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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.
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- biology of cell development and senescence;
- nerve and muscle cell biology;
- cellular basis of diseases.

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MAIN LECTURES

EXPLOITATION OF SMN ROLE IN NEURON DEVELOPMENT USING A *C. ELEGANS* MODEL

E. Di Schiavi, P. Santonicola, F. Cieri, G. Zampi, I. Matino

IBBR, CNR, Naples, Italy
E-mail: elia.dischiavi@cnr.it

Spinal muscular atrophy (SMA) is a neuromuscular disorder characterized by the degeneration of lower spinal cord motor neurons (MNs), which leads to progressive muscle atrophy and death of young patients, if not treated. SMA is caused by mutations of the Survival of Motor Neuron gene, *Smn1*. Most of pre-clinical studies have been performed in post-natal animals, but recent studies support the hypothesis that SMA, particularly in its most severe forms, present with pre-symptomatic and prenatal developmental phenotypes. However, SMN role during development remains to be understood and we still do not know where and when the disease first manifests. Three treatments are currently in the clinics for SMA patients: Spinraza, Zolgensma and Evrysdi. All treatments work by increasing the levels of full-length SMN protein with a limited and very early therapeutic time window, again suggesting the need of understanding developmental components to disease pathogenesis. To understand the neurodevelopmental consequences of SMN depletion we took advantage of *C. elegans* as a model for SMA. *C. elegans* represents an excellent model organism to study neurodevelopment. We used an alternative genetic model developed in our lab to efficiently reduce the function of *smn-1,* the *C. elegans* homolog of *Smn1,* specifically in 19 MNs. The silencing of *smn-1* caused an age-dependent neurodegeneration that resulted in altered locomotion and neuron death[1]. These results provide strong evidence that our model is a powerful and unique tool to study SMA, that allows the study of *smn-1* role in neuron development. Indeed, we could demonstrate that *smn-1* plays a role in neurogenesis and axogenesis of MNs, and that different subclasses of neurons are differentially affected. Moreover, using genetic manipulations[2], pharmacological treatments, and phenotypic analysis, we identified the precise time-window during development capable of rescuing SMN function. Our results demonstrate that we can successfully study neuron development role in SMA in a whole living animal and deliver major progresses in defining new combinatorial therapies to prevent *Smn1*-related neurodevelopment defects.

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STEM CELL-BASED EMBRYO MODELS: CHALLENGES AND OPPORTUNITIES FOR BASIC AND TRANSLATIONAL RESEARCH

F. Amoroso[1,2], A. Coppola[1,2], C. D'Aniello[1], A. Fico[1], E.J. Patriarca[1], G. Minchiotti[1]

[1]Institute of Genetics and Biophysics, 'A. Buzzati-Traverso', CNR, Naples, Italy; [2]Department of Precision Medicine, University of Campania Luigi Vanvitelli, Naples, Italy
E-mail: gabriella.minchiotti@igb.cnr.it

Stem cell-based embryo models have emerged as pioneering experimental models to study early mammalian embryogenesis using pluripotent stem cells. These models provide invaluable tools to study fundamental aspects of embryonic development and serve as platforms for disease modeling and drug screening[1]. Among the stem-cell based embryo models are gastruloids. Gastruloids are aggregates of defined numbers of embryonic stem cells that, under defined culture conditions, undergo symmetry breaking and recapitulate crucial events of gastrulation and axis formation[2]. I will introduce this topic and present some recent works of our group on the relationship between pluripotency and competence to develop gastruloids[3] and how we can manipulate gastruloids to ask questions about how genes and cells interact to build an embryo[4,5].

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WHAT HAPPENS WHEN SPERM MEETS THE EGG: NEW INSIGHTS INTO THE FERTILIZATION PROCESS

L. Santella[1], N. Limatola[1], J.T. Chun[2]

[1]Department of Research Infrastructures for Marine Biological Resources, Stazione Zoologica Anton Dohrn, Napoli; [2]Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Napoli, Italy
E-mail: santella@szn.it

Fertilization depends on the specific recognition and binding of the sperm with the egg plasma membrane. Upon fusion, the fertilizing sperm triggers a precise spatiotemporal series of structural and physiological changes in the activated egg, which is crucial for successful embryonic development. In starfish, the immature oocytes in the gonad arrested at the prophase of the first meiotic division (the germinal vesicle stage) are prone to polyspermy. Only after the maturation process stimulated *in vitro* by the maturing hormone 1-methyladenine can the starfish egg respond to only one sperm with a normal Ca^{2+} response to accomplish successful fertilization and embryonic development. Our findings have shown that the optimal fertilizable conditions of eggs are achieved during the maturation process owing to a restructuring of the egg surface and actin filaments in the outer cytoplasmic region of the oocyte as a result of the intermixing of the nuclear and cytoplasmic components following the breakdown of the nuclear envelope[1-7]. In sea urchins, our new results have highlighted that the integrity of the vitelline layer of the eggs, as well as the structural organization and dynamics of the egg's cortical actin cytoskeleton, are crucial for a normal fertilization response and the entry of only one

sperm[8-11]. New insights on the role of the vitelline layer of the eggs for the species-specific recognition of acrosome-reacted sea urchin spermatozoa will also be discussed at the meetings, defying the prevailing view[12].

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NOVEL ADVANCEMENTS IN HUMAN EMBRYO CULTURE TO OPTIMIZE SINGLE BLASTOCYST TRANSFER IN ASSISTED REPRODUCTIVE TREATMENTS

R. Sciorio

Lab Director: Fertility Medicine and Gynaecological Endocrinology Unit, Department Woman-Mother-Child, Lausanne University Hospital, CHUV, Lausanne, Switzerland
E-mail: sciorioromualdo@hotmail.com

Human infertility is a major global public health issue estimated to affect one out of six couples. In the last four decades the assisted reproductive technology (ART) field has witnessed outstanding advances, resulting in continuously improving pregnancy rate and diminishing complications, in particular reduced incidence of multiple births. These improvements are secondary to advanced knowledge on embryonic physiology and metabolism, resulting in the ability to design new and improved culture conditions. Indeed, the incubator represents only a surrogate of the oviduct and uterus, and the culture conditions are only imitating the physiological environment of the female reproductive tract. *In vivo*, the embryo travels through a dynamic and changing environment from the oviduct to the uterus, while *in vitro* the embryo is cultured in a static fashion. Importantly, while culture media play a critical role in optimize embryo development, a large host of additional factors are equally important. Additional potential variables, including but not limited to pH, temperature, osmolality, gas concentrations, light exposure need to be carefully controlled to prevent stress and permit optimal implantation potential[1,2,3.] This lecture will provide an overview of how different current culture conditions and novel advancement in embryo culture may affect oocyte and embryo viability, with the goal to increase blastocyst formation and implantation potential.

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ADVANCED NANOTOOLS TO ENHANCE REGENERATION IN CNIDARIAN MODEL ORGANISMS

C. Tortiglione

Istituto di Scienze Applicate e Sistemi Intelligenti "E. Caianiello", CNR, Pozzuoli, Italy
E-mail claudia.tortiglione@cnr.it

Tissue regeneration is one of the most fascinating biological capabilities of multicellular organisms. During animal evolution this capability has been progressively lost while primitive organisms such as cnidarian are able to regenerate amputated body parts starting from tiny piece of tissues. After injury, various intracellular pathways and intercellular communication must be activated to establish a new tissue integrity and homeostasis. Alongside biochemical and genetic networks, in the last decade physical stimulations (light, heat, electrical fields) have gained important role as biophysical master regulators, controlling cell behaviours and driving proliferation, differentiation, migration processes. Here I will describe the use of nanoparticles of different chemical composition and activable through diverse physical stimulations as innovative tools to modulate the regeneration capacity of the freshwater polyp *Hydra vulgaris* and the sea anemone *Nematostella vectensis*. I will present the possibility to integrate animal, cellular, molecular and biochemical approaches to test *in vivo* these novel nanotools, from toxicity to regeneration and reproductive capacity and to uncover the mechanism underlying the cell and animal responses, opening the path to novel approaches for the optical and thermal modulation of various biologic functions.

ORAL COMMUNICATIONS

IMMUNOMODULATORY AND PROTECTIVE EFFECTS OF EXTRACTS FROM GREEN LEAVES AND RHIZOMES OF *P. OCEANICA* (L.) DELILE ON RAW 264.7 MACROPHAGES AND A HUMAN BLOOD-BRAIN BARRIER MODEL

G. Abruscato[1], M. Mauro[1], R. Chiarelli[1], A. Vizzini[1,2], V. Arizza[1,2], M. Vazzana[1,2], F.Gosselet[3], P.Candela[3], Luparello[1,2]

[1]Department of Biological,Chemical and Pharmaceutical Sciences and Technologies, University of Palermo,Palermo, Italy; [2]NBFC, National Biodiversity Future Center, Palermo, Italy; [3]Blood-Brain Barrier Laboratory, University of Artois Lens, France
E-mail: giulia.abruscato@unipa.it

Bioactive compounds from marine biodiversity exert several beneficial effects on human health (*e.g.,* anti-inflammatory and antioxidant). In particular, extracts derived from green leaves (GLE) and rhizomes (RE) of *P. oceanica* have been shown to exert antitumoral activity *in vitro* against HepG2 cells[1]. Their prominent polyphenolic content prompted us to assess the potential anti-inflammatory effect on LPS-treated mouse RAW 264.7 macrophages and TNFα-treated endothelial cells of an *in vitro* model of human blood brain barrier (BBB)[2] . No cytotoxic effect and a reduction of nitrite production by LPS-treated macrophages were found after 24 h-treatments with increasing concentrations of both extracts. A differential immuno-modulatory activity of both extracts was revealed by qPCR and Western blot assays. Subsequently, shifting the focus from the peripheral level, to investigate their potential anti-inflammatory effect at the central nervous system level, an *in vitro* model of inflamed BBB consisting of a human endothelial cells/pericytes co-culture exposed to TNFα[3], was used. Even though both extracts appeared ineffective in reducing inflammation, interestingly they did not alter BBB integrity but played a protective role reducing the TNFα-induced permeability alteration and counteracting the release of nitrites. Noteworthy, only RE up-regulated both mRNA and protein expression of molecular markers of tight and adherens junctions, leading to a recovery of protein delocalization after exposure to TNFα. These results prompt further investigation to detail the potential immunomodulatory role of GLE and RE and to unveil the molecular cascade responsible for the observed beneficial effect on BBB integrity.

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VASOSTATIN 1 MODULATES AUTISM-LIKE BEHAVIORS AND HIPPOCAMPAL NEUROINFLAMMATION IN BTBR MICE

R. Alò[1], E. Avolio[1], I. Olivito[1], F. Bosco[2], L. Guarnieri[2], S.K. Mahata[3,4], D. Minervini[1], A. Leo[2], M.C. Cerra[5], R. Citraro[2], R.M. Facciolo[1]

[1]Comparative Neuroanatomy Lab, [5]Organ and System Physiology Lab-Department of Biology, Ecology and Earth Sciences, University of Calabria, Italy; [2]Section of Pharmacology, Department of Health Sciences-School of Medicine, Research Center FAS@UMG, Department of Health Sciences, University "Magna Graecia" of Catanzaro, Italy; [3]VA San Diego Healthcare System, San Diego, CA, USA; [4]Department of Medicine, University of California, San Diego, La Jolla, CA, USA
E-mail: raffaella.alo@unical.it

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that causes behavioral impairments along with cardiometabolic disease, immune system dysregulation and neuroinflammation[1,2]. Vasostatin 1 (VS1), the most conserved fragment of chromogranin A, is implicated in the inhibition of angiogenesis and inflammation[3]. In the present study, we evaluated the effects of an intraperitoneal treatment of VS1 for 4 weeks on social and cognitive deficits, and on repetitive behaviors of an idiopathic autism model (BTBR). Furthermore, expression profiles of some pro-inflammatory cytokines (IL-1β, IL6) and NF-kB were investigated in the hippocampus, a brain region involved in social and cognitive deficits[4]. Interestingly, VS1 reversed sociability deficits in BTBR by increasing time spent in the chamber with the stranger ($p<0.001$) and sniffing it ($p<0.001$) rather than the novel object in the three-chamber test. VS1 also reduced self-grooming behaviors ($p<0.001$) as well as rescuing memory impairments as indicated in the novel object recognition test by a higher discrimination index ($p<0.001$) with respect to BTBR treated with a saline solution. Such behavioral effects were related to reduced expression levels of both IL-6 and IL-1β ($p<0.001$) as well as NF-kB ($p<0.01$) in the hippocampus of BTBR mice treated with VS1. This is a first study highlighting a potential therapeutic role of VS1 as indicated by its involvement in decreasing the pro-inflammatory response with consequent improvement of the behavioral deficits typical of ASD.

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A LIGHT ON ASTHENOZOOSPERMIA: DOES THE NEAR-INFRARED PHOTOBIOMODULATION THERAPY (PBM-t) OFFER A SUPPORTIVE APPROACH?

A. Amaroli[1], S.Stigliani[2], S. Ravera[3], E. Maccarini[2], P. Anserini[2], P. Scaruffi[2]

[1]Earth, Environmental and Life Sciences Department, University of Genoa; [2]Physiopathology of Human Reproduction, IRCCS Ospedale Policlinico San Martino, Genoa; [3]Experimental Medicine Department, University of Genoa, Italy
E-mail: andrea.amaroli@unige.it

Sperm motility is a crucial factor in male fertility and is influenced by energy consumption mainly associated with mitochondrial oxidative phosphorylation[1,2]. Stimulation of mitochondrial photoreceptors with near-infrared wavelengths was characterized in *in vitro* and *in vivo* models[3,4]. We collected semen samples from asthenospermic (n=70) and normospermic (n=17) men. Each asthenospermic sample was divided into 5 aliquots: 1 was used as untreated control and 4 were irradiated with an 810 nm laser device at different powers: 0.25W, 0.5W, 1W, and 2W (60 sec, 1 cm^2). Sperm motility was assessed immediately, 30 min and 60 min after exposure. Wilcoxon paired samples test and one-way ANOVA were used. One W was the most effective output in increasing the progressive motility of asthenospermic samples compared to the control ($p<0.0001$). The maximum effect was obtained immediately after PBM-t ($p<0.0001$) and kept high within 30 min with a slow decrease after 60 min ($p<0.0001$). Time physiologically decreased vitality ($p<0.001$) but less in the PBM-t samples ($p<0.01$). Chromatin condensation remained stable after PBM-t ($p>0.05$). Asthenospermic samples displayed an impairment of 80% in oxygen consumption and ATP production, and a slight inefficiency of the oxidative phosphorylation with respect to normospermic samples ($p<0.0001$). PBM-t partially restored the functionality of aerobic metabolism ($p<0.001$) by a complete recovery of oxidative phosphorylation efficiency. No sample accumulated malondialdehyde, a lipidic peroxidation marker.
In conclusion, 810 nm laser irradiation of sperm significantly improves progressive motility through increased mitochondrial energetic metabolism without harmful oxidative stress, nor negative impact on membrane integrity and chromatin condensation.

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HUMAN ADIPOSE STEM CELLS: EVALUATION OF THE IMPACT OF DONORS' AGING ON CELL PROPERTIES AND IMPAIRMENTS

L. Barone[1], F. Rossi[1], A. Bruno[1,2], L. Valdatta[1], R. Papait[1], C. Pagiatakis[1], G. Bernardini[1], R. Gornati[1]

[1]Department of Biotechnology and Life Sciences, University of Insubria, Varese; [2]Laboratory of Innate Immunology, IRCCS MultiMedica, Milan, Italy
E-mail: rosalba.gornati@uninsubria.it

Aging is defined as the accumulation of alterations in cells and tissues that increase the risk of disease[1] and represent an increasing burden for the health care system, especially in those countries with the highest longevity. This leads to an expanding request for new therapeutic strategies, and in this scenario the use of mesenchymal stem cells (MSCs) and their secretome for regenerative medicine applications has proved to hold great promise for the treatment of several pathological conditions[2]. We focused on adipose stem cells (ASCs), a subset of MSCs, which, being often isolated from old subjects, could be affected by age-dependent senescence and impairments; thus, we assessed the potential impact of aging on ASC regenerative properties. To do this, we isolated ASCs from different donor's age groups, evaluating the impact on several biological processes affected by cell senescence, among which proliferation, oxidative stress, β-galactosidase and telomerase activity. Furthermore, we conducted the mRNA analysis of those genes whose expression is associated with metabolism and cellular homeostasis maintenance. The preliminary results suggested that ASCs are not deeply affected by donor's age, indeed cells isolated from subjects belonging to all four groups exhibited comparable proliferation rate and telomerase activity. Although also the results on mRNA expression were comparable throughout all four age groups, an altered expression was observed in those genes involved in the epigenetic regulation of gene expression and in those related to the glycolytic pathway, suggesting an interesting correlation between aging and ASC metabolic profile, for which future metabolomic and transcriptomic studies will be needed.

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SYNAPTO-GLIOSOMAL CHARACTERIZATION IN A ZEBRAFISH MODEL OF ALEXANDER DISEASE

D. Bellitto[1], M. Bozzo[1], E. Serafini[1], M. Balbi[2], M. Milanese[3], E. Fedele[3], S. Candiani[1]

[1]Department of Earth, Environment and Life Sciences, University of Genova; [2]Department of Experimental Medicine, University of Genova; [3] Department of Pharmacy, University of Genova, Italy
E-mail: deianira.bellitto@edu.unige.it

Alexander disease (AxD) is a rare genetic pathology affecting astrocytes[1]. One of the most intriguing and less investigated aspect of this diseases regards potential alterations in synapto-gliosomal environment. Existing literature indicates that both *in vitro* and *in vivo* models of AxD manifest neuronal loss, attributed to compromised glutamate buffering by AxD-afflicted astrocytes[2,3,4]. In this work, we report the first synapto-gliosomal preparation and characterization in a zebrafish model of Alexander disease (zAxD)[5]. Our findings corroborate a diminished glutamate release in the zAxD model. Additionally, we extend our investigation to elucidate the inhibitory neurotransmission in the synapto-gliosomal preparation by examining GABA release, thus providing fresh perspectives for comprehending this rare disease.

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A NEW APPROACH METHODOLOGY TO EVALUATE THE BIOLOGICAL IMPACT OF SILVER NANOMATERIALS IN HUMAN LUNG

R. Bengalli[1], G. Motta[1], M. Gualtieri[1], P. Mantecca[1]

[1]POLARIS Research Center, University of Milano-Bicocca, Milano, Italy
E-mail: rossella.bengalli@unimib.it

Nowadays, nanomaterials (NMs) find extensive application across various commercial products. This in parallel increases concerns regarding potential hazard to human health. New Approach Methodologies (NAMs) are emerging *in vitro*, *in chemico* and *in silico* tools that allows collecting informative toxicological data crucial for next generation risk assessment (NGRA) of NMs without the use of animals. Moreover, according to the Safe and Sustainable by Design (SSbD) framework[1], the use of NAM needs to be prioritized for the hazard assessment of chemical and materials, including NMs. Here we present a NAM-based framework designed to identify and evaluate the hazard associated with new antibacterial silver (Ag) nanoparticles (NPs), designed accordingly to the SSbD principle and used for the coating of antibacterial textiles. To assess the potential hazard of these NMs, we used an *in vitro* model representative of the inhalation route. Alveolar cells (A549) were co-cultured with THP-1 differentiated in macrophages and exposed at the air liquid interface (ALI) to Ag NPs with different coatings. For realistic exposure scenarios, environmental monitoring data measured at a manufacturing site, during an industrial process to produce coated textiles, were used as inputs to calculate the alveolar retained doses through MPPD modelling. Exposure to aerosolized NPs was performed by the Vitrocell® Cloud Alpha 12 system. The exposure doses (ng/cm^2) used are equivalent to a chronic exposure of 1, 6 and 12 months. The biological effects in terms of cell viability, cytokine release, and genes expression were evaluated 24 h after the exposure. Whole-Genome transcriptomics changes were also evaluated to determine the relevant pathways induced by NPs. Altogether, the results suggest the lack of significant hazard for the doses representative of realistic exposure conditions, but contribute in pointing out the molecular signature of possible adverse effects, according to the NM physico-chemical properties.
Funding: This work was funded by the EU H2020-projects ASINA (GA nr 862444) and INTEGRANO (GA nr 101138414).

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HOW DOES ZEBRAFISH KEEP TRANSPOSABLE ELEMENTS UNDER CONTROL?

M.A. Biscotti[1], E. Tittarelli[1], F. Carducci[1], M. Barucca[1], A. Canapa[1], E. Carotti[1]

[1]Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy
E-mail: m.a.biscotti@univpm.it

Transposable elements (TEs) are dynamic components in eukaryotic genomes and play a significant role in speciation. In sarcopterygians, the transcriptional activity of these mobile elements is regulated by Krüppel box-associated zinc finger proteins (KRAB-ZFPs) through the recruitment of NuRD complex. This system exhibited transcriptional activity also in actinopterygians, even though genes encoding crucial proteins as TRIM28 and KRAB-ZNF are missing. This study hypothesizes that TRIM33 could serve as a replacement for TRIM28 in *Danio rerio* and suggests an evolutionary relationship between fish specific ZNFs, named KRAB-like ZNF and cyprinid specific N-terminal zinc-associated domain (FiNZ) ZNFs, with sarcopterygian KRAB-ZFPs. Moreover, through co-immunoprecipitation analyses, we evaluated the interaction of TRIM33 with the KRAB-like and FiNZ ZFPs in zebrafish. The analyses of the expression profiles of TEs and genes encoding for proteins involved in their control from zygote to larvae of zebrafish development highlighted that this machinery targets mainly LTR young copies. Our results potentially represent a fresh groundwork to deeply understand the evolutionary mechanisms underpinning the adaptation of ray-finned fish.

EXPLORING THE IMPACT OF PET NANOPLASTIC DISPERSION IN WATER ON THE ACTIVATION OF THE INNATE IMMUNE RESPONSE IN *HIRUDO VERBANA*

C. Bon[1], N. Baranzini[1,2], L. Pulze[1,2], D. Tessaro[3], A. Grimaldi[1,2]

[1]Department of Biotechnologies and Life Sciences, University of Insubria, Varese; [2]ILFARM S.R.L., Varese; [3] Department CMIG "G.Natta" Polytechnic University of Milan, Italy

Plastics are among the most used synthetic materials worldwide, employed in different fields, such as textile, healthcare and telecommunications industries. As a result, this massive production leads to the production of tons of waste every year.[1] When plastic debris diffuse in both terrestrial and aquatic environments, undergo biotic and abiotic processes of degradation, leading to the formation of small plastic particles, namely microplastics (MPs) and nanoplastics (NPs), posing a threat for living organism, humans included, due to their dimensions, which allow them to be easily eaten, entering the trophic chain[2]. Among all the types of plastics on the market, polyethylene terephthalate (PET) is one of the most diffuse synthetic thermoplastic materials, in fact, has been pointed out as the third most used plastic in the packaging industry[1]. Considering the amount of PET waste discarded in the environment, the focus of this project was to evaluate the effects of PET NPs diffusion in water on living organisms, using as animal model the medicinal leech *Hirudo verbana*, already used in previous studies on polypropylene[3]. Leeches were divided into three groups and exposed to the concentrations of 0,05, 0,5 and 5 mg/L of PET NPs, mimicking the real conditions of plastic pollution in aquatic areas. Different timings of treatment were taken into account, in order to analyze the immediate effects in shorter timings and delayed effects after chronic exposition to NPs. In the samples obtained it was possible to observe morphological changes in the body wall of leeches, such as an increase in blood vessels number, strictly linked to the activation of the immune response and of other defence mechanisms already studied in leeches. This led to the conclusion that PET NPs induce an inflammatory and an immune response, after a short period of exposure, whereas longer exposure end in a chronicization of these mechanisms.

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THE USE OF ZEBRAFISH EMBRYOS TO EXPLORE DIFFERENT NATURAL POLYPHENOL APPLICATIONS

C. Bragato[1], A. Persico[1], A. Pavan[1], G. Ferreres[2], T. Tzanov[2], A. Colombo[1], M. Brindisi[3], H. Lange[1], P. Mantecca[1]

[1]Department of Earth and Env. Sciences, University of Milano-Bicocca, Milano, Italy; [2]Department of Mol. and Ind. Biotechnology, Universitat Politècnica de Catalunya, Terrassa, Spain; [3]Department of Pharm. Sciences, University of Federico II, Napoli, Italy
E-mail: cinzia.bragato@unimib.it

Lignin and tannins are natural polyphenols abundantly present on Earth. These aromatic biopolymers have gained interest in different fields due to their attractive properties[1-3], especially in the form of nanocapsules (NCs) and nanoparticles (NPs)[4]. Zebrafish embryos can be efficiently employed to investigate their biomedical applications, not only to test these natural nano-formulations in a living organism, but also to evaluate their interactions, toxicity, and behavior in the biological milieu[5]. Two case studies are here reported. The first is based on demonstrating in an inducible zebrafish embryo model of Alzheimer's Disease (AD) that a multifunctional tannin-based carrier system is suitable for *in vivo* MRI and difference-fluorescence imaging. The second project aims at testing the anti-inflammatory properties of sonochemically nano-formulated pristine lignin (LigNPs) and enzymatically-phenolated lignin (PheLigNPs)[6], in two different zebrafish inflammatory models. The data obtained demonstrated that the NCs and NPs related to each project showed negligible toxicity, besides the capacity to induce a positive response during an inflammatory event, increasing the recruitment of cytokines to accelerate their chemotactic function. Moreover, the lignin NPs played a role in the resolution of wounds, favoring the regeneration process. The results pave the way for further evaluations to exploit the wide applicability of natural (nano)polyphenols, making them extremely interesting for the medical sector, considering their biocompatibility, anti-inflammatory, and pro-healing properties.

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DISSECTING THE EMBRYONIC ZEBRAFISH BRAIN AT A SINGLE CELL LEVEL TO UNVEIL THE HIDDEN SECRETS OF MICROGLIA-NEURON INTERACTIONS.

S. Ricci[1], Q. Lo Giudice[2], J.Y. Bertrand[3], V. Franceschini[1], P. Cacialli[1]

[1]Department of Biological, Geological and Environmental Sciences (BIGEA), University of Bologna, Bologna, Italy; [2]Department of Neuroscience, University of Geneva, Switzerland; [3]Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland
E-mail: pietro.cacialli@unibo.it

The purpose of the present study is to identify the molecular pathways that modulate microglia-neuron interactions during embryonic development. It is known that microglia plays a crucial role during early stages of vertebrate development[1,2], however further studies need to decipher how it influences and interacts with other brain cells. Recently, in zebrafish embryonic brain, we identified a lysosomal gene, *ifi30*, which is specifically expressed in microglia. First, we generated a new transgenic line *Tg*(*ifi30:mcherry)* to trace the origin of *ifi30*-expressing cells. Next, we used morpholino strategy and a knock-out zebrafish model, characterized in our previous study [3] to study the loss of function during embryonic neurogenesis. Using single-cell transcriptomics we identified how the loss of *ifi30* modified the genetic landscape of brain cells during embryogenesis. Our zebrafish model presented: synaptic dysfunction, impaired neurogenesis, altered autophagy and reduction of movement. These phenotypes were rescued by overexpressing *ifi30* specifically in microglia.

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DECIPHERING THE *IN VITRO* TOXICITY OF BIOGENERATED SILVER NANOPARTICLE IN BREAST CANCER: A MULTIOMICS ANALYSIS

C. D'Amico[1], M. Buttacavoli[1], S. Marino[1], E. Peri[1], F. Vaglica[1], S. Feo[1], F. Baldi[2], P. Cancemi[1]

[1]Department of Biological Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo; [2]Department of Molecular Science and Nanosystems, Cà Foscari University of Venice, Italy
E-mail: patrizia.cancemi@unipa.it

Silver nanoparticles (AgNPs), have attracted much attention especially in biomedical field, where nanomaterials are expected to improve cancer diagnosis and therapy. The biological activity of AgNPs depends on several morphological and physicochemical characteristics. Among the proposed routes for AgNPs synthesis, more emphasis has been made on the so called green synthesis, especially biogenic route which utilise microorganisms (bacteria and fungi) for their synthesis. In this route, the nanoparticles size is generally smaller than those produced by chemical routes and stabilized by carbohydrates/proteins. In this study, we analysed the anticancer activity and the mechanism of action of biogenerated AgNPs in the breast cancer cell line SKBR-3. The AgNPs, embedded in the extracted exopolysaccharide (EPS) of K. oxytoca, were used to induce toxicity in SKBR-3 cells[1]. The AgNPs uptake

occurs through endocytosis, as verified by TEM analysis and, as suggested by colony formation assay, the effects are significant after 1h of treatment. After sequential centrifugation of treated cells, higher Ag concentration in mitochondria was detected, suggesting a prominent role of mitochondria in AgNPs-toxicity. Transcriptomic results, efficiently explained the detected cellular effects, which included oxidative stress and cell death. In particular, after 6 h of treatment, a significant upregulation of pathways involved in metal ions response, inflammation and heat shock was detected, while after 24 h of treatment a significant downregulation of cell cycle and replication pathways was found. Among the differentially expressed proteins identified in our proteomic investigation, PRDXs were chosen for western blot validation for their involvement in carcinogenesis and in drug resistance. Concluding, the obtained results show the complexity of AgNPs action both at transcriptional and post-translational levels.

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EVALUATION OF MATERIAL BIOCOMPATIBILITY FOR MANUFACTURING OVARIAN TISSUE DYNAMIC CULTURE DEVICES

A. Candela, V. Genovese, A. Travaglione, V. De Gregorio, V. Barbato, R. Talevi, R. Gualtieri

Department of Biology, University of Naples Federico II, Napoli, Italy
E-mail: andrea.candela@unina.it

The 3D printing techniques in tissue engineering are becoming more attractive due to their ability to manufacture device prototypes using cost-effective polymers[1]. Ovarian tissue is sensitive to chemical toxicants that could be leached into the medium by culture device materials, necessitating careful material selection[2,3]. However, evaluating material impact on ovarian tissue culture is time-consuming due to histological and viability analyses required. In this sense, we aimed to develop a rapid and reliable toxicity test that could predict the biocompatibility of materials for manufacturing ovarian tissue culture devices. Samples of various materials were tested as follow: 1) the first step involved co-incubation of leachable-containing medium with bull spermatozoa evaluating kinetics parameters, through Sperm Class Analyzer® CASA System; 2) bovine ovarian tissue cultured 7 days in the bioreactors manufactured with selected materials were evaluated in terms of follicle progression and viability. Results showed minimal adverse effects on sperm kinetics for materials such as medical/food-grade polypropylene, untreated polyamide, dental resins, and polycarbonate, compared to conventional polystyrene. Conversely, ABS-like resin and low-density polyethylene exhibited a lower biocompatibility in terms of sperm motility and kinetics. Subsequent validation using dynamic culture devices demonstrated improved follicle development and viability with less toxic materials compared to conventional dishes. Conversely, the most toxic material resulted in the absence of viable follicles after 7 days. Even though the bovine is a widely accepted reproductive model, it is essential to validate the findings on humans. Overall, the biocompatibility assay on leachable-containing media through computer-assisted sperm motility represents a rapid and reliable tool for selection of the suitable materials for manufacturing devices to culture reproductive tissue.

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COCAINE EFFECTS ON FISH REPRODUCTION: A HISTOLOGICAL, BIOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY

F. Carrella, A. Lallo, T. Chianese, A. Mileo, A. Capaldo

Department of Biology, University of Naples Federico II, Naples, Italy
E-mail: anna.capaldo@unina.it

Many studies showed that drugs of abuse disrupt the endocrine system, inducing the endocrine glands to either over- or underproduce, with mechanisms that are often not yet fully understood[1]. In mammals, cocaine influences the activity of HPA axis, resulting in increased levels of ACTH and cortisol, and of HPG axis, resulting in reduced LH release, male hypogonadism, and disruption of menstrual cycle in females[2]. Moreover, in males, decreased diameter of seminiferous tubules, lowering of the seminiferous epithelium and impaired motility of the sperms were observed. In females, where data are more limited, a delay in puberty, ovulation failure in sexually mature individuals and alterations of the mitotic spindle of oocytes were observed. Little is known about the effects of cocaine on non-mammalian reproduction; for example, a decrease in fertilization rates of sea urchin *Echinometra lucunter*, and defects in the formation and maturation of ovarian follicles, follicle apoptosis and adult lethality in *Drosophila melanogaster*[3]. In our study, the effect of an environmentally relevant concentration of cocaine (20 ng/ L), on the ovaries of *Anguilla anguilla* was evaluated, by means of the morphology of the ovaries, the presence and distribution of enzymes involved in oogenesis and serum cortisol, FSH, and LH levels. The eels exposed to cocaine showed a smaller follicular area and a higher percentage of connective tissue than controls, as well as many previtellogenic oocytes compared with controls having numerous fully vitellogenic and early vitellogenic oocytes. In addition, the presence and location of 3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase, and P450 aromatase differed in the two groups. Finally, cocaine exposure decreased FSH and LH levels, and increased cortisol levels. These findings suggest a potential impact of cocaine on reproduction in the European eel *A. anguilla*.

