IN VITRO EFFECTS OF POLYSTYRENE NANOPLASTICS ON HUMAN SPERM MOTILITY AND MITOCHONDRIAL ACTIVITY

EFEITOS IN VITRO DOS NANOPLÁSTICOS DE POLIESTIRENO SOBRE A MOTILIDADE E A ATIVIDADE MITOCONDRIAL DE ESPERMATOZOIDES HUMANOS

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ABSTRACT

It is estimated that approximately 350 million tons of plastic become waste through physical and chemical processes, generating microplastics and nanoplastics. Considering the increasing global production of plastics and their potential environmental and human health impacts, this study evaluated the effects of polystyrene nanoplastics (PS-NP) on human sperm parameters in vitro. Spermatozoa from healthy volunteers (n=7) were collected and included only if the samples met the World Health Organization's normality criteria. Cells were isolated using the swim-up technique in BWW medium and exposed to PS-NP at concentrations of 2, 20, and 200 μg/mL, along with a control group, for 30 and 120 minutes. Sperm motility and mitochondrial activity were assessed. The results showed that intermediate and high concentrations significantly reduced motility and mitochondrial functionality in a concentration- and time-dependent manner. These findings suggest that PS-NPs may impair critical functions for male fertility, highlighting the need for further studies to clarify the underlying mechanisms and reproductive health risks.

KEYWORDS: Plastic; gamete; mitochondria; reproduction.

RESUMO

É estimado que cerca de 350 milhões de toneladas de plástico se tornam resíduos, por meio de processos físicos e químicos, formando microplásticos e nanoplásticos. Considerando a crescente produção global de plásticos e seus potenciais impactos ambientais e à saúde humana, o presente estudo avaliou os efeitos do nanoplástico de poliestireno (NP-PS) sobre parâmetros espermáticos humanos *in vitro*. Espermatozoides de voluntários saudáveis (n=7) foram obtidos e incluídos apenas se a amostra se enquadrasse dentro dos padrões de normalidade estabelecidos pela Organização Mundial da Saúde. As células foram isoladas por *swim-up* em meio BWW e expostas a NP-PS em concentrações de 2, 20 e 200 μg/mL, além de grupo controle, por 30 e 120 minutos. A motilidade e atividade mitocondrial foram avaliadas. Os resultados mostraram que concentrações intermediárias e altas

reduziram significativamente a motilidade e a funcionalidade mitocondrial, com efeitos dependentes de concentração e tempo. Estes achados indicam que NP-PS pode comprometer funções essenciais à fertilidade masculina, reforçando a necessidade de estudos adicionais sobre seus mecanismos e riscos à saúde reprodutiva.
PALAVRAS-CHAVE: Plástico; gameta; mitocôndria; reprodução.

INTRODUCTION

The development of synthetic plastics began in the early twentieth century with Leo Hendrik Baekeland's invention of Bakelite, the first fully synthetic polymer. Since then, the plastic industry has expanded exponentially, producing hundreds of polymer types—such as polyethylene, vinyl acetate, and polystyrene (PS)—for various industrial and domestic applications[1]. Global plastic waste generation reached approximately 400 million tons in 2022, with PS accounting for about 5.3% of this total due to its extensive use in packaging, appliances, construction, and consumer goods[2]. While the versatility and durability of plastics revolutionized modern life, these same properties contribute to their persistence in the environment[3].

As large plastic materials degrade, they form microplastics and nanoplastics (NPs), which can enter biological systems via inhalation, ingestion, or dermal absorption. PS-derived nanoparticles (PS-NP) are particularly concerning due to their size-dependent interactions with cells and tissues[4,5]. Inhaled NPs can reach the alveolar region of the lungs, cross thin epithelial barriers, and disseminate systemically, leading to inflammation and oxidative stress. Ingested particles can alter intestinal redox balance and lipid metabolism, while dermal exposure through cosmetic products may trigger local irritation or systemic absorption. Thus, human exposure to NPs is widespread, raising concerns about chronic health effects[6,7].

Recent studies have highlighted the potential of PS-NP to disrupt reproductive function. Experimental evidence shows that PS-NP exposure impairs spermatogenesis in mice, alters testicular morphology, reduces sperm concentration and motility, and affects sex hormone levels. These findings suggest that PS-NP induces oxidative stress, inflammation, and apoptosis in male reproductive tissues,

which may ultimately compromise fertility[8,9]. Despite growing evidence of its toxicity, the mechanisms underlying PS-NP effects on human sperm physiology remain insufficiently understood.

