Research in Veterinary Science 197 (2025) 105949

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Effect of polystyrene nanoplastics on in vitro maturation of pig cumulus-encosed oocytes

Marcella Spinaci [a,*], Sofia Dindo [a], Nadia Govoni [a], Laura Tovar [a], Alessandro Marino Volsa [b], Cinzia Cappannari [a], Diego Bucci [a], Jose Manuel Ortiz-Rodriguez [a]

[a] Department of Veterinary Medical Sciences (DIMEVET), University of Bologna, Bologna, Italy
[b] Department of Physiology, International Excellence Campus for Higher Education and Research (Campus Mare Nostrum), University of Murcia, Murcia, Spain

ARTICLE INFO

Keywords:
Nanoplastics
Oocyte
Nuclear maturation
Cytoplasmic maturation
Oocyte developmental competence
ROS
Cumulus cells steroidogenesis

ABSTRACT

In recent years, concern has been increasing over the impact of environmental nanoplastics (NPs) contamination on both human and animal health, particularly regarding potential effects on reproductive systems. Nevertheless, current knowledge about the hazards posed by nanoplastics to mammalian gametes remains limited. In this study we evaluated the impact of increasing concentrations of polystyrene nanoplastics (PS-NPs)(5, 50, 100 and 200 μg/mL) on female gamete, using an in vitro model of pig oocyte maturation (IVM). Nuclear maturation, cytoplasmic maturation and developmental competence of oocytes, intracellular oocyte levels of glutathione (GSH) and reactive oxygen species (ROS), as well as steroidogenic activity of cumulus cells (CCs) were evaluated. Exposure to PS-NPs during IVM did not affect CCs steroidogenesis, oocyte nuclear maturation and oocyte cytoplasmic maturation in term of both fertilization parameters after IVF and blastocyst rate after parthenogenic activation. Nevertheless, PS-NPs significantly increased oocyte ROS levels at all the concentrations tested and compromised oocyte developmental competence, as indicated by reduced blastomere number per blastocyst. Our results confirm that PS-NPs may interfere with oocyte maturation and highlights the need to assess NPs exposure as an emerging environmental factor with potential implications for both animal and human fertility.

1. Introduction

Plastic pollution has become an increasingly severe environmental issue due to plastic widespread use, mainly as disposable goods, and inadequate waste management. In the environment, discarded plastics can be slowly fragmented into smaller particles by physical forces, UV-light, temperature changes and biological degradation (Gewert et al., 2015). The resulting breakdown products may be classified, depending on the diameter of plastic fragments, into microplastics (MPs, 5 mm to 100 nm in diameter) and nanoplastics (NPs, diameter smaller than 100 nm) (EFSA, 2016).

A growing body of evidence has demonstrated the harmful health effects of micro- and nanoplastics (MNPs) on living organisms, including humans, through oxidative stress, inflammation, immune dysfunction, alteration of cellular and energy metabolism, tissue degeneration, impaired cell proliferation, and carcinogenicity (Ali et al., 2024).

In recent years, the toxic effects of MNPs exposure on mammal reproductive system have been a cause of growing concern as a potentially serious risk to fertility. However, few studies have been conducted on the effect of these substances on mammalian oocyte, most of which used mice as experimental model (Volsa et al., 2025).

It has been suggested that NPs exposure may pose a greater hazard than MPs due to their possible higher concentration in the environment and to their smaller size which enable them to pass more easly through biological barriers (Gaylarde et al., 2021). NPs have been reported to penetrate granulosa and cumulus cells and to be internalized into oocytes cytoplasm (He et al., 2024; Merlo et al., 2025; Park et al., 2022; Xue et al., 2024; Yang et al., 2022; Zeng et al., 2023). Various disturbing effect have been observed after NPs exposure during oocyte in vitro maturation (IVM) such as impaired meiotic progression and chromosome organization, impaired mitochondrial activity, increased oxidative stress, apoptosis and DNA damage (He et al., 2024; Park et al., 2022; Xue et al., 2024). However, the data obtained are not always consistent and conflicting results have been obtained (Xue et al., 2024; Yang et al., 2022).

The aim of this study was to investigate the impact of PS-NPs on the

* Corresponding author at: Department of Veterinary Medical Sciences, Via Tolara di Sopra, 50, 40064 Ozzano dell'Emilia (Bologna), University of Bologna, Italy.
E-mail address: marcella.spinaci@unibo.it (M. Spinaci).

https://doi.org/10.1016/j.rvsc.2025.105949
Received 1 July 2025; Received in revised form 31 October 2025; Accepted 1 November 2025
Available online 7 November 2025

in vitro maturation of cumulus enclosed oocytes using pig as an animal model, in accordance with the 3Rs principle. The evaluation focused on nuclear maturation, cytoplasmic maturation and developmental competence of oocytes after parthenogenetic activation, oocyte intracellular levels of glutathione (GSH) and reactive oxygen species (ROS), as well as the steroidogenic activity of cumulus cells (CCs).

2. Materials and methods

All the chemicals, unless otherwise specified, were purchased from Sigma-Aldrich (Merck, Italy).

2.1. Oocytes collection, in vitro maturation (IVM) and experimental design

Ovaries of pre-pubertal gilts were collected at a local abattoir and transported to the laboratory within 2 h. Cumulus-oocyte complexes (COCs) were aspirated from follicles 3–6 mm in diameter with an 18-gauge needle fixed to a 10 mL disposable syringe. The oocytes COCs with dark and uniform cytoplasms and several compact cumulus cell layers were washes three times in IVM medium consisting of NCSU 37 (Petters and Wells, 1993) supplemented with 0.57 mM cysteine, 1 mM glutamine, 5 μg/mL insulin, 10 ng/mL epidermal growth factor (EGF), 50 μM β-mercaptoethanol and 10 % porcine follicular fluid. Groups of around 50 COCs were transferred to a Nunc 4-well multidish containing 500 μL of IVM medium per well and cultured at 39 °C in a humidified incubator with an atmosphere of 5 % CO2 in air. For the first 22 h IVM the medium was supplemented with 1.0 mM db-cAMP and 0.12 IU/mL Pluset (Carlier, Italy). Then COCs were incubated for an additional 22 h in the same medium without supplementations (Funahashi et al., 1997).