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mSOD1NSC-34 MOTONEURON-LIKE CELLS DERIVED EXTRACELLULAR VESICLES MODULATE THE NEUROINFLAMMATION IN MICROGLIAL CELLS *VIA* CX3CR1/TGFβRII AXIS

E. Carata[1], M. Muci[1], S. Di Giulio[1], S. Mariano[1], E. Panzarini[1]

[1]Department of Biological Sciences and Technologies, University of Salento, Lecce, Italy
E-mail: elisabetta.carata@unisalento.it

In recent years, numerous studies have demonstrated the correlation between amyotrophic lateral sclerosis (ALS) and neuroinflammation in the central nervous system (CNS). Increasing attention has been paid to the non-neuronal cells that constitute the cortical microenvironment providing metabolic support to motor neurons (MNs) and their axons. Microglia cells are the main inflammatory effector in the CNS and can exhibit activation status: a neurotoxic (pro-inflammatory) M1 phenotype and a neuroprotective (anti-inflammatory) M2 phenotype[1]. Recent evidence has suggested that many proteins associated with ALS are present in extracellular vesicles (EVs) and move between neuronal and glial cells, contributing to the spread and propagation of the disease[2]. Here, we evaluate the response of BV2 microglial cells to the presence of EVs released by NSC-34 mSOD1 MNs-like cells in the conditioned medium. The EVs released from NSC-34 in the culture medium were isolated through differential centrifugation, which allowed to obtain two fractions, one containing small vesicles (sEVs, diameter <200 nm) and the other containing large vesicles (lEVs, diameter >200 nm. BV2 cells were incubated with the EVs fractions for 12, 24 and 48 h. to evaluate 1) the inflammation status of the microglia through RT-PCR of IL-1β, IL-6, IL- 4, IL-10, and TNFα, and 2) the expression of proteins involved in inflammasome activation (IL-β and caspase 1), cell death (caspase 3), recruitment of glial cells (CXCR1), transforming growth factor-β2 (TGF-β2)-TGF-β type II receptor and an important regulator of innate immunity (MIF). The response of BV-2 cells depends on the type of SOD1 mutation, and the highest effect is mediated by sEVs. Our results suggest a significative role of the CX3CR1/TGFβRII axis in driving the activation of microglial cells.

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UNRAVELLING POTENTIAL VITELLOGENIN RECEPTORS FROM AN EXTENSIVE CHARACTERIZATION OF LOW DENSITY LIPOPROTEIN RECEPTOR SUPERFAMILY IN THE AMPHIBIAN *CYNOPS ORIENTALIS*

F. Carducci[1], C. Spinsante[1], E. Carotti[1], A. Canapa[1], D. Bizzaro[1], M. A. Biscotti[1], M. Barucca[1]

[1]Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy
E-mail: f.carducci@univpm.it

The Low Density Lipoprotein receptors (LDLRs) gene family is constituted by a total of 15 receptors: Very Low-Density Lipoprotein Receptors (VLDLR) or Lipoprotein Receptor 8 (LR8), LDLR, Sorting-Related receptor with A-type repeats (SORLA), and 12 LDL Receptor-related Proteins (LRPs): LRP1, LRP1B, LRP2, LRP3, LRP4, LRP5, LRP6, LRP8, LRP10, LRP11, LRP12, LRP13[1]. The main functions performed by the most of these proteins are the transduction of key signals during embryonic development and the regulation of cholesterol homeostasis[2]. Moreover, in oviparous animals, a key role is played by the VTGR, also known as VLDL receptor, that facilitates the uptake of vitellogenin. In tetrapods, knowledge concerning genes encoding these proteins are restricted to few taxa[3]. In line with these premises, here we report an extensive characterization of receptors belonging to this gene family in the amphibian *Cynops orientalis*. A maximum-likelihood phylogenetic analysis was conducted on 161 sequences belonging to 11 genera of vertebrates, revealing that the expansion of this gene superfamily occurred in the vertebrate common ancestor and remained conserved throughout the evolutionary lineages of vertebrates. In addition, secondary structure predictions and gene expression analyses were conducted in *C. orientalis*. Finally, the findings here obtained allowed to propose LRP8, together with VLDLR, as potential Vtg receptor.

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GENOTOXIC STRESS INDUCED BY MECHANICAL FORCE IN CELLULAR MODELS OF PARKINSON'S DISEASE

M. Catanesi[1], C. Milanese[2], P.G. Mastroberardino[1,2,3]

[1]Department of Life, Health, and Environmental Sciences, University of L'Aquila, L'Aquila, Italy; [2]IFOM-ETS, the AIRC Institute for Molecular Oncology, Milano, Italy; [3]Erasmus MC Rotterdam, 3015CN Rotterdam, the Netherlands

Increasingly, studies are shedding light on the correlation between changes in brain age-related stiffness and the onset of Parkinson's disease (PD). It is hypothesized that these alterations of cell mechanobiology perturb brain parenchyma homeostasis and contribute to neuronal degeneration. However, the molecular mechanisms that regulate these phenomena are poorly understood. The aim of this work is to elucidate how perturbations in brain stiffness mechanistically alter cytoskeleton organization and nuclear morphology, which directly reflect cell mechanobiology properties. Here we examined how these processes relate to DNA damage, a recognized hallmark of aging and neurodegeneration. We leveraged an experimental setup based on differentiated neuroblastoma cells grown of substrates with different stiffness. We exposed these cell lines to stressors that are relevant for PD pathology, that is 6-OHDA, rotenone, and alpha-synuclein. As readout measures, we monitored nuclear shape parameters and aberrations. We also observed chromatin organization under different conditions with histone H3k9me3 and cytoskeleton conformation with F-Actin. Following these experiments, we evaluated sensitivity to DNA damage through analysis based on 53bp1/γH2AX colocalization. We found that the effect of stressors on indirect measures of mechanical stress such as nuclear shape, chromatin, and cytoskeleton architecture depends on the substrate stiffness. Moreover, harder substrates increase cell sensitivity to exogenous insults, particularly at the level of DNA damage. These findings are very relevant given that natural aging, *i.e*. PD main risk factor, is parallel by changes in brain stiffness, and mechanistically, we demonstrated that these effects rely on the YAP pathway. In conclusion, our results offer new mechanistic insights into how cellular mechanical alterations, a poorly recognized age-related factor in neurological diseases, contribute to neurotoxicity in Parkinson's disease.

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EFFECTS OF GLYPHOSATE EXPOSURE ON NON-TUMOR HUMAN PROSTATIC CELLS.

T. Chianese, G. Trinchese, M. De Falco, G. Rosato, L. Rosati, R. Scudiero

Department of Biology, University Federico II, Napoli, Italy
E-mail: teresa.chianese2@unina.it

The herbicide Glyphosate (Gly) is a contaminant of soil, water, and crops; it reaches animals, including humans, through the food chain, posing serious risks to their health. Gly is considered a potential endocrine disruptor (EDC), able of altering estrogen homeostasis by modifying the expression/localization of estrogen receptors (ERα, ERβ)[1]. This study aims to verify the action of Gly on the PNT1A cell line, a non-tumor human prostate epithelial cell line. Cells were treated with different concentrations of Gly at different time intervals. Cell viability and toxicity were assessed using MTT and LDH assays; cell viability decreased as the Gly concentration increased, while cell toxicity increased. The tests allowed us to establish two doses of Gly to be used for further investigations (3.5×10^{-4} M and 3.5×10^{-3} M). Comet assay and Western blot showed that Gly-induced cell death occurred through apoptosis. Indeed, cells treated for 24 h showed a higher rate of DNA fragmentation and increased levels of the pro-apoptotic proteins Bax and Bak and the concomitant decrease in the anti-apoptotic protein Bcl-2 and inactivated Caspase 3; the changes were more evident in cells treated with the higher dose of Gly. Apoptosis could be due to alterations in mitochondrial metabolism, therefore the efficiency of mitochondria was studied using the Seahorse analyser and the Cell Mito Stress Test kit. Impaired mitochondrial function was observed in Gly-treated cells. It is likely that Gly-treated cells with are struggling and trying to compensate by working at high efficiency, as confirmed by the marked reduction in mitochondrial proton leakage and spare respiratory capacity, both indicative of the cells inability to adapt bioenergetically in response to conditions of stress. Finally, the results of immunofluorescence analysis demonstrated that Gly acted as an EDC resulting in the activation and nuclear translocation of both ERs; the latter occurred regardless of dose, faster than the specific hormone, and persisted throughout treatment. In conclusion, the results collected show that in non-tumor prostate cells the herbicide causes cell apoptosis, mitochondria dysfunction and activation of ERs.

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PROTEIN PHOSPHORYLATION ORCHESTRATES SELECTIVE AUTOPHAGY TO SECURE ORGANELLE HOMEOSTASIS

M. Nanni[1], R. De Cegli[2], M. Bordi[3], G. Milletti[4], C. Di Malta[2], E. Ziviani[5], S. Zanivan[6], S. Ismail[7], T. Persichini[8], M. Colasanti[8], F. Cecconi[3,9], V. Cianfanelli[8]

[1]Department of Pathology, University of California San Francisco (UCSF), San Francisco, USA; [2]Telethon Institute of Genetics and Medicine, Naples, Italy; [3]Unit of Cell Stress and Survival, Danish Cancer Institute, Copenhagen, Denmark; [4]DNA Replication and Cancer Group, Danish Cancer Institute, Copenhagen, Denmark; [5]Department of Biology, University of Padova, Padova, Italy; [6]Cancer Research UK Scotland Institute, Glasgow, UK; [7]Biochemistry, Molecular and Structural Biology, Department of Chemistry, KU Leuven, Belgium; [8]Department of Sciences, University Roma Tre, Rome, Italy; [9]Department of Basic Biotechnological Sciences, Intensive Care and Perioperative Clinics Research, Catholic University of the Sacred Heart, Rome, Italy
E-mail: valentina.cianfanelli@uniroma3.it

Because organelle homeostasis is crucial for the proper functioning of the cell, it relies on a tight balance between biogenesis and degradation. Selective autophagy is one of the main pathways involved in the clearance of damaged or old organelles[1-3]. However, how selective autophagy is regulated and coordinated with organelle biogenesis is poorly understood. We identify a protein kinase-phosphatase network which impacts organelle mass by regulating both biogenesis and selective autophagy. Upon organelle damage or aging, this regulatory axis determines the timing and amplitude of the autophagy response, by regulating both the early and late steps of the process. Also, it controls a program of organelle biogenesis. Importantly, the coordinated regulation of degradation and biogenesis is relevant to the organelle homeostasis and cell survival. To identify this signaling network we used unbiased approaches, namely last generation liquid chromatography coupled to mass-spectrometry, and transcriptomics. Importantly, the regulators that we identified may be potential therapeutic targets in the medical conditions resulting from impaired balance between degradation and biogenesis of organelles, such as neurodegenerative diseases, cancer and resistance to chemotherapy.

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THE 7 STAGES OF NEURONAL DEVELOPMENT *IN VITRO:* MORPHOLOGICAL AND MOLECULAR SIGNATURES FROM BIRTH TO SENESCENCE

V. Ciraci[1], E. Saccone[1], N. Gualandi[2], O.M. Roggero[1], E. Tongiorgi[1]

[1]Department of Life Sciences, University of Trieste; [2]Lab. of Epigenomics, Department of Medicine, University of Udine, Udine, Italy
E-mail: viviana.ciraci@units.it

Neuronal development is a multifaceted and complex biological process which can be divided into distinct stages. The first description of the development of rat hippocampal neurons (HPNs) *in*

vitro refers to Dotti (1988), who identified 5 distinct stages of development, starting from days is vitro 0 to 7 (DIV0-7)[1]. In 2006, Horton described a new phase at DIV7-10[2]. Baj (2014), developed a staging system for the development of mouse HPNs *in vitro,* adding a sixth stage (DIV12-15)[3]. Although the processes occurring during these stages are well studied, no biomarkers have been identified to delineate the transition between stages. In this study, to search for new non-canonical genes that could mark the different developmental stages, we carried out a bioinformatic analysis of a public dataset (GSE113680) of RNA expression arrays reporting the differentially expressed genes (DEGs) at DIV0, 1, 3, 6, 12, and 15 in cultured mouse HPNs. We identified 5 DEGs with unique expression pattern for stage 1-2 (DIV0), stage 3 (DIV1-3), stage 4 (DIV4-6), stage 6 (DIV11-17). The best candidate genes have been selected for real-time PCR validation. Moreover, we have characterized a new stage 7 (DIV18-21), corresponding to the cell senescence. Immunofluorescence to detect gH2AX, a biomarker for DNA double-strand breaks occurring during senescence, in cultured mouse hip neurons at different DIVs (6, 9, 12, 15, 18 and 21) showed a significant increase ($p<0.0001$) in the number of gH2AX foci at DIV18 and DIV21. In addition, we analyzed the accumulation of the transcription factor GATA4, a key activator of the senescence-associated secretory phenotype (SASP) genes, which increases upon senescence induction. Our results showed that GATA4 accumulation increased significantly from DIV18 ($p<0.0001$). These results suggest a new stage with a timepoint at DIV18 representing the senescence stage.

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POLYPHENOLS TO IMPROVE DIET-INDUCED OVARY INFLAMMATION: A STUDY ON ZEBRAFISH (*DANIO RERIO*)

E. Coccia[1], R.Imperatore[1], G. Orso[1], R.Sciarrillo[1], A. Falzarano[1], S.H. Hoseinifar[2], G. Ashouri[1], M. Paolucci[1]

[1]Department of Sciences and Technologies, University of Sannio, Benevento, Italy; [2]Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
E-mail: elecoccia@unisannio.it

Infertility is an increasing worldwide problem and 50% of cases concern females who often do not suffer from pathologies of the reproductive system[1]. It has recently been observed that female infertility is related to lifestyle risk factors, including diet-induced inflammation, alcohol and stress[2]. Chronic inflammatory diseases and oxidative stress have an important role in ovarian function due to the increase in free radicals which increase systemic inflammatory biomarkers, such as TNFα and interleukin resulting in cellular necrosis and/or apoptosis[2,3]. Polyphenols are important natural compounds highly used as nutraceuticals and food supplements as they are involved in anti-inflammatory and anti-oxidative mechanisms[4]. The aim of the current study was to investigate the ability of polyphenols to counteract ovarian alterations induced by a pro-inflammatory diet in zebrafish, widely employed as animal model in biomedicine[5]. Zebrafish were divided in four experimental groups: control group, Inflamed Group (IG; k-carrageenan 0.1% supplementation), IG treated with polyphenols and a group treated only with polyphenols. Ovarian morphology, inflammatory status and oxidative stress were examined by histology and immunohistochemistry. The ovary of the IG showed histopathological alterations, such as atretic follicles and membrane folding, which were prevented by polyphenol treatment. The morphological alterations induced by the inflammatory diet were accompanied by an increase in proinflammatory factors and a reduction in antioxidant enzymes (TNFα and SOD/CAT, respectively). Polyphenols significantly reduced TNFα expression and increased antioxidant enzymes.These preliminary results indicate polyphenols as possible dietary supplementation to counteract or reduce the increasingly widespread problem of female infertility.

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EXPLORING PATHOGENIC MECHANISMS OF BBSOAS NEURODEVELOPMENTAL DISORDER: INSIGHTS FROM THE ADULT MOUSE DENTATE GYRUS NEUROGENIC NICHE

S. Bonzano[1,2], E.Dallorto[1,2,5], E. Hidisoglu[3], A.Marcantoni[3], M. Sassoe'-Pognetto[4], P. Peretto[1,2], M. Studer[5], S. De Marchis[1,2]

[1]Neuroscience Institute Cavalieri Ottolenghi (NICO), Orbassano (Turin), Italy; [2]Department of Life Sciences and Systems Biology (DBIOS), University of Turin, Italy; *[3]Department of Drug Science and Technology, University of Turin, Italy*; *[4]Department of Neurosciences Rita Levi Montalcini, University of Turin, Italy; Institute of Biology Valrose (iBV), Univ. Côte d'Azur, CNRS, Inserm, Nice, France*
E-mail: silvia.demarchis@unito.it

Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS; OMIN#615722) is a rare neurodevelopmental disorder caused by mutations in the NR2F1 gene, a transcriptional regulator with pleiotropic functions in brain development[1]. *NR2F1* mutations often result in haploinsufficiency or dominant negative effects, but their biological consequences remain poorly understood. The spectrum of BBSOAS includes intellectual disability (ID), visual impairment and autistic traits. Interestingly, alterations in postnatal dentate gyrus (DG) hippocampal neurogenesis have been reported in several animal models of autism or ID[2]. In our laboratory, we are investigating the role of NR2F1 in the adult DG by combining mouse genetics, genome-wide and *in silico* analyses with neuroanatomical and imaging approaches[3,4]. Our study revealed a novel regulatory role for NR2F1 in mitochondria. Loss of NR2F1 function in the adult mouse DG results in reduced mitochondrial mass, mitochondrial fragmentation and downregulation of key mitochondrial proteins in newborn neurons, affecting their genesis, survival and integration[4]. *Nr2f1* heterozygous mice, in parallel with the dysregulation of several nuclear-encoded mitochondrial genes and proteins, show impaired morphology, altered circuit activation and reduced synaptic inhibition in mature DG granule neurons. These findings underscore hippocampal dysfunction in BBSOAS and reveal potential mechanisms contributing to the associated cognitive impairment.

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CELLULAR ALTERATIONS INDUCED IN GILLS OF MARINE MUSSELS DUE TO DEXAMETHASONE EXPOSURE, AND MITIGATION POTENTIAL OF ULVANS

G. De Marco[1], A. Cristaldi[2], M.C. Eliso[1], D. Spagnuolo[1], G. Oliveri Conti[2], M. Galati[1], B. Billè[1], M. Terranova[1], V. Parrino[1], G. Genovese[1], T. Cappello[1], M. Ferrante[2], M. Maisano[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina; [2]Department of Medical, Surgical and Advanced Technologies "G.F. Ingrassia", University of Catania, Italy
E-mail: giuseppe.demarco@unime.it

Mussel farms represent a dominant sector of European aquaculture. However, the facilities located in coastal areas are daily threatened by the continuous release of emerging contaminants such as pharmaceutical active compounds (PhACs). Dexamethasone (DEX) ranks as one of the most widely used steroid anti-inflammatory drugs worldwide, both in veterinary and human medical settings. The effect of realistic concentrations of DEX (4 ng/L-2 µg/L) was evaluated on gills of *Mytilus galloprovincialis* during a 12-day exposure. Chemical analysis revealed the ability of mussels to uptake DEX during exposure above 400 ng/L DEX. Moreover, changes in branchial mucopolysaccharide levels and alteration in the antioxidant system (enzymatic and non-enzymatic) were recorded. The use of a multi-biomarker approaches (including histochemical, metabolomic, biochemical, and molecular tools) revealed DEX-induced alterations in different branchial cellular pathways like energy metabolism, protein turnover, osmoregulation and cholinergic neurotransmission. Once assessed DEX concentrations with the most significant effects (400 ng/L), a second experimental exposure was carried out using ulvans (ULV), a group of polysaccharides extracted by algae belonging to the genera *Ulva spp*. with well-known antioxidant properties[1]. Therefore, mussel specimens were treated to 100 mg/L of ULV, both individually and combined with 400 ng/L DEX for 12 days. Preliminary results regarding ULV treatment revealed a reduction in lipid peroxidation and detoxifying activity, coupled to a partial mitigation of DEX neurotoxic effects. Overall, the use of ULV represents a promising tool to ameliorate the harmful effects caused by emerging contaminants in the mussel's farms.

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DIFFERENTIATION AND FUNCTIONING OF THE LATERAL LINE ORGAN IN ZEBRAFISH REQUIRE SMPX ACTIVITY

A. Diana, A. Ghilardi, L. Del Giacco

Department of Bioscinces, Università degli Studi di Milano, Milano, Italy
E-mail: luca.delgiacco@unimi.it

The small muscle protein, X-linked (*SMPX*) gene encodes a cytoskeleton-associated protein, highly expressed in the inner ear hair cells (HCs), possibly regulating auditory function. Mutations in *SMPX* have been associated with hearing loss in humans and, in line with this, Smpx-deficient animal models, namely zebrafish and mouse, showed significant impairment of inner ear HCs development, maintenance, and functioning[1,2]. Here, we uncovered *smpx* expression in the neuromast mechanosensory HCs of the lateral line organ of zebrafish larvae and, by means of loss-of-function experiments, *via* both morpholino-mediated gene knockdown and CRISPR/Cas9 F0 gene knockout, showed that the lack of Smpx led to fewer properly differentiated and functional neuromasts. Additionally, the kinocilia of Smpx-deficient neuromast HCs appeared structurally and numerically altered, with a significant reduction in the mechanotransduction activity of the neuromast HCs. In summary, this work highlights the importance of Smpx in lateral line development and, specifically, in proper HCs differentiation and/or maintenance, and in the mechanotransduction process carried out by the neuromast HCs[3]. Because lateral line HCs are both functionally and structurally analogous to the cochlear HCs, the neuromasts might represent an invaluable—and easily accessible—tool to dissect the role of Smpx in HCs development/functioning and shed light on the underlying mechanisms involved in hearing loss.

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AMBRA1 PHOSPHORYLATION BY CDK1 AND PLK1 REGULATES MITOTIC SPINDLE ORIENTATION

F. Faienza[1,2], F. Polverino[3], D. Gargano[4], V. Cianfanelli[5,6], J. Dengjel[7], M. Barisic[8], G. Guarguaglini[3], S. Di Bartolomeo[4], F. Cecconi[2,6]

[1]Department of Biology, University of Rome Tor Vergata, Rome, Italy; [2]Cell Stress and Survival Group, Danish Cancer Institute, Copenhagen, Denmark; [3]Institute of Molecular Biology and Pathology, CNR, Rome, Italy; [4]Department of Biosciences and Territory, University of Molise, Pesche, Italy; [5]Department of Science, University "ROMA TRE", Rome, Italy; [6]Università Cattolica del Sacro Cuore, Rome, Italy; [7] Department of Biology, University of Fribourg, Fribourg, Switzerland; [8]Cell Division and Cytoskeleton, Danish Cancer Institute, Copenhagen, Denmark
E-mail: sabrina.dibartolomeo@unimol.it

Since its discovery in 2007, AMBRA1 has emerged as a crucial signaling molecule in several signaling pathways ranging from autophagy to mitophagy, cell death and cell proliferation[1,2]. Indeed, AMBRA1 deficiency has been associated with unbalanced cell proliferation and displacement of several regulators of differentiation during morphogenesis, and the excessive proliferation rate of AMBRA1-deficient cells has been linked to enhanced susceptibility to form tumoral masses in mice[3]. AMBRA1 is able to regulate cellular proliferation by promoting de-phosphorylation and degradation of the c-Myc proto-oncogene, thus linking AMBRA1 to cell cycle regulation[3]. Recently, an additional role of AMBRA1 in coordinating cell cycle progression and genomic stability through regulating CyclinD stability has been elucidated[4]. Here we show that AMBRA1 is phosphorylated during mitosis on multiple sites by the two mitotic kinases CDK1 and PLK1[5]. Moreover, we demonstrate that AMBRA1 phosphorylation at mitosis is required for a proper spindle function and orientation, driven by NUMA1 protein. We show that the localization and dynamics of NUMA1 are strictly dependent on AMBRA1 pres-

ence, phosphorylation and binding ability[5]. Since spindle orientation is critical for tissue morphogenesis and differentiation, our findings could account for an additional role of AMBRA1 in development and cancer ontogenesis.

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RECENT ADVANCES IN D-ASPARTATE SIGNALING IN TESTICULAR ACTIVITY

S. Falvo[1], G. Grillo[1], D. Latino[1], A. Santillo[1], M.M. Di Fiore[1], M. Venditti[2], S. Minucci[2], G. Chieffi Baccari[1]

[1]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania L. Vanvitelli, Caserta; [2]Department of Experimental Medicine, University of Campania 'Luigi Vanvitelli', Napoli, Italy
E-mail: sara.falvo@unicampania.it

Accumulating evidence points to the key role of D-aspartic acid (D-Asp) in vertebrate reproduction*[1]*. Since its involvement in testosterone biosynthesis was discovered about 30 years ago many studies have been carried out to elucidate the action mechanism of D-Asp in testicular function. Oral administration of D-Asp in rats induced increased levels of testosterone through activation of the hypothalamic-pituitary-gonadal axis resulting in activation of spermatogenesis[1]. *In vitro* experiments on mouse cell lines GC1 (B spermatogonia), GC2 (a stage between preleptotene spermatocyte and round spermatids), and TM4 (Sertoli cells) allowed us to evidence a direct effect of D-Asp on germ cell multiplication/maturation and Sertoli proliferation. In this study, we report the signaling pathways activated by D-Asp in both germ cells and Sertoli cells. D-Asp promoted spermatogenesis by directly stimulating spermatogonial proliferation and spermatocyte maturation *via* AMPAR/NMDAR-mediated ERK-AKT pathways with a consequent increase of PCNA-Aurora B protein expression in spermatogonia and, PCNA, p-H3, and SYCP3 protein expressions in spermatocytes. Further D-Asp actively participated in cytoskeleton remodeling occurring during mitosis in GC1 cells triggering prolyl endopeptidase (PREP) and Dishevelled-Associated-Activator of Morphogenesis1 (DAAM1) protein expressions. In GC-2 cells, D-Asp affects mitochondrial functionality suggesting its participation in the metabolic shift occurring during meiosis. This hypothesis was supported by the up-regulation of mitochondrial biogenesis (PGC1-α, NRF1, TFAM) and fusion (MFN1) markers in these cells. D-Asp induced proliferation of TM4 cells via ERK-AKT pathways with consequent increase of PCNA protein expression. Further, in these cells, the amino acid enhanced the functionality of both mitochondria and ER. Finally, D-Asp reduced the apoptotic process in germ cells and Sertoli cells. In conclusion, the present study provided novel insights into the mechanisms underlying the contribution of D-Asp in spermatogenesis progression.

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CONFOCAL HISTOLOGY OF THE MOUSE OVARY: A TOOL FOR 3D DIGITAL RECONSTRUCTION

G. Fiorentino[1], A. Fantinato[1], G. Brazzale[1], D. Cimadomo[2], A. Vaiarelli[2], F.M. Ubaldi[2], L. Rienzi[2,3], S. Garagna[1], M. Zuccotti[1]

[1]Department of Biology and Biotechnology Lazzaro Spallanzani, University of Pavia; [2]IVIRMA Global Research Alliance, Genera, Clinica Valle Giulia, Rome; [3]Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy
E-mail: andrea.fantinato01@universitadipavia.it

Most of our knowledge on mammalian folliculogenesis derives from histological approaches which cause loss of 3D integrity and limit image acquisition to the sections surface, thus hindering the production of an accurate 3D digital model of the ovary. In this study we propose an innovative high-resolution technique that exploits the eosin Y fluorescent property combined with confocal microscopy analysis of 30 µm-thick sections of the mouse ovary. This approach, which we named "Confocal histology", allowed us to obtain cytofunctional information on all the maturing follicle types and on their enclosed oocytes. Prepubertal 25-day-old CD1 mouse ovaries were fixed in Bouin and sectioned in 30 µm-thick sections stained with 0.5% eosin Y and analyzed with a confocal microscope (SP8, Leica). Eosin, an anionic dye, binds to arginine, histidine, lysine, and tryptophan protein residues through its carboxylic and phenolic groups[1], providing proteins localization. On the confocal images, eosin strong fluorescent signal allowed to (i) identify blood vessels, (ii) distinguish all follicle types, from primordial to preovulatory, and (iii) classify oocytes into developmentally competent SN (surrounded nucleolus) oocytes, with a ring of condensed chromatin around the nucleolus, or non-developmentally competent NSN *(*non-surrounded nucleolus) oocytes, in which chromatin is dispersed throughout the nucleus[2]. Prospectively, Confocal histology, combined with 3D volume rendering tools, may contribute to the 3D digital morpho-functional reconstruction of the ovary, improving our understanding of this organ, during differentiation or adulthood, under normal conditions or in pathologies associated with infertility.

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LRRK2, A KINASE IMPLICATED IN CANCER AND NEURODEGENERATION, PARTICIPATES IN DNA REPAIR

S. Farina[1,2], S. Barnhoorn[1], C. E. Payan Gomez[3], S. Pigliacelli[2], M. Sette[2], P.G. Mastroberardino[1,2,4]

[1]Department of Molecular Genetics, Erasmus Medical Center, Rotterdam, Netherlands; [2]Dipartimento di Medicina Clinica, Sanità Pubblica, Scienze della Vita e dell'Ambiente, Universitá degli Studi dell'Aquila, L'Aquila, Italy; [3]Universidad Nacional de Colombia, Bogotá, Colombia; [4]IFOM, Istituto Fondazione di Oncologia Molecolare ETS, Milano, Italy
E-mail: s.farina@erasmusmc.nl

LRRK2 is a large multidomain protein with Ser-Thr kinase activity[1]. At present, LRRK2 function has been predominantly related to endocytosis regulation *via* Rab proteins; the role of this kinase, however, is incompletely understood and some evidence points to the involvement in alternative processes such as maintenance of mitochondrial DNA integrity. A more in depth understanding of

LRRK2 biological function is highly relevant given that its variants have been correlated with neoplastic progression in mammary carcinoma, lung tumorigenesis, and renal cell carcinoma, as well as with the development of neurodegenerative disorders such as Parkinson's disease.[1-4] Here we interrogated whether LRRK2 may participate in nuclear DNA repair given that accumulation of DNA damage is a hallmark of aging, that is the principal risk factor for both cancer and neurodegeneration. Canonical pathway analysis on a phosphor-proteomic data set from LRRK2 mutant cells revealed that several proteins involved in DNA repair are LRRK2 substrates. Consistently, we observed that X-ray induced DNA damage increases LRRK2 transcription *via* an ATM mediated mechanism. Bioinformatics and cell biology studies revealed that LRRK2 transcription upon damage is mediated by the ATM substrate TRIM28. We also found that LRRK2 participates to DNA damage because its depletion *via* silencing experiments significantly affects DNA damage induced recruitment of different DDR key players such as ATM, MDC1, RNF168 and 53BP1. Consistently, dysregulated DDR is also observed in cells harbouring LRRK2 mutations causing constitutive activation of the its kinase activity. Taken together, our data indicates for the first time that LRRK2 transcription is augmented upon DNA damage and that the protein participated in DDR efficiency.

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miRNAs SECRETED BY CUMULUS CELLS AND THEIR ROLE IN THE ACQUISITION OF THE MOUSE OOCYTE DEVELOPMENTAL COMPETENCE

G. Fiorentino[1], V. Merico[1], M. Zanoni[1], S. Comincini[1], D. Sproviero[2], M. Garofalo[3], S. Gagliardi[3], C. Cereda[4], C.J. Lin[5], F. Innocenti[6], M. Taggi[6], A Vaiarelli[6], F.M. Ubaldi[6], L. Rienzi[6,7], D. Cimadomo[6], S. Garagna[1], M. Zuccotti[1]

[1]Department of Biology and Biotechnology Lazzaro Spallanzani, University of Pavia, Pavia, Italy; [2]IFOM, IFOM-The FIRC Institute of Molecular Oncology, Milan, Italy; [3]Molecular Biology and Transcriptomics Unit, IRCCS Mondino Foundation, Pavia, Italy; [4]Department of Pediatrics, Buzzi Children's Hospital, Milan, Italy; [5]Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK; [6]IVIRMA Global Research Alliance, Genera, Clinica Valle Giulia, Rome, Italy; [7]Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy
E-mail: giulia.fiorentino@unipv.it

Cumulus cells (CCs) interact bidirectionally with the oocyte and contribute to the acquisition of its developmental competence by mechanisms that are still under investigation. We previously reported that cumulus-denuded fully-grown mouse oocytes (DOs) cultured on a feeder layer of CCs (FL-CCs) isolated from developmentally competent oocytes (FL-SN-CCs) can develop to blastocyst, whereas those matured on FL-CCs from incompetent oocytes (FL-NSN-CCs) stop at the 2-cell stage[1]. Using this co-culture platform, here, we focused on the potential regulatory role of CC-secreted extracellular vesicles (EVs) and their miRNA content in the acquisition of the oocyte developmental competence. Imaging flow cytometry combined with confocal microscopy showed that, during the 15-hr co-culture of DOs with FL-CCs, both FL-SN-CCs and FL-NSN-CCs released EVs, most of which <200 nm in size, and capable to cross the zona pellucida and reach the ooplasm. Subsequent NGS analysis showed 74 differentially expressed miRNAs in FL-SN-CCs *vs.* FL-NSN-CCs EVs (43 up- and 31 down-regulated). For 7 of these miRNAs, *in silico* functional analysis identified 71 potential target genes: 23 were involved in follicle growth, 24 in meiosis resumption, 1 in fertilization, and 23 in the acquisition of oocyte developmental competence. Overall, these results suggest CC-EVs as signaling mediators between CCs and the enclosed oocyte, contributing to its proper maturation.

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DELORAZEPAM TREATMENT EFFECTS ON EARLY EMBRYONIC DEVELOPMENT: A COMPARATIVE STUDY OF TWO MODEL ORGANISMS

C. Fogliano, C.M. Motta, C. Agnisola, R. Carotenuto, B. Avallone

Department of Biology, University of Naples Federico II, Napoli, Italy.
E-mail: chiara.fogliano@unina.it

In recent decades, technological advances in water pollution assessments allowed the identification of new environmental contaminants, including pharmaceuticals. The presence of benzodiazepines in the environment, especially after the recent COVID-19 pandemic, is reported to be steadily increasing, comporting growing concerns due to the potentially high impact they exert on the aquatic biota[1]. These psychoactive drugs are found in all environmental matrices and are very effective, even at very low concentrations, since binding with high affinity to GABA and TSPO receptors, highly conserved in invertebrates and vertebrates[2]. In recent years, our attention focused on the effects of delorazepam exposure on *Xenopus laevis* early embryogenesis, demonstrating cytological, physiological and biochemical consequences. To expand knowledge of delorazepam's harmful effects, the same concentrations used for *Xenopus* were tested on the early developmental stages of *Artemia salina* nauplii, an excellent bioindicator model in toxicity studies[3]. Similar effects on embryo development were observed in the two models: accelerated hatching, increased mortality, altered growth rate, and morphological anomalies, with severe damage to the eye. A decrease in locomotory activity was also registered. Curiously, alterations in lipid reserve consumption were observed in both models, indicative of interference with the yolk consumption mechanism and possible cause of delayed development progression. In conclusion, exposure to this psychotropic drug seems to interfere with the same pathways, inducing similar alterations in two evolutionary distant species. Once again, our study underlines the threat represented by the presence of this pharmaceutical in nature and also looks at the possible harmful consequences of human use by young people or pregnant women.

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FROM IMMUNE DEFENCE TO DEVELOPMENT: UNVEILING THE ROLES OF THE COMPLEMENT SYSTEM IN GASTROPODS

N. Franchi[1], G. Sales[2], A. Vezzi[2], S. Sacchi[1], D. Malagoli[1]

[1]Department of Life Sciences, University of Modena and Reggio Emilia, Modena; [2]Department of Biology, University of Padova, Italy
E-mail: nicola.franchi@unimore.it

The Complement System (CS), a known effector of humoral innate immunity in vertebrates, exhibits functions that transcend immune response, extending to crucial developmental mechanisms. There is emerging evidence that the CS can dictate which cells to preserve or eliminate during development, although the molecular mechanisms underlying this selection remain undefined. A recent theory suggests that complement regulators determine cell fate during development and homeostasis in a process akin to 'non-self' recognition. In the absence of such regulators, component 3 of the CS (C3) may act as the principal mediator in cell elimination, facilitating interactions with phagocytic cells. Mapping the localisation of C3 and its regulators in developing tissues is therefore essential to assess the actual developmental role of CS. The most evolutionary conserved CS components have been studied here in the Gastropod *Pomacea canaliculata*. By analyzing tissue-specific Nanopore transcriptomes, the expression of the C3 gene and its protein localization in selected tissues across various developmental stages, we aim to explore whether CS non-canonical functions are present in gastropods. The data collected are delineating the sequence variability of the C3 regulators and confirming the presence of C3 in non-immune contexts during the development of *P. canaliculata*. These findings suggest that the 'non-canonical' role proposed for the CS may actually be the primordial function underpinning its evolutionary conservation.

BIOLOGICAL EFFECTS OF ENVIRONMENTAL DOSES OF DEXAMETHASONE ON THE REPRODUCTIVE SYSTEM OF MUSSEL *MYTILUS GALLOPROVINCIALIS*

M. Galati[1], G. De Marco[1], B. Billè[1], A. Cristaldi[2], M.C. Eliso[1], M. Terranova[1], G. Oliveri Conti[2], M. Ferrante[2], M. Maisano[1], T. Cappello[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina; [2]Department of Medical, Surgical and Advanced Technologies "G.F. Ingrassia", University of Catania, Italy
E-mail: mariachiara.galati@studenti.unime.it

Dexamethasone (Dex), an anti-inflammatory synthetic glucocorticoid, has been widely employed in the SARS Cov-2 pandemic for treatment of acute respiratory distress syndrome. The high use of Dex led to its increased presence in various environmental matrices due to the unsuitability of wastewater treatment plants to block it, worsening its distribution in aquatic ecosystems[1]. This has raised concerns on the potential of Dex to affect non-target organisms, and since its known involvement on the reproductive health, the attention of this study was focused on its probable role as endocrine disruptor in the gonochorous mussel *Mytilus galloprovincialis*. By a multi-biomarker approach, the impact of environmental doses of Dex (C1: 4 ng/L to C4: 2 µg/L) was evaluated at different time-points (T0, T3, T6, T12) during an exposure of 12 days, to unveil their effects on the gonads of both sexes. Chemical analyses revealed the highest tissual levels of Dex at C4. The histomorphological data showed an inflammatory response for both sexes as revealed by the presence of hemocytes along the gonadal connective tissue, with a greater extent in males at C4, where hemocyte infiltration was found inside the follicles. The Periodic Acid Schiff[2] reaction showed alteration in glycogen reserve. Metabolomics[3] described changes at T12 on cellular energy demands with raise of acetoacetate, lactate, mytilitol, and free amino acids. Dysregulation of osmotic balance due to increased organic osmolytes such as betaine was also noticed. The results suggested that the Dex exposes the reproductive system of non-target marine species to a danger that can be translated into a risk for the fitness and reproductive health of mussels, with serious ecological repercussions due to their key role within intertidal ecosystems.

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REPRODUCTIVE TOXICITY RISK ASSESSMENT: NEW STRATEGIES IN INVESTIGATING GAMETE QUALITY

A. Gallo[1], R. Boni[2], E. Tosti[1]

[1]Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Napoli; [2]Department of Sciences, University of Basilicata, Potenza, Italy
E-mail: alessandra.gallo@szn.it

The embryo's developmental fate is principally dictated by the oocyte and sperm competence, which is strictly influenced by the surrounding environment. In externally fertilizing species, gametes are directly exposed to environmental stress, which, leading to gamete quality disturbance, can have carry-over effects into the following embryo stage[1]. Despite that, the current approaches to risk assessment in reproductive toxicity with marine invertebrates focused on one life-history stage, commonly spermatozoa and embryo through sperm cell toxicity and embryotoxicity tests, respectively. The reproductive toxicity risk assessment process aims to expand these traditional approaches with new ones. In this framework, we have developed a multi-integrated approach, which combines traditional tests, with innovative bioassays along with gamete quality assessment[2]. The ovotoxicity test has been set up to investigate the potential effects of stressors on female gamete fertilization competence considering that the reproductive success of living organisms also relies on oocyte competence. Moreover, a novel approach based on fluorescence spectroscopy has been developed to assess different physiological parameters employed to evaluate gamete quality in marine invertebrates widely used as animal models. The developed method represents a suitable alternative to those used so far for dye-loaded gametes analysis since it is easy to perform and allows a rapid and sensitive assessment of several parameters including viability, mitochondrial activity, intracellular reactive oxygen species, lipid peroxidation, and intracellular pH in oocytes and spermatozoa of marine invertebrates providing markers of gamete quality and, hence, their reproductive potential assessment[3,4]. This approach represents a valuable tool to investigate the toxicity and action mechanism of environmental stressors in the framework of reproductive risk assessment[5].

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NEW INSIGHT INTO MORPHOLOGICAL AND MACROMOLECULAR COMPOSITION OF *MUSTELUS MUSTELUS* SPERMATOCYST

G. Chemello[1,2], L. J. De Santis[1], E. Trotta [1,2], M. Zarantoniello[1], V. Notarstefano[1], F. Maradonna[1,2], I. Olivotto[1], E. Giorgini[1], O. Carnevali[1,2], G. Gioacchini[1]

[1]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona; [2]Istituto Nazionale Biostrutture e Biosistemi, Consorzio Interuniversitario (INBB), Rome, Italy
E-mail: giorgia.gioacchini@univpm.it

Elasmobranchs are characterized by ancestral reproductive systems which offer insights into vertebrate reproductive evolution. Despite their unchanged design over 400 million years, they have evolved complex mechanisms ensuring reproductive success. However, human activities have led to a significant decline in elasmobranch populations worldwide[1]. In the Mediterranean basin, the smooth-hound shark (*Mustelus mustelus*) is one of the shark species that are considered vulnerable to human activities[2]. Conservation efforts necessitate a thorough understanding of its reproductive strategy. This study focused on the Adriatic area from where mature male specimens of the smooth-hound shark were captured and analyzed. This study provides, for the first time, a histological detailed description of testicular development in the smooth-hound shark, identifying seven phases of the spermatogenesis process examined also at the macromolecular level through Fourier Transform InfraRed Imaging. Histological analysis showed consistent structural and cellular features similar to those previously documented in spermatocysts of other elasmobranch species. The evolution and migration of both germinative and Sertoli cells were described at each phase, revealing their close connection. Furthermore, the analysis revealed varying expression levels of lipids, proteins, and DNA characteristic of germinative cells and Sertoli cells for each spermatogenesis stage. This research provided new information on spermatogenesis in the common smooth-hound shark, essential for achieving the conservation goal against population decline and anthropogenic pressures.

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MITOCHONDRIAL DNA COPY NUMBER VARIATION IN RESPONSE TO CHEMICAL POLLUTANTS IN WILDLIFE FISH SPECIES

M. Giuga [1,2], V. Ferrito[2], G.S. Calogero[2] , M. Sprovieri[3], A.M. Pappalardo[2]

[1]Institute for the study of Anthropogenic impacts and Sustainability in marine environment (IAS–CNR) Genova; [2]Department Biol., Geol. Envir. Sciences, University of Catania, Italy; [3]Institute of Marine Sciences (ISMAR-CNR) Venezia, Italy
E-mail: marta.giuga@phd.unict.it

The persistence, ecotoxicity and biogeochemical cicle[1] of Mercury (Hg) represent a serious threat to marine ecosystem. Due the pro-oxidant nature of many heavy metal and the mitochondrial genome vulnerability to Reactive Oxygen Species, this study aims to validate the variation of mitochondrial DNA copy number (mtDNAcn) as biomarker of oxidative stress related to chemical contaminants in marine environment[2]. Three target fish species (*Mullus barbatus, Diplodus annularis* and *Pagellus erythrinus*) were collected along the south-east coast of Sicily: in Augusta bay, one of the most contaminated area in Mediterranean sea interested by past Hg imputs[3], and in a control area (CTR). Specimens of each species, showing respectively the higher and the lower levels of Hg bioaccumulation in the two sites, were selected for detecting the relative mtDNAcn using qPCR. The species, having different ecology, exhibited different bioaccumulation rate suggesting the complexity of xenobiotics transfer along the trophic web[2]. However, all the species sampled in Augusta bioaccumulated higher level of Hg compared to those of the CTR confirming the role of sink of the bay sediments[3]. Concerning the variation of mtDNAcn, while *M. barbatus* and *P. erythrinus* collected in Augusta showed a drastic reduction of the biomarker in comparison to their control groups, *D. annularis* showed an incredible mtDNAcn increase suggesting an higher resilience of the species compared to the others of the same area. These results are discussed in the light of the mitochondrial dynamics (fission and fusion)[4] triggered by environmental toxicants. In conclusion, the three target species could be considered as good wildlife organisms for marine monitoring and the implementation of the mtDNAcn could be suggested as a valid tool for the early warning in aquatic system.

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CORRELATION BETWEEN AUTOPHAGY AND APOPTOSIS IN HUMAN GLIOBLASTOMA CANCER STEM CELLS: ROLE OF M2 RECEPTOR AND mTOR COMPLEX-1

C. Guerriero[1], M. Manfredelli[1], A. Iazzulino[1,2], L. Conti[3], C. Dallanoce[4], D. Trisciuoglio[3], A. M. Tata[1,5,6]

[1]Department of Biology and Biotechnologies "C. Darwin", Sapienza University of Rome; [2]Institute of Molecular Biology and Pathology, National Research Council, Rome; [3]Department of Cellular, Computational and Integrative Biology, CIBIO, University of Trento; [4]Department of Pharmaceutical Sciences, University of Milan; [5]Research Centre of Neurobiology "D. Bovet", Sapienza University of Rome; [6]Consortium Interuniversity Biotechnologies (CIB), University of Ferrara, Italy
E-mail: claudia.guerriero@uniroma1.it

Although autophagy is a pro-survival process of tumor cells, it can promote cell death in particular conditions and when regulated by specific signals[1]. We previously demonstrated that the selective stimulation of the M2 muscarinic receptor subtype (M2 mAChR) negatively controls cell proliferation and survival, causing oxidative stress and cytotoxic and genotoxic effects in GBM cell lines and GBM stem cells (GSCs). In this work, we have evaluated whether autophagy was induced upstream of the observed pro-apoptotic processes induced by M2 mAChR activation by the orthosteric ago-

nist APE or the dualsteric agonist N-8-Iper. Our data indicate that activation of M2 mAChR by N-8-Iper promotes autophagy in both U251 and GB7 cell lines, as suggested by the increased protein expression level of LC3B-II and observation, by fluorescence microscopy, of intracellular LC3B accumulation in EGFP-LC3B and in mRFP-EGFP-LC3B transfected cell lines. The autophagy induction by M2 mAChR is regulated by the decreased activity of the PI3K/AKT/mTORC1 pathway and the upregulated expression of pAMPK[2]. Downstream of the activation of autophagy, an increase in apoptosis was also observed in both cell lines after treatment with the two M2 agonists. These data suggest that N-8-Iper treatment causes an increase in autophagy followed by apoptosis in both cell lines. In contrast, the absence of autophagy in GSCs treated with APE seems to indicate that cell death could be triggered by alternative mechanisms to those observed for N-8-Iper.

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GLYCOHISTOCHEMICAL RESPONSE IN MOLLUSCS FOLLOWING METAL TREATMENTS

M.V. Guglielmi[1], M. Mastrodonato[2], C. De Blasi[3], D. Semeraro[2], I. Ferrandino[4], G. Scillitani[2]

[1]Institute for Water Research (IRSA), Italian National Research Council (CNR), Taranto; [2]Department of Biosciences, Biotechnology and Environment, University of Bari Aldo Moro, Bari; [3]CoNISMa (Consorzio Nazionale Interuniversitario per le Scienze del Mare), Rome; [4]Department of Biology, University of Naples Federico II, Naples, Italy
E-mail: marcovito.guglielmi@irsa.cnr.it

Molluscs revealed good models to study the effects of metal pollution. In particular, mucin composition and secretion results affected by treatments and can be used as a biomarker to assess presence and effects of metals in both aquatic and terrestrial environments. Here we report the results of our studies on marine bivalves and terrestrial snail. Aluminum (Al) was administered to both the clam *Chamelea gallina* (CG) and the snail *Eobania vermiculata* (EV) and mucins were studied in situ by histochemical and lectin-histochemical methods. Treatments resulted in significant reduction of secretion in both the gills and the foot of CG[1] as well as in the foot of EV[2]. The diversity of oligosaccharidic chains in mucins was significantly lowered with reduction in acidic sulphated, GalNAc, GlcNAc, mannosylated, and fucosylated residues. These changes could potentially lead to variations in acidity, viscosity, and functionality of the mucus, thereby potentially influencing organismal well-being and survival. In another study we administered lanthanum (La) to the clam *Ruditapes philippinarum* and evaluated alteration in mucous secretion by the mantle. A great variability in the composition of the mucus resulted in the different folds of the mantle. This variability leads to differential responses of each area to La treatment. In the outer epithelium, facing the valves, there is a marked increase in GalNAc and GlcNAc residues, particularly at low dosages of La. In the central and inner folds, towards the gills, there is an increase in other residues, with a notable rise in fucose. In conclusion, this study underscores the significance of comprehending glycohistochemical responses of molluscs to metal treatments, shedding light on potential implications for their survival and ecological interactions in polluted environments.

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PROTECTIVE EFFECTS OF POLYPHENOLS ON DIET-INDUCED MUSCLE DEGENERATION IN ZEBRAFISH (*DANIO RERIO*)

R. Imperatore[1], G. Orso[1], E.Coccia[1], R. Sciarrillo[1], A. Falzarano[1], S.H. Hoseinifar[2], G. Ashouri[1], M. Paolucci[1]

[1]Department of Sciences and Technologies, University of Sannio, Benevento, Italy; [2]Department of Fisheries, University of Agricultural Sciences and Natural Resources, Gorgan, Iran
E-mail:rimperatore@unisannio.it

Aging is a gradual process that begins in early adulthood and reduce different body functions causing health problems such as muscle mass declines[1]. This process has multifaceted linked features that can be influenced by external factors, such as unhealthy diets and degenerative diseases, which can induce chronic inflammation and oxidative stress resulting in dysfunctions such as sarcopenia[2]. Polyphenols, found abundantly in plants, exhibit anti-inflammatory and antioxidant properties, making them promising for preventing/treating chronic diseases like obesity and degenerative conditions[3]. The zebrafish (*Danio rerio*) is a crucial model to study the interplay of inflammation and aging[4]. Using IHC and WB analysis, we evaluated the hypothesis that polyphenols can prevent skeletal muscle degeneration induced by a proinflammatory diet. Zebrafish were divided in five experimental groups: Ctrl, Inflamed (k-carrageenan 0.1% supplementation) and PV pre-, co- or post-treated groups. Muscle morphology, inflammation and oxidative stress were examined using IHC. WB was employed to identify inflammatory cell pathways and to investigate muscle structural proteins. Inflammation induced by k-carrageenan was characterized by morphological alterations of skeletal muscle like that induced by aging, increase of TNFα and COX2 and alteration of the anti-oxidative enzymes SOD and CAT. The diet enrichment with PV prevents and partially counteract muscle fibres alteration inducing a reduction of TNFα and COX2 expression, maybe acting on the NF-kB signaling pathway by inhibition of IkB phosphorylation, as observed by WB analysis. Moreover, muscle structural proteins analysis showed as diet-induced inflammation alters muscle structure which is restored by PV treatment. Overall, the results of the study open the way to the use of PV to prevent or counteract the muscle atrophy induced by aging.

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GLIOBLASTOMA MULTIFORME CELL COMMUNICATION IS MEDIATED BY EXTRACELLULAR VESICLES DURING TEMOZOLOMIDE TREATMENT

D. Vardanyan[1], M. Karimova[1], S. Tacconi[2], L. Dini[1]

[1]Department of Biology and Biotechnologies Charles Darwin, the Sapienza University of Rome, Italy; [2]CarMeN Laboratory (UMR INSERM 1060/INRA 1397), Lyon-Sud Faculty of Medicine, University of Lyon, Pierre-Bénite, France
E-mail: mariana.karimova@uniroma1.it

Glioblastoma multiforme (GBM) is the most common malignant primary brain tumour, and is characterised by high aggressiveness, heterogeneity, and resistance to therapy. Despite the combination of surgical resection of the tumour, radiotherapy, and chemotherapy with temozolomide (TMZ), the estimated median survival is only 15 months[1]. Recently, extracellular vesicles (EVs) have received increasing attention for their multiple roles in cell-cell communication. EVs are membrane-enclosed nanoscale vesicles that can modulate the behaviour of recipient cells by transporting biologically active molecules between cells[2]. In this study, we focused on EVs derived from TMZ-sensitive and TMZ-resistant GBM cell lines. We characterised GBM-derived EVs using electron microscopy, atomic force microscopy, and proteomic analysis, which revealed differences not only between EVs isolated from different GBM cell lines, but also between EVs isolated from TMZ-treated and untreated cells. Based on the proteomics data, we focused on a resistant (U251MG) and a sensitive (U87MG) cell line to study the effect of EVs treatment. Analysis of migration and cell death rates was performed after treating both GBM cell lines with EVs isolated from the same cell line and EVs isolated from the different cell line. In particular, the treatment of sensitive GBM cells with EVs isolated from resistant cells and vice versa showed that EVs do indeed transfer resistance or sensitivity information to TMZ to the recipient GBM cells. Interestingly, when EVs isolated from a sensitive cell line were incubated with resistant GBM cells, an increase in migration activity and cell death markers was observed. This research provides new insights into the variability of GBM-derived EVs and their multiple effects on recipient cells.

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REGENERATION OF ZEBRAFISH RETINA FOLLOWING TOXIC INJURY

A. La Pietra, A.R. Bianchi, T. Capriello, A. De Maio, I. Ferrandino

Department of Biology, University of Naples Federico II, Napoli, Italy
E-mail: alessandra.lapietra@unina.it

The zebrafish retina is anatomically and functionally similar to that human, so it's widely used for eye diseases research. However, unlike humans, the zebrafish retina is naturally able to regenerate after damage[1]. Regeneration begins with Müller glia, which in the presence of damage are stimulated to dedifferentiate, re-entering the cell cycle, and giving rise to neuronal progenitor cells. These migrate to the damaged retina and differentiate into the missing cell types[2]. This study aim to evaluate if the zebrafish retina can regenerate following toxic injury caused by exposure to aluminum, known for its negative effects on the central nervous system[3,4]. Adults of zebrafish were exposed to 11 mg/L of aluminum for 10, 15, and 20 days. Hematoxylin-eosin staining allowed to observe degeneration and regeneration of retina. Damage occurred at 10 and 15 days, followed by regeneration at 20 days of exposure. Neurodegeneration by Fluoro Jade B was observed at 15 days of exposure, while at 20 days it returned to a condition similar to the control. The nuclear PARP1 and PARP2, with molecular of 113 kDa and 70 kDa respectively were identified. The highest expression of both enzymes was evidenced after 10 days of exposure. At this time the presence of oxidative DNA damage can be hypothesized. Furthermore, by quantitative Real Time-PCR, an up-regulation of *pax6a*, *pax2a*, *ngn1*[5], and *notch1a*[6] genes involved in the regeneration process was observed at 10 and 15 days of exposure. The results confirmed that aluminum causes alterations and neurodegeneration in zebrafish retina at 10 and 15 days, but at 20 days of exposure the tissue damage, apoptosis, PARP and gene expression levels return in condition similar to control, indicating a damage repair. To sum up, data showed that zebrafish are able to adapt following exposure to a toxic metal and trigger repair mechanisms that lead to the regeneration of damaged tissues.

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THE CELL ISLAND NICHE OF THE COLONIAL ASCIDIAN *BOTRYLLUS SCHLOSSERI* IS TEMPORARY AND DYNAMIC THROUGH THE ASEXUAL CYCLE

F. La Torre[1], V. Vanni[2], D. Asnicar[3], F. Caicci[1], L. Manni[1]

[1]Dipartimento di Biologia, Università di Padova, Padova, Italy; [2]Living Systems Institute, University of Exeter, Exeter, United Kingdom; [3]Huntsman Marine Science Centre, St Andrews, NB, Canada

The colonial tunicate *Botryllus schlosseri* has developed remarkable stem cell-mediated regenerative capabilities in response to environmental pressures. These unique capabilities among chordates are evident through asexual reproduction *via* budding and whole-body regeneration[1]. In a colony there are three generations of individuals (zooids): adults, primary buds and secondary buds. Cyclically adult zooids are resorbed while buds mature into the new adult generation, allowing colony growth. Adults possess stem cell niches. Cell islands[2] are in the ventral body wall and consist of various cell types including stem cells and phagocytes. This niche is transient due to the physiological turnover of adults. This study aims to investigate the dynamics of the cell islands in fixed and *in vivo* organisms. 54 whole-mounted colonies at different phases of the asexual cycle were used to count the cell islands, which are visible in fixed colonies under a stereomicroscope, along with the number of primary buds on each individual. Three living colonies were injected with fluorescent *Escherichia coli* bioparticles: after ingesting bioparticles, circulating phagocytes became fluorescent, enabling the detection and the counting of cell islands throughout the various blastogenetic phases. Overall, the data revealed that the number of cell islands decreases with the adult degeneration approaching and may differ among genetically

identical zooids within the same colony. Within a zooid, cell islands are asymmetrically distributed, and their number correlates positively with the number of primary buds. These findings demonstrate the dynamic nature of this stem cell niche and confirm the stem cell ability to circulate in the colony and home in the forming niches, highlighting the unique stemness properties of these organisms.

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HUMAN AMNIOTIC FLUID STEM CELLS-DERIVED SECRETOME EXERTS NEUROPROTECTIVE EFFECTS IN AN ISCHEMIA/REPERFUSION MODEL

E. Maceroni[1], V. Castelli[1], M. Pace[1], E. Colaianni[1], M. d'Angelo[1], A. Cimini[1,2]

[1]Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy; [2]Sbarro Institute for Cancer Research and Molecular Medicine and Center for Biotechnology, Temple University, Philadelphia, USA
E-mail: emaceroni@unite.it

Stem cells provide a basis for developing new therapeutic strategies to treat a range of human conditions[1]. Current discoveries in regenerative medicine have promoted the search for new sources of stem cells with beneficial properties; in particular, amniotic fluid represents a valuable stem cell resource. In the past decade, the stem cell secretome and, consequently, the paracrine effect have been of particular interest[2]. Despite the increasing interest in human amniotic fluid stem cells, there is limited knowledge about their secretome characteristics and potential neuroprotective mechanisms in various conditions, including stroke[3]. To get more insight into amniotic fluid cells' therapeutic potential, we examined the signal transduction pathways activated by the secretome derived from human amniotic fluid stem cells (hAFSCs) in an ischemia/reperfusion (I/R) *in vitro* stroke model using Western blot. Additionally, we analyzed the miRNA expression in the exosomal fraction of the conditioned medium. The hAFSCs-derived secretome activated pro-survival and anti-apoptotic pathways. The microRNA analysis in the exosomal component revealed 16 overexpressed miRNAs involved in regulating coherent signaling pathways. Specifically, we analysed the pathways of relevance in ischemia/reperfusion, such as neurotrophin signaling, and those related to neuroprotection and neuronal cell death. The findings strongly suggest the neuroprotective effects of hAFSCs-conditioned medium in the *in vitro* stroke model. This neuroprotection may be achieved, at least in part, through the modulation and activation of pro-survival processes, possibly due to the activity of secreted miRNAs.

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ADDRESSING THE TOXICITY OF PFOA ON ZEBRAFISH LARVAL DEVELOPMENT: EXPLORING *BACILLUS SUBTILIS NATTO* AS A MITIGATING AGENT

F. Maradonna[1], C. Giommi [1], M. Lombó[1,2] O. Carnevali[1]

[1]Dipartimento Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona, Italy; [2]Departamento de Biología Molecular, Universidad de León, León, Spain
E-mail: f.maradonna@univpm.it

Perfluorooctanoic acid (PFOA) is a well-known perfluorinated compound used in an increasing variety of consumer and industrial products, including food packaging, fire-retardant foams, cosmetics and surface protectors, which exposure causes development delays, endocrine disruption, liver toxicity, tumorigenicity, and other adverse effects[1]. In zebrafish, previous studies demonstrated that exposure to PFOA during development leads to immune system, heartbeat rate, and locomotor behavior alterations[2]. Noteworthy, evidence suggests that probiotics can efficiently reduce the toxicity caused by exposure to environmental pollutants[3]. Therefore, this study aims to determine the toxic effects caused by PFOA exposure during zebrafish early development and the mitigating capacity of *Bacillus subtilis natto* on PFOA toxicity. In the experimental trial, control and exposed larvae were reared starting from hatching until 21 days post fertilization (dpf) with PFOA (50 and 100 mg/L) alone or in presence of *B.subtilis*, administered *via* the diet (10^7 CFU/larvae/day). Morphometric analyses reveal significant impacts on development following exposure to PFOA. Both concentrations caused a reduction in standard length at 21 dpf, coupled with an increase in head length. Noteworthy was the profound skeletal malformation observed in larvae exposed to 50 mg/L PFOA, along with a notable decrease in eye size diameter. Remarkably, supplementation with probiotics exerted a positive effect on the growth of untreated larvae. Furthermore, when administered alongside PFOA, it effectively mitigated the toxic effects, showcasing a particular strong recovery on larvae exposed to the lower dose. These morphological alterations correlated with changes in the expression of genes associated with growth and ossification processes. Collectively, these findings underscore the potential of probiotics, particularly *B. subtilis*, in ameliorating PFOA-induced toxicity during larval development.

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PLASMONIC NANOPARTICLES AND OPTICAL HYPERTERMIA TO STIMULATE TISSUE REGENERATION

N. Dell'Aversano[1], M L. Amenta[2], M. Iencharelli[1], M. Blasio[1], V. De Felice[1], M. Moros[3], M. Rippa[1], A. Tino[1], C. Tortiglione[1], V. Marchesano[1]

[1]Istituto di Scienze Applicate e Sistemi Intelligenti CNR, Pozzuoli, Italy; [2]Istituto di Bioscienze e Biorisorse, CNR, Napoli, Italy; [3]Instituto de Nanociencia y Materiales de Aragón (INMA) Zaragoza, Spain
E-mail: valentina.marchesano@cnr.it

Optical hyperthermia mediated by plasmonic nanomaterials is a non-invasive technique allowing the controlled increase of tem-

perature into biological tissues. Many factors influence the therapeutic effects of heat treatment timing, repetition, and pulsing. Several studies suggest heat-based therapies for cancer treatment and for regenerative medicine to enhance wound healing and tissue regeneration. Photothermal agents, as gold nanoparticles (AuNPs), have been used as nano-hotspot to selectively generate heat in a spatiotemporal fashion (photothermal therapy)[1]. Cnidarians are excellent model organisms for studying tissue regeneration, thanks to their ability to regenerate missing body parts. The effects induced by heat and light irradiation on *Hydra vulgaris* have been recently investigated[2-3], opening interesting scenario on the possibility to develop new nanodevices to enhance their regenerative potential. AuNPs, due to plasmonic features, can release precise heat doses under nir-infrared irradiation (NIR) and in *Hydra* they have been shown able to induce diverse responses[4], ranging from cell ablation to programmed cell death or thermo tolerance, by simply tuning NP shape, size and their thermal properties. Tuning NIR irradiation and AuNPs dose, treated polyps capability to regenerate missing heads under photostimulation has been dissected at whole animal, cellular and molecular levels, and compared to exposure to external macroscopic heat sources, suggesting new application of hyperthermia mediated by AuNPs to enhance tissue regeneration[5]. Results reveal the action of heat on animal physiology and open new perspectives for the development of technologies based on hyperthermia for regenerative medicine.