Global reports indicate a substantial decline in sperm counts over recent decades[10], reinforcing the urgency to investigate environmental factors contributing to male infertility. Lifestyle habits, endocrine disruptors, and environmental contaminants have been implicated in this decline[11], but the impact of emerging pollutants such as PS-NP remains largely unexplored. Therefore, this study aimed to evaluate the in vitro effects of different concentrations of PS-NP on mitochondrial activity in human spermatozoa, providing insights into potential mechanisms of reproductive toxicity associated with nanoplastic exposure.

MATERIAL AND METHODS

POLYSTYRENE NANOPLASTICS (PS-NP)

The polystyrene nanopalastics (PS-NP) solution was obtained and characterized in collaboration with the Faculty of Philosophy, Sciences and Letters of Ribeirão Preto (FFCLRP, University of São Paulo, USP). The particles exhibited a hydrodynamic radius of 180 nm and a zeta potential of –27 mV.

PARTICIPANTS AND EXPERIMENTAL DESIGN

This study was approved by the Research Ethics Committee of the Federal University of Triângulo Mineiro (CEP-UFTM; CAAE 71552323.7.0000.5154). Seven healthy male volunteers were recruited at UFTM after being informed about the study's objectives, procedures, potential risks and benefits, and their right to withdraw at any time without prejudice. Inclusion criteria comprised biological males aged 20–39 years, non-smokers, with a body mass index (BMI) ≤ 24.99, and ejaculatory abstinence between 3 and 7 days. Participants had no history of chronic disease, sexually transmitted infection (STI), inflammatory or infectious illness in the preceding 90 days, and no medication use during the same period. After signing two copies of the informed consent form, volunteers were provided with sterile

collection containers and instructed to collect semen samples by masturbation after genital hygiene, avoiding any sexual contact. Samples were to be delivered within 15 minutes of collection to the General Pathology Laboratory, along with collection time and additional information.

Semen was first evaluated macroscopically for volume and liquefaction time. Only samples within the reference limits established by the World Health Organization[12] were included: volume ≥ 1.5 mL, homogeneous white/gray opalescent appearance, complete liquefaction within 30 minutes, and normal viscosity (drop formation upon sample flow).

Viable spermatozoa were isolated by the swim-up technique using BWW medium[13]. Cell suspensions (2×10^6 spermatozoa per well) were then placed into 96-well plates and exposed to PS-NP at three concentrations (2, 20, and 200 µg/mL) or to control medium (n = 7/group). Incubations were carried out at 37°C with 5% CO_2 for 30 and 120 minutes. Following exposure, sperm cells were analyzed as described below. All experiments were performed in duplicate, and unused or non-compliant samples were discarded.

SPERM QUALITY EVALUATION

After exposure of the cells to the compound, a small aliquot containing spermatozoa was transferred to a Neubauer chamber, and 100 sperm cells were counted under a light microscope and classified as progressively motile, non-progressively motile, or immotile.

Mitochondrial activity was assessed by selective incorporation of 3,3'-diaminobenzidine tetrahydrochloride (DAB) into active mitochondria within the sperm midpiece. Samples were incubated in DAB solution (1 mg/mL in PBS) at 37°C for 1 hour, followed by smear preparation and fixation in 10% formaldehyde. Under light microscopy (100× magnification), 100 spermatozoa were classified as DAB I (fully stained midpiece), DAB II (partially stained), or DAB III (unstained).

Quantitative data were analyzed using GraphPad Prism (version 9.5). Data distribution and homogeneity of variances were assessed prior to statistical testing.

Depending on normality, comparisons were performed using one-way ANOVA followed by Tukey–Kramer post hoc test, or by the non-parametric Kruskal–Wallis test followed by Dunn's post hoc test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

SAMPLE CHARACTERISTICS

The volunteers were aged between 20 and 32 years. After the swim-up procedure, sperm concentration ranged from approximately 6.5×10^6 to 82×10^6 spermatozoa/mL, with a mean value of 24.6×10^6 spermatozoa/mL (Table 1).

Table 1. Sample characteristics before PS-NP exposure.

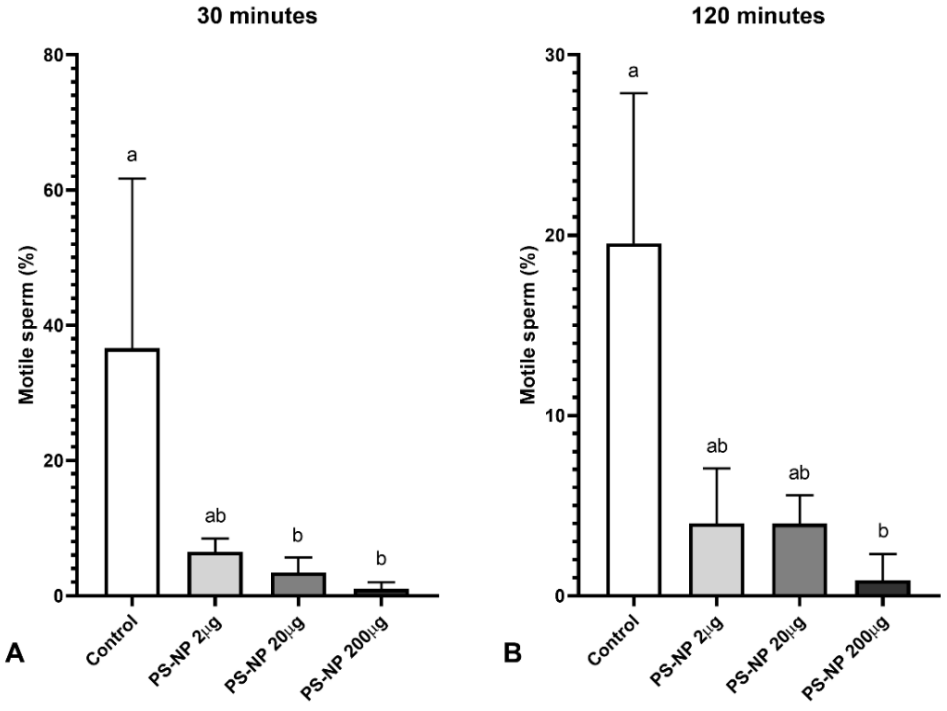
Participant number	Age	Sperm concentration (after swim-up) / mL
1	22	8.000.000
2	32	30.250.000
3	20	7.500.000
4	23	24.000.000
5	23	14.200.000
6	25	6.500.000
7	30	81.750.000

SPERM QUALITY EVALUATION

The sperm motility data were presented in figure 1. After 30 minutes of NP-PS exposure, the percentage of motile spermatozoa was significantly reduced at concentrations of 20 μg/mL and 200 μg/mL compared to the Control group ($p = 0.038$ and $p = 0.0009$, respectively). The 2 μg/mL group did not differ significantly from the other experimental groups. After 120 minutes of exposure, only the 200 μg/mL concentration significantly impaired sperm motility compared to the Control group ($p = 0.0008$). At this time point, the 2 μg/mL and 20 μg/mL groups remained statistically similar to the other groups. At both exposure times, no significant

differences were observed between the Control group and the 2 μg/mL PS-NP group.

Figure 1. Sperm motility after in vitro exposure to 2, 20, and 200 μg/mL of PS-NP for 30 and 120 minutes.



(A) Percentage of motile sperm after 30 minutes of exposure to PS-NP. (B) Percentage of motile sperm after 120 minutes of exposure to PS-NP. Data are presented as mean ± standard deviation. Kruskal–Wallis test followed by Dunn's post hoc test (n = 7/group). a, b Different letters indicate statistically significant differences between groups ($p < 0.05$).

