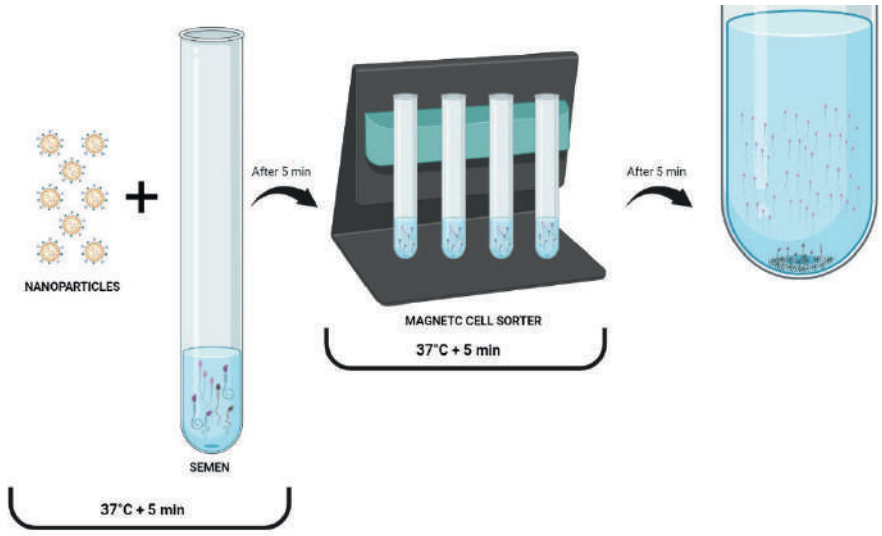


Figure 1. Schematic Diagram of Nanopurification of Semen

Performance of the MACS® system is restricted to less than 10^9 sperm for one factor in sperm viability, like apoptosis. The use of MACS® by the swine industry is thus hindered as a result (Durfey et al., 2019). The amount of boar semen used in postcervical artificial insemination is often increased to 50 milliliters and contains between one and one and a half million sperm. Extended amounts of 80-100 mL of swine semen containing $1.5\text{-}3.0\text{x}10^9$ spermatozoa are considered to be typical for conventional artificial insemination, as stated by Schulze et al., (2019). Due to the fact that the dosage necessary to enhance fertility after AI is only $500\text{x}10^6$, the aforementioned constraint presents less of a concern in horse reproduction (Samper, 2009).

Human sperm with a significantly higher motility and better cryosurvival rate after freezing and thawing are the result of MACS® separation. Oocytes that have been sorted have a better chance of being penetrated (Said et al., 2008). Paasch et al., (2003) found that both the magnetic field and the

separation columns had no appreciable impact on sperm viability in their experiments. The sperm count did not decrease, and neither did their motility. When compared to annexin-positive sperm, annexin-negative sperm had significantly higher increasing motile speeds after separation.

In a human study, Said et al., (2008) found that MACS® screening for non-apoptotic sperm subpopulations resulted in better sperm morphology. Lower sperm deformity index scores were observed, and fewer sperm had midpiece abnormalities, acrosomal defects, tail defects, or persisting cytoplasmic droplets. MACS® has several benefits when used to sort sperm, including cheap cost, convenience of use, high sensitivity, high specificity, quick response kinetics, and the production of stable suspensions. Annexin V, a cell protein, reacts immunospecifically with the phospholipid phosphatidylserine, resulting in a highly sensitive and specific assay. One of the few limitations of MACS is that the microbeads are so small. Miltenyi Biotec GmbH's MACS® Microbeads and Columns (MiniMACS® column) can produce this powerful magnetic field without compromising cell viability or performance. The tiny magnet needs a magnetic field of around 1 Tesla to preserve the tagged cells. Using MACS® to isolate a non-apoptotic fraction improves the overall quality and fertilization potential of the recovered sperm. MACS® can sort a large number of cells by surface marker expression. Apoptotic sperm aren't the only thing in an ejaculate that has to be filtered out if you want better outcomes. Leukocytes, debris, and plasma are all examples of these components. Thus, MACS® can enhance current methods of preparation (Said et al., 2008). For instance, double density gradient centrifugation paired with MACS® has been shown to be the most effective procedure for sperm selection in human fertility trials (Gil et al., 2013).

Separation of motile and high-quality spermatozoa and an improved conception rate were also reported by Feugang et al., (2015) using Fe3O4 NPs coated with PNA/PSA lectins for nanopurification of swine semen. Improved semen quality with more viable cells was seen by Farini et al., (2016) when synthetic DNA (deoxyribonucleic acid) aptamers were associated with spermatozoa with damaged membranes using avidin-coated superparamagnetic Fe3O4 NPs. Researchers Durfey et al., (2017, 2019) found that using the nanoselection technology to produce purified semen led to sperm with excellent structural and functional features without negatively impacting fertility. In addition, it did not compromise the well-being or growth of future generations.

Successful removal of apoptotic spermatozoa from ejaculate by MACS® has been reported in stallions by da Silva et al., (2010). Caspase activation, sperm motility, and membrane integrity were all negatively affected in spermatozoa that had connected to annexin V-conjugated microbeads, as predicted. In addition, MACS® did not alter the sperm's natural form in any way. In this investigation, only 46.3% of the sperm were retrieved using MACS®. More study is needed to determine if MACS® can strengthen sperm quality in infertile stallions and freeze-thawing of sperm (da Silva et al., 2010).

2. Magnetic nanoparticles removal early acrosome-reacted spermatozoa

Normal sperm undergo an acrosome response when they encounter the zona pellucida of an egg during fertilization in the fallopian tube. Yousef et al., (2020) and Leemans et al., (2019) found that sperm with premature acrosome reactions are unable to fertilize an egg because their reactions occur too early or in the wrong places. Acrosome injuries caused by osmotic, mechanical, or cryogenic pressure may cause premature acrosome reactions, and these injuries can be inherited. The inner acrosome membrane, combined with the remaining unfused component of the plasma membrane, creates the new outer sperm membrane in sperm that have responded prematurely with their acrosomes or in sperm that have damaged acrosomes. This happens in sperm that are either healthy or injured (Leemans et al., 2019).

Specific carbohydrates are released from the inner acrosomal membrane after premature capacitation of spermatozoa, an acrosomal damage, or acrosomal reaction reaction (Lone, 2016). Head-to-head agglutination of spermatozoa may be triggered by the interaction of these particular carbohydrates with lectins. The rate of conception drops when this happens because fewer sperm are able to reach the egg. Carbohydrates on the epithelial membrane of the fallopian tube and the zona pellucida of the egg may interact with lectins, glycoproteins on the surface of sperm. Lectin-carbohydrate interplays are used by the oviduct to choose sperm with optimal shape and performance. Pisum sativum agglutinin (PSA) and Peanut agglutinin from Arachis hypogaea (PNA) are two examples of lectins that have a high affinity for carbohydrates found exclusively in the inner acrosomal membrane (Feugang et al., 2015). Accurate indications of acrosomal damage in mammalian sperm include the lectins PSA and PNA. Yousef et al., (2020) and Odhiambo et al., (2014) propose that PNA lectins or MNP coated with PSA may be used to exclude sperm with a damaged acrosome.