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THE MEDICINAL LEECH *HIRUDO VERBANA* AS INNOVATIVE INVERTEBRATE MODEL TO INVESTIGATE THE REGENERATIVE POTENTIAL OF HUMAN MESENCHYMAL STEM CELLS SOLUBLE FACTORS

G. Marcolli[1], N. Baranzini[1,2], L. Pulze[1,2], N. Paris[1], L. Barone[1], F. Rossi[1], S. Sadia[1], R. Gornati[1], A. Grimaldi[1,2]

[1]Department of Biotechnology and Life Sciences, University of Insubria; [2]ILFARM S.r.l., Varese, Italy
E-mail: gmarcolli@studenti.uninsubria.it

Although various sophisticated approaches have been developed to mimic multicellular interactions in three-dimensional systems, they still fail to faithfully replicate an entire living organism. Therefore, in accordance to the European (2010/63/EU) and Italian (26/2014) Directives, the use of alternative animal models has become a fundamental step in providing useful information in several research fields. In this context, the medicinal leech *Hirudo verbana*, due to the lack of ethical restrictions associated with *in vivo* research, is starting to show promise as complementary model for pre-clinical biomedical studies[1]. Indeed, despite their simple body organization and the relatively low genetic complexity, leeches exhibit biological processes, cellular responses, and tissue organization extremely similar to those found in vertebrates[2,3]. Considering the aforementioned, in the current work we propose to use medicinal leeches to investigate the potential of soluble factors released from human Mesenchymal Stem Cells (hMSCs). Morphological, immunohistochemical, and molecular analyses have been performed on injured animals to assess the promotion of cellular invasion and vessel growth. Both of these processes are crucial for ensuring adequate vascularization, necessary for cell survival, and tissue regeneration, preventing the formation of hypertrophic scars. Our results confirmed an improved ability in restoring injured tissues and a significant reduction of healing time[4,5] in animals treated with hMSCs soluble factors, thus confirming this cell-free approach as a novel solution for the treatment of multiple chronic diseases.

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R-FETAX: TERATOGENIC AND NEUROBEHAVIORAL EFFECTS OF ETHANOL

E. Menegola[1], F. Metruccio[2], M. Battistoni[1], R. Bacchetta[1], F. Di Renzo[1]

[1]Department of Environmental Science and Policy Università degli Studi di Milano; [2]ICPS, ASST Fatebenefratelli Sacco, Milan, Italy
E-mail: elena.menegola@unimi.it

Ethanol (Eth) consumption in pregnancy is correlated to a wide spectrum of effects, classified as Fetal Alcohol Spectrum Disorders (FASD), including morphological defects, developmental delays and neurobehavioral effects both in humans and in experimental species. High Eth concentrations (0.6-3% v/v) induced in amphibian *Xenopus laevis* exposed from midblastula to not feeding tadpole (FETAX protocol) malformations and lethal effects. Aim of the present work was the evaluation of multiple morphological and neurobehavioral effects of Eth exposure (Eth 0.1-3% v/v) using R-FETAX protocol. Samples, obtained by natural mating, were exposed during different specific developmental windows (organogenetic period, sensitive for morphological abnormalities; neurodevelopmental windows, sensitive to behavioral alterations). Extra groups, exposed during the whole test (classical FETAX exposure) or for 4 h before the end of the test (acute exposure) were performed. Samples were monitored for lethal effects during the full six-day test period. At the end of the test, external morphology and developmental degree were evaluated. The neurobehavioral swimming test was applied only on not-malformed tadpoles. Effects were modelled using PROAST software: dose-relation curves were obtained and benchmark dose level derived, setting response at levels used as point of departure for risk assessment. Overall data showed dose- and stage- specific effects miming the Eth-induced effects observed in humans, suggesting no safe amount of alcohol use during development.

AQUAPORIN-9 (AQP9) PLAYS A ROLE IN THE SYSTEMIC DISORDERS UNDERLYING THE WOLMAN DISEASE

D. Mentino*, P. Gena*, N. Zagaria, S. Garra, M. Mastrodonato, G. Calamita

Department of Biosciences, Biotechnologies and Environments, University of Bari "Aldo Moro", Bari, Italy
**Equal contribution to the work.*
E-mail: donatella.mentino@uniba.it

Lysosomal acid lipase deficiency (LAL-D) is an autosomal recessive disease due to mutations in the lysosomal acid lipase (LIPA) gene[1]. Reduced lysosomal acid lipase activity leads to a progressive accumulation of cholesterol esters and triglycerides in hepatocytes, adrenal glands, intestine, and macrophage-monocyte system cells throughout the body. LAL-D induces two clinical severity spectra, an infantile form called Wolman disease (WD) and a less aggressive form known as cholesterol ester storage disease (CESD) [2]. To date, there is no effective therapy against LAL-D. In this study, a transgenic mouse model of CESD was used to characterize the LAL-D phenotype and to verify the relevance of AQP9, an aquaporin highly expressed in liver[3] and leukocytes with roles in the metabolic homeostasis and inflammation[4,5]. Compared to healthy control mice, CESD mice revealed early onset of hepatic steatosis already from the 9th day of postnatal life with rapid progression to severe microvesicular steatosis at the 90th day associated with a strong infiltration of neutrophils and Kupffer cells in liver parenchyma. From weaning, AQP9 mRNA and protein levels were significantly reduced until the 90th day of postnatal life, at which time the overall reduction of this AQP was less marked, probably due to the progressive infiltration of Kupffer cells and neutrophils expressing AQP9. Consistent with these results, genetic AQP9 ablation in a murine model of CESD/WD significantly improved the LAL-D phenotype indicating important roles for AQP9 in the disease and its potential relevance as drug target in treating the clinical disorder.

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NEW ANIMAL MODEL SYSTEMS IN THE STUDY OF AGEING AND LONGEVITY

M. Iannello, M. Martini, G. Piccinini, L. Milani

Department of Biological, Geological and Environmental Sciences (BiGeA), University of Bologna, Italy
E-mail: liliana.milani@unibo.it

Ageing is the progressive decline of tissue function that ultimately results in death[1]. This depends on the decrease in postmitotic cell function and on the inability to replace these cells with stem cell replication. The mechanisms underlying ageing and, consequently, lifespan are multiple and have both genetic and environmental components. Errors in replication and transcription, alteration of the nuclear architecture, shortening of telomeres, and incorrect protein folding lead to ageing and decreased longevity. Organisms have evolved different mechanisms that counteract damage accumulation, but when repair mechanisms are not sufficient cell death is induced to the point of determining organism death. Since studies on ageing in classical animal models are evidently insufficient for understanding certain aspects underlying ageing and lifespan, new model systems are being investigated to proceed further. In this concern, we studied bivalve molluscs and birds. By comparing genes of short- and long-lived species inside the two classes, we searched for signals of convergent evolution. Bivalves show an extraordinary wide range of lifespan -between 1 and 507 years-. We found that in long-lived bivalves some genes showing convergent evolution have already been associated with ageing and longevity in model organisms, suggesting a shared underlying gene network for longevity in metazoans; for other genes that were not, a possible role in longevity can be suggested and worth further investigation[2]. Birds have a lifespan ranging from 3 to 84 years[3], although the highly energetic metabolism required by active flight. We found that convergently conserved genes in long-lived birds are involved in chromosome segregation during cell division, and constraints on genes associated with efficient cell cycle regulation offer a potential explanation for their delayed ageing. Even if we could not find a way to live longer, by studying new model systems we could understand deeply causes and consequences of ageing, and possibly how to slow down the onset of age-related diseases.

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IMPACT OF BISPHENOL A ON PROSTATE CELL PHYSIOLOGY

A. Mileo[1], F. Carrella[1], S. Boccia[1], R. Pasca[1], F. Bost[2,3], C. Hinault[2,3,4], M. De Falco[1,5]

[1]Department of Biology, University of Naples Federico II, Napoli, Italy; [2]Université Côte d'Azur, INSERM U1065, C3M, Bâtiment Universitaire Archimed, Nice, France; [3]Équipe Labelisée Ligue Nationale Contre le Cancer; [4]Université Côte d'Azur, CHU, INSERM U1065, C3M, 06202 Nice, France; [5]National Institute of Biostructures and Biosystems (INBB), Rome, Italy

Bisphenol A (BPA) is a synthetic compound 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 lids. Recently, BPA massive use gained attention, becoming a concern issue for the human health, specifically reproduction, due to chronic exposure and its capability to bind Estrogen (ERs -ERα, ERβ) and Androgen receptor (AR), also at low dosage. Prostate is an accessory gland that play a key role in male reproductive system. AR, as well ERs are involved in prostate development and in the maintenance of prostatic physiology. BPA xenoestrogenic activity together with its widespreade environmental presence may lead to an inappropriate activation of ERs or AR pathways in prostate gland during all the life or may interfere with specific therapeutic strategies against prostate cancer.[1-3] In this work, we showed the effects of BPA on two cancer prostate cell lines: DU145 and LNCAP (androgen independent, androgen dependent, respectively), on cell proliferation, cell migration and oxidative stress. BPA increased cell proliferation at low concentra-

tion (10^{-9} M) for both cell lines, demonstrating its non monotonic action. Using a specific ER inhibitor, this phenomenon was stopped in each cell line, suggesting an ER involvement in cell proliferation. Then, BPA treatment increased cell migration, especially in DU145 cells. Furthermore, BPA activated NRF2 pathways: genes such as HO-1, CAT and SOD was upregulated after BPA treatment. To sum up, BPA can affect prostate cancer cell behaviour, increasing cell proliferation and migration and inducing oxidative stress, *via* estrogen pathways acting as xenoestrogenic compound.

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POLYSTYRENE MICROBEADS RELEASE VOLATILE COMPOUNDS: EFFECT ON *ARTEMIA SALINA* EARLY DEVELOPMENT

C.M. Motta[1], M. Trifuoggi[2], M. Toscanesi[2], G. Fasciolo[1], P. Venditti[1], C. Fogliano[1], A. Raggio[3], R. Romano[4*], B. Avallone[1], R. Carotenuto[1]

*[1]Department of Biology, University of Naples Federico II, Napoli; Department of Chemistry, University of Naples Federico II, Napoli; [3]Stazione Zoologica Anton Dohrn, Naples, Italy and NBFC, National Biodiversity Future Center, Palermo; [4]Department of Science and Technology, University Parthenope, Napoli, Italy. * International PhD Programme, UNESCO Chair "Environment, Resources and Sustainable Development*
E-mail: mottacm@unina.it

Polystyrene is one of the most diffused contaminants worldwide. Once released in the environment, it degrades into micro and nanofragments that severely impact vegetal and animal organisms. A plethora of toxic effects have been reported on different organs and tissues[2]. In the present work, we examined the effects of 3 μm polystyrene beads MP on the early development of the model species *Artemia salina*. The first objective was to assess whether beads release volatile compounds[1] (VOCs) in culture seawater. Then, the effects of beads/VOCs were determined at the level of oxidative stress[1] and morphofunctional damage, in nauplii obtained from intact and dechorionated cysts[2]. Results demonstrated that MP releases significant amounts of ethylbenzene, xylene, benzaldehyde, and styrene, at concentrations that may result in cytotoxicity and genotoxicity. Nauplii exposed to MP/VOCs showed reduced hatching and delayed development, probably due to the significant oxidative stress that occurred during embryogenesis. Significant damage was observed at the level of the gut brush border, as indicated by the reduced presence of glcNAc while yolk was poor in galNAc, and its resorption was markedly delayed. The effects of MB ingestion were also determined and excluded in specific experiments in which the nauplii were exposed to beads after hatching. In conclusion, two important pieces of evidence emerge: polystyrene toxicity in early nauplii is due to the release of chemicals, and second, the yolk platelets must be added among targets. If confirmed, these results indicate that microplastic toxicity mechanisms should be reconsidered.

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CYTOTOXIC AND GENOTOXIC EFFECTS OF POLYSTYRENE MICROPLASTICS ON SEA URCHIN SPERMATOZOA

F. Mottola, M. Carannante, A. Barretta, I. Palmieri, L. Rocco[1]

[1]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania L. Vanvitelli, Caserta, Italy
E-mail: lucia.rocco@unicampania.it

Plastic pollution is a significant concern, with hundreds of tons of plastic products entering the environment annually[1]. Plastic degrades into microplastics (MPs), ranging from 1 μm to 5 mm, due to various processes like UV radiation and human activities[2]. The main worry with MPs exposure is their potential to accumulate and biomagnify in organisms. Among MPs, polystyrene microplastics (PS-MPs) are prominent pollutants in marine ecosystems[3]. The effects of PS-MPs on marine and terrestrial organisms have been well documented and mainly include impacts on the reproductive system[4]. The reproductive toxicity and genotoxicity of MPs raises concerns also for human, particularly considering the recent discovery of plastic micro fragments in human seminal fluid[5].This study aimed to assess the cytotoxic and genotoxic effects of PS-MPs on sea urchin sperm. Spermatozoa were exposed to 50 μg/mL of PS-MP for 30 min. Cytotoxic analysis was conducted using eosin vitality tests and through observation of sperm motility and morphology. The results highlighted that PS-MPs cause a significant reduction in sperm viability and motility, without inducing alteration in sperm morphology. Genotoxicity analysis conducted through the TUNEL technique, and the NitroBlue Tetrazolium chloride test (NBT), revealed increased DNA fragmentation and reactive oxygen species (ROS) production after PS-MPs exposure. Moreover, head-to-head agglutination of spermatozoa was observed in the sample treated with PS-MPs, indicating the MPs capacity to adhere to the surface of sperm cells and form aggregates with MPs on other sperm cells. This phenomenon inhibits movement and reduces reproductive potential. The observations arising from this study suggest that PS-MPs may detrimentally affect the quality of sea urchin sperm, potentially influencing reproductive processes.

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EARLY-LIFE FLUOXETINE-EXPOSURE INDUCES SEROTONERGIC CIRCUITRY DEVELOPMENTAL ABNORMALITIES AND ANXIETY-LIKE BEHAVIOR IN MICE

S. Nazzi[1], M. Picchi[1], S. Migliarini[1], N. Barsotti[1], M. Pasqualetti[1,2]

[1]Department of Biology, University of Pisa; [2]CNCS@UniTn, IIT, Rovereto, Italy
E-mail: serena.nazzi@biologia.unipi.it

The serotonergic system innervates the entire central nervous system serving vital roles in physiological and behavioral functions. In line, dysfunction of this system can lead to the onset of neu-

ropsychiatric disorders. Genetically disruption of 5-HT brain production underscored the importance of maintaining serotonin balance in the adult brain for proper axonal connections[1,2]. Recently we have shown that peri-physiological fluctuation of 5-HT homeostasis induced by the antidepressant fluoxetine treatment produces modification of the serotonergic wiring in hippocampus (HP) and prefrontal cortex (PFC) of adult mice, which is reversible after treatment suspension[3,4]. However, there is limited understanding of how serotonergic fibers react to clinically relevant changes in 5-HT levels that may occur early in life. We therefore explored whether 5-HT fluctuations within the peri-physiological range during development can remodel 5-HT fibers and impact behavior. To this aim, we administered fluoxetine to *Tph2*^{GFP} knock-in mice *via* maternal exposure from embryonic day 0 (E0) up to P21. Our findings revealed that developmental fluoxetine exposure induces a "behavioral paradox" exacerbating anxiety- and depression-like traits, opposite effects to that expected following antidepressant administration. In addition, combining confocal imaging and 3D modeling, we observed already at P0 a dramatic alteration of 5-HT fiber density in both PFC and HP which persisted over the whole treatment duration. Intriguingly, upon treatment suspension, the 5-HT fibers remodeling induced early in life resulted to be permanent, as opposed to what observed when fluoxetine was administered to adults[3,4]. Taken together these results suggest that 5-HT level fluctuation during early-life temporal windows impact serotonergic wiring development irreversibly and produce anxiety-like related behavior.

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THREE-DIMENSIONAL MICRO-SCAFFOLD EFFECTS, DEVELOPMENTAL AND MATRIX STIFFNESS IMPACT ON MESENCHYMAL STEM CELLS AND MESOTHELIOMA CELLS

S. Oliveto,[1,2], P. Ritter[2,3], M.T. Raimondi[3] , S. Biffo[1,2]

[1]DBS, University of Milan; [2]National Institute of Molecular Genetics, INGM, Milan; [3]Department of Electronics, Information and Bioengineering, Politecnico di Milano, Milan, Italy
E-mail: oliveto@ingm.org

The cell's microenvironment is crucial for its behaviour and fate, comprising a network of matrix components, neighboring cells, factors and physical cues. These signals influence cellular activities like differentiation, migration and developmental patterning. Cellular interactions within this environment guide tissue development and organogenesis[1]. Cells' ability to adapt to their surroundings is fundamental, impacting processes like embryonic development and tissue regeneration. In this context, three-dimensional (3D) cultures provide a platform for studying cell-cell and cell-ECM interactions[2], which are essential for regulating cell fate and function *in vivo*, mimicking aspects of the developmental microenvironment. Here, we explore the impact of 3D culture environments, specifically Nichoids[3], on cell behaviour and gene expression patterns in two distinct contexts: mesenchymal stem cells (MSCs) and mesothelioma cells. Nichoids provide a unique microstructured scaffold that influences cell phenotype, affecting ECM and developmental processes. Comparative analyses reveal significant gene expression differences, especially in ECM assembly and cell adhesion pathways. Cells in Nichoids show increased proliferation and gene expression related to ECM assembly, including heightened expression of COL1A1 and COL5A1 and indicating potential changes in matrix stiffness and signaling. In summary our work highlights Nichoids' impact on MSCs behaviour and mesothelioma cells plasticity, revealing potential in development, regenerative medicine, and cancer research. Considering the microenvironment is crucial for stem cell studies, developmental biology, therapy and disease modeling. This underscores the importance of considering the microenvironment in stem cell studies and its implications for developmental biology, therapeutic applications and disease modeling.

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KETOSIS MODULATES NEURAL NETWORK AND BDNF EXPRESSION IN PRIMARY PFC AND HIP NEURONAL CULTURES OF BTBR MICE

I. Olivito[1], A. Piscioneri[3], S. Morelli[3], D. Minervini[1], E. Avolio[1], R. Alò[1], M.C. Cerra[2], L. De Bartolo[3#], R.M. Facciolo[1#]

[1]Comparative Neuroanatomy Laboratory; [2]Laboratory of Organ and System Physiology, Department of Biology, Ecology and Earth Sciences (DiBEST); [3]Institute of Membrane Technology, National Research Council of Italy, ITM-CNR, University of Calabria, Arcavacata di Rende, Cosenza, Italy
[#]These authors contributed equally to this work.
E-mail: ilaria.olivito@gmail.com

Autism Spectrum Disorder (ASD) is a multifaceted condition characterized by social deficits, repetitive behaviors, and often co-occurring psychiatric and medical conditions[1]. Effective therapeutic interventions are urgently needed given its prevalence and complex etiology[2]. Ketosis emerged as a promising strategy for alleviating ASD symptoms[3,4], although its underlying mechanisms remain elusive. Here, we conducted morphological characterization of primary neuronal cultures from the prefrontal cortex (PFC) and hippocampus (HIP) of BTBR and B6 mice, evaluating brain-derived neurotrophic factor (BDNF) expression at day 7, 21, and 28 of *in vitro* neuronal growth (DIV). PFC and HIP neurons of BTBR exhibited cytoskeletal and synaptic alterations compared to B6 neuronal cultures. Additionally, an altered pattern of BDNF expression was observed, with HIP neurons displaying reduced BDNF levels, significantly varying at DIV 7 ($p<0.001$) and 21 ($p<0.05$), while PFC neurons consistently exhibited reduction compared to neurons from B6 mice at DIV 7, 21, and 28 ($p<0.05$; $p<0.001$; $p<0.01$, respectively). Notably, inducing a ketogenic state, a beneficial modulation of the neural network was reported, thus stabilizing compromised connections, and significantly increasing BDNF levels ($p<0.001$) in both HIP and PFC neuronal cultures of BTBR. These findings underscore the potential of ketosis as a therapeutic strategy for ASD through its effects on neural network stability and BDNF expression modulation.

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MODELING RARE NEURODEVELOPMENTAL DISORDERS: PITT HOPKINS SYNDROME AND *DE NOVO* PATHOGENETIC VARIANTS OF THE *TCF4* GENE

M. Orefice[1], B. Cimbalo[1], A. Vitobello[2], C. Gabellini[1], M. Ori[1]

[1]Department of Biology, University of Pisa, Italy; [2]University of Dijon, France
E-mail: michela.ori@unipi.it

TCF4 loss-of-function variants cause Pitt-Hopkins Syndrome (PTHS), which is characterized by intellectual disability, a wide mouth, distinctive facial features, and intermittent hyperventilation followed by apnea[1]. Pathogenic missense variants primarily cluster within the C-terminal bHLH domain, which is essential for dimerization and DNA binding. Through exome sequencing, we identified *de novo* missense variants in TCF4 in three individuals who did not display the typical hallmarks of PTHS. To study the *in vivo* cellular and molecular mechanisms responsible for the central nervous system and craniofacial alterations observed in PTHS, as well as the functional role of these *de novo* TCF4 missense variants, we are using Danio rerio (zebrafish) as an *in vivo* model system. We are examining the function of the *de novo* TCF4 variants during brain and craniofacial development by overexpressing them in zebrafish embryos. In parallel, using a gene-editing approach, we are creating a zebrafish model that carries the same *de novo* TCF4 mutation. Additionally, we have recently created a zebrafish TCF4 loss-of-function line using the CRISPR/Cas9 technique, providing a new *in vivo* model for PTHS. Comparing the transcriptomes of zebrafish embryos with the *de novo* TCF4 mutation (Met/Lys), those with the TCF4 loss-of-function mutation, and those with TCF4 overexpression will help uncover new aspects of TCF4's role in brain and craniofacial development. This approach may also identify new TCF4 druggable targets. Our models could further contribute to the development of gene therapy approaches by testing functional rescue strategies that modulate TCF4 gene dosage and stage-specific requirements to correct the PTHS phenotype. This approach also allows us to evaluate potential side effects when reintroducing TCF4 activity in a TCF4 loss-of-function model. The project is supported by the Telethon Foundation grant GMR22T1071 (Prof. Ori) for 2023-2025

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EPIGENETIC REGULATION OF ENHANCERS REGULATING AGING

C. Pagiatakis[1], S. Serio[1], A. Romanò[1], H. Manahil[1], R. Gornati[1], G. Bernardini[1], G. Condorelli, R. Papait[1]

[1]Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy
E-mail: christina.pagiatakis@uninsubria.it

Ageing is associated with a progressive decline of numerous physiological processes, which is accompanied by an increased risk of development of several diseases such as cancer, cardiovascular disease and neurodegenerative disorders[1]. The mechanisms that link aging with the onset of age-related diseases remain largely unknown[2]. Amongst these diseases, ageing is associated with a significant risk of heart failure whereby myocardial function is decreased. Myocardial ageing is associated with a metabolic switch in cardiomyocytes, with increased glycolysis and a decrease in oxidative phosphorylation, resulting in a severe energy deficit impacting cardiomyocyte contractility. In order to better understand the mechanisms underlying this metabolic switch, we investigated the role of epigenetic mechanisms in this context, by analysing three key histone modifications (H3K27ac, H3K27me3 and H3K4me1) and their impact on metabolic remodelling in the aged heart. We found an activation of a conserved set of enhancers that contribute to these transcriptional alterations: activation of the enhancer region of *Hk2* was found to be a key regulator of this metabolic switch in the aged heart, a process which is regulated by the transcriptional coactivator p300. We found that pharmacological inhibition of p300 blunts aging onset–associated upregulation of gene expression, leading to improvements in cardiac function. Our results show that epigenetic dysregulation of enhancers involved in the glycolysis pathway could potentially be targeted to treat heart failure in the elderly[3].

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COMPARATIVE TOXICITY OF COPPER-BASED NANOPARTICLES ON ZEBRAFISH EMBRYOS

R. Pecoraro[1], E.M. Scalisi[1], S. Indelicato[1], A. Scalisi[1], G. Coco[1], S. Cartelli[1], R. Lo Faro[1], F. Capparucci[2], R. Fiorenza[3], M.V. Brundo[1]

[1]Department of Biological, Geological and Environmental Sciences, University of Catania; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina; [3]Department of Chemical Sciences, University of Catania, Italy
E-mail: roberta.pecoraro@unict.it

Nanotoxicology aims to define toxic effects of engineered nanomaterials (ENMs) and nanoparticles (ENPs) on living organism and environment. Main ENPs investigated in toxicological studies are metal NPs (such as silver, gold, copper). Copper-based NPs (Cu-NPs) have been shown to catalyze a deal of reactions due to their environmentally good character, low cost and efficiency[1]. They have been used in the production of sensors, catalysts, antimicrobial agents[2,3]. ENPs have peculiar properties based on quantum size effects and large surface area to volume ratio compared to larger particles of the same material, but their use raised concerns since they can interact with living organisms. Cu-NPs with two oxidation states (CuO and Cu_2O NPs) have been tested on zebrafish embryos by performing the most frequent method for acute toxicity identification carried out in Europe[4]. Cu-NPs have been synthetized at Department of Chemical Sciences (University of Catania). Aqueous exposure was performed on zebrafish eggs coming from the Sicilian Centre for Experimental Fish Pathology (University of Messina) as described in the guideline[4]. Embryos were daily observed to verify four endpoints. The response to the biomarkers of exposure was evaluated by immunofluorescence protocol. No morphological alteration in the exposed larvae to the lowest concentrations of both NPs, but the highest concentration of Cu_2O NPs caused a major percentage of embryos' death (>20%) compared to the lower ones and controls. A slight immunopositivity has been found in the exposed larvae compared to controls.

Further studies could confirm these results, adding more details, also to hypothesize a possible scenario about chronic exposure.

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MOLECULAR ANALYSES TO ASSESS POLLUTION-DRIVEN EPIGENETIC ALTERATIONS IN SPERM UNDETECTABLE BY SPERMIOGRAM AND TRANSGENERATIONAL EFFECTS IN YOUNG MEN LIVING IN THE LAND OF FIRES

M. Piscopo[1], C. Marinaro[1], G. Lettieri[1], G. Ferrero[2], R. Festa[1], S. Tarallo[3], C. Moriello[1], F. Marra[1],T. Notari[4], A. Giarra[5], B. Pardini[3], A. Marano[5], M. Trifuoggi[5], A. Naccarati[3], L. Montano[6]

[1]Department of Biology, University of Naples Federico II, Naples; [2]Department of Clinical and Biological Sciences, University of Turin;[3]Italian Institute for Genomic Medicine, c/o IRCCS, Turin; [4]Check-Up PolyDiagnostic and Research Laboratory, Andrology Unit, Salerno; [5]Department of Chemical Sciences, University of Naples Federico II, Naples;[6]Andrology Unit and Service of Lifestyle Medicine in UroAndrology, (ASL) Salerno, Coordination Unit Eco-FoodFertility Project, S. Francesco di Assisi Hospital, Oliveto Citra, Salerno, Italy
E-mail: marina.piscopo@unina.it

The WHO 2023 report estimated 17.5% of couple infertility globally and a recent meta-analysis has recorded a global decrease in total sperm count of 62.3% from 1973 to 2018[1]. Spermatozoa are particularly sensitive to environmental contaminants and are considered early sentinels of general and reproductive health of organisms. We present, within the Ecofoodfertility project, a multilevel molecular profiling by small RNA sequencing and Sperm Nuclear Basic Protein (SNBP) analysis of male germ cells from healthy young subjects residing in low (*Valley of Sele,* VSL) and high-polluted (*Land of Fires,* LF) areas in Campania Region. Sperm motility and concentration were comparable in VSL and LF subjects but those from LF had a higher concentration of immature/immune cells, a lower protamine/histone ratio, a reduced ability of SNBP to protect DNA from oxidative damage, and an altered Cu/Zn ratio in sperm[2]. Sperm levels of 32 microRNAs involved in intraflagellar transport, oxidative stress response and spermatogenesis were different between VSL and LF[2]. We also found a different excess of Cu and Cr in the sperm of fathers and sons and a worsening of the SNBP properties and seminal antioxidant activity of sons[3]. This comprehensive analysis provides new insights into epigenetic alterations in sperm due to pollution not detectable by spermiogram.

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A NEW NANODELIVERY TOOL FOR TARGETED TEMOZOLOMIDE DELIVERY TO GLIOBLASTOMA CELLS

M. Prisco[1], J. Pisano[2], T. Barra[3], A. Falanga[4], R. Bellavita[3], S. Galdiero[3], S. Valiante[1]

[1]Department of Biology, University of Napoli Federico II, Napoli; [2]Ecosustainable Marine Biotechnology Department, Stazione Zoologica Anton Dohrn, Napoli; [3]Department of Pharmacy, School of Medicine, University of Naples Federico II, Napoli; [4]Department of Agricultural Science, University of Naples Federico II, Portici, Italy

Glioblastoma multiforme (GBM) is the most common primary brain cancer in adults and the most lethal subtype of astrocytoma[1], with a very poor prognosis. The treatment of choice of GBM is a combination of radiotherapy and chemotherapy with temozolomide (TMZ, an alkylating agent), increasing the 2-year survival rate from 10.4 to 26.5%[2]. TMZ has been proven to be highly cytotoxic to GBM cancer cells, but it suffers from limitations including short half-life time and fast degradation in the blood so that only a small fraction can cross the blood-brain barrier (BBB) and reach the target cells[3]: hence TMZ requires multiple high doses exposing the patient to severe side effects due to systemic toxicity. The current challenge is the use of nanocarriers or functionalization towards the more effective BBB crossing to improve drug action[4]; we tested a dynamic nanoplatform (DNP) based on self-assembling peptides whose surface will be functionalized to have a targeted and controlled release of TMZ. The internalization of the DNP is favored by the gH625 peptide and the targeting to GBM cells by a peptide binding the overexpressed EGFRvIII receptor. We have evaluated the effect of TMZ transported by nanocarrier on U118 MG and U87 MG cell lines at different concentrations and different times, compared with the free TMZ[5]. Our DNP could be a promising strategy for the delivery of TMZ, allowing an increased effect of the drug on cell viability at lower concentrations than the free TMZ.

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MOLECULAR PROCESSES UNDERLYING HYPERTROPHY IN ICEFISH AS PROMISING TOOLS TO TRANSLATE IN HUMANS MODELS.

N. Romano[1], E. Messina[2], M. Ceci[1], R. Gaetani[2], M. Mercola[3]

[1]Department of Ecology and Biology, University of Tuscia, Viterbo, Italy; [2]Department of Molecular Medicine, University Sapienza, Roma, Italy; [3]Department of Medicine and the Cardiovascular Institute, University of Stanford, Palo Alto, CA, USA
E-mail: nromano@unitus.it

Pathological hypertrophic remodelling is typically irreversible and shows a reduced number of fibres and slow repolarization of the left ventricle. Potential therapeutic targets for treating hypertrophy and heart failure are focused on the knowledge of the signal molecules involved[1] or the metabolism pathways in the process.[2]

Humans and fish share the mechanisms of some pathological pathways that lead to cardiac hypertrophy and miRNAs are particularly key regulators of cardiac size.[3,4] Antarctic icefish (*Chionodraco hamatus*, Channichthyidae-Notothenioids) are invaluable for answering some main biological questions. They are able to survive to -1.8 °C in seawater and are the only cold-blood vertebrates that lack haemoglobin; possess a vascularisation enhancement and a hypertrophic heart.[5] The heart can displace large systolic volumes at a low rate and relatively low pressure, with large ventricular fillings (high ventricular compliance). The analysis of RNA extracted from the ventricular portion of the icefish heart has revealed a downregulation of miRNAs (*i.e*. 1,133a,b) similar to the fish and mammalian models. These miRNAs seem to control cardiac hypertrophy.[6,7] Contemporary, the immunohistochemical analysis reveals the expression of embryonic genes such as GATA4, WT1, NFAT2[8] and also RACK1, which we have identified as a key molecule in hypertrophy-activated genes in fish. These preliminary results indicate a promising pipeline among fish and human-3D-organoid culture models. Identifying novel therapeutic targets using innovative approaches is critical to developing new therapies.

