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Microplastics have a negative effect on sperm and oocytes in vitro

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An annual increase in global plastic production, in addition to poor waste management, represents a massive contamination of the environment. These plastics pollutants can break down to small particles, and when fragmented to a size smaller than 5 mm, are called microplastics (MPs). A decline in fertility was already pointed out by WHO as a major health issue and, considering the lack of studies relating MPs and reproduction, for better understanding of its effects, more studies are necessary. Therefore, the present study aimed to investigate if MPs can affect gametes in vitro. Bovine frozen semen (N = 4 bulls) were thawed and submitted to swim-up separation. 5x106 sperms/mL were then incubated with a range of sizes of polystyrene (PS) beads (SURF-CAL™ particle size standards): 0.05, 0.1, 0.3, and 1.1 µm (1.2 million beads/mL), and CellRox (2 µL/mL) using FERT-TALP for 2 hours at 38.5ºC, 5% CO2, 95% O2. Every 30 minutes an aliquot was collected, checked for motility (N=9 replicates), spread in a SuperFrost slide and fixed with 4% paraformaldehyde (PFA). Fixed samples were stained with Hoechst33342 and FITC-PNA, to check acrosome integrity (FITC-PNA, N=4), bead attachment (N=3 replicates) and oxidative stress (CellRox; N=2 replicates) by fluorescence microscopy. Oocytes were isolated from bovine ovaries by follicle aspiration and only those with a homogeneous cytoplasm and at least three layers of cumulus cells were selected. Oocytes were randomly assigned to 3 groups: (1) 0.3 µm PS beads (1.2 million beads/mL; N=46); (2) 1.1 µm PS beads (1.2 million beads/mL; N=64), and (3) control (N=52) no beads, and matured for 24h at 38.5ºC, 5% CO2, 95% O2; three replicates were performed. COCs were then washed, denuded by pipetting, fixed in 4% PFA, and stained with Hoechst33342 (5 µg/mL) for nuclear stage checking. Oocytes were classified as mature (MT), degenerating (DG), or broken zona pellucida (BZP). All data was analyzed for normality using the Shapiro-Wilk test, a two-way ANOVA was used and the differences checked using Tukey HSD. Both 0.3 and 1.1 µm beads attached to the sperm surface in 6.9±4.8 and 2.1±18% of sperm counted, respectively. Even though a small attachment of beads was identified, they did not affect sperm motility (p>0.05). However, sperm incubated with 1.1 µm beads had reduced acrosome integrity at 2h compared to the control (32.3±6.5 vs 59.1±20.5, respectively, p=0.001). The results for oxidative stress indicate a small increase of ROS production on sperm incubated with MPs, but no significant differences were detected and more replicates need to be done. Oocytes matured in the presence of MPs had a reduced maturation rate (67.5±12.5%, 41.9 ± 18.1 and 37.6 ±20.0%, for control, 0.3 and 1.1 µm, respectively). It was also shown that MPs promoted an increase in oocytes with BZP (2.6±4.4%, 17.8 ± 3.4 and 23.4 ±11.4%, for control, 0.3 and 1.1 µm, respectively; p=0.03). Proteomics of oocytes is currently in progress to better understand the molecular mechanisms by which MPs are damaging oocytes. We have shown, for the first time, that PS MPs exert a negative effect on both male and female gametes in vitro, demonstrating that MPs should be treated as concerning environment toxicants.

Keywords: microplastics, infertility, reproduction, polystyrene