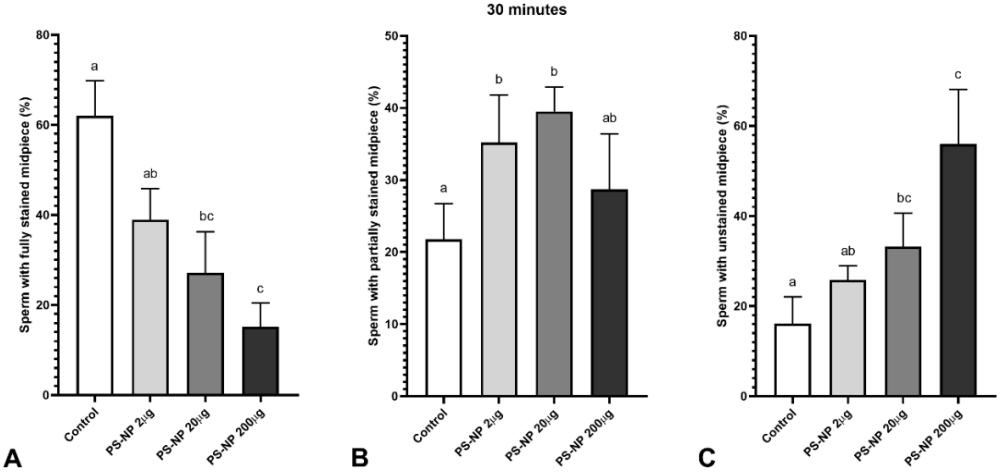
Regarding mitochondrial activity, after 30 minutes of in vitro exposure to NP-PS, concentrations of 20 μg/mL and 200 μg/mL significantly reduced the percentage of spermatozoa with fully stained midpieces compared to the Control group ($p = 0.0108$ and $p = 0.0001$, respectively - figure 2A). No significant difference was observed between the Control and NP-PS 2 μg/mL groups. The PS-NP 2 μg/mL and 20 μg/mL groups were statistically similar, as were the 20 μg/mL and 200 μg/mL groups.

Exposure to 2 μg/mL and 20 μg/mL NP-PS for 30 minutes increased the percentage of spermatozoa with partially stained midpieces ($p = 0.0252$ and $p = 0.0010$, respectively - figure 2B), compared to the Control group. No significant

difference was found between the Control and 200 µg/mL groups, nor between the NP-PS 2 µg/mL and 20 µg/mL groups.

An increase in the percentage of unstained spermatozoa was observed after exposure to NP-PS at 20 µg/mL and 200 µg/mL for 30 minutes ($p = 0.0398$ and $p = 0.0001$, respectively - figure 2C), compared to the Control group. No significant differences were detected between the Control and 2 µg/mL groups, nor between the 2 µg/mL and 20 µg/mL, or 20 µg/mL and 200 µg/mL groups.

Figure 2. Mitochondrial activity after in vitro exposure to different concentrations of PS-NP for 30 minutes.



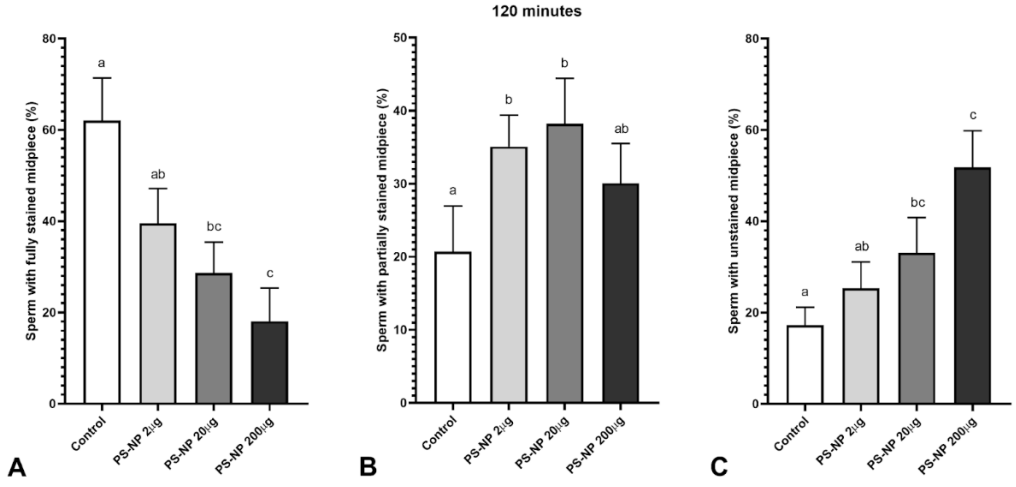
(A) Percentage of spermatozoa with a fully DAB-stained midpiece, indicating complete mitochondrial activity. (B) Percentage of spermatozoa with a partially DAB-stained midpiece, indicating partial mitochondrial activity. (C) Percentage of spermatozoa with an unstained midpiece, indicating absence of mitochondrial activity. Data are presented as mean ± standard deviation. Kruskal–Wallis test followed by Dunn's post hoc test (n = 7/group). a, b Different letters indicate statistically significant differences between groups ($p < 0.05$).

