TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2026, vol. 11, No 2(21): 44–53

ULTRASONOGRAPHIC AND MORPHOMETRIC CHARACTERIZATION OF EMBRYONIC DEVELOPMENT FOLLOWING MATERNAL EXPOSURE TO POLYSTYRENE MICROPLASTICS

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(Submitted: 12 January 2026; Accepted 24 March 2026: Published: 30 June 2026)

ABSTRACT

Microplastics (MPs) are widespread environmental contaminants, yet their effects on reproductive and early ontogenetic development remain insufficiently understood. The present study aimed to evaluate the effects of chronic pregestational and gestational exposure to polystyrene microplastics (PS-MPs) with particle sizes of 1 μm (G1) and 5 μm (G5) on embryonic and early postnatal development in Wistar rats, using combined ultrasonographic and morphometric assessment.

Juvenile animals were subjected to a 30-day oral exposure to PS-MPs at a dose of 0.1 mg/day suspended in drinking water until sexual maturity. After mating, exposure in females continued throughout pregnancy, allowing the modeling of combined pregestational and gestational exposure.

Ultrasonographic evaluation on gestational day 10 revealed no significant intergroup differences, suggesting unaffected early implantation and initial embryonic development. In contrast, gestational day 18 demonstrated statistically significant reductions in the main ultrasonographic parameters in exposed groups compared to controls, with the most pronounced alterations observed in G1, indicating a size-dependent effect during late gestation.

Postnatal morphometric analysis demonstrated a tendency toward lower body weight and body length in offspring from exposed groups on postnatal days 2 and 21 (PND 2 and PND 21), while the physiological growth pattern remained preserved. Smaller particles (1 μm) induced more pronounced alterations than 5 μm particles.

In conclusion, maternal exposure to PS-MPs was associated with measurable, phase-dependent changes in embryonic and postnatal development in Wistar rats, with effects tending to be more pronounced following exposure to smaller particles and during late gestation. These findings suggest that particle size may influence the biological activity of MPs and support the need for further investigation into their potential long-term reproductive effects.

Key words: polystyrene microplastics (PS-MPs), maternal exposure, ultrasonography, embryonic development, postnatal development, reproductive toxicity, Wistar rats.

Introduction

Microplastics (MPs; <5 mm) and nanoplastics (NPs; <1000 nm), collectively referred to as micro- and nanoplastics (MNPs), have emerged as ubiquitous environmental contaminants originating from the degradation of larger plastic materials or from direct industrial and consumer sources. Due to their persistence and widespread distribution, they have been detected in air, water, soil, and the food chain, leading to continuous exposure in both humans and animals (Jiang *et al*., 2020; Park & Park, 2021). Increasing plastic production and the limited efficiency of waste management further exacerbate their environmental burden (Fletcher *et al*., 2023). In recent years, growing evidence has indicated that MNPs can cross biological barriers and reach the reproductive system and placenta. The first report of plastic particles in human placental tissue by Ragusa *et al.* (2021) raised concerns regarding potential prenatal exposure and its implications for fetal development. Subsequent studies have demonstrated an increasing frequency of MP detection in placental tissue over recent decades, supporting the hypothesis of rising in utero exposure (Weingrill *et al*., 2023). Experimental rodent models have further confirmed that MNPs can accumulate in the placenta and impair its physiological function, including maternal–fetal exchange (Dusza *et al*., 2022; Wang *et al*., 2025).

A growing body of evidence demonstrates that exposure to polystyrene microplastics (PS-MPs) induces reproductive toxicity in both male and female animals, including impaired spermatogenesis, altered ovarian function, and disrupted embryonic development. Reported mechanisms include oxidative stress, inflammatory responses, mitochondrial dysfunction, and impairment of reproductive barriers, all of which may compromise fertility and normal gestation (Xie *et al*., 2020; Liu *et al*., 2022a; Liu *et al*., 2022b; Gao *et al*., 2023; Wen *et al*., 2023). However, despite the increasing number of publications, most available data are based on endpoint biochemical or histological analyses, while information on in vivo embryonic developmental dynamics remains limited. Even less is known about the effects of chronic pregestational exposure initiated during juvenile life and continued throughout pregnancy on subsequent embryonic and postnatal development.

In this context, the present study aimed to evaluate the effects of chronic exposure to PS-MPs of 1 μm (G1) and 5 μm (G5) on embryonic and early postnatal development in Wistar rats, by combining pregestational and gestational exposure with in vivo ultrasonographic monitoring of pregnancy and postnatal morphometric assessment of offspring. Comparison between the two particle sizes further allows evaluation of a potential size-dependent effect on reproductive and early developmental processes.

Materials and Methods

Animals

A total of 36 juvenile Wistar rats (18 females and 18 males) obtained from a licensed breeder EB-BEA (Slivnitsa, Bulgaria) were used in the study. The animals were housed under standard laboratory conditions (temperature 22 ± 1 °C, relative humidity 50 ± 5%, 12 h light/dark cycle) with ad libitum access to standard chow and water. All experimental procedures were conducted in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes and were approved by the Institutional Ethics Committee (Approval No. 425/24 February 2025).

Experimental design and exposure

The animals were allocated into three experimental groups: a control group (Co), a group exposed to 1 µm PS-MPs (G1), and a group exposed to 5 µm PS-MPs (G5). MPs were administered orally via drinking water at a dose of 0.1 mg/animal/day. Prior to administration, the suspension was sonicated for 30 minutes to ensure homogeneous particle dispersion. The dose and exposure regimen were based on our previously published in vivo rodent studies reporting that low-dose chronic oral PS-MP exposure induces reproductive and developmental effects without overt systemic toxicity (Kanzova et al., 2026a; Kanzova et al., 2026b), and were selected to model environmentally relevant chronic exposure.

Exposure began at the juvenile stage and continued for 30 days until sexual maturity, thereby modeling chronic pregestational exposure. Subsequently, animals were mated within their respective groups (Co × Co, G1 × G1, and G5 × G5) at a 1:1 female-to-male ratio to ensure that offspring originated from parents with identical exposure status. Due to natural variability in the timing of fertilization, gestational day 0 (GD0) was defined as the first day following the end of the mating period. In females, exposure was continued throughout pregnancy, enabling the assessment of combined pregestational and gestational effects. The overall experimental design and timeline of exposure, mating, pregnancy monitoring, and postnatal assessments are summarized in Figure 1.

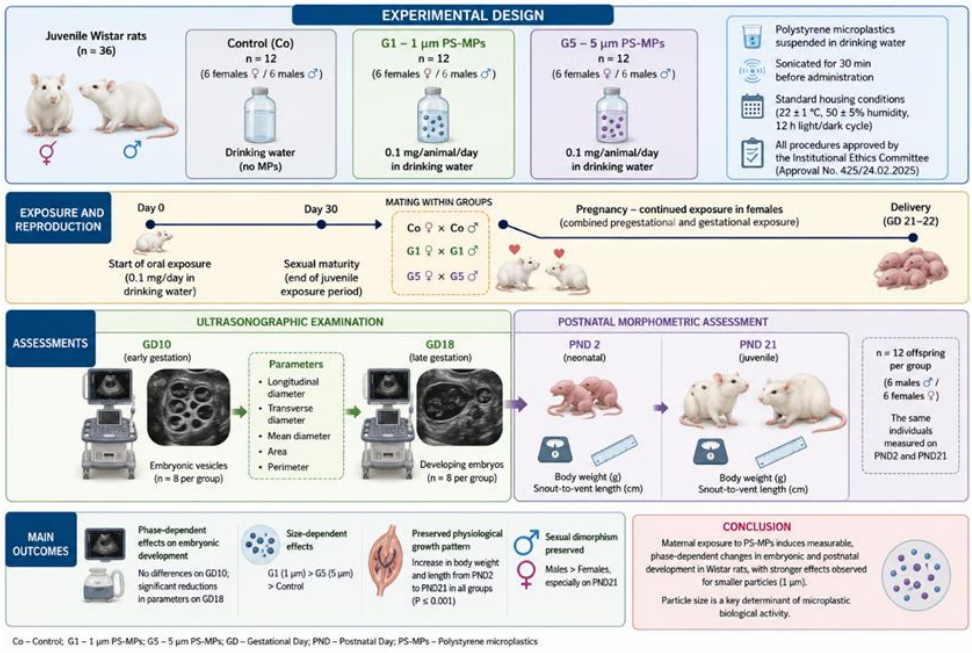

Figure 1: Experimental design of maternal exposure to PS-MPs and developmental assessments.