During the in vitro maturation period (44 h), COCs were exposed to 0 (Control, Ctr), 5, 50, 100, 200 μg/mL of 100 nm Polystyrene Nanoplastics (PS-NPs) (Sigma Aldrich, catalogue N. 43,302). The particles were in aqueous suspension (10 % WT) with a density of 1.05 g/cm^3. The examined concentrations were chosen based on those tested during oocyte IVM in literature (He et al., 2024; Merlo et al., 2025; Park et al., 2022; Xue et al., 2024).

2.2. Evaluation of nuclear maturation

At the end of the maturation period cumulus cells were removed from the oocytes by gentle repeated pipetting and oocytes were mounted on microscope slides, fixed in acetic acid/ ethanol (1:3 *v*/v) for 24 h and then stained with 1 % (*w/v*) Lacmoid. The oocytes were observed under a phase contrast microscope to evaluate the meiotic stage achieved. Oocytes with a nuclear morphology corresponding to metaphase-II stage (MII) were considered mature (Mattioli et al., 1994). A total of 1074 oocytes were examined across five replicates.

2.3. Evaluation of cytoplasmic maturation

At the end of the maturation period cytoplasmic maturation was assessed by evaluating:

a) Insemination parameters and ability of oocytes to sustain male pronucleus formation after in vitro fertilization (IVF).

The oocytes were fertilized with frozen boar semen (Inseme S.P.A., Modena, Italy). Straws were thawed in a water bath at 37 °C under agitation for 30 s and immediately diluted, at the same temperature, in Beltsville Thawing Solution (BTS) at a dilution rate 1:3. After 1 h, semen was washed twice with BTS and finally resuspended in IVF medium which consisted of Brackett and Oliphant (1975) supplemented with 12 % fetal calf serum (Gibco, Invitrogen, Italy) and 0.7 mg/mL caffeine. Forty-five to fifty oocytes, after being denuded above described, were washed twice in IVF medium and transferred to 500 μL of the same medium containing 1.25 × 10^6 sperm/mL. After 1 h of gamete coincubation at 39 °C in a humidified atmosphere of 5 % CO_2 in air, oocytes were transferred to fresh IVF medium previously equilibrated under 5 % CO2 and cultured for 17 h until fixation as above described. After staining with 1 % Lacmoid, the oocytes were observed under a phase contrast microscope and the following parameters were evaluated: penetration rate (number of oocytes penetrated/total inseminated), monospermy rate (number of oocytes containing only one sperm head-male pronucleus/total penetrated) and the ability of oocytes to sustain male pronucleus formation (number of penetrated oocytes with a female and at least one male pronucleus/total penetrated). Degenerated and immature oocytes were not counted. A total of 1009 oocytes were examined across five replicates.

b) Developmental competence of parthenotes after 7 days of in vitro culture.

Oocytes exposed to 0, 50, 100, 200 μg/mL of PS-NPs during IVM were denuded as described above, washed three times in IVF medium and then parthenogenetically activated according to the method described by Boquest et al. (2002) slightly modified (Spinaci et al., 2019) to avoid possible sperm-related effects. Briefly, the oocytes were transferred to IVF medium containing 5 mM ionomycin for 5 min, then washed twice and incubated in NCSU-23 (Petters and Wells, 1993) containing 2 mM 6-dimethylaminopurine (6-DMAP) for 3 h at 39 °C. Presumptive parthenotes were washed twice in NCSU-23 and cultured in groups of around 50 in 500 μL of the same medium. On day 5 post-activation, 250 μL of the medium were replaced with fresh pre-equilibrated NCSU-23 containing 20 % (*v*/v) FCS to reach a final FCS concentration of 10 % (v/v). At Day 2 post-activation, the number of embryos that reached the 2- to 4-cell stage was assessed and the cleavage rate (cleaved embryos/total activated oocytes) was calculated. At Day 7 post-activation, blastocyst rate and number of blastocyst nuclei were determined by fixing and staining parthenotes as above described for oocytes. Embryos with at least 20 blastomeres and a clearly visible blastocoel were considered as blastocysts. A total of 804 activated oocytes were evaluated across four replicates.

2.4. Detection of oocyte GSH and ROS levels

Intracellular GSH and ROS levels of oocytes at the end of IVM in presence of NPs (0, 5, 50, 100, 200 μg/mL) were determined as previously described (Spinaci et al., 2019). From each treatment group, oocytes were incubated in the dark for 30 min at 39 °C in PBS/0.1 % (wt/vol) PVA supplemented with 10 μM CellTracker Blue (4-chloromethyl-6,8-difluoro-7-hydroxycoumarin; CMF2HC; Invitrogen, Italy) or 10 μM H2DCFDA (Invitrogen). Following incubation, the oocytes were washed in PBS/0.1 % (*w/v*) PVA, placed into 10 μL droplets, and fluorescence was evaluated under a Nikon Eclipse E 600 epifluorescence microscope (Nikon Europe BV, Badhoeverdop, The Netherlands). The images of fluorescent oocytes were analysed with Image J software (public domain). Relative oocyte fluorescence was measured by normalizing the oocyte fluorescence with the background and with each oocyte area. Four independent experiments were performed (415 oocytes for GSH analysis and 427 oocytes for ROS analysis).

