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University of Modena and Reggio Emilia

The conference will take place in Modena on 10-13 June 2025
at Complex of San Geminiano

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European Journal of Histochemistry

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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 for 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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MAIN LECTURES

TO TARGET AND TO PROTECT: NANOMEDICINE IN PRECISE DELIVERY TO SPECIFIC TARGETS

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Nanomedicine offers unparalleled potential for the targeted delivery of therapeutic agents to specific sites or cells within the body. This approach has shown particular promise in overcoming physiological barriers such as the blood–brain barrier, enabling the effective delivery of drugs to the brain. The success of nanomedicine-based vaccines for treating and preventing SARS-CoV-2 infection has highlighted the potential of nanocarriers in delivering nucleic acids to intracellular targets. A growing understanding of intracellular trafficking mechanisms is central to these advances, as these mechanisms determine the fate and efficacy of delivered therapeutics.

This talk will explore how nanomedicine can achieve precise delivery to both extracellular and intracellular targets, focusing on brain delivery and the lessons learned from recent vaccine developments.

THE PHYLOGENETIC DIMENSION OF HUMAN EVOLUTION

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The concept of the «Upper Paleolithic Revolution» (last 50-100 ka) is traditionally considered as marking a transition from the Middle to Upper Paleolithic, characterized by increased morphological variability in tool types (Aurignacian, Gravettian, Solutrean, Magdalenian), human art (Lion-Man, Venus of Willendorf, Lascaux cave paintings), and intentional burials. However, growing evidence highlights complex behaviors appearing earlier and elsewhere than the Upper Paleolithic Revolution, even among Neanderthals and *Homo heidelbergensis*. Examples include complex spatial organization (Bruniquel cave), the use of glue for tools, musical instruments (Divje Babe bone-flute), stencils and cave paintings (La Pasiega), and Krapina talons. Older evidence of intentional structures (like ‘engineered wood logs at Kalambo Falls, Zambia, 476 ka) and use of plant resources for intentional cooking (Gesher Benot Ya’aqov, 800 ka) further challenge the idea of a sudden «cultural modernity» exclusive to *Homo sapiens*. The difficulty in preserving and dating older organic materials like garments and shoes blurs the argument in favor and against the Upper Paleolithic revolution and shrouds the origin of cultural modernity in mystery. We will delve into the phylogenetic dimension of these changes, discussing how evolutionary rates of traits like brain size and shape can be analyzed, and noted along the long-cherished, well-known pattern of increasing brain size in *H. sapiens*. We will show the development of brain asymmetry, specifically the «Yakovlevian torque,» which is linked to cognitive abilities and lateralization. While *H. sapiens* shows the highest

evolutionary rate for asymmetry, a significant positive shift in the evolution of asymmetry is found in the clade including *H. heidelbergensis*, *H. neanderthalensis*, besides living humans. Evidence of handedness in stone tool production and the birth of symbolic culture (pigments, ornamentation) is intriguingly linked to increased brain asymmetry in Middle Stone Age humans. We will show that cultural modernity links to cognitive networks in the brain. The prefrontal cortex (PFC) and posterior parietal cortex (PPC) are highlighted as connected areas in primates supporting higher-order cognition. Cortical areas under selection in *H. sapiens* are consistent with the location of the default mode network (DMN), which is involved in functions like deconstructing surroundings into mental symbols and reconstructing internal worlds. The results suggest a real evolution towards increased cognitive abilities in anthropoid primates.

THE ROLE OF MOLECULAR INNOVATIONS AND EXTREME INTER-INDIVIDUAL GENETIC DIVERSITY IN DRIVING ADAPTATION IN MODEL ORGANISMS

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For many decades, our understanding of biological systems in several widespread and ecologically significant metazoan taxa has been hindered by the lack of genomic data. This has largely confined molecular investigations in non-model organisms to a limited set of evolutionarily conserved biological pathways shared with classical laboratory models, significantly underestimating the importance of evolutionary innovations that have led to the emergence of alternative adaptive strategies in major animal phyla (such as Mollusca) which diverged hundreds of millions of years ago from traditional model systems like vertebrates, *Drosophila melanogaster* and *Caenorhabditis elegans*. Today, the ability to sequence complete genomes at relatively low cost for virtually any organism has revolutionized this landscape, enabling the detection of hidden molecular signatures that often underlie unexpected taxonomic distributions of biological pathways. By lifting the lid on this Pandora’s box, comparative genomics is revealing that evolution has much more frequently than expected led to independent lineage-specific expansions, reductions, or even complete losses of gene families, as well as to the acquisition of orphan genes with novel functions, with important implications for the adaptation to specific environmental niches. At the same time, another long-overlooked aspect that is increasingly emerging in several metazoans is their remarkable level of interindividual genetic variability, which extends well beyond standard heterozygosity and includes gene presence/absence variation. Strikingly, in many cases, the dispensable fraction of animal pangenomes appears to be strongly enriched in genes involved in survival processes and defense systems, thereby offering a possible explanation for the extraordinary capacity of certain species to adapt rapidly in response to environmental stress and colonize new niches. In addition to its biological relevance, this remarkable yet still underestimated diversity has important implications for the interpretation of the unexpected variability commonly encountered in experimental studies of non-model organisms.

PARMIGIANO REGGIANO PDO CHEESE: HEALTHY ROLE IN HUMAN NUTRITION

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Parmigiano Reggiano (PR) is a raw-milk, hard cooked, long-ripened cheese, produced in a restricted area in northern Italy. This type of cheese has been suggested to bear by positive nutritional and health effects, which could be related to the cheese matrix, i.e. the components interaction and the unique structure of the product, rather than single nutrients alone¹. The interrelationship among cheese microbiota, cheesemaking process, ageing time and the specific peptides' profile of the ripened cheese before and after *in vitro* digestion is known and the potential influence of these peptides on human gut microbiota has been evaluated^{2,3}. The potential anti-diabetic activity of PR cheese was demonstrated over ripening time, strengthening the functional and healthy role of this cheese in human nutrition⁴. Studies allowed to speculate a potential prebiotic effect of PR, in the light of the consensus definition of prebiotic as "a substrate that is selectively utilized by host microorganisms conferring a health benefit"⁵, that expands the concept of prebiotic also to non-carbohydrates substances. Proteins content, 32,4% is characterised by a high biological value because contain high concentrations of all the essential amino acids. Parmigiano Reggiano is an important source of calcium and phosphorus and trace elements (selenium and chromium)⁶. 40-month ripened PR is a source of selenium and chromium, allowing to claim the health properties ascribed to food sources of selenium and chromium according to European Regulation 432/2012⁷. The overall nutritional value of Parmigiano Reggiano PDO could be a starting point to consider it as a functional food in human nutrition.

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ORAL COMMUNICATIONS

MODULATION OF MITOCHONDRIAL METABOLISM UNDERLIES VISIBLE/NIR LIGHT-INDUCED RESCUE OF GLUTAMATE EFFLUX IN ROTENONE-DAMAGED SYNAPTOSOMES

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Mitochondrial dysfunction in energy metabolism and glutamate release critically contribute to the pathogenesis of neurodegenerative disorders such as Alzheimer's and Parkinson's disease. This study investigated the effects of photobiomodulation (PBM) on cortical nerve terminals, called synaptosomes, applying the ENEA Trio diode laser system (Garda Laser S.A.S) at wavelengths of 450, 635, 810, 940, and 1064 nm, with powers of 0.1 W and 1 W for 60 seconds over an irradiated area of 1 cm². Oxygen consumption, ATP synthesis, lipid peroxidation, and glutamate release were analysed under physiological conditions or following rotenone-induced mitochondrial dysfunction. The results show that PBM modulates mitochondrial function in a wavelength- and dose-dependent manner. Specifically, irradiation at 635 nm caused an uncoupling between energy production and respiration, inducing lipid peroxidation, while the other wavelengths preserved mitochondrial efficiency without an increment in oxidative damage. Among them, 810 nm exhibited the most pronounced effects: at 1 W, it strongly increased ATP synthesis and dramatically evoked glutamate release. Conversely, stimulation at 0.1 W led to a more moderate ATP response and a glutamate efflux comparable to a near-physiological stimulation. Under Complex I inhibition by rotenone, PBM partially restored energy metabolism, suggesting a compensatory contribution from Complex II in sustaining oxidative phosphorylation. Moreover, under conditions of mitochondrial impairment, depolarisation promoted dysregulated glutamate efflux, which was partially attenuated by PBM at 810 nm and 0.1 W. These findings demonstrate that PBM acts directly on presynaptic mitochondria, enhancing ATP production and regulating glutamatergic transmission via mechanisms tightly linked to respiratory chain activity. Thus, PBM represents a promising therapeutic strategy to modulate neuronal communication in neurodegenerative conditions associated with mitochondrial impairment.

A TUNICATE MODEL OF BRAIN TURNOVER: STEMNESS AND DISEASE-LINKED GENES IN *BOTRYLLUS SCHLOSSERI*

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Understanding how stem cells regulate neural regeneration and

degeneration is central to tackling human neurodegenerative diseases. However, the molecular and cellular mechanisms underlying these processes remain poorly understood, particularly in adult systems. In this study, we investigated the colonial tunicate *Botryllus schlosseri*, a close invertebrate relative of vertebrates, which exhibits a unique life cycle of weekly tissue turnover and simultaneous brain degeneration and regeneration. This natural phenomenon allows us to study neurogenesis and neurodegeneration within the same organism and time frame, providing a powerful comparative model. Using single-cell transcriptomics, histology, RNA-FISH, behavioral experiments, and electrophysiological recordings, we identified the cellular diversity of the *Botryllus* brain including prospective neural stem cells and neuronal progenitors. Moreover, we identified conserved markers associated with human neurodegenerative diseases (e.g., APP, PARK2). Our findings reveal age- and cycle-dependent changes in brain cell number and function, implicating stem cell-derived regeneration in the maintenance of brain structure and activity. This work establishes *B. schlosseri* as a robust model for exploring evolutionarily conserved neural repair mechanisms and offers novel insights into genes and pathways with potential therapeutic relevance for neurodegeneration in humans.

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HEALTH RISKS OF POLYSTYRENE MICRO- AND NANOPLASTICS: AN *IN VITRO* STUDY ON HUMAN MESENCHYMAL STEM CELLS

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Polystyrene (PS) is one of the most commonly used plastic polymers in the world and one of the main components of plastic debris that are widely released in the ecosystem. Toxicity of micro and nano-plastics in aquatic life is well-documented, however, information in terrestrial species, and particularly in humans, is scarce, and very little is known about the impact of environment contaminants on stem cells. Herein, we used dental pulp stem cells to investigate cytotoxicity following their exposure to PS particles of 1 µm (PS1µm) and 100 nm (PS100nm) of diameter, evaluating also the potential release of substances in the culture medium after long incubation. Employing microscopy and molecular techniques we assessed the toxicity, the uptake, the cytoskeleton organization, and the modulation of genes involved in oxidative stress, inflammation, stemness, and senescence. Our results highlighted that, despite the cells being engulfed by PS, mainly internalized by endocytosis, cell viability was not affected by direct exposure but rather from the compounds released after their stay for a month in the culture medium. This finding was supported by atomic force observations which established that PS, after a month, appeared mushy and more clustered. Furthermore, in our experimental con-

ditions, gene expression did not result altered; however, we observed a decrease of actomyosin cortex complexes and an increment of irregular and granular phalloidin staining, sign of impaired actin polymerization. In conclusion, plastic persistence represents a threat due to the compounds released when PS are internalized in the human body, coming in contact with cells, particularly stem cells. With this work we believe to have contributed to better comprehending the mechanisms of plastic toxicity but also addressed to raise awareness on the risks of the exorbitant production, use, and abandonment of plastic in the environment.

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IN VITRO NEW APPROACH METHODOLOGIES (NAMs) FOR THE HAZARD ASSESSMENT OF NANOMATERIALS

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The extensive use of nanomaterials (NMs) in several applications poses concern about their potential hazard on the environment and human health. One of the main goals of the proactive European Safe and Sustainable by Design (SSbD) framework is to minimize the hazard of novel chemicals and products, including NMs and nano-enabled products, throughout their life cycle. The framework promotes the use of new approach methodologies (NAMs), such as *in vitro* and *in silico* methods, for assessing the NMs toxicity, but specific testing strategies are not yet well defined. In this work the potential hazards of copper and zinc oxide (CuO, ZnO) and silver (Ag) nanoparticles (NPs), used as antibacterial agents in the coating of textiles, were evaluated at each stage of the NMs life cycle. The importance of assessing NPs release, exposure routes and the level of complexity of the biological models is emphasised. After a physico-chemical characterization of the NMs, *in vitro* toxicological tests were performed based on an Adverse Outcome Pathways (AOPs) oriented approach. Classical submerged monocultures and co-cultures epithelial models exposed at the air liquid interface (ALI) were adopted for hazard assessment at synthesis and manufacturing phases, respectively. Data from monoculture models showed that NPs induce toxic responses and trigger key events (KEs) depending on the different NPs' properties. *In vitro* tests resembling realistic occupational inhalation doses revealed no significant cytotoxic and inflammatory responses. Also, transcriptomic analyses to screen differently expressed genes (DEGs) were performed on co-culture models exposed at the ALI to AgNPs human relevant doses. For skin contact exposure, Epiderm™ 3D *in vitro* model was used for the use phase according to the Skin Irritation test (OECD TG 439). Results showed that AgNPs are generally non-irritant to intact skin while CuO and ZnO NPs can cause irritation, especially under acidic sweat conditions. Overall, the study confirms that SSbD is a powerful strategy to guide safer innovation in nanotechnology and that performing hazard evaluation with NAMs and according to the AOP-approach may improve the understanding of the potential impact of next-generation NMs on human and environmental health, enhancing NMs safety and acceptance.

Acknowledgements

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FUNCTIONAL RESPONSES OF *MYTILUS GALLO-PROVINCIALIS* (LAMARK, 1919) AFTER SIMULATION OF DIESEL-OIL SPILL EXPOSURE

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Oil spills are one of the major causes of marine pollution, triggering severe impact on marine ecosystems. In this context, studying and investigating the responses of marine organisms is useful to monitor the health of the aquatic ecosystem. This work delves into the responses of the sentinel organism *Mytilus galloprovincialis* after short-term exposure to different concentrations of a hydrocarbon mixture (HC) simulating realistic oil spill scenarios, through a multi-comprehensive approach. Different concentrations of HC significantly affected the phagocytic activity of hemocytes. Antioxidant and inflammatory enzymes evaluated in the hemolymph and digestive gland were also modulated following HC exposure. Simultaneously, the activity of chaperonins HSP70 and HSC70 increased, suggesting their involvement in maintaining homeostasis. The tubular structure of the digestive gland, evaluated by histomorphological analysis, was also compromised after hydrocarbon exposure, leading hemocyte infiltration after exposure to all the concentrations, atrophy after exposure to low and high concentrations and alteration of epithelium after exposure to medium and high concentrations. Furthermore, Automated Ribosomal Intergenic Spaces (ARISA) and 16S rRNA gene sequencing analyses were also performed on hemolymph and digestive gland showing that different concentration of HC caused a shift in the microbial community favoring the growth of several known degrading bacterial genera. These results show how the evaluation of the responses of the sentinel organism *M. galloprovincialis* from different points of view is important to better understand the potential impacts of a hydrocarbon spill on marine ecosystem.

EFFECTS OF NOISE EXPOSURE ON A SESSILE INVERTEBRATE, THE ASCIDIAN *CLAVELINA LEPADIFORMIS*

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Noise is now considered an emerging pollutant that can affect different aspects of animal life. In particular, it is known that exposure to high frequency sounds can damage ciliated sensory cells

and impair their development with consequences on animal behavior. *Clavelina lepadiformis* is an ascidian belonging to the aplousobranchia clade, common in the shallow waters of the Mediterranean Sea, such in harbors where the maritime traffic and noise pollution are particularly intense. We exposed adults of *C. clavelina* carrying developing embryos for 24 h to a pink noise (higher intensities at 63-125 Hz), at different levels (120-180dB), in tanks using an underwater speaker. Then, we analyzed the effects on site-selection behaviors of the developed larvae by analyzing the spatial distribution of juveniles attached on the bottom of the tanks. Moreover, we performed a functional, ultrastructural and immuno-histological analysis of the sensory cells of the juveniles in order to identify alterations caused by chronic noise exposure. The results indicated that larvae avoided to settle near the noise source revealing that they can sense the sounds. Moreover, juveniles exposed to noise presented altered responses to sensory cells stimulation and impaired development of ciliated sensory structures as suggested by immunostaining and SEM analysis. These results suggest that studies on noise effects on invertebrate fauna deserve further considerations.

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AXIAL ELONGATION FROM TAIL BUD CELLS IN AMPHIOXUS EMBRYOS

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Following the completion of gastrulation, vertebrate embryos elongate their body axis primarily through the addition of cells to the posterior dorsal mesoderm and spinal cord. These cells are derived from a population of progenitors located at the posterior pole of the embryo, within a structure known as the tail bud. The behaviour of these progenitors is regulated by the combination of multiple signalling pathways, most notably Wnt and BMP signalling. Despite differences in morphology across vertebrate classes, this system appears broadly conserved and is generally regarded as a vertebrate novelty. However, the structure and function of the tailbud in invertebrate chordates remain poorly characterised, limiting our understanding of its evolutionary origins. In this study, we investigate the tailbud of developing amphioxus, an early-branching chordate that, owing to its relatively slow morphological and genomic evolution, represent a good proxy for the ancestral vertebrate. Using a combination of bulk and single-cell RNA sequencing, hybridisation chain reaction, and EdU labelling, we present a morpho-molecular characterisation of posterior cell populations in amphioxus embryos. Furthermore, we examine the genetic regulation of axial elongation dynamics, providing insights into cellular behaviour following Wnt pathway inhibition.

HUMAN CUMULUS CELLS RELEASE EXTRACELLULAR VESICLES CONTAINING miRNAs WITH A ROLE IN THE ACQUISITION OF OOCYTE DEVELOPMENTAL COMPETENCE

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The bidirectional communication between the oocyte and surrounding cumulus cells (CCs) within the cumulus-oocyte complex plays a key role in determining gamete quality and developmental competence. We previously demonstrated that germinal vesicle (GV) mouse oocytes matured to metaphase II (MII) on a feeder layer of CCs (FL-CCs) from competent oocytes can develop to the blastocyst stage, whereas those cultured on FL-CCs from incompetent oocytes arrest at the two-cell stage¹. Both FL-CCs release extracellular vesicles (EVs) containing 74 differentially expressed miRNAs of which 7 regulate 71 genes specifically involved in crucial functions of oocyte and follicle development². Here we translated our platform to the human model creating FL of CCs derived from either competent (BL-hFL-CCs) or incompetent (NoBL-hFL-CCs) human oocytes collected individually from women undergoing intracytoplasmic sperm injection. miRNome microarray comparison of EVs released by BL-hFL-CCs vs. NoBL-hFL-CCs revealed 90 differentially expressed miRNAs ($p < 0.05$), with 4 up- and 86 downregulated. Among them, 23 miRNAs are known to be involved in ovarian functions. *In silico* functional analysis identified target genes, 55 of which are in common to the group of the 71 genes described in mouse and are implicated in meiosis resumption, fertilization, follicle growth, and acquisition of oocyte developmental competence. These findings suggest a possible convergence in the molecular mechanisms underpinning the acquisition of mammalian oocyte developmental competence.

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SMYD3 CONTROLS MEIOTIC PROGRESSION AND OOCYTE COMPETENCE THROUGH SOMATIC–GERMINAL INTERPLAY IN THE OVINE FOLLICLE

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The success of late oogenesis requires a precise coordination between intra-oocyte signals and somatic cues under gonadotropin control. In this study, we identified SMYD3 as a critical modulator of meiotic cell cycle in the ovine Early Antral follicles using a 3D follicle-enclosed *in vitro* maturation (FEO-IVM) system.¹ SMYD3 inhibition induced a prompt resumption of meiosis through CDC25A activation in the absence of any gonadotropin stimulation thus indicating a clear role of methyltransferases in the maintenance of meiotic arrest. Notably, SMYD3 interferes also with early (MAPK1/3 and PDE5A pathways) and late (metabolic coupling between oocyte and cumulus cells) hCG-mediated mechanisms leading meiotic resumption by reducing dramatically the developmental competence of MII oocytes. In summary, this study identifies SMYD3 as a central epigenetic regulator of oocyte maturation. Its precise temporal modulation is essential for meiotic arrest, nuclear and cytoplasmic maturation, and inter-compartmental communication. Disruption of SMYD3 activity compromises oocyte quality and developmental competence, highlighting its pivotal role in ensuring the successful completion of oocyte maturation.

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MITOCHONDRIAL DYNAMICS IN T CELL PHYSIOLOGY

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Mitochondrial dynamics deeply affect cell physiology and are strictly regulated by mitochondria-shaping proteins, which balance mitochondria fission and fusion processes in response to cell needs. In recent years, we contributed to the field, demonstrating how DRP1-dependent mitochondrial fission is crucial in orchestrating processes determining T cell differentiation, homeostasis, efficiency and life, as well as an efficient immune-surveillance against solid tumors. In this scenario, we showed how the checkpoint inhibitor PD1-signaling is capable of directly hindering

mitochondrial fragmentation by downregulating DRP1, thus affecting the anti-tumoral activity of T cells in solid tumor microenvironment. We are now investigating the molecular mechanisms of this negative regulation, with the aim of modulating and by-passing it, through mitochondria, to therapeutically improve T cell efficiency and tumoral defense.

INTERORGAN CROSSTALK IN AMYOTROPHIC LATERAL SCLEROSIS: HOW mSOD1 NSC-34 MOTOR NEURON-LIKE CELLS' MICROENVIRONMENT MODULATES IMMUNE RESPONSE, MUSCLE DIFFERENTIATION, AND INTESTINAL INFLAMMATION

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Interorgan communication is important to maintain metabolic homeostasis under physiological conditions. This crosstalk is mediated by soluble molecules such as interleukins, cytokines, or other metabolites, but recently expanded to include extracellular vesicles (EVs). In Amyotrophic Lateral Sclerosis (ALS), it is widely suggested that the brain, immune system, skeletal muscle, and the gut interact and influence disease progression and symptoms. Here, we investigated the effect of the microenvironment generated by NSC-34 mSOD1 MN-like cells on Raw 264.7 macrophage polarization, C2C12 myoblasts-myotubes differentiation, and Caco-2 enterocytes behavior by co-culture experiments. We selected the mutations mSOD1G85R and mSOD1G37R, as previous experiments demonstrated the capability to burst into a significant inflammatory microenvironment. EVs released by NSC-34 mSOD1G85R MN-like cells influence the myoblasts-myotubes differentiation of C2C12 cells as demonstrated by analysing the expression of key genes involved in myoblast differentiation (MyoD and MyoG), maturation (MyoG and Mrf4), and muscle atrophy processes (Atrogin 1 and Murf1). Raw 264.7 macrophages were co-cultured for 24 h with MNs using a transwell system. We observed the activation of the MIF-NF- κ B-IBA1 axis driving in Raw 264.7 macrophages the increase of pro-inflammatory IL-1 β , IL-6, TNF- α and IL-8 transcripts, and in NSC-34 the increase of IL-6, IL-8 and IL-4 transcripts. Finally, NF- κ B as well as IL-1R, IL-1 β and IL-4 expression increase in Caco-2 cells cultured for 24 h with mSOD1 NSC-34; moreover, the induction of autophagy in Caco-2 cells has been observed only in the presence of NSC-34 mSOD1G37R. The results suggest the complex role of neuroinflammation in the progression of ALS, and EVs are confirmed as key mediators in the modulation of the immune response.

HISTOLOGICAL CHARACTERIZATION OF OVARIAN DEVELOPMENT IN BLUE CRAB (*CALLINECTES SAPIDUS*) DURING SPAWNING SEASON IN THE NORTH-CENTRAL ADRIATIC SEA

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The Atlantic blue crab (*Callinectes sapidus* Rathbun, 1896) is a euryhaline and eurythermal species native to the Atlantic coasts of the Americas. Although its widespread distribution across the Mediterranean basin is well documented, information on its reproductive patterns remains limited. This study presented the microscopical characterization of ovarian developmental stages in female blue crabs along the north-central Italian Adriatic coast, within the spawning period. Samples were collected off the coast of Ancona, in collaboration with local fishermen, between September and November 2023. Sex and biometric parameters were recorded, and ovary samples collected to characterize sex ratio, size distribution, gonadal stage, and gonadosomatic index (GSI) in female specimens. Quantification of eggs in ovigerous females was also performed. Data showed females as the dominant sex throughout the sampling period, with males being extremely rare. The collapse of female catches in early November, alongside the consistent presence of ovigerous females and strong female-biased sex ratio, supported the hypothesis that identified this area as a spawning ground. These findings align with reproductive patterns observed in the species' native range and other Mediterranean areas. GSI values associated to macroscopic and histological examination of ovaries supported that the end of the reproductive season corresponded to mid-November. Although no data were collected in August due to the fishing ban in the sampling area, the presence of non-ovigerous females with ovaries at the late primary growth (IPG) oocyte stage suggested that reproduction had likely begun in August. Finally, as expected, the number of eggs produced by a single female was positively correlated with female size and GSI values, and thus with the peak of the reproductive season. These results highlighted that fecundity in this species is related to female size and raised a new question regarding the factors that determine the onset of sexual maturity at varying sizes.