Ultrasonographic examination

Transabdominal ultrasonography was performed using a Mindray DP-10 ultrasound system equipped with a convex transducer (6.5–8.0 MHz). Examinations were conducted on gestational days 10 and 18 (GD10 and GD18) under gentle manual restraint without anesthesia.

For ultrasonographic analysis, eight embryonic vesicles per experimental group were randomly selected at GD10 and GD18. Embryonic vesicles were treated as independent observational units for morphometric analysis.

Morphometric parameters of embryonic vesicles were assessed, including longitudinal and transverse diameters, mean diameter, area, and perimeter.

Morphometric analysis of offspring

Morphometric assessment of offspring was performed on postnatal day 2 (PND 2) and postnatal day 21 (PND 21). A total of 12 offspring per experimental group (6 males and 6 females) were included in the analysis. Offspring were selected from litters born on the same day to ensure uniform postnatal age at the time of assessment. In the control group, offspring were derived from four litters delivered on the same day. In the G1 group, offspring originated from three litters delivered on the same day. In the G5 group, offspring were derived from four litters delivered on the same day. From each litter, offspring were randomly selected to ensure balanced representation across litters. All pups included in the analysis had comparable body size at PND2, indicating minimal baseline variability between groups. Body weight (g) and snout-to-vent length (SVL, cm) were measured using a calibrated laboratory balance and a graduated ruler under standardized conditions. All measurements were performed by the same investigator to minimize inter-observer variability.

The same offspring were longitudinally followed and measured at both PND2 and PND21.

Statistical analysis

Data were analyzed using IBM SPSS Statistics (version 26.0; IBM Corp., Armonk, NY, USA). Morphometric data from embryonic vesicles were analyzed using two-way repeated measures ANOVA for comparisons between gestational days (GD10 vs GD18) within each experimental group. Postnatal morphometric data were analyzed using Paired-Samples T-test for within-group comparisons between PND2 and PND21. Sex- and group-related differences were evaluated using two-way repeated measures ANOVA followed by Tukey's HSD post hoc test. Statistical significance was set at $p < 0.05$. Litter was considered the experimental unit for offspring analysis, while repeated measurements were performed on the same individuals at PND2 and PND21.

Results and Discussion

The present study provides one of the first combined ultrasonographic and morphometric in vivo evaluations of embryonic and early postnatal development following maternal exposure to PS-MPs in Wistar rats. The integration of real-time ultrasonographic monitoring with postnatal morphometric assessment allows a more comprehensive characterization of gestational development and developmental toxicity associated with chronic exposure. Furthermore, the study contributes novel data regarding the size-dependent biological effects of PS-MPs during prenatal and early postnatal periods.

All pregnant females delivered successfully between gestational days 21 and 22, indicating that exposure to PS-MPs did not prevent conception or completion of pregnancy under the present experimental conditions.

Ultrasonographic examination performed on gestational day 10 demonstrated clearly detectable embryonic vesicles in all experimental groups (Figure 2).

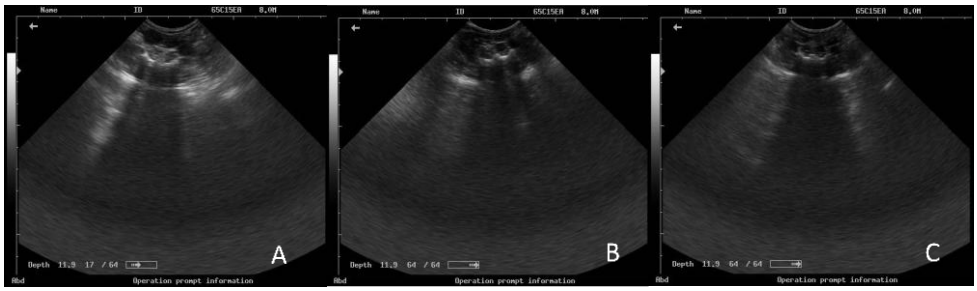

Figure 2: Ultrasonographic image of embryonic vesicles in pregnant Wistar rats on gestational day 10 following exposure to PS-MPs. (A–CoD10; B–G1D10; C–G5D10)

At this stage, no statistically significant intergroup differences were observed in longitudinal diameter, transverse diameter, mean vesicle diameter, vesicle area, or vesicle perimeter (Table 1). These findings suggest that early implantation and initial embryonic vesicle formation were not markedly affected by exposure to PS-MPs during the preimplantation and early gestational periods.