2.5. Evaluation of cumulus cell steroidogenesis

Spent IVM media of both the first and the second day of COCs culture in presence of different concentrations of PS-NPs (0, 5, 50, 100, 200 μg/mL) were collected, centrifuged at 900g for 5 min and the supernatants were stored at −20 °C until assayed for progesterone (P4) and estradiol-17β (E2) by validated radioimmunoassays (Galeati et al., 2016). At the end of the maturation period, cumulus cells were removed from oocytes by gentle repeated pipetting and counted using a Thoma's hemocytometer. For P4, the intra-coefficient of variation was 3.4 % and assay

sensitivity was 5.09 pg/tube. The intra-coefficient of variation for E2 was 5.4 % and assay sensitivity was 0.41 pg/tube. Steroid concentrations are expressed as ng/10^6 cells.

2.6. Statistical analyses

Statistical analyses were performed using R (version 4.2.2). Values are expressed as mean ± standard deviation (SD) and level of significance was at $p < 0.05$.

Data on nuclear maturation, IVF trials, blastocyst formation and cumulus cell steroidogenesis were analysed using a general linear model with binomial distribution and a Tukey post-hoc test was subsequently run to determine differences between treatments. Data on blastomere number were analysed using a Poisson distribution and a Tukey post-hoc test was subsequently run to determine differences between treatments. Data on GSH and ROS intracellular levels, after being tested for normality and homogeneity of variances through Shapiro-Wilk test and Levene tests, were analysed using Non-parametric Kruskal-Wallis Test and Wilcoxon test was subsequently used to assess differences between treatments.

3. Results

3.1. Effect of PS-NPs on nuclear and cytoplasmic maturation

The percentage of oocytes reaching the metaphase II (MII) stage at the end of IVM was similar ($P \geq 0.05$) between the experimental groups (0, 5, 50, 100, 200 μg/mL PS-NPs)(Table 1).

After IVF of oocytes exposed to PS-NPs during IVM, no significant differences in the percentages of penetrated oocyte, monospermic oocytes and penetrated oocytes with at least one male pronucleus were recorded (Table 2).

The addition of PS-NPs to IVM medium at all the concentrations tested (50, 100, 200 μg/mL) did not influence, after parthenogenic activation, the cleavage rate and the percentage of oocytes that developed to blastocyst stage (Fig. 1 left panel). However, a dose dependent significant decrease in the mean number of blastomere per blastocyst was recorded (Mean ± SD for 0, 50, 100 and 200 μg/mL PS-NPs: 56.8 ± 21.3, 48.6 ± 16.8, 47.4 ± 18.7, 45.0 ± 17.3; respectively) ($p < 0.05$) (Fig. 1 right panel).

3.2. Effect of PS-NPs on GSH and ROS levels

The oocyte GSH levels were not significantly affected by the exposure to PS-NPs at any of the concentrations tested during IVM (Fig. 2, left panel).

Nevertheless, the presence of PS-NPs during IVM significantly increased intracellular ROS levels in exposed oocytes ($p < 0.01$) (Mean ± SD for 0, 5, 50, 100 and 200 μg/mL PS-NPs: 100 ± 33.1, 115.9 ± 38.3, 118.8 ± 35.2, 128.1 ± 39.2, 128.5 ± 39.0 a.u, respectively) (Fig. 2, right panel).

3.3. Effect of PS-NPs on cumulus cell steroidogenesis

Cumulus cell steroidogenesis was assayed by quantification of E2 and P4 in spent IVM media of both the first and the second day of culture in presence of different concentrations of PS-NPs (0, 5, 50, 100, 200 μg/mL). Steroid production by COCs is shown in Fig. 3.

None of the PS-NPs concentrations tested influenced the production of E2 and P4 by cumulus cells both on the first and the second day of culture (Fig. 3). However, it is worth noting that P4 production was significantly increased ($P < 0.001$) at 44 h of culture compared to 22 h, irrespective of PS-NP presence (Fig. 3, right panel).

Table 1
Effect of different concentrations of PS-NPs on the percentage of oocytes in metaphase II at the end of IVM period. Data represent the mean ± SD of five replicates repeated in different experiments.

	PS-NPs (μg/mL)				
	0	5	50	100	200
MII (%)	93.2 ± 4.3	92.0 ± 1.6	91.9 ± 4.5	93.9 ± 2.5	91.8 ± 3.5
Oocytes (n°)	213	228	216	214	203

Table 2
Effect of the addition of increasing concentrations of PS-NPs during IVM on insemination parameters after IVF with frozen-thawed semen. Penetration rate: number of oocytes penetrated/total inseminated; Monospermy rate: number of oocytes containing only one sperm head-male pronucleus/total penetrated; Male pronucleus formation: number of penetrated oocytes with a female and at least one male pronucleus/total penetrated. Data represent the mean ± SD of five replicates repeated in different experiments.

	PS-NPs (μg/mL)				
	0	5	50	100	200
Penetration rate	49.2 ± 12.5	48.8 ± 19.8	49.7 ± 14.5	47.1 ± 11.8	49.5 ± 7.8
Monospermy rate	75.4 ± 7.0	80.4 ± 15.6	84.1 ± 10.1	80.6 ± 19.5	84.6 ± 8.6
Male pronuclear formation	97.3 ± 4.0	97.8 ± 5.0	97.1 ± 6.4	98.3 ± 3.7	98.9 ± 2.4
Oocytes (n°)	195	212	201	205	196

4. Discussion

In recent years, growing concern has emerged about the potential harmful effects of NPs on mammalian reproductive processes, particularly regarding their impact on gametes. This study aimed to investigate the impact of PS-NPs exposure on female gamete utilizing an in vitro porcine oocyte maturation model, suggested as a reliable model for human oocytes (Santos et al., 2014).