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POLYSTYRENE TRIGGERS CELL STRESS IN MUSSELS: ANALYSIS OF MORPHOFUNCTIONAL AND PHYSIOLOGICAL EFFECTS

R. Romano[1-2], L. Rosati[3], G. Napolitano[1-2], F. Ferrigno[1], G.F. Russo[1-2], C.M. Motta[3], R. Scudiero[3], P. Simoniello[1-2]

[1]Department of Sciences and Technology, University Parthenope, Napoli; [2]International PhD Programme UNESCO Chair "Environment, Resources and Sustainable Development" Napoli; [3]Department of Biology, University of Naples Federico II, Napoli, Italy
E-mail: rossana.romano001@studenti.uniparthenope.it

Plastic materials were designed to improve human living conditions but have now become a risk to the environment and the safety of the planet[1]. Plastic is present in all matrices (water, air and soil) representing a threatening pollution problem[2-3] due to the generation of micro and nano fragments. In this work, we determined the effects of polystyrene micro (MPs, 5 µm) and nanoparticles (NPs, 0.1 µm) on *Mytilus galloprovincialis*. Exposition times (1, 3, or 11 days) and concentrations were environmentally realistic[4]. Histological and immunohistochemical analyses were conducted to detect interferences with different organs, namely gills, responsible for feeding and breeding, digestive glands, and gonads, all considered biological indicators of environmental pollution. Results demonstrated that both MPs and NPs trigger marked cytotoxic effects, in particular on gills. The lamellar structure is altered, and the stress condition is evidenced by the increase in the number of goblet cells and by the alterations of parameters linked to oxidative stress condition, such as reactive oxygen species levels, oxidative damage to lipids, *in vitro* susceptibility to oxidants, and total antioxidant capacity. Together, this evidence indicates a severe impairment of gill function. In conclusion, plastic reduction in the oceans is an issue that can no longer be postponed; the first step seems to be a more conscious use of this material[5].

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THE EFFECT OF VARIATION IN FUNCTIONAL TRADE-OFFS ON CARNIVORES' CRANIAL MORPHOLOGICAL DIVERSITY

G. Sansalone[1,2,3], S. Wroe[2], G. Coates[2], M.R.G. Attard[2,4], C. Fruciano[3,5,6]

[1]University of Modena and Reggio Emilia, Department of Life Sciences, Modena, Italy; [2]Function, Evolution and Anatomy Research Lab, University of New England, Armidale, NSW, Australia; [3]Institute for Marine Biological Resources and Biotechnology (CNR-IRBIM), National Research Council. Messina, Italy; [4]British Antarctic Survey, Cambridge, UK; [5]National Biodiversity Future Center, Palermo, Italy; [6]Department of Biological, Geological and Environmental Sciences, University of Catania, Italy

Functional trade-offs are fundamentally affecting numerous anatomical structures and can influence morphological and functional evolution. Our study, employing morpho-functional landscape modelling on the skulls of 132 species of carnivores, focused on the macroevolutionary implications of the classical trade-off between bite force and bite speed. We discovered that the pace of evolution in form (morphology) is decoupled from the pace of evolution in function. Moreover, we found that speed can be optimised using a wider variety of theoretical morphologies compared to optimising for force, which can be obtained by a smaller set of morphologies. Remarkably, this pattern observed in theoretical morphologies is closely matched by the pattern observed in actual morphological variation in our sample. Notably, our study did not identify differences between placentals and marsupials, which are classically expected to have distinct patterns of morphological evolution due to their very different development. Instead, we show that these two groups have pursued similar evolutionary paths and show similar relationships between form and function. This lends support to the notion that adaptations driven by force are more likely to be biomechanically constrained (as they are produced by a smaller set of morphologies) rather than influenced by different developmental schemes. Given the pervasiveness of functional trade-offs in biological systems, these patterns may be widespread and could shed light on the uneven distribution of morphological and functional diversity across the tree of life.

THE INFLUENCE OF TiO_2 ON CHORIONALLANTOIC MEMBRANE AND EMBRYO DEVELOPMENT OF *GALLUS GALLUS DOMESTICUS*

E.M. Scalisi[1], R. Pecoraro[1], A. Scalisi[1], R. Fiorenza[2], A. Salvaggio[3], G. Impellizzeri[4], M.V. Brundo[1]

[1]Department of Biological, Geological and Environmental Science, University of Catania; [2]Department of Chemical Sciences University of Catania; [3]Exp. Zooprophylatic Institute of Sicily "A. Mirri", Palermo; [4]CNR-IMM, Catania, Italy.
E-mail: elenamaria.scalisi@unict.it

The vasculogenesis process occurs by *de novo* production of endothelial cells (ECs). The new network of ECs will serve as the basis for angiogenesis that allow the growth of new capillaries from it[1]. During the embryonic development, ECs modify their shape in response to angiogenic stimuli to maintain the vascular homeostasis. The chicken chorioallantoic membrane (CAM), has long been used to study the angiogenesis *in vivo*. It's a membrane highly vascularized that surrounds the developing chicken embryo. We have used the CAM of *G. g. domesticus*[2] in order to evaluate the influence of Titanium Dioxide (TiO_2) on CAM's angiogenesis and embryo development. The TiO_2 powder synthetized using the sol–gel technique were provide by CNR-IMM of Catania. The working solutions of 0.3 and 0.03 mg/ml TiO_2 have inoculated in the embryonated eggs, and a control group was also included. Each experimental group was sacrificed at 6th, 9th and 12th day after fertilization respectively. A window was made in the shell to visualize the underlying CAM vessels and embryo, than taken. A correct embryonic development for the exposed group of 0.3 mg/ml as well as the control has been showed, furthermore the vessel's growth was increasing from 6th to 12th days. Instead, the embryos exposed to 0.03 mg/ml showed alteration with visceral ectopia and in the CAM the number of vessel was lower. On the 12th day, in the 0.3 mg/ml group compared to the 0.03 mg/ml group a well vascular architecture of the CAM and an higher positivity for CD51/CD61 biomarker has been observed. The CD51/CD61 is a marker of immature and angiogenic blood vessels. Our results suggest that the TiO_2 has not inhibited the formation of blood vessels in a dose-dependent manner, on the contrary an lower concentration can improve its ability to be distributed in embryo and invade the CAM contributing in its toxicity.

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A COMPARATIVE ANALYSIS OF THE MICROBIOME OF GUT, LIVER, AND TESTIS IN AN OVIPAROUS LIZARD *PODARCIS SICULUS*

R. Sciarrillo[1], A. Falzarano[1], V. Gallicchio[2], R. Imperatore[1], E. Coccia[1], M. Paolucci[1]

[1]Department of Science and Technologies, University of Sannio, Benevento; [2]Vascular Surgery, Hospital of National Importance San Giuseppe Moscati, Avellino, Italy, Italy
E-mail: sciarrillo@unisannio.it

As high-throughput sequencing becomes more accessible, studies of the microbiome are expanding beyond humans and mammals to non-model organism systems[1]. The purpose of this study was to compare combinations of hypervariable regions (V2, V3, V4, V6-7, V8, and V9) to optimize the analysis of the microbiome gut, liver, and testis in an oviparous lizard *Podarcis siculus.* We collected male lizards around Naples without significant differences in body mass that were transported to the laboratory and dissected separately the whole gut, liver and testis, which were stored at -20°C until DNA extraction and sequencing. DNA extractions were performed using commercial Qiagen DNeasy® tissue kit, DNA sample quality was checked using the Qubit 2.0™ fluorimetric method. 16S rRNA genes were amplified by PCR using primers for the hypervariable regions according to the Ion 16S Metagenomics kit. The resulting libraries were amplified using the Ion Plus Fragment Library kit. The purified libraries were pooled at an equimolar concentration of 40 pM and prepared for sequencing with the Ion Torrent™ system, Ion S5™, using the Ion 520™ Chip kit. The patterns were then sequenced on Ion GeneStudio™ S5 and analyzed using Ion Reporter™ software, with the MicroSEQ™ ID 16S rRNA database and the Greengenes database[2]. The reads were grouped into three bins: at the family level: 90-97% match, at the genus level : >97-99% identity, and at the species level: ≥ 99%. Reads of several common bacterial families were not detected in some amplicons; particularly by V9, which did not detect Bacteroidaceae in the gut. At the phylum level, in the gut, Proteobacteria (20.22%),Firmicutes (38.03%), Bacteroidetes (37.58%), Actinobacteria (0.16%) were four identified dominant phyla; in the liver, Proteobacteria (74.57%), Firmicutes (5.02%), Bacteroidetes (2.15%), Actinobacteria (15.44%), in testis, Proteobacteria (57.37%), Firmicutes (10.05%), Bacteroidetes (6.19%), Actinobacteria (20.98%).

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METALLIC ENGINEERED ZERO VALENT NANOPARTICLES ALTER MUCOUS SECRETIONS OF EMBRYONIC EPIDERMIS IN THE POOL FROG *PELOPHYLAX LESSONAE*

D. Semeraro[1], G. Scillitani[1], M.V. Guglielmi[2], D. Mentino[3], M. Mastrodonato[1]

[1]Department of Biosciences, Biotechnology and Environment, University of Bari Aldo Moro, Bari; [2]Institute for Water Research (IRSA), National Research Council (CNR), Taranto; [3]Department of Precision and Regenerative Medicine and Ionian Area, University of Bari Aldo Moro, Bari, Italy
E-mail: daniela.semeraro@uniba.it

The assessment of potential risk of emerging xenobiotics requires the use of good experimental models, to evaluate the effects on both health and ecosystems. On the other hand, histopathological biomarkers are good tools for detecting and characterizing the effects of acute and chronic exposure to toxic and carcinogenic agents[1,2]. Many of them have been studied in the skin, since it is the main organ exposed to environmental stressors[3,4]. We assessed the effects of treatments on the secretion of the embryonic epidermis of the pool frog with engineered zero valent nanoparticles (NPs) of Fe, Co, and Ni at increasing concentration (LC_{50}, and $2xLC_{50}$). Embryos at Gosner's stage 21 were considered. Five cell types were observed: basal cells (BC), ciliated cells (CC), goblet cells (GC), small secretory cells (SSC) and ionocytes (IC). Secretory vesicles were observed in GCs, CCs and SSCs. In controls, standard histochemical techniques (PAS, Alcian Blue pH 2.5, Alcian Blue pH 1) revealed sialomucins in CCs, SSCs, and GCs, the latter showing also small amounts of sulphated glycans. Lectin-histo-

chemical techniques (WGA, PNA, SBA, DBA, SNA, MAA, ConA, LTA, AAA, UEA-I) indicated the presence of glycosaminylated, sialylated and fucosylated residuals in the same cell types, with small amount of galactosylated residuals in GCs and SSCs only. In treatments, a general increase of acidic, galactosylated and fucosylated residuals was observed, whereas glycosaminylated residuals decreased. It is concluded that NPs induce modifications of glycopatterns in epidermic mucins that could result in altered functionality of the protective role of mucus. Poll frog embryos confirm as a good model for ecotoxicological studies.

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AXIAL EXTENSION AND CELL DIFFERENTIATION IN DEVELOPING CEPHALOCHORDATES

E. Serafini[1], M. Bozzo[1], J. E. Carvalho[2], D. Bellitto[1], M. Pestarino[1], M. Schubert[2], S. Candiani[1]

[1]Department of Earth, Environment and Life Sciences, University of Genoa, Italy; [2]Laboratoire de Biologie du Développement de Villefranche sur-Mer (LBDV), Institut de la Mer de Villefranche, Sorbonne Université, CNRS, Villefranche-sur-Mer, France
E-mail: emanuele.serafini@edu.unige.it; matteo.bozzo@unige.it; candiani@unige.it

In vertebrates, the posterior spinal cord and somites are generated by bi-potent stem cells called axial progenitors that emerge at the end of gastrulation and are localized in the tail bud[1]. Cephalochordates (amphioxus) are considered the best model for studying the evolution of chordates and possess a vertebrate-like tail bud which is thought to produce the posterior tissues[2], contributing to axial extension of the embryo. While cell proliferation is known to drive neural cell type diversification[3], the role of proliferating cells to the development of the posterior amphioxus nervous system, including their origin, fate and dynamics, remains largely unexplored. Here, we present a combination of RNA-seq, hybridization chain reaction, and EdU labeling to investigate the genetic regulation and cellular dynamics involved in axial extension and cell differentiation in the posterior part of the amphioxus embryo.

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UNRAVELING THE MECHANISMS OF SYSTEMIC IRON REGULATION AND THEIR EFFECTS ON THE CENTRAL NERVOUS SYSTEM

M. Sette[1], C. Milanese[2], S. Barnhoorn[3], S. Pigliacelli[1], C. Payán-Gómez[4], P.G. Mastroberardino[1,2,3]

[1]Department of Life, Health, and Environmental Sciences, University of L'Aquila, Italy; [2]IFOM-ETS, the AIRC Institute of Molecular Oncology, Milan, Italy; [3]Department of Molecular Genetics, Erasmus MC, Rotterdam, the Netherlands; [4]Universidad Nacional de Colombia, Sede de La Paz, Colombia
E-mail: martina.sette@guest.univaq.it

Iron is critical for several physiological processes and its metabolism is finely tuned. The hormone hepcidin is the master systemic iron regulator. Hepcidin function, however, is complex and not limited to iron metabolism[1]. In fact, this molecule was originally described as an antimicrobic peptide. In this study, we performed in depth analyses to further characterize the biological role of hepcidin.

Transcriptome bioinformatic analysis on cells exposed to exogenous hepcidin unraveled a role for the transcription factor SOX2 and cell biology experiments confirmed that hepcidin stimulates SOX2 transcription in different, unrelated cell types. Consistently with SOX2 role in maintaining pluripotency and stemness[2], hepcidin delays differentiation. SOX2 has been also implicated in maintenance of genome fidelity[3] and accordingly we found that hepcidin favors nucleotide excision repair and DNA damage correction. SOX2 also participates to the regulation of innate immunity promoting recognition of cytosolic DNA and transcription of inflammatory genes[4]. We demonstrated that hepcidin-mediated SOX2 upregulation elicits a transcriptional inflammatory response upon administration of dsDNA, independently from the major cGAS-STING DNA sensing mechanism. We finally tested the hypothesis that peripheral production of hepcidin, as it happens in the gut during experimental colitis[5], may alter iron metabolism in the central nervous system. We show that treatment with dextran sodium sulfate increases iron levels in mouse brain, therefore establishing a connection between peripheral and central regulation of iron homeostasis. Collectively, these studies unravel novel biological mechanisms related to hepcidin with important implication in health and disease.

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UNVEILING PRIMATE BACULUM MORPHOLOGY: INSIGHTS FROM ADVANCED COMPARATIVE TECHNIQUES

F. Spani[1], M. Carosi[2], M. P. Morigi[3,4], M. Bettuzzi[3,4], M. Scalici[2,5], M. Colasanti[2]

[1]Faculty of Science and Technology for Sustainable Development and One Health, Università Campus Bio-Medico di Roma, Rome; [2]Department of Sciences, Roma Tre University, Rome; [3]Department of Physics and Astronomy "Augusto Righi", University of Bologna, Bologna; [4]National Institute for Nuclear Physics, Section of Bologna, Bologna; [5]National Biodiversity Future Center, Università di Palermo, Palermo, Italy
E-mail: f.spani@unicampus.it

The study delves into the intricate relationship between animal morphology and its significance in evolutionary biology, particularly focusing on the *baculum* (penile bone) in primates. Animal morphology encompasses various aspects such as size, shape, texture, and colour, which reflect both evolutionary history and current adaptation. The research applied advanced non-landmark-based methodologies, including micro-CT scanning, to analyse the shape of primate *bacula*, crucial in comprehending the evolutionary forces driving divergence. A dataset comprising specimens from various sources[1,2], including fresh cadaver samples and museum collections, was compiled for analysis, totalling 95 specimens from 55 species and 3 subspecies. The study employed stochastic character mapping on primate phylogeny to analyse the distribution of *baculum* diversified anatomical position inside the penis, qualitative morphological discrete types, and a continuous measure of morphological complexity. The results indicated a strong phylogenetic signal for *baculum* anatomical position, suggesting as ancestral condition the filling of the entire penis length in Strepsirrhini, evolving into distally placed *bacula* in Haplorrhini. Qualitative morphological analysis suggested stick-shaped *bacula* as ancestral, with transitions to pear-shaped and Y-shaped *bacula* observed. The α-shape technique[3] provided a continuous measure of *baculum* shape complexity, corroborating trends observed in discrete morphological types. Overall, the study contributes to our understanding of primate *baculum* morphology and evolutionary history, highlighting the importance of advanced morphometric techniques in elucidating evolutionary patterns of copulatory structures.

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INVESTIGATING THE PATHOGENETIC MECHANISMS OF THE SLC6A1-ASSOCIATED DISORDERS BY ZEBRAFISH KNOCKOUT MODELS

C. Tesoriero[1], E. Cannone[2], F. Greco[1], F. Ghirotto[1], A. Vettori[1]

[1]Department of Biotechnology, University of Verona, Verona; [2]Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy
E-mail: chiara.tesoriero@univr.it

Since 2015, SLC6A1 gene mutations have emerged as a common cause of childhood disorders characterized by a broad spectrum of clinical manifestations, including intellectual disabilities and epilepsy[1]. The SLC6A1 gene encodes for the GAT-1 transporter, which reuptakes the inhibitory neurotransmitter GABA in the central nervous system. In pathological conditions, the global protein function is compromised, and the balance of excitation/inhibition is impaired. Despite the progress, the pathogenetic mechanisms underlying the SLC6A1 disorders are still unclear. Moreover, not all patients respond positively to the therapeutic options available so far. Since zebrafish (*Danio rerio*) has become an important model for studying neurodevelopmental disorders[2], two independent knock-out (KO) zebrafish mutant lines - named slc6a1a^{av1} and slc6a1b^{av2} - were generated in our lab. In both cases, the out-of-frame modifications lead to a putative non-functional truncated protein. Following qPCR analysis, a significant decrease in mRNA levels was observed in both homozygous and double heterozygous (Slc6a1a$^{wt/av1}$Slc6a1b$^{wt/av2}$) mutant lines. Therefore, these lines were considered for further phenotypical and functional characterization. Although all the KO zebrafish animals are vital and fertile, the depletion of the Slc6a1b gene increased the mortality rate of the zebrafish embryos. Additionally, length measurement of KO mutant embryos revealed the presence of a generalized growth delay in Slc6a1a homozygous mutants, whereas, in Slc6a1b$^{av2/av2}$ and the double heterozygous mutants, statistically significant differences were found only up to 5 days post-fertilization. At the same time point, the behavioral analysis pointed out different alterations of the locomotor activity after light/dark stimuli exposition, thus suggesting the presence of seizure-like behaviors. On this basis, our zebrafish mutants may provide a promising model to study *in vivo* the neurological effects associated with alterations of the slc6a1 gene.

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A SHORT-TERM HIGH-FAT DIET ALTERS RAT TESTICULAR ACTIVITY AND BLOOD-TESTIS BARRIER INTEGRITY THROUGH THE SIRT1/NRF2/MAPKs SIGNALING PATHWAYS

M. Venditti[1], S. Falvo[2], A. Santillo[2], G. Chieffi Baccari[2], S. Minucci[1]

[1]Department of Experimental Medicine, University of Campania L. Vanvitelli, Napoli; [2]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania L. Vanvitelli, Caserta, Italy
E-mail: massimo.venditti@unicampania.it

It has been estimated that obesity and overweight affect more than 1.9 billion adults worldwide, rising from epidemic to pandemic states. Besides the well-known comorbidities associated with obesity, numerous papers demonstrated the positive correlation between growing BMI and sub-infertility[1]. Most of the studies focused on the impact of obesity on testicular activity using experimental models fed with a long-term high-fat diet (HFD)[2], while just a few papers used a different approach, with a short-term HFD (st-HFD)[3], that is correlated to an overweight condition. This study expands the knowledge on the impact of an st-HFD on rat testicular activity, to obtain parameters to be used to monitor the progression of infertility related to being overweight, even at the early stages before it progresses to obesity, which is considered the real "pathological state". Five weeks of HFD results in reduced steroidogenesis, increased apoptosis of spermatogenic cells, and altered spermatogenesis. Further, the compromission of the BTB integrity was evidenced, as revealed by the downregulation of structural proteins (N-Cadherin, ZO-1, occludin, connexin 43, and

VANGL2) and the phosphorylation of regulative kinases (Src and FAK). At the molecular level, the impairment of mitochondrial dynamics, and the dysregulation of the SIRT1/NRF2/MAPKs signaling pathways, were proved. Interestingly, no change in the levels of pro-inflammatory markers (TNFα, NF-kB, and IL-6) was observed. The combined data confirmed that overweight is a less severe state than obesity. Furthermore, understanding the molecular mechanisms behind the association between metabolic disorders and male fertility could improve the possibility of identifying novel targets to prevent and treat fertility disorders related to overweight/obesity.

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TOMATO AND OLIVE BIOACTIVE COMPOUNDS MODULATE ZEBRAFISH TAIL REGENERATION PROCESS

S. Zugaro[1], T. Silvestrini[2], M. Minacori[3], A. Cimini[1], M. Eufemi[1], M. Perugini[2], E. Benedetti[1]

[1]Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila; [2]Department of Bioscience and Agro-Food and Environmental Technology, University of Teramo, Teramo; [3]Department of Biochemical Science "A. Rossi Fanelli", University of Rome "La Sapienza", Sapienza, Rome, Italy
E-mail: zugarosilvana@gmail.com/silvana.zugaro@guest.univaq.it

Tomato and Olives Bioactive Compounds (TOBC) have antioxidant, anti-tumor, anti-inflammatory, and immune-boosting properties. The present study aims to evaluate the modulation of inflammatory response by TOBC in a zebrafish tail-amputated model *in vivo*. We performed caudal fin amputation on 3-day-post-fertilization larvae in zebrafish line Tg(mpx: GFP). First, we tested two TOBC concentrations, 1 mg/L, and 2 mg/L, and we monitored neutrophil recruitment using in time-lapse microscope. Through this first data set, we selected 6 h post-amputation (Hpa) as the first endpoint. At 6 Hpa we analyzed the expression levels of *tnf-α, il-1β*, and *irf8*. Both concentrations of TOBC decreased *tnf-α,* while only 1 mg/L increased the expression levels of *il-1β* and *irf8*, confirming that 1mg/L was the best concentration of TOBC to modulate the inflammatory process. This ability was also confirmed by neutrophil count. Subsequently, we tested the concentration of 1 mg/L of TOBC at 24 and 48 Hpa. Confocal microscopy still showed a sustained presence of neutrophils in treated larvae, paralleled by an increased expression of *tnf-α* at 24 Hpa. *tnf-a* is the principal interleukin produced by macrophages, as already demonstrated by Nguyen-Chi *et al*., (2017). This together with the sustained level of *irf8*, which promotes the development of macrophages, supports the rise of macrophage recruitment on the site of phlogosis. Anyway, in all endpoints selected TOBC treatment maintained high levels of *il-1β*. These preliminary data indicate a beneficial effect of TOBC on the inflammatory process during caudal fin regeneration.

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POSTERS

POLYSTYRENE EXPOSURE INDUCES INFLAMMATION IN THE DIGESTIVE GLAND OF BIVALVE MOLLUSC *MYTILUS GALLOPROVINCIALIS*

A. Acquisto[1-3], R. Romano[1-2], L. Rosati[3], M. Karam[1], F. Ferrigno[1], C.M. Motta[3], P. Simoniello[1]

[1]Department of Sciences and Technology, University Parthenope, Napoli; [2]International PhD Programme UNESCO Chair "Environment, Resources and Sustainable Development" Napoli; [3]Department of Biology, University of Naples Federico II, Napoli, Italy
E-mail: a.acquisto@studenti.unina.it

In the last decades, human activity has caused a serious problem of plastic pollution in coastal and marine environments worldwide. Plastic contamination comes in various shapes and sizes, ranging from large mega plastics down to tiny micro (MPs) and nanoplastics (NPs), potentially bioavailable to many organisms[1]. In this work, *Mytilus galloprovincialis* was used as a model organism to evaluate the cytotoxic effects of MPs and NPs on the digestive glands of mussels. *Mytilus galloprovincialis* has been exposed to 5 (MPs) or 0,1 µm (NPs), polystyrene beads for 1, 3 and 11 days, at environmental concentrations[2]. Digestive glands were fixed in Bouin's and processed for light microscopy[3]. Histological investigations showed severe alterations in connectives (collagen deposition), epithelial hyperplasia and regression of tubular tissue. The haemocyte infiltrates indicate the activation of the immune system and the onset of an inflammatory process. This aspect was confirmed by the increased number and altered distribution of goblet cells and by the presence of vacuoles in the epithelial tissue after exposure. In conclusion, MPs and NPs alter mussels' digestive and detoxification processes, negatively impacting on their health status.

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INVOLVEMENT OF THE SIRT/NRF2/MAPKS PATHWAYS IN THE TESTICULAR DYSFUNCTION INDUCED BY TYPE 1 DIABETES

M.R. Ambruosi[1,2], M.Z. Romano[2], A. Biasi[2], S. Minucci[2], M. Venditti[2]

[1]Department of Biology, University of Naples Federico II, Napoli; [2]Department of Experimental Medicine, University of Campania L. Vanvitelli, Napoli, Italy
E-mail: ma.ambruosi@studenti.unina.it

Nowadays, growing evidence highlights the correlation between metabolic disorders, including type 1 diabetes (T1D) and male infertility[1], *via* impaired steroidogenesis and spermatogenesis, with a consequent reduction of sperm quality. Earlier studies have already shown that TD1 induced oxidative stress and, subsequently, increased apoptosis and alterations in testicular cells[2]. In this study, a T1D rat model, obtained by treatment with streptozotocin, was used to confirm previous reports and to analyze additional

parameters of testicular activity. Data showed that T1D altered both testicular somatic and germ cells, as evidenced by enhanced apoptosis, impaired steroidogenesis, Leydig cell maturity, and compromised spermatogenesis. Furthermore, for a broader picture of the underlying molecular pathways of the T1D effects on testicular function, the contribution of some key factors, notoriously involved in the oxidative stress, namely SIRT1, NRF2, and the MAPKs p38/JNK, was analyzed. Results showed a reduction in SIRT1 protein level in T1D rat testis as compared to the control and, interestingly, the reduced nuclear translocation of NRF2 was evidenced. Considering that NRF2 acts as a transcription factor that activates the expression of genes encoding for antioxidant enzymes, comprising HO-1 and SOD, the T1D-induced oxidative stress may be due to the deregulation of the SIRT1/NRF2 pathway. Finally, data showed that T1D induced the phosphorylation, and thus the activation, of testicular p38, and JNK, which were positively associated with the oxidative stress status, as well as the enhanced apoptosis observed in the testis of T1D rats. The combined data encourages further studies not only to better understand the molecular mechanisms, but also on the development of strategies to be used in preventing/mitigating the effects of T1D, and other metabolic disorders, on human male fertility.

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THE ROLE OF BRAIN-DERIVED NEUROTROPHIC FACTOR IN A BTBR MOUSE MODEL OF AUTISM

F. Ammannito[1], V. Castelli[1], M. Scenna[1], E. Maceroni[1], D. Marinidis[1], M. d'Angelo[1], A. Cimini[1,2]

[1]Department of Life, Health and Environmental Science, University of L'Aquila, Italy; [2]Sbarro Institute for Cancer Research and Molecular Medicine, Department of Biology, Temple University, Philadelphia, USA
E-mail: fabrizio.ammannito@guest.univaq.it

Autism and autism spectrum disorder (ASD) are neurodevelopmental disorders characterized by deficits in social communication and the presence of restricted and repetitive patterns of behavior[1]. The BTBR T+ tf/J (BTBR) mouse model is widely recognized in autism research, exhibiting behavioural deficits that mimic the core deficits of autism[2]. Brain-Derived Neurotrophic Factor (BDNF), a crucial player in cerebral and behavioural plasticity, and in neurodevelopment[3]. Cerebral areas mainly involved in ASD are the hippocampus, prefrontal cortex, and amygdala, which are linked to behavioural patterns and impairments[4]. Based on this evidence, our work aimed to evaluate the potential effects of BDNF in this disorder using BTBR mice. Specifically, the neurotrophin was administered intranasally, a minimally invasive route of administration. To assess the 'core symptoms' of ASD, behavioural tests were performed such as the Marble burying[5], the Hole board test, and the social interaction test[6,7]. Subsequently, immunohistological studies were performed on the brain area involved. Interestingly, the results obtained showed a significant improvement in core symptoms in BTBR mice treated early with BDNF, confirmed by morphological analysis. In addition, we noted significant differences between the sexes.

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EVALUATING THE IMPACTS OF NANOPLASTICS AND MICROPLASTICS: A *CAENORHABDITIS ELEGANS* PERSPECTIVE

E. Schifano, S. Augello, S. Fortuna, L. Dini, D. Uccelletti

Department of Biology and Biotechnologies Charles Darwin, the Sapienza University of Rome, Rome, Italy
E-mail: simone.augello@uniroma1.it.

Nanoplastics (NPs) and microplastics (MPs) have emerged as significant environmental contaminants, deriving from the fragmentation of larger plastic objects or direct production as microbeads for personal care products and synthetic materials. Their ubiquitous presence in aquatic and terrestrial ecosystems raises concerns regarding their uptake and ingestion by living organisms, with poorly understood consequences for both human health and ecosystem integrity. These particles induce oxidative stress, inflammation, altered metabolism and cellular damage, disrupting tissue and organismic homeostasis in various animal species and human cells. The ability of NPs/MPs to carry contaminants, toxic chemicals, pesticides, and bioactive compounds, such as endocrine disrupting chemicals, present an additional risk to ecosystems. In aquatic ecosystems, microplastics play an important role in facilitating the transport of alien species, a phenomenon known as 'biological rafting', that can compromise ecosystem functioning[1]. However, the full impact of these plastic particles on living organisms is not completely understood. This study exploits the multifaceted effects of NPs and MPs on the model organism *Caenorhabditis elegans*. Renowned for its important role in developmental biology, toxicology and molecular biology research, *C. elegans*' well-characterised biology, short lifespan and genetic manipulability make it a valuable tool for studying the effects of environmental pollutants[2]. We investigated the responses of nematodes to different concentrations of MPs and NPs and found a dose-dependent reduction in *C. elegans* lifespan and fertility following exposure to these plastic particles, highlighting the potential threat they pose at the organismal level. We also investigated the underlying molecular mechanisms involved in these responses and identified alterations in pathways involved in oxidative stress responses and immunity, such as insulin/insulin-like growth factor signalling.

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

N. Baranzini[1,2], G. Marcolli[1], C. Bon[1], L. Pulze[1,2], D. Rotondo[3], D. Gualandris[3], A. Calisi[3], F. Dondero[3], A. Grimaldi[1,2]

[1]Department of Biotechnology and Life Sciences, University of Insubria, Varese; [2]ILFARM S.r.l., Varese; [3]Department of Science and Technological Innovation, University of Eastern Piedmont, Alessandria, Italy
E-mail: nicolo.baranzini@uninsubria.it

Per- and poly-fluoroalkyl substances (PFAS) encompass a broad array of synthetically produced fluorinated compounds extensively used in many industrial fields, from the manufacturing of plastics items to the synthesis of firefighting aqueous film forming foams, food packaging, non-stick cookware, and cosmetics[1]. Although PFAS enhanced people everyday life, as already observed for other chemicals, their massive use and the consequent abundant release improved the risk of bioaccumulation. Indeed, PFAS not only were identified in many environmental matrices, but also can accumulate inside living organisms. In this context, despite various studies were performed, little data have been collected in particular on a new emerging PFAS generation, introduced on the global market in substitution of oldest compounds, whose dangerous potential has been already demonstrated[2,3]. Based on this evidence, here we propose the leech *Hirudo verbana* to investigate the effects of different PFAS (GenX, PFMoBa, PFOA and PFMOPrA) on freshwater organisms, testing two concentrations (0.6 and 229 μM). Morphological, immunohistochemical and molecular assays revealed a diverse modulation of both cellular and molecular innate immune response, in relation to the fluorinated chemical examined. In addition, in order to evaluate a possible activation of the oxidative metabolism, the levels of expression of the superoxide dismutase (SOD) and glutathione-s-transferase (GST) have been analyzed. As already observed for plastics[4], not only the medicinal leech represents a valid freshwater model to assess the impact of pollutants, but also this work allows to deepen the current knowledge on PFAS the potential effects.

