Implication of nano and microplastics in reproduction: understanding oocyte vulnerability

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ABSTRACT

The widespread environmental dispersion of nano- and microplastics (NMPs) has sparked serious concerns about their potential impact on human and animal health. NMPs are the result of plastics breaking down due to various chemical, physical, and biological processes. Numerous studies have identified and confirmed the harmful effects of NMPs on the female reproductive system. Specifically, NMPs trigger apoptosis and pyroptosis in granulosa cells by activating the NLRP3/caspase pathway and disrupting the Wnt signaling pathway. These processes contribute to uterine fibrosis and reduce the number of ovarian follicles. Additionally, some research suggests that NMPs may impair oocyte maturation, reduce oocyte quality, and compromise overall ovarian function. This review aims to synthesize the currently limited knowledge on the effects of NMPs on granulosa cells and oocytes.

KEYWORDS

Nano-microplastics (NMPs), oocyte, granulosa cells, oxidative stress, inflammation, apoptosis.

Introduction

The widespread release of nano- and microplastics (NMPs) into the environment is raising serious concerns about their potential effects on human and animal health. NMPs are formed when plastics degrade into particles smaller than 5 micrometers and 100 nanometers, respectively micro- and nanoplastics [1], under various chemical, physical, and biological conditions. The pervasive presence of NMPs raises serious concerns about their potential impacts on human health, as these particles are extremely challenging to remove from the environment [2,3].

Research has shown that NMPs can cross biological barriers, such as the placenta [4], and induce toxic effects in various organs and tissues. Numerous studies indicate that NMPs can adversely affect oocytes and granulosa cells (GCs) through mechanisms involving oxidative stress and endocrine disruption, ultimately impairing fertility and development. In females, NMPs have been shown to accumulate in the ovaries, thereby reducing follicle count and ovarian size, with dramatic consequences on fertility in terms of reduced embryo production and pregnancy rates.

Exposure to NMPs tends to have more pronounced reproductive effects in females than in males [5]. This raises serious concerns for future generations, as transgenerational effects in offspring have also been observed [6]. Despite these findings, the underlying mechanisms of NMP toxicity in oocytes and GCs, particularly in mammals, remain poorly understood. The present work examines the mechanisms underlying NMP toxicity in the ovarian microenvironment and its long-term implications.

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Search methods

The PubMed database was examined for peer-reviewed original articles. The following keywords were searched: 'Nanoplastics, 'Microplastics', 'Human granulosa cells', 'Oocytes', 'Fertility', 'mammals', and 'oxidative stress'. These keywords were combined with other search phrases relevant to the topic.

The effects of NMPs on oocytes

Research on the impact of NMPs on the mammalian female reproductive system is limited, with the majority of studies focusing primarily on rodent models such as mice and rats. NMPs have been found to accumulate in mouse ovary, uterus, and blood [7-9].

Exposure to NMPs has been linked to microstructural abnormalities in the mammalian female reproductive system, including dilated oviducts, an increase in corpus luteum and ovarian cysts, a thinner granular layer of secondary follicles, and a decrease in the number of developing follicles [7,10-12].

Rat ovarian GCs are permeable to bigger NMPs, which

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causes an increase in fibrosis markers [7] and a marked drop in serum levels of anti-Müllerian hormone and E2 expression. Moreover, follicle growth appeared to be slowed and abnormalities in the estrous cycle have been reported. After exposure to NMPs, mice showed dilated oviducts, ovarian cysts, a higher number of corpora lutea, a thinner granulosa layer in secondary follicles, fewer growing follicles, increased ovarian collagen and fibronectin accumulation, and GC apoptosis [5]. MNPs were also found to influence oocyte maturation and function, leading to reduced fertility potential [13]. Finally, NMPs deeply affected the abundance of cytoskeletal proteins in rat ovaries, e.g. α-microtubulin, which plays a key role in oocyte-GC synchronization [8].