Table 1: Ultrasonographic assessment of embryonic vesicle development in Wistar rats after maternal exposure to PS-MPs

Group	Longitudinal diameter, (cm)	Transverse diameter, (cm)	Mean vesicle diameter, (cm)	Vesicle area, (cm^2)	Vesicle perimeter, (cm)
CoD10	0.49 ± 0.06	0.64 ± 0.04	0.57 ± 0.05	0.25 ± 0.05	1.80 ± 0.16
G1D10	0.48 ± 0.04	0.59 ± 0.08	0.54 ± 0.05	0.23 ± 0.04	1.70 ± 0.17
G5D10	0.46 ± 0.03	0.62 ± 0.04	0.54 ± 0.03	0.22 ± 0.03	1.70 ± 0.11
CoD18	1.35 ± 0.05 ###	2.09 ± 0.13 ###	1.73 ± 0.08 ###	2.23 ± 0.18 ###	5.48 ± 0.24 ###
G1D18	1.11 ± 0.06 ###,***	1.73 ± 0.18 ###,***	1.42 ± 0.12 ###,***	1.51 ± 0.24 ###,***	4.51 ± 0.39 ###,***
G5D18	1.23 ± 0.07 ###,*,†††	1.99 ± 0.17 ###,†	1.63 ± 0.11 ###,†††	1.98 ± 0.24 ###,†††	5.17 ± 0.35 ###,††

*Note: Data are presented as mean ± SD. ### indicates significant differences between Day 10 and Day 18 within each experimental group. *, **, *** indicate differences versus control group ($P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively). †, ††, ††† indicate size-dependent differences between G1 and G5 ($P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively).*

In contrast, on gestational day 18 a statistically significant increase in all ultrasonographic parameters was observed compared to day 10 within each experimental group ($P \leq 0.001$), confirming normal embryonic development and growth during gestation (Figure 3). However, clear differences between the groups were identified at the end of pregnancy.

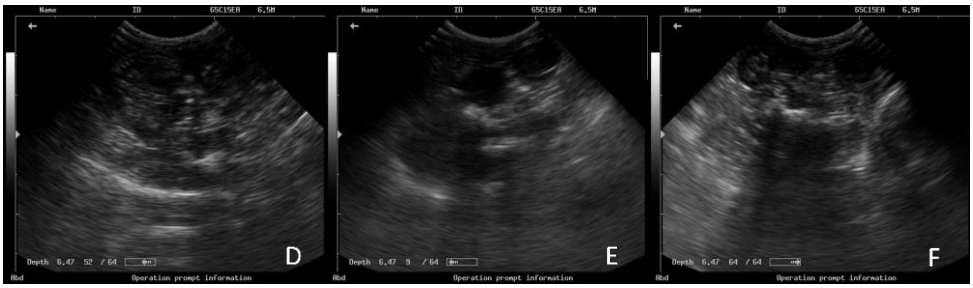

Figure 3: Ultrasonographic image of developing embryos in pregnant Wistar rats on gestational day 18 following exposure to PS-MPs. (D–CoD18; E–G1D18; F–G5D18)

Animals in the G1 group, exposed to 1 μm PS-MPs, exhibited significantly lower longitudinal diameter, transverse diameter, mean vesicle diameter, area, and perimeter of embryonic vesicles compared to the control group ($P \leq 0.001$). These results indicate an adverse effect of smaller particles on embryonic development.

The G5 group, exposed to 5 μm PS-MPs, exhibited intermediate values between Co and G1. The statistically significant differences observed between G1 and G5 ($P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$) indicate a size-dependent effect of MPs on reproductive parameters. Embryonic structures in the G5 group were larger than those in G1, suggesting that smaller particles exhibit higher biological activity and a greater potential for tissue penetration.

Postnatal morphometric analysis of offspring on postnatal days 2 and 21 (PND 2 and PND 21) demonstrated a statistically significant increase in both body weight and body length across all groups ($P \leq 0.001$), reflecting normal postnatal development (Table 2). Representative images supporting these morphometric findings are presented in Figures 4 and 5. However, offspring from the experimental groups showed a tendency toward lower body weight and body length compared to controls.

were assessed using two-way repeated measures ANOVA followed by post hoc comparisons.