3. Magnetic nanoparticle elimination of ubiquitinated spermatozoa

In MNP-assisted sperm selection, ubiquitin, a regulatory chaperone molecule, may be used as a marker, as revealed by Štiavnická et al., (2017). The reproductive organs of both sexes are common sites of ubiquitination (Lone, 2016). When it comes to X chromosome silencing, cell signaling, and gene transcription during gametogenesis, fertilization, and early embryonic development, mono-ubiquitination plays a key role (Odhiambo et al., 2014). Polyubiquitination, in which several ubiquitin chains are attached to the internal lysine residues of a substrate protein, is a stable covalent post-translational modification. Štiavnická et al., (2017) shown that poly-ubiquitination is crucial to the ubiquitin-proteasome system's role in protein turnover. According to the findings of study conducted by Odhiambo et al., (2014), ubiquitin in mammalian epididymal fluid helps in the turnover and aggregation of epididymal proteins that are secretory. This, in turn, assists in the maturation of sperm by encasing damaged or dead sperm, which helps the maturation process.

Epididymal epithelial cells use an apocrine secretory pathway to release ubiquitin as a quality control marker when sperm move through the epididymis. This happens as the sperm go through the epididymis. Epididymal epithelial cells are responsible for the release of unconjugated ubiquitin as well as the enzymes that are necessary for its covalent attachment to certain proteins found on the surface of damaged sperm. Ubiquitination of defective sperm triggers their phagocytosis and elimination. The ejaculate still contains ubiquitinated defective sperm (Štiavnická et al., 2017). That's why ubiquitination may cause morphological abnormalities, DNA fragmentation, acrosomal damage, dysregulated and ectopic accumulation of fertility-associated proteins, or all three in bull sperm. Odhiambo et al., (2014) found a negative correlation between ubiquitination of sperm and male fertility in various mammalian species. These data support the use of ubiquitin as a reliable spermatozoa marker. To eliminate incorrectly ubiquitinated spermatozoa, we used MNP coated with an anti-ubiquitin monoclonal antibody (Štiavnická et al., 2017).

Measurements of sperm quality were unaffected by the application of magnetic nanoparticles coated with PNA lectins and sex-specific components, as reported by Morris et al., (2018). Sperm treated with NPs demonstrated less DNA damage and had a higher conception rate (80%). Bull sperm nuclear integrity and structural surface damage were shown to be correlated with increasing ubiquitination (Zhang et al., 2018). Updated MACS techniques utilizing NPs coated with anti-ubiquitin antibodies

effectively removed morphologically damaged sperm from bull sperm by pushing sperm that were ubiquitinated and connected to NPs downward into the magnetic field.

4. A two-step procedure for removing spermatozoa that have undergone apoptosis and acrosome reaction

In two separate studies using the same methodology, researchers treated porcine sperm with magnetic nanoparticles (MNP) conjugated to the lectins PNA/PSA and MNP conjugated to annexin V (Durfey et al.,2019). By altering the acrosome of the sperm, lectin-MNP was used to remove the sperm. The nuclear diameter of these lectin-MNP conjugates is 14±0.4 nm, as determined by transmission electron microscopy (TEM). Annexin V-MNP was used to remove the apoptotic and dead spermatozoa. The results of this Annexin V-MNP conjugates TEM show that the nuclear diameter is about 7.1±0.2 nm. Freshly collected porcine semen was combined with lectin MNPs or Annexin V-MNP to produce 3-4x10^9 spermatozoa/80 mL per insemination dose. Gentle rotation was performed during the 10-15 min incubation period at 37°C. The sperm sample was exposed to a 12,000 gauss neodymium magnet for 10 min at room temperature after being exposed for 15 to 20 min to capture all unbound and sperm-bound MNP. Following Durfey et al. (2019), the unbound sperm were eluted into fresh tubes.

In a two-step process, the spermatozoa that were still alive and had their acrosomes intact were separated. In the first phase, 87.5 g of Annexin V-MNP was added to the enlarged semen sample, and the mixture was incubated at 37 degrees Celsius for 30 minutes to kill off any apoptotic or dead spermatozoa. There were between 1.6 and 2.0x10^9 spermatozoa in 40 mL of the concentrated semen sample. The second step involves combining the non-apoptotic, nano-selected spermatozoa with 87.5 gr of lectin-MNP and incubating the mixture for the optimal incubation time of 30 minutes to remove any spermatozoon that have undergone a change in their sperm acrosome (Durfey et al., 2019). To extract viable, apoptosis-free spermatozoa, one must use a new tube.

The synergistic and supplementary benefits of the two-step nanopurification method are evident. At each nanoselection stage, the fraction of total motile, forward, and fast spermatozoa is statistically significantly higher compared to the control sample, while the fraction of static spermatozoa is statistically significantly lower. MNP conjugates may be able to reduce the number of faulty spermatozoa since there are fewer of them. There is a significant relationship between the first phase of the

two-step nanopurification process and the resulting fast sperm motility, total sperm motility, curvilinear velocity (VCL), average path velocity (VAP), and straight-line velocity (VSL), The directional parameters improve to varying degrees after each elimination stage. The second stage significantly reduced the lateral head amplitude of the spermatozoa, while simultaneously increasing their beating frequency, straightness (STR), and linearity (LIN), in comparison to the control specimen and the specimens in which only the first step was conducted. Evidence like this highlights the need for a sequential removal technique to effectively filter out defective spermatozoon from sperm specimen (Durfey et al., 2019).

Sperm kinematic qualities are indicators of how well sperm will swim through the uterus and oviduct. Both male fertility and the rate of fertilization are positively correlated with these factors. However, the viability of the enriched samples was not improved compared to the controls after being enriched with better nano-selected sperm. To survive freezing conditions and be fertilized, the nano-selected sperm maintain a more stable plasma membrane, a greater mitochondrial membrane potential and a lower ROS level. These measures of sperm vitality do not vary from those of control sperm. Nano-selected semen samples did not increase the reproductive rate of gilts in field experiments. Concerns about potential danger or toxicity may be disregarded since there was no difference between the control and nano-selected groups in terms of litter size, weight, developmental pace, or offspring health. No significant associations between sperm treatment with MNP and offspring were found by Durfey et al., (2019).