An important implication of NMPs is related to the observation that they can also be assumed indirectly through the diet. Indeed, it has been demonstrated that consumption of seafood containing NMPs [14] may negatively impact reproduction, food intake, and the endocrine system [15]. In 2019, Cong *et al*. found decreased oocyte production among the many consequences of 120 days of exposure to 10-μm polystyrene microspheres in *Oryzias melastigma* [16]. Studies in *Oryzias latipes* [17] and oysters [18] have similarly revealed a decline in the quantity and caliber of eggs and oocytes. NMPs have also been shown to induce oxidative stress and damage to DNA. They also reduced the growth of human extravillous trophoblastic cells, triggered apoptosis, and produced cell cycle arrest at the G2/M phase [19].

NMP exposure induces alteration in ROS production and DNA damage in GCs and oocytes

Reactive oxygen species (ROS) are highly reactive oxygen-containing molecules, including hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and superoxide (O_2•–) [20]. These molecules are involved in various biological processes, acting both as mediators of cell signaling and as contributors to oxidative damage in cells and tissues. ROS are typically produced as byproducts of cellular metabolism during oxidative phosphorylation.

It has been observed that NMPs accumulate in organs and induce endocrine and reproductive system dysfunctions, typically by inducing apoptosis and oxidative stress [21-23]. Numerous investigations in rainbow trout (*Oncorhynchus mykiss*) and zebrafish have demonstrated that NMPs raise oxidative stress in the gonads, with detrimental effects on the reproductive system [24-26]. When mouse oocytes were exposed to NMPs, a decrease in calcium and reduced glutathione abundance in the endoplasmic reticulum were reported in association with an increase in ROS [9].

Furthermore, it was noted that antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD) were less active [8] after NMP exposure. In mouse ovaries, NMPs induced oxidative stress, an inflammatory response demonstrated by increased levels of IL-6, IL-10, and TNF-α, which leads to decreased egg quality, in turn demonstrated by a reduced number of ovulated oocytes showing the first polar body and a decline in oocyte survival [9] (Figure 1).

ROS signaling is important for female reproductive functions, affecting oocyte quality and early embryo development in particular [3]. Considering that exposure to NMPs leads to an increase in ROS content at systemic level [27], NMP detrimental effects are also related to the imbalance in redox homeostasis in the reproductive system [9]. ROS increase can cause changes in the expression levels of various proteins and inflammatory factors, which in turn activate related signaling pathways, ultimately leading to ovarian dysfunction and a decrease in oocyte quality. Indeed, increased ROS production, as well as histological abnormalities, e.g. the presence of micronuclei in the oocyte nucleus and cytoplasmic vacuolization in somatic and germinal cells, has been described in ovaries exposed to NMPs [17,28]. Accordingly, GCs isolated from rats exposed to NMPs were characterized by reduced activity of the antioxidant enzymes, that is SOD, CAT, and GPx (glutathione peroxidase), which may be considered a consequence of ROS accumulation [29].

Grechi *et al*. observed that bovine oocytes exposed to NMPs during *in vitro* maturation were characterized by a reduced IVM rate and presented damage in the zona pellucida [30]. Applying proteome analysis to examine oocytes exposed to NMPs, these authors found an upregulation in the expression of proteins implicated in the oxidative stress response, including TXN, GPX1, ATP5IF1, PADI6, and SNRPD2 [30], and the up-regulation of proteins crucial for spindle formation, nuclear maturation, and the arrangement of microtubules and organelles. This evidence suggests that NMP exposure induces oxidative stress and DNA damage during oocyte growth and maturation *in vitro*.

WNT pathway activation caused by NMPs induces fibrosis

In ovaries of mice exposed to NMPs, the establishment of a condition of oxidative stress induces the expression of proteins involved in the Wnt/β-Catenin signaling pathway and of fibrosis markers [7]. The Wnt/β-Catenin signaling pathway controls the processes of embryonic development, cell division, and apoptosis — all vital processes for maintaining tissue and organ homeostasis. Recent literature has demonstrated that NMPs induce ovarian fibrosis through the Wnt/β-Catenin signaling pathway, which in turn increases the expression of fibrotic markers, namely, transforming growth factor-β (TGF-β), fibronectin, and α-smooth muscle actin, or α-SMA, in ovarian GCs [7]. Activation of the Wnt/β-Catenin signaling pathway has been also associated with a variety of morphological and functional alterations in the reproductive system, including lower oocyte counts, impaired follicular growth, GC mortality, decreased ovarian reserve function, and ovarian and uterine fibrosis (Figure 1).