As an initial step of this study, we examined the effect of 100 nm PS-NPs exposure during IVM on nuclear maturation of porcine oocytes under our culture condition. PS-NPs addition, at all the concentrations tested (5, 50, 100, 200 μg/mL) did not affect the percentage of oocytes reaching MII stage compared to control group. Similarly, no impact on oocyte meiosis was recorded in bovine after IVM in presence of 50 nm PS-NPs (10 μg/ml) (Yang et al., 2022) and in mice oocytes cultured with 25 nm PS-NPs at different concentrations (50, 100, 200 μg/mL) (Xue et al., 2024). However, our results are in contrast with a recent study in which the presence of 100 nm PS-NPs during pig IVM negatively influenced oocyte nuclear maturation, starting from 50 μg/mL concentration, inducing a reduction of the percentage of polar body extrusion associated with spindle abnormality, misaligned chromosomes and DNA damage (He et al., 2024). Similar negative effects on oocyte nuclear maturation have also been reported both in mice when IVM was performed in presence of 100 μg/mL PS-NPs (diameter 50 nm) (Park et al., 2022) and in bovine oocytes exposed to either 100 μg/mL PS-NPs (diameter 100 nm) or 70 μg/mL of smaller PS-NPs (30 nm) (Merlo et al., 2025). The discrepancy between these observations could be attributed to differences in experimental conditions, culture systems, and the quality and selection of the biological material used across studies. Specifically, when comparing our porcine IVM system with that of He et al. (2024), it is worth highlighting that our system better supports nuclear maturation, with over 93 % of control oocytes (non-exposed to PS-NPs) reaching the MII stage, compared to less than 80 % reported by He et al. (2024). It can be hypothesized that the culture conditions and/or the initial oocyte quality in He's study (2024) may have heightened oocyte susceptibility to PS-NPs adverse effect. Indeed, in that study PS-NPs were shown to induce oocyte ferroptosis by increasing oxidative stress and altering lipid metabolism, ultimately impairing oocyte maturation. Alternatively, the conditions used in our

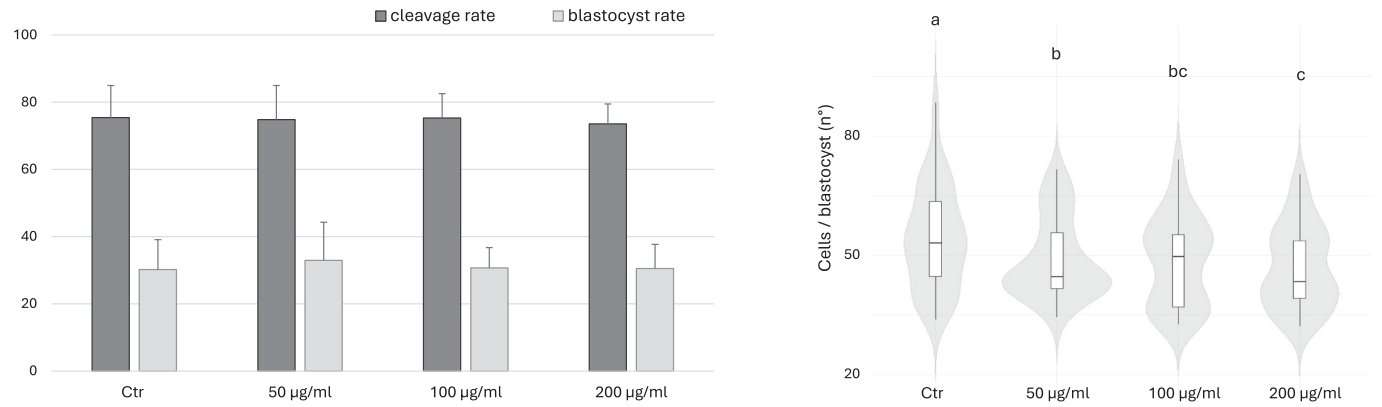

Fig. 1. Effect of PS-NPs addition (0, 50, 100, 200 µg/mL) during IVM on developmental competence of parthenotes after 7 days of in vitro culture (four replicates repeated in different experiments; total number of activated oocytes = 804)). Left panel: Effect of PS-NPs on cleavage and blastocyst rate (mean ± SD). Right panel: violin plot representing the effect of PS-NPs on the number of blastomeres per blastocyst. Central lines (within the box) represent the median; Box edges represent first quartile (25 %) and third quartile (75 %); Whiskers represent values within 1.5 × IQR (interquartile range). Different letters within same graph represent significant difference for $p < 0.05$ among treatments.