5. Current studies with sperm nano purification

Metallic nanoparticles	*Species*	*Mechanism of action/results*	*References*
Fe_3O_4 (Iron Oxide) Nanoparticles	Buck	Sperm selection on Angora buck semen using nanoparticles at three distinct temperatures (37°C, 21°C, and 4°C). In comparison to the control group, total motility, LIN, VCL, VSL, and acrosome integrity parameters were all higher at 37oC. On the other hand, at 21oC, the control group had higher values for LIN, STR, VCL, VSL, VAP, wobble, and plasma membrane-acrosome integrity (PMAI). There were changes, although they were insignificant, between the group that was exposed to 4oC and the control group.	Alemdar and Tirpan, 2022
	Bull	Antibodies against ubiquitin-coated (Abs) IONPs for reducing oxidative damage to living spermatozoa obtained from unprocessed semen. In Groups II, III, and IV, IONPs-Abs complex was added in the following ratios: 1:1 (0.5 g/ml), 1:2 (1.0 g/ml), and 1:4 (2.0 g/ml), respectively. In order to reduce oxidative stress, IONPs conjugated with anti-ubiquitin Abs at a concentration of 2.0 g/ml may be an efficient way to remove damaged or dead spermatozoa from buffalo ejaculates.	Bisla et al., 2020
	Boar	Samples were classified into 4 groups based on their levels of motility: >90% (1), 80–90% (2), 70–80% (3), and <70% (4).Although there was a similar pattern in the sperm motility character measured by VCL, VSL, VAP, and LIN. The motility enhancement was more obvious in the group of sperm with less than 70% motility.	Chung and Son, 2016
	Buffalo	The efficacy of the swim-up approach and nano-purification in removing dead or damaged spermatozoa was compared in different concentrations of semen. The current study's findings showed that nano-purification utilizing anti-ubiquitin particles eliminated defective or dead spermatozoa at the lag stage considerably more successfully than swim up procedure.	Din et al., 2018

Boar	Studied magnetic nanoparticle conjugates to eliminate non-viable spermatozoa and evaluate their motility and vitality. According to research, it is possible to increase male fertility by successfully removing moribund (static) spermatozoa without harming their viability.	Durfey et al., 2017
Boar	According to research, moribund sperm, also known as static sperm, may be successfully eliminated without negatively impacting viability, resulting in an increase in male fertility. The findings demonstrate that magnetic nanoselection is beneficial for high-throughput targeted removal of damaged sperm as well as simple and rapid enrichment of sperm dosages with highly mobile, viable, and fertile sperm. Therefore, magnetic nanoselection, which involves removing abnormal sperm from seminal fluid, has the potential to improve male fertility. This is especially true during times of heat stress in the summer.	Durfey et al., 2019
Bull	Using Cell-SELEX, it was possible to identify sperm cells that had suffered heat damage by using single-stranded DNA aptamers with the ideal combination of affinity and specificity. First, aptamers that bind to the membrane of heat-damaged spermatozoa were isolated; these aptamers contain two conserved motifs with a total length of 6 nucleotides. Then, synthetic biotin-labeled aptamers with the conserved motif were used to find membrane-damaged cells and separate them from healthy cells by using superparamagnetic iron oxide nanoparticles (SPION) coated with avidin. By dramatically increasing the proportion of viable sperm, this method improved sperm quality without decreasing the rate of blastocyst separation. Both asexual and sex-selected sperm suspensions performed well when treated with this method.	Farini et al., 2016
Boar	Improved Reproductive Performance with Lectin-Functionalized Magnetic Iron Oxide Nanoparticles. Sperm motility was considerably enhanced by nanopurification. The viability of the piglets and the ability of sperm to fertilize them were not adversely affected by the magnetic nanoparticles utilized in this early investigation. Semen fertility may have positive improvements, with potential for application in gender selection.	Feugang et al., 2015

Bull	The goal of this study was to see how well a nanoparticle-based magnetic purification method improved the viability and fertilization ability of sperm samples in vitro and in vivo by getting rid of the 30% of the sample that was made up of bad spermatozoa. Therefore, this study describes the successful implementation of a new nanotechnology to improve artificial insemination in cattle through field trials. The offspring of males whose spermatozoa were nanopurified to remove all traces of PNA and ubiquitin looked perfectly healthy. In the first year of this artificial intelligence field study, heifers conceived with both the non-purified control semen and the artificial intelligence showed normal fertility.	Odhiambo et al., 2014
Camel	This study set out to determine whether a magnetic nanoparticle-based sperm purification approach was adequate for removing damaged and apoptotic camel spermatozoa from dosages of cryopreserved semen liquefied using a protease. These results suggest that protease-based liquefaction of sperm before cryopreservation, followed by magnetic nanopurification after thawing, is promising to decrease the percentage of damaged and dead sperm and increase the fertilization efficiency of camelid sperm.	Rateb, 2021
Buffalo	A negative fertility marker is employed in this test to exclude any damaged or dead sperm that may have been present. Assays using hypoosmotic (HOS) and fluorescein-conjugated Pisum sativum agglutinin (FITC-PSA) demonstrate the effects of this substance. The success of this treatment is supported by evidence such as Ca2+-regulatory mechanisms, depolarization of the sperm membrane, a reduction in the quantity of free radicals, and an in vitro fertility test. An antibody concentration of 1.0 g/ml that was biotinylated with IONPs proved to be the most effective method for increasing the number of viable zona-bound sperm that were present in the ejaculate.	Rautela et al., 2022

Conclusion

Nano-based methods for mammalian sperm purification using MNPs coated with biomarkers are promising and offer new opportunities for developing easy, effective, and noninvasive methods, as stated by Odhiambo et al. (2014), and Yousef et al. (2020). Research by Durfey et al. (2019) suggests that MNPs may be utilized to selectively filter out damaged sperm from a semen sample in a high-throughput setting. The method is easily incorporated into semen cryopreservation protocols, is low-cost, does not call for extensive manipulation of semen, employs commercially available components, is highly sensitive and specific, requires little to no labor, does not impede fertilization or the establishment of a healthy pregnancy, and does not increase the risk of infections in the reproductive tract. MNP's use in spermatology has grown during the last several years. There have been several scholarly articles written on the subject, however not all of them can be considered scientifically sound. Manufacturers of MNPs state that various strains of their product have several potential uses. However, these initiatives frequently rely on research that has not been reviewed by experts in the field. Future research is required to separate fact from fiction.

References

Aitken, R. J., & Baker, M. A. (2013). Causes and consequences of apoptosis in spermatozoa; contributions to infertility and impacts on development. *International Journal of Developmental Biology*, *57*(2-3-4), 265-272.

Alemdar, H., & Tırpan, M. B. (2022). A novel approach to sperm selection: Nanoparticle-based purification improves quality of Angora cryopreserved buck's semen. Journal of the Hellenic Veterinary Medical Society, *73*(4), 4881-4890.

Arias, M. E., Andara, K., Briones, E., & Felmer, R. (2017). Bovine sperm separation by Swim-up and density gradients (Percoll and BoviPure): Effect on sperm quality, function and gene expression. *Reproductive biology*, *17*(2), 126-132.

Bisla, A., Rautela, R., Yadav, V., Saini, G., Singh, P., Ngou, A. A., ... & Srivastava, N. (2021). Synthesis of iron oxide nanoparticles–antiubiquitin antibodies conjugates for depletion of dead/damaged spermatozoa from buffalo (Bubalus bubalis) semen. *Biotechnology and Applied Biochemistry*, *68*(6), 1453-1468.

Bisla, A., Rautela, R., Yadav, V., Singh, P., Kumar, A., Ghosh, S., ... & Srivastava, N. (2020). Nano-purification of raw semen minimises oxidative stress with improvement in post-thaw quality of buffalo spermatozoa. Andrologia, 52(9), e13709.