After 120 minutes of in vitro exposure, PS-NP concentrations of 20 µg/mL and 200 µg/mL significantly reduced the percentage of spermatozoa with fully stained midpieces compared to the Control group ($p = 0.0108$ and $p = 0.0001$, respectively - figure 3A). No significant differences were observed between the Control and 2 µg/mL groups, between the 2 µg/mL and 20 µg/mL groups, or between the 20 µg/mL and 200 µg/mL groups. However, the 2 µg/mL group differed significantly from the 200 µg/mL group ($p = 0.0342$).

At the same time point, PS-NP concentrations of 2 μg/mL and 20 μg/mL increased the percentage of spermatozoa with partially stained midpieces compared to the Control group ($p = 0.0193$ and $p = 0.0013$, respectively - figure 3B). No significant differences were observed between the Control and 200 μg/mL groups, nor between the 2 μg/mL and 20 μg/mL groups.

Exposure to PS-NP for 120 minutes also resulted in an increase in the percentage of unstained spermatozoa at 20 μg/mL and 200 μg/mL compared to the Control group ($p = 0.0457$ and $p = 0.0001$, respectively - figure 3C). No significant differences were found between the Control and 2 μg/mL groups, nor between the 2 μg/mL and 20 μg/mL groups, or between the 20 μg/mL and 200 μg/mL groups.

Figure 3. Mitochondrial activity after in vitro exposure to different concentrations of PS-NP for 120 minutes.



(A) Percentage of spermatozoa with a fully DAB-stained midpiece, indicating complete mitochondrial activity. (B) Percentage of spermatozoa with a partially DAB-stained midpiece, indicating partial mitochondrial activity. (C) Percentage of spermatozoa with an unstained midpiece, indicating absence of mitochondrial activity. Data are presented as mean ± standard deviation. Kruskal–Wallis test followed by Dunn's post hoc test (n = 7/group). a, b Different letters indicate statistically significant differences between groups ($p < 0.05$).

DISCUSSION

The widespread use of plastics has increased due to their physicochemical stability, high resistance to degradation, and low production costs[14]. Consequently, nanoplastic pollution has attracted growing scientific attention, particularly due to its ability to penetrate the human body. Exposure to PS-NP, the focus of this study, has been associated with intestinal cell inflammation and apoptosis, induction of oxidative stress[15], disruption of cholesterol metabolism and placental coagulation pathways[16], and respiratory health risks[7]. However, the effects of PS-NP on the male reproductive system, especially on human spermatozoa, remain poorly understood.

In this study, spermatozoa were isolated from semen using the swim-up technique in BWW medium[13], a method commonly applied to samples with normal sperm concentration ($\geq 15 \times 10^6$ sperm/mL). This technique selects motile sperm based on their ability to swim out of the seminal plasma into the culture medium, thereby normalizing sperm quality across samples[12]. Post swim-up, a considerable variation in sperm concentration among volunteers was observed (6.5×10^6 to 82×10^6 sperm/mL), with younger participants showing lower post-processing concentrations, possibly due to reduced semen quality compared to older volunteers (30–32 years). Similar trends have been reported in Brazilian men, where retrospective analysis of semen parameters between 1995 and 2018 revealed significant declines in total motile sperm count, normal morphology, and post-processing sperm concentration[17], highlighting a long-term reduction in male reproductive parameters.

Sperm motility is a key determinant of male fertility, as it enables sperm to migrate through the female reproductive tract, overcoming physical and chemical barriers to reach the oocyte[12,18]. Sperm are classified as progressively motile (active movement in straight or large circular paths), non-progressively motile (movement without forward progression), or immotile (no movement). In our analyses, in vitro exposure to PS-NP for 30 minutes at 20 μg/mL and 200 μg/mL significantly reduced the percentage of motile sperm, whereas at 120 minutes, only the 200 μg/mL

concentration had a similar effect. These findings are consistent with Contino et al. (2023)[19], who reported reduced sperm motility after exposure to PS-NP at 0.5 and 1 μg/mL for 30 minutes. The lack of effect at 2 μg/mL in the present study, compared to Contino et al., likely reflects methodological differences, as the latter employed a highly sensitive video-based CASA system, whereas our study used conventional light microscopy.