THE INTAKE OF MILK FROM DIFFERENT ANIMALS IMPACTS THE SPERMATOGENESIS PROCESS IN RATS

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Reproduction is a fundamental biological process for the preservation of species, governed by numerous environmental and nutritional factors¹. Diet plays a crucial role in modulating fertility, influencing key parameters such as semen quality and spermatogenesis efficiency. This study explored the impact of feeding milk from four different mammals (donkey, human, goat and cow milk) on spermatogenesis in *Rattus norvegicus* (Wistar strain), using histomorphological and biochemical approaches. Our findings

revealed a significant increase in the number of spermatozoa in the lumen of the seminiferous tubules of rats fed with donkey and human milk, compared to controls (rats that have not received any milk) and the other two experimental groups. This effect seems to be linked to the peculiar nutritional profile of donkey milk, which is low in fat and calories but rich in polyunsaturated fatty acids (PUFAs), that are known for their beneficial effects on cell membrane fluidity, sperm quality, hormone synthesis, and protection from oxidative stress². The presence of essential minerals, high levels of α -lactalbumin and β -lactoglobulin, and a high lysozyme content could further contribute to an optimal testicular microenvironment for spermatogenesis. The Western blot analysis showed a significant increase in the expression of the enzyme 17 β -hydroxysteroid dehydrogenase a key enzyme in testosterone biosynthesis across all experimental classes. This was particularly evident in rats treated with donkey milk, with the exception of those fed with cow milk. In the latter, histological analysis revealed a marked deposition of fibrotic collagen in testis, suggesting a potential inflammatory state, in line with what has been observed in other tissues³. Overall, the data obtained suggest a positive effect of donkey and human milk on testicular function and spermatogenesis, paving the way for future studies on the nutraceutical use of specific foods to support male fertility.

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MODULATION OF BROMODOMAIN AND EXTRA-TERMINAL DOMAIN (BET) PROTEINS DRIVES NEURONAL DIFFERENTIATION: INVOLVEMENT OF CHOLESTEROL METABOLISM AND AUTOPHAGY

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Neuronal differentiation is essential for nervous system development and plasticity, and involves the expression of specific proteins that drive both morphological and functional changes. Notably, neuronal differentiation is tightly linked to dynamic changes in cholesterol homeostasis. Epigenetic mechanisms play crucial roles in regulating differentiation in various cell types. In this context, Bromodomain and Extra-Terminal domain (BET) proteins act as epigenetic readers by recognizing acetylated histones and regulating gene expression. Although emerging evidence suggests that BET proteins may influence cell fate in diverse physiological conditions, their precise role in neuronal differentiation remains to be fully elucidated. To investigate this, we employed a loss-of-function strategy using JQ1, a selective BET inhibitor. Our findings demonstrate that BET inhibition promotes neurogenesis in cell culture models of neuronal differentiation. This pro-differentiating effect is associated with the modulation of key proteins involved in cholesterol metabolism. Furthermore, we observed that autophagy is required for JQ1-induced neuronal differentiation, suggesting a coordinated mechanism supporting

proper neuronal development and survival. *In vivo*, BET inhibition markedly increased the number of Doublecortin-positive neurons in the mouse subventricular zone and subgranular zone of the hippocampal dentate gyrus, indicating enhanced adult neurogenesis. Taken together, these results identify BET proteins as critical epigenetic regulators of neuronal differentiation and propose BET inhibition as a putative therapeutic strategy for neurological disorders characterized by impaired neurogenesis.

ROLE OF REELIN AND CAJAL-RETZIUS CELLS IN DEVELOPMENT OF THE CEREBRAL CORTEX

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The cerebral cortex is among the most complex structure of the mammalian brain. Mammalian corticogenesis ultimately leads to the formation of different cortical areas with peculiar cytoarchitectonic. A key factor in this process is the secreted glycoprotein Reelin, which plays a crucial role in organizing cortical structure, determining the final positioning into cortical layers of differentiating neural precursors. Reelin is secreted by the Cajal-Retzius cells, the first differentiated neurons in the mammalian cortex. Human Reelin variants are associated with epilepsy, autism, and cerebellar and cortical abnormalities. We are interested in unravelling Reelin and CR cells' role on cortical development and evolution. Constitutive Reelin knock-out murine models have elucidated major aspects of its function, but broad deletion masks region-specific effects. We took advantage of the *Cre-lox* system to generate conditional knock-out, enabling spatially and temporally restricted Reelin deletion in the telencephalon. In a mouse model where Reelin is deleted in the dorsal telencephalon only starting from E11, we observed only minimal defects in cortical layer-specific markers expression. In contrast, when Reelin is deleted in the whole telencephalon starting as early as E9.5, we observed a significant decrease in Reelin expression associated to an evident structural disorganization, and even a reversal of cortical layering. Moreover, using a transgenic line, we show that Reelin is expressed in pallial differentiated neurons and in specific subpallial domains, of the teleost fish *Danio rerio* during development. Studying Reelin expression and function in the non-laminated zebrafish pallium is instrumental to understand Reelin role on vertebrate telencephalon development. These results emphasize the spatial and temporal specific role of Reelin in modulating cortical development and suggest for Reelin a conserved role of Reelin in vertebrate brain evolution.

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GEOMETRIC MORPHOMETRIC ANALYSIS OF MULLIDAE SCALE OUTLINES: A STUDY OF INDIVIDUAL AND SPECIES-SPECIFIC SHAPE VARIABILITY

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The elasmoid scales of teleosts are structures of bony tissue located in the skin of the animal, with variable shapes across different species and different body regions of a single individual. To study this variability and to identify new criteria for discriminating species within the Mullidae family, a total of 339 scales were collected from various body regions of three goatfish specimens belonging to two different species, *Mullus barbatus* and *M. surmuletus*, of similar size (TL 21.8±3.3 cm). Morphological variations among scales were quantified through elliptical Fourier analysis applied to the outlines, followed by principal component analysis. The analyses were performed through the package Momocs in R environment. It was observed that, within a single individual, scales can be categorized into two generalized types: broad and short scales, and long and thin scales, which correlate with the curvature of the body. In particular, the latter are specific to more curved regions of the skin. The shape of the scales appears conserved between the two conspecific specimens but is distinctly different between the two species. This study highlighted that scale shape can, at least partially, be correlated with aspects of fish anatomy and body shape, providing interesting insights into their mechanical properties and into using them as a proxy to hypothesize tridimensionality in fish fossils. Conversely, interspecies variability seems a promising tool for taxonomic or systematic studies. This is particularly interesting as the outline-based geometric morphometric approach presented here is likely to be more easily automatized, lacking the process of landmark choice and positioning typical of the more commonly used landmark based geometric morphometrics.

HISTOLOGICAL AND CELLULAR RESPONSES OF TWO AQUATIC SPECIES EXPOSED TO GADOLINIUM

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The use of gadolinium (Gd) in the clinical imaging has led to its wide release in hospital sewage, posing an high concern for aquatic biota. Regarding its harmful biological effects, poor information exist on the uptake and cellular toxic mechanisms that Gd causes. This study focused on the histological and cellular effects of two forms of Gd (Gd oxide nanoparticles, Gd₂O₃; Gd chloride, GdCl₃)

on marine organisms. Bivalves (mussel *Mytilus galloprovincialis*) and fishes (sea bass *Dicentrarchus labrax*) were exposed to two doses of GdCl₃ and Gd₂O₃ (C1: 1 µg/L; C2: 10 µg/L) for 28 days (mussels) and 7 days (sea bass). Histological, molecular, and biochemical assays were applied on gills of both species. A relevant immune cell (haemocyte for mussels, eosinophilic and granulated cells for sea bass) infiltration in the gills, coupled with a rise in acid mucopolysaccharides cells, suggests a possible involvement of these cells in the Gd uptake. A general downregulation in the expression of antioxidant genes (SOD, CAT, GST) mixed to an enhanced lipid peroxidation, mainly in samples exposed to 10 µg/L of GdCl₃, indicates the Gd ability to interfere with the Ca²⁺ metabolism and other metal ions. The rise in gills of lactate dehydrogenase and acetylcholinesterase activity highlights a possible interference in mitochondrial activity and vesicles release. These results offer useful information in regard to the cellular mechanisms altered by Gd, supporting the use of new technologies for the wastewater treatment.

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POTENTIAL STRATEGIES FOR RESCUING CELLULAR AND BEHAVIORAL ENDOPHENOTYPES ASSOCIATED WITH THE AUTISM-LINKED R451C MUTATION IN NEUROLIGIN3

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Neuroligins are among the risk genes for autism spectrum disorders, being involved in the functional maturation of the synapse. In particular the substitution Arg451Cys (R451C) in the synaptic adhesion protein Neuroligin3 (NLGN3) promotes a partial misfolding of the extracellular domain leading to retention in the endoplasmic reticulum (ER) and reduced trafficking of R451C NLGN3 to the cell surface. By *in vitro* studies, through the screening an FDA-approved drug library we have selected glucocorticoid compounds favoring the exit of full-length R451C NLGN3 from the ER and improving stability and trafficking of the mutant protein to the cell surface. These results were consistent in HEK-293 cells overexpressing the mutant protein and in differentiated neurons derived from the staminal niches of the adult hippocampus of R451C knock-in mice¹. The adult monogenic mouse model for autism, expressing R451C NLGN3, is characterized by reduced NLGN3 protein levels and autism-like behaviors, such as social deficits^{2,3}. We have further characterized social behavior at three developmental stages: adolescence, young adulthood, and adulthood, in comparison to age-matched wild-type controls. Our findings revealed significant impairments in social habituation and social novelty preference in the R451C NLGN3 mice starting from adolescence when exposed to an unfamiliar conspecific. Additionally, abnormal aggression was observed exclusively in adult mutant mice. Since oxytocin has been proposed as a potential treatment for psychiatric disorders involving social impairments⁴, we administered acute intranasal treatment and examined its impact on social behavior in the R451C NLGN3 adult male mice.

Our results showed that oxytocin treatment rescued the social deficits and reduced the aggression behaviors associated to the R451C NLGN3 mutation.

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GADOLINIUM IMPAIRS MALE STEROIDOGENESIS: IN VIVO AND IN VITRO EVIDENCE

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The increasing use of gadolinium (Gd) in industrial and medical fields made it a hazardous environmental pollutant. Once ingested through water and/or food, Gd may potentially have toxic effects on all body districts. For the first time this study investigates the effects of Gd on the testicular activity. Adult male rats were allowed to drink GdCl₃ or Gd₂O₃ (10-20-40 mg/Kg bw) for 4 weeks. Following Gd treatment, a significant decrease in steroidogenic-related proteins (StAR, 3 β -HSD, 17 β -HSD) expression, testosterone and DHT levels, and spermatozoa number were observed. To clarify the cellular mechanisms underlying Gd-induced damage, we exposed mouse Leydig (TM3) cells to increasing concentrations (5-1000 μ M) of GdCl₃ or Gd₂O₃ for 24 h. The *in vitro* results showed a dose-dependent decrease in cell viability and also confirmed that both forms of Gd inhibited steroidogenesis-related proteins expression. Steroidogenesis is a multistep process taking place in mitochondria and endoplasmic reticulum, and Mitochondria-Associated Endoplasmic Reticulum Membranes (MAMs) play a key role. We found a decrease in mitochondrial membrane potential as well as in mitochondrial biogenesis (PGC1- α , NRF1, TFAM) and MAM (GRP75, VDAC) markers expression. Finally, the decrease in catalase, SOD1/2 protein levels evidenced an antioxidant system impairment in Gd-treated TM3 cells. The increase in BAX and Cytochrome C protein levels indicated apoptosis activation, due to the oxidative stress. In conclusion, our study highlights the effects and the relative intracellular mechanisms induced by Gd on the testicular function, laying the foundation for further research to understand its impact on male fertility.

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AN ARTIFICIAL INTELLIGENCE TOOL FOR PREDICTING BLASTULATION BASED ON THE OBSERVATION OF CYTOPLASMIC MOVEMENTS IN EARLY HUMAN EMBRYOS

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We are searching for molecular and cytological non-invasive markers predictive of mammalian oocyte and pre-implantation embryo developmental competence. By using time-lapse monitoring (TLM) we recently showed that cytoplasmic movements (CM) occurring during mouse oocytes GV-to-MII transition¹ and human preimplantation development² could be a valid non-invasive cytological marker of development to blastocyst. In the present study, images of 6052 human oocytes, fertilized by ICSI and cultured in TLM, were analysed using optical flow algorithms (Lucas-Kanade, Farneback) to generate CM time series. To predict blastulation of early embryos, data from the first 24hr (pre-cleavage) or 72hr (up to 8-cell) were used to train three AI models: ROCKET, LSTM-FCN or ConvTran. Analysing the first 24hr, models reached respectively a balanced accuracy of 64%, 63% or 67%, a sensitivity of 67%, 78% or 89% and an F1 Score of 62%, 64% or 69%. Analysis of 72hr improved balanced accuracy to 74%, 76% or 77%, sensitivity to 89%, 85% or 91% and F1 Score to 74%, 75% or 76%. Interestingly, TLM brought up a difference in the intensity of CM already at the very beginning of post-fertilization events, when the 1-cell embryo is about to cleave. These results reveal that the observation and AI-powered analysis of CM can unveil previously hidden cytoplasmic dynamics governing early development and be predictive of embryo developmental competence, paving the way for clinical applications.

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HEMOCYTE PROLIFERATION IN *POMACEA CANALICULATA* TISSUES: INSIGHTS FROM HEMOLYMPH COLLECTION AND TENTACLE REGENERATION

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In the absence of panhemocyte markers, the proliferation and maturation of molluscan hemocytes are poorly understood. *Pomacea canaliculata*, a freshwater snail, is emerging as a valuable immunobiological model, due to available omics resources, genome editing and documented hemocyte-based responses such as hemolymph repopulation and organ regeneration. Here, the expression of *Pc*-hemocyanin (Hc) (hemocyte marker), *Pc*-TGase2 and *Pc*-Hhex (hemocyte activation-/proliferation-related genes) was analyzed by RT-qPCR and FISH in tissue-resident hemocytes during hemolymph repopulation and organ regeneration. All the 3 target molecules were detected in the control posterior kidney (PK) “hemocyte islets” (potential hemopoietic sites) and in the anterior kidney (AK), which also contains hemocytes but no “hemocyte islets”. The expression of *Pc*-Hc, *Pc*-TGase2 and *Pc*-Hhex was quantified in the PK and AK at 18 and 24 h after a hemolymph withdrawal. *Pc*-Hc decreased in both organs, suggesting hemocyte mobilization, while *Pc*-TGase2 and *Pc*-Hhex expression increased significantly only in the PK, supporting its involvement in hemocyte proliferation and recovery. Hemocytes are also actively involved in the early stages of cephalic tentacle regeneration (CTR). FISH experiments on the regenerating blastema at 12 h post-amputation revealed infiltrating hemocytes expressing *Pc*-Hc, *Pc*-TGase2, and *Pc*-Hhex. Local upregulation of only *Pc*-Hc and *Pc*-TGase2, compared to intact tentacle, was confirmed by RT-qPCR, suggesting that hemocytes migrating into blastema may actively proliferate and contribute to clotting, immune defense and CTR. These results suggest that hemopoiesis in *P. canaliculata* may occur at multiple sites, depending on the nature of the stimulus. Whether proliferation is driven by resident hematopoietic precursors, circulating hemocytes, or both remains to be elucidated.

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INVESTIGATING THE MORPHOLOGY AND ULTRASTRUCTURE OF IMPG2-RELATED RETINAL DEGENERATION USING ZEBRAFISH KNOCK-OUT MODEL

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Retinitis pigmentosa (RP) is one of the most prevalent inherited retinal dystrophies, characterized by the progressive degeneration of photoreceptors. Recent studies have identified nonsense muta-

tions in the *interphotoreceptor matrix proteoglycan 2 (IMPG2)* gene as causative of autosomal recessive forms of RP in humans. *IMPG2* encodes a proteoglycan localized in the interphotoreceptor matrix (IPM), which surrounds the outer segments and ellipsoids of retinal photoreceptors and likely contributes to IPM integrity through interactions with other extracellular matrix components. We employed zebrafish as a model organism to investigate how *IMPG2* loss compromises extracellular matrix stability and leads to photoreceptor degeneration and functional retinal decline. Due to its retinal architecture, which closely resembles the human macula, zebrafish offer a powerful system for studying retinal diseases. We characterized the zebrafish *IMPG2* paralogues, *imp2a* and *imp2b*, and examined the morphological and ultrastructural consequences of gene loss in 7-day-post-fertilization (dpf) knock-out mutants. Our analyses revealed marked alterations in ocular morphology associated with the absence of these genes, highlighting their essential role in eye development and suggesting that matrix disorganization may drive disease onset. Ultimately, this study aims to validate the *imp2*-deficient zebrafish line as a model for screening therapeutic agents capable of preserving interphotoreceptor matrix integrity, with promising implications for treating a wide range of retinal degenerative diseases.

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SYNAPSE OR SURVIVE? COMPLEMENT REGULATORS IN THE GASTROPOD GANGLION TRANSCRIPTOME REVEAL CONSERVED MECHANISMS OF NEURAL PRUNING

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The Complement System (CS), a fundamental component of innate immunity, is increasingly recognized for its roles in the development and remodeling of the nervous system. In vertebrates, complement regulators such as CSMD1 modulate synaptic pruning and neuronal survival by controlling the opsonizing activity of C3 on self-tissues. The absence or dysfunction of these regulators leads to inappropriate C3 deposition and activation of phagocytic pathways, resulting in aberrant neuronal elimination. In this study, we present the first ganglion-specific transcriptome of the gastropod *Pomacea canaliculata*, generated using long-read Nanopore sequencing. Our analysis revealed the expression of multiple complement regulatory transcripts, including several specifically enriched in ganglionic tissue. These candidates exhibit structural and sequence features suggestive of functional homology with vertebrate CSMD1. The identification of such regulators in a lophotrochozoan model supports the hypothesis that complement-mediated synaptic remodeling predates vertebrates and may

represent an ancient, conserved mechanism for neural circuit refinement. These findings open new avenues for comparative studies on the neuro-immune interface and highlight the evolutionary depth of complement-based developmental control.

RARE-EARTH ELEMENTS (REE): A POTENTIAL HAZARD TO NON-TARGET ORGANISMS?

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Gadolinium (Gd), a rare-earth element (REE), is a lanthanide with ferromagnetic properties, used in electronics and medicine as a contrast agent for magnetic resonance imaging. As such, it is excreted entirely from the body and, therefore, the high solubility and the inability to mitigate its efflux by wastewater treatment plants determines its release and persistence in marine waters, closer to the drains of these factories. This raises concerns about the welfare of non-target organisms. Therefore, the aim of the study was to evaluate the temporal course of biological responses (T0, T7, T15, T28) to 1 µg/L and 10 µg/L of GdCl₃ and Gd₂O₃, taking the digestive gland as the target organ since it is predisposed to detoxifying activity in *Mytilus galloprovincialis*. Using a multi-bioindicator approach, the presence of hemocytes around the digestive tubules was observed at histological level. The analysis of the activity of enzymes involved in the antioxidant (SOD, CAT) and detoxifying (GST) processes showed alterations mainly affecting the GdCl₃ form, data supported by an increase in LPO. The qPCR expression relating to the *Cu/Zn* and *Mn SOD*, *CAT* and *GST* genes at T28 was characterised by a decrease for the exposure to GdCl₃ and an increase by Gd₂O₃. The data obtained consolidate the role of Gd as an emerging micropollutant, demonstrating its potential risk for non-target biota.

Acknowledgements

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DISCOVERY OF POTENTIAL BIOMARKERS IN MULTIPLE SCLEROSIS VIA CEREBROSPINAL FLUID PROTEOMIC PROFILING

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Multiple Sclerosis (MS) constitutes a long-term condition characterized as a multifaceted disorder, shaped by a variety of influences, where the interaction between genetic factors and environmental conditions plays a significant role in the initiation of the

disease. Understanding these differences can assist in anticipating the course of the disease and provide insights into the diverse responses of MS patients to a range of therapeutic strategies. The identification of key biomarkers enhances the precision of diagnosing patients, informs treatment approaches, and aids in evaluating potential outcomes. These biomarkers, including cytokines, chemokines, and RNAs, demonstrate the multifaceted involvement of the immune system in the pathophysiology and advancement of multiple sclerosis (MS). Unfortunately, none of them demonstrate specificity for MS. Here we define a proteomics MS signature by using mass spectrometry in cerebrospinal fluid (CSF) of MS patients ($n=15$) compared to neurological controls (NC) ($n=12$). A total of 964 proteins were identified. Among these, 72 were exclusively found in MS patients, while 46 proteins were differentially expressed between NC and MS. The protein profiles of individuals with multiple sclerosis include unique components such as proteolytic enzymes, factors that aid in cell adhesion, and proteins linked to stress and immune responses. Moreover, the results showed that IGFBP-2 levels increased, whereas IGF-2 levels decreased. Such a lack of equilibrium might weaken the neuroprotective functions of IGF-1, which could accelerate neurodegenerative processes. By employing machine learning, the investigation aimed to identify which proteins within the proteomic dataset could more effectively distinguish multiple sclerosis (MS) from normal controls (NC). This research highlighted proteins that can successfully differentiate between MS and NC.

The biomarkers related to proteomics that have been identified here contribute essential knowledge for effectively managing multiple sclerosis. IGFBP2 was validated as promising MS biomarkers in CSF.

UNVEALING THE BPA-INDUCED EPITHELIAL BARRIER DYSFUNCTION AND PROBIOTIC-DRIVEN EPITHELIAL RESTORATION IN HUMAN GUT ORGANIDS

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Endocrine Disrupting Chemicals (EDCs) are pervasive environmental pollutants that perturb cellular signaling, disrupting tissue architecture and function. Bisphenol A (BPA), a widely used plasticizer, is implicated in intestinal epithelial damage and homeostatic imbalance. In this study, we employed 3D human intestinal organoids derived from biopsies, physiologically relevant models recapitulating the human gut structure and formation. The aim of the present study is to investigate BPA-induced toxicity and the mitigating effects of the probiotic formulation SLAB51. Organoids were exposed to BPA at 0.56 pM, 56 pM, and 56 µM for four days, revealing clear impairment of morphogenesis, indicative of altered epithelial architecture. Co-administration of 10⁸ CFU of SLAB51, the most effective dose, notably preserved organoid morphology at 56 pM BPA. Spectroscopic profiling via

Fourier Transformed Infrared Spectroscopy showed BPA-induced accumulation of saturated fatty acids, while immunohistochemistry revealed reduced E-Cadherin signal suggesting loss of epithelial polarity and compromised barrier formation. SLAB51 co-treatment counteracted these alterations, restoring lipid homeostasis. In addition, SLAB51 mitigated junctional protein level, thus supporting epithelial integrity. Ongoing assays aim to further assess intestinal barrier functionality and stem cell-driven proliferation. This study provides morphological and molecular insights into EDC-induced toxicity in human gut organoids, offering a new insight on the role of targeted probiotics to restore epithelial differentiation.

D-ASPARTATE COUNTERACTS THE TESTICULAR DAMAGE INDUCED BY POLYSTYRENE MICROPLASTICS

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Polystyrene microplastics (PS-MPs) are a focus of attention among environmental pollutants, as all organisms are constantly exposed to them, and their impact on human health is inevitable. Recent studies demonstrated that PS-MPs penetrate the testicular parenchyma in mice and rat models, altering reproductive function. PS-MPs can cause inflammation, oxidative stress, apoptosis, and activation of autophagy in the testicular tissue with consequent damage to steroidogenesis and spermatogenesis. This study evaluated the potential role of D-Aspartate (D-Asp) in alleviating the PS-MPs-induced damage to the rat testis. D-Asp, an endogenous amino acid in vertebrate testis, possesses various biochemical and pharmacological properties, including antioxidant and anti-apoptotic ones. Furthermore, D-Asp activates steroidogenesis and spermatogenesis by promoting the expression of steroidogenic enzymes and proteins involved in germ cell proliferation. The experimental design consisted of D-Asp oral treatment, performed contemporaneously or given at different times with PS-MPs, to adult rats. The results showed that PS-MP adverse effects on testicular activity were reversed by D-Asp treatment, suggesting that D-Asp alleviates PS-MP toxicity. Mechanistically, D-Asp inhibited testicular oxidative stress by modulating the protein levels of CAT, SOD1, SOD2 and 4-HNE, TBARS levels, and reduced apoptosis, as suggested by CYT C analysis and TUNEL assay. Furthermore, D-Asp administration mitigated PS-MPs-induced autophagy activation by modulating the expression of LC3BI, LC3BII, and p62 proteins. Finally, the analysis of steroidogenic (StAR, 3 β -HSD, and 17 β -HSD) and spermatogenic (PCNA, SYCP3) markers, as well as sperm parameters, indicates that the amino acid counteracts PS-MPs damage by restoring normal steroidogenesis and spermatogenesis.