Brinsko, S. P., Blanchard, T. L., Rigby, S. L., Love, C. C., & Varner, D. D. (2003). Effects of dead spermatozoa on motion characteristics and membrane integrity of live spermatozoa in fresh and cooled-stored equine semen. *Theriogenology*, *59*(3-4), 735-742.

Cortés-Gutiérrez, E. I., Dávila-Rodríguez, M. I., López-Fernández, C., Fernández, J. L., & Gosálvez, J. (2007). Evaluación del daño en el DNA espermático. *Actas urológicas españolas*, *31*(2), 120-131.

Chung, K. H., & Son, J. H. (2016). Improvement of boar semen quality by sperm selection using magnetic nano-particles. Journal of Life Science, 26(8), 943-947.

da Silva, M. C., Pinto, C. R. F., Young, J. M., & Cole, K. (2010). 8 The Use of Annexin V Magnetic-Activated Cell Sorting to Separate Apoptotic Sperm from the Ejaculate of Stallions. *Reproduction, Fertility and Development*, *23*(1), 110-111.

Daneshmandpour, Y., Pashazadeh, F., Ansari, F., Hosseinifard, H., Nouri, M., Yousefi, M., & Sakhinia, E. (2019). The comparative effect of magnetic activated cell sorting, density gradient centrifugation and swim up on assisted reproduction outcomes, sperm DNA fragmentation, and aneuploidy: a systematic review and meta-analysis. *Meta Gene*, *22*, 100607.

Degheidy, T., Abdelfattah, H., Seif, A., Albuz, F. K., Gazi, S., & Abbas, S. (2015). Magnetic activated cell sorting: an effective method for reduction of sperm DNA fragmentation in varicocele men prior to assisted reproductive techniques. *Andrologia*, *47*(8), 892-896.

Durfey, C. L., Burnett, D. D., Liao, S. F., Steadman, C. S., Crenshaw, M. A., Clemente, H. J., ... & Feugang, J. M. (2017). Nanotechnology-based selection of boar spermatozoa: growth development and health assessments of produced offspring. *Livestock science*, *205*, 137-142.

Durfey, C. L., Swistek, S. E., Liao, S. F., Crenshaw, M. A., Clemente, H. J., Thirumalai, R. V., ... & Feugang, J. M. (2019). Nanotechnology-based approach for safer enrichment of semen with best spermatozoa. *Journal of animal science and biotechnology*, *10*(1), 1-12.

Farini, V. L., Camaño, C. V., Ybarra, G., Viale, D. L., Vichera, G., Yakisich, J. S., & Radrizzani, M. (2016). Improvement of bovine semen quality by removal of membrane-damaged sperm cells with DNA aptamers and magnetic nanoparticles. *Journal of biotechnology*, *229*, 33-41.

Feugang, J. M. (2017). Novel agents for sperm purification, sorting, and imaging. *Molecular Reproduction and Development*, *84*(9), 832-841.

Feugang, J., Liao, S., Crenshaw, M., Clemente, H., Willard, S., & Ryan, P. (2015). Lectin-functionalized magnetic iron oxide nanoparticles for reproductive improvement. *JFIV Reprod Med Genet*, *3*(145), 17-19.

Galarza, D. A., Lopez-Sebastian, A., Woelders, H., Blesbois, E., & Santiago-Moreno, J. (2018). Sephadex filtration as successful alternative to density-gradient centrifugation procedures for ram sperm selection with improved kinetics. *Animal reproduction science*, *192*, 261-270.

Gil, M., Sar-Shalom, V., Melendez Sivira, Y., Carreras, R., & Checa, M. A. (2013). Sperm selection using magnetic activated cell sorting (MACS) in assisted reproduction: a systematic review and meta-analysis. *Journal of assisted reproduction and genetics*, *30*, 479-485.

Gopinathan, A., Sivaselvam, S. N., Karthickeyan, S. M. K., & Kulasekar, K. (2016). Discard percentage of semen and disposal pattern of crossbred jersey bulls in Tamil Nadu. *Shalnax Int J Vet Sci*, *4*, 17-21.

Huang, Z., & Tang, F. (2004). Preparation, structure, and magnetic properties of polystyrene coated by Fe3O4 nanoparticles. *Journal of Colloid and Interface Science*, *275*(1), 142-147.

Kumar, A., Prasad, J. K., Mustapha, A. R., Amin, B. Y., Din, O., Katiyar, R., ... & Ghosh, S. K. (2018). Reduction of dissolved oxygen in semen extender with nitrogen gassing reduces oxidative stress and improves post-thaw semen quality of bulls. *Animal reproduction science*, *197*, 162-169.

Lan, Z., & Yang, W. X. (2012). Nanoparticles and spermatogenesis: how do nanoparticles affect spermatogenesis and penetrate the blood–testis barrier. *Nanomedicine*, *7*(4), 579-596.

Leemans, B., Stout, T. A., De Schauwer, C., Heras, S., Nelis, H., Hoogewijs, M., ... & Gadella, B. M. (2019). Update on mammalian sperm capacitation: how much does the horse differ from other species?. *Reproduction*, *157*(5), R181-R197.

Lindenberg, M., Kopp, S., & Dressman, J. B. (2004). Classification of orally administered drugs on the World Health Organization Model list of Essential Medicines according to the biopharmaceutics classification system. *European Journal of Pharmaceutics and Biopharmaceutics*, *58*(2), 265-278.

Lone, F. H. (2016). Nanotechnology based semen purification: a panacea. *Veterinary Med. Open J*, *1*, e1-e2.

Manda, S., Makkena, S., Srilatha, C. H., Rao, K. B., & Naidu, K. S. (2016). Semen discarded during different stages of cryopreservation in Ongole (Bos indicus) bulls. *Journal of Veterinary Science and Technology*, *7*(2).

Morris, L. H., de Haan, T., Landriscina, L. G., Wilsher, S., & Gibb, Z. (2018). The effects of nanoparticle semen purification on semen quality parameters in stallions. *Journal of Equine Veterinary Science*, *66*, 75.

Nongbua, T., Johannisson, A., Edman, A., & Morrell, J. M. (2017). Effects of single layer centrifugation (SLC) on bull spermatozoa prior to freezing on post-thaw semen characteristics. *Reproduction in domestic animals*, *52*(4), 596-602.

Odhiambo, J. F., DeJarnette, J. M., Geary, T. W., Kennedy, C. E., Suarez, S. S., Sutovsky, M., & Sutovsky, P. (2014). Increased conception rates in beef cattle inseminated with nanopurified bull semen. *Biology of reproduction*, *91*(4), 97-1.

Öztürk, A.E. and Ömür, A.D. (2022). Curent Approaches to the Use of Nanotechnology in Reproductive Biotechnologies: Spermatological Researches, In The Trend in Nanomaterials, Synthesis and Applications , İstanbul: Efe Academy Publishing, pp.29-46.

Paasch, U., Grunewald, S., Fitzl, G., & Glander, H. J. (2003). Deterioration of plasma membrane is associated with activated caspases in human spermatozoa. *Journal of andrology*, *24*(2), 246-252.

Rappa, K. L., Rodriguez, H. F., Hakkarainen, G. C., Anchan, R. M., Mutter, G. L., & Asghar, W. (2016). Sperm processing for advanced reproductive technologies: Where are we today?. *Biotechnology advances*, *34*(5), 578-587.

Rateb, S. A. (2021). Purification of cryopreserved camel spermatozoa following protease-based semen liquefaction by lectin-functionalized DNA-defrag

magnetic nanoparticles. Reproduction in Domestic Animals, 56(1), 183-192.

Rautela, R., Bisla, A., Ngou, A. A., Kumar, A., Ghosh, S. K., & Srivastava, N. (2020). Comparative efficacy of conventional stains for evaluation of plasmalemma and acrosome integrity of buffalo spermatozoa. *Indian Journal of Animal Sciences*, *90*(2), 00-00.

Romany, L., Garrido, N., Cobo, A., Aparicio-Ruiz, B., Serra, V., & Meseguer, M. (2017). Obstetric and perinatal outcome of babies born from sperm selected by MACS from a randomized controlled trial. *Journal of assisted reproduction and genetics*, *34*, 201-207.

Said, T. M., Agarwal, A., Zborowski, M., Grunewald, S., Glander, H. J., & Paasch, U. (2008). ANDROLOGY LAB CORNER*: utility of magnetic cell separation as a molecular sperm preparation technique. *Journal of andrology*, *29*(2), 134-142.

Sakkas, D., & Alvarez, J. G. (2010). Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. *Fertility and sterility*, *93*(4), 1027-1036.

Samper, J. C. (2009). Artificial insemination with fresh and cooled semen. *Equine Breeding Management and Artificial Insemination*, 165-174.

Schulze, M., Nitsche-Melkus, E., Jakop, U., Jung, M., & Waberski, D. (2019). New trends in production management in European pig AI centers. *Theriogenology*, *137*, 88-92.

Sieme, H., Martinsson, G., Rauterberg, H., Walter, K., Aurich, C., Petzoldt, R., & Klug, E. (2003). Application of techniques for sperm selection in fresh and frozen-thawed stallion semen. *Reproduction in Domestic Animals*, *38*(2), 134-140.

Štiavnická, M., Abril-Parreño, L., Nevoral, J., Králíčková, M., & García-Álvarez, O. (2017). Non-invasive approaches to epigenetic-based sperm selection. *Medical science monitor: international medical journal of experimental and clinical research*, *23*, 4677.

Tiwar, R., Mishra, G. K., Shukla, M. K., Singh, R. B., Saxena, S. K., & Siddiqu, M. (2011). Seasonal variations in semen production of Murrah buffalo bulls. *The Indian Journal of Animal Reproduction*, *32*(2), 5-7.

Valcarce, D. G., Herráez, M. P., Chereguini, O., Rodríguez, C., & Robles, V. (2016). Selection of nonapoptotic sperm by magnetic-activated cell sorting in Senegalese sole (Solea senegalensis). *Theriogenology*, *86*(5), 1195-1202.

Yousef, M. S., López-Lorente, A. I., Diaz-Jimenez, M., Consuegra, C., Dorado, J., Pereira, B., ... & Hidalgo, M. (2020). Nano-depletion of acrosome-damaged donkey sperm by using lectin peanut agglutinin (PNA)-magnetic nanoparticles. *Theriogenology*, *151*, 103-111.

Zhang, J., Su, J., Hu, S., Zhang, J., Ding, R., Guo, J., ... & Li, X. (2018). Correlation between ubiquitination and defects of bull spermatozoa and removal of defective spermatozoa using anti-ubiquitin antibody-coated magnetized beads. *Animal reproduction science*, *192*, 44-52.

Chapter 8

Nanotechnology in the Treatment of Infertility

Salih Narlıçay[1]

Mehmet Buğra Kıvrak[2]

Abstract

With the increasing reproductive issues in all living beings worldwide, numerous problems related to fertility have emerged. Concurrently, the rising global population rates have led to a situation where the available food resources cannot meet the demand. Environmental factors, genetics, age, nutrition, stress, and global changes are some of the reasons that lead to infertility problems. Efforts are still underway to find methods in vivo or in vitro studies to eliminate reproductive problems by preventing structural abnormalities or changes in germ cells. In recent years, some nanoparticles have been utilized to address infertility issues caused by hormonal imbalances, metabolic disorders, or abnormalities in gamete cells, aiming to perform manipulations that could positively impact fertilization. Nanoparticles, used at non-toxic levels, have shown positive responses in fertility. Particularly in spermatological studies, nanoantioxidants have been observed to reduce reactive oxygen species, consequently preventing oxidative stress. Additionally, they are reported to benefit motility and sperm viability, while preserving DNA and gene expression. This section provides important insights into the potential of nanobiotechnology in addressing infertility issues in the years to come.

1. Introduction

Particles with a length or width of less than 100 nm in colloidal structures are referred to as Nanoparticles (NPs) (Khan et al., 2019). Depending on the type of material used, nanoparticles are classified into four categories: metallic nanoparticles (Au, Ag, Cu, Fe, Zn NPs), metal and metal oxide nanoparticles (FeO, VO, AlO, ZnO NPs), semiconductor nanoparticles (ZnS,

1 Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Turkey, ORCID: 0000-0001-8043-3807

2 Assist. Prof., Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Turkey, 0000-0002-4772-874X
mbkivrak@cumhuriyet.edu.tr

https://doi.org/10.58830/ozgur.pub203.c941

CdSe, ZnSe, CdS NPs), and carbon-based (C) nanoparticles (Hong et al., 2022). These nanoparticles, which are produced in extremely small sizes, are also classified based on their dimensions (0D, 1D, 2D, and 3D) (Tiwari et al., 2012). Nowadays, researchers have discovered that nano-sized particles can alter the structural properties of a material. Recently, it has been reported that nano-sized particles are used in approximately 2000 products, and this number is rapidly increasing. These nanotechnological products are widely employed in biomedical, industrial, and agricultural fields. It is known that nanotechnology is used particularly in textile products, healthcare, sports equipment, the food industry, and many other products (Vance et al., 2015). The increasing utilization of nanotechnology in the past 30 years has raised the exposure level to nanomaterials. However, even though it may not be immediate, these products' positive or negative effects on living organisms are not yet fully understood. The current implications of this situation have been subject to investigation by some studies (Kwon et al.,2008; Nazar et al., 2016; Hong et al., 2017; Iftikhar et al., 2021; Klein et al., 2022; Öztürk and Ömür, 2022).

Nanomaterials, which have a multidisciplinary nature, have paved the way for a scientific discipline called nanobiotechnology with their applications in various fields of biology. It has been reported that various nanoparticles are used in the fields of biomedicine and veterinary science (Barkalina et al., 2014; Rath et al., 2015; Feugang, 2017; Falchi et al., 2018; Jain et al., 2018; Remião et al., 2018).