Animal studies corroborate the detrimental effects of PS-NP on sperm motility[20]. Observed impaired motility in BALB/C male mice exposed for four months to NP-PS at 0.1–5 mg/L, while Ebrahim et al. (2024)[21] reported reduced motility in adult Wistar rats after 60-day exposure to 3–10 mg/kg/day PS-NP. Despite differences in exposure duration, methodology, and concentration, these studies collectively indicate that NP-PS compromises sperm function, either via systemic bioaccumulation in vivo or direct effects in vitro, even at relatively low concentrations.

Mitochondria are essential organelles for sperm function, providing the ATP necessary for motility and fertilization. Structural or functional mitochondrial impairments can severely reduce sperm motility and fertilizing capacity[22]. In the present study, PS-NP exposure for 30 and 120 minutes induced concentration-dependent alterations in mitochondrial activity. Specifically, 20 μg/mL and 200 μg/mL reduced the proportion of sperm with fully stained midpieces, increased the proportion of unstained sperm, and 2 μg/mL and 20 μg/mL increased partially stained midpieces. These results align with Contino et al. (2023)[19], who observed similar mitochondrial impairments across concentrations from 0.1 to 1 μg/mL. Collectively, these findings indicate that PS-NP affects mitochondrial functionality, which in turn directly influences sperm motility. Impaired motility may hinder sperm transport to the oocyte, potentially compromising fertilization.

It is important to note that this study was based on a small sample size, which limits its statistical power and the generalizability of its findings. Nonetheless, the results are consistent with previous reports and provide valuable preliminary evidence that warrants further investigation. Although the reduced cohort increases

susceptibility to individual variability and requires cautious interpretation, the data remain robust and contribute meaningfully to the current understanding of microplastic toxicity.

CONCLUSION

In vitro exposure of human spermatozoa to polystyrene nanoplastics for 30 and 120 minutes significantly impaired motility, likely due to mitochondrial damage. Further studies with larger sample sizes are needed to elucidate the mechanisms and broader effects of PS-NP on human sperm.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this work.

REFERENCES

1. Fenichell S. Plastic: The Making of a Synthetic Century. New York: HarperBusiness, 1996.

2. Houssini K, Li J, Tan, Q. Complexities of the global plastics supply chain revealed in a trade-linked material flow analysis. Communications Earth & Environment. 2025; 6: 257. https://doi.org/10.1038/s43247-025-02169-5.

3. Plastics give and plastics take. Nature Reviews Materials. 2022; 7: 67. https://doi.org/10.1038/s41578-022-00419-y.

4. Rodrigues, ACB, de Jesus, GP, Waked, D, Gomes, GL, Silva, TM, Yariwake, VY, da Silva, MP, Magaldi, AJ, Veras, MM. Scientific Evidence about the Risks of Micro and Nanoplastics (MNPLs) to Human Health and Their Exposure Routes through the Environment. Toxics. 2022; 10(6): 308. https://doi.org/10.3390/toxics10060308.

5. Montes-Burgos K, Walczyk D, Hole P, Smith J, Lynch I, Dawson K. Characterisation of nanoparticle size and state prior to nanotoxicological studies. Journal of Nanoparticle Research. 2010; 12(1): 47-53. https://doi.org/10.1007/s11051-009-9774-z.

6. Winiarska E, Jutel M, Zemelka-Wiacek M. The potential impact of nano- and microplastics on human health: Understanding human health risks. Environmental Research. 2024; 251: 118535. https://doi.org/10.1016/j.envres.2024.118535.

7. Oliveira CRF, Maestri G, Tochetto G, Oliveira JL, Stiegelmaier E, Fischer TV, Immich APS. Nanoplastics: Unveiling Contamination Routes and Toxicological Implications for Human Health. Cuttent Analytical Chemistry. 2024; 21(3): 175 - 190. https://doi.org/10.2174/0115734110305991240523073849.

8. Han H, Chang Z, Xu B, Ding L, Yang H, He T, Du X, Pei X, Fu X. Integrated transcriptomic and metabolomic analysis reveals the underlying mechanisms for male reproductive toxicity of polystyrene nanoplastics in mouse spermatocyte-derived GC-2spd(ts) cells. Toxicology in Vitro. 2024; 100: 105893. https://doi.org/10.1016/j.tiv.2024.105893.