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DEVELOPMENTAL DELAYS INDUCED BY PARTICULATE EXPOSURE FROM URBAN AND RURAL SOURCES: PRELIMINARY RESULTS

M. Battistoni[1], R. Vecchi[2], G. Valli[2], S. Valentini[2], C. Colombi[3], B. Biffi[3], E. Cuccia[3], F. Di Renzo[1], R. Bacchetta[1], E. Menegola[1]

[1]Department of Environmental Science and Policy Università degli Studi di Milano; [2]Department of Physics Università degli Studi di Milano; [3]ARPA Lombardia, Italy
E-mail: maria.battistoni@unimi.it

The effects on development of PM_{10} collected from two different locations (urban, Milano city; rural, Bertonico, Lodi) were tested on *X. laevis* samples (R-FETAX protocol). At both sites, PM_{10} daily samples were collected on PTFE filters. Natural fertilized embryos were exposed during the whole test period (from mid-blastula to tadpole) to extracts obtained by brushing and washing filters in deionized water, diluted 1:10 in FETAX solution. Embryotoxicity tests were also performed on control PTFE water extracts. Samples were monitored for lethal effects during the full six-day test period. At the end of the test, tadpoles were observed under a dissecting microscope to evaluate any morphological alteration. The developmental degree to evaluate old- and young-for-age phenotypes (YFA-OFA) was determined according to the previously described developmental scoring system. Tadpole length was measured in order to evaluate small- and large-for-age phenotypes (SFA- LFA). Data were modelled using PROAST software package (Bench-Mark approach) to describe effects considering chemical characterization in terms of mass concentration, elements, ions, and carbonaceous components. Preliminary results show that neither lethal nor malformative effects were recorded after the exposure to the control and test extracts. YFA phenotypes were dose-dependent relatively to PM10 mass, S, NO_3^-, NH_4^+. Effects were more pronounced in tadpoles exposed to extracts from the rural site, suggesting a role of rural environmental components (proximity to farms and agriculture activities, mixture effects?) that need further investigations.

TYPE 1 DIABETES IMPAIRS RAT SPERMATOGENESIS INDUCING OXIDATIVE STRESS

A. Biasi, M. Venditti, M.Z. Romano, S. Minucci

Department of Experimental Medicine, University of Campania L. Vanvitelli, Napoli, Italy
E-mail: alessandra.biasi@unicampania.it

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

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THE POTENTIAL EMBRYOTOXICITY OF THE PESTICIDE THIRAM IN SEA URCHIN *ARBACIA LIXULA*

B. Billè[1], M.C. Eliso [2], G. Genovese [1], D. Iannazzo [2], T. Cappello[1], M. Morabito[1], M. Maisano[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina; [2]Department of Investigative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples; [3]Department of Engineering, University of Messina, Italy
E-mail: barbara.bille@studenti.unime.it

Dithiocarbamates (DTCs) are a group of chemical compounds mainly used in agriculture as insecticides, herbicides and fungicides to protect fruits and vegetables. Due to their wide use, these compounds easily reach the aquatic environment and become potential pollutants for biota. Among the DTCs, thiram is most abundant in the soil and aquatic environments[1]. In recent years, this pesticide has gained particular attention because it is listed as a category 1 potential endocrine disruptor by the European Union[2], and it acts as a pro-oxidant inducing the formation of reactive oxygen species[3]. For this reason, several studies focused the attention on its mechanism of toxicity in order to assess and predict the toxicological effects on non-target organisms. In light of this, within the SAMOTHRACE project, the effects of thiram were assessed on the embryo development of sea urchin *Arbacia lixula* (Linnaeus, 1758) exposed for 72 h post fertilization (hpf) to four different environmental concentrations of this pesticide (0,24 µg/L - 1,2 µg/L – 6 µg/L - 30 µg/L). Preliminary results showed developmental impairment already at the lowest thiram dose tested, highlighting the importance to deeply investigate on the mechanism of toxicity exerted by this pesticide, and on the potential use of products obtained from algal biomass wastes to mitigate the toxic effects of thiram on aquatic organisms.

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BETA-CARYOPHYLLENE AS A THERAPEUTIC AGENT IN 2D AND 3D *IN VITRO* MODELS OF HEPATIC STEATOSIS: POTENTIAL INVOLVEMENT OF EXTRACELLULAR VESICLES

S. Boumya[1], R. Scandiffio[1], F. Barbero[2], E. Cottone[1], S. Bonzano[1], C. Zungrone[1], B. Mognetti[1], S. De Marchis[1], M.E. Maffei[1], I. Fenoglio[2], P. Bovolin[1]

[1]Department of Life Sciences and Systems Biology and [2]Department of Chemistry, University of Turin, Italy
E-mail: sara.boumya@unito.it

Non-alcoholic fatty liver disease (NAFLD) is a prevalent liver disorder characterized by accumulation of triglycerides within hepatocytes, leading to oxidative stress and inflammation. Despite its high prevalence, there is currently no approved pharmacological therapy specifically targeting NAFLD.[1] In this study, we explored the therapeutic potential of (E)-β- caryophyllene (BCP), a natural product derived from several plants. To investigate the effects of BCP, we developed 2D and 3D *in vitro* models of liver steatosis using HepG2 human hepatoma cells. Steatosis was induced by incubating both spheroids and monolayer cells with a pathophysiological concentration of a free fatty acid mixture (FFAm) in serum-free MEM for 24 h. Through a fluorescent-based lipid quantification assay and gas chromatography-mass spectrometry (GC-MS) analysis, we demonstrated that BCP effectively decreases lipid accumulation in steatotic conditions. Notably, BCP altered the typical steatotic lipid profile by specifically reducing saturated fatty acids. Pharmacological studies revealed that BCP action is mediated by multiple receptors: CB2 cannabinoid receptor, peroxisome proliferator-activated receptor α (PPARα), and γ (PPARγ). Moreover, we observed that BCP is internalized by HepG2 cells, with a peak uptake occurring at 2 h, suggesting direct interaction of BCP with intracellular receptors. Since stressed hepatocytes can modify the released Extracellular Vesicles (EVs), which play a crucial role in intercellular communication, we isolated HepG2-derived EVs from spheroid conditioned media using high-speed centrifugation. Interestingly, nanoparticle tracking analysis (NTA) showed that BCP treatment significantly reduced the size and the number of released EVs in steatotic conditions. In conclusion, this study highlights BCP as a promising candidate for NAFLD therapy, implicating its multifaceted effects on lipid metabolism and intercellular communication *via* EVs.

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IDENTIFICATION OF miRNAs RELEASED BY HUMAN CUMULUS CELLS AND THEIR ROLE IN THE ACQUISITION OF THE OOCYTE DEVELOPMENTAL COMPETENCE

G. Brazzale[1], G. Fiorentino[1], V. Merico[1], M. Zanoni[1], F. Innocenti[2], M. Taggi[2], A. Vaiarelli[2], F.M. Ubaldi[2], L. Rienzi[2,3], D. Cimadomo[2], S. Garagna[1], M. Zuccotti[1]

[1] Department of Biology and Biotechnology Lazzaro Spallanzani, University of Pavia; [2]IVIRMA Global Research Alliance, Genera, Clinica Valle Giulia, Rome; [3]Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy
E-mail: giulia.brazzale01@universitadipavia.it

The communication between the oocyte and cumulus cells (CCs) within the cumulus-oocyte-complex (COC) significantly influences gamete quality and developmental competence. Previously, we showed that germinal vesicle (GV) mouse oocytes, when matured to metaphase II (MII) on a feeder layer of CCs (FL-CCs) derived from incompetent oocytes, interrupt their development at the two-cell stage; on the contrary, the blastocyst stage is reached when cultured on FL-CCs from competent oocytes[1]. Also, we found that FL-CCs release into the medium extracellular vesicles (EVs) containing 74 differentially expressed miRNAs[2]. Here, we present the translation of this culture platform from the mouse to the human model to identify EVs-enclosed miRNAs released by human FL-CCs (hFL-CCs). hFL-CCs were produced from patient-specific COCs classified as competent or incompetent when >30% or <30% of their oocytes reached blastocyst, respectively. EVs were first isolated from the medium of the two experimental conditions, the miRNAs extracted and NGS analysis performed with the Illumina Genome Analyzer and the NextSeq 500/550 High

Output v2.5 kit. This analysis identified 25 miRNAs differentially expressed (19 up- and 6 down-regulated). Then, NGS data was further confirmed using *q*-RT-PCR testing the expression level of 4 of these 25 miRNAs. Importantly, these miRNAs are known for their role in folliculogenesis, suggesting them as promising candidates for decoding the somatic contribution to the acquisition of the oocyte developmental competence. Our next aim is to produce FLs using CCs derived only from individual COCs. This would allow for a direct correlation between the EVs miRNA profile and the oocyte's ability to complete pre-implantation development.

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HOW DID TRANSPOSABLE ELEMENTS SHAPE *XENOPUS LAEVIS* SUBGENOMES? GENOME LANDSCAPE AND TRANSCRIPTIONAL CONTRIBUTION DURING DEVELOPMENT

E. Carotti[1], E. Tittarelli[1], F. Carducci[1], M. Barucca[1], A. Canapa[1], M.A. Biscotti[1]

[1]Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy
E-mail: e.carotti@univpm.it

Amphibians, especially *Xenopus* species, are valuable models in various fields such as developmental and cell biology, infectious disease, ecology, pharmacology, environmental health, and biological diversity. Although polyploidy is rare in vertebrates, the African clawed frog *Xenopus laevis* presents a tetraploid karyotype, derived by two diploid progenitor species around 17-18 Mya leading to L (long chromosome) and S (short chromosome) subgenomes. A detailed exploration of *Xenopus* repetitive composition revealed a role of distinct families of transposable elements (TEs) located at pericentromeric and telomeric regions in the stability of its chromosomes. In this regard we investigated the fraction of these mobile elements in *X. laevis* genome and evaluated the quantitative impact of each TE class. Moreover, we explored the transcriptional activity of TEs in six developmental stages as well as of genes encoding proteins involved in their control considering both subgenomes. Comparisons at genome and transcriptome levels allowed to increase knowledge on the impact of the repetitive fraction in the African clawed frog.

THE EFFECTS OF AMYLOID-β AND MAGNETITE NANOPARTICLES ON CULTURED HUMAN ASTROCYTES

L. Carpinelli, V. D'Ezio, R. Proietti, A. Sperati, A. Presaghi, V. Cianfanelli, M. Colasanti, T. Persichini

Department of Sciences, University Roma Tre, Rome, Italy
E-mail: ludovica.carpinelli@uniroma3.it

Air pollution has been identified as a potential contributing factor in the increased prevalence of neurodegenerative diseases such as Alzheimer's and Parkinson's. Recent studies have indicated that in urban areas with high levels of traffic and industrialisation (*e.g.*, Mexico City), the incidence of dementia in younger individuals has increased[1]. Among the traffic-related air pollutants (TRAPs), magnetite nanoparticles (MNPs) can be inhaled and directly reach the brain, where they can promote the formation of reactive oxygen species (ROS) and induce oxidative stress, a condition often associated with Alzheimer's disease (AD). In the brain, astrocytes can efficiently counteract oxidative stress through the activation of an antioxidant response[2], thereby exerting protective or detrimental effects on neurons. In order to investigate the biological impact of MNPs in the context of neurodegenerative diseases, we employed normal human astrocytes cultured in the presence or absence of amyloid (Aβ). Our findings indicate that MNPs treatment resulted in a significant and dose-dependent reduction in cell viability of astrocytes. Moreover, oligomeric and fibrillar forms of Aβ1-42 peptide exert distinct effects on astrocyte viability. The oligomeric form of Aβ was found to reduce astrocyte viability in a dose-dependent manner, whereas the fibrillar form of Aβ reduced it by 50% at all concentrations tested. Furthermore, the co-treatment of MNPs with the oligomeric form of Aβ induced a further significant reduction in astrocyte viability when compared to Aβ alone. Finally, the antioxidant response was also examined. The results indicate that astrocytes exposed to MNPs+Aβ can up-regulate the expression of System Xc^- and CD44 proteins by two-fold. The present findings collectively suggest that MNPs pollution can intensify the toxic impact of Aβ on astrocytes and neuronal cells, affecting their viability and redox state.

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COMPARATIVE ANALYSIS OF *SEPIA OFFICINALIS* EMBRYO DEVELOPMENT: ASSESSING THE INFLUENCE OF FARMED AND WILD ENVIRONMENTS IN THE ADRIATIC SEA

G. Chemello[1], E. Trotta[1], V. Faraoni[1], C. Fini[1], V. Notarstefano[1], O. Carnevali[1], G. Gioacchini[1]

[1]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona, Italy
E-mail: g.chemello@univpm.it

In the Adriatic Sea, exploitation of *Sepia officinalis* has approached sustainable limits. This species relies on embryo and juvenile survival for population stability since adults die at the end of the reproductive season. Embryos are highly susceptible to environmental factors and fishing techniques[1] These last affect the fate of eggs laid in the cages during the operation of net cleaning. To prevent stock collapse, exploring alternative farming methods is crucial. The present study aimed to evaluate the possibility to maintain cuttlefish embryos under farmed condition during their development before their release in the aquatic environment. Embryos developmental rate and health status were compared between embryos kept under farmed and wild condition until the hatching. Cuttlefish eggs were collected from the costal waters of the central Adriatic Sea and divided into three groups. The first group was immediately sampled, embryos were classified and histologically examined, biometric parameters were registered, and the presence of microplastics in embryos was investigated. The second and third groups were maintained under wild and farmed conditions respectively until hatching. Embryos were sampled after 14 days (period of time that led to the hatching of embryos) to monitor their development and health status through histological analysis and molecular approach to evaluate the expression of genes involved in stress response. Microplastics have been detected in embryos of *Sepia officinalis*, with no evident differences

observed between farmed and wild condition and between the beginning and the end of the trial. The maintenance of embryos under farmed conditions did not compromise embryonic development or hatching percentage; rather, farmed conditions appeared to favor the health status compared to wild embryos as suggested by the gene expression pattern observed related to stress response and oxygen consumption. Further research is needed to explore how microplastics may affect hatchling survival.

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A POTION OF ETERNAL YOUTH: THE VOGHERA SWEET PEPPER

F. De Luca[1], F. Gola[1], A. Azzalin[1], C. Casali[1], L. Gaiaschi[1], G. Milanesi[1], R. Vicini[2], P. Rossi[1], M. G. Bottone[1]

[1]Department of Biology and Biotechnology, University of Pavia; [2]Bio Basic Europe S.R.L.; Pavia, Italy
E-mail: fabrizio.deluca@unipv.it

Aging is a biological process characterized by the unstoppable decline of physiological functions that affect the entire organism[1]. Recently, increased attention has been directed towards various natural compounds such as carotenoids, tocopherols, flavonoids, and vitamins (such as A, C, D, and E), owing to their potential beneficial biological effects in preventing or treating age-related issues. In this context, sweet peppers (*Capsicum annuum*) are rich in bioactive compounds, including polyphenols, β-carotene, and vitamin C, the amounts of which vary depending on the type of pepper and its degree of maturity[2,3]. Voghera pepper (VP) is a native Lombardy variety of vegetable cultivated in Italy, between the provinces of Alessandria and Pavia, by a limited number of producers as a niche product, known for its high content of vitamin C and carotenoids and its literature-reported role in protecting against oxidative damage[4], suggesting the potential importance of these pepper varieties as anti-aging vegetables in the diet. Therefore, we evaluated the anti-aging effect of VP extract on both *in vitro* and *in vivo* models, using young and old (i) Normal Human Diploid Fibroblasts (NHDF) and (ii) C57BL/6 mice. Our results clearly demonstrate a protective effect of Voghera pepper on aged NHDF cells, preserving youthful ultrastructural and morphological features in old treated cells. Moreover, this pepper extract modulates the expression levels of specific proliferation (PCNA) and senescence (p16 and p53) markers. Additionally, our pepper extract exhibited a potential *in vivo* effect, preserving the body weight of older mice and regulating water and food intake in this animal model of aging. Taken together, these findings support the idea that Voghera pepper supplementation could be a promising natural strategy to prevent physiological aging.

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IMPACT OF CADMIUM ON *DANIO RERIO'S* SENSE OF SMELL

P. Denre[1], C. Fogliano[1], G. Miccoli[1], P. Simoniello[2], R. Romano[2*], C. Calas[1], R. Sasso[1], L. Rosati[1], B. Avallone[1], R. Carotenuto[1], C.M. Motta[1]

[1]Department of Biology, University of Naples Federico II, Napoli; [2]Department of Science and Technology, University Parthenope, Napoli, Italy
**International PhD Programme, UNESCO Chair "Environment, Resources and Sustainable Development*
E-mail: p.denre@studenti.unina.it

Anthropic activities have significantly increased cadmium availability, which has become a significant stressor in aquatic ecosystems[1]. Present at high concentrations in a variety of water bodies, it is bioaccumulated and biomagnified in the food chain. Multiple toxic effects have been reported on different organs and tissues[2] while information on interferences on sensory systems is relatively scanty[3] though the senses are essential for survival. This study investigated the effects of a short exposure (96 h) to 50 μM cadmium chloride on the olfactory organ of adult zebrafish. Cyto-anatomy of lamellae was assessed by light microscopy and immunocytochemistry techniques. At the same time, functionality was tested by behavioral odour recognition test[4] that was carried out in a labyrinth glass tank in which food was added at the end of a mandatory path. Investigations were extended to animals exposed to cadmium but let recover for six days in clean water. Results demonstrated that the lamellar epithelium is profoundly affected by the metal: the lamina propria becomes oedematous and the epithelium thickens, showing increased numbers of crypt and rodlet cells. Apoptotic cells became numerous, and the cilia of non-sensory cells appeared collapsed. The animals showed reduced performance in behavioral tests being unable to reach the food in five successive trials. After recovery, lamellar damage was reduced and performance in behavioral tests improved. Our data demonstrate that cadmium impairs the sense of smell, and that recovery can occur if the insult ends. Ongoing studies will explore the role played in the observed response by visual information gathered in the labyrinth and memory. Data highlight the significant impact that cadmium pollution can exert on so far neglected aspects of animal survival.

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IMPACT OF ANXIOLYTIC TREATMENT ON NEURAL DEVELOPMENT: FIRST EVIDENCE ON *XENOPUS LAEVIS* EMBRYOS

S. Di Marino, C. Fogliano, B. Avallone, C.M. Motta, R. Carotenuto

[1]Department of Biology, University of Naples Federico II, Napoli, Italy
E-mail: sim.dimarino@studenti.unina.it; chiara.fogliano@unina.it

Anxiolytics, such as benzodiazepine, are prescribed to pregnant women for acute and ongoing treatment. These medications can cross the placental barrier, potentially affecting fetal and brain

development. Exposure to these drugs during pregnancy might disrupt neurotransmitter systems in the brain, leading to enduring effects on the offspring's neurodevelopment[1]. Delorazepam is a benzodiazepine which binds to the GABA-A receptor, enhancing the activity of the GABA neurotransmitter, which, in early embryogenesis, acts as a developmental signal involved in regulating cell migration, axonal outgrowth, and synaptogenesis, and as a coordinating mediator of intercellular communication in the fetus[2]. Higher levels of GABA, resulting from prenatal exposure to anxiolytics, could potentially lead to changes in brain structure and function. In this preliminary study, a modified version of FETAX[3] test was employed by exposing *Xenopus laevis* embryos, from stage 4-8 cells to three increasing doses of delorazepam: the aim was to understand if and how treatment with these common drugs can reshape neural growth during early embryonic development. Using optical and electronic microscopy techniques, morphometric tests and gene expression of the main markers of brain function and development (BDNF, GAPDH, otx2, sox3) important variations were highlighted starting from the minimum dose administered. Considering that major signaling pathways in brain patterning are highly conserved between frogs and mammals[4], the results raise alarm bells on the possible risk of neurodevelopment problems following exposure to anxiolytics in the early stages of embryonic development.

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R-FETAX: TERATOGENIC AND NEUROBEHAVIORAL EFFECTS OF CAFFEINE

F. Di Renzo[1], F. Metruccio[2], M. Battistoni[1], R. Bacchetta[1], E. Menegola[1]

[1]Department of Environmental Science and Policy Università degli Studi di Milano; [2]ICPS, ASST Fatebenefratelli Sacco, Milan, Italy
E-mail: francesca.direnzo@unimi.it

Caffeine (CAF) consumption in pregnancy is a common condition with safety under debate because it is suspected to induce side effects both for mother and for conceptuses. Aim of the present work was the evaluation of morphological and neurobehavioral effects of CAF exposure (0.005-1 mg/mL; 0.005 mg/mL approximatively corresponds to blood CAF levels reached after two Italian coffees or one American coffee or one energy drink) using the amphibian *X. laevis* model (R-FETAX protocol). Samples, obtained by natural mating, were exposed during different specific developmental windows (organogenetic period, sensitive for morphological abnormalities; neurodevelopmental windows, sensitive to behavioral alterations). Extra groups, exposed during the whole test (classical FETAX exposure) or for 4 h before the end of the test (acute exposure) were performed. Samples were monitored for lethal effects during the full six-day test period. At the end of the test, external morphology and developmental degree were evaluated. The neurobehavioral swimming test was applied only on not-malformed tadpoles. Effects were modelled using PROAST software: dose-relation curves were obtained and benchmark dose level derived, setting response at levels used as point of departure for risk assessment. Overall data showed dose- and stage- specific effects of CAF exposure.

THE *IN VIVO* IMPACT OF SMPX MUTATIONS DURING DEVELOPMENT AND DISEASE

A. Diana[1], A. Ghilardi[1], R. Bacchetta[2], N. Santo[3], M. Ascagni[3], L. Prosperi[1], L. Del Giacco[1]

[1]Department of Biosciences, Università degli Studi di Milano, Milano; [2]Department of Environmental Science and Policy, Università degli Studi di Milano; [3]Unitech NOLIMITS, Università degli Studi di Milano, Italy
E-mail: alberto.diana@unimi.it

The last decade has witnessed the identification of several families affected by hereditary non-syndromic hearing loss (NSHL) caused by mutations in the *SMPX* gene and the loss of function has been suggested as the underlying mechanism[1]. In the attempt to confirm this hypothesis we generated an Smpx-deficient zebrafish model, pointing out its crucial role in proper inner ear development. Indeed, a marked decrease in the number of kinocilia together with structural alterations of the stereocilia and the kinocilium itself in the hair cells of the inner ear were observed. We also report the impairment of the mechanotransduction by the hair cells, making *SMPX* a potential key player in the construction of the machinery necessary for sound detection. This wealth of data provides the first possible explanation for hearing loss in *SMPX*-mutated patients. Additionally, we observed a clear muscular phenotype consisting of the defective organization and functioning of muscle fibers, in which *smpx* is also expressed[2], strongly suggesting a potential role for the protein in the development of muscle fibers. A preliminary analysis in the *smpx*^{crispant} embryos generated *via* CRISPR/Cas9 technology was also performed, uncovering that the V-shaped patterning of Vinculin, the only known interactor of Smpx[3], was completely misshaped in the myotendinous junctions with a proportion of Vinculin mislocalized within the myotome. This piece of evidence highlights the need for more in-depth analyses in search for possible correlations between *SMPX* mutations and muscular disorders in humans, thus potentially turning this non-syndromic hearing loss-associated gene into the genetic cause of dysfunctions characterized by more than one symptom, making *SMPX* a novel syndromic gene.

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ULTRASTRUCTURAL INVESTIGATIONS OF THE CEREBRAL CORTEX IN MOUSE MODELS OF BBSOAS

I. Esposito[1], C. Fogliano[1], M. Gammella[1], M. Bertacchi[2], M. Studer[2], B. Avallone[1]

[1]Department of Biology, University of Naples Federico II, Napoli, Italy; [2] Université Côte d'Azur (UCA), CNRS, Inserm, Institute of Biology Valrose (iBV), Nice
E-mail: ilaria.esposito25@studenti.unina.it

The Bosch-Boonstra-Schaaf Optic Atrophy Syndrome (BBSOAS; OMIM 615722; ORPHA 401777) is a neurodevelopmental disease caused by mutations in the NR2F1 gene, a transcriptional regulator known for its multiple key roles in brain development and postnatal brain plasticity[1]. The clinical phenotypes are heterogeneous and include optic nerve atrophy or hypoplasia, cortical visual impairment, intellectual disability, motor impairment, and autism spec-

trum disorder. With an estimated prevalence between 1 in 100,000 to 250,000 people worldwide, BBSOAS has been diagnosed in more than 300 patients[2]. However, more cases are being reported yearly, suggesting that this frequency might be underestimated. There is currently no cure for BBSOAS, but with early intervention and appropriate therapies, it is possible to improve the quality of life of affected children. To contribute to our understanding of the pathophysiology of the disease, this study focuses on electron microscopy analysis of brain tissues using a mouse model of BBSOAS. Both juvenile and adult Nr2f1-het mice were examined ultrastructurally to detect any changes in the thickness and shape of the myelin fiber and the size of the mitochondria in the cerebral cortex. The results indicate a reduction in the thickness of the myelin sheath and loss of its characteristic multilaminar structure, a reduction in neurofilaments in the axoplasm, and abnormalities in the mitochondrial morphology. Our results align with what was previously reported at the optic nerve level[3] and highlight new alterations that open novel perspectives for future investigations on the pathogenesis of BBSOAS in other brain areas.

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OILS FROM SICILIAN WHITE AND RED GRAPE SEEDS EXHIBIT POTENTIAL ANTI-TUMORAL AND ANTI-DIABETIC PROPERTIES: *IN VITRO* PRELIMINARY STUDIES

D. Ganci[1], G. Abruscato[1], A. Sanfilippo[1], F. Bellistrì[1], M. Mauro[1,2], M. Vazzana[1,2], V. Arizza[1,2], C. Luparello[1,2]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo; [2]NFBC, National Biodiversity Future Center, Palermo, Italy
E-mail: daniela.ganci01@unipa.it

Vitis vinifera (Linneo, 1753), possesses numerous health-promoting properties, such as antioxidant and anti-aging effects[1]. Here, we evaluated the possible antiproliferative and cytotoxic activity of white (Catarratto/Insolia/Grillo mix) and red grape (Sangiovese) seed oils on colon (CaCo-2) and liver (HepG2) cancer cells. Differentiated non-tumoral CaCo-2 cells were tested in parallel. MTT assays[2] showed that Caco-2 cells were more sensitive than HepG2 cells to the viability-restraining effect of the oils, especially the white one, and the IC_{50} values were evaluated. Interestingly, both oils exerted no toxic effect to differentiated Caco-2 cells, suggesting their possible biocompatibility with the normal intestinal microenvironment. Further investigation on cell cycle perturbation upon 24h treatments with IC_{50} of white grape seed oil revealed an increase of the sub-G_0G_1 fraction in both cancer cell lines, suggesting the occurrence of DNA fragmentation. Whether this aspect is linked to the promotion of apoptosis is still an object of study. A second line of research was focused on the evaluation of the impact of 24 h-exposure to the minimum non-inhibitory concentration of seed oils on glucose metabolism in HepG2 cells, which, although tumoral, maintain many differentiated hepatic functions. PAS reaction and glucose uptake assays[3] showed that both oils acted as potential anti-diabetic supplements, determining the increase of glucose consumption and intracellular glycogen accumulation. The preliminary data obtained in both lines of research represent a good starting point for a deeper molecular investigation on the beneficial effects of grape seed oils and their applications.

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THE USE OF ARTIFICIAL INTELLIGENCE IN ASSISTED REPRODUCTION: SPERM SELECTION

F. Gardini[1], F. Barbarulo[2], C. Bianco[1], C. Scarica[2], S. Moreno[1]

[1]Department of Science, University "Roma Tre", Rome; [2]European Hospital – New Fertility Group, Rome, Italy

The World Health Organization (WHO) estimates that 9% of couples struggle with infertility and that male factor contributes to 50% of the issues[1]. In assisted reproductive technology (ART), a crucial step is to select competent spermatozoa for intracytoplasmic sperm injection (ICSI). However, the methods mimicking natural selection processes occurring in the female reproductive tract – *e.g.*, swim-up, centrifugation on density gradient, selection based on morphology - are time-consuming, subjective, and may cause damage to the sperm[2]. We addressed the potential of artificial intelligence (AI) algorithms in sperm cells selection, thanks to large-data processing capabilities, objectivity and improvement over time, as more robust datasets become available for training. AI-based image analysis allows recognition of patterns unperceivable to the eye[1]. Multiple tools have been developed, such as computer-aided sperm analysis (CASA), which can assess large amounts of the sperm cells, though unable to perform single-sperm analysis. In the present study, we employed Sperm ID (SiD), an individual-sperm identification system, assisting the embryologist during ICSI. This novel AI tool detects motile spermatozoa, using a digitizer connected to a microscope. Evaluation of morphological and motility features is used by SiD to rapidly identify the best spermatozoa in the sample, assigning a color to each spermatozoon (green, yellow or red), based on its quality[3]. Samples from sixteen volunteers were analyzed by evaluating the average concentration of sperm selected by the software and comparing them with the values of the classic spermiogram. It was possible to show that the SiD correctly recognizes spermatozoa, based on their motility, but this is also influenced by morphology and sperm concentration in the starting sample. However, the system is unable to analyze all the spermatozoa present in an observation field. While the system needs to be further tested to recommend AI employment in sperm selection, we support the hypothesis that such applications are most promising in the field of ART.

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CHARACTERIZATION OF ETS FORMATION IN *CHERAX QUADRICARINATUS*

C. Gargano[1], C. Luparello[1], M. Mauro[1], R. Chiarelli[1], F. Dumas[1], R. Alduina[1], A. Vizzini[1], V. Arizza[1], M. Vazzana[1]

[1]Department of STeBiCeF, University of Palermo, Italy
E-mail: claudio.gargano@unipa.it

Extracellular Trap formation regulated cell death (ETosis) is an alternative process firstly characterized in neutrophils (NETosis)[1] inducing the release of chromatin from cell nucleus because of the infection entrapping and killing microorganisms[2] in the extracellular environment as an innate immune response. In the last decade that process was further described as a highly conserved mechanism in few plants, invertebrates and vertebrates acting against bacteria, fungi or viruses[3] therefore, termed as ETosis because of the same chromatin trap formation (ETs) in other cell types[4]. ETosis is involved in other responses including entrapment of cancer cells, unspecific triggering related to stress conditions. Therefore, several autoimmune diseases such as thrombosis, gout or tumor metastases are related to unregulated ETosis modulation[5]. The aim of this study is to describe the process of ETs formation in a crustacean species using hemocytes from *Cherax quadricarinatus* (Crustacea; Decapoda). ETs production was stimulated using LPS, PMA and *S. aureus* at various times with selected concentrations[7]. The evidence of ETs release in hemocytes from *Cherax* differs in structure as it has been demonstrated depending on the stimuli used and the concentrations used[8]. It has been observed that those hemocytes can perform ETosis with antimicrobial function against Gram+ bacteria and also react to molecules derived from a Gram- strain. Thus, ETosis was firstly described in *C. quadricarinatus* highlighting promising further knowledge on ETs formation and its modulators. Finally, a better characterization of that process would contribute to future studies to understand diseases as a consequence of ETosis activation in mammals.

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ENHANCED FOLLICLE GROWTH AND HEALTH ASSOCIATED WITH EXTRACELLULAR MATRIX REMODELING IN BOVINE OVARIAN TISSUE THROUGH DYNAMIC CULTURE

V. Genovese, V. De Gregorio, A. Travaglione, A. Candela, M. Cimmino, R. Gualtieri, R. Talevi

Department of Biology, University of Naples Federico II, Napoli, Italy
E-mail: vincenzo.genovese@unina.it

In this study, we investigated whether the dynamic culture in a Perifusion Bioreactor (PB) improves the follicle growth, health and the remodeling of the extracellular matrix (ECM). Over the past two decades, *in vitro* folliculogenesis has shown a limited success in large mammals[1]. The dynamic culture in bioreactors emerges as a reliable method for obtaining improved follicle development, viability and quality compared to static culture (Conventional dish CD)2. However, less attention has been given to ECM remodeling, despite its crucial role in cell–cell, and cell-ECM interactions involved to follicular development. In this perspective, bovine ovarian cortical tissue strips were cultured for 14 days in PB and CD. The thickness and degree of maturation of collagen fibers were assessed through PicroSirius red (PSR) staining under polarized light[3]. The follicle stages and quality were evaluated through histology (hematoxylin-eosin staining), and viability (live-dead assay). Analysis of PSR-stained samples revealed significantly higher production of green (newly synthesized) and yellow (low assembly) collagen fibers in PB compared to CD, indicating improved ECM remodeling. The ECM remodeling in PB culture was associated with a significantly higher percentage of secondary follicles, increased follicle quality, and viability. Our findings suggest that the continuous nutrient supply, oxygenation, catabolite removal, and biomechanical stimulation during dynamic PB culture promote the deposition of newly synthesized collagen, thereby enhancing follicle growth and health. While the bovine is a reliable model for human folliculogenesis, confirming these findings with human ovarian tissue is essential. The use of dynamic ovarian tissue culture could enhance *in vitro* folliculogenesis outcomes, increasing the growth of healthy secondary follicles, essential to generate mature oocytes for fertility preservation and assisted reproduction techniques.