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DOUBLY UNIPARENTAL INHERITANCE OF MITOCHONDRIA IN BIVALVES AS A MODEL TO INVESTIGATE THE DYNAMICS OF MITOCHONDRIAL SEGREGATION DURING ANIMAL EMBRYO DEVELOPMENT

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The mechanisms that undergo mitochondrial segregation during animal cell divisions involve different cytoskeletal proteins, but it is less clear how they are regulated and to what extent an active selection is present. To investigate such matter, we leveraged an exception to strictly maternal mitochondrial inheritance that is present in some bivalve species: Doubly Uniparental Inheritance (DUI). In such species, due to maternal factors, paternal mitochondria in embryos show a sex-specific segregation path: in male embryos, they aggregate and localize at the cleavage furrow in the blastomeres of the D-lineage, eventually ending up in germ cells; in females, they are randomly dispersed across blastomeres. Leveraging the fact that some females of the DUI species *Mytilus galloprovincialis* produce offspring with extreme sex-ratio distortions, we sampled eggs producing either female-biased or male-biased progenies. Through total RNA-Seq, we identified nearly 250 genes differentially transcribed between the two. We tested whether these genes overlapped or interacted with genes we found showing convergent stronger purifying selection in 6 independent occurrences of DUI in Bivalvia. We obtained a network of interacting proteins enriched for microtubule-based movement functions, interestingly coherent with putative roles in the localization of paternal mitochondria. By identifying specific candidates responsible for DUI, we help the characterization of a unique exception that is however also useful to investigate overall mechanisms of mitochondrial segregation in the germline and during embryo divisions.

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CXCR1/2 INHIBITION MODULATES INSULIN RESISTANCE IN ADIPOCYTE AND HEPATOCYTE MODELS

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Insulin resistance (IR) is a central feature of type 2 diabetes (T2D), largely driven by chronic low-grade inflammation, where tumor

necrosis factor alpha (TNF α) is a key disruptor of insulin signaling in metabolic tissues such as adipose tissue and liver. Recent evidence has highlighted the chemokine receptors CXCR1 and CXCR2 as critical modulators of inflammation and insulin sensitivity; however, their specific role in the context of TNF α -induced IR remains poorly defined. In this study, we investigated the contribution of the TNF α -CXCR1/2 axis to IR by employing *in vitro* models of adipocytes and hepatocytes, which were challenged with TNF α to induce an inflammatory and IR phenotype. Pharmacological inhibition of CXCR1/2 using Ladarixin, as well as genetic silencing and antibody-mediated neutralization, were applied to evaluate their therapeutic impact. Our results demonstrate that TNF α treatment led to impaired insulin signaling, as evidenced by decreased Akt phosphorylation, reduced GLUT4 and GLUT2 expression, and elevated JNK activation and CXCL1 secretion. Notably, CXCR1/2 inhibition restored insulin responsiveness by improving glucose uptake, enhancing IRS1/2 and IGF expression, and normalizing mitochondrial respiration and glycolytic flux. In hepatocytes, Ladarixin treatment further promoted nuclear translocation of PPAR α and reduced lipid droplet accumulation and lipolysis, indicating improved metabolic balance. Collectively, these findings reveal a pivotal role for CXCR1/2 in mediating TNF α -induced insulin resistance and suggest that targeting this chemokine axis may offer a promising therapeutic avenue to restore insulin sensitivity in both hepatic and adipose tissues under inflammatory conditions associated with T2D.

IS MORPHOLOGICAL HETEROGENEITY OF EXTRACELLULAR VESICLES FROM TEMOZOLOMIDE-TREATED GLIOBLASTOMA MULTIFORME CELLS DRIVEN BY LIPIDS?

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Glioblastoma multiforme (GBM) poses a major challenge in health care being the most common malignant brain tumour in adults. One of the causes of the tumour's unresponsiveness to therapy is the establishment of the tumour microenvironment in which extracellular vesicles (EVs) play an integral role. EVs are nano- and micro-sized membrane-enclosed vesicles that are released by almost all types of cells. EVs carry bioactive cargo and can be internalized in recipient cells provoking a specific response. Formerly, we reported significant differences between EVs derived from four GBM cell lines (U87MG, U251MG, U373MG, T98G). We showed that treatment of GBM cells with a chemotherapeutic drug temozolomide (TMZ) altered the size, number, and protein cargo of released EVs, as well as the functional response in recipient GBM cells in terms of cell migration and cell death. Since, proteomics (LC-MS/MS) of EVs released from TMZ-treated cells showed an increase in the proteins involved in lipid biology, we performed an in-depth investigation of the EVs morphology and the role of lipids in the EVs-mediated cell-cell communication. Cryogenic electron microscopy indicated a great morphological heterogeneity (*i.e.* unilamellar, multilamellar vesicles; shape, membrane thickness) within EVs population and a modulation of their relative number. The dual role of lipids, as structural and functional molecules, was also evidenced by the fact that the

inactivation of proteins and/or RNAs present in EVs did not alter the EVs-mediated cell death. Therefore, we characterised neutral lipids by thin-layer chromatography indicating that TMZ-treatment affects lipid metabolism and profile in GBM cells and in the conditioned media. The data acquired so far will be extended to the analysis of GBM-EVs. Since the role of lipids in EVs is one of the most overlooked aspects, our preliminary data provides an initial valuable insight into the different contribution of EVs in cell communication.

INTERACTION BETWEEN MICROPLASTICS AND BISPHENOL A DURING EARLY STAGES OF ZEBRAFISH DEVELOPMENT

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Plastic in the environment represents one of the main problems in recent decades. Due to atmospheric phenomena, particularly the action of UV rays, plastic degrades into smaller fragments releasing microplastics which have a hydrophobic surface and can be carriers for other pollutants¹. Bisphenol A (BPA) is a plasticizer and one of the most prevalent environmental pollutants. It is known to be endocrine disruptor, and its toxicity has already been widely demonstrated². Our previous study showed how polystyrene microplastics (PS-MPs) of 1 μm and 3 μm in size interfere with zebrafish development, causing phenotypical alterations, apoptosis, an increase of heart rate and disorders in redox homeostasis³. Based on these data, the present study analyzed how PS-MPs affect the toxicity of BPA during early stages of zebrafish development. Zebrafish embryos were exposed to PS-MPs 1 mgL^{-1} and to BPA 25 μM both alone and in combination for 72 h and toxicity parameters, oxidative stress and expression of genes involved in development were analysed. The results showed that PS-MPs did not affect the hatching rate unlike BPA, which was observed to slow down the hatching process. Spontaneous movements were reduced during the single exposure of PS-MPs and BPA while the heart rate was accelerated with PS-MPs and decelerated in presence of BPA. During co-exposure, PS-MPs and BPA acted antagonistically. In fact, PS-MPs reduced the negative effects caused by BPA. Instead, PS-MPs and BPA caused alteration in redox homeostasis both alone and in co-exposure with synergistic action. Finally, the gene expression was down-regulated in the presence of PS-MPs and BPA alone, while an antagonistic effect of the two pollutants was found during co-exposure. These data highlight how microplastics affect BPA toxicity, but further investigations are needed to understand the mechanism of interactions between the two environmental pollutants.

Acknowledgements

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PHOTOBIO-MODULATION WITH VISIBLE/NIR LIGHT IMPROVES ASTHENOZOOSPERMIC SPERM METABOLISM VIA MITOCHONDRIA-NOS INTERPLAY OR MILD-THERMAL-MEDIATED EFFECTS

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Male infertility is a growing global concern, with asthenozoospermia representing a major contributing factor. In this study, the efficacy and safety of photobiomodulation (PBM) using visible and near-infrared light were tested on asthenozoospermic spermatozoa by evaluating mitochondrial energy metabolism (ATP, AMP, ATP/AMP ratio), oxidative stress damage (malondialdehyde, 8-hydroxy-2'-deoxyguanosine), and nitric oxide (NO) levels. Semen samples from 20 patients were irradiated with the ENEA Trio diode laser system (Garda Laser S.A.S) for 60 seconds over a 1 cm² area at five wavelengths (450, 635, 810, 940, 1064 nm) and four power outputs (0.25–0.50–1.00–2.00 W). The sample size was calculated based on prior data.¹ Statistical significance was assessed using the Shapiro-Wilk normality test, one-way ANOVA, and Sidak's post-hoc test for multiple comparisons. Results revealed a wavelength- and dose-dependent modulation of sperm bioenergetics: 810 nm at 1.00 W yielded the highest ATP/AMP ratio increase without inducing oxidative stress, while 635 nm significantly impaired mitochondrial activity and increased NO levels. These effects are attributed to mitochondrial chromophores stimulation (e.g., cytochromes and flavins), leading to enhanced oxidative phosphorylation (OxPhos), and potentially to photoacceptive sites within nitric oxide synthase. At higher wavelengths (940–1064 nm), local thermal interactions with lipids and water may alter membrane fluidity and mitochondrial function. NO analysis suggests a biphasic mechanism: low levels enhance OxPhos, whereas high levels (as with 635 nm) may inhibit complex IV, leading to energetic collapse. Therapeutic effects continued up to 60 min post-treatment without detectable DNA or membrane damage. In summary, PBM emerges as a safe and promising strategy to enhance sperm function by modulating mitochondrial metabolism and redox homeostasis.

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ORGANOID TECHNOLOGY: A NEW WAY TO MODEL ISCHEMIC STROKE

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Ischemic stroke (IS) is a major global neurological emergency, ranking as the second leading cause of death and the most common cause of disability.^{1,2} It is characterized by rapid disruption of brain blood flow, and it can cause irreversible damage to brain cells

leading to permanent disability or death. Currently, treatment options are limited and fail to completely prevent or reverse brain damage. Cerebral organoids are three-dimensional models that can reproduce the human brain's cellular composition, structure, and neuronal connectivity. In this work, we explore the use of hiPSC brain organoids as a model for studying ischemic stroke. Organoids were developed at different times to mimic the completed maturation of the human brain. Once the characterization of the organoids was completed, they were subjected to oxygen-glucose deprivation (OGD) as a model of IS. Viability and LDH assays were performed to evaluate the OGD effect. This study offers a valid platform for evaluating the efficacy of novel therapeutic approaches. In particular, the neuroprotective potential of neurotrophins was investigated, given their ability to promote synaptic regeneration and support functional recovery. The efficacy of neurotrophins was assessed in 50-day-old organoids subjected to OGD at various time points. Following OGD, organoids were treated with different concentrations of neurotrophins at different times. Overall, this study establishes a human-based *in vitro* model of IS, demonstrating significant cell death following OGD, which is reduced by neurotrophin treatment.

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COMPARATIVE ANALYSIS OF CANNABINOID RECEPTOR 1 LOCALIZATION IN SPERMATOZOA: FROM INVERTEBRATES TO MAMMALS

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The endocannabinoid system (ECS) is a deeply conserved regulatory network present across metazoans, playing essential roles in reproduction. Cannabinoid receptor 1 (CB1), a central ECS component, is implicated in sperm motility, capacitation, and acrosome reaction. However, its precise localization in spermatozoa remains controversial, with conflicting reports describing its presence in different sperm compartments. We hypothesized that CB1 localization in spermatozoa is evolutionarily conserved and correlates with species-specific chromatin compaction mechanisms. To achieve high-resolution visualization of CB1, we employed confocal and Airyscan super-resolution microscopy following immunocytochemistry on spermatozoa from diverse species with varying fertilization strategies and chromatin packaging modes: sea urchin, zebrafish, rooster, mouse, bull, ram, and human. In all species analyzed, CB1 was consistently localized along the sperm principal piece of flagellum, suggesting a conserved role in motility-related processes. Remarkably, CB1 was detected in the sperm head only in species displaying protamine-mediated chromatin compaction specifically in roosters (acrosomal localization) and

mammals (intracellular localization). No head localization was observed in sea urchin or zebrafish, where histones remain the primary chromatin component. In mammals, CB1 was found in a dotted pattern along both the tail and midpiece, and as discrete intracellular spots in the head, still present after acrosome reaction. These findings propose a conserved role for CB1 in mammalian sperm chromatin dynamics.

HEARING UNDERWATER: THE IMPACT OF NOISE ON THE MECHANORECEPTION OF THE COLONIAL ASCIDIAN *BOTRYLLUS SCHOSSERI*

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Although underwater noise is considered a highly pervasive pollutant in our basins, its effects on marine invertebrates are mostly unknown. Sessile animals, such as ascidians, cannot escape underwater noise generated by maritime traffic; nonetheless, they are provided with a variety of mechanoreceptors, possibly affected by noise. Among these receptors, there are both epidermal peripheral neurons (primary receptor cells), and the secondary sensory cells of the coronal organ considered homologues to vertebrate hair cells of the inner ear and the lateral line system. Both ascidian primary and secondary mechanoreceptor cells are involved in controlling the oral siphon activity, so in the physiological control of feeding and respiration, and in defense responses. Here, we present our results on the effects of anthropogenic underwater noise on the colonial ascidian *Botryllus schlosseri*. We exposed colonies, sampled in the Venetian Lagoon close to Chioggia (Italy) to noise (peak bands 63-125 Hz), mimicking the low frequency maritime traffic noise. After measurements of the lagoon soundscape, we tested noise levels (138.36-163 dB) comparable to those produced by boats passing close to Chioggia. To verify the effects induced by treatments, we used behavioral assays testing the mechanoreceptor ability to detect stimuli, and physiological assays. Results show that noise has negative effects on mechanoreceptors, reducing their sensitivity. The study evidences the necessity to monitor this pollutant for reaching the Good Environmental Status of European basins.

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EXPLOITING CALCOCO2-DRIVEN MITOCHONDRIA-NUCLEUS INTERPLAY TO OVERCOME MEDULLOBLASTOMA THERAPY RESISTANCE

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Medulloblastoma is a malignant developmental tumor that arises in the cerebellum, typically affecting children, and originates from immature neural cells. Recent progresses in genomics, singlecell sequencing, and novel tumor models have updated the classification and stratification of MB into 4 subgroups (Wingless, Sonic Hedgehog, Group3 and Group4), highlighting its complex intratumoral cellular diversity. The current standard of care includes neurosurgical resection, craniospinal irradiation, and cisplatin-based chemotherapy. To date, the role of mitophagy and whether mitochondria dysfunction could contribute to MB onset and aggressiveness is completely unknown. Here we identified a novel pronocogenic role for a mitophagy receptor CALCOCO2 as a regulator of MB cancer stem cells malignant phenotype. In details, we discovered 1) CALCOCO2 as a novel subgroup-specific oncogene in MB G3, whose upregulation acts as an initiating event in the development of MB G3; 2) a dual cytoplasmic-nuclear role for CALCOCO2 as crucial for regulating MB resilience to radiotherapy, highlighting its potential as a critical mediator in the cellular mechanisms that govern radioresistance; By unraveling the mechanisms underlying CALCOCO2's involvement in treatment resistance and metastasis, our research offers the opportunity to identify novel therapeutic targets and to develop personalized treatment to target specific vulnerabilities in MB G3 tumors.

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A LUNG CO-CULTURE MODEL TO EXPLORE THE INFLAMMATORY EFFECTS DETERMINED BY COMBINED EXPOSURE TO PM_{2.5}, SARS-CoV-2 AND NANO-ANTIVIRAL AGENTS

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Literature suggests that exposure to fine particulate matter (PM_{2.5}) may influence susceptibility and severity of viral infection in humans. PM_{2.5} may facilitate viral infection, thereby enhancing the pathogenicity of respiratory viruses¹. However, the underlying

mechanisms are not fully understood. The possibility to assess the biological responses in *in vitro* models resembling the *in vivo* cellular interplay at tissue level is of primary importance. This approach allows exploring the possible risk factors underlying the onset and spread of viral infections in relation to exposure to air pollution. Moreover, novel antiviral agents are being developed to prevent or fight against viral infections. In this perspective, silver (Ag)-based nanoparticles (Ag-NPs) are among the most exploited ones. In the present research an advanced experimental *in vitro* model has been set up to study the interplay among PM_{2.5} exposure, SARS-CoV-2 infection and Ag-NPs antiviral activity in human lung cells. A co-culture of differentiated macrophage-like THP-1 cells (dTHP-1) and A549 alveolar cells was exposed for 24 h to PM_{2.5} in combination or not with viral particles (inactivated SARS-CoV-2) to explore their possible synergistic effects. Then, the cytotoxic effects, immune response and inflammatory pathway were studied both in A549 monoculture and in the co-culture to determine whether dTHP-1 may increase the A549 response to PM_{2.5} and SARS-CoV-2. Our findings suggest that dTHP-1 cells may influence the response of epithelial cells to PM_{2.5} and SARS, exacerbating the inflammatory response caused by the viral infection. Ag-NPs doped with curcumin (AgCUR) were then administered to cells for additional 24 h to verify the anti-inflammatory or antiviral effects. Preliminary results are reported. These results deepen the understanding of the additional risk posed by PM in promoting and worsening virus-mediated respiratory disease in exposed populations and furnish a useful platform to test the efficacy and safety of novel nan antimicrobials.

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CROSS-TAXONOMIC INSIGHTS INTO LIFESPAN EVOLUTION: UNCOVERING GENETIC DETERMINANTS IN MAMMALS AND BIRDS

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Mammals are one of the most studied animal classes because of their widely diverse phenotypes and life history traits. Additionally, since humans are members of this group, results found in the class can be more easily transferred to our species. We focused our attention on lifespan, which ranges in mammals from 0.8 to 211 years. Leveraging this variability, we investigated patterns of molecular evolution along the mammalian phylogenetic tree for nearly 18,000 protein-coding genes, trying to associate independent evolutions of exceptionally long or short lifespans to signals of molecular evolutionary convergence. To do so, we exploited the ever-increasing number of genomes freely available online, in particular using the genomes published by the Zoonomia project, which produced a whole genome alignment for 241 placental mammals (80% of the extant families), a dataset exceptionally well-curated and documented. After removing confounding systematic factors, such as body mass and phylogenetic inertia,

our analysis revealed convergent evolutionary signatures in a subset of orthologous genes. Then, we placed these results into a broader comparative framework. Specifically, we compared them with findings from our previous similar study on birds. This approach aimed to identify possible recurrent molecular signals and candidate genes shared across both classes, suggesting the presence of common genetic background underlying extreme longevity. This comparative, cross-taxonomic approach underscores the value of integrating large-scale genomic data with evolutionary models to uncover the genetic bases of complex life history traits. Our results not only propose novel candidate genes for future functional validation but also emphasise the unique potential of comparative genomics to shed light on the molecular foundations of ageing.

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AVIAN LIFESPAN NETWORK REVEALS SHARED MECHANISMS AND NEW KEY PLAYERS IN ANIMAL LONGEVITY

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Lifespan is a highly variable life trait across the Tree of Life, governed by complex and multifactorial mechanisms. While some conserved pathways regulating longevity have been identified in various species, the molecular basis of this phenotype is far from being understood. In this context, the adoption of new model species and methods of investigation may offer opportunities to explore the molecular underpinnings of longevity in animals. In this study, we investigated the genomic resources of 141 birds and we analyzed the molecular evolution underlying extremely long- and short lifespans. We found that birds with similar lifespans exhibit convergent evolution in specific genes regardless of body mass and phylogenetic relationship, enabling the construction of a "lifespan network" of protein-protein interactions. This network highlights the interplay between metabolism and cell cycle control as key processes in avian lifespan regulation. This lifespan network not only provides evidence for shared mechanisms of lifespan regulation across different organisms but also enables the identification of new candidates for studying aging, particularly in humans. By integrating multiple evolutionary signals from both extremes of the lifespan distribution, our results show the power of evolutionary and comparative approaches in studying complex traits like longevity, providing new insights into aging research.

LESS IS MORE: PHYSIOLOGICAL DOSES OF VITAMIN B12 DRIVE IMPROVED RECOVERY COMPARED TO HIGH DOSE IN A H₂O₂-STRESSED NEURAL-LIKE SH-SY5Y CELL MODEL

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Vitamin B12 (VitB12) plays a crucial role in neural homeostasis and is commonly administered at high dose in cases of deficiency-related neural impairment. However, the dose-dependent effects and mechanisms of VitB12 in neural recovery remain underexplored¹. Using an *in vitro* neural-like model of differentiated SH-SY5Y cells exposed to H₂O₂, we compared recovery following supplementation with physiological versus high doses of VitB12. The physiological dose significantly enhanced cell survival, neurite outgrowth, and mitochondrial function. It also prompted earlier activation of antioxidant defences, suggesting a more rapid cellular adaptation to oxidative stress. Furthermore, single-cell Raman spectroscopy (SCRS) revealed lipid remodelling, while Oil Red O (ORO) staining highlighted lipid droplet formation during recovery with physiological VitB12, indicating late-stage mechanisms for managing oxidative damage. These results support the notion that a physiological dose of VitB12 promotes more effective recovery after H₂O₂ insult than a high dose by engaging timely antioxidant mechanisms and modulating lipid metabolism.

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DEVELOPMENTAL TOXICITY OF CuSO₄ ALONE AND IN MIXTURE WITH ZnSO₄, ON XENOPUS LAEVIS EMBRYOS (R-FETAX): PRELIMINARY RESULTS

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Trace essential metals, including Cu and Zn, are important pollutants in aerosols and aquatic environments. Preliminary results on the effects of Po Valley particulate matter extracts suggest a possi-

ble correlation between Cu/ Zn levels and *Xenopus laevis* developmental delays. The first aim of the present study was to evaluate stage-dependent embryotoxic effects of Cu (CuSO₄), considering lethality, teratogenicity (gross and ultrastructural morphology, evaluated using optical and scanning electron microscopy, respectively), developmental delays (FETAX-score method) and neurobehavioral development (swimming test). R-FETAX was applied, selecting three exposure windows: i) gastrulation (Nieuwkoop-Faber stages NF 10-13), ii) neurulation-neurogenesis (NF 13-40), iii) neuron migration/ innate neuromotor reaction (NF 40- 46). Additional groups exposed during the whole test period (NF 10-46) were included. CuSO₄ concentrations (0- 10- 20- 40 μM) were selected based on the literature and previous range-finding tests. Considering that metals are usually present in the natural environment as complex mixtures, the second aim of this work was to evaluate mixtures of CuSO₄ (0- 2.5- 5- 10- 20- 40 μM) and ZnSO₄ (0- 2.5- 5- 10- 20- 40 μM), using the fix-and-moving ratio approach. Mixture groups were exposed throughout the entire test period (NF 10-46). Data were modelled using the PROAST software package (www.proastweb.rivm.nl). Results suggest that CuSO₄ induces dose- and stage-dependent responses. The effects of CuSO₄ and ZnSO₄ mixture resulted in a complex picture, indicating the need for more detailed experiments.

EFFECTS OF DIFFERENT DIETARY PATTERNS ON BONE HEALTH: MOUSE MODELS

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Bone is a dynamic tissue that undergoes continuous remodeling to maintain its shape and structural integrity.¹ Bone remodeling, essential for maintaining bone health, involves the resorption of mineralized bone by osteoclasts, followed by the formation of new bone matrix by osteoblasts, which is subsequently mineralized. Various factors, including diet, influence this process. The aim of this study is to evaluate the effects of diets on femurs of mice fed for 16 or 20 weeks with normal diet (ND16w and ND20w) or Western diet (WD16w and WD20w) and for 20 weeks with their combinations with ketogenic diet (KD) (WD+ND20w, ND+KD20w, WD+KD20w). Micro-CT analysis on femoral cancellous bone revealed decreased bone volume fraction and trabecular thickness in mice fed a combined WD+KD20w compared to WD20w. Cortical bone thickness was significantly lower in mice fed WD16w and WD20w compared to those fed a ND16w and ND20w, as well as, in mice fed WD20w and WD+KD20w compared to WD+ND20w. Histological analysis revealed that the number of osteoclasts per bone perimeter on cancellous bone strongly increases in WD+KD20w compared with ND20w and WD20w. In addition, a combined KD produced a decrease of the number of osteoblasts in the cancellous bone. Whereas, in cortical bone, comparing WD+KD20w with WD20w an increase was observed, this suggests that the KD may have differential effects depending on the baseline condition. In conclusion, we demonstrated that the KD diet causes an alteration of bone remodeling by shifting it towards an increase in the number of osteoclasts and a reduction in osteoblasts. The combination of the normal diet and

the Western diet reduces the detrimental effects on bone of the Western diet alone.