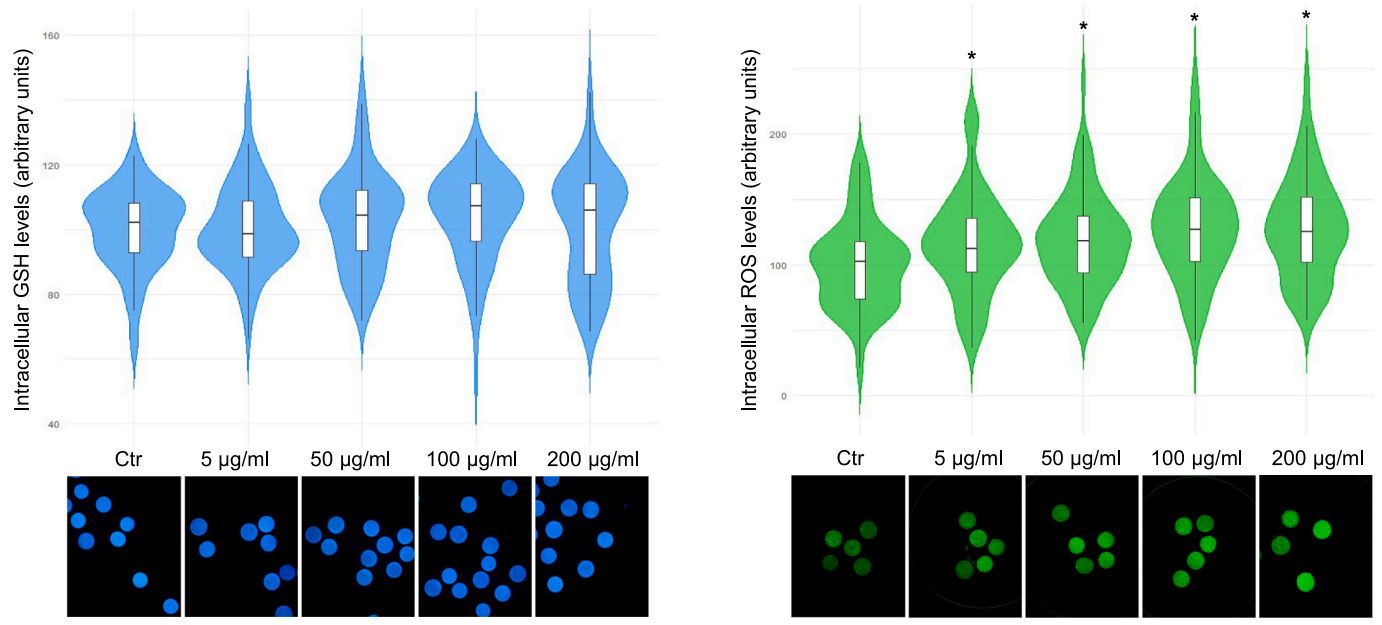

Fig. 2. Upper panel. Violin plots for intracellular GSH levels (left panel) and ROS levels (right panel) of oocytes matured in presence of PS-NPs (0, 5, 50, 100, 200 µg/mL) dyed with CellTracker Blue or H2DCFDA respectively. * indicates significant difference compared to control ($p < 0.05$). The experiment was replicated four times (415 oocytes for GSH analysis and 427 oocytes for ROS analysis). Lower panel. Representative epifluorescent microphotographic images of porcine oocytes matured in presence of PS-NPs stained with CellTracker Blue (left panel) to detect intracellular GSH levels or with H2DCFDA (right panel) to detect intracellular ROS levels. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

study may have mitigated or masked the toxic effects of PS-NPs.

The ability of oocytes to decondense sperm head sustaining male pronucleus formation after IVF and the developmental competence of parthenotes after 7 days of in vitro culture were used as parameters of proper cytoplasmic maturation.

As far as fertilizing parameters concerns, as observed for nuclear maturation, even the highest dose tested of PS-NPs was ineffective in altering penetration rate, monospermy rate and the ability of oocytes to sustain male pronucleus formation. Our results are in line with those of Merlo et al. (2025) on bovine oocytes matured in presence of 100 nm PS-NPs, at a similar range of concentration tested in our study, as they did not observe any significant impact on fertilization parameters apart from a reduced ability to form male pronucleus. Interestingly, that study reported a stronger effect on fertilization parameters caused by 30 nm PS-NPs, with a decrease of penetration rate at 70 µg/mL, confirming an increasing harmful effect related to the size of the particles (Merlo et al., 2025). It is well established that GSH is involved in reducing protamine disulfide bonds within the sperm head allowing the DNA to decondense and form the male pronucleus (Yoshida et al., 1993). The lack of any influence of PS-NPs on intracellular GSH levels we recorded is consistent with the preservation of the ability of exposed oocytes to decondense sperm nuclei. It should be taken into account that, in our model, pig IVM was performed in NCSU 37 medium supplemented with β-mercaptoethanol, cysteine and EGF, molecules often added to pig IVM media as have been demonstrated to induce a reduction in ROS levels and an increase in GSH content of porcine oocyte, improving the maturation of this gamete (Abeydeera et al., 1998; Abeydeera et al., 1999; Sawai et al., 1997). Therefore, the potential toxic effects of PS-NPs may have been masked by the activity of the system supplementations, potentially explaining the discrepancy between our results and those of Merlo et al.

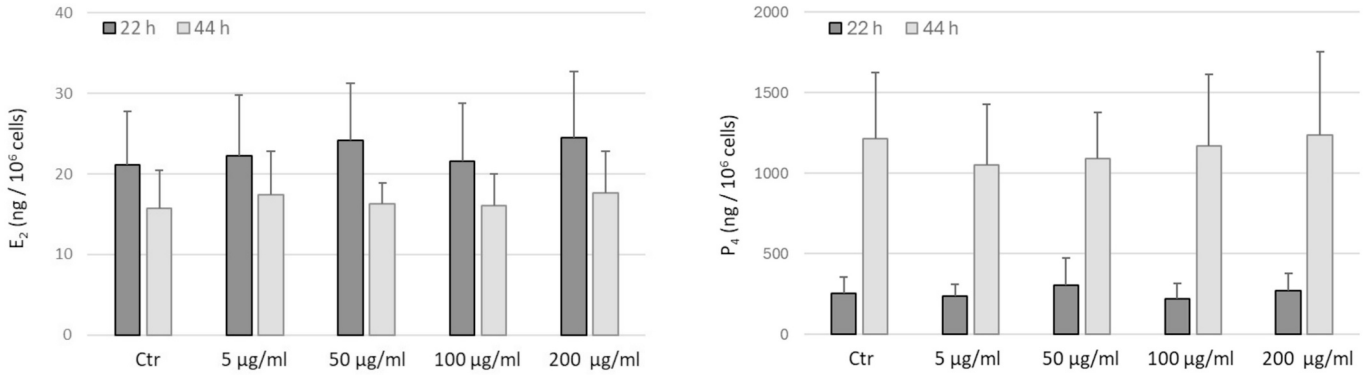

Fig. 3. Effect of PS-NPs (0, 5, 50, 100, 200, µg/mL) on E2 (left panel) and P4 (right panel) production by porcine cumulus cells evaluated in the spent IVM media after 22 h and 44 h of IVM. Data represent mean ± SD of 5 independent experiments.

