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European Journal of Histochemistry
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The *European Journal of Histochemistry* was founded in 1954 by Maffo Vialli and published till 1979 under the title of *Rivista di Istochimica Normale e Patologica*, from 1980 to 1990 as *Basic and Applied Histochemistry* and in 1991 as *European Journal of Basic and Applied Histochemistry*. It is now published under the auspices of the University of Pavia, Italy.
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table of contents

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MAIN LECTURES

ENDOCRINE DISRUPTORS AND DEVELOPMENT

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Endocrine disruptors (ED) are a diverse ensemble of chemicals (persistent pollutants, pesticides, plasticizers, plant toxins, etc.) that cause adverse health by altering the endocrine signalling network[1]. Since hormone are critical to regulate developmental processes, the prenatal lifestages are highly susceptible to ED exposure levels that may be of little concern for adults[2]. ED are involved in the pathogenesis of congenital anomalies (*e.g.*, hypospadias) and mainly in the developmental origins of adult diseases, including metabolic syndrome[3]. A main question is how ED may affect development. With the help of the Adverse Outcome Pathway conceptual framework[4], we can highlight some major clusters of mechanisms: i. direct alteration of relevant endocrine signalling, such as the impaired steroid signalling leading to cryptorchidism and hypospadias, or the inhibition of thyroperoxidase leading to impaired neurodevelopment; ii. interfering with the utilization of biological factors essential for prenatal development, such as iodine or retinoids[5]; iii. altered endocrine regulation of placental function leading to adverse embryo-foetal effects; iv. epigenetic reprogramming[6] leading to the altered expression of key factors for cell replication or differentiation and increased risk of long-term effects, *e.g.*, cancer, obesity. The search for ED mechanisms supports an evidence-based risk assessment of ED environmental exposures as well as a better understanding of the pathogenesis of human developmental disorders.

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THE GENETIC AND ENVIRONMENTAL ARCHITECTURE OF BRAIN DISEASES: LOCALIZING THE "WHERE" AND "WHEN" THROUGH BRAIN ORGANOIDS AT SINGLE CELL RESOLUTION

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ABSTRACTS

THE CHALLENGE OF TEACHING PRACTICAL MORPHOLOGY CLASSES IN THE COVID-19 ERA

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Teaching in the academic year 2020/21 was heavily affected by the COVID-19 pandemic, with students having restricted or no access to the classrooms and laboratories of their universities. Distance learning replaced traditional classes and presented challenges to teachers and students as well. While theoretical lectures could be delivered through online platforms maintaining their traditional structure, practicals posed a harder challenge. Since online learning is becoming more and more important regardless of medical emergencies, we present here the experience of our group in delivering remote laboratory classes for the courses of Histology and Comparative Anatomy, hoping it will be helpful for other educators. We aimed at giving our students a laboratory experience as engaging and formative as possible. We purchased a slide digitization system that allowed us to generate high-quality zoomable digital slides that our students could access through the web. This way, each student could actively explore each specimen at their own pace, helped by the teachers' pin notes, and even take snapshots. Overall, the experience proved to be as close as possible to the use of a real microscope and was generally very positively evaluated by our students in the satisfaction surveys. Concerning Comparative Anatomy practicals, we organized interactive streaming sessions where the students could provide themselves with inexpensive, easily obtainable specimens and perform dissections with their own hands under the supervision of the teachers as if in the laboratory. We also resorted to online 3D anatomical models, which proved to be superior to their physical counterparts. In sum, we believed the tools developed to face this challenging situation proved to be good surrogates of the activities performed in normal times. Moreover, every tool used to explain the subject to students is always available online for the students to help them during the preparation of the exam. Although the outcome of this teaching approach will be clearer in the next years, we intend to keep using some of the new tools to flank traditional teaching once the pandemic is over.

DIFFERENTIATION IMBALANCE IN HIPSC-DERIVED NEURONS CARRYING A WDR62 *DE NOVO* MUTATION

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Genome editing technologies offer promising solutions to unravel the basic mechanism that underlie genetic disorders. Moreover, the increasing application of both human induced pluripotent stem (hiPS) cells and CRISPR-Cas9 technology in disease modelling have paved the way towards the deep knowledge of neurodevelopmental disorders such as microcephaly[1]. Primary microcephaly (MCPH) is a rare neurodevelopmental disorder. Several MCPH genes are involved in diverse molecular mechanism crucial for the regulation of neocortical size during corticogenesis[2]. Mutations in WDR62 (MCPH2) cause the second most common form of MCPH and severe structural cortical malformations suggesting a key role in corticogenetic processes. Indeed, WDR62 is a centrosome-associated protein expressed in the primary germinal zone and thus implicated in maintaining the neural progenitor pool during neocorticogenesis. Recently, a novel biallelic truncating mutation in WDR62 (D955AfsX112) has been identified in two MCPH-affected siblings[3]. Initiating from patient-derived iPS cells, we successfully performed CRISPR-Cas9 genome editing, thus restoring the genetic alteration and obtaining isogenic lines as gold standard control. To delineate the functional repercussions of this alteration during the dynamic process of corticogenesis, we applied – both to patient-derived and one isogenic line – an *in vitro* Fin vitro directed neural differentiation protocol. Indeed, we focused on the main neuronal populations – neural progenitors, deep and upper layer neurons – recapitulating the main stages of neocortex development. As result, in mutant-derived lines we observed cell cycle alterations and generation timing impairment, potentially explaining incorrect layering events during patient's neocortical development, at the base of MCPH etiology.

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HUMAN NEURAL STEM CELLS TO UNCOVER TORCH-RELATED MICROCEPHALY

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Zika virus (ZIKV) outbreak posed an urgent need to unravel the molecular mechanisms involved of microcephaly induced by neurotropic pathogens of the TORCH group. Here, we aim to investigate the events during TORCH viral infection, using innovative human neural progenitor populations, called neuroepithelial stem (NES) cells, derived both from human developing tissue and induced pluripotent stem cells[1]. We focused on two main targets affecting cell cycle progression of NES cells. pTBK1, a kinase involved in the antiviral innate immune response, results delocalized from centrosomes to mitochondria, following ZIKV infection[1]. To verify whether the same mechanism could be involved in other TORCH pathogen infections, we exposed NES cