## Proceedings of the 69th Congress of the Italian Embryological Group-Italian Society of Development and Cell Biology (GEI-SIBSC)

11-14 June 2024

Jointly organised by
University of Naples Federico II,
University of Naples "Parthenope"
University of Campania "Luigi Vanvitelli"

The conference will take place in Naples on 11-14 June 2024 at the two historical venues: Complex of Saints Marcellinus and Festus, in the Historic Centre, and Villa Doria D'Angri, on the Posillipo hill.

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## Published by PAGEPress, Pavia, Italy

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PAGEPress s.r.l.

via A. Cavagna Sangiuliani 5, 27100 Pavia, Italy Phone: +39.0382.1549020 - Fax: +39.0382.1727454

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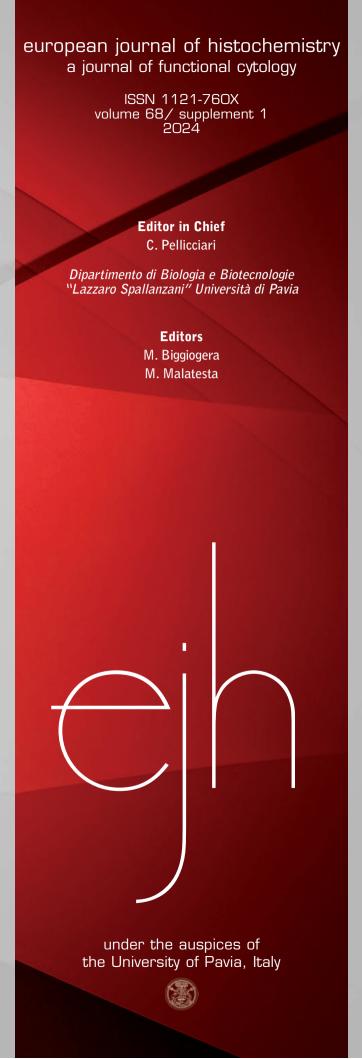
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Reg. Tribunale di Pavia n. 289/23.2.1984.

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The European Journal of Histochemistry was founded in 1954 by Maffo Vialli and published till 1979 under the title of Rivista di Istochimica Normale e Patologica, from 1980 to 1990 as Basic and Applied Histochemistry and in 1991 as European Journal of Basic and Applied Histochemistry. It is now published under the auspices of the University of Pavia, Italy. The European Journal of Histochemistry is the official organ of the Italian Society of Histochemistry and a member of the journal subcommittee of the International Federation of Societies Histochemistry and Cytochemistry (IFSHC), and has been an influential cytology journal for over 60 years, publishing research articles on functional cytology and histology in animals and plants.

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2022 Impact factor: 2.0. ©JCR Clarivate Analytics



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ISSN 1121-760X volume 68/supplement 1 2024

# EXPOSURE TO PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) INDUCES A SIGNIFICANT INFLAMMATORY RESPONSE IN THE MEDICINAL LEECH HIRUDO VERBANA

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Per- and poly-fluoroalkyl substances (PFAS) encompass a broad array of synthetically produced fluorinated compounds extensively used in many industrial fields, from the manufacturing of plastics items to the synthesis of firefighting aqueous film forming foams, food packaging, non-stick cookware, and cosmetics1. Although PFAS enhanced people everyday life, as already observed for other chemicals, their massive use and the consequent abundant release improved the risk of bioaccumulation. Indeed, PFAS not only were identified in many environmental matrices, but also can accumulate inside living organisms. In this context, despite various studies were performed, little data have been collected in particular on a new emerging PFAS generation, introduced on the global market in substitution of oldest compounds, whose dangerous potential has been already demonstrated<sup>2,3</sup>. Based on this evidence, here we propose the leech *Hirudo* verbana to investigate the effects of different PFAS (GenX, PFMoBa, PFOA and PFMOPrA) on freshwater organisms, testing two concentrations (0.6 and 229 µM). Morphological, immunohistochemical and molecular assays revealed a diverse modulation of both cellular and molecular innate immune response, in relation to the fluorinated chemical examined. In addition, in order to evaluate a possible activation of the oxidative metabolism, the levels of expression of the superoxide dismutase (SOD) and glutathione-stransferase (GST) have been analyzed. As already observed for plastics4, not only the medicinal leech represents a valid freshwater model to assess the impact of pollutants, but also this work allows to deepen the current knowledge on PFAS the potential effects.