(2025), who observed in bovine oocytes treated with 100 nm PS-NPs a reduced ability to decondense the sperm nucleus, in parallel with decreased GSH levels. However, a species-specific susceptibility cannot be excluded.

Oxidative stress is recognized as one of the most detrimental factors impairing oocyte maturation (Combelles et al., 2009), and several studies have identified it as a key toxic mechanism by which NPs exert their harmful effects on oocytes (He et al., 2024; Park et al., 2022). Consistent with these findings, our results revealed a significant rise in ROS levels in oocytes following PS-NPs exposure during IVM, in comparison to the control group. An inverse relationship between oocytes intracellular ROS and GSH levels has been observed in bovine oocytes exposed in vitro to PS-NPs (Merlo et al., 2025) and in oocytes obtained from orally MPs-treated mice (Liu et al., 2022; Zhang et al., 2023). By contrast, in our study PS-NPs significantly increased the intracellular levels of ROS without affecting GSH levels. These findings suggest that, in our model, the ROS increase may not have been strong enough to markedly deplete oocyte GSH as part of the antioxidant defence system, which may explain the absence of detectable impairment in oocyte decondensing activity that we observed, as discussed above.

Since no effect was recorded on fertilizing parameters, to further investigate if PS-NPs can impair a proper cytoplasmic maturation, the developmental competence of IVM-exposed oocytes was assessed after parthenogenetic activation to avoid possible sperm-related effects on IVF. As far as we know, this is the first study on the impact of PS-NPs exposure during IVM on the subsequent developmental competence of mammalian oocytes. We observed that both embryo cleavage and blastocyst rate were not affected by PS-NPs treatment during IVM at all the concentrations tested (50, 100, 200 µg/mL). Our findings agree with those obtained in mouse after parthenogenetic activation of MII oocyte collected from female orally exposed to PS-MPs, as no significant difference were observed in cleavage and blastocysts rate (Liu et al., 2022). Nevertheless, in our study PS-NPs induced a dose dependent reduction in the number of blastomere per blastocyst. Therefore, the exposure of oocytes during IVM to PS-NPs compromised the acquisition of a proper cytoplasmic maturation, which subsequently led to a reduction in blastocyst quality, possibly interfering with embryonic cell proliferation, despite the absence of PS-NPs during the embryo culture stage. Further studies are needed to elucidate the underlying mechanism through which PS-NPs exert this detrimental effect. It has to be stressed that exposing oocytes and embryos to PS-NPs throughout all stages of in vitro culture (maturation, fertilization, embryo development) would more closely mimic in vivo condition and could potentially result in more pronounced detrimental effects. This aspect warrants further investigation. Indeed, the addition of 100 µg/mL PS-NPs to the culture medium of bovine embryos significantly impaired both 8-cell embryo and blastocyst rates (Barbato et al., 2020). Moreover, it has been recently demonstrated that Polymethylmethacrylate-NPs added during mouse embryo culture rapidly enter into embryos negatively affecting, by ROS-dependent mechanisms, embryo quality, blastocyst formation and implantation rate in pseudopregnat mice (You et al., 2024).

Concerning the effects on cumulus cell steroidogenesis, irrespective to PS-NPs treatment, P4 production increased during the second day of culture likely due to cumulus cell differentiation/luteinization as already observed (Mateo-Otero et al., 2022; Spinaci et al., 2019; Spinaci et al., 2020). PS-NPs at all concentration tested did not alter the pattern of P4 and E2 secretion by CCs after 22 and 44 h of culture suggesting that PS-NPs do not affect steroidogenesis of cumulus cells. Nevertheless, disturbing effect of PS-NPs on the in vitro secretion of steroid hormones by the mural granulosa have been reported, although the obtained results are not always consistent with one another. Indeed, Basini et al. (2021), reported a negative influence of 100 nm PS-NPs on porcine granulosa cells steroidogenesis in vitro, with E2 secretion being stimulated and P4 secretion inhibited, while a significant reduction in E2 levels was recorded when mouse primary granulosa cells were exposed to 25 nm PS-NPs (Xue et al., 2024). However, it is important to note that these studies utilized plated granulosa cells for their research, while our model involved cumulus-oocyte complexes (COCs) during IVM. In fact, cumulus cells and mural granulosa cells are anatomically and functionally distinct: cumulus cells play a crucial role in supporting oocyte growth and development, while mural granulosa cells mainly exert endocrine functions and support follicle growth (Li et al., 2000). Furthermore, it has been demonstrated that oocytes secrete factors that regulate steroid production by both cumulus and granulosa cells (Coskun et al., 1995; Li et al., 2000). Therefore, based on these differences, our findings on cumulus cell steroidogenesis cannot be directly compared to those obtained on cultured granulosa cells.

In conclusion, our results indicate that the exposure to PS-NPs during IVM, even if it does not affect nuclear maturation, fertilization parameters and embryo cleavage, causes an increase of oocyte ROS levels and impairs oocyte developmental competence in term of blastocyst cellularity.

Although the findings from our in vitro porcine model cannot be directly extrapolated to other species or to humans, they contribute to a growing body of evidence suggesting that (PS-NPs) may interfere with oocyte maturation and reinforce the need to consider NPs exposure as an emerging environmental factor with potential implications for both animal and human fertility.