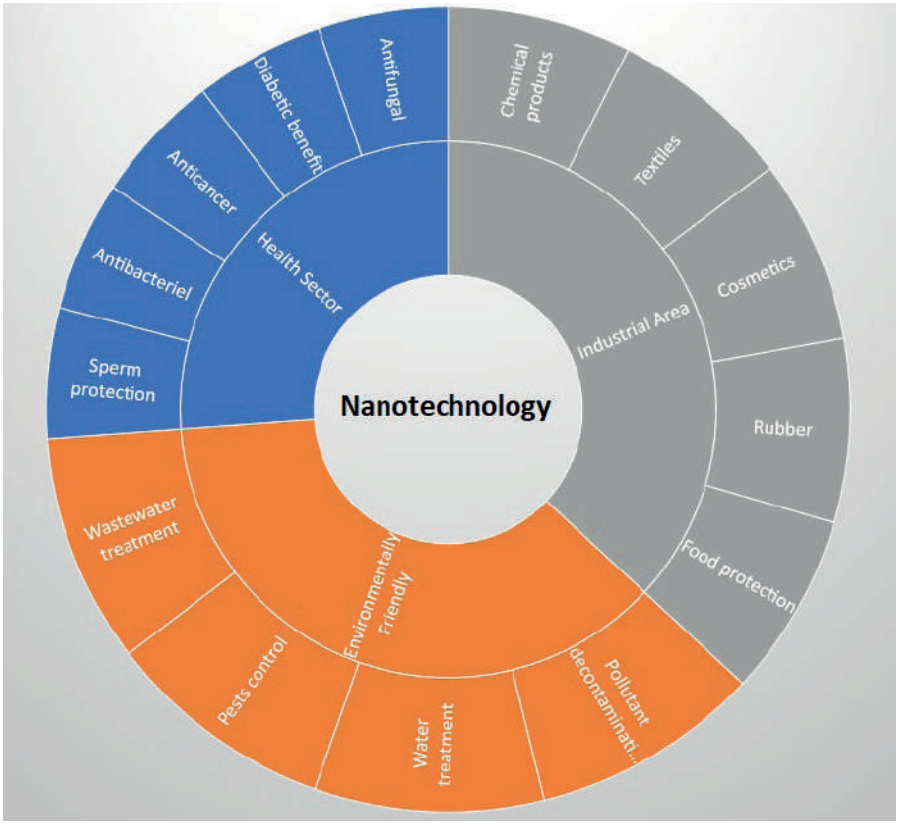


Figure 1. Current usage areas of Nanotechnology

With the rapid advancement of nanobiotechnology, nanoparticles, both physically, chemically, and biologically, are now increasingly present in the natural environment. According to recent research, it has been reported that nanoparticles can penetrate cells and lead to an increase in reactive oxygen species (ROS). Consequently, this may enhance intracellular oxidative stress, potentially disrupting biological structures and normal cellular functions (Nel et al., 2006). In some studies, the toxic effect of nanoparticles has been described to induce apoptosis or apoptosis-related tissue inflammation and perturb cellular redox status (Foldbjerg et al., 2009; Ahamed et al., 2010; Li et al., 2010). Finally, researchers have reported some nanoparticles to cross the blood-testis, placental, and blood-brain barriers, accumulating in different cells (Lan and Yang, 2012; Baghirov et al., 2016; Muoth et al., 2016).

In this section, the dose of nanoparticles administered to the living body through oral, parenteral, and inhalation routes, as well as their effects on fertility, will be discussed.

2. Use of Nanotechnology in Infertility Treatment

Approximately 15% of married couples worldwide experience infertility issues. Half of these infertility problems stem from males and the other half from females (Tahmasbpour et al., 2014). In pets, precise information about infertility rates has not been provided. We believe that urgent research should be conducted in this regard. Infertility problems, which are half caused by males, can arise either congenitally or due to environmental factors later in life.

The initial empirical studies have investigated the presence of the toxic effects of nanoparticles due to their small size and wide distribution. Research has been conducted in the veterinary field on reproduction and fertility, examining nanotoxicity effects (Jha et al., 2014; Falchi et al., 2018). Some researchers have reported that their studies in the field of reproductive biotechnology have preserved male fertility and sperm motility (Feugang et al., 2012; Odhiambo et al., 2014; Feugang et al., 2015; Falchi et al 2016; Durfey et al., 2017). One of the common causes of male infertility is asthenospermia. Astenospermia refers to an infertility problem where the motility (forward movement) of spermatozoa is below normal values (Nowicka-Bauer and Nixon, 2020). Progesterone hormone is commonly preferred in the treatment of asthenospermia. This hormone binds to mitochondrial membrane receptors, increasing the mitochondrial membrane potential, and assisting spermatozoa in gaining motility (Tantibhedhyangkul et al., 2014). The researchers focusing on this information obtained semen samples from 20 cases of asthenospermia. For the treatment, they used solid lipid nanoparticles to deliver the progesterone hormone. As a result, it has been shown to increase acrosomal reaction, sperm capacitation, and motility, as well as enhance the expression of protein kinase A, protein tyrosine kinase, P38MAPK, and SPACA1 genes in intracellular signaling pathways. It appears that progesterone-loaded solid lipid nanoparticles can be considered a significant and potent factor in sperm capacitation and acrosome reaction (Baranizadeh et al, 2022). Fifteen semen samples from asthenospermia cases were pooled and divided into 10 equal parts. They were then incubated with nanoliposomes containing testosterone, catalase, resveratrol, and resveratrol-catalase for 45 minutes. Before and after freezing, all spermatological parameters, sperm DNA, and gene expression were evaluated. They reported that nanoliposomes facilitated the delivery of antioxidants and testosterone into the cells. The nanoliposomes were found to enhance the effectiveness of antioxidants, leading to improvements in spermatological parameters and DNA integrity before and after freezing (Mohammadzadeh et al., 2021).

The researchers observed that sperm obtained from bulls could interact with polyvinyl alcohol-coated iron oxide nanoparticles after a 2-hour incubation. Following the investigations, they reported that the motility of spermatozoa improved, and their fertilization potential was preserved (Ben-David Makhluf et al., 2006). In another study conducted in humans and fish, conjugated iron oxide nanoparticles, utilized with a magnetic-activated cell sorting technique, facilitated increased usage for molecular-based targeting and removal (Gil et al., 2013; Valcarce et al., 2016). However, the spermatozoa capacity used in the study remains limited to 10^9 (Miltenyi et al., 1990; Feugang et al., 2019). In a similar study, researchers used iron oxide nanoparticles coated with a specific lectin or annexin-V to detect acrosome-reacted or abnormal spermatozoa in bull and pig sperm samples. Through this study, spermatozoa with impaired fertilization ability were eliminated. Spermatozoa with plasma membrane damage and mitochondrial membrane damage, exposed to oxidative stress, remained at low levels. Healthy offspring were obtained from mothers by performing simple and safe nanopurification, allowing for a normal gestation period (Odhiambo et al., 2014; Sutovsky, 2015; Durfey et al.,2017; Durfey et al.,2019).