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EXPOSURE TO HIGH FAT DIET AND DICHLORO-BIPHENYLETHYLENE ALTER LIVER PARENCHYMA AND INTRACELLULAR ORGANELLE'S STRUCTURE AND FUNCTION IN MALE WISTAR RAT MODEL

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Chronic exposure to a high fat diet (HFD) and environmental pollutants can cause liver damage and steatosis involving oxidative stress and organelle dysfunction. This study aimed to compare the effects of HFD and the environmental pollutant dichlorobiphenylethylene (DDE), a persistent metabolite of dichlorodiphenylethane on mitochondrial and endoplasmic reticulum (ER) alteration in rat liver. Additionally, the combined effects of HFD and DDE exposure were also examined. Four groups of rats were used: two groups fed with a standard diet (10% fat) were daily orally administered with vehicle (ND) or DDE at a dose of 10 mg/Kg bw (ND+DDE) for four weeks. Other two groups were fed with HFD (45% fat) and received vehicle (HDF) or DDE (HFD+DDE). Liver histology was examined by using Haemallume & Eosin. Mitochondria and ER morphology was detected by electron microscopy. Spectrophotometric assays were used to evaluate oxidative stress markers. Protein levels related to mitochondrial dynamics, ER stress, and inflammation were tested by western blotting. To evaluate apoptosis process, caspase-3 immunostaining and activity were performed. Results indicated that both HFD and DDE treatments alter liver parenchyma. HFD leads to lipid accumulation in hepatocytes, characteristic of steatosis, whereas DDE caused cell vacuolization and the formation of inflammatory foci. Both HFD and DDE exerted pro-oxidant effects as revealed by enhancement of ROS and lipid peroxides. They also promote increases in the levels of dynamic related protein 1, index of mitochondrial fragmentation, and the C/EBP homologous protein, index of ER stress. The two treatments also induce apoptosis by enhancing caspase-3 activity. Notably, the combined treatment with HFD and DDE did not produce additive effects; instead, the alterations observed in the liver and organelles were like those caused by each stressor, individually administered. This suggests that HFD and DDE may activate overlapping pathways that do not necessarily exacerbate each other's effects when combined. In conclusion, both HFD and DDE exposure induce alteration of liver structure and functionality by affecting organelle's function and by promoting inflammation, and apoptosis processes. These findings highlight the potential mechanistic links between dietary and environmental stressors in the pathogenesis of liver injury for developing strategies to prevent or mitigate liver diseases associated with lifestyle and environmental factors.

BISPHENOL A AND PROSTATE DYSFUNCTION: A HIDDEN HAZARD TO HUMAN HEALTH

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Bisphenol A (BPA) is a well-known Endocrine Disrupting Chemical (EDC) widely used to make materials in polycarbonate plastic and epoxy resins as well as plastic bottle, food packaging, household appliances, food cans, and metal jar lids¹. In the last decades, BPA massive use gained attention, becoming a concern issue for the human health, especially for the reproductive system due to its capability to mimic the actions of sexual hormones, such as 17- β -Oestradiol, Testosterone and Dihydrotestosterone, activating or inhibiting hormone-related pathways². In this work, we showed the effects of low dosage Bisphenol A (BPA) on normal prostate cell line (PNT1A) focusing our attention on interaction between BPA and Androgen Receptor (AR), that plays a crucial role in prostate development, maintenance, and function³. Our results highlighted the BPA antiandrogenic effect after 72 h of treatment: indeed, 1 nM BPA treatment induced a reduction of cell metabolic activity that was correlated with a decrease of cell proliferation rate. Moreover, 1nM BPA blocked AR in the cytoplasm, modulating the expression of genes AR-related, such as, Cyclin D1, and Ki67. Blocking AR with its specific inhibitor Enzalutamide (ENZA), the BPA effect was partially reduced, suggesting its capability to interact with other classes of receptors. To sum up, BPA exposure up to 72 h showed its antiandrogenic action, creating prone conditions to the genesis of hormone-dependent pathologies.

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MICROGLIA AND FLUOXETINE: NEW MECHANISMS OF PLASTICITY AND REORGANIZATION OF SEROTONERGIC SYSTEM

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Serotonin is a key neurotransmitter essential for functioning of central nervous system, influencing mood, sleep, appetite, and brain development. During development, serotonin shapes neural networks, modulating synaptic circuits and brain plasticity. Dysregulation of serotonergic signaling has been linked to neuropsychiatric disorders such as depression, which are often treated with selective serotonin reuptake inhibitors (SSRIs) that increase serotonin availability. Though, SSRIs therapeutic effect has a

delayed appearance, suggesting that rather than immediate serotonin level changes it is related to neural plasticity processes. Our lab has shown that the serotonergic system is highly plastic in key mood-regulating brain regions, such as the hippocampus and medial prefrontal cortex, , adapting to serotonergic homeostasis changes¹⁻⁴. This plasticity results from fluoxetine-induced serotonergic axon morphology changes and its antidepressant effects. Recent attention has focused on microglia, central nervous system immune cells, which also regulate synaptic plasticity and neural network reorganization. This study explores whether microglia mediate serotonergic fiber re-modelling during chronic fluoxetine treatment, via the serotonin 2B receptor (HTR2B). To this aim, *Tph2^{GFP}* knock-in mice, in which serotonergic circuitry is highlighted by GFP, were treated with fluoxetine and the HTR2B antagonist RS127445 for a 30-day period. Serotonergic fibers 3D-analyses revealed that HTR2B blockade through RS127445 hampers fluoxetine-induced serotonergic axon changes. To further confirm the HTR2B involvement in mediating fluoxetine effect, we are investigating whether HTR2B agonist BW723C86 treatment can mimic fluoxetine effect. The expected results could advance our understanding of the fluoxetine action mechanism and could have high translational value, contributing to the development of more targeted therapies for neuropsychiatric disorders.

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FAM46C IS REQUIRED FOR PROPER DIFFERENTIATION AND FUNCTIONALITY OF CD4⁺ iTREG CELLS

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FAM46C is a well-characterized tumour suppressor gene, initially identified in multiple myeloma and now recognized as a pan-cancer tumour suppressor. FAM46C belongs to a gene family that emerged evolutionarily in metazoans and vertebrates, suggesting a conserved role in complex multicellular processes. Despite its well-documented role in pathology, the precise mechanisms underlying its function remain under debate. Our laboratory has proposed a model in which FAM46C acts as a master regulator of intracellular trafficking dynamics, thereby influencing protein secretion and indirectly inhibiting autophagy. Beyond its role in cancer, FAM46C has been implicated in key developmental processes, including spermatid maturation and plasma cell differentiation. Here, we present the first evidence of its involvement in CD4⁺ T cell biology. We demonstrate that FAM46C is highly expressed in CD4⁺ regulatory T (Treg) cells, and that its expression during Treg differentiation is critical for maintaining their suppressive function. Specifically, we show that reduced FAM46C levels during *in vitro*-induced Treg (iTreg) differentiation impair the secretion of specific cytokines, without affecting their transcript levels, ultimately leading to diminished suppression of CD4⁺ T cell proliferation. These findings suggest that FAM46C functions as a post-transcriptional regulator of cytokine secretion in Treg cells, expanding its functional repertoire beyond cancer

and development. Ongoing studies aim to elucidate the precise molecular mechanisms underlying this regulation.

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EXPLORING EPIGENETIC REGULATION IN THE AGING PROCESS: INSIGHTS AND IMPLICATIONS

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Aging is a complex biological process marked by a gradual and time-dependent decline in tissue and organ function, increasing susceptibility to various chronic diseases. While much research has focused on the genetic determinants of aging, recent attention has shifted toward non-genetic factors, particularly epigenetic mechanisms. These mechanisms are gaining interest for their critical role in shaping the transcriptional programs associated with aging and mediating environmental influences such as diet, exercise, toxins, and stress. The highly dynamic nature of chromatin is essential for regulating gene expression, and its remodeling can lead to aberrant transcription patterns that disrupt cellular homeostasis¹. Although several studies have highlighted the connection between epigenetic alterations and aging in different tissue contexts, detailed insights remain limited. Our current findings reveal a key role for epigenetic regulation of enhancer activity by the histone acetyltransferase p300 in the aged heart, highlighting how these enhancer regions control the expression of genes involved in glycolysis, ultimately contributing to age-related decline in cardiac function². Other tissues that are highly impacted by aging are liver and muscle. In the liver, significant loss of regenerative capacity, decline in hepatocyte numbers and gradual alterations in structure and function have been observed³. In muscle, age-related degeneration leads to a progressive loss of mass, strength, and function⁴. In both cases, however, the underlying epigenomic modifications governing such changes are largely unknown. Therefore, we aim to investigate age-related changes in the epigenetic landscape of these tissues to better understand the interplay between epigenomic and transcriptomic dynamics governing aging-associated gene expression changes.

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BET PROTEINS MODULATE REDOX METABOLISM AND MITOCHONDRIAL HOMEOSTASIS: IMPLICATIONS FOR NIEMANN-PICK TYPE C DISEASE

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Bromodomain and Extra-Terminal (BET) proteins are epigenetic readers that bind to acetylated lysines, promoting chromatin relaxation and subsequent gene transcription. Their importance has emerged in various tissues including the brain, where these proteins were found to be deeply involved in the regulation of neurotransmitter metabolism, neurotrophic factor expression and neuroinflammatory pathways, thus strongly contributing to the central nervous system homeostasis. Additionally, BET proteins have emerged as key targets in counteracting various neurological disorders, considering their profound entanglement with organelle homeostasis, redox balance, autophagy and cholesterol metabolism. Particularly, Niemann Pick type C disease (NPCD) is a rare genetic disorder caused by mutations on *NPC1* and *NPC2* genes provoking intra-lysosomal cholesterol overload, autophagy derangements and mitochondrial aberrations. The onset and severity of this disease may vary depending on the degree of disturbances in cholesterol trafficking, including cerebellar ataxia, seizures, dementia and overall neurological decline. Current therapeutic strategies rely on palliative care and disease modifying drugs, thus highlighting the need to find new treatment strategies that can arrest NPCD progression. The aim of this study was to dissect the effects of BET proteins inhibition by JQ1 in NPCD patients-derived fibroblasts, with particular interest towards oxidative stress. Our data indicated that JQ1 treatment displayed antioxidant activity when evaluating oxidative damage markers upon macromolecules. This event was attributed to both a boost in the expression and activity of enzymes involved in reactive oxygen species (ROS) scavenging and to the downregulation of enzymatic modulatory subunits involved in pro-oxidant functions. Amongst other beneficial effects exerted by JQ1, we observed a rescue in mitochondrial connectivity, suggesting a recovery in morphological features and functionality presumably dependent on both the clearance of damaged organelle and their biogenesis. Collectively, these results highlight the potential of BET proteins inhibition as a novel therapeutic strategy to mitigate redox alteration NPCD.

TRANSGENIC ZEBRAFISH EMBRYOS FOR *IN VIVO* EVALUATION OF NANOCARRIER-DELIVERED PACLITAXEL

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Zebrafish represent a well-established *in vivo* model for investigat-

ing the behaviour, safety, and design of nanocarrier-based drug delivery systems. In this study, we examined the potential of liposomal formulations for the delivery of Paclitaxel (PTX), a widely used chemotherapeutic agent whose clinical application is often limited by chemotherapy-induced peripheral neuropathy (CIPN). Exploiting the genetic and physiological similarities between zebrafish and humans, we compared the effects of free PTX with those of PTX encapsulated in various liposomal systems. The high degree of genetic conservation -with approximately 70% of human genes having a zebrafish orthologue- enables the study of molecular pathways involved in drug-induced toxicity and oxidative stress. Initially, a transgenic zebrafish model was used to define the systemic response to increasing concentrations of PTX, guiding the selection of doses for subsequent liposomal encapsulation, both with and without functional modifications. We then evaluated the ability of these PTX-loaded liposomes to mitigate PTX-induced toxicity through molecular, histochemical, and behavioural analyses, highlighting their potential to reduce adverse effects while maintaining therapeutic efficacy.

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NANOPARTICLE-BASED CARRIERS TESTED IN EMBRYO MODELS

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This study is conducted within the framework of the National Center for Gene Therapy and Drugs based on RNA Technology – PNRR Spoke 8 "Platforms for RNA/DNA delivery", Task 8.2.2 "In embryo models", which aims to develop and validate delivery systems for RNA/DNA-based therapeutics. mRNA-based approaches offer great promise for precision and regenerative medicine, but achieving efficient and specific delivery during early development remains a major challenge. To address this, we use the zebrafish (*Danio rerio*) embryos as a model to assess nanoparticles (NPs) biodistribution, targeting and toxicity *in vivo*. We first established a benchmark protocol using CAP1 eGFP mRNA (Tebubio) encapsulated in Lipofectamine™ 2000 (Invitrogen), injected in the yolk, near the Duct of Cuvier at 48 h post fertilization (hpf). Fluorescence was observed in the heart and pectoral fins up to 72 h post injection (hpi), providing a reference for evaluating novel formulations under developmental conditions. To explore alternatives to liver-targeting carriers, we tested different formulations of poly(lipoic acid) NPs, differing in core chemistry (glycerol, octane diol, TEG derivatives) and properly labelled. These were injected into Tg(fli1:EGFP)^{y1} zebrafish embryos to monitor vascular biodistribution via confocal microscopy, showing no toxicity or developmental impairment. Ongoing experiments involve the same NPs loaded with mRNA to

evaluate delivery capacity. Additionally, labelled lipid nanoparticles, functionalized with various targeting peptides, were tested in Tg(tg:EGFP-my17:EGFP)ia300 embryos. Despite their presence in the heart and caudal vessels by 1 dpi, specific targeting to cardiac tissue was not confirmed. Parallel studies in synthetic mouse embryos are ongoing to broaden model applicability. This embryo-based platform offers a real-time, ethically sustainable strategy to evaluate RNA/DNA nanocarriers in a developmental context, in line with PNRR's objectives.

UNCOVERING NOVEL DEVELOPMENTAL ROLES FOR TCF4 IN THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM TO ELUCIDATE UNDERSTUDIED ASPECTS OF PITT-HOPKINS SYNDROME

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TCF4 loss-of-function variants cause Pitt-Hopkins syndrome (PHS), a rare neurodevelopmental disorder characterised by intellectual disability, distinctive facial features, intermittent hyperventilation followed by apnoea, motor incoordination and intestinal constipation. This plethora of symptoms reflects the widespread distribution of TCF4 in different embryonic cell types. Previous studies have focused on TCF4 function in a subset of brain areas involved in cognition, while its role in several other brain regions remains unexplored. Similarly, the biological causes of craniofacial dysmorphism, muscle hypotonia, motor incoordination and gastrointestinal constipation are unknown. Elucidating these aspects is essential to understand the requirement for TCF4 during development and to plan interventions that could have a significant impact on patients' quality of life. To this end, we have recently generated a new model of PHS in zebrafish using the CRISPR/Cas9 approach. *Tcf4*^{+/-} embryos were studied at different developmental stages to analyse craniofacial morphogenesis, central and peripheral nervous system development, neuro-muscular development and enteric nervous system development. Characterisation of heterozygous embryos revealed phenotypic impairments that may resemble the characteristics of Pitt-Hopkins patients. To assess functional impairment in *tcf4*^{+/-} embryos, we also perform behavioural tests to assess larval motor activity and investigate enteric nervous system functionality by visualising and quantifying *in vivo* gut peristalsis and gut mobility. A transcriptomic analysis of *tcf4*^{+/-} embryos and embryos overexpressing *tcf4* is underway to reveal novel tissue-specific *tcf4* targets. We hope that this project will disclose new developmental roles for *tcf4* and pave the way for planning new intervention strategies to improve the quality of life of PHS patients.

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INHIBITION OF *FOSILA* ATTENUATES ASTROGLIOSIS AND NEURONAL CELL DEATH IN THE EMBRYONIC BRAIN OF A NEW TAY-SACHS DISEASE ZEBRAFISH MODEL

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Lysosomal storage disorders (LSDs) are rare metabolic diseases that are caused by deficient lysosomal enzyme activity. The GM2 gangliosidosis, a subclass of LSDs, are a group of related illnesses brought on by a deficiency active β -hexosaminidase (Hex). Gangliosides are a type of glycosphingolipids involved in the formation of lipid rafts in the cellular plasma membrane. Tay-Sachs disease (TSD) is characterized by autosomal-recessive mutations in the hexa gene, with the accumulation of GM2 in the lysosomes of neuronal cells. The incidence is 1:320000 live births. The progression of the disease is variable, depending by the form. Infantile TSD is a lethal form, patients die before the age 3-5 years. These patients present macrocephaly, mental retardation, visual and motor system dysfunction. The specific cellular and molecular mechanisms underlying the rapid progression of the disease have not yet been elucidated. In our study, we generated a new zebrafish animal model to determine the impact of hexa-deficiency during brain development. Morphant and *hexa*^{-/-} mutant (generated by CRISPR/Cas9 system) presented macrocephaly, visual and motor dysfunctions, associated to astrogliosis, vacuolated macrophages and loss of neurons in the embryonic brain of hexa-deficient animals. In addition, we used RNA-seq approach to identify the altered pathways in the brain of our zebrafish model. We identified *fosIla* transcription factor as a potential target, the inhibition of which allowed to rescue the pathological phenotypes of *hexa*^{-/-} mutant. This study delves into the intricate mechanisms governing embryonic development, aiming to elucidate the molecular choreography orchestrating the formation of complex biological structures. Through a comprehensive examination of gene regulatory networks, signalling pathways, and epigenetic modifications, we unveil the dynamic interplay that shapes cellular fate and tissue patterning during embryogenesis. Furthermore, we explore how disruptions in these processes can lead to developmental abnormalities and disease pathogenesis.

HISTOLOGICAL, MOLECULAR AND CHEMICAL EVALUATIONS OF DOG TESTICLES FROM HIGHLY POLLUTED AREAS IN CAMPANIA REGION

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In Campania Region an area known as "the Land of Fires" is con-

sidered an open-air dump due to illegal toxic waste disposal, raising concerns about environmental and reproductive health.¹ Considering that humans and dogs share the same environment, leading to exposure to the same pollutants such as Heavy Metals (HMs), this study aimed to investigate the correlation between HMs exposure and testicular diseases in dogs. Fifty puberal male stray dogs living in high polluted areas in CR were enrolled for the study. A total of 100 testicular samples were collected and analysed by histological, molecular and chemical methods. H&E stain assessed baseline morphology while immunofluorescence (IF) allowed to evaluate the localization of Connexin-43, α -SMA, and Vimentin. Immunohistochemistry (IHC) and Western Blot (WB) analyses were used to evaluate 17- β -HSD, P450 aromatase, and PCNA. Heavy metals (Pb, Cd, Hg, As, Sn, and depleted U) were quantified by Inductively Coupled Plasma Mass Spectrometry. Based on histological evaluation, samples were classified into four groups: A) complete spermatogenesis, B) incomplete, C) absent and D) neoplasia. IF revealed altered localization of junctional and cytoskeletal proteins in groups B–D. IHC and WB showed significant downregulation of 17- β -HSD and upregulation of P450 aromatase in groups C-D while PCNA levels were unchanged. Chemical analysis revealed a dose-dependent accumulation of HMs, with highest concentrations in group D (91%) followed by C (36%), B (31%), and A (27%). All neoplastic testes (D) showed aberrant accumulation of depleted U. These findings suggest that environmental HMs exposure may alter testicular architecture and enzyme expression, contributing to spermatogenic failure and neoplastic transformation in canine testicles.

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EXTRACELLULAR MATRIX AS A PROMISING TOOL TO REGENERATE THE HEART

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Previously, it has been demonstrated that zebrafish (ZF) injured hearts can regenerate efficiently in *ex vivo* culture using a cocktail of growth factors (GFs) in approximately 40 days.¹⁻³ The epicardial and endocardial cells seem to be key elements driving this regeneration.^{2,3} Here, cryo- or mechanically damaged hearts of ZF have been used as a model for regeneration by utilising various types of different biocompatible connective supports (ECM1, ECM2, ECM3), supplemented with GFs in *ex vivo* cultures. After 15 days of culture, epicardial- and endocardial-derived cells, along with cardiomyocytes, have been analysed using histology, immunohistochemistry, and qRT-PCR for the expression of key genes (miR1, miR133a, miR133b, miR499; GATA4, WT1, NFAT2, cTNT, etc.). Under stimulation by growth factors, epicardial cells (GATA4+/WT1+/miR133a+) and endocardial cells (GATA4+/NFAT2+/miR133b+) are the cells with trans-differentiation capabilities, able to move in and out of the tissue. In general, epicardial and endocardial cells were effectively stimulated by the biocompatible supports supplemented with a specific cocktail of growth factors. Moreover, the ECM3 support/GFs resulted in the

best biocompatible device in *ex vivo* heart regeneration, showing comparability to conditions observed *in vivo*. The reason for this success in *ex vivo* regeneration- considering that the heart has regenerated in a culture plate- is that connective supports regulate the release of growth factors, thus driving regeneration. These promising results encourage further research to utilise ZF as an alternative model for the future translation of knowledge into mammals in both *in vivo* and *in vitro* contexts.

Acknowledgements

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COMPARATIVE CYTOTOXIC EFFECTS OF MICRO AND NANOPLASTICS ON THE REPRODUCTIVE PROCESS OF MYTILUS GALLOPROVINCIALIS

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Micro and nanoplastics are emerging contaminants of global concern due to their persistence in marine environments and the potential to interfere with biological processes in aquatic organisms. Their small size facilitates cellular uptake, raising concerns about their impact on reproductive health, particularly in filter-feeding invertebrates. In this study, we compare the cytotoxic effects of polystyrene microplastics (MPs) and nanoplastics (NPs) on the ovaries of *Mytilus galloprovincialis* after exposure to environmentally relevant concentrations for 1, 3, and 11 days. Histological and immunohistochemical analyses revealed diffuse follicular atresia in exposed mussels. Hemocyte infiltration implied altered immune activation. No significant structural changes were found in collagen organisation and PCNA expression. MPs, but not NPs, disrupt glycosylation patterns in ovarian cells of *M. galloprovincialis*. Analyses on cortex alterations in oocytes are currently ongoing. Referring to the possible mechanisms leading to the observed tissular alterations, MNPs exposure also disturbed the physiological ovarian redox homeostasis by targeting mitochondria. In particular, we found an increase in reactive oxygen species production and lipid peroxidation, accompanied by a reduction in the mitochondrial superoxide dismutase 2 protein levels as a key enzyme involved in intracellular antioxidant defence. The distinct immune and cytological responses between MPs and NPs suggest size-dependent mechanisms of toxicity. These findings highlight potential reproductive risks posed by micro- and nanoplastics in marine invertebrates and underscore the need for further investigation.

VALIDATION OF CONDITION INDICES TO ASSESS HEALTH OF FARMED GILTHEAD SEABREAM

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Condition indices are often used to assess animal health and welfare, including those of fish in aquaculture settings. However, reliability as indicators of physiological status needs to be validated against direct biomarkers. In this study, we tested the ability of four commonly used body condition indices (CI) to predict oxidative stress responses in a population of farmed gilthead seabream (*Sparus aurata*). We measured Hepatosomatic Index (HSI), Fulton's Condition Factor (K), Relative Condition Factor (Krel), and Scaled Mass Index (SMI) and the activity of two key antioxidant enzymes, catalase (CAT) and glutathione S-transferase (GST), in liver and gill tissues. A multivariate analysis of variance (MANOVA) was conducted to assess whether the condition indices could explain significant variation in the antioxidant response. When an enzyme appeared to be a potential biomarker of physiological condition, we performed a multivariate linear model followed by ANOVA to evaluate the effect of each index, and a principal component analysis (PCA) to compare adjusted R² and effect sizes across models. Results show that both HSI and SMI were significantly associated with the antioxidant enzyme activity, in particular GST in gill, indicating that they better reflect the internal physiological condition of the fish compared to K and Krel. Among them, SMI provided the best model fit and highest explanatory power for enzyme variation. These findings support the use of SMI, in combination with more traditional indices like K, as a reliable, non-invasive approach to evaluate the health and welfare of farmed fish. Incorporating such validated indices into routine monitoring protocols may offer a more accurate understanding of the physiological state of aquaculture stocks, thereby promoting better management practices and improved animal welfare.