CRediT authorship contribution statement

Marcella Spinaci: Writing – original draft, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Sofia Dindo:** Writing – review & editing, Investigation. **Nadia Govoni:** Writing – review & editing, Investigation. **Laura Tovar:** Writing – review & editing, Investigation. **Alessandro Marino Volsa:** Writing –

review & editing, Investigation. **Cinzia Cappannari:** Writing – review & editing, Investigation, Data curation. **Diego Bucci:** Writing – review & editing, Validation, Formal analysis, Data curation. **Jose Manuel Ortiz-Rodriguez:** Writing – review & editing, Visualization, Investigation, Data curation.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT as an AI-assisted tool to refine the language and clarity of our English article. After using this tool, the authors thoroughly reviewed and edited the content to ensure accuracy and appropriateness, taking full responsibility for the final published version of the article.

Declaration of competing interest

Authors declare that there are no conflicts of interest.

Data availability

The datasets generated and/or analysed during the current study are available at the following link: https://amsacta.unibo.it/id/eprint/8372

Acknowledgments

The authors wish to thank 'La Regione Emilia-Romagna - Settore Prevenzione Collettiva e Sanita' Pubblica' for funding Volsa AM's fellowship and Mr. Danilo Matteuzzi and Mr. Roberto Giusti for kindly conferring swine ovaries from a local slaughterhouse to the laboratory.

References

Abeydeera, L.R., Wang, W.H., Cantley, T.C., Prather, R.S., Day, B.N., 1998. Presence of beta-mercaptoethanol can increase the glutathione content of pig oocytes matured in vitro and the rate of blastocyst development after in vitro fertilization. Theriogenology 50, 747–756. https://doi.org/10.1017/S0967199499000581.

Abeydeera, L.R., Wang, W.H., Cantley, T.C., Prather, R.S., Day, B.N., 1999. Glutathione content and embryo development after in vitro fertilisation of pig oocytes matured in the presence of a thiol compound and various concentrations of cysteine. Zygote 7, 203–210. https://doi.org/10.1017/s0967199499000581.

Ali, N., Katsouli, J., Marczylo, E.L., Gant, T.W., Wright, S., Bernardino de la Serna, J., 2024. The potential impacts of micro-and-nano plastics on various organ systems in humans. EBioMedicine 99, 104901. https://doi.org/10.1016/j.ebiom.2023.104901.

Barbato, V., Talevi, R., Gualtieri, R., Pallotta, M.M., Di Nardo, M., Costanzo, V., Catapano, G., Capriglione, T., 2020. Polystyrene nanoparticles may affect cell mitosis and compromise early embryo development in mammals. Theriogenology 145, 18–23. https://doi.org/10.1016/j.theriogenology.2020.01.007.

Basini, G., Bussolati, S., Andriani, L., Grolli, S., Ramoni, R., Bertini, S., Iemmi, T., Menozzi, A., Berni, P., Grasselli, F., 2021. Nanoplastics impair in vitro swine granulosa cell functions. Domest. Anim. Endocrinol. 76, 106611. https://doi.org/10.1016/j.domaniend.2021.106611.

Boquest, A.C., Grupen, C.G., Harrison, S.J., McIlfatrick, S.M., Ashman, R.J., D'Apice, A.J. F., Nottle, M.B., 2002. Production of cloned pigs from cultured fetal fibroblast cells. Biol. Reprod. 66, 1283e7. https://doi.org/10.1095/biolreprod66.5.1283.

Brackett, B.G., Oliphant, G., 1975. Capacitation of rabbit spermatozoa in vitro. Biol. Reprod. 12, 260–274. https://doi.org/10.1095/biolreprod12.2.260.

Combelles, C.M., Gupta, S., Agarwal, A., 2009. Could oxidative stress influence the in-vitro maturation of oocytes? Reprod. Biomed. Online 18 (6), 864–880. https://doi.org/10.1016/s1472-6483(10)60038-7.

Coskun, S., Uzumcu, M., Lin, Y.C., Friedman, C.I., Alak, B.M., 1995. Regulation of cumulus cell steroidogenesis by the porcine oocyte and preliminary characterization of oocyte-produced factor(s). Biol. Reprod. 53, 670–675. https://doi.org/10.1095/biolreprod53.3.670.

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2016. Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA J. 14 (6), e04501. https://doi.org/10.2903/j.efsa.2016.4501.

Funahashi, H., Cantley, T., Day, B.N., 1997. Synchronization of meiosis in porcine oocytes by exposure to dibutyryl cyclic adenosine monophosphate improves developmental competence following in vitro fertilization. Biol. Reprod. 57, 49–53. https://doi.org/10.1095/biolreprod57.1.49.

Galeati, G., Giaretta, E., Zannoni, A., Bucci, D., Tamanini, C., Forni, M., Spinaci, M., 2016. Embelin supplementation of in vitro maturation medium does not influence nuclear and cytoplasmic maturation of pig oocytes. J. Physiol. Pharmacol. 67 (4), 513–519.

Gaylarde, C.C., Baptista Neto, J.A., da Fonseca, E.M., 2021. Nanoplastics in aquatic systems - are they more hazardous than microplastics. Environ. Pollut. 272, 115950. https://doi.org/10.1016/j.envpol.2020.115950.

Gewert, B., Plassmann, M.M., MacLeod, M., 2015. Pathways for degradation of plastic polymers floating in the marine environment. Environ Sci Process Impacts 17, 1513–1521. https://doi.org/10.1039/c5em00207a.

He, Y., Yu, T., Li, H., Sun, Q., Chen, M., Lin, Y., Dai, J., Wang, W., Li, Q., Ju, S., 2024. Polystyrene nanoplastic exposure actives ferroptosis by oxidative stress-induced lipid peroxidation in porcine oocytes during maturation. J. Anim. Sci. Biotechnol. 15 (1), 117. https://doi.org/10.1186/s40104-024-01077-6.