Sex-dependent differences were also observed, with male animals showing higher body weight and larger size compared to females, particularly on PND 21. These differences were present in both control and experimental groups, suggesting preservation of physiological sexual dimorphism regardless of PS-MP exposure.

Notably, animals in the G5 group exhibited morphometric parameters closer to control values compared to the G1 group, further supporting the presence of a size-dependent toxic effect. Smaller microplastic particles are likely to cross the placental barrier more easily and interact more extensively with developing fetal tissues due to their higher surface area-to-volume ratio and greater cellular internalization capacity.

Table 2: Postnatal morphometric assessment of offspring following maternal exposure to PS-MPs

Group	PND 2 SVL, (cm)	PND 21 SVL, (cm)	PND 2 Weight, (g)	PND 21 Weight, (g)
♂Co	5.37±0.12	10.18±0.31###	8.67±0.82	41.83±2.14###
♂G1	5.12±0.12	9.61±0.39###	8.16±0.41	39.83±1.94###
♂G5	5.22±0.08	9.95±0.34###	8.33±0.52	40.17±1.94###
♀Co	5.05±0.05	9.60±0.46###	7.67±0.52	36.50±1.76###
♀G1	4.68±0.12	9.27±0.47###	7.33±0.52	34.17±1.83###
♀G5	4.80±0.09	9.47±0.21###	7.33±0.52	35.50±1.87###
Statistically Significant Difference	*Co♂ vs G1♂ (P≤0.01); Co♂ vs Co♀ (P≤ 0.001); Co♂ vs G1♀ (P≤0.001); Co♂ vs G5♀ (P≤0.001); G1♂ vs G1♀ (P≤0.001); G1♂ vs G5♀ (P≤0.001); G5♂ vs G1♀ (P≤0.001); G5♂ vs G5♀ (P≤0.001); Co♀ vs G1♀ (P≤0.001); Co♀ vs G5♀ (P≤0.01);*	*Co♂ vs G1♀ (P≤0.01); Co♂ vs G5♀ (P≤0.05); Co♀ vs G1♀ (P≤0.05); Co♀ vs G5♀ (P≤0.01);*	*Co♂ vs Co♀ (P≤ 0.05); Co♂ vs G1♀ (P≤0.01); Co♂ vs G5♀ (P≤0.01); G5♂ vs G1♀ (P≤0.05); G5♂ vs G5♀ (P≤0.05);*	*Co♂ vs Co♀ (P≤ 0.001); Co♂ vs G1♀ (P≤0.001); Co♂ vs G5♀ (P≤0.001); G1♂ vs Co♀ (P≤0.05); G1♂ vs G1♀ (P≤0.001); G1♂ vs G5♀ (P≤0.01);*

Note: Data are presented as mean ± SD. ### indicates significant differences between PND 2 and PND 21 within the same group (P ≤ 0.001). Sex- and group-related differences are indicated in the table and

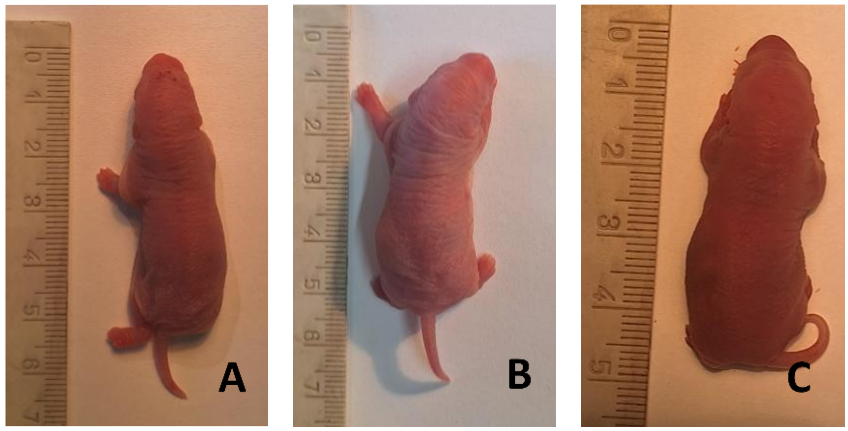

Figure 4: Representative images of neonatal rats (PND 2) used for morphometric assessment following maternal exposure to PS-MPs.