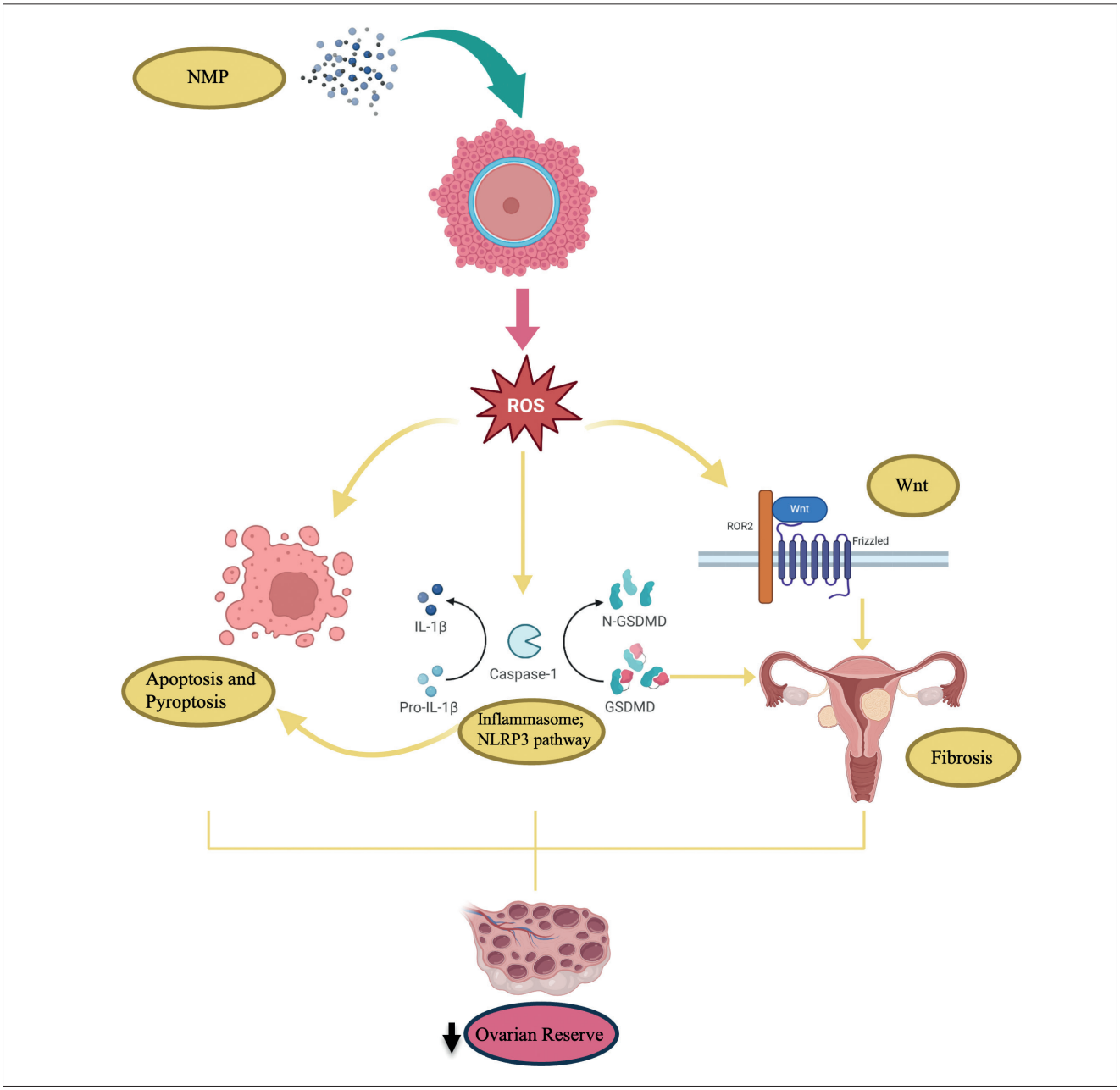
Moreover, NMPs cause uterine fibrosis through TLR4 and Notch signaling pathway activation. When these pathways are activated, more uterine fibrotic collagen and proteins are produced, which results in the creation of ROS [31]. Furthermore, NMPs increase the expression of HMGB1 and acetyl-HMGB1, which stimulates the TLR4/NOX2 receptors and encourages oxidative stress [31,32]. These results are corroborated by Wu *et al*.,

who showed that ROS-induced endometrial thinning and uterine fibrosis in female mice treated with NMPs was mediated by the Notch and TLR4 signaling pathways. Moreover, in these animals, fibrotic tissue development was facilitated by TGF-β [31].