9. Gao X, Xu K, Du W, Wang S, Jiang M, Wang Y, Han Q, Chen M. Comparing the effects and mechanisms of exposure to polystyrene nanoplastics with different functional groups on the male reproductive system. The Science of The Total Environment. 2024; 922: 171299. https://doi.org/10.1016/j.scitotenv.2024.171299.

10. Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Jolles M, Pinotti R, Swan SH. Temporal trends in sperm count: a systematic review and meta-regression analysis of samples collected globally in the 20th and 21st centuries. Human Reproduction Update. 2022; 29(2): 157–176. https://doi.org/10.1093/humupd/dmac035.

11. Green, MP, Harvey, AJ, Finger, BJ, Tarulli, GA. Endocrine disrupting chemicals: Impacts on human fertility and fecundity during the peri-conception period. Environmental Research. 2021; 194: 110694. https://doi.org/10.1016/j.envres.2020.110694.

12. World Health Organization. WHO laboratory manual for the examination and processing of human semen. World Health Organization; 2021. https://www.who.int/publications/i/item/9789240030787.

13. Biggers JD, Whitten WK, Whittingham DG. Thoughts on embryo culture conditions. Reproductive BioMedicine Online. 2002; 4: 30-38. https://doi.org/10.1016/S1472-6483(12)60009-1.

14. Schirinzini GF, Pérez-Pomeda I, Sanchís J, Rossini C, Farré M, Barceló D. Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells. Environmental Research. 2017; 159: 579–587. https://doi.org/10.1016/j.envres.2017.08.043.

15. Hou Z, Meng R, Chen G, Lai T, Qing R, Hao S, Deng J, Wang B. Distinct accumulation of nanoplastics in human intestinal organoids. Science of The Total Environment. 2022; 83: 155811. https://doi.org/10.1016/j.scitotenv.2022.155811.

16. Chen G, Xiong S, Jing S, van Gestel, CAV, van Straalen NM, Roelofs D, Sun L, Qiu H. Maternal exposure to polystyrene nanoparticles retarded fetal growth and triggered metabolic disorders of placenta and fetus in mice. The Science of The Total Environment. 2022; 854: 158666. https://doi.org/10.1016/j.scitotenv.2022.158666.

17. Siqueira S, Ropelle AC, Nascimento JAA, Fazano FAT, Bahamondes LG, Gabiatti JR, Costa-Paiva L, Baccaro LF. Changes in seminal parameters among Brazilian men between 1995 and 2018. Scientific Reports. 2020; 10(1): 6430. https://doi.org/10.1038/s41598-020-63468-9.

18. Dcunha R, Hussein RS, Ananda H, Kumari S, Adiga SK, Kannan N, Zhao Y, Kalthur G. Current Insights and Latest Updates in Sperm Motility and Associated Applications in Assisted Reproduction. Reproductive Sciences. 2020; 29(1): 7–25. https://doi.org/10.1007/s43032-020-00408-y.

19. Contino M, Ferruggia G, Stefania I, Pecorato R, Scalisi EM, Bracchiatta G, Gragotto J, Salvaggio A, Brundo MV. In Vitro Nano-Polystyrene Toxicity: Metabolic Dysfunctions and Cytoprotective Responses of Human Spermatozoa. Biology. 2023; 12(4): 624. https://doi.org/10.3390/biology12040624.

20. Zhang X, Wu Y, Fu Z, He S, Shi L, Xu H, Shi X, Yang Y, Zhu Y, Wang Y, Qiu H, Li H, Li J. Toxicity to the Male Reproductive System after Exposure to Polystyrene Nanoplastics: A Macrogenomic and Metabolomic Analysis. Toxics. 2024; 12(8): 531. https://doi.org/10.3390/toxics12080531.

21. Ebrahim MM, El-Dakdoky MH, El-Shafiey SH, Amer AS. Oral exposure to polystyrene nanoplastics altered the hypothalamic-pituitary-testicular axis role in hormonal regulation, inducing reproductive toxicity in albino rats. Birth Defects Research. 2024; 116(6): e2368. https://doi.org/10.1002/bdr2.2368.

22. Lahnsteiner F, Mansour N, Berger B. Seminal plasma proteins prolong the viability of rainbow trout (Oncorynchus mykiss) spermatozoa. Theriogenology. 2004; 62(5): 801-808. https://doi.org/10.1016/j.theriogenology.2003.12.001.