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INVESTIGATING THE PATHOGENESIS OF AUTOSOMAL DOMINANT TUBULOINTERSTITIAL KIDNEY DISEASE (ADTKD) THROUGH ZEBRAFISH MODELS

F. Ghirotto, C. Tesoriero, F. Greco, A. Vettori

Department of Biotechnology, Laboratory of Neurogenetic and Translational Biology, University of Verona, Italy
E-mail: andrea.vettori@univr.it

Autosomal Dominant Tubulointerstitial Kidney Disease (ADTKD) encompasses a group of clinical disorders characterized by tubular damage and interstitial fibrosis, leading to chronic kidney disease (CKD) and eventual end-stage renal disease (ESRD) in affected individuals. While several genes have been implicated in ADTKD, including *UMOD*, *MUC1*, *HNF1B*, and *SEC61A1*, mutations in the *REN* gene encoding preprorenin represent a common cause[1]. Preprorenin, synthesized by juxtaglomerular (JG) cells, undergoes proteolytic cleavage to form mature renin, a crucial component of the renin-angiotensin-aldosterone system (RAAS) involved in blood pressure regulation and electrolyte balance[2]. ADTKD-*REN* is characterized by a relative decrease in renin levels and accumulation of mutant renin protein within the Endoplasmic Reticulum (ER), triggering the Unfolded Protein Response (UPR) and subsequent pathological processes. Furthermore, ER stress conditions in ADTKD may lead to dysregulation of ER-resident proteins, including those recognized by KDEL receptors (KDELRs), such as KDELR3[3]. Emerging evidence suggests an upregulation of KDELR3 in ADTKD models, hinting at its potential role in disease progression. However, the specific functions of KDELR3 during UPR and in ADTKD remain poorly understood[4].To address these gaps we generated zebrafish

transgenic lines expressing altered forms of the human *REN* gene, including L16R, E48K, and L381P variants, to model ADTKD. Moreover, a kdelr3 knock-out zebrafish line has also been generated to investigate the involvement of KDELR3 in ADTKD pathology, particularly in conjunction with the renin pathological mutation L381P. Through comprehensive morphological and behavioral characterization of these zebrafish models, we aim to elucidate the molecular mechanisms driving ADTKD pathogenesis, potentially identifying novel therapeutic targets for this debilitating disease.

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POLYSTYRENE MICROPLASTICS IMPAIR STEROIDOGENESIS BY INDUCING MITOCHONDRION-ENDOPLASMIC RETICULUM DYSREGULATION

G. Grillo[1], S. Falvo[1], D. Latino[1], G. Chieffi Baccari[1], M. Venditti[2], M.M. Di Fiore[1], S. Minucci[2], A. Santillo[1]

[1]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania L. Vanvitelli, Caserta; [2]Department of Experimental Medicine, University of Campania 'Luigi Vanvitelli', Napoli, Italy
E-mail: giulia.grillo@unicampania.it

Polystyrene microplastics (PS-MP) exposure has been found to cause testosterone deficiency and spermatogenic impairment[1]; however, the mechanism by which this happens remains unclear. Using a murine cell line, TM3, our study aims to investigate the cellular response induced by PS-MP in Leydig cells (LC), the testicular site of testosterone synthesis; this process begins with the translocation of cholesterol in mitochondria, by StAR protein[2], and completes in the endoplasmic reticulum (ER). First, we found that exposure *in vitro* for 24 h to increasing concentrations of PS-MP from 0 to 200 µg/mL caused a dose-dependent reduction in viability in TM3 cells, confirming the cytotoxic effect of PS-MP on LC. Furthermore, we found that PS-MP inhibited the protein expression levels of StAR, as well as 3β-hydroxysteroid dehydrogenase, and 17β-hydroxysteroid dehydrogenase, two enzymes playing a key role in steroidogenesis pathway. Both ERK1/2 and Akt pathways resulted downregulated by PS-MPs in TM3 cells, suggesting that PS-MP could interfere with LC function inhibiting the kinase pathway signaling. The PS-MP-caused oxidative stress decreased antioxidant defense in TM3 cells and forces them to trigger autophagic and apoptotic processes. Mitochondrial dysfunction was also observed, as evidenced by the decrease in mitochondrial membrane potential, ATP, and calcium levels. Finally, activation of ER stress and alterations in Mitochondrial-Associated Endoplasmic Reticulum Membrane (MAMs) were induced by PS-MP treatment. These findings suggest that PS-MP impair steroidogenesis through mitochondrion-endoplasmic reticulum dysregulation. Our study highlights, for the first time, the intracellular mechanism underlying the PS-MP effects on LC and adds new knowledge to the action mechanism of PS-MP on the male reproductive system.

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TIO_2 BROOKITE NANOPARTICLES IN AQUATIC MICROENVIRONMENTS AFFECT SPERM MOTILITY IN MARINE INVERTEBRATES

S. Indelicato[1], R. Pecoraro[1], E.M. Scalisi[1], G. Coco[1], R. Lo Faro[1], S. Cartelli[1], A. Scalisi[1], R. Fiorenza[2], A. Salvaggio[3], M.V. Brundo[1]

[1]Department of Biological, Geological and Environmental Sciences, University of Catania, Catania; [2]Department of Chemical Sciences, University of Catania, Catania; [3]Experimental of Zooprophylactic Institute of Sicily "A. Mirri", Palermo, Italy
E-mail: stefania.indelicato@phd.unict.it

The field of nanotechnology has been booming in recent years due to the versatility of nanomaterials, which are now widely used in medical, industrial and pharmaceutical fields. With the continuous and uncontrolled release of chemical wastes and discharge byproducts into the outdoor environment, there is a growing need to monitor their possible negative impacts on the aquatic microenvironment and particularly on free-living stages such as gametes, embryos and larvae[1]. According to some studies, TiO□-NPs have been found to be able to penetrate biological membranes and cause severe damage to maturing germ cells, probably by inducing ROS[2] production and consequently triggering processes of programmed cell death and mitochondrial malfunction. In this study, spermatozoa of *Mytilus galloprovincialis,* were exposed to different concentrations of TiO_2 in the brookite phase in order to assess potential damaging effects on structure and metabolism. Although no structural damage was observed, exposure to NPs appears to have disrupted sperm motility. Fluorescence analysis for assessment of oxidative stress was positive for all concentrations tested, thus suggesting a possible correlation. These, constitute preliminary data and therefore more investigation should be done in this field precisely to clarify the impact of NPs on the male reproductive system in order to minimize any toxicity and preserve fertility.

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DO MICROPLASTICS MODIFY THE MORPHOPHYSIOLOGY OF GILLS AND GONADS IN THE MUSSEL *MYTILUS GALLOPROVINCIALIS*?

T. Chianese[1], A. Lallo[1], N. Russo[1], A. Locascio[2], R. Scudiero[1], L. Rosati[1]
[1]Department of Biology, University Federico II, Napoli; [2]SZN, Napoli, Italy
E-mail: a.lallo@studenti.unina.it

Plastic contamination is a contemporary pollution problem; this historical moment is called *The plastic Era*[1]. Plastic undergoes fragmentation to form microplastics (MPs), that accumulate at different trophic levels. Aquatic organisms ingest MPs, that, binding other pollutants, facilitate their uptake into living organisms, in what is called the *Trojan horse effect*. This study investigated the effects of polystyrene MPs in the gills and gonads of *Mytilus galloprovincialis*, a good bioindicator of the marine environment and widely exploited as food. Animals were exposed for 48 and 72h to 2 concentrations of 5µm MPs (0.5 and 1µg/mL), alone or conju-

gated with bisphenol A (BPA), an endocrine disrupting water contaminant. The analyses showed alteration of gills and gonads in all treated animals, regardless of MPs concentration, conjugation with BPA and exposure time. In the gills, alterations in septum and lamellae, presence of granules and infiltration of hemolymphatic cells were found; increased levels of HSP70 and p63 and increased positive cells for the PCNA immunological signal were observed. PCNA is involved in cell cycle regulation, DNA synthesis and repair, HSP70 in protein folding and inflammation, p63 in tissue growth and development; their increase is indicative of cell stress. In the gonads, alterations in the germinal epithelium were demonstrated, with the detachment of cells from each other in males, and degeneration of oocytes in females. The alteration of chromatin profile in spermatozoa was evident. Mucus cells increased in number in both the gills and gonads of treated animals, particularly at the higher MPs concentration. In all organs examined, the analysis of the functionality of the antioxidant machinery showed the occurrence of cellular stress conditions, indifferent to BPA conjugation, but greater with the highest MPs concentration tested. In conclusion, the results clearly demonstrate the stressogenic effect of MPs. The Trojan horse effect for BPA does not seem to occur; further morpho-functional and molecular investigations are needed to resolve any doubts.

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D-ASPARTATE UPREGULATES STEROIDOGENESIS BY MODULATING MITOCHONDRIA DYNAMICS AND MITOCHONDRIA-ASSOCIATED ENDOPLASMIC RETICULUM MEMBRANES (MAMs)

D. Latino[1], S. Falvo[1], G. Grillo[1], A. Santillo[1], M. Venditti[2], S. Minucci[2], G. Chieffi Baccari[1], M.M. Di Fiore[1]

[1]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Caserta; [2]Department of Experimental Medicine, Section Human Physiology and Integrated Biological Functions, University of Campania "Luigi Vanvitelli", Naples, Italy
E-mail: debora.latino@unicampania.it

MAMs mediate communication between the Endoplasmic Reticulum (ER) and Mitochondria, and they play a fundamental role in steroidogenesis. The study aims to understand how D-aspartate (D-Asp), a stimulator of testosterone biosynthesis and spermatogenesis, affects the mechanism of steroidogenesis in rat testes and TM3 Leydig cells. The results indicate that D-Asp improves steroidogenesis efficiency in rat testis by acting on mitochondria and MAMs, by improving lipid transfer and calcium signaling from the ER to the mitochondria, as well as by activating fusion and biogenesis in the mitochondrial compartment. Following 15 days of oral administration of D-Asp to rats, there was an increase in the protein levels of ATAD3A, FACL4, and SOAT1, markers of lipid transfer, as well as VDAC and GRP75, markers of calcium signaling. D-Asp plays an active role also in mitochondrial dynamics, by inducing increased expression levels of proteins involved in fusion (MFN1,MFN2, and OPA1) and biogenesis (NRF1, TFAM), as well as mitochondrial mass (TOMM20) while decreasing the expression level of DRP1, a mitochondrial fission marker. Immunofluorescent signals of ATAD3A, MFN1/2, TFAM, and TOMM20 confirmed their localization in Leydig cells, and they were increased in D-Asp-treated rat testes, compared to the control. We also observed an increase in the expression levels of proteins involved in mitochondrial fusion, biogenesis, calcium signaling, and lipid transfer between ER and mitochondria in TM3 Leydig cells treated with 200 mM D-Asp, compared to untreated cells. These results demonstrate the involvement of D-Asp in steroidogenesis, acting at multiple stages on both MAMs and mitochondrial dynamics. However, further effort is needed to understand the mechanisms underlying the contribution of D-Asp to steroidogenesis and spermatogenesis.

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ENVIRONMENTAL POLLUTION AND MALE REPRODUCTIVE HEALTH: FINDINGS FROM A PRELIMINARY STUDY IN VALLEY OF SACCO RIVER (LAZIO REGION, ITALY)

G. Lettieri[1], C. Marinaro[1], P. Perrone[2], S. Capone[3], S. Pappalardo[4], L. Montano[5], M. Piscopo[1]

[1]Department of Biology, University of Naples Federico II, Napoli; [2]Department of Precision Medicine, School of Medicine, University of Campania Luigi Vanvitelli; [3]Institute for Microelectronics and Microsystems (IMM), National Research Council of Italy (CNR), Lecce; [4]Reproduction and Fertility Center, Rome;[5]Andrology Unit and Service of Lifestyle Medicine in UroAndrology, Local Health Authority (ASL) Salerno, Italy
E-mail: gennarole@outlook.com

In recent decades there has been a decline in the semen quality; the causes may be various, but the pollution seems to be the most significant variable. In fact, the spermatozoon is very sensitive to environmental changes and is considered an early sentinel of organism health state. The present study, within the Ecofoodfertility project, evaluates the effects of environmental pollution in the *Valley of Sacco River* (VSR) (Lazio region, Italy) on human semen using classical and molecular approaches. This is an area with industrial chemical production that contaminated the environment with various pollutants, in particular Volatile Organic Compounds (VOCs). We evaluated the presence of these VOCs in the semen of healthy young males of this area. In addition, molecular analyses to assess the content of sperm nuclear basic proteins (SNBPs) and their ability to bind DNA were performed. The evaluation of the electrophoretic pattern of the SNBPs, i.e protamines and histones, by acetic acid urea polyacrylamide gel electrophoresis (AU-PAGE), revealed alterations in the ratio of protamines/histones. Furthermore, the SNBP from VSR area showed a reduced ability to bind DNA by Electrophoretic Mobility Shift Assay (EMSA), regardless of their protamine/histone ratio. Finally, we found several severe alterations in sperm morphology, motility and count. The data were compared to control group living in a low environmental impact area, *Valley of Sele* (VSL) in Salerno province[1]. Our results provide preliminary indications of a possible correlation between the observed alterations and the presence of specific VOCs and can provide a different view of the state of semen that cannot be provided by the classic spermiogram.

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ROLE OF PHOSPHODIESTERASE-5A IN HEPATOCARCINOGENESIS AND POSSIBLE IMPLICATIONS OF HYPOXIA: *IN VITRO* STUDIES

M.G. Armillotta[1], L. Lizzi[1], M. Saliola[2], M. Giorgi[2], M. Massimi[1]

[1]Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila; [2]Department of Biology and Biotechnology Charles Darwin, Sapienza University of Rome, Italy
E-mail: mara.massimi@univaq.it

Phosphodiesterase-5 (PDE5) is one of the main enzymes responsible for controlling intracellular cGMP levels, the expression of which is dysregulated in several tumors, including hepatocarcinoma. In normal rat liver, PDE5A expression remains confined to a few hepatocytes around the central vein, a zone physiologically hypoxic, while it increases with loss of zonation in cancerous liver tissues[1]. In addition, single murine PDE5 isoforms show a differential intracellular localization when transfected in *K. lactis* yeast. While PDE5A1 and A3 isoforms are localized to the nucleus and cytoplasm and seem to favor a transition towards fermentative metabolism in yeast, the PDE5A2 isoform preferentially localizes in mitochondria, where it influences the balance of cyclic nucleotides and redox cofactors[2]. The present research aimed to shed light on the possible involvement of PDE5 in hepatocarcinogenesis and on the role played by hypoxia, as a recurrent factor in cancer, and possibly involved in PDE5 overexpression. PDE5 expression and activity levels increase in tumor cells, being higher in HepG2 and Huh7 cell lines compared to the more differentiated HepaRG cells. PDE5 expression also increases in cells cultured under hypoxic conditions, either real (1% O2) or simulated ($CoCl_2$ or DFOM), as well as in the presence of exogenous hydrogen peroxide, which is the mitochondrial ROS that guides hypoxic responses. These findings suggest that hypoxic conditions, or other conditions that cause a stabilization of HIF-1α, lead to increased PDE5 intracellular levels. The induction of PDE5 may thus depend both on events occurring downstream of HIF activation and on mitochondrial ROS. In conclusion, PDE5 may have a new, undisclosed function, sharing a role with hypoxia in carcinogenesis and in the selection of a more aggressive cellular phenotype. A direct involvement in promoting the Warburg effect cannot be ruled out, although further studies are needed to confirm this hypothesis.

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HEMOLYMPH WITHDRAWAL REVEALS A NEW BIOLOGICAL CONTEXT TO STUDY HEMOCYTE PROLIFERATION AND FUNCTIONS IN THE SNAIL *POMACEA CANALICULATA*

C. Losi[1], M. Montanari[1], S. Sacchi[1], D. Malagoli[1,2]

[1]Department of Life Sciences, University of Modena and Reggio Emilia, Modena; [2]NBFC, National Biodiversity Future Center, Palermo, Italy
E-mail: 283436@studenti.unimore.it; davide.malagoli@unimore.it

Hemocytes are key cells in invertebrate immunity, but there's increasing evidence that they are also involved in tissue homeostasis and development. The laboratory bred and genetically tractable freshwater snail, *Pomacea canaliculata*, offers the possibility of multiple hemolymph sampling and its hemocytes have been classified into Group I (GI) (small and blast-like cells) and Group II (GII) (large cells). The only functional difference observed between GI and GII cells is represented by phagocytic activity, only detected in GII agranular hemocytes. The number of circulating hemocytes and the proportion of GI and GII populations (GII/GI ratio) are conserved between individuals, suggesting the presence of control mechanisms over circulating hemocyte populations and possible cell-specific functions. In this view, hemocyte populations were assessed by flow cytometry 1.5, 3, 6, 9, 18, 24 or 48 h after a previous hemolymph collection. GII/GI was lower at 6, 9 and 18 h time points, and returned to baseline conditions at 24 h, when the number of hemocytes was significantly higher than at time 0. Time 0 conditions were reached 48 h after the first collection. To investigate whether *P. canaliculata* hemocytes might be associated with biological functions other than immunity, the expression of PcKi-67 (cell proliferation) and PcIL-17 (immunity, hematopoiesis and homeostasis) was examined at 18 h (minimal GII/GI) and 24 h (normal GII/GI). qPCR experiments showed that regardless of GII/GI ratio and in the absence of immune challenge, PcKi-67 and PcIL-17 expression levels were constant, suggesting that circulating hemocytes might be able to proliferate and physiologically intervene in tissue homeostasis and signaling. These data support the hypothesis of a control on the proportion of hemocyte populations and the possibility to rapidly restore it. They also indicate in hemolymph repopulation a biological context for studying hemocyte turnover and for exploring the possible and as yet unknown distinct biological roles of hemocyte subpopulations in the absence of direct immune stimulation.

MICRO AND NANOPARTICLES AFFECT REPRODUCTIVE PROCESS IN THE MUSSEL *MYTILUS GALLOPROVINCIALIS*

G. Maisto[1], R. Romano[1-2], L. Rosati[3], F. Ferrigno[1], G.F. Russo[1-2], C.M. Motta[3], P. Simoniello[1-2]

[1]Department of Sciences and Technology, University Parthenope, Napoli;[2]International PhD Programme UNESCO Chair "Environment, Resources and Sustainable Development" Napoli; [3]Department of Biology, University of Naples Federico II, Napoli, Italy
E-mail: gelsomina.maisto001@studenti.uniparthenope.it

Reproductive apparatus represents a target tissue of different environmental chemical pollutants, such as drugs and heavy metals. These xenobiotics can interfere with the fertility and reproductive processes in terrestrial and aquatic organisms, both invertebrates and vertebrates. Being able to mimic endocrine factors, they may interfere with gonad differentiation, gametogenesis, gamete quality and sustainability of their resources[1]. In the last decades, micro (MPs) and nano plastics (NPs)[2] have become one of the most threatening risks for marine organisms, due to their ability to penetrate *via* the body surface or to be ingested, mistaken for prey. In this study, we examined the effects of polystyrene micro and nanoparticles on the ovaries of *M. galloprovincialis*. Mussels were exposed to MPs (5 μm) or NPs (0.1 μm), as described in a previous study[3]. Ovaries were processed for light microscopy analyses. Morpho-functional alterations were detected with histological and histochemical staining. Results showed that MPs and NPs affect follicle and oocyte organisation, increase the number of atretic follicles and determine the presence of haemocyte infiltrates. These effects appeared very early, after 1 day of exposure to the contam-

inants; later, after 3 and 11 days of exposure, a significant deposition of collagen was detected within the ovarian connectives. Ongoing investigations will clarify the effects as a function of the exposure time. In conclusion, MPs and NPs alter ovarian follicles in *Mytilus galloprovincialis* with evident consequences on fecundity. Mussels are important species for the stability of coastal ecosystems, and, in addition, their economic impact is not negligible. For these reasons, a fast and drastic reduction of plastics in water would be desirable.

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SPERM CHROMATIN REMODELING DURING EPIDIDYMAL TRANSIT DEFINE A PATERNAL EPIGENETIC SIGNATURE POTENTIALLY IMPLICATED IN EMBRYO DEVELOPMENT

F. Manfrevola[1], T, Chioccarelli[1], V. Nittoli[2], V.G. Mele[1], R, Chianese[1], C. Ambriosino[2,3], S. Fasano[1], G. Cobellis[1]

[1]Department of Experimental Medicine, University of Campania L. Vanvitelli; [2]Biogem Scarl, Institute of Genetic Research; [3]Department of Science and Technology, University of Sannio, Italy
E-mail: francesco.manfrevola@unicampania.it

During spermiogenesis a genome-wide histone displacement, molecularly orchestrated by histone H4 acetylation, facilitates histone-to-protamines exchange in elongated spermatids[1]. Despite this massive chromatin remodeling, a few amount of histones is retained in mature spermatozoa (SPZs) and condenses genomic regions containing housekeeping and paternally-expressed imprinted genes, as well as genes associated to embryo development and spermatogenesis[2-3]. Additionally to testicular histone displacement wave, little is known on chromatin remodeling events occurring in SPZs during epididymal transit. Here, using mouse SPZs collected from caput and cauda epididymis, we studied epididymal remodeling of histone-based sperm chromatin. Our results demonstrate that a further wave of histone displacement occurs in SPZs during epididymal maturation. This event co-occurs with a qualitative remodeling of H3-enriched genomic regions, mechanistically mediated by SIRT1 and histone H4 acetylation. Indeed, H3-ChIP-seq data highlight a number of peaks differentially present in caput (n. 181) vs cauda (n.80) SPZs and an impressive change in sperm H3-associated loci. Gene ontology enrichment analysis reveals the involvement of cauda retained H3-associated loci in biological pathways regulating embryo development. Furthermore, *in vitro* treatment of caput SPZs with a selective inhibitor of SIRT1 deacetylase, promoted H4 acetylation, histone displacement and sperm nuclear resizing, thus revealing the involvement of SIRT1 in remodeling of histone-based chromatin of SPZs. Our data demonstrate a significant remodeling of histone-enriched loci during epididymal transit highlighting: i) the potential implications of paternally transmitted epigenetic signature in the regulation of embryonic development, and ii) the nuclear size as potential marker of sperm quality.

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IN VIVO MODELS TO UNVEIL NEW NKTR FUNCTIONS ASSOCIATED WITH AN UNDIAGNOSED RARE NEURODEVELOPMENTAL DISORDER

S. Marchetta[1], M. Orefice[1], C. Soldo[1], M. Denti[2], A. Cariboni[3], M. Ori[1]

[1] Department of Biology, University of Pisa; [2]CIBIO, University of Trento; [3]University of Milan, Italy
E-mail: michela.ori@unipi.it

Exome sequencing data identified two patients with a rare neurodevelopmental syndrome characterized by developmental delay, microcephaly, and dysmorphic facial features. The patients carry a homozygous nonsense mutation in the *NKTR* (Natural Killer Triggering Receptor) gene. *NKTR* encodes a protein initially identified as a membrane co-receptor involved in the target recognition of natural killer cells[1], but its role in neurodevelopment is unknown.We showed the expression of mouse and zebrafish *NKTR* orthologs in brain and neural crest cell (NCC)-derived structures during embryogenesis. *NKTR* expression overlaps with Alcian blue staining and *Sox9* expression in chondrogenic structures of the developing mouse head, suggesting a potential role for NKTR in craniofacial development. To model the human syndrome associated with the NKTR mutation, we used two loss-of-function approaches in zebrafish embryos. The injection of a translation-blocking morpholino in zebrafish embryos caused defects in head cartilage formation and altered expression of NCC markers *dlx2* and *crestin.* To further validate our findings, we designed a CRISPR/Cas13d-based knockdown strategy in zebrafish embryos to induce degradation of nktr mRNA. qPCR analysis showed a decrease in nktr expression in nktr crispants, with a tunable effect depending on the sgRNA dosage during injection. The craniofacial defects observed in nktr crispants were similar to, but less severe than, those in nktr morphants. Among the craniofacial cartilages, the most affected were the jaw and palatoquadrate cartilages. We are now characterizing our new models during craniofacial and brain development at the molecular and cellular levels. By combining NKTR-knockdown models (both in cells and zebrafish) with RNA sequencing and in silico analyses, we aim to elucidate the molecular mechanisms underlying genetic disorders due to NKTR loss of function and to identify potential drug targets for future therapies. The project is supported by the PRIN-PNRR2022 program (Acronym: MONAD, Coordinator M. Ori) for 2023-2025.

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IMPACT OF HEAVY METALS MIXTURE ON THE REPRODUCTIVE SYSTEM OF *MYTILUS GALLOPROVINCIALIS*: A MOLECULAR MECHANISM

C. Marinaro[1], G. Lettieri[1], T. Chianese[1], A. R. Bianchi[1], D. Palatucci[1], A. Zarrelli[2], L. Rosati[1], A. De Maio[1], M. Piscopo[1]

[1]Department of Biology and [2]Department of Chemical Sciences, University of Naples Federico II, Naples, Italy
E-mail: carmelamarinaro9@gmail.com

Human activities such as industrialization, urbanisation and land development (agricultural and mining) have severe impacts on the environmental and organism health. Heavy metals, that are the most common pollutants, resulted toxic to the reproductive system

of all organisms. However, invertebrate infertility has been poorly investigated and in particular molecular, cellular and toxicological studies are also limited. In order to explore these aspects, in the present work, we exposed *Mytilus galloprovincialis* to three individual metal chlorides ($CuCl_2$ 15 μM, $CdCl_2$ 1.5 μM, $NiCl_2$ 15 μM) and their mixture for 24 h, evaluating the effects on the protamine-like proteins (PLs), sperm DNA and on their interaction in the formation of sperm chromatin. Under all exposure conditions, but particularly after exposure to the metals mixture, relevant alterations in the electrophoretic pattern of PLs, by AU-PAGE and SDS-PAGE, and in fluorescence spectroscopy measurements were shown. Furthermore, changes in DNA binding of these proteins were also observed by Electrophoretic Mobility Shift Assay (EMSA) and through their release from sperm nuclei. In addition, there was evidence of increased accessibility of micrococcal nuclease to sperm chromatin, which was also confirmed by toluidine blue staining. Moreover, morphological analyses indicated severe gonadal impairments which were also supported by increased PARP expression, by Western blotting, and sperm DNA fragmentation, by comet assay. Finally, the high-expression of stress genes, *gst*, *hsp70* and *mt10*, in gonadal tissue, showed that exposure to this metals mixture was more harmful than exposure to the individual metals tested. Although many aspects of the toxicological mechanism of heavy metals are still unclear, the present results suggest that these metals and in particular their mixture could have a negative impact on the reproductive health of *M. galloprovincialis*.

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FIRST EVIDENCE OF METAL-CONJUGATED POLYSTYRENE MICROPLASTICS IMPACT ON THE *XENOPUS LAEVIS* DEVELOPMENT

R. Mastrantone[1]. C. Fogliano[1], M. Trifuoggi[2], M. Toscanesi[2], B. Avallone[1], C.M. Motta[1], R. Carotenuto[1]

[1]Department of Biology and [2]Department of Chemical Sciences, University of Naples Federico II, Napoli, Italy
E-mail: r.mastrantone@studenti.unina.it

Microplastics (MPs), the small plastic particles no larger than 5 mm in size, are composed of different polymers derived mainly from petroleum (PVC, PET, PE, PP or PS)[1]. They can be classified into primary, intentionally produced, and secondary, mainly from the fragmentation of larger plastic objects through physical, chemical and biological processes[2]. Metals have a ubiquitous distribution and, as MPs, are bioaccumulated and biomagnified in the food chain. Multiple health effects are associated with exposure to heavy metals, with varying degrees of severity and conditions, including cancer[4]. Here we studied the effects of 5 μm polystyrene MPs conjugated to various metals (Cd, Cu, Pb and mix) commonly present in water and which could magnify the activity of MPs on aquatic organisms. First, the solutions were analyzed by gas chromatography to determine whether MPs release volatile organic compounds (VOCs). The FETAX test was performed on *X. laevis* embryos to verify the teratogenic potential of solutions containing metal-conjugated MPs and compare it to MPs alone. Preliminary results showed that the beads released significant amounts of VOCs. Treatment with MPs alone or conjugated did not affect survival, but heavily interfered with proper embryonic development by altering the expression of some early embryonic development genes, thus modifying the correct embryonic phenotype. VOCs are probably mainly responsible for the observed toxic effects; ongoing studies are highlighting the mechanism of action of individual VOCs and characterizing the effects they exert on different aspects of embryonic development.

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KETOGENIC DIET MITIGATES NEUROINFLAMATION AND MYELINIZATION IN A MOUSE MODEL OF IDIOPHATIC AUTISM (BTBR)

D. Minervini[1], I. Olivito[1], E. Avolio[1], C. Rocca[2], T. Angelone[2], A. De Bartolo[2], I.D. Perrotta[3], R.M. Facciolo[1], M.C. Cerra[4], R. Alò[1]

[1]Comparative Neuroanatomy Laboratory; [2]Laboratory of Cellular and Molecular Cardiovascular Pathophysiology; [3]Centre for Microscopy and Microanalysis; [4]Laboratory of Organ and System Physiology, Department of Biology, Ecology and Earth Sciences (DiBEST), University of Calabria, Arcavacata di Rende, Cosenza, Italy
E-mail: damiana.minervini@unical.it

Autism spectrum disorder (ASD) is a complex neurodevelopmental disease, characterized by impaired social interaction, communication deficits and stereotyped behaviors. Such abnormal behaviors have been correlated to immune dysregulation, neuroinflammation and oxidative stress[1], together with hypomyelination in different ASD mouse model including BTBR T+ Itpr3tf/J[2,3]. Since ASD involves many comorbidities, i.e. gastrointestinal disorders, different dietary interventions such as ketogenic diet (KD) is beginning to be used as a promising strategy to mitigate symptoms. In addition, studies have identified a stronger connectivity of the cerebellum (CB) with cortical regions involved in sensory and motor processes, whereas a weaker connectivity was reported for cognitive and socio-emotional regions, particularly prefrontal cortex (PFC) of ASD patients[4]. On this basis, the present study aimed to investigate the effects of KD on neuroinflammation and myelinization in PFC and CB of BTBR and C57BL/6J (C57), which were fed either a control diet (CD) or KD for 5 weeks. As expected, KD reduced the expression levels of pro-inflammatory cytokines, such as IL-6 and IL-1β, in both PFC[5] ($p<0.01$ and $p<0.05$, respectively) and CB ($p<0.05$ and $p<0.05$, respectively) of BTBR. Interestingly, reduced neuroinflammatory factors were accompanied by an improved myelination of BTBR neurons ($p<0.001$) as revealed by TEM analysis. Overall, these data suggest that KD may contribute to mitigate ASD-like conditions by affecting myelination and neuroinflammation in PFC and CB of a mouse model of idiopathic autism.

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COMPARATIVE EFFECTS OF CONDENSED AND HYDROLYSABLE TANNINS ON EMBRYONIC DEVELOPMENT OF ZEBRAFISH

T. Mobilio[1], R. Imperatore[2], A. La Pietra[1], E. Coccia[2], M. Paolucci[2], I. Ferrandino[1]

[1]Department of Biology, University of Naples Federico II, Napoli; [2]Department of Science and Technologies, University of Sannio, Benevento, Italy
E-mail: 29teresamobilio@gmail.com

Tannins are natural phenolic compounds with remarkable antioxidant, anti-inflammatory and antimicrobial activities. They are classified as condensed (CTs) or hydrolysable tannins (HTs). HTs are composed of ellagic or gallic acids with a sugar core, while CTs are flavonoid polymers[1,2]. Their different chemical structure results in distinct bioactive properties[2]. Indeed, several studies report that tannins can also cause acute toxicity in animals and antinutritional effects[3]. The purpose of this study was to compare the effects of CTs and HTs at different concentrations on zebrafish development to identify the range of concentrations with toxic effects. Zebrafish embryos were exposed to CTs and HTs at two different concentration ranges (5, 10, 20 μgL^{-1} and 5, 10, 20 mgL^{-1}) for 72h of treatment. The toxicity parameters were observed up to 72h of treatment. The uptake of CTs and HTs by zebrafish larvae was assessed by HPLC analysis. The qRT-PCR analysis was performed to evaluate gene expression of *cd63*, *zhe1* and *klf4*, involved in the hatching process of zebrafish. Treatments with CTs and HTs at 5, 10, and 20 μgL^{-1} were not toxic compared with the control group. Otherwise, at 5, 10, and 20 mgL^{-1} HTs induced a delayed in hatching starting from 48h of treatment, while CTs at 72h showed a delayed in hatching only at the concentration of 20 mgL^{-1}. The analysis of *cd63*, *zhe1* and *klf4* genes showed a down-regulation in the group exposed to HTs compared to control group confirming the hatching data. The differences found in hatching could result from different metabolization or accumulation of CTs and HTs by zebrafish embryos. In fact, their uptake increased with the exposure concentration, and it is interesting to note that in the group exposed to HTs 5 mgL^{-1} the uptake was considerably higher than in all the others. In conclusion, it is important to define the optimal doses of CTs and HTs to test them as beneficial substances.

