The impact of nanoplastics on mammalian reproductive function: an *in vitro* study with gametes, embryos and placental cells

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Plastic pollution is a growing global environmental challenge. In the environment, plastics break down into massive amounts of microplastics (MPLs, <5 mm) and nanoplastics (NPLs, <1 μm). Their widespread distribution, persistence, and minute size facilitate their uptake by living organisms, raising concerns about their potential harmful effects on ecosystems, wildlife, and human health. Given that reproductive function is highly susceptible to pollutants, exposure to MPLs and NPLs may negatively impact the fertility of current individuals and pose risks to future generations.

To elucidate the impact of NPLs on various mammalian reproductive cellular components, we exposed CD-1 mouse gametes and preimplantation embryos and human placental cells (JEG-3 and BeWo) to polystyrene NPLs *in vitro* (100 nm; 100 μg/ml). We found that NPLs adhered to sperm plasma membranes, leading to a significant reduction in motility and membrane integrity and compromising acrosome reaction. However, no significant increases in oxidative stress or DNA fragmentation were observed. NPLs also attached to the zona pellucida of oocytes and embryos, but their slow internalization prevented adverse effects on oocyte maturation and embryonic development, with oxidative stress levels remaining stable. Notably, rapid NPLs internalization occurred in zona pellucida-free embryos, triggering toxicity characterized by increased oxidative stress, impaired embryonic development, and diminished blastocyst quality. Placental cells rapidly trafficked NPLs to lysosomes. NPLs internalization did not disturb metabolic efficiency, but had a genotoxic effect, resulting in an increased frequency of DNA double-strand breaks and micronuclei in both cell lines.

In conclusion, our results indicate that gametes, embryos and placental cells are vulnerable to NPLs exposure, although the zona pellucida acts as a crucial protective barrier for oocytes and embryos. These findings emphasize the potential threats of plastic pollution to reproductive health and highlight the need for further research into the long-term consequences of MNPLs exposure on fertility and embryo-fetal development.

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