The sperm cells obtained from broiler breed roosters can be damaged during cryopreservation. They have added soy lecithin in nanoparticle form (nano-SL) to the diluent at different ratios to enable minimal damage to germ cells during cryopreservation. The sperm samples, which underwent a freezing process, were examined after thawing. When the sperm parameters were checked, it was determined that the motility and viability rates of the sperm samples containing 1% nano-SL were higher compared to the other groups. In addition, the obtained sperm samples were used for artificial insemination in chickens to examine the hatchability rate. After this process, it was also determined that the sperm samples containing 1% nano-SL had the highest fertility rate (Sun et al., 2021). Another study on a nanoparticle containing lecithin was conducted in goat. The utilization of a Tris-based diluent containing 2% nano-SL for cryopreservation of goat sperm positively impacted the sperm parameters and motility rates compared to the other groups. They have also reported positive results in the sperm used for in vitro fertilization. (Nadri et al., 2019).

Afifi et al. (2015) investigated sperm parameters, oxidative stress, and testosterone levels in rats with experimentally induced Diabetes mellitus. For the treatment, they used zinc oxide (ZnONP) in nanoparticle form or a combination of ZnONPs with insulin. The results indicated a decrease in sperm motility and an increase in the presence of abnormal spermatozoa in rats with diabetes mellitus. They found that only ZnONPs or ZnONPs

given in combination with insulin positively responded to the treatment, leading to an increase in sperm count and motility rate in the sperm cells. When interpreting these findings, it has been demonstrated that ZnONPs mitigate the harmful effects caused by diabetes.

3. Conclusion

In light of this information, we would like to report that nano-based particles, when administered in appropriate doses, yield positive responses in infertility. It would be accurate to say that there is a positive correlation in studies on fertility when using nanoparticles in sperm obtained from living organisms. In addition to the ease and effectiveness of the procedures, the easy integration of these processes into sperm cryopreservation protocols is of significant importance. Furthermore, we observe the need for an increase in studies on the female reproductive system, particularly in the areas of in vivo and in vitro fertilization (embryo production) processes. Furthermore, we observe the need for an increase in studies on the Due to their low costs and easy availability, we believe that nanoparticles should be further explored and utilized in more studies by experts. While initial studies in the reproductive field reported toxic effects, recent research has disproved this hypothesis, indicating that nanoparticles are not toxic in this context. However, it is not sufficient to state that there are still reliable results scientifically. Indeed, continuous and diligent research will be crucial in obtaining evidence-based information and revealing the truth.

References

Afifi, M., Almaghrabi, O. A., & Kadasa, N. M. (2015). Ameliorative effect of zinc oxide nanoparticles on antioxidants and sperm characteristics in streptozotocin-induced diabetic rat testes. BioMed Research International, 2015.

Ahamed, M., Posgai, R., Gorey, T. J., Nielsen, M., Hussain, S. M., & Rowe, J. J. (2010). Silver nanoparticles induced heat shock protein 70, oxidative stress and apoptosis in Drosophila melanogaster. Toxicology and applied pharmacology, 242(3), 263-269.

Baghirov, H., Karaman, D., Viitala, T., Duchanoy, A., Lou, Y. R., Mamaeva, V., ... & Rosenholm, J. M. (2016). Feasibility study of the permeability and uptake of mesoporous silica nanoparticles across the blood-brain barrier. PLoS One, 11(8), e0160705.

Baranizadeh, K., Mahboobian, M. M., Amiri, I., Tavilani, H., & Shafiee, G. (2022). Effects of progesterone nanoparticles on the sperm capacitation and acrosome reaction in asthenozoospermia men. Andrologia, 54(1), e14258.

Barkalina, N., Jones, C., Kashir, J., Coote, S., Huang, X., Morrison, R., ... & Coward, K. (2014). Effects of mesoporous silica nanoparticles upon the function of mammalian sperm in vitro. Nanomedicine: Nanotechnology, Biology and Medicine, 10(4), 859-870.

Ben-David Makhluf, S., Qasem, R., Rubinstein, S., Gedanken, A., & Breitbart, H. (2006). Loading magnetic nanoparticles into sperm cells does not affect their functionality. Langmuir, 22(23), 9480-9482.

Durfey, C. L., Burnett, D. D., Liao, S. F., Steadman, C. S., Crenshaw, M. A., Clemente, H. J., ... & Feugang, J. M. (2017). Nanotechnology-based selection of boar spermatozoa: growth development and health assessments of produced offspring. Livestock science, 205, 137-142.

Durfey, C. L., Burnett, D. D., Liao, S. F., Steadman, C. S., Crenshaw, M. A., Clemente, H. J., ... & Feugang, J. M. (2017). Nanotechnology-based selection of boar spermatozoa: growth development and health assessments of produced offspring. Livestock science, 205, 137-142.

Durfey, C. L., Swistek, S. E., Liao, S. F., Crenshaw, M. A., Clemente, H. J., Thirumalai, R. V., ... & Feugang, J. M. (2019). Nanotechnology-based approach for safer enrichment of semen with best spermatozoa. Journal of animal science and biotechnology, 10(1), 1-12.

Falchi, L., Bogliolo, L., Galleri, G., Ariu, F., Zedda, M. T., Pinna, A., ... & Ledda, S. (2016). Cerium dioxide nanoparticles did not alter the functional and morphologic characteristics of ram sperm during short-term exposure. Theriogenology, 85(7), 1274-1281.

Falchi, L., Khalil, W. A., Hassan, M., & Marei, W. F. (2018). Perspectives of nanotechnology in male fertility and sperm function. International Journal of Veterinary Science and Medicine, 6(2), 265-269.

Feugang, J. M. (2017). Novel agents for sperm purification, sorting, and imaging. Molecular Reproduction and Development, 84(9), 832-841.

Feugang, J. M., Rhoads, C. E., Mustapha, P. A., Tardif, S., Parrish, J. J., Willard, S. T., & Ryan, P. L. (2019). Treatment of boar sperm with nanoparticles for improved fertility. Theriogenology, 137, 75-81.

Feugang, J. M., Youngblood, R. C., Greene, J. M., Fahad, A. S., Monroe, W. A., Willard, S. T., & Ryan, P. L. (2012). Application of quantum dot nanoparticles for potential non-invasive bio-imaging of mammalian spermatozoa. Journal of Nanobiotechnology, 10, 1-8.

Feugang, J., Liao, S., Crenshaw, M., Clemente, H., Willard, S., & Ryan, P. (2015). Lectin-functionalized magnetic iron oxide nanoparticles for reproductive improvement. JFIV Reprod Med Genet, 3(145), 17-19.

Foldbjerg, R., Olesen, P., Hougaard, M., Dang, D. A., Hoffmann, H. J., & Autrup, H. (2009). PVP-coated silver nanoparticles and silver ions induce reactive oxygen species, apoptosis and necrosis in THP-1 monocytes. Toxicology letters, 190(2), 156-162.

Gil, M., Sar-Shalom, V., Melendez Sivira, Y., Carreras, R., & Checa, M. A. (2013). Sperm selection using magnetic activated cell sorting (MACS) in assisted reproduction: a systematic review and meta-analysis. Journal of assisted reproduction and genetics, 30, 479-485.

Hong, F., Yu, X., Wu, N., & Zhang, Y. Q. (2017). Progress of in vivo studies on the systemic toxicities induced by titanium dioxide nanoparticles. Toxicology research, 6(2), 115-133.