NMP exposure induces apoptosis and pyroptosis in human granulosa cells and oocytes

Ovaries of mice exposed to NMPs are also characterized by increased expression of pro-apoptotic proteins in concomitance with lower levels of anti-apoptotic factors [7]. In this context, the multiprotein complex NOD-like receptor thermal protein domain-associated protein 3 (NLRP3) is implicated in apoptosis and inflammation and acts as a defense mechanism against endogenous damage, microbial invasion, and toxic stimuli. Recent literature has demonstrated that in GCs from mice exposed to NMPs, a condition of oxidative stress is responsible for activation of not only elements of Wnt/β-Catenin signaling [7], but also the NLRP3/Caspase-1 [10] pathway [33], and these changes have been described as the possible mechanism underlying the decline of ovarian reserve. Moreover, ovarian dysfunction and GC apoptosis [7,10] seem to be related to a rise in the apoptosis-associated speck-like protein [34], which is part of the NLRP3/caspase-1 signaling pathway and has been found to

Figure 1 NMPs enter the oocytes and cause an increase in ROS. An excessive increase in ROS leads to cell death, activation of the NLRP3 pathway, which activates the inflammasome and induces cell death by pyroptosis, and activation of the Wnt pathway. The Wnt/NLRP3 pathways cause fibrosis in the female reproductive system. All these pathways lead to a decrease in the ovarian reserve.



increase upon oxidative stress and NMP exposure [10].

Zhang *et al*. found that injection of NMPs lowered oocyte quality and hindered oocyte maturation by increasing ROS levels and inducing apoptosis [13]. Another study revealed that after 28 days of exposure, NMPs induced apoptosis and damage to DNA in the germ cells of *C. elegans*. Furthermore, it was noted [13,35] that there was upregulation of HUS-1 expression as well as pro-apoptotic genes such as *cep-1*, *egl-1*, *p53*, *ced-3*, and *ced-4*, which are implicated in germ line apoptosis [35,36]. Similarly, human GCs exposed to NMPs displayed an increase in apoptosis. Finally, numerous lines of evidence suggest that NMPs activate the inflammasome, which in turn initiates pyroptosis, as a consequence of increased activation of the NLRP3/Caspase-1 signaling pathway [10,37]. Pyroptosis is an alternative program of cell death [38], which occurs during inflammatory processes and causes the flattening of the cytoplasm [39]. The latter is related to the formation of pores in the plasma membrane that allow the passage of IL-1β/IL-18 [40]. Through ion influx, oxidative stress induces cell lysis and releases IL-18, IL-1β, and other inflammatory cytokines [41,42]. Interleukins that are inert, including IL-1β and IL-18, become active when the NLRP3 signaling pathway is activated [33]. In the final event, water enters through the pores, causing cell swelling, osmotic lysis, and the subsequent release of IL-1β and IL-18. While activation of caspase 1 is crucial for the host defense systems because it inhibits pathogen growth and host survival, its overstimulation can lead to pathological inflammation [41,43]. In human GCs, it has been demonstrated that NMP-induced oxidative stress may activate the NLRP3/Caspase-1 signaling pathway [10]. Therefore, it can be speculated that the primary causes of the decline in ovarian reserve are oocyte apoptosis and GC pyroptosis [10] (Figure 1).

Conclusion

NMPs are among the most widespread and dangerous contaminants, with severe toxic effects in animals and humans [44], due to their ability to accumulate in all organs, including the ovaries. Moreover, NMPs play a crucial role in the toxicity to GCs and oocytes, with dramatic consequences on fertility. In particular, the exposure to NMPs causes an increase in ROS generation, apoptosis and pyroptosis in the ovarian microenvironment, which seem to be the causative mechanism underlying diminished ovarian reserve.

However, very few studies have been conducted so far, thus current understanding of the reproductive toxicity associated with NMPs is still limited and requires further investigations.

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