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#### DEVELOPMENTAL DELAYS INDUCED BY PARTICU-LATE EXPOSURE FROM URBAN AND RURAL SOURCES: PRELIMINARY RESULTS

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The effects on development of  $PM_{10}$  collected from two different locations (urban, Milano city; rural, Bertonico, Lodi) were tested on *X. laevis* samples (R-FETAX protocol). At both sites,  $PM_{10}$ 

daily samples were collected on PTFE filters. Natural fertilized embryos were exposed during the whole test period (from midblastula to tadpole) to extracts obtained by brushing and washing filters in deionized water, diluted 1:10 in FETAX solution. Embryotoxicity tests were also performed on control PTFE water extracts. Samples were monitored for lethal effects during the full six-day test period. At the end of the test, tadpoles were observed under a dissecting microscope to evaluate any morphological alteration. The developmental degree to evaluate old- and youngfor-age phenotypes (YFA-OFA) was determined according to the previously described developmental scoring system. Tadpole length was measured in order to evaluate small- and large-for-age phenotypes (SFA- LFA). Data were modelled using PROAST software package (Bench-Mark approach) to describe effects considering chemical characterization in terms of mass concentration, elements, ions, and carbonaceous components. Preliminary results show that neither lethal nor malformative effects were recorded after the exposure to the control and test extracts. YFA phenotypes were dose-dependent relatively to PM10 mass, S, NO<sub>3</sub>-, NH<sub>4</sub>+. Effects were more pronounced in tadpoles exposed to extracts from the rural site, suggesting a role of rural environmental components (proximity to farms and agriculture activities, mixture effects?) that need further investigations.

# TYPE 1 DIABETES IMPAIRS RAT SPERMATOGENESIS INDUCING OXIDATIVE STRESS

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Type 1 diabetes (T1D) is frequently correlated with diminished male reproductive capacity, leading to aberrant spermatogenesis and diminished sperm parameters1. The intricate nature of maintaining reproductive homeostasis, coupled with the involvement of numerous factors, renders gametogenesis vulnerable to being compromised, thereby substantially diminishing gamete quality and fertilization capability<sup>2</sup>. Approximately 50% of such cases involve male infertility, primarily characterized by suboptimal sperm attributes including quantity, morphology, and motility<sup>3</sup>. In this study, ten adult male rats were divided into two groups: a control group and a T1D group, induced by a single intraperitoneal injection of streptozotocin. Results showed that T1D induces oxidative stress, as highlighted by increased TBARS levels, decreased activity of the antioxidant enzymes SOD and CAT, as well as increased levels of 4-HNE, a prominent by-product of lipid peroxidation. In addition, the impairment of the blood-testis barrier integrity was evidenced, as revealed by diminished levels of structural proteins (N-Cadherin, ZO-1, occludin, connexin 43, and VANGL2) alongside modified phosphorylation status of regulatory kinases (Src and FAK). Finally, data showed that T1D induced testicular inflammation and pyroptosis, as confirmed by increased levels of some markers, such as NF-κB and NLRP3. Interestingly, immunofluorescence analysis revealed that NLRP3 localized at the center of the developing acrosome of round spermatids in the control testis while, in T1D animals, its localization appeared wider in the same cells, and the fluorescent signal significantly increased. The combined data led us to confirm that T1D has detrimental effects on rat testicular activity. Moreover, a better comprehension of the molecular mechanisms underlying the association between metabolic disorders and male fertility could help to identify novel targets to prevent and treat fertility disorders related to T1D.