Li, R., Norman, R.J., Armstrong, D.T., Gilchrist, R.B., 2000. Oocyte-secreted factor(s) determine functional differences between bovine mural granulosa cells and cumulus cells. Biol. Reprod. 63 (3), 839–845. https://doi.org/10.1095/biolreprod63.3.839.

Liu, Z., Zhuan, Q., Zhang, L., Meng, L., Fu, X., Hou, Y., 2022. Polystyrene microplastics induced female reproductive toxicity in mice. J. Hazard. Mater. 424 (Pt C), 127629. https://doi.org/10.1016/j.jhazmat.2021.127629.

Mateo-Otero, Y., Yeste, M., Roca, J., Llavanera, M., Bucci, D., Galeati, G., Spinaci, M., Barranco, I., 2022. Seminal extracellular vesicles subsets modulate gene expression in cumulus cells of porcine in vitro matured oocytes. Sci. Rep. 12 (1), 19096. https://doi.org/10.1038/s41598-022-22004-7.

Mattioli, M., Galeati, G., Barboni, B., Seren, E., 1994. Concentration of cyclic AMP during the maturation of pig oocytes in vivo and in vitro. J. Reprod. Fertil. 100 (2), 403–409. https://doi.org/10.1530/jrf.0.1000403.

Merlo, B., Volsa, A.M., Tovar, L., Gaiani, M., Gugole, P.M., Attolini, E., Iacono, E., 2025. Effects of polystyrene nanoparticles on bovine oocyte in vitro maturation. Theriogenology 244, 117482. https://doi.org/10.1016/j.theriogenology.2025.117482.

Park, S., Jeon, H.J., Choi, D.Y., Oh, J.S., 2022. Polystyrene nanoparticles incorporate into the endoplasmic reticulum and disturb translation during meiotic maturation in mouse oocytes. Toxicol. In Vitro 82, 105380. https://doi.org/10.1016/j.tiv.2022.105380.

Petters, R.M., Wells, K.D., 1993. Culture of pig embryos. J. Reprod. Fertil. 48, 61–73.

Santos, R.R., Schoevers, E.J., Roelen, B.A., 2014. Usefulness of bovine and porcine IVM/IVF models for reproductive toxicology. Reprod. Biol. Endocrinol. 12, 117. https://doi.org/10.1186/1477-7827-12-117.

Sawai, K., Funahashi, H., Niwa, K., 1997. Stage-specific requirement of cysteine during in vitro maturation of porcine oocytes for glutathione synthesis associated with male pronuclear formation. Biol. Reprod. 57 (1), 1–6. https://doi.org/10.1095/biolreprod57.1.1.

Spinaci, M., Bucci, D., Muccilli, V., Cardullo, N., Nerozzi, C., Galeati, G., 2019. A polyphenol-rich extract from an oenological oak-derived tannin influences in vitro maturation of porcine oocytes. Theriogenology 129, 82–89. https://doi.org/10.1016/j.theriogenology.2019.02.017.

Spinaci, M., Nerozzi, C., Tamanini, C., Bucci, D., Galeati, G., 2020. Glyphosate and its formulation roundup impair pig oocyte maturation. Sci. Rep. 10 (1), 12007. https://doi.org/10.1038/s41598-020-68813-6.

Volsa, A.M., Iacono, E., Merlo, B., 2025. Micro-nanoplastics pollution and mammalian fertility: a systematic review and meta-analysis. Theriogenology 238, 117369. https://doi.org/10.1016/j.theriogenology.2025.117369.

Xue, Y., Cheng, X., Ma, Z.Q., Wang, H.P., Zhou, C., Li, J., Zhang, D.L., Hu, L.L., Cui, Y.F., Huang, J., Luo, T., Zheng, L.P., 2024. Polystyrene nanoplastics induce apoptosis, autophagy, and steroidogenesis disruption in granulosa cells to reduce oocyte quality and fertility by inhibiting the PI3K/AKT pathway in female mice. J. Nanobiotechnol. 22 (1), 460. https://doi.org/10.1186/s12951-024-02735-7.

Yang, J.Y., Birza, M.J.J., Kamstra, J.H., Aardema, H., 2022. First evidence of nanoplastic uptake by the maturing oocyte. Anim. Reprod. 19 (2 spe), e22203.

Yoshida, M., Ishigaki, K., Nagai, T., Chikyu, M., Pursel, V.G., 1993. Glutathione concentration during maturation and after fertilization in pig oocytes: relevance to the ability of oocytes to form male pronucleus. Biol. Reprod. 49 (1), 89–94. https://doi.org/10.1095/biolreprod49.1.89.

You, H.J., Jo, Y.J., Kim, G., Kwon, J., Yoon, S.B., Youn, C., Kim, Y., Kang, M.J., Cho, W. S., Kim, J.S., 2024. Disruption of early embryonic development in mice by polymethylmethacrylate nanoplastics in an oxidative stress mechanism. Chemosphere 361, 142407. https://doi.org/10.1016/j.chemosphere.2024.142407.

Zeng, L., Zhou, C., Xu, W., Huang, Y., Wang, W., Ma, Z., Huang, J., Li, J., Hu, L., Xue, Y., Luo, T., Zheng, L., 2023. The ovarian-related effects of polystyrene nanoplastics on human ovarian granulosa cells and female mice. Ecotoxicol. Environ. Saf. 257, 114941. https://doi.org/10.1016/j.ecoenv.2023.114941.

Zhang, Y., Wang, X., Zhao, Y., Zhao, J., Yu, T., Yao, Y., Zhao, R., Yu, R., Liu, J., Su, J., 2023. Reproductive toxicity of microplastics in female mice and their offspring from induction of oxidative stress. Environ. Pollut. 327, 121482. https://doi.org/10.1016/j.envpol.2023.121482.