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IMMUNE-NEUROENDOCRINE CROSSTALK IN *POMACEA CANALICULATA*: INSIGHTS FROM THE EXPRESSION OF ALLOGRAFT INFLAMMATORY FACTOR-1 (PC-AIF1)

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The immune and neuroendocrine systems share a wide range of signaling molecules, including cytokines that act at the interface between immune responses and nervous system function. Allograft Inflammatory Factor1 (AIF1), is a conserved cytokine expressed by phagocytic cells (e.g., macrophages, immunocompetent cells and microglia) in both vertebrate and invertebrate mod-

els. Despite the known roles of AIF-1 among mollusks, the expression and function of AIF1 in the freshwater gastropod *Pomacea canaliculata* (Pc-AIF1, LOC1125660762) remain poorly characterized. To gain a better understanding of Pc-AIF1 involvement in immune and nervous functions, we analyzed all available *P. canaliculata* RNA-seq datasets deposited in the NCBI SRA database. Our *in silico* analysis revealed that Pc-AIF1 is predominantly expressed in hemocytes, ganglia and during the early phases of sensory organ regeneration. Expression patterns were validated via fluorescence *in situ* hybridization (FISH), which detected Pc-AIF1 in circulating hemocytes, tissue-resident hemocytes (e.g., in the posterior kidney), and in the blastema of regenerating cephalic tentacles. We further quantified Pc-AIF1 transcript levels using RT-qPCR in the cerebral ganglia at 24 and 48 h post-amputation (hpa) of cephalic tentacle. Notably, we observed a significant upregulation of Pc-AIF1 only at 48 hpa in both the ipsilateral and contralateral ganglia to the injury site. Our findings confirm that also in *P. canaliculata* AIF1 is a molecule found in various immune-related cells and tissues, including neuro-immune interactions. The data also suggests that the cerebral ganglia of *P. canaliculata* respond to a peripheral sensory injury by increasing the expression of immune-related mediators, such as Pc-AIF-1. Ongoing molecular and morphological analyses aim to determine the role of hemocytes, microglial-like cells and/or neurons in the neuro-immune crosstalk during cephalic tentacle regeneration.

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THE COOL SHAPE: HOW MODERN HUMANS PACKED 90 BILLION NEURONS IN THEIR BRAIN

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It is paradoxical that the most intelligent species on the planet is still far from fully comprehend how such outstanding cognitive capacities were acquired. It is widely accepted that our cognitive capacities depend on the presence of 86 billion neurons in our brain, however, what is far from being understood is how we managed to move from a 22-26 billion neurons brain typical of great apes. Nonetheless, it is still unsolved how we managed to handle the increased metabolic cost and related thermal loading of packing 60 more billion neurons in our brain. Modern humans display a unique globular brain with tall forebrain. This globular shape is recognisable among primates and other members of the genus *Homo* as well, suggesting that selection favoured the emergence of such shape. However, it is possible that this globular shape may bring in some undesired features such as lowered capacity to dissipate heat. Here, combining 3D shape analysis with cutting edge simulations of brain heat dissipation via Finite Elements Analysis, we show that, surprisingly, modern humans brain it is not the

hottest among primates, whereas great apes do (Chimps, Gorillas). Furthermore, we show a marked shift in brain shape occurring along the australopithecines linked to significantly reduced brain temperature. This suggests that the selection for specific brain shapes played a critical role in the evolution of higher cognitive capabilities, allowing our lineage to evolve a brain shape capable of more efficiently dissipate heat and, hence, ultimately capable of greatly increase the total neuron number.

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TiO₂ NANOPARTICLES DEREGULATE SHBG GENE EXPRESSION PATTERN IN ADULT MALE OF *DANIO RERIO*

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Sex hormone-binding globulin (SHBG) is a protein produced in the liver of vertebrates, which binds steroids in the bloodstream to prevent their rapid metabolic degradation and at the same time regulates their bioavailability in target tissues¹. In addition to its high affinity for natural steroids, SHBG has shown affinity to environmental contaminants that act as endocrine disruptors. Since SHBG is found in *D. rerio*, a small freshwater fish employed in the ecotoxicological assays, we have evaluated the ability of TiO₂-NPs to influence the SHBG production and then the physiological activity of the endocrine system. TiO₂-NPs are commonly used in commerce, as a result their risk assessment is necessary. In our study, adult males of *Danio rerio* were exposed to TiO₂-NPs (1 mg/L, 2 mg/L, and 4 mg/L) for 30 days, and a control group has been also included. Daily the fishes were monitored and at the end of the exposure, all fishes were anesthetized and dissected. We have found an increase in the gene expression of SHBG in the gut by qRT-PCR, especially in the exposed group of 4mg/L compared to others exposed groups. Moreover, an evaluation of crystal structure of the SHBG thanks to Fingerprint for Ligand and Protein (FLAP), highlighted a large pocket in which a hydrophobic area is mainly involved in the binding to TiO₂-NPs. Therefore, the presence of TiO₂-NPs has induced also in gut the expression of SHBG and in this way they can act as endocrine disruptor similar to others contaminant like phthalates and bisphenol AF (BPAF)². Today, the expression of SHBG in the gut has been showed during the development of *D. rerio* but our results underline that also in adult stage this nanoparticles can cause a deregulation of the SHBG gene expression pattern.

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THE POWER OF NEAR-INFRARED PHOTOBIMODULATION THERAPY ON RESCUE *IN VITRO* MATURATION OF HUMAN OOCYTES

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We aimed to investigate how photobiomodulation therapy (PBM-t) with laser at 810 nm wavelength impacts rescue *in vitro* maturation (r-IVM) of human immature oocytes (germinal vesicles, GV and metaphase I oocytes, MI) after controlled ovarian stimulation of women undergoing assisted reproduction technology (ART) treatments. Inclusion criteria: women < 40 years old without a female factor of infertility. After follicle aspiration 36 h post trigger of final oocyte maturation and cumulus-oocyte complexes incubation for 2 h in G-IVFTM medium (Vitrolife) at 37°C, 6% CO₂, 5% O₂, the oocytes were denuded. Immature oocytes were randomized into two groups: the control group and the test group that received laser treatment with 1.0 W for 1 min at the fluence of 60 J/cm² (Garda Laser). Oocyte nuclear maturation and morphology were assessed at 1-2-4-6 h of IVM in G-1TM medium (Vitrolife). Oximetric and luminometric analyses were performed to evaluate the oxidative phosphorylation (OxPhos). The Chi-square and one-way ANOVA tests were used for IVM rates and metabolic analyses, respectively. A total of 137 oocytes (71 VG and 66 MI) from 60 patients were analyzed. The immature oocytes underwent r-IVM after PBM-t (31 VG from 24 patients and 33 MI from 29 patients) or without PBM-t (40 VG from 31 patients and 33 MI from 24 patients). No increase in oocyte degeneration rate was observed in the PBM-t group. The effect on maturation was obtained 1 h after irradiation and kept higher in the PBM-t group within 4 h for VG and 6 h for MI oocytes compared to controls. The same behavior was observed in 38 sibling VG (20 after PBM-t and 18 controls) from 14 patients and 28 siblings MI (14 after PBM-t and 14 controls) from 10 patients. Biochemical data showed that ATP synthesis and oxygen consumption increased by approximately 64% immediately after laser administration, reaching a maximum of 112% after 30 min, and remained unchanged even 1 h after treatment. The OxPhos increment was not associated with an uncoupling between oxygen consumption and ATP synthesis. In conclusion, laser irradiation at 810 nm improves cell cycle progression after r-IVM of immature oocytes through increased mitochondrial energetic metabolism.

THYROID RESPONSE TO PERIPHERAL ENDOCRINE FACTORS: NEUROPEPTIDE Y INTERSECTS THYROID FUNCTION VIA HYPOTHALAMIC-PITUITARY-THYROID AXIS IN THE REPTILE *PODARCIS SICULUS*

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Neuropeptides, produced by neurons from proneuropeptide, are small signalling molecules that bind to and activate G-protein-coupled receptors to modulate complex homeostatic mechanisms and behaviour in animals. NPY is most conserved peptide among vertebrate species and that non-mammalian species share a common ancestor that diverged from mammals in their NPY sequence. Regarding the role and regulation of NPY in reptiles, there are very few studies in relation to their phylogenetic and ecological importance, being reptiles the first organisms to adapt to a terrestrial way of life. Since NPY lies at the intersection of nutrition, metabolism and homeostasis, this neuropeptide appears to be a promising candidate for the regulation of thyroid function via the hypothalamic-pituitary-thyroid axis of the lizard *Podarcis siculus*, along with other peripheral endocrine factors such as leptin¹, substance P², galanin³ and endothelin-1⁴, given that NPY-IR fibers were found around the follicles, and immunoreactivity was localized in the cellular apices of the thyrocytes of the lizard *Podarcis siculus*⁵. Our study aimed to explore the presence of NPY in the diencephalon of lizard and investigated the effect of NPY on the hypothalamic-pituitary-thyroid axis, along with peripheral regulation of thyroid hormones after intraperitoneally administration of NPY in the lizard. Our results show the expression of NPY in the diencephalon region near the third ventricle. Besides, NPY stimulates energy balance by suppressing TRH and stimulating thyroid follicular cells and modulates pituitary TSH secretion. Our study helps fill a gap in current research in reptilian endocrinology regarding the effects of energy balance on metabolism and thyroid function.

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THE IMPACT OF MARINE NOISE POLLUTION ON CEPHALOCHORDATES

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In recent decades, human activities have significantly increased marine noise, posing a growing threat to ecosystems. Anthropogenic underwater noise is known to affect survival, development, physiology and behavior of many marine species. However, most research has focused on sound perception in mammals and fish, while invertebrate bioacoustics and their responses remain largely understudied. Cephalochordates (amphioxus) are a subphylum of marine deuterostome invertebrates with anatomical features resembling those of vertebrates. Notably, these organisms possess two types of sensory cells, some believed to be homologous to vertebrate hair cells, making amphioxus an ideal model for studying the effects of noise pollution. In this study, we examined the impact of marine noise on various biochemical parameters and the filter-feeding activity of *Branchiostoma lanceolatum*. Laboratory tests used two soundtracks of maritime traffic noise: one artificial and one from real underwater recordings. Amphioxus were exposed to noise for 1 or 24 hours or kept in silence. Responses were measured immediately and after 24 hours of recovery. Our findings reveal the harmful effects of anthropogenic marine noise on amphioxus, highlighting the need for further investigation into the vulnerability of invertebrates to acoustic pollution.

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THE GUT-BRAIN AXIS AS A THERAPEUTIC TARGET IN NEUROLIGIN3 R451C KNOCK-IN MOUSE MODEL OF AUTISM: ROLE OF *LACTIPLANTIBACILLUS (LPB.) PLANTARUM*

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Autism spectrum disorders (ASDs) involve central nervous system (CNS), immune and gastrointestinal (GI) dysfunctions, linked via the gut-brain axis¹. In the Neuroligin3 R451C knock-in (KI) monogenic mouse model of autism^{2,3}, 5-weeks of a food-borne *Lactiplantibacillus (Lpb.) plantarum* supplementation improved impaired social behavior and restored synaptic and gut markers. We assessed the effectiveness of *Lpb. plantarum* on impaired social behavior of KI mice through the three chambers test, observing a rescue to WT mice values on sociability index. We analyzed both excitatory and inhibitory synaptic proteins levels (PSD-95, Gephyrin), finding a rescue in their expression to the WT values after *Lpb. plantarum* intake. We also analyzed gut

microbiota composition, finding an increase in gut-supporter taxa in KI mice fed with *Lpb. plantarum*. Finally, we assessed intestinal permeability by analyzing through rt-PCR the gene expression of tight junctions (Claudin-1, Claudin-3 and Zonulin-1), observing a beneficial effect of *Lpb. plantarum* administration in KI mice, in colon, cecum and ileum gut regions. These findings support microbiota-targeted therapies for ASDs.

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HISTOLOGICAL AND HISTOCHEMICAL BIOMARKERS IN PIPEFISH *SYNGNATHUS ABASTER* FROM DIFFERENT AREAS OF THE IONIAN SEA

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Histological and histochemical alterations in the gills¹ of the black-striped pipefish, were tested as possible biomarkers of environmental stressors in the context of the project “HEASY”. Samples from the different areas of the Ionian Sea, the highly impacted Mar Piccolo basin and the Marine Protected Area of Porto Cesareo, were compared. Histochemical (Periodic Acid-Schiff and High Iron Diamine-Alcian Blue) and lectin-histochemical (WGA, PNA, SBA, DBA, UEA, AAA, sialidase PNA and SBA) techniques were used to observe the morphology of gills and characterize their secreted mucins. Qualitative and quantitative variations of the mucins were analyzed by statistical tests. All the gills of individuals from Mar Piccolo were infested by the protozoa *Trichodina sp.*. Morphologically, hyperplasia of mucous cells and a large space between lamellar epithelium and lamellar capillary were observed. In the Porto Cesareo individuals, gills mucous secretion was predominantly carboxylated and weakly sulphated. Sulphation decreased in the Cervaro samples, as well as GlcNAc β 1,4 residues. On the other hand, a significant increase of Gal β 1,3GalNAc, GalNAc β Gal and Fuca1,6GlcNAc- β NAsn, Fuca1,3, Fuca1,4 was observed in the Cervaro samples. After sialidase, differences in PNA- and SBA- lectin binding increased in Porto Cesareo, indicating an amount of residues masked by sialic acid. No positivity for UEA-I lectin was detected. The observed differences could be linked to the different environmental conditions and anthropogenic impacts in the two sampled areas.

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PRELIMINARY ANALYSIS OF BACULUM DEVELOPMENT IN THE HATINH LANGUR (*TRACHYPITHECUS HATINHENSIS*)

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In primates, the *baculum (os penis)* is a variable skeletal structure reflecting developmental and reproductive adaptations. However, data on its ontogeny and morphology are scarce in Asian colobines¹. This study investigates the development of the *baculum* in the Hatinh langur (*Trachypithecus hatinhensis*) using 3D micro-computed tomography (micro-CT) to acquire high-resolution morphometric data. Micro-CT scans allowed the extraction of quantitative parameters² -length, volume, and refinement coefficient- as well as the Vacuity index (V_i), a measure of internal porosity linked to ossification dynamics³. Alpha-shape analysis was applied to 3D models to obtain a standardized descriptor of *baculum* shape and surface complexity⁴. Six wild male specimens were examined, five of known age. Morphometric variables were modelled in relation to chronological age using multiple statistical functions (linear, logarithmic, sigmoidal, and polynomial), selected through fit criteria including R^2 , AIC, BIC, and ANOVA. This comparative approach enabled the identification of the best-fitting model for each parameter. Sigmoidal models best described the nonlinear ontogenetic trajectories of length and volume; the refinement coefficient followed a linear pattern, while V_i was best approximated by a second-degree polynomial curve. Based on these models, the age of the unknown specimen was estimated through inverse function calculation, yielding a mean age of 2.73 ± 0.79 years. The refinement coefficient was excluded from the final calculation due to its lower predictive reliability and reduced ontogenetic sensitivity. These findings contribute to a more detailed developmental profile of the *baculum* in colobines and demonstrate the potential of digital morphometrics as a tool for inferring biological age, with possible applications in museum studies, conservation biology, and developmental anatomy.

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EMERGING ROLE OF EXTRACELLULAR LIPID DROPLETS IN CELL-TO-CELL COMMUNICATION

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Knowledge of intercellular communication is continuously advancing, capturing the attention of the scientific community. Extracellular vesicles (EVs) are a key player in this process, serving as an essential mechanism for cell-to-cell communication. As research progresses, the heterogeneity of EVs is becoming more evident, revealing new and diverse EV populations. While the structural and functional similarities between lipid droplets (LDs) and EVs are increasingly understood and the evidence on their dynamic roles is growing, LDs, traditionally seen as intracellular lipid storage organelles, have yet to be fully recognized as potential mediators of intercellular communication. The dysregulation of LD dynamics is linked to several pathological conditions, including obesity. Obesity is associated with systemic inflammation and recent evidence suggests that resident Lipid Associated/TREM2 macrophages (LAM) in adipose tissue have a protective role in reducing lipotoxicity and adipose tissue hypertrophy. In this study, fatty acid (FFA) overload (palmitate + oleate) in THP-1 and primary human macrophages induces a LAM/TREM2-like phenotype marked by increased expression of TREM2, CD9, FABP4, CD36, PLIN2, and ABCA1, as well as IL-10 secretion, indicating an immunosuppressive state. This led to robust LD accumulation and activation of lipogenic enzymes. Notably, high-resolution microscopy revealed the presence of large extracellular lipid droplets (ELDs) released from FFA-loaded macrophages, with LDs pushing out the plasma membrane, highlighting a potential non-EV export mechanism. Isolation of large and small EV fractions from LAM showed that only large EVs contained TAG-rich, single-membrane particles and as confirmed by DLS, a distinct vesicle population absent in controls. We also identified CD81 clustering at LD release sites, implicating tetraspanin-enriched microdomains in LD secretion. Functionally, radiolabelled ¹⁴C-palmitate tracing demonstrated that large EVs, but not small EVs, carried TAG in recipient macrophages and activated them toward a LAM phenotype. Together, these findings positioned ELDs as a novel mechanism of lipid exchange and intercellular communication alongside EVs.

SNAP25 CLEAVAGE BY BOTULINUM NEUROTOXIN-A REPROGRAMS SCHWANN CELLS INTO A PRO-REGENERATIVE PHENOTYPE

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Botulinum neurotoxin A (BoNT/A) is classically recognized for its ability to cleave SNAP25, thereby inhibiting vesicular exocytosis at the neuromuscular junction. We previously demonstrated, in an *in vivo* model of neuropathy, that BoNT/A promotes peripheral

nerve regeneration by targeting Schwann cells (SCs). The cleavage of SNAP25 in SCs inhibits the autocrine release of acetylcholine, a known negative regulator of proliferation, thereby sustaining the proliferative state of the SCs (1-4). This study further investigated the molecular mechanisms underlying this regenerative effect. *In vitro*, BoNT/A increases the expression of SV2c (its cognate receptor), reduces levels of SNAP25 (its canonical substrate), but does not alter SNAP23, indicating a high degree of target specificity. BoNT/A-treated SCs, triggers a proliferative state, maintaining SC plasticity and exhibiting increased expression of pro-proliferative markers. Notably, both *in vitro* and *ex vivo* experiments show increased nuclear localization of c-Jun and elevated SV2c expression, confirming BoNT/A uptake and the activation of a regeneration-associated transcriptional profile. BoNT/A also significantly promotes neurite outgrowth from dorsal root ganglia (DRG) neurons by increased expression of pro-NGF in SCs. In parallel, it increases MMP9 expression and vesicle dynamics, facilitating extracellular matrix remodeling and enhancing the neurotrophic environment. Collectively, these findings uncover a novel mechanism by which BoNT/A-mediated SNAP25 cleavage in Schwann cells lifts inhibitory signaling constraints, sustains a reparative state, and creates a permissive environment for axonal regrowth, significantly supporting peripheral nerve regeneration.

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DISSECTING THE ROLE OF 11-KETOTESTOSTERONE AND CORTISOL IN MALE REPRODUCTION: INSIGHTS FROM *hsd11b2* AND *cyp11c1* ZEBRAFISH MUTANT LINES

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The enzyme 11 β -hydroxysteroid dehydrogenase type 2 (Hsd11b2) inactivates glucocorticoids (GCs), regulating their bioavailability. In teleost, it also catalyses the synthesis of 11-ketotestosterone (11-KT), the main active androgen. Hence, *hsd11b2* zebrafish mutants show reproductive defects. Of note, silencing *Cyp11c1* enzyme causes the absence of 11-KT and GCs synthesis, leading to even worse reproductive defects. Since *hsd11b2* and *cyp11c1* mutant lines offer a valuable system to dissect the roles of 11-KT and cortisol, this work investigates how these steroids affect male reproduction. Our data indicate that lack of 11-KT is a major driver of reproductive impairment. Courtship behavior and eggs counts evidenced reduced reproductive success in *hsd11b2* males mated with WT females, although their sperm can fertilize eggs *in vitro*. Sperm analysis showed a significant reduction in total sperm count, while viability and motility were preserved. In contrast, *cyp11c1* males completely failed to induce spawning, and presented decreased quantity and motility of the released sperm. Transcriptomic analysis of *hsd11b2* testes revealed downregula-

tion of genes involved in microtubule formation and sperm flagellum composition, suggesting an impaired progression of spermatogenesis. Upregulated genes mainly encoded steroidogenic enzymes and the similar expression pattern also in *cyp11c1* testes appeared consistent with lack of negative feedback mechanisms. Interestingly, while *cyp11c1* show more type A and B spermatogonia, likely unable to proceed with maturation, the cortisol present in *hsd11b2* mutants seem to mitigate this early-stage arrest. Indeed, testes immunofluorescence revealed altered distribution of germ cell developmental stages mainly in *cyp11c1* males. Overall, our data evidence the role of 11-KT and GCs to ensure proper male fertility in zebrafish.

GUT INFLAMMATION IN TRICHOHEPATOENTERIC SYNDROME: THE ROLE OF TTC37 AND ITS INTERPLAY WITH STAT3

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Trichohepatoenteric Syndrome (THES) is an ultra-rare, multisystem disorder caused by biallelic mutations in the *TTC37* or *SKIV2L* genes, which encode essential subunits of the SKI complex, a cofactor for the RNA exosome. The hallmark of THES is intractable diarrhea, often necessitating parenteral nutrition for survival. In addition, many patients exhibit symptoms resembling inflammatory bowel disease. THES is a life-threatening condition, with mortality mainly attributed to intestinal failure and infections. Despite its clinical implications, no curative treatment exists, and management is limited to supportive care. How mutations in the *TTC37* gene lead to such severe impairment in quality of life and high mortality is unclear. Current literature mainly consists of clinical data and case reports, without shedding light on the molecular mechanisms connecting these mutations to THES enteropathy. This gap in understanding makes impossible to identify therapeutic targets for a condition that urgently requires effective treatments. To elucidate the mechanism behind the pathophysiology of THES, we developed a *ttc37* knockout zebrafish line to phenocopy the patient condition. Transcriptomic analysis of mutants revealed significant alterations in the expression of genes related to cell cycle, apoptosis and immune response, specifically in intestinal tissue. The disruption of gut homeostasis in *ttc37* knockout mutants triggers severe gut inflammation and morphological alterations. Even more compelling, both larvae and adults showed pronounced alteration in the STAT3 pathway, a crucial regulator of inflammation, suggesting that STAT3 signalling may play a pivotal role in the gut condition of THES patients. In conclusion, we hypothesize that the intestinal disease in THES could be driven by chronic gut inflammation and reduced regenerative capacity of the intestinal epithelium. Our preliminary findings suggest a previously unrecognized crosstalk between *TTC37* and *STAT3*, that could explain the observed intestinal phenotype and could represent an interesting target for patient treatment.

THERMAL STRESS TRIGGERS TRANSPOSABLE ELEMENTS EXPRESSION IN THE ANTARCTIC FISH *TREMATOMUS BERNACCHII*

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Transposable elements (TEs) are mobile DNA sequences recognized as key players in genome regulation, architecture, and evolution. Their activity is controlled by host silencing mechanisms. However, environmental stressors can destabilize these regulatory pathways, leading to variations in TE mobilization. Nowadays, it is recognized the dual role of TEs, which act not only as genomic parasites but also as sources of genetic variability contributing to rapid adaptation to changing environments¹. The TEs activity in relation to environmental changes in fish is well known in literature². In this study, we explored the TEs behavior in *Trematomus bernacchii*, an Antarctic fish adapted to stable conditions. *T. bernacchii* is a model species for investigating the biochemical response to abiotic stressors. Considering the current impacts of climate change, our aim was to understand how this species copes with thermal stress at molecular level. We examined the differentially expressed TEs in two tissues, liver and gills, in specimens exposed to higher temperatures (+1 °C and +3 °C) over periods of 5 and 15 days compared to control temperature of 0°C. Our findings revealed that TEs activity has different behavior in gills and liver under heat stress. To recognize whether these changes depend on the way the fish regulates its genome, we also assessed the expression of genes involved in TE silencing. Genes known to be part of TE repression pathways, showed different expression patterns depending on temperature conditions and tissues. Overall, this study suggests a tissue-specific response to heat stress in *T. bernacchii*. TEs and their regulatory machinery respond dynamically, reflecting the first signs of stress adaptation in this cold-specialized species.

Acknowledgements

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EMBRYOGENESIS IN *XENOPUS LAEVIS*: TRANSPOSABLE ELEMENT IMPACT ACROSS TWO SUBGENOMES

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The African clawed frog, *Xenopus laevis*, has an allotetraploid genome comprising two subgenomes: L (Long chromosomes) and S (Short chromosomes). While the L subgenome maintains conserved synteny with the diploid relative *X. tropicalis*, the S

subgenome has experienced extensive structural rearrangements and gene loss, resulting in marked differences in gene content and transposable element (TE) composition between the two subgenomes. This asymmetric evolutionary history is also reflected at the functional level, with disparities in both gene expression and TE activity. TEs are mobile genetic elements that contribute significantly to genome evolution and gene regulation. However, their mobility poses potential risks to genome stability, and is therefore tightly regulated by host defense systems, including the nucleosome remodeling and deacetylase (NuRD) complex and Argonaute proteins, which act primarily through modulation of heterochromatin. During early embryogenesis, particularly at the maternal-to-zygotic transition, the chromatin landscape becomes transiently permissive to transcription, allowing some TEs to escape silencing. Nonetheless, recent evidence suggests that TE reactivation during this window is not a random consequence of global chromatin remodeling, but rather a tightly orchestrated, class- and stage-specific process, indicating a nuanced regulatory mechanism. Building on this framework, we examined the transcriptional dynamics of TEs across six developmental stages in *X. laevis*. While the global expression profile of transcribed TEs remained largely consistent with their genomic abundance throughout development, subgenome-specific analyses revealed distinct transcriptional signatures. In particular, LTR retroelements predominated in the L subgenome, whereas the S subgenome exhibited a bias toward LINE retroelements, primarily represented by evolutionarily young copies. Interestingly, genes encoding components of chromatin-silencing machinery were actively expressed in both subgenomes, suggesting that TE repression mechanisms are functionally engaged during embryogenesis and have evolved in a balanced manner across both genomic compartments.

NEUROTOXIC EFFECTS OF THERMAL VARIATION ON THE NERVOUS SYSTEM AND EYE OF ADULT ZEBRAFISH

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Neurotoxicity refers to any adverse effect on the chemistry, structure, or function of the nervous system, triggered by chemical or physical influences. Environmental changes may induce neurotoxic responses in animals, with aquatic species particularly vulnerable due to the susceptibility of their ecosystems to chemical and physical alterations. Our research has shown that adult zebrafish (*Danio rerio*), a widely used model in neuroscience, respond sensitively to thermal variation. Chronic exposure to low (18°C) or high (34°C) temperatures for 21 days—compared to the control temperature of 26°C—significantly altered brain proteomic profiles, lipid composition, and behaviour, both in individual fish and in small shoals. Notably, similar behavioural and molecular effects were also evident after shorter (4-day) exposures. Importantly, these effects extended beyond the brain. Proteomic analysis of the eye revealed that high-temperature exposure upregulated the sirtuin signalling pathway and downregulated proteins involved in oxidative phos-

phorylation, electron transport, and ATP synthesis. In the brain, these changes were accompanied by reduced expression of synaptic and neurotransmitter-related proteins, while in the eye, they corresponded with decreased phototransduction proteins. These findings demonstrate that thermal stress can have broad neurotoxic consequences, impairing both central and visual nervous system function. They highlight the zebrafish as a powerful model for studying temperature-induced neurotoxicity and emphasise the ecological relevance of environmental temperature stability for aquatic organisms.

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FROM WASTE TO WONDER: *IN VITRO* EFFECT OF FOLLICULAR FLUID IN ENHANCING ENDOMETRIAL RECEPTIVITY

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In assisted reproductive technologies (ART), the follicular fluid (FF) is currently discarded as laboratory waste following oocyte retrieval and selection. However, the FF consists of plasma exudate containing hormones, cytokines, growth factors, metabolites and extracellular vesicles (EVs) necessary for the crosstalk between oocyte and somatic cells¹. Moreover, has been shown that FF composition reflect oocyte quality and ovarian function. This study explores the hypothesis that FF could contribute to enhancing endometrial receptivity and facilitate embryo implantation, a critical factor for successful embryo implantation. With this aim, receptive (RL95-2) and non-receptive (HEC-1-A) endometrial cell lines¹, were used to tested the ability of FF samples, collected from patients undergoing to ART, to stimulate cell migration via wound healing assays. A significant increase in motility was observed, at both 6 and 24 h of treatment, especially in HEC-1-A cells, alongside with a concomitant downregulation of E-cadherin expression. These results suggest the induction of epithelial-mesenchymal transition (EMT), a process analogous to the physiological mesenchymal-epithelial transition (MET), observed during implantation. Clusterin (CLU), C-reactive protein (CRP), and matrix metalloproteinases (MMP-2 and MMP-9) were identified as potential mediators of this effect. Our findings support the hypothesis that FF, may influence endometrial receptivity by promoting cell migration and EMT. This study provides a foundation for future research on FF-based approaches to enhance uterine receptivity and improve outcomes in assisted reproductive technology (ART).

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CRACKED TRACKS: UNRAVELING MICROTUBULE DYNAMICS IN TESTICULAR AND SPERM IMPAIRMENT IN A RAT MODEL OF TYPE 1 DIABETES

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Type 1 diabetes (T1D), through chronic hyperglycemia and oxidative stress, is increasingly recognized as a contributing factor to male fertility¹. This study investigated how T1D-induced oxidative stress affects testicular microtubule (MT) dynamics, microtubule-associated proteins (MAPs), and sperm physiology. A streptozotocin-induced model of T1D in adult Wistar rats (65 mg/kg, i.p.) was used to evaluate the expression and localization of key MAPs -MARK4, MAP1A, DYNLL1, PREP, and RSPH6A -in both testis and spermatozoa². Diabetic rats exhibited significant downregulation and mis-localization of these MAPs, associated with MT disorganization and impaired germ cell maturation. Sperm analysis revealed reduced concentration, motility, and viability, alongside increased morphological abnormalities, DNA fragmentation, apoptosis, and chromatin remodelling anomalies, indicating defective spermatogenesis. Elevated levels of 4-hydroxynonenal (4-HNE), a lipid peroxidation marker, were detected in sperm, particularly localized in the acrosomal and flagellar regions, coupled with mitochondrial dysfunction and reduced ATP production. Intracellular Ca²⁺ levels were decreased, together with CatSper and VDAC3 expression, compromising calcium signalling and motility. Additionally, altered tubulin acetylation -due to decreased ATAT1 and increased HDAC6 expression-, further impaired flagellar function. Notably, the expression and localization of PREP, RSPH6A, and DNAL1 -MAPs crucial for axonemal structure and sperm motility- were significantly reduced in T1D rats. Overall, these findings highlight MAPs as key mediators in the crosstalk between oxidative stress and cytoskeletal integrity, offering new insight for basic research into the molecular mechanisms of diabetic male subfertility and potential therapeutic targeting.

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MULTISENSORY INTEGRATION OF SOCIAL CUES IN THE VENTROMEDIAL PREFRONTAL CORTEX

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The ability to attract and reproduce with a suitable mate is a key driver of evolution. Mate selection depends on social interaction, and animals have evolved diverse behavioural strategies that rely on multimodal communication. Despite the importance of multisensory cues in courtship, the neural circuits responsible for integrating these signals remain poorly understood. In rodents, males use a combination of olfactory (e.g., pheromones) and acoustic (ultrasonic vocalizations, USVs) cues to court females. Recent studies show that female mice prefer USVs from unfamiliar males only when paired with sexual odors, suggesting cross-modal integration. However, where and how this sensory information converges in the brain remains unclear. We first confirmed through behavioural preference tests that female mice favor multimodal cues, but only within a social context. To explore the brain's response to unimodal and multimodal stimuli, we conducted whole-brain analysis of immediate-early gene (IEG) expression, using tissue clearing and light-sheet microscopy. Our results revealed that sexual odors predominantly drive brain activation, with the ventromedial prefrontal cortex (vmPFC) specifically responding to multisensory social cues. To further investigate vmPFC involvement, we used miniscope recording to perform one-photon functional imaging in awake animals. We found that vmPFC neurons respond to both unimodal and multimodal cues, with a distinct subset selectively activated by combined sensory inputs. By performing chemogenetic manipulation of vmPFC neurons, we are now investigating their causal role in shaping female preference for multisensory social stimuli. Together, these findings highlight the vmPFC as a key hub for integrating social cues from different modalities, suggesting its potential role in shaping mate preference.

POSTERS

KETOGENIC DIET PROMOTES MOTOR AND ANXIETY BEHAVIORS VIA ACTIVATION OF THE MYELIN BASIC PROTEIN IN BTBR MICE

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Recently, ketogenic diet (KD) has shown to reduce core symptoms and neuroinflammation in a mouse model of autism (BTBR)¹. Moreover, emerging evidence is beginning to suggest a key role for the cerebellum in the pathophysiology of autism spectrum disorder (ASD) as a result, among others, of structural cerebellar abnormalities, including atypical connectivity². On this basis, both BTBR and C57BL/6 (B6) mice were exposed to standard chow diet (CD) or KD for 5 weeks and both motor/exploratory and anxiety behaviors were checked. Contextually, levels of classical isoforms (21.5-, 18.5/17- and 14-kDa) of the myelin basic protein (MBP) plus TNF- α proinflammatory cytokine were measured in the cerebellum. Performances in the Modified Beam Walking test were significantly decreased ($p < 0.001$) in BTBR mice compared to B6, while KD improved motor behaviors by increasing ($p < 0.001$) the time and number of laps and reduced anxiety by increasing the number of pecking. Additionally, KD-fed BTBRs improved exploration in the Open Field Test as indicated by an enhanced ($p < 0.001$) length of the path traveled and a reduced ($p < 0.001$) average speed. Interestingly, KD led to an upregulation ($p < 0.05$) of 18.5/17-kDa MBP isoform in the cerebellum of BTBR mice, while TNF- α levels were downregulated ($p < 0.05$). These data suggest that KD may play an important role in neuroprotection of the cerebellum, similarly to other works dealing with cerebral ischemia and neurodegenerative diseases³. In this work, the activation of cerebellar myelination could lead to a reinforcement of neuronal functions that can in turn improve motor and anxiety behaviors, crucial to enhance social communication in ASD.

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FIGHTING REPRODUCTIVE DAMAGE: HOW MELATONIN SHIELDS THE TESTIS FROM ZEAREALENONE

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The progressive decline in male fertility is a growing concern, with many cases remaining idiopathic. Environmental pollutants, including mycotoxins, have emerged as key contributors to impaired reproductive health. Among them, zearalenone (ZEN), a non-steroidal compound produced by *Fusarium* species, structurally mimics β -estradiol and binds estrogen receptors. Beyond its estrogenic action, ZEN increases oxidative stress by elevating reactive oxygen species (ROS) and impairing antioxidant defenses, ultimately affecting spermatogenesis. Nevertheless, the precise molecular mechanisms by which ZEN impairs spermatogenesis remain incompletely understood. In this context, the present study investigates the impact of ZEN in adult male Wistar rat testis and explores the protective role of melatonin (MLT), a pineal hormone with well-documented antioxidant and anti-inflammatory properties. Adult rats were randomly assigned to four experimental groups: control, ZEN-treated (100 μ g/kg body weight, i.p.), MLT-treated (4 mg/L in drinking water), and ZEN+MLT co-treated. Treatments were administered daily for 30 consecutive days. ZEN exposure resulted in disruption of the hypothalamic-pituitary-testis axis, elevated oxidative stress, increased apoptosis, and pronounced alterations in spermatogenesis and testicular histoarchitecture, ultimately impairing sperm quality. Notably, co-treatment with MLT effectively mitigated these effects by scavenging ROS and modulating key cellular pathways involved in oxidative stress response, steroidogenesis, and apoptosis. This study offers novel insights into the molecular pathways disrupted by ZEN in the testis and underscores the protective potential of melatonin, thereby contributing to the advancement of targeted interventions aimed at preserving male reproductive health.

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DEVELOPMENT OF A 3D PLATFORM FOR DRUG SCREENING

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Ischemic stroke is a multifactorial neurological disorder with high mortality and is the leading cause of disability and cognitive impairment in Western countries¹. Brain organoids are innovative 3D cellular models that replicate the complexity of human brain

structure and composition, offering a promising tool to study ischemic injury. In this study, we developed and characterized an *in vitro* stroke model using cerebral organoids derived from human induced pluripotent stem cells (hiPSCs), generated at different time points and assessed for maturation using specific markers. To simulate stroke conditions, various durations of oxygen and glucose deprivation (OGD) followed by reperfusion (R) were tested, selecting a sublethal condition to study cellular responses. The model was analyzed using Western blotting, dPCR, immunofluorescence, and electrophysiology. Proteomic profiling by 2D SDS-PAGE was also performed to identify differentially expressed proteins². This 3D platform enables drug screening in a physiologically relevant context, and the multi-omics approach supports the identification of molecular mechanisms and therapeutic targets for ischemic brain injury.

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PRELIMINARY STUDIES ON THE ROLE OF BIOACTIVE MOLECULES IN THE MODULATION OF THE ETOSIS PROCESS

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Extracellular trap formation, known as ETosis, represents a key innate immune strategy in vertebrate and invertebrate organisms, activated to combat pathogen invasion. However, it has also been linked to the onset of autoimmune disorders, chronic inflammation and cancer^{1,2}. Our study investigated the effects of natural products on the modulation of ETosis and inflammation in the invertebrate species *C. quadricarinatus* and *A. lixula*, as well as in murine RAW 264.7 macrophages. The invertebrates were immunostimulated with *S. aureus* and co-treated with extracts from leaves (GLE) and rhizomes (RE) of *P. oceanica*. Quantification and evaluation using fluorescence microscopy showed a decrease in extracellular trap (ET) release following co-treatments. RAW 264.7 cells were immunostimulated with *E. coli* lipopolysaccharide and co-treated with GLE and RE and polyphenols extracted from olive mill wastewater (OMW). ET formation was monitored by quantifying extracellular DNA (exDNA) in control and treated conditions. The release of exDNA decreased following the co-treatment with GLE and mainly OMW. ELISA assays for TNF- α and IL-6 secretion also confirmed the anti-inflammatory activity

of both extracts. Preliminary MTT assay and Griess reactions were performed using polyphenols from OMW in order to evaluate the dose-response effect and the anti-inflammatory potential of the preparation. The data collected thus far provide an excellent basis for an in-depth molecular study on the modulation of ETosis and inflammatory processes.

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BIOINFORMATIC PIPELINE FOR THE EXTRACTION OF MOLECULAR MARKERS FROM NANOPORE TRANSCRIPTOMIC DATA IN *POMACEA CANALICULATA*

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Pomacea canaliculata is a freshwater pulmonate gastropod known for its high invasiveness, its role as an intermediate host for human parasites, and its remarkable regenerative capacity in adulthood. Despite the increasing interest in this species, molecular-level knowledge remains limited, and currently available cellular markers are scarce. Thanks to advances in bioinformatics and the integration of artificial intelligence-based tools, the analysis of large transcriptomic datasets has become significantly more accessible, efficient, and accurate. In this study, we developed an integrated bioinformatic pipeline, combining custom scripts with tools reported in the literature, with the aim of identifying cell-specific markers across different tissues of *P. canaliculata*. Key steps in the pipeline include the generation of subtractive databases to isolate tissue-specific components, filtering of sequences based on predicted subcellular localization, and identification through comparison with annotated public databases. This approach enabled the generation of refined datasets containing candidate sequences as potential molecular markers, selected according to filtering criteria defined within the pipeline. Additionally, transcriptomes were assembled to serve as valuable resources for future biological and molecular studies on this species.

IMPACT OF COCAINE ON HUMAN COLORECTAL ADENOCARCINOMA CELLS

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Drug addiction is one of the most global issues in the 21st century.

One of the most widely used drugs is cocaine (COC), whose production reached 1982 tons in 2020. 0.4% of the global population aged 15-64 used COC at least once in their life¹. The high tendency of cocaine to cause addiction depends on its ability to cross the blood-brain barrier and inhibit the reuptake of the monoamines norepinephrine (NE), 5-hydroxytryptamine (5-HT), and especially dopamine (DA), from the synaptic cleft². While much scientific evidence confirms the involvement of the endocannabinoid system (eCB), and mainly of the cannabinoid receptor 1 (CB1) in the effects of COC on the nervous central system³, there is no data about the eCB modulation by COC in other compartments, especially in the intestinal tract. eCB is a widely preserved signalling system, whose main function is to maintain homeostasis; CB1 is widely present in peripheral tissues, as well as in the nervous system; it is involved in the modulation of neurotransmission, neurodevelopment, embryonic development, reproduction, and the activity of the gastrointestinal system, among others⁴. Our study was aimed to verify if COC could affect CB1 expression in human colorectal adenocarcinoma cell line (CACO2). Firstly, we tested the COC at several environmental dosages (from 350 to 0 nM) highlighting the COC non-monotonic effect: COC positively affected the metabolic activity of CACO2 cell line at concentration of 1nM, as demonstrated by MTT Assay. Moreover, exposure to COC for 24 h downregulated the expression of CB1. Although these data are preliminary and require further studies, they display for the first time that COC influences CB1 expression as well as cell metabolic activity in CACO2 cells. These phenomena may play a key role in the process known as *leaky gut*, considered one of the triggering or aggravating causes of several intestinal diseases.

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FAM134B-MEDIATED ER-PHAGY REGULATES ER REMODELING DURING SKELETAL MUSCLE DIFFERENTIATION

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Skeletal muscle develops through myogenesis, a highly regulated process in which myoblasts proliferate, differentiate, and fuse to form multinucleated myotubes. This transition requires remodeling of intracellular organelles, particularly the endoplasmic reticulum (ER) to meet the growing demands of protein folding, calcium storage, and lipid metabolism. We generated two different stable lines of C2C12 cells where the ER was highlighted in red (RFP) or green (GFP) through lentiviral infection of KDEL plasmids and observed heterotypic fusion of the ER membranes during myotubes formation. Notably, we observed a significant activation of the autophagy machinery during myogenesis by western blotting and confocal analyses. Particularly, we identified discrete

portions of ER within autophagosome-like structures in myotubes and observed an increase of ER-phagy flux during cell differentiation. ER-phagy, a form of selective autophagy, plays a critical role in reshaping the ER by degrading damaged or excess fragments, maintaining ER homeostasis and enabling structural adaptation to developmental cues. Its physiological relevance is highlighted by the progressive decline of ER and autophagy functions during aging and their severe impairment in muscle pathologies such as in Duchenne and Ullrich muscular dystrophies. Through MS analysis we identified six ER-phagy receptors involved; one of them, namely Fam134b, showed significant downregulation upon differentiation. We demonstrated the pivotal role of Fam134b in preserving ER protein homeostasis during myoblast differentiation and highlight how disruptions in ER-phagy can hinder myogenesis¹. Disrupting this balance leads to ER stress and impaired differentiation, highlighting a key role for ER-phagy in muscle development and its potential involvement in muscular dystrophies.

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PRELIMINARY REMARKS ON THE MITOCHONDRIAL DNA COPY NUMBER VARIATION IN *CALLINECTES SAPIDUS* FROM CONTAMINATED AND CONTROL SITES

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Mitochondrial DNA is highly susceptible to stress-induced changes, due to the lack of protective histones but especially because it is in the proximity of ROS generation sites. This feature has made mitochondrial DNA copy number (mtDNAcn) variation a potential biomarker of exposure to environmental contaminants and other stress-related disorders. In this study, mtDNAcn variation was assessed for the first time in individuals of the invasive alien blue crab *Callinectes sapidus* sampled in the highly polluted industrial area of Augusta and in the Natural Reserve of Vendicari (Sicily). *C. sapidus* is a highly adaptable species, able to thrive in a wide range of environmental conditions and habitats, from freshwater to estuarine and marine¹. This ecological plasticity has facilitated its successful establishment and spread in different Mediterranean regions². Here, the relative mtDNAcn for this species was evaluated using real-time PCR of a fragment of the mitochondrial *COI* and nuclear *Rpl12* genes. An increase in mtDNAcn was observed in samples from the polluted site confirming data obtained on several species already studied³. These results highlight how environmental contaminants can alter the mitochondrial genome and how *C. sapidus* can be considered as a potential bioindicator species of environmental quality.

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ELECTRON MICROSCOPY AND WESTERN BLOTTING ANALYSIS OF EXTRACELLULAR VESICLES AND PBMCS REVEALS DIFFERENCES IN MULTIPLE SCLEROSIS PATIENTS

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The growing body of evidence underscores the significant role of extracellular vesicles (EVs) in cell-to-cell signaling in autoimmune disorders, notably Multiple Sclerosis (MS). Although the precise origins of MS remain elusive, its molecular underpinnings are well-characterized, identifying peripheral blood mononuclear cells (PBMCs) as major contributors to the disease progression and neuroinflammation. To comprehensively assess the role of EVs in MS, we used a combined approach of electron microscopy (EM) and western blot analysis. This allowed us to investigate both the morphology and molecular content of serum-derived EVs, as well as the detailed ultrastructure of PBMCs obtained from MS patients and healthy controls. Our exploratory analysis successfully identified significant differences between the patient and control groups. EM imaging revealed that PBMCs from MS patients displayed more numerous pseudopods, large vesicles at the cell surface, and endoplasmic vesicles, indicating an activated state. Furthermore, PBMCs exhibited increased multivesicular bodies, amorphous material around vesicles, numerous plasma membrane extensions with associated large vesicles, and autophagosomal vacuoles containing undigested material. Analysis of EV cargo identified dysregulated levels of several molecules in MS patients, including GANAB, IFI35, Cortactin, Septin 2, Cofilin 1, and ARHGDI1A, suggesting both their involvement as inflammatory signals in the context of altered vesicular dynamics and the possibility to use it as biomarker. Our findings highlight the utility of integrating EM and Western blot analysis of PBMCs and their released vesicles as a valuable strategy for deepening the understanding of the physiopathology of MS.

PFOA EXPOSURE IN HEPG2 CELLS: UNVEILING MOLECULAR TARGETS OF LIVER TOXICITY AT OCCUPATIONALLY RELEVANT DOSES

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Until the early 2000s, perfluorooctanoic acid (PFOA), a long-chain PFAS, was widely used in industrial processes and consumer products. Despite being gradually replaced by short-chain PFAS due to growing evidence of its toxicity, and despite its classification as a possible human carcinogen (Zham *et al.*, 2024) and restrictions on its use and production, PFOA is still detectable in environmental matrices (water, soil, and air) and in human bioflu-

ids, particularly serum. Since the liver is a major target organ for PFOA accumulation and toxicity, this study aimed to identify novel molecular targets and biochemical mechanisms underlying its toxic effects, using the human liver-derived HepG2 cell line. Cells were exposed for 24 h to a wide range of PFOA concentrations (0.5-400 µM), mimicking both occupational and non-occupational exposure. Cell viability, assessed by MTT assay, revealed a dose-dependent reduction, with an IC50 of 176.3 µM. For subsequent analyses, sub-cytotoxic concentrations (10-100 µM) were selected. A significant increase in cytosolic reactive oxygen species (ROS) was observed starting from 10 µM, while mitochondrial ROS increased up to 50 µM. DNA damage, measured by comet assay, peaked at 50 µM, then decreased at higher concentrations. A significant rise in late apoptotic cells was observed from 25 µM onward. Gene expression analysis revealed a marked downregulation of GPX4, involved in protection against lipid peroxidation, and PARP1, a key enzyme in DNA repair and inflammation pathways, starting from 25 µM. These findings indicate that PFOA alters redox balance, induces genotoxicity, and promotes apoptosis even at sub-lethal concentrations. Ongoing studies will further elucidate how PFOA interferes with critical cellular processes, supporting the establishment of safety thresholds to mitigate risks associated with occupational exposure to this persistent environmental contaminant.

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THE SHORT-TERM EFFECTS OF POLYSTYRENE MICROPLASTICS ON THE REPRODUCTIVE FUNCTION OF MYTILUS GALLOPROVINCIALIS: MORPHOLOGICAL AND METABOLOMIC EVIDENCE

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Microplastic (MP) pollution is one of the most significant environmental challenges worldwide¹. Plastic's persistence and fragmentation capacity generate microscopic particles that can accumulate in aquatic ecosystems, potentially causing toxic effects on aquatic organisms and, indirectly, humans². In this study, we evaluated the cytotoxic and metabolic effects associated with a short-term (48 h) exposure to polystyrene MP (5 µm) in the gonads of the bivalve *Mytilus galloprovincialis*, a model species widely used for environmental biomonitoring due to its wide distribution and relevance, both ecological and commercial. Morphological analyses, performed using specific histological techniques, revealed significant alterations in the structure of sperm cysts in a dose-dependent manner, leading to impaired germ cell interactions and disorganization of gonadal tissue. These changes are caused by both physical damage from direct contact with the particles and the initiation of oxidative stress. Furthermore, metabolomic analysis, based on proton nuclear magnetic resonance (¹H NMR)-based metabolomics, combined with chemometrics, allowed for the identification of time-dependent changes in the metabolic profile of the exposed gonads, revealing an imbalance in energy and osmoregulatory pathways. Finally, we observed altered chromatin folding in spermatozoa, suggesting a potential impact on fertility and reproductive success. Our findings suggest that even brief

exposures to MP can significantly impair the reproductive health of mussels, negatively affecting the fertilisation capacity of spermatozoa.