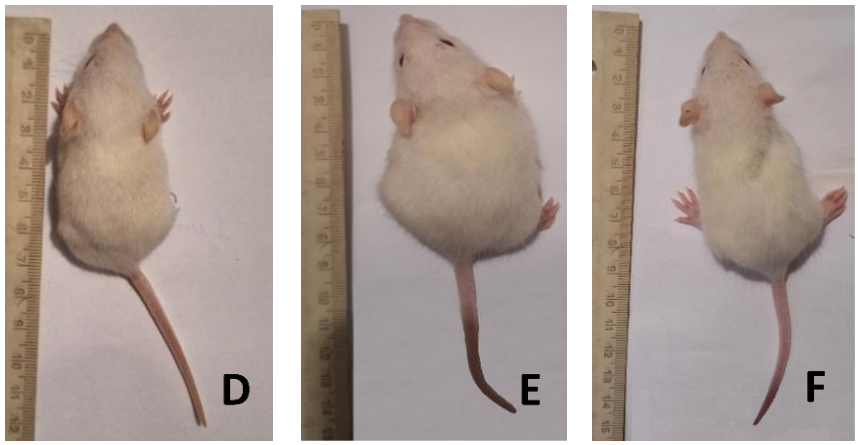

Figure 5: Representative images of juvenile rats (PND 21) demonstrating growth differences following maternal exposure to PS-MPs.

The results of the present study demonstrate that maternal exposure to PS-MPs induces phase-dependent alterations in embryonic development, whereby early gestational stages (day 10) remain relatively unaffected, while a significant reduction in ultrasonographic parameters of embryonic structures is observed in the later stage of pregnancy (day 18). A similar pattern, in which adverse effects become more pronounced in later stages of gestation, has been reported following maternal exposure to polystyrene MNPs, including placental dysfunction, impaired placental metabolism, and adverse effects on fetal development (Aghaei *et al*., 2022; Dibbon *et al*., 2024; Zhang *et al*., 2024).

In line with this, previous studies have reported that maternal exposure to MPs may be associated with delayed postnatal growth and alterations in metabolic development of offspring, which corresponds to the trends toward lower morphometric parameters observed in the exposed groups in the present study (Luo *et al*., 2019).

A key finding of the present study is the clear size-dependent effect, whereby smaller PS-MPs (1 μm) are associated with more pronounced alterations compared to larger particles (5 μm). This type of size dependency has been widely described in the literature, with smaller particles demonstrating a greater potential for tissue translocation, crossing of biological barriers, and accumulation in placental and fetal structures (Fournier *et al*., 2020; Dibbon *et al*., 2024; Wang *et al*., 2025).

Additionally, experimental evidence indicates that smaller PS-MPs and NPs can cross the placental barrier and accumulate in placental and fetal tissues, where they are associated with alterations in placental morphology, vascularization, and fetal development (Wang *et al*., 2025). This is consistent with the more pronounced effects observed in the present study in the group exposed to 1 μm particles.

The literature also reports that exposure to PS-MPs may be associated with disturbances in oxidative status, reproductive toxicity, and developmental alterations (Campanale *et al*., 2020; Hu *et al*., 2021). Experimental studies, including those in mice and female rats, have demonstrated changes in oxidative stress markers following PS-MP exposure, supporting a potential role of oxidative imbalance as a contributing mechanism to the observed biological effects (Andreeva *et al*., 2024; Kanzova *et al*., 2026). These processes have been proposed as possible mechanistic bases for

the observed reproductive and ontogenetic alterations, although such molecular markers were not directly assessed in the present study.

Despite the observed intergroup differences, postnatal development in all cases followed a physiological growth pattern between PND 2 and PND 21, indicating that PS-MP exposure at the applied dose does not completely disrupt growth processes, but is instead associated with subtle alterations in their dynamics.

In conclusion, maternal exposure to PS-MPs was associated with measurable, phase-dependent changes in embryonic and postnatal development in Wistar rats, with effects tending to be more pronounced following exposure to smaller particles and during late gestation. These findings suggest that particle size may influence the biological activity of MPs and support the need for further investigation into their potential long-term reproductive effects.

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