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CAENORHABDITIS ELEGANS MODEL TO EXPLORE THE DIFFERENTIAL EFFECTS OF PALMITIC ACID AND OLEIC ACID ON LIPOTOXICITY

C. Moliterni[1], E. Schifano[1], S. Taccon[2], D. Uccelletti[1], L. Dini[1]

[1]Department of Biology and Biotechnology "C. Darwin", Sapienza University of Rome, Rome, Italy; [2]CarMeN Laboratory (UMR INSERM 1060/INRA 1397), HCL, Lyon 1 University, Pierre-Bénite, France
E-mail: camilla.moliterni@uniroma1.it.

Lipotoxicity, the detrimental effects of lipid accumulation, poses a significant challenge in understanding metabolic disorders and exploring potential therapeutic interventions[1]. This study delves into the distinct impacts of Palmitic Acid (PA) and Oleic Acid (OA) on lipotoxicity using the nematode *Caenorhabditis elegans*, a well-established model system for lipid metabolism. Our investigation reveals that PA elicits greater lipotoxicity compared to OA in *C. elegans*, as evidenced by the accumulation of lipid droplets (LDs) with altered morphology and LDs dynamics. Mechanistically, PA and OA differently alter the expression of diacylglycerol acyltransferase 1 (DGAT1) and diacylglycerol acyltransferase 2 (DGAT2), suggesting the implication of these enzymes in differential effects mediated by PA and OA on LDs, in agreement with data observed in hepatic cells[2]. Furthermore, PA-induced lipotoxicity is associated with endoplasmic reticulum (ER) stress induction, reduced lifespan and impaired fertility. In contrast, OA treatment extends C. elegans lifespan and enhances fertility, potentially through modulation of longevity, involving the DAF-2 receptor and the DAF-16 transcriptional factor, belonging to the Insulin/Insulin-like (IIS) signalling pathway. Additionally, OA promotes the accumulation of glutathione S-transferase 4 (GST-4) and antioxidant gene expression, mitigating oxidative stress. Moreover, OA enhances TAG synthesis efficiency and augments antioxidant defences. Stimulation of fatty acid oxidation *via* peroxisome proliferator-acrescue receptor alpha (PPARα) agonists rescues nematodes from PA-induced lipotoxicity. In summary, our findings underscore the differential effects of PA and OA on lipid metabolism and provide valuable insights for possible therapeutic approaches.

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NSC-34 G85R SOD1-DERIVED EXTRACELLULAR VESICLES IMPACT MYOBLAST DIFFERENTIATION OF C2C12 CELLS

M. Muci, E. Carata, E. Panzarini

Department of Biological Sciences and Technologies, University of Salento, Lecce, Italy
E-mail: marco.muci@unisalento.it

Amyotrophic Lateral Sclerosis (ALS) is characterized by the degeneration and loss of function of upper and lower motor neurons (MNs), leading to chronic and progressive muscle atrophy, paralysis and ultimately resulting in death. Muscle atrophy in ALS involves the dysregulation of multiple cellular processes, including disruptions in protein degradation pathways, as well as impaired protein synthesis. Moreover, oxidative stress and inflammation in the microenvironment exacerbate muscle atrophy by promoting protein breakdown and slowing down muscle regeneration[1]. Recent observations indicate that MNs themselves are implicated in releasing extracellular vesicles (EVs) containing toxic molecules alongside soluble factors, that may impair the proper differentiation of skeletal muscle cells[2]. Previous results achieved by us demonstrated that NSC-34 mSOD1 G85R-derived EVs induce an inflammatory microenvironment[3]. Here, we cultured C2C12 myoblast cells and exposed them to NSC-34 mSOD1 G85R-derived EVs. EVs were added in the C2C12 cells culture medium, at three different stages of myoblast differentiation: start (T0-at first differentiation day), midpoint (T3-at third differentiation day), and conclusion (T5-at fifth differentiation day followed by 1 day of culture). The expression levels of differentiation and maturation markers (MyoG, MyoD, and Mrf4) and muscle atrophy markers (Atrogin1 and Murf1) evaluated by qPCR analysis high-

light the modulation of myoblast differentiation in a time-dependent manner. The addition of EVs at T0 induces a slowdown of the differentiation process; whereas a decrease in the Myo D gene expression and an increase in the gene expression of myotubes maturation (Myo G and Mrf 4) were observed at T3; finally, the addition of EVs at T5 induces the expression of Atrogin 1 and Murf 1 increase at T5, suggesting that EVs trigger damage to muscle cells. Further studies are necessary to obtain a more comprehensive understanding of the underlying mechanisms involving EVs in driving muscle atrophy in ALS.

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THE MEDICINAL LEECH AS A MODEL TO STUDY THE EFFECTS OF NANO AND MICROPLASTICS ON TISSUE REGENERATION

N. Paris[1], C. Bon[1], A. Maretti[1], N. Baranzini[1,2], L. Pulze[1,2], L. Izzo[1], S. Pragliola[3], A. Grimaldi[1,2]

[1] Department of Biotechnology and Life Sciences, University of Insubria, Varese; [2]ILFARM S.r.l., Varese; [3]Department of Chemistry and Biology, University of Salerno and INSTM Research Unit, Salerno, Italy
E-mail: nparis@studenti.uninsubria.it

Plastics are a heterogeneous group of man-made compounds that offer a wide range of applications in the industrial, civil and commercial sectors due to their unique characteristics of lightness, strength and malleability. However, these materials are difficult to dispose of and recycle and have a negative environmental impact. In fact, when plastics are dispersed in nature, they do not decompose into biodegradable molecules or compounds; rather, they degrade into tiny particles known as microplastics (MP) and nanoplastics (NP)[1]. These particles have been shown to have harmful effects on both terrestrial and aquatic ecosystems, as they can accumulate in tissues and enter the trophic chain. With regard to the various types of plastic, polypropylene (PP)[2] is one of the most prevalent and extensively utilised in the food and textile industries for disposable packaging and the production of surgical masks. However, few studies have been conducted on its toxicological effects. Given the extensive distribution and consequent abundant presence of PP waste products in terrestrial and aquatic ecosystems, there is a pressing need to investigate the toxicity of PP-MPs and NPs on living organisms. The objective of this project was to assess the impact of PP-MP and NP on tissue regeneration processes in the freshwater invertebrate model *Hirudo verbana*[3]. Following injury, leeches were exposed to NP and MP and examined at 24 h, 48 h, 72 h and 1 week post-injury. Samples were analysed using light and electron microscopy and immunofluorescence techniques. Our results demonstrate that, in leeches subjected to injury and treated with PP-MP and NP, the formation of fibrotic tissue at the site of injury is observed. This effect might be attributed to the excessive deposition of collagen, which impairs the correct muscle tissue regeneration.

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NEW INSIGHTS IN THE EPITHELIAL-TO-MESENCHYMAL TRANSITION IN BREAST CANCER PROGRESSION

E. Peri[1], M. Buttacavoli[2], C. D'Amico[1], S. Marino[1], F. Vaglica[1], I. Pucci-Minafra[3], E. Roz[4], S. Feo[1,3], P. Cancemi[1,3]

[1]STEBICEF Department, University of Palermo, Palermo; [2]BIND Department, University of Palermo, Palermo; [3]Experimental Center of Oncobiology, Palermo; [4]Oncological Hospital La Maddalena, Palermo, Italy
E-mail: emanuela.peri@unipa.it

The epithelial-to-mesenchymal transition (EMT) is a complex process pivotal in embryonic development, wound healing process but also in tumor progression, particularly in breast cancer (BC), a heterogeneous disease in which patients diagnosed at the same stage of the disease show different clinical responses and survival periods. In BC progression, the EMT enhances cell survival and helps cancer cells acquire metastatic behaviours by losing epithelial phenotypic characteristics and acquiring mesenchymal traits[1,2]. To better understand the biological significance of the EMT in BC, western blot analysis was performed on BC biopsy fragments (n=95), focusing on Vimentin and E-cadherin expression, markers of the mesenchymal and the epithelial phenotype respectively. A high variability of expression of both proteins was detected among patients, also in terms of the presence of different forms. Interestingly, quantitative analysis revealed a positive correlation between Vimentin and E-cadherin expression levels suggesting an incomplete EMT pattern could occur. Moreover, to establish if the sera of BC patients were differently able to affect cell migration, the MDA-MB-231 cells were used as a prototype of a highly proliferative and metastatic tumor model. The migratory ability was investigated by wound healing assay at different time stages of treatment with 1% sera from BC patients (n=15) compared to 1% Fetal Bovine Serum (FBS) treatment. Interestingly, the migratory capability of MDA-MB-231 cells was differentially affected by sera treatment, although even the FBS treated cells showed complete wound closure within 24 h. The correlation between the migratory ability and the EMT pattern should be evaluated to clarify the nature and aggressiveness of BC and how these hallmarks can be used for patients' stratification. Further studies on the connection between EMT markers and BC will contribute to identifying the clinical significance of this heterogeneity.

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INSIGHTS INTO REPRODUCTIVE DYNAMICS AND AGE STRUCTURE OF EUROPEAN ANCHOVY (*ENGRAULIS ENCRASICOLUS*) IN THE SOUTHERN TYRRHENIAN SEA

M. Peruzzi[1], D.Ciorciaro[1-2], M. Karam[1], R. Romano[1-2], F. Ferrigno[1], L. Appolloni[1], P. Simoniello[1-2], G.F. Russo[1-2]

[1]Department of Sciences and Technology, University Parthenope, Napoli, Italy; [2]PhD Programme UNESCO Chair "Environment, Resources and Sustainable Development".
E-mail: miriana.peruzzi@studenti.uniparthenope.it

The European anchovy (*Engraulis encrasicolus*) stands out as one of the most important fisheries resources in Campania region (Southern Italy). With the aim of promoting the sustainable

exploitation of this fish stock, the study explores: species reproductive characteristics, their age structure and the relationships with environmental factors, highlighting the importance of ecosystem consideration for a sustainable use of marine biological resources. Monthly samples of anchovy, coming from professional fisherman of Campania region (south Tyrrhenian Sea) were collected throughout the period 2022-2023. For each sample, individual biometrics and macroscopic reproductive phases (evaluated through gonads investigation[2]) were assessed, confirmed by a sub sample microscopic analysis. Monthly distribution of the Gonado-Somatic Index (GSI) has been calculated[3] to identify the seasonality of the reproductive cycle. Age and growth were estimated by analysing the saggitta otoliths extracted from each length-class identified[3], allowing for the estimation of the size-at-first maturity (L_{50}) and the length-at-age distribution. Finally, the biological indices have been correlated with the oceanographic parameters extrapolated for the study area. Our findings revealed that the spawning season of this species extends from May to September and that the sharp increase of the Sea Surface Temperature is probably the trigger for the beginning of the spawning. The value of size at first maturity and the analysis of age structure showed that juveniles (less than 1 year) could be active spawners supporting the need for a more consideration of nursery areas and minimum landing size in the management of this resource along the area.

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LOCALIZATION OF GERMLINE DETERMINANTS AND MITOCHONDRIAL MARKERS DURING OOGENESIS IN *POECILIA RETICULATA* (*CYPRINODONTIFORMES)*

G. Piccinini[1]*, M. Iannello[1]*, M. Lazzari[1], P. Cacialli[1], V. Franceschini[1], M.G. Maurizii[1], L. Milani[1]

*[1]Department of Biological, Geological, and Environmental Sciences, University of Bologna, Italy. *Equal contribution*
E-mail: giovanni.piccinini5@unibo.it

A set of homologous genes is associated to germline determination and differentiation in almost all Metazoa[1]. However, modes and times of their expression are variable, implying that investigation of their expression patterns in a diverse set of animals is necessary to thoroughly characterize animal germline. Interests also focused on mitochondria interactions with germline determinants, both functionally and positionally[2]. Roles of mitochondria in germline-related molecular pathways have been assessed[3], but mechanisms and degree of involvement remain obscure. In light of this, we aimed to characterize the expression of multiple markers during oogenesis of the viviparous guppy *Poecilia reticulata*, expanding germline knowledge in fish, so far mostly limited to the distantly related zebrafish. At confocal microscopy, the expression of the germline marker Vasa during early oocyte differentiation was characterized by an evident continuous perinuclear localization. Perinuclear cytoplasmic granules (nuage) are a common feature of animal germ cells. However, scaffold proteins often involved in nuage assembly (TDRKH and TDRD7) did not reveal patterns similar to Vasa in our samples, suggesting granules may be assembled by different factors. Nuage can be intimately associated to mitochondria, thus we looked for the expression of mitochondrial outer membrane markers. The ubiquitous TOMM20 showed peak expression in late oogonia and early oocytes, with a diffused perinuclear position. Interestingly, the same stages were the only sites where we could observe PLD6, a protein with roles in mitochondrial fusion and germline-related piRNA pathway. PLD6 was expressed in strongly stained perinuclear granules, suggesting that: (1) mitochondrial network dynamics may be enriched for fusion in that specific time and space; (2) the piRNA pathway, a genomic defence from retrotransposon mobilization, may be enriched in the sensible stage of meiosis onset.

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THE MEDICINAL LEECH *HV*RNASET2 ENZYME AS A PROMISING MOLECULE DURING TISSUE REGENERATION

L. Pulze[1,2], G. Marcolli[1], A. Grimaldi[1,2], F. Acquati[1,2], P. Campomenosi[1], N. Baranzini[1,2]

[1]Department of Biotechnology and Life Sciences, University of Insubria, Varese; [2] Italian Leech Farm (ILFARM) Srl, Varese, Italy
E-mail: laura.pulze@uninsubria.it

In biotechnological research, the identification of new molecules to advance regenerative therapies is of primary interest. A crucial aspect of wound repair and healing involves fibroblasts activation and proliferation. These cells play a pivotal role in tissue remodeling. Firstly, they are the main producers of collagen and other extracellular matrix components; moreover, they physically support other cells in performing their biological functions[1]. Additionally, fibroblasts exhibit remarkable versatility, taking part in the expression of molecular effectors, such as growth factors and cytokines, and resulting directly implicated in the regulation of tissue homeostasis and regeneration. Of note, these cellular and molecular regulators, involved in tissue remodelling and regeneration, are highly evolutionary conserved, allowing to use low-complexity eukaryotic species as emerging experimental models. Indeed, during the last years, the leech *Hirudo verbana* has been promoted as a novel invertebrate model for studying muscle regeneration[2]. In this context, our previous data demonstrated that the *Hv*RNASET2 enzyme, identified in the leech *H. verbana*, showed a marked ability to induce collagen 1 production in human MRC5 fibroblasts, with a significant increase in COL1α1 gene expression[3]. Moreover, the injection of *Hv*RNASET2 in the leech body wall stimulated, in a short time, fibroblast proliferation and activation, resulting in the deposition of spatially-organized collagen fibers that form a robust scaffold[4]. Based on these findings, here we analyzed the molecular mechanisms through which *Hv*RNASET2 regulates this process, with a focus on identifying all its potential interactions with the specific effectors involved in collagen pathway and tissue remodeling. In particular, we focused our attention on TGFβ-SMAD3 signaling pathway.

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DOG AS A SENTINEL FOR MALE INFERTILITY RISK: MORPHOLOGICAL ANALYSIS OF TESTIS RELATED TO ENVIRONMENTAL POLLUTION IN CAMPANIA REGION

L. Riccio[1], E. Di Napoli[1], M. Piscopo[2], L. Montano[3], N. Ambrosio[4], A. Spada[4], M. De Falco[2], O. Paciello[1], L. Rosati[2]

[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II; [2]Department of Biology, University of Naples Federico II; [3]Andrology Unit and Service of Lifestyle Medicine in UroAndrology, ASL Salerno, Oliveto Citra Hospital; [4]ASL Napoli 2 Nord, DS 45 Caivano, Italy
E-mail: lorenzo.riccio@unina.it

Endocrine-disrupting chemicals (EDCs) impair testicular steroidogenic enzymes, Sertoli and Leydig cells function, and integrity of the blood-testis barrier (BTB) [1]. Humans and dogs share the same environment, resulting in exposure to ubiquitously distributed EDCs. In this view dogs can be used as sentinels for human infertility risk to demonstrate if a direct cause-effect exists[2]. Our study aimed to evaluate the testicular histology of dogs from different areas of the Campania Region, correlating the findings with EDCs levels from environmental biomonitoring activities. We examined 45 puberal male dogs from 3 levels of EDCs impact area: 15 dogs from low impact area (group A), 15 dogs from medium impact area (group B), and 15 dogs from high impact area (group C). For each case, both testicles were collected and processed for routine histology. By histomorphometric analysis, we evaluated the morphology of seminiferous tubules and interstitial microenvironment; by immunohistochemistry, we evaluated key enzymes and proteins for proper spermatogenesis: 17-β-HSD, indicative of potential interference with the endocrine axis; Connexin-43, indicative of BTB loss integrity and PCNA, indicative of alterations in spermatogonia proliferation. Our results showed that cases from most contaminated areas had, according to the literature, histomorphological alterations correlated to EDCs exposure. The altered levels and localization of enzymes, BTB proteins, and markers of proliferation seen in our cases draw attention to the abnormal involvement of estrogenic-like mechanisms which could be associated with EDCs exposure. While considering the study limits, our study represents the first case of histological comparison between dog testis from different areas of the Campania region.

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EXPRESSION OF RSPH6A IN THE FIRST WAVE OF RAT SPERMATOGENESIS AND OXIDATIVE STRESS CONDITIONS: ATTENUATION BY MELATONIN

M.Z. Romano[1], M. Venditti[1], I. Messaoudi[2], S. Minucci[1]

[1]Department of Experimental Medicine, University of Campania L. Vanvitelli, Napoli, Italy; [2]Higher Institute of Biotechnology, University of Monastir, Monastir, Tunisia
E-mail: mariazelinda.romano@unicampania.it

Exposure to environmental pollutants, in addition to genetic and hormonal causes, is one of the main causes of infertility. Among the environmental contaminants, cadmium (Cd), due to its intrinsic toxicity and endocrine disruptor activity[1], can alter male fertility with implications on spermatozoa (SPZ) quantity and quality, including motility. Several factors are involved in sperm motility, among them is the radial spoke head 6 homolog A (RSPH6A), a testis/SPZ-specific protein located in the flagellar axoneme. Our previous studies showed a compromised expression and localization of RSPH6A in human SPZ from patients affected by myotonic dystrophy type 1, as well as in human SPZ exposed to a prooxidant environment. Here is reported the expression and localization of RSPH6A protein during the first wave of rat spermatogenesis and in oxidative stress conditions. Western blot results showed the absence of the protein at 7 and 14 postnatal days (PND), while its significant increase was observed from 21 PND, concomitantly to the appearance of I spermatocytes (SPC), up to 60 PND. Immunofluorescence analysis revealed that RPSH6A localization is restricted to I and II SPC, spermatids, and luminal SPZ. In addition, *in vivo* treatments with Cd and/or melatonin (MLT), an antioxidant free radical scavenger, administered alone or in combination, were performed to evaluate the effects of their exposure on the expression and localization of RSPH6A in the rat testis and SPZ. Results showed a significant decrease in RSPH6A protein level in both the testis and SPZ of Cd-treated rats. Interestingly, when MLT was given together with Cd, it can counteract its damaging effects on testis and mature gametes. The combined data suggest that RSPH6A contributes to the onset of fertility by acting on sperm motility, raising the possibility of using it as a new marker for fertility in the general population.

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EFFECTS OF BISPHENOL A ON TRANSPOSABLE ELEMENT ACTIVITY IN ZEBRAFISH EMBRYO

E. Tittarelli[1], E. Carotti[1], F. Carducci[1], M. Barucca[1], A. Canapa[1], M.A. Biscotti[1]

[1]Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy
E-mail: edith.tittarelli@iusspavia.it

Bisphenol A (BPA) is a synthetic monomer used in the production of polycarbonate plastics, epoxy resin linings of canned foods and beverage containers, dental sealants, and thermal receipt paper. It is known that BPA is an estrogenic compound and its ubiquitous dispersion poses an environmental problem as it is released as a contaminant from numerous products, raising concerns due to its potential adverse effects on aquatic organisms and human health. To study the effect of BPA on aquatic species, many studies employing the model organism *Danio rerio* have been conducted. Transposable elements constitute a significant portion of eucaryotic genomes and play pivotal roles in genome evolution, gene regulation, and genomic diversity. Moreover, several papers have reported a TE response in relation to environmental organic pollutants. In the zebrafish genome, transposable elements (TEs) constitute a significant portion of the genomic landscape, 55% of total genome. Information about their activity in response to BPA exposure in zebrafish embryos are scarce. In this context, we investigated the activity of TEs and genes involved in their silencing mechanisms in zebrafish embryos exposed to different BPA concentrations (0.1, 1 and 4 ppm). Regarding the total TE transcriptional contribution, a slight increase was observed when embryos were exposed to higher BPA concentrations. This finding was in line with the expression levels of silencing genes. Interestingly, LTR and SINE retroelements showed significant changes in their transcriptional activity in all tested conditions.

ENVIRONMENTAL TEMPERATURE VARIATION AFFECTS BRAIN LIPID COMPOSITION IN ADULT ZEBRAFISH (*DANIO RERIO*)

E. Maffioli[1], S. Nonnis[1,2] ,F.Grassi Scalvini[1], A. Negri[1], G. Tedeschi[1,2], F. Frabetti[3], M. Toni[4]

[1]Department of Veterinary Medicine and Animal Science (DIVAS), Università degli Studi di Milano, Lodi; [2]CRC "Innovation for Well-Being and Environment" (I-WE), Università degli Studi di Milano, Milano; [3]Department of Medical and Surgical Sciences - DIMEC, University of Bologna, Bologna; [4]Department of Biology and Biotechnologies "Charles Darwin", Sapienza University, Rome, Italy
E-mail: mattia.toni@uniroma1.it

Fish, being heterothermic animals, are particularly susceptible to thermal fluctuations. Recent investigations by our research team revealed that acute (4 days) or chronic (21 days) thermal treatments at 18°C or 34°C significantly affect the nervous system of adult zebrafish, resulting in notable changes in both brain proteome and behaviour when compared to the control condition (26°C)[1-4]. Proteomic analyses unveiled altered expression patterns of proteins implicated in critical cellular processes such as cytoskeletal organisation, mitochondrial regulation, synapse function, and neurotransmitter release at the two thermal extremes, supporting a neurotoxic effect of temperature variation[5-6]. These neural impairments manifest with observed alterations in locomotor activity and cognitive functions at both 18°C and 34°C. Notably, distinct effects were observed, with anxiety-like behaviours prevalent at 18°C and reduced anxiety and heightened boldness at 34°C. In this study, lipidomic analyses were conducted to explore whether chronic thermal treatment also perturbs the brain's lipid composition. Consistent with prior investigations, the findings indicate disparate impacts of low and high temperatures, with exposure to 34°C demonstrating a more pronounced effect characterised by significant elevations in ceramide, sphingomyelin, and ganglioside levels, alongside reductions in phosphatidylethanolamine and monogalactosyl diglyceride levels. The lipidomic profile further corroborates the neurotoxic consequences of thermal variation in zebrafish.

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EFFECTS OF SINGLE EMBRYO CULTURE IN A CONFINED ENVIRONMENT ON THE DEVELOPMENTAL COMPETENCE OF BOVINE EMBRYOS

A. Travaglione, A. Candela, V. De Gregorio, V. Genovese, M. Cimmino, R. Talevi, R. Gualtieri

Department of Biology, University of Naples Federico II, Napoli, Italy;
E-mail: angela.travaglione@unina.it

In mammals, *in vitro* developmental rates in single embryo culture are suboptimal compared with group culture[1], due to a lower concentration of autocrine signalling molecules (ASM) that exert positive effects on embryo development[2]. Attempts to increase the ASM concentrations during single embryo culture by reducing the volume of the drops under oil were hampered by the detrimental effects of unwanted solute exchanges between oil and medium. Currently, there is a trend toward individual embryo culture that could allow the determination of embryo competence through metabolomic, proteomic and non-invasive preimplantation genetic tests on spent media[3]. Our study explores the effect of different culture condition, focusing on the influence of confined microwell single culture (MS) and semi-confined microwell group culture (MG) compared to conventional group culture (GC). We analyze blastocyst rates, stages, and quality in terms of cell number and percentage of TUNEL-positive cells. Our results, indicate that blastocyst rates in MS were significantly higher compared to MG. MS culture shows non-detrimental outcomes compared to GC. Notably, the percentages of TUNEL-positive cells are significantly lower in MS and MG compared to GC. Our findings demonstrate for the first time that bovine embryos singularly cultured for 7 days in microwells in an extremely reduced medium volume had improved blastocyst rates and competence. Confined culture (MS) in microwells could increase the concentration of positive autocrine embryotrophic factors without increasing toxic exchanges between the oil and the medium. Overall, our findings support the feasibility and effectiveness of individual embryo culture as a promising approach for optimizing *in vitro* embryo development.

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UNRAVELING THE MOLECULAR MECHANISMS OF MICROPLASTICS INTERNALIZATION AND THEIR INTRACELLULAR IMPACT

A. Trianni[1], A. Brossa[2], F. Catalano[3], A. Saraceni[1], S. Bongiovanni[1], G.R. Merlo[2], E. Cottone[1], P. Bovolin[1]

[1]Department of Life Sciences and Systems Biology, University of Turin, Turin; [2]Department of Molecular Biotechnology and Health Sciences, University of Turin; [3] Electron Microscopy Facility, Italian Institute of Technology, Genoa, Italy

In recent years, microplastics (MPs) in our environment has become a critical concern. These tiny plastic particles, measuring less than 5 millimeters, result from the fragmentation of larger plastic debris due to UV-light-induced oxidation, mechanical abrasion, and temperature fluctuations. Nowadays plastics, have led us into what many refer to as "Plasticene" since their mid-20th century

discovery[1]. Despite widespread recognition of the detrimental effects of MPs, including impacts on fertility, hepatic function, immune response, and gene expression patterns, understanding their cellular-level effects remains challenging due to their diverse characteristics, including size, composition, and target specificity. In this work we aim to elucidate the molecular mechanisms underlying the internalization of MPs of different sizes (5 and 0.5 microns), using distinct cell cultures: GT1-7 (human hypothalamic neurons) and 3T3-L1 (murine pre-adipocytes). First, we assessed the toxicity of both types of MPs and we found no significant effects on cell viability for either cell line, except for a slight reduction at the highest concentration in GT1-7 cells. Using fluorescence microscopy, we found that larger particles exhibit minimal cellular uptake compared to their smaller counterparts. To understand the predominant internalization mechanism, we conducted inhibition experiments on macropinocytosis, clathrin-dependent and caveolin-dependent endocytosis *via* flow cytometry. Then, combining optical and electron microscopy techniques, we examined the intracellular localization of MPs. This allowed us to identify interactions between MPs and intracellular organelles, such as lysosomes and mitochondria, potentially compromising cellular health. Through this research we hope to enhance our understanding of MPs actions at the cellular level. Furthermore, our findings will inform strategies for mitigating the impact of MPs on both human health and the environment.

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NEW BIOMARKERS FOR THE ASSESSMENT OF THE PRESENCE OF MICROPLASTICS IN LOGGERHEAD SEA TURTLE EMBRYOS (*CARETTA CARETTA*)

E. Trotta[1], G. Chemello[1], V. Notarstefano[1], L. Papetti[2], L. Di Renzo[3], M. Matiddi[4], C. Silvestri[4], O. Carnevali[1], G. Gioacchini[1]

[1]Università Politecnica delle Marche; [2]CRTM tartAmare Onlus; [3]Istituto Zooprofilattico Dell'Abruzzo e Del Molise "G. Caporale"; [4]Istituto Superiore per La Ricerca e La Protezione Ambientale (ISPRA), Italy.
Email: e.trotta@pm.univpm.it

The loggerhead sea turtle (*Caretta caretta*) faces various anthropogenic threats during its long life cycle. The potential toxicity of contaminants, particularly marine litter, garnering significant scientific attention due to its potential impact on the survival of this species worldwide[1]. Therefore, *C. caretta* has been selected as the official bioindicator for Descriptor 10 "Marine Litter" within the European Union's Marine Strategy Framework Directive[1]. Microplastic (MPs) presence may impair the health status of sea turtles, yet during their embryonic phase, which is one of the most vulnerable stages of their lifecycle. Hence, this study aimed to examine the presence of MPs in unhatched embryos. Out of 180 unhatched eggs collected in nests laid along the Tuscany coast (Italy) were sampled, only 17 embryos at stage 30 of development were suitable for MPs extraction and subsequent analysis. MPs were extracted via alkaline digestion, and Raman microspectroscopy was conducted to characterize them in terms of abundance, size, shape, color, and polymer composition. All identified MPs were smaller than 5µm,predominantly consisting of spheres and fragments, with the main polymers being Acrylonitrile butadiene styrene, polyvinyl chloride, and polyethylene. Moreover, the study investigated the effects of MPs on embryo health by evaluating selected biomarkers indicative of stress (melanomacrophages, MMs, and cortisol BP levels in livers), inflammatory processes (IL-1β level in livers), and exposure to contaminants (CYP4501A1 level in livers) *via* histology and immunofluorescence. Statistical correlation analysis confirmed the effects of MPs on embryo health status, particularly highlighting the strong correlation between MPs presence and MMs and IL-1β expression. Further studies could enhance understanding of the potential impacts of MPs and associated pollutants on embryonic development and establish standardized protocols for future assessments.

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NEW PROTEOMIC INSIGHTS INTO MULTIPLE SCLEROSIS FROM CEREBROSPINAL FLUID

F. Vaglica[1], M. Buttacavoli[1], S. D. Scilabra[3], M. Lo Pinto[3], E. Peri[1], C. D'Amico[1], S. Marino[1], F. Geraci[1], G. Salemi[2], P. Cancemi[1]

[1]STEBICEF Department, University of Palermo, Italy; [2]BIND Department, University of Palermo; [3]Proteomics Group, Department of Research, ISMETT-IRCCS, Ri.MED Foundation, Palermo, Italy
E-mail: fabiola.vaglica@unipa.it

Multiple sclerosis (MS) stands as the foremost chronic inflammatory and autoimmune disorder of the central nervous system (CNS). In MS, the disruption of the blood-brain barrier (BBB) facilitates the infiltration of immunocompetent cells into the CNS parenchyma, leading to neuroinflammation and neuronal damage.[1] Although the environmental and genetic factors encourage its development, the cause is not known yet. Gelatinases MMP-2 and MMP-9 have been implicated in various aspects of MS pathology, including BBB breakdown.[2] We previously investigated the differential activity of gelatinases *via* zymographic analysis in cerebrospinal fluids (CSFs), comparing 49 MS patients to 27 individuals diagnosed with other neurological disorders (referred to as neurological controls, NC). Surprisingly, reduced activity of MMPs was observed in MS patients compared to NC, with a significantly lower expression of MMP-2 active form in MS. Further exploration of the clinical implications of these findings is warranted. For instance, preliminary assessments of the inflammatory status of MS patients by evaluating C-reactive protein (CRP) levels was performed, to establish potential correlations with MMP activity. Here a proteomic analysis to compare CSF samples from 15 MS patients and 12 NC, was performed. A total of 892 proteins were identified. Among the differentially expressed proteins an interesting upregulation of IGFBP-2 in MS patients and a concomitant downregulation of IGF-1 was detected. IGF-1 in the CNS acts as a potent trophic factor for neurons and glial cells promoting cell proliferation, survival, and differentiation. Moreover, IGF-1 has been shown to be a potent neuroprotective agent that supports the survival and production of myelin by oligodendrocytes. The complex regulation of IGF-1 in CNS is not yet fully defined and deserves thorough exploration, because it has potential in treating of MS.

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