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NEUROGLOBIN REGULATES CHOLESTEROL METABOLISM IN GLIAL CELLS: A NOVEL MECHANISM FOR NEUROPROTECTION?

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Astrocytes plays a crucial role in maintaining brain homeostasis by providing neurons with essential metabolic support, including energy substrates and molecules, such as cholesterol, required for neuronal survival and function. In this study, we investigated whether the overexpression of neuroglobin (NGB), an O₂-binding globin able to modulate cell metabolism and enhance neuronal resilience under stress conditions, may influence cholesterol metabolism in the U373 astrocytic cell line. The main results show that NGB overexpression significantly upregulated proteins and enzymes belonging to cholesterol regulatory network, resulting in increased intracellular cholesterol content. However, co-culture experiments revealed that this NGB-driven metabolic rearrangement failed to exert neuroprotection in a neuronal cell model of Parkinson’s disease. Collectively, these results broaden the understanding of NGB as a metabolic modulator in glial cells. Further investigation is needed to better comprehend the physiological relevance of NGB-induced modulation of glial cholesterol metabolism as well as its potential implication in neuropathological conditions.

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EXPLORING THE NEUROPROTECTIVE AND ANTIOXIDANT PROPERTIES OF VOGHERA PEPPER IN THE CEREBELLUM DURING AGING: AN *IN VIVO* APPROACH

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Aging is a universal and inevitable process, characterized by the progressive decline of physical, cognitive, and biological functions, which can ultimately lead to the onset of specific age-related conditions, e.g. neurodegeneration. Previous *in vitro* research has demonstrated that extracts from the Voghera sweet pepper, a traditional Italian agricultural product, are capable of modulating oxidative stress, counteracting cellular senescence, and enhancing cell proliferation in both young and aged human fibroblasts. Building upon this *in vitro* evidence, we investigated the *in vivo* effects of phytotherapeutic supplementation with extracts derived from both the fruit body and the by-products (placenta, seeds, and pedicel) of the Voghera pepper in a murine aging model. Specifically, we assessed potential morphological alterations in the cerebellum of mice at two different life stages (two and eighteen months old), employing Nissl staining. Additionally, we examined changes in the expression of oxidative stress-related markers, *i.e.* SOD1 and SOD2, in the cerebellar cortex, using an immunohistochemical approach and bright-field microscopy. Our *in vivo* results revealed a slight attenuation of age-related structural degeneration, along with significant modulation of the oxidative stress response, in the cerebellar cortex of mice treated with both extracts. These outcomes suggest that both the edible and waste components of the Voghera pepper possess antioxidant and potentially anti-aging properties. Consequently, their supplementation may contribute to the prevention of age-related structural and functional brain impairments.

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ENVIRONMENTAL EXPOSURE TO PSYCHOACTIVE SUBSTANCES: EFFECTS ON *XENOPUS LAEVIS* EMBRYONIC DEVELOPMENT

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Clonazepam, a benzodiazepine with sedative properties, is widely prescribed for anxiety disorders and epilepsy, also in pediatric populations. Frequently used alongside other psychoactive substances such as caffeine and nicotine, clonazepam and its metabolites are excreted into wastewater and ultimately reach aquatic environments. There, residues of clonazepam, caffeine, and nico-

tine have all been detected, persisting as environmental microcontaminants at concentrations ranging from ng/L to µg/L.^{1,2} Their increasing presence raises concerns about potential ecotoxicological effects. To evaluate their developmental toxicity, we used *Xenopus laevis* embryos, a recognized model in environmental risk assessment. A modified FETAX protocol was employed, exposing embryos at the 4-8 cell stage better to simulate environmentally relevant exposure scenarios.³ A range of concentrations, reflecting those found in surface and wastewater, was tested to assess potential dose-dependent effects. In this preliminary experiment, the three psychoactive substances, clonazepam, caffeine, and nicotine, were tested individually to establish baseline effects for each compound. At developmental stages 45/46, standard FETAX endpoints (mortality, growth, malformations) were evaluated, alongside heart rate as an additional parameter.⁴ Observed outcomes included significant cephalic and abdominal edema, intestinal malformations, bent tail phenotype, and alterations in dorsal pigmentation. LC₅₀, EC₅₀, and the Teratogenic Index (TI) were calculated, revealing a notable teratogenic potential. Future studies will assess combined exposures to investigate possible synergistic or additive effects. Nonetheless, these preliminary results already highlight significant developmental risks linked to psychoactive contaminants. Given the absence of specific regulatory thresholds, further research is needed to guide environmental protection efforts and safeguard ecosystem and human health.

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DEVELOPMENTAL TOXICITY AND CYTOTOXIC/ GENOTOXIC POTENTIAL OF PM₁₀ EXTRACTS USING *XENOPUS LAEVIS* EMBRYOS AND A549 LUNG EPITHELIAL CELLS

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Particulate matter, including PM₁₀, poses significant health and environmental risks. While human studies highlight associations with adverse prenatal outcomes, such as low birth weight and preterm births, experimental animal data on PM₁₀ remain limited. This study assessed the developmental toxicity and cytotoxicity/genotoxicity potential of PM₁₀ extracts collected from a rural site in the Po Valley (Bertonico, northern Italy), using *Xenopus laevis* embryos (R-FETAX) and A549 lung epithelial cells. PM₁₀ samples were chemically characterized, focusing on PM₁₀ and key toxic components (NO₃⁻, SO₄⁻, NH₄⁺, OC, EC, Al, Si, Ti, V, Mn, Fe, Cu, Zn, Rb, Pb, and Sr). Embryos/cells were exposed to sample extracts diluted 1:10 to assess developmental toxicity and, in the cell model, cytotoxicity and genotoxicity. R-FETAX results showed no lethal effects or major malformations, while statistically significant developmental delays were observed

in groups exposed to some extracts. Delays correlated both with PM₁₀ and the considered analytes, including Zn and Cu. Minimal cytotoxic and genotoxic responses were observed in A549 exposed cells. Results suggest that, even in rural settings, PM₁₀ can impair embryonic development without cytotoxic or genotoxic effects *in vitro*, demonstrating the effectiveness of the FETAX model for detecting subtle developmental toxicity induced by complex particulate mixtures.

MULTIPLE ENVIRONMENTAL STRESSORS: A THREAT TO THE REPRODUCTIVE HEALTH OF MUSSEL *MYTILUS GALLOPROVINCIALIS*?

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Ecotoxicological analyses performed on the species mussel *Mytilus galloprovincialis*, cultivated at the mussel farm S.A.Co.M. located in the Natural Reserve of the Lagoon of Capo Peloro (Messina, Italy), showed the potential action on male and female reproductive health of multiple environmental stressors due to climate change. Through a multi-biomarker approach, potential biomarkers useful for the assessment of ecosystem risk were identified. In fact, histological analysis underlined coherence between the autumn seasonality and the reproductive period, while histochemical (dPAS/PAS) and metabolomic (proton nuclear magnetic resonance, ¹H NMR) evaluations showed differences between the two sexes in the storage of energy reserves, such as glycogen. The Schmorl's method demonstrated the threshold of immune vigilance, not highlighting accumulation of melanin, as supported by the enzymatic results about the activity of catalase (CAT), glutathione *S*-transferase (GST) and levels of malondialdehyde (MDA). Current data remark that in the field there is a basal divergence in metabolite concentration for the target species subjected to multiple stresses, but also that sexual maturation remains synchronous for both sexes at least during the selected season in the natural environmental conditions.

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MOLECULAR INSIGHTS INTO THE GLUCOSE-LOWERING EFFECTS OF SICILIAN RED AND WHITE GRAPE SEED OILS ON HEPG2 CELLS

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Grape seed oil, a by-product of wine production, is rich in polyunsaturated fatty acids and antioxidant polyphenols with well-documented metabolic benefits. In preliminary experiments¹, HepG2 liver cells were exposed for 24 h to non-cytotoxic concentrations

of seed oil obtained from Sicilian white (WGSO) and red (RGSO) grapes. This increased intracellular glycogen content, as demonstrated by PAS staining, and decreased extracellular glucose levels, suggesting improved glucose utilization. To further investigate these effects, experiments were performed using the fluorescent glucose analog 2-NBDG, which revealed a significant enhancement in glucose uptake with both WGSO and RGSO, comparable to insulin stimulation. In parallel, Western blot analysis was conducted to examine the expression of glucose transporters GLUT-2 and GLUT-4, the transcription factor HNF1 α , and key regulators of insulin-dependent (IRS-1, AKT, PKC ζ) and insulin-independent (AMPK) signaling pathways. WGSO selectively upregulated GLUT-4 and activated the AKT pathway, whereas RGSO induced a broader effect by increasing both GLUT-2 and GLUT-4, upregulating HNF1 α , and activating both PI3K/AKT/PKC ζ and AMPK pathways, promoting GLUT-4 translocation. Our results suggest that grape seed oil may help regulate glucose metabolism, especially in cases of insulin resistance, making it relevant for managing diabetes. The repurposing of this wine by-product enhances its value and promotes practices, encouraging further research into its therapeutic potential.

Acknowledgements

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3D CELL MODELS: A VALUABLE TOOL TO STUDY AUTOPHAGY ROLE IN GLIOBLASTOMA BIOLOGY

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Glioblastoma (GBM) is the most common and aggressive adult brain tumor, with poor median survival and limited therapeutic options. One of the major limitations to the treatment and resolution of GBM is the presence within the tumor mass of a small subpopulation of cells called glioma initial glioma cells (GICs). GICs, harboring potent tumor-initiating capability and sustaining tumor-growth, are responsible for the high degree of morphological, molecular and cellular heterogeneity that characterizes GBM.¹ Autophagy is a cytoprotective mechanism that is often deregulated in human diseases, especially in cancer. The relevance of autophagy in GBM has yet to be fully clarified, although a growing body of evidence suggests that suppression of autophagy is correlated with gliomagenesis.² In order to overcome the weakness of 2D cell models in investigating tumor biology, we are setting protocols for generating reliable 3D *in vitro* models, enriched in GICs and that hopefully recapitulate the tumor features. In detail, we are generating both tumor spheres and tumoroids from GBM cell lines or primary cells from patients. We are characterizing the GBM 3D models for proliferation rate, expression of specific markers and autophagy and apoptosis occurrence. 3D models will

allow us to in-depth investigate the autophagy role in GBM biology and to correlate it to tumor features such as stemness/differentiation degree, proliferation proficiency and drug-response proficiency. Autophagy-defective GBM models will be also employed to assign a role to specific autophagy master regulators in GBM tumorigenesis.

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IN VIVO EVALUATION OF CYTOTOXIC AND GENOTOXIC RESPONSES INDUCED BY TITANIUM DIOXIDE NANOPARTICLES (TiO₂-NPS) IN GOLDFISH (CARASSIUS AURATUS)

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Titanium dioxide nanoparticles (TiO₂-NPs) are widely used in industrial and consumer products,¹ raising concerns about their ecotoxicological effects, especially in aquatic environments. With the expansion of nanotechnology, artificial particles are increasingly released into the environment and, due to their structure, often different from natural substances, they tend to persist and degrade slowly, posing risks to organisms lacking natural defense mechanisms.² Once released, it can be absorbed by aquatic organisms and enter the food chain. Prior studies, such as Rocco et al.³, have shown that TiO₂-NPs exposure causes DNA damage and genomic instability in zebrafish. This study aimed to assess *in vivo* cytotoxic and genotoxic effects in goldfish (*Carassius auratus*), exposed to 10 μ g/L TiO₂-NPs for 14 and 21 days. A control group (NC) was maintained without exposure. Three assays were performed: eosin Y staining for cell viability, NBT test for ROS production, and TUNEL test for DNA fragmentation. The results showed a significant reduction in cell viability, especially at the end of the longest exposure period (21 days), with a decrease of 78.6% compared to 96% observed in NC. In addition, a significant production of ROS was observed in the treated samples, with an increase of 27.98% at 14 days and 36% at 21 days. Similarly, a significant increase in DNA fragmentation was recorded, equal to 24% at 14 days and 28% at 21 days, compared to controls, which show a fragmentation of 13% and 15%, respectively. Furthermore, another aspect observed was the color change of the livery of the exposed specimens, which showed a change from a reddish-orange to blackish shades, suggesting a possible visible effect of prolonged exposure. These data indicate that exposure to TiO₂-NPs causes cytotoxic and genotoxic effects in goldfish, effects that become more evident with the prolongation of exposure time. The increase in oxidative stress, DNA damage and the granting of cell viability suggests that these nanoparticles, can have a significant biological impact. The consistency between the results of the three tests strengthens the hypothesis of a potential ecotoxicological risk and underlines the importance of a more rigorous regulatory monitoring of the release of TiO₂-NPs in environment.

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RAMAN MICROSCOPY UNVEILS POLYSTYRENE NANOPARTICLES IN ZEBRAFISH AND THEIR ROLE IN OCULAR STRESS

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Nanoplastics (NP) pollution poses a growing environmental threat, with potential toxic effects on aquatic organisms. In this study, Raman microscopy was employed to monitor 150 nm polystyrene (PS) bioaccumulation during zebrafish early developmental stages. Raman imaging enabled the detection of PS in eye at 96 h post-fertilization (hpf), as previously reported in the literature.¹ PS induced proteins structural changes as demonstrated from the identification of amide I and amide III peaks, at 1663 cm⁻¹ and 1250 cm⁻¹ respectively, detected exclusively in treated samples. These peaks, according to the literature, are closely associated with inflammatory processes and oxidative stress.² The eyes revealed alterations in pigmentation patterns that could relate to inflammatory and oxidative stress processes. This hypothesis is supported by RT-PCR results, which show increased gene expression of inflammation- and oxidative stress-related genes, such as *il-1β*, *sod1*, *cat*, and *gstm*. Particularly noteworthy is the increased expression of the *rpe65c* gene, specific to the retinal pigment epithelium and essential for visual function. Dysregulation of *rpe65c* has been associated with ocular diseases in various experimental models, highlighting the potential impact of polystyrene nanoparticles on eye physiology.³

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NEUROTOXICITY INDUCED BY CuO NANOPARTICLES IN A SALTWATER MICROCRUSTACEAN

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Research on nanoparticles (NPs) has made progress through their use in a variety of fields¹, but their effects on human health are still not fully understood. Assessing the toxicological risk associated with xenobiotics may not be easy. However, as the use of nano-sized materials increases, there is a growing need to monitor their possible effects on exposed organisms. Nanomaterials can easily accumulate in the environment and they can interact with biological systems, pushing the scientific community to investigate the main mechanisms of action so as to allow their safe production and consumption in order to preserve health and the environment. CuO nanoparticles have found wide application in various fields of science and technology, including electronics, agriculture, medicine, solar energy². Several evidences have shown that exposure to copper oxide nanoparticles (CuO-NPs) can have harmful effects on the metabolism of exposed organisms as they are stable and characterized by peculiar properties that make them able to accumulate in the environment. In this study, juvenile specimens of *A. salina* were exposed to different concentrations of CuO-NPs in order to evaluate potential neurotoxic damage. Although exposure did not have a negative impact on the viability of the specimens, the immunofluorescence assay has suggested a positivity for Acetylcholinesterase (AChE) as a biomarker of neurotoxicity.

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EMBRYOTOXIC EFFECTS OF ZnO NANOPARTICLES IN ZEBRAFISH: INFLUENCE OF THE TESTING CONDITIONS

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Zinc oxide nanoparticles (ZnO NPs) are widely used as additives in various materials such as ceramics, cosmetics, food, electronics, paints, and rubber. One of the key benefits is its antimicrobial activity, mainly due to the redox properties of elemental zinc in nano form. In the Integrano project, sonochemically produced ZnO NPs are used to develop antimicrobial textile coatings. To align with a Safe and Sustainable by Design (SSbD) approach, the project evaluates the potential hazards of these nanomaterials at different stages of their lifecycle, assessing toxicity with zebrafish using the Fish Embryo Toxicity (FET) test (OECD 236/2013). Given that the fate, transport, and toxicity of NPs are heavily influenced by their intrinsic physicochemical properties and water chemistry parameters – such as pH, ionic strength (IS), and natural organic matter – we investigated how IS affects the properties and toxicity of ZnO NPs. ZnO nanopowder was dispersed in standard

and diluted (1:10 in MilliQ water) FET solution. DLS techniques assessed the NPs' hydrodynamic behavior and surface charge, while ICP-OES analyzed dissolution. Zebrafish embryos at 3 h post fertilization (hpf) were exposed to increasing concentrations of freshly prepared ZnO NPs until 96 hpf. Lethality was recorded every 24 h, hatching was evaluated from 48 hpf, and malformations and morphometric parameters were analyzed at 96 hpf. The results showed that the simulated lowIS improved the suspensions stability by decreasing the NP hydrodynamic size compared to standard IS conditions. These physicochemical changes were linked to increased embryotoxicity, evidenced by higher rates of lethality and malformations. Conversely, the inhibition of embryo hatching -the main effect observed under standard test conditions- was partially alleviated under lowIS, as indicated by nearly a ten-fold increase in the Hatching Concentration 50. These evidences suggest that decreasing the IS of the aqueous media, may improve the NP bioavailability for developing tissues, increasing the lethal and teratogenic potential of ZnO NPs. Altered embryo hatching can be considered a sensible marker for ZnO NPs effects in fish, although its sensitivity varies according to the water salinity. Further researches are taking place to characterize the mechanisms beyond these effects.

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SCREENING POSSIBLE SAFE HARBOR LANDING SITES FOR REPRODUCIBLE TRANSGENESIS IN THE ANNUAL KILLIFISH *NOTHOBRANCHIUS FURZERI*

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The African annual killifish *Nothobranchius furzeri* is an emerging model organism in the fields of aging, regeneration and developmental biology. Its appeal lies in its naturally compressed lifespan and unique embryonic development, which evolved as an adaptation to seasonal habitats. Annual killifish embryos exhibit dispersion and reaggregation of blastomeres after epiboly and prior to axis formation, and they can enter diapause at three distinct developmental stages. Annual killifish are genetically tractable, but the identification of safe genomic loci for transgenesis remains a challenge. Consequently, most transgenic approaches in killifish have relied on random integration and identifying suitable locations for universal and permissive genomic integration has become highly desirable. Notably, transgene expression from randomly inserted cassettes is frequently repressed following the blastomere dispersion phase. This repression may be attributed to the species' unique embryonic development or, more likely, to position effects at the integration site. To address this, the nested Thermal Asymmetric Interlaced (TAIL)-PCR protocol was employed to map genomic loci of previous well-established Tol2-based transgenic lines. TAIL-PCR products were sequenced, and, in some cases, the individual transgene integration was independently validated using specific primers. Both intergenic and intragenic putative insertions were identified. A GFP reporter construct was then inserted into the mapped regions using CRISPR/Cas9 to confirm these loci as possible safe harbor sites, with the downstream goal of generating a stable *attP* landing site line suitable for

phiC31 integrase-mediated transgenesis. Preliminary data showed that transgene expression began to fade in GFP-positive embryos after the early stages of development. Further investigation of mapped regions is required to confirm whether efficient transgene expression is position-dependent or if other repressive mechanisms could be responsible for the observed rate of transgene repression in *N. furzeri*.

FIRST EVIDENCE OF CONTROL MECHANISMS OVER CIRCULATING HEMOCYTE POPULATIONS AFTER HEMOLYMPH COLLECTION, IN THE SNAIL *POMACEA CANALICULATA*

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Hemocytes are key cells in mollusc immunity, homeostasis and development, but no specific markers are available and little is known on how their number is controlled. The laboratory bred snail *Pomacea canaliculata* offers the possibility to stimulate hematopoiesis by multiple hemolymph samplings and its hemocytes have been categorized into small and blast-like Group I (GI) and large Group II (GII). In single individuals the number of circulating hemocytes (CH) and the GII/GI ratio are stable, suggesting the existence of a control over CH populations (CHP) and cell-specific functions. In this regard, CHP were assessed by flow cytometry (FC) 1.5, 3, 6, 9, 18, 24 or 48 h after a previous hemolymph collection. GII/GI ratio was the lowest at 18h and recovered at 24 h, when the total hemocyte number was significantly higher, returning to baseline after 48h. On this basis, DNA content was evaluated by FC to check for active mitosis in CH. Interestingly, FC showed that CH had varying amounts of DNA not ascribable to cell cycle and that there was a positive correlation between DNA-content and cell size. In agreement with the data on GII/GI ratio, high-DNA-content CH significantly decreased after 18h and returned to baseline after 24h. FC data on DNA content might be explained by cell endocycling, a phenomenon so far described in gastropod neurons and other invertebrate cell types, but not in molluscan hemocytes. Consistent with this hypothesis, preliminary RT-PCR and western blot data showed the expression of PCNA, a key eukaryotic DNA replication and repair factor, in control CH. These data suggest that *P. canaliculata* controls CHP composition and its hemocytes might exhibit localized DNA amplification, especially in larger cells, confirming that hemolymph repopulation is a promising biological context for studying CHP dynamics and hemocyte turnover.

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DEVELOPMENT OF *IN VITRO* MODELS FOR THE STUDY OF RETINAL DISEASES

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Hereditary Hemorrhagic Telangiectasia (HHT) is a vascular disorder of genetic origin that affects over 1.4 million individuals worldwide.¹ Mutations in the endothelial surface receptors *ENG* (HHT type 1) and *ALK1* (HHT type 2) account for more than 90% of cases. Phenotypically, HHT is characterized by the formation of arteriovenous malformations (AVMs), which are direct connections between arteries and veins that bypass the capillary network. Larger AVMs typically occur in the liver, lungs, or brain, and can lead to severe complications, including heart failure, stroke, or death. Dysregulated angiogenesis plays a central role in disease pathogenesis. Vascular Endothelial Growth Factor (VEGF) has emerged as a key factor in this context, with elevated levels reported in both tissues and plasma of HHT patients. Although anti-VEGF therapies have shown some clinical benefits, they are often limited by significant side effects.² Familial Exudative Vitreoretinopathy (FEVR) is a rare inherited disorder primarily affecting retinal vascular development.³ It results in incomplete vascularization of the peripheral retina and poor vascular differentiation. In severe cases, retinal ischemia can trigger secondary neovascularization, which may lead to complications such as retinal detachment and retinal dysplasia. To date, five genes have been associated with FEVR, including *LRP5* (3 ALIAS 4). To explore new, safe, and effective therapeutic strategies, we are developing *in vitro* models of HHT type 2 and FEVR using RNA interference technology. These models are being validated and used to evaluate the effects of candidate compounds. Validation and functional assessment are being performed through qPCR, Western Blotting and tube formation assays.

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IMPACT OF POLYSTYRENE MICRO- AND NANOSPHERES ON 3D *IN VITRO* INTESTINAL BARRIER: ROLE OF SIZE AND SURFACE FUNCTIONAL GROUPS

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Micro- and nanoplastics (MNPs) have become ubiquitous environmental contaminants, with increasing evidence of their accumulation in the food chain and potential ingestion by humans. The gastrointestinal tract represents the primary site of exposure through ingestion of contaminated food and water, yet the cellular and molecular effects of MNPs on the intestinal epithelium remain incompletely understood. In this study, we investigated the impact of fluorescent carboxylate-modified polystyrene beads (PS-COOH) (200 and 40 nm, respectively green and red) and amine-modified polystyrene beads (PS-NH₂) (200 nm, red) on an *in vitro* gastrointestinal barrier model developed with a co-culture Caco-2, HT29-MTX and Raji B cell lines, to best represent the different cell types present in the human gastrointestinal barrier. The particles were characterized in terms of size, zeta potential, and stability in culture media. Functional assessment of the epithelial barrier was performed by measuring transepithelial electrical resistance (TEER), Scanning Electron Microscopy (SEM), Wheat germ agglutinin (WGA) conjugates staining and expression of tight junction proteins (ZO-1). Our findings indicate that particle size and surface chemistry critically affect cellular uptake and barrier integrity. All tested particles were internalized by the three cell types, suggesting a high potential for epithelial penetration. Carboxylated nanospheres were more cytotoxic than their micro-sized counterparts, while amine-modified microspheres caused less cytotoxicity than carboxylated microspheres. Moreover, the exposure to all polystyrene particles caused significant alterations in TEER values and the expression of ZO-1 tight junction protein was reduced; in particular, in presence of nanospheres. Our results suggest that the size and surface properties of these particles play a key role in modulating their impact on the intestinal epithelium, underlining the need for further investigation into their long-term effects on intestinal health and their broader implications for human health.