Hong, X., Shao, N., Yin, L., Li, C., Tao, G., Sun, Y., ... & Zhou, Z. (2022). Exposure to zinc oxide nanoparticles affects testicular structure, reproductive development and spermatogenesis in parental and offspring male rats. Annals of Translational Medicine, 10(13).

Iftikhar, M., Noureen, A., Uzair, M., Jabeen, F., Abdel Daim, M., & Cappello, T. (2021). Perspectives of nanoparticles in male infertility: evidence for induced abnormalities in sperm production. International Journal of Environmental Research and Public Health, 18(4), 1758.

Jain, S., Park, S. B., Pillai, S. R., Ryan, P. L., Willard, S. T., & Feugang, J. M. (2018). Applications of fluorescent quantum dots for reproductive medicine and disease detection. Unraveling the Safety Profile of Nanoscale Particles and Materials—From Biomedical to Environmental Applications.

Jha, R. K., Jha, P. K., Chaudhury, K., Rana, S. V., & Guha, S. K. (2014). An emerging interface between life science and nanotechnology: present status and prospects of reproductive healthcare aided by nano-biotechnology. Nano reviews, 5(1), 22762.

Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. Arabian journal of chemistry, 12(7), 908-931.

Klein, J. P., Mery, L., Boudard, D., Ravel, C., Cottier, M., & Bitounis, D. (2022). Impact of Nanoparticles on Male Fertility: What Do We Really Know? A Systematic Review. International Journal of Molecular Sciences, 24(1), 576.

Kwon, J. T., Hwang, S. K., Jin, H., Kim, D. S., Minai-Tehrani, A., Yoon, H. J., ... & Cho, M. H. (2008). Body distribution of inhaled fluorescent magnetic nanoparticles in the mice. Journal of occupational health, 50(1), 1-6.

Lan, Z., & Yang, W. X. (2012). Nanoparticles and spermatogenesis: how do nanoparticles affect spermatogenesis and penetrate the blood–testis barrier. Nanomedicine, 7(4), 579-596.

Li, P. W., Kuo, T. H., Chang, J. H., Yeh, J. M., & Chan, W. H. (2010). Induction of cytotoxicity and apoptosis in mouse blastocysts by silver nanoparticles. Toxicology letters, 197(2), 82-87.

Miltenyi, S., Müller, W., Weichel, W., & Radbruch, A. (1990). High gradient magnetic cell separation with MACS. Cytometry: The Journal of the International Society for Analytical Cytology, 11(2), 231-238.

Mohammadzadeh, M., Hamishehkar, H., Vatanparast, M., Akhavan Sales, Z., Nabi, A., Mazaheri, F., ... & Talebi, A. R. (2021). The effect of testosterone and antioxidants nanoliposomes on gene expressions and sperm parameters in asthenospermic individuals. Drug Development and Industrial Pharmacy, 47(11), 1733-1743.

Muoth, C., Aengenheister, L., Kucki, M., Wick, P., & Buerki-Thurnherr, T. (2016). Nanoparticle transport across the placental barrier: pushing the field forward!. Nanomedicine, 11(8), 941-957.

Nadri, T., Towhidi, A., Zeinoaldini, S., Martínez-Pastor, F., Mousavi, M., Noei, R., ... & Sangcheshmeh, A. M. (2019). Lecithin nanoparticles enhance the cryosurvival of caprine sperm. Theriogenology, 133, 38-44.

Nazar, M., Talebi, A. R., Sharifabad, M. H., Abbasi, A., Khoradmehr, A., & Danafar, A. H. (2016). Acute and chronic effects of gold nanoparticles on sperm parameters and chromatin structure in Mice. International Journal of Reproductive BioMedicine, 14(10), 637.

Nel, A., Xia, T., Madler, L., & Li, N. (2006). Toxic potential of materials at the nanolevel. science, 311(5761), 622-627.

Nowicka-Bauer, K., & Nixon, B. (2020). Molecular changes induced by oxidative stress that impair human sperm motility. Antioxidants, 9(2), 134.

Odhiambo, J. F., DeJarnette, J. M., Geary, T. W., Kennedy, C. E., Suarez, S. S., Sutovsky, M., & Sutovsky, P. (2014). Increased conception rates in beef

cattle inseminated with nanopurified bull semen. Biology of reproduction, 91(4), 97-1.

Odhiambo, J. F., DeJarnette, J. M., Geary, T. W., Kennedy, C. E., Suarez, S. S., Sutovsky, M., & Sutovsky, P. (2014). Increased conception rates in beef cattle inseminated with nanopurified bull semen. Biology of reproduction, 91(4), 97-1.

Öztürk, A. L. İ., & Ömür, A. (2022). Curent Approaches to the Use of Nanotechnology in Reproductive Biotechnologies: Spermatological Researches.

Rath, D., Tiedemann, D., Gamrad, L., Johnson, L. A., Klein, S., Kues, W., ... & Barcikowski, S. (2015). Sex-sorted boar sperm–an update on related production methods. Reproduction in domestic animals, 50, 56-60.

Remião, M. H., Segatto, N. V., Pohlmann, A., Guterres, S. S., Seixas, F. K., & Collares, T. (2018). The potential of nanotechnology in medically assisted reproduction. Frontiers in Pharmacology, 8, 994.

Sun, L., He, M., Wu, C., Zhang, S., Dai, J., & Zhang, D. (2021). Beneficial influence of soybean lecithin nanoparticles on rooster frozen–thawed semen quality and fertility. Animals, 11(6), 1769.

Sutovsky, P. (2015). New approaches to boar semen evaluation, processing and improvement. Reproduction in domestic animals, 50, 11-19.

Tahmasbpour, E., Balasubramanian, D., & Agarwal, A. (2014). A multi-faceted approach to understanding male infertility: gene mutations, molecular defects and assisted reproductive techniques (ART). Journal of assisted reproduction and genetics, 31, 1115-1137.

Tantibhedhyangkul, J., Hawkins, K. C., Dai, Q., Mu, K., Dunn, C. N., Miller, S. E., & Price, T. M. (2014). Expression of a mitochondrial progesterone receptor in human spermatozoa correlates with a progestin-dependent increase in mitochondrial membrane potential. Andrology, 2(6), 875-883.

Tiwari, J. N., Tiwari, R. N., & Kim, K. S. (2012). Zero-dimensional, one-dimensional, two-dimensional and three-dimensional nanostructured materials for advanced electrochemical energy devices. Progress in Materials Science, 57(4), 724-803.

Valcarce, D. G., Herráez, M. P., Chereguini, O., Rodríguez, C., & Robles, V. (2016). Selection of nonapoptotic sperm by magnetic-activated cell sorting in Senegalese sole (Solea senegalensis). Theriogenology, 86(5), 1195-1202.

Vance, M. E., Kuiken, T., Vejerano, E. P., McGinnis, S. P., Hochella Jr, M. F., Rejeski, D., & Hull, M. S. (2015). Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. Beilstein journal of nanotechnology, 6(1), 1769-1780.

Nanotechnology in Reproduction

Editor: Dr. Ali Erdem Öztürk

ÖZGÜR YAYINLARI

ISBN 978-975-447-692-7