DECELLULARIZED HUMAN LUNG MATRIX FOR TUMOR MICROENVIRONMENT MODELLING

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Decellularized extracellular matrix (d-ECM) is a promising biological scaffold for *in vitro* cancer research, due to its ability to replicate the native microenvironment and maintain key biomechanical properties. An effective decellularization process must

remove all cellular material while preserving the ECM's structure and composition. Human tissue-derived d-ECM offers relevant biochemical and structural cues, making it valuable for disease modelling. In this study, we developed an *in vitro* model to investigate the pulmonary metastatic microenvironment induced by breast cancer. Human lung biopsies from pathological donors were snap-frozen, sectioned at 100 μ m, and decellularized using 1% SDS and 1% Triton X-100 for 24 h. To prevent contamination, decellularized scaffolds were treated with an antibiotic-antimycotic solution and sterilized with UV light. H&E staining confirmed successful cell removal while maintaining ECM integrity. The lung-derived d-ECM was reseeded with normal human lung fibroblasts, recreating a native-like microenvironment. Human lung fibroblasts were then stimulated with breast cancer-conditioned medium to study how stromal cells remodel the ECM and support tumor invasion. This model offers a reliable platform to explore cell-matrix interactions and the stromal role in cancer progression, providing new insights for preclinical oncology research.

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NEUROBIOLOGICAL EFFECTS OF HESPERIDIN IN THE IDIOPATHIC AUTISM MODEL BTBR

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Hesperidin (HSP), a major flavonoid of citrus fruits, has been shown to exhibit a wide range of beneficial effects, including antioxidant, anti-inflammatory and neuroprotective effects¹ along with regulating lipid and glucose metabolism². Due to its neuroprotective and anti-inflammatory properties, hesperidin was selected in the present study to evaluate its effectiveness in different neurobiological activities of a mouse model of autism (BTBR)³. With this aim, we treated both BTBR and C57BL/6J (C57) mice with a standard chow diet (CD, 2019 Teklad Global Diet - 9% fat, 19% protein, 44.9% carbohydrate; Envigo RMS, Udine, Italy) \pm HSP (\geq 80%, Sigma-Aldrich Inc.) supplementation of 100 mg/kg⁴ for four weeks. Behavioral performances began during the last week of treatment, while cortical and hippocampal thickness, plus metabolic profiles were checked at the end of the treatment. Firstly, HSP supplementation significantly reduced repetitive behaviors ($p < 0.001$) while improving discrimination index of memory ($p < 0.001$) and sociability index ($p < 0.05$) in BTBR. Moreover, latency to dark were increased in both BTBR ($p < 0.01$) and C57 ($p < 0.001$) treated with HSP rather than mice that received only CD. Histological analyses mostly revealed that the thickness of the lateral cortex and CA3 of the hippocampus were strongly increased ($p < 0.001$) in the HSP-treated groups. Interestingly, such modifications were linked to a general reduction of some biochemical parameters, such as LDL cholesterol levels ($p < 0.05$) in BTBR mice treated with CD+HPS. These findings suggest for the first time that HSP may exert neuroprotective

effects and improve several autism-like symptoms, strengthening its potential as a therapeutic dietary supplement for neurodevelopmental disorders such as ASD.

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ACTION OF PROPRANOLOL ON RESPIRATORY ACTIVITY IN GOLDFISH

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Oxygen levels in water constantly fluctuate due to natural processes or human activities. Some aquatic organisms, such as goldfish (*Carassius auratus*), can survive in hypoxic and even anoxic conditions due to their ability to enter in a hypometabolic state, saving energy¹. In recent years, environmental drug pollution has increased exponentially, mainly due to anthropogenic activities. Propranolol, one of the most prevalent drugs in the aquatic environment, is a β -blocker, capable of binding β -adrenergic receptors and used to treat cardiovascular diseases. Several studies have reported the effects of propranolol on fish, which could reduce physiological response to hypoxia due to the presence of β -adrenergic receptors in the gills. Therefore, we studied the changes in the histology of gills and oxidative metabolism of goldfish induced by propranolol at concentrations of 3 ng/L in both normoxia and hypoxia for 19 days. Haematoxylin-eosin and Mallory's trichrome staining revealed the aggregation of primary lamellae and the suffering of secondary lamellae in the group exposed to propranolol alone and in the group co-exposed. Instead, PAS staining showed an increase in mucosal cell number in both treatments. Biochemical analyses showed that propranolol attenuated the reduction induced by hypoxia in mitochondrial cytochrome oxidase (COX) activity², an effect not found when fish are exposed to the drug alone. Regarding the oxidoreductive balance, the increase in total antioxidant capacity (TAC) that occurred during hypoxia is slightly attenuated in animals exposed to both hypoxia and the drug. In contrast, the amount of reactive oxygen species (ROS) and the protein-bound carbonyls were significantly increased in the group exposed to both hypoxia and the drug. Although there was an increase in antioxidant defenses during co-exposure, propranolol alone was unable to attenuate ROS formation and damage to macromolecules, also inhibiting the strategy of decreased metabolism following hypoxia. Therefore, propranolol has a negative influence on the adaptations of goldfish and seriously impairs aquatic environmental health.

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“PRIMING TEMPERATURE” PHENOMENON IN MUSSEL MYTILUS GALLOPROVINCIALIS EXPOSED TO ACUTE THERMAL STRESS: CELLULAR AND TISSUE RESPONSE

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In recent years, coastal marine ecosystems have come under pressure from both human and nature. Global warming, and specifically the gradual increase in temperature from one year to the next, is becoming increasingly widespread, with adverse effects on the survival of marine organisms. The Mediterranean mussel *Mytilus galloprovincialis* is a species of bivalve mollusc in the Mytilidae family. This species was chosen to study the “priming temperature” phenomenon and its response to exposure to different temperatures (T) at different time intervals. In order to understand the response of mussels to increasing T, a mortality curve was drawn starting from 28°C and rising to 42°C. This curve showed the LT50 value at around 36°C. This first step enabled us to select the T useful to carry out an acute exposure of mussels, with a control group maintained at 18°C and three groups exposed to 28°C, 30°C and 32°C, respectively, for a period of two h each, to then maintained at the control T of 18°C. After 24 h, mussels from each treated group were exposed to a higher T of 36°C for 2 h. The aim of this experiment was to understand the effectiveness of the “priming temperature” to adapt mussels to temperature fluctuations. Biochemical enzymatic analyses were carried out to monitor the response of mussels after acute thermal exposure. A multi-marker approach was applied to study the enzymatic activity of catalase (CAT), glutathione S-transferase (GST), and acetylcholinesterase (AChE), as well as malondialdehyde (MDA) levels on digestive glands and gills. Histological investigations on digestive glands, gills, and gonads of mussels from all groups were also performed to monitor their tissue response to acute thermal exposure. This study will enable us to study the chronic exposure of this model species to high temperature waves and confirm their resistance to this ecological stress factor.

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IMPACT ON ZEBRAFISH CELLS OF CADMIUM AND POLYSTYRENE MICROPLASTICS CO-EXPOSURE: AN IN VITRO APPROACH

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Environmental pollution has been a central topic in public discussions for several years due to its potential harmful effects on ecosystems and human health. The accumulation of plastic in the environment has become a growing threat to marine organisms’

health, as plastic breaks down into smaller fragments like microplastics (MP) and nanoplastics (NP), which can infiltrate any part of the body of exposed organisms with harmful effects.¹ Though the mechanism of action remains unclear, it is known that these substances can create an inflammatory-oxidative environment that triggers the formation of oxidant species capable of inducing apoptosis.² Recent studies also suggest that microplastics can act as vectors for other toxic substances, leading to synergistic toxic effects.³ Among the most widespread environmental contaminants are heavy metals, with cadmium (Cd²⁺) being one of the most prevalent. Exposure to Cd²⁺ and its accumulation through the food chain pose a significant threat to all organisms.⁴ This study aimed to evaluate the cytotoxic and genotoxic effects of PS-MPs and Cd²⁺ *in vitro*, both individually and in combination, on *Danio rerio* blood cells. The cells were exposed to 70 µg/mL of PS-MPs and 0.1 µg/L of Cd²⁺ for 30, 60, and 90 min. Cytotoxicity was assessed using the eosin viability test, revealing a significant reduction in cell viability due to PS-MP and Cd²⁺ exposure. To evaluate genotoxicity, the NitroBlue tetrazolium chloride (NBT) test was used to detect oxidative stress and the production of ROS. Results showed a significant increase in ROS after exposure to both PS-MP and Cd²⁺, either alone or combined. Additionally, RAPD-PCR was conducted to assess genomic template stability (GTS), revealing genotoxic effects. We can be deduced that PS-MP and Cd²⁺ have cytotoxic and genotoxic activity capable of causing DNA damage in zebrafish through the production of ROS, reducing cell viability, which, in turn, induce oxidative stress with consequent activation of the apoptotic pathway. However, the co-exposure to Cd²⁺ and PS-MPs induced a lower cytotoxic and genotoxic response compared to single exposures, suggesting a potential interaction between the substances that modulates their reactivity.

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MITOCHONDRIAL INHERITANCE AND PHENOTYPIC LANDSCAPE OF BIVALVE GERMLINE

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Despite the essential role of mitochondria for the organism correct functioning, little is known about the mechanisms regulating their transmission across generations. In several bivalve species, a peculiar mode of mitochondrial inheritance^{1,2} generates naturally heteroplasmic organisms, a characteristic that can be extremely useful in the study of mitochondrial biology. *In silico* analyses on bivalve species suggest the ubiquitin-proteasome pathway¹ and mitochondrial membrane potential² as shared molecular mechanisms involved in mitochondrial selection and inheritance. The aim of this project is to experimentally test some working hypotheses using multiple techniques on *Mytilus galloprovincialis* and *Ruditapes philippinarum*. We immunolocalized proteins in the

ubiquitin pathway (prohibitin and poly-ubiquitin) in early embryos by confocal microscopy. We used Raman spectroscopy to identify potential markers for diverse cellular compartments and organelles, and differentiate germline and somatic cell lineages. We found ubiquitin in the sperm midbody and a strong concentration of prohibitin at the CD and D blastomeres of 2 and 4-cell embryos, respectively. Also, we identified the ratio between some Raman bands as potential markers to identify cell types and differentiation stage. Overall, our findings support a role of the ubiquitin-proteasome pathway in the differential segregation of sperm mitochondria in bivalves and suggest a concentration of mitochondria in germline blastomeres. We also confirm the potential of Raman spectroscopy in the characterization of germline development and mitochondrial inheritance.

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CYTOTOXIC EFFECT OF BEETLE-DERIVED CANTHARIDIN ON GLIOMA CELLS

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Cantharidin (CTD) is a beetle's derived terpene with several healing properties whose exploitation is limited due to its toxicity. Here, we have investigated the anti-tumoral effect of CTD on glioblastoma (GB), the most common type of primary malignant brain tumor characterized by a very severe prognosis. The objective of this study is twofold: first, to analyze the cellular mechanisms underlying the cytotoxic effects of CTD in GB cells, and second, to identify a method to mitigate its significant side effects. The initial evaluation assessed CTD's impact on GB cells, revealing high toxicity and a dose-dependent reduction in cell viability. CTD also increased reactive oxygen species (ROS) levels, an effect being mitigated by the ROS scavenger N-acetylcysteine. The study further shows that CTD inhibits the NRF2-driven antioxidant response, as evidenced by results with or without the Nrf2 activator DMF. It is hypothesized that increased ROS and Nrf2 inhibition may be the primary cause of CTD-induced toxicity, and this hypothesis will be tested in future studies. However, CTD was also found to be cytotoxic to normal human glial cells, thus indicating its side effects. To overcome these limitations and to increase CTD specificity, it was necessary to identify possible tumor markers as potential targets to selectively direct the effects of CTD. One way to make cantharidin toxicity selective is through advanced drug-delivery systems like Antibody-Drug Conjugates (ADCs). ADCs link an antibody's specificity to a cytotoxic drug, targeting tumor cells by binding to specific markers, then internalizing and releasing the drug. Potential targets for ADCs in this context include: 1) HER/erbB receptor proteins such as EGFR,

Her2, or Her3; 2) CD44, a cell surface glycoprotein involved in cancer progression; and 3) System XC- (cys/glu antiporter), which helps cancer cells resist oxidative stress. These proteins are expressed at significantly higher levels in glioma cells. We are also evaluating the intracellular trafficking of antibodies after they bind to their targets. This is crucial for understanding how ADCs work.

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FROM FRY TO ADULT: HISTOLOGICAL INSIGHTS INTO SPLEEN MATURATION IN FARMED SEA BREAM

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The spleen appeared in jawed vertebrates' evolution alongside the development of adaptive immunity. During teleost ontogeny, it initially serves as a site of erythropoiesis and later grows into one of the main lymphoid organs in adults, together with kidney and thymus. The spleen also plays a role in blood filtration and the regulation of circulating erythrocytes, acting as a storage organ. These various functions coexist, and although the terms 'white pulp' and 'red pulp' are used in fish as they are in mammals, the teleost spleen lacks clear demarcation between the two regions. Immune cells, erythrocytes, and melano-macrophages are dispersed and intermixed with specifically structured blood vessels, such as sinusoids, and ellipsoids. The relative abundance of these structures can vary in the fish spleen, adapting to factors such as age, nutritional status, parasitic infection, and environmental stress. This likely affects the shape and size of the organ, although few studies have explored this aspect. In this study, spleen morphology and histology were documented in farmed *Sparus aurata* Linnaeus, 1758, from 6 cm to 29 cm TL, over 24 months of growth in an off-shore aquaculture facility in the Ligurian Sea. The body condition and the histopathology of other organs were assessed in the same specimens to evaluate the general health of the fish. Although the fish appeared healthy throughout the study, some individual variation was observed, along with a slight deterioration of condition over time. This allowed us to correlate the optimal condition of juvenile and young adult *S. aurata* with a relatively smaller spleen and a reduced abundance of melano-macrophage centers.

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SEA URCHIN EMBRYO AS AN EXPERIMENTAL MODEL FOR PREDICTIVE STUDIES OF VANADIUM-INDUCED TOXIC EFFECTS UNDER CLIMATE CHANGE CONDITIONS

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The study of ecotoxicity induced by vanadium (V) represents an area of increasing interest due to the growing use of V in both the industrial and pharmaceutical areas. This leads to its introduction into water environments, marking a developing problem, especially since rising global temperatures appear to intensify its toxic properties. Cytotoxicological approaches carried out on whole marine embryos represent a valid research tool since they grow directly in contact with the pollutants and are equipped with highly responsive cells to stressors. Here, we discuss the detrimental impact on *Paracentrotus lividus* sea urchin embryos resulting from the combination of V and higher temperatures, reflecting the effects of climate variation. The results demonstrate the remodeling of embryonic architecture at the morphometric level, revealing developmental delays and anomalies. These malformations involve variations in the total skeletal mass due to the almost total absence of the skeleton, with the exception of small calcareous aggregates. Furthermore, both a modulation in total tissue remodeling enzymatic activities and a variation in the amount of three MMP-like gelatinases (MMP-2, -9, and -14) were observed. This research demonstrates that climate change significantly increases the harmful effects of V, emphasizing the necessity for comprehensive toxicity assessments in environmental evaluations.

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THERAPEUTIC EFFECTS OF A NEUROTROPHIN IN AUTISM SPECTRUM DISORDER: EVIDENCE FROM IN VIVO AND IN VITRO MODELS

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Autism spectrum disorders (ASDs) are neurodevelopmental conditions frequently associated with impairments in social, communicative, and language skills.¹ An increasing body of evidence from both ASD patients and animal models suggests that alterations in neurotrophic factors may lead to dysregulation of proteins involved in synaptic plasticity.² The aim of this study is to evaluate the efficacy of a neurotrophin in autism models, using both *in vivo* and *in vitro* approaches. The BTBR T+Itpr3tf/J mouse strain, a well-established model of autism, was selected for *in vivo* experiments. The neurotrophin was administered intranasally, offering a minimally invasive delivery method. To assess the

effects of the neurotrophin on stereotyped and repetitive behaviors in the BTBR strain, marble buried test and hole board test were performed, while the social interaction test was then carried out to analyze the outcome of the neurotrophin on the social behavior. Notably, exogenous neurotrophin administration was found to improve stereotyped behaviors and social interactions. For the *in vitro* component, human-induced pluripotent stem cells (hiPSCs) were generated from Peripheral Blood Mononuclear Cells (PBMCs) of ASD patients and healthy controls. The establishment of the hiPSC cell line will provide the opportunity to continue the research by generating cerebral organoids and testing the effects of the neurotrophin in this model.

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MICROSCOPICAL BIOMARKERS OF ENVIRONMENTAL STRESS IN THE BLACK GOBY *GOBIUS NIGER*

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Individuals of the black goby were sampled from the station "SVAM" of the second inlet of Mar Piccolo, Taranto, in the context of the project "HEASY". Histopathological changes were used as biomarkers of environmental stressors¹. Gills, liver, spleen and intestine were selected as target organs. All the examined individuals presented alterations. Gills showed hyperplasia of the epithelial cells, resulting in fusion of some secondary lamellae and blood congestion. Parasitic infestation of the liver and/or intestine was recorded in all individuals. Hepatic lipidosis, a symptom of lipid metabolism disorder², was observed in 80% of the individuals, especially in case of parasitic liver infection. Nematode infection, caused hepatocytes necrosis, blood-filled dilated sinusoids² and increase of melanomacrophage centers³ in both livers and spleen². The intestinal villi near the parasitic infestation showed rupture and loss of the epithelial layer, with consequent loss of its secretory function. The observed alterations make good biomarkers of the health of the organisms and the presence of environmental stressors and thus the water conditions. It would be appropriate to increase monitoring plans and implement immediate remediation to ensure the survival of the species for ecological and commercial purposes.

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EFFECTS OF DEXAMETHASONE ON NEUROTRANSMISSION IN MUSSEL GILLS

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Pharmaceutically active compounds (PhACs) are a major class of emerging contaminants. Among them, dexamethasone (DEX), a synthetic glucocorticoid used for its anti-inflammatory effects, is frequently detected in coastal waters with doses reaching the ng/L range. DEX is known to disrupt several biological pathways (e.g., protein turn-over, ROS and energy metabolism) but few information exists on its effect on the neurotransmission system of invertebrates. In this study, the impact of realistic doses of DEX (4 to 2000 ng/L) was assessed on the branchial neurotransmission system of mussel *Mytilus galloprovincialis* for a 12-day period, considering three sampling time-points (3, 6 and 12 days). The gills were selected for their crucial role in gas exchange, filtration, and osmoregulation, all of them regulated by neurochemical signalling. Thus, disturbances in neurotransmission can impair vital functions of organisms. The cholinergic (choline acetyltransferase, ChAT; acetylcholinesterase, AChE; acetylcholine, ACh), serotonergic (serotonin, 5-HT; its receptor, 5-HT₃R), and dopaminergic (tyrosine hydroxylase, TH) systems were evaluated using a multi-biomarker approach combining enzymatic, immunohistochemical and metabolomic analyses. DEX induced an early and marked alteration in all the gill neurotransmission systems, showing dose-dependent effects. The cholinergic system appeared severely impaired, as witnessed by AChE inhibited activity, drop in the immunopositivity of ChAT and AChE, and rise in ACh levels. A relevant reduction of immunopositivity was also reported both in serotonergic and dopaminergic systems. Furthermore, alterations in osmolytes (taurine, betaine, homarine) indicated an impaired osmoregulation, suggesting also a possible disturbances in membrane integrity. This study highlights the risk posed by environmental doses of DEX on mussel gills functions, confirming its neurotoxic impact on non-target aquatic invertebrates.

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ROTENONE-INDUCED PEROXISOMAL ALTERATIONS IN BV-2 MICROGLIAL CELL LINE

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Parkinson’s disease (PD) is a neurodegenerative disorder marked by a progressive decline in motor skills, accompanied by non-motor symptoms. Despite multifactorial etiology, common pathological mechanisms are recognized, including mitochondrial dysfunction, oxidative stress, α -synuclein buildup, lipid dysmetabolism, and neuroinflammation. These processes culminate with selective loss of nigral dopaminergic neurons. Preclinical PD models, involving administration of rotenone, a mitochondrial complex I inhibitor, are commonly used to replicate dopaminergic neuron degeneration and aggregate formation. Peroxisomes are ubiquitous cytoplasmic organelles, crucially involved in lipid metabolism, reactive oxygen species detoxification and inflammatory signaling. As our group and other authors have previously demonstrated, peroxisomal dysfunction is involved in neurodegeneration conditions, showing mitochondrial deficits. Interestingly, changes in peroxisomes reportedly lead to increased mitochondrial proliferation, however it remains unclear whether mitochondrial alterations can trigger an adaptive response in peroxisomes¹. The present study aims at investigating the involvement of peroxisomes in PD, focusing on the relatively unexplored role of microglia. To address this issue, BV-2 microglial cell line treated with rotenone was utilized to mimic a Parkinsonian cell phenotype. Immunofluorescence analysis shows enhanced expression of inflammation markers IBA-1, IL-6, along with α -synuclein aggregation. Even peroxisomal markers (PMP-70, ACOX1) are increased, and we also observed a peculiar intracellular localization of peroxisomes, related to abnormal α -tubulin distribution. Our results indicate a marked activation of peroxisomes, which may partially compensate for mitochondrial dysfunction, thereby supporting the functional relationship between the two organelles.

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PHARMACOLOGICAL INHIBITION OF BET PROTEINS REDUCES OXIDATIVE STRESS AND INFLAMMATION IN A CELLULAR MODEL OF RETT SYNDROME

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Rett syndrome (RTT) is a severe X-linked neurodevelopmental disorder primarily caused by mutations in *Mecp2* gene. While initially considered a neurological condition, RTT is now recognized as a multisystem disorder, with redox imbalance and inflammation significantly contributing to disease progression. Recent studies highlighted the role of Bromodomain and Extra-Terminal domain (BET) proteins in the epigenetic control of inflammation and redox metabolism, identifying them as valuable therapeutic targets for several chronic diseases. Therefore, this study aimed to evaluate the effects of the BET inhibitor JQ1 on cultured fibroblasts derived from RTT patients. Our results demonstrate that JQ1 significantly reduces oxidative stress markers in RTT fibroblasts. Notably, BET inhibition normalizes the expression of certain mediators involved in both anti- and pro-oxidant systems, counteracting redox disbalance. Moreover, BET blockade reduces the levels of pro-inflammatory cytokines expression levels. Overall, these findings suggest that selective inhibition of BET proteins could represent a promising pharmacological approach to mitigate cellular abnormalities associated with RTT. Notably, several JQ1-derivatives with improved pharmacokinetics successfully entered clinical trials, pointing to strong candidacy for future *in vivo* validation.

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GADOLINIUM AS A NEW EMERGING MICROPOLLUTANT: A RISK FOR MARINE BIOTA?

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Gadolinium (Gd) is a rare-earth element (REE) that has been widely exploited since the previous century due to its great versatility in technological, agricultural and medical applications. Nowadays, the ever-increasing demand for this lanthanide, especially in the most industrialized countries, has led to an alteration of the geogenic concentration in various environmental matrices, mainly aquatic ones. The increasing human activities, combined with poorly performing waste disposal facilities for this class of compounds, has led to the worsening of Gd concentrations in marine ecosystems where wastewater collects. The aim of this work was therefore

to evaluate the potential toxic effects of GdCl₃ and Gd₂O₃ on the male and female reproductive health of the marine mussel *Mytilus galloprovincialis*. To this aim, a 28-day chronic exposure was set-up, considering the temporal trend of biological responses (T0, T7, T15, T28) of mussels challenged with 1 and 10 µg/L of both forms of Gd. Histological analyses of gonads confirmed the impact of Gd, highlighting the presence of hemocytes in the connective tissue underlying the follicles of both sexes. Preliminary data obtained from ¹H NMR-based metabolomics allowed to have a comprehensive overview of the metabolic state of mussel gonads in response to the different experimental conditions, while histochemical investigations, carried out using the dPAS/PAS method, showed the reserves of glycogen, an essential metabolite useful for the correct gametogenesis. The results collected to date support the hypothesis that Gd may present itself as an emerging risk for non-target marine organisms.

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TRAF6 REGULATES AMBRA1 UBIQUITINATION INTERCONNECTING AUTOPHAGY AND UBIQUITIN PROTEASOME SYSTEM

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Autophagy and Ubiquitin Proteasome System (UPS) are degradative pathways both ensuring cellular homeostasis. For many years these mechanisms have been considered distinct but, nowadays it is clear protein ubiquitination is a common feature to target specific substrates. Moreover, tightly and reciprocal regulations among these pathways have been now extensively described. In this context, AMBRA1 is a positive regulator of both autophagy and UPS, binding and modulating the activity of different E3-ubiquitin ligases. Here, we show a novel molecular mechanism by which the E3-ubiquitin ligase TRAF6 mediates the poly-K63 ubiquitination of AMBRA1 on lysine 2 during autophagy response, thus connecting autophagy, ubiquitin proteasome system and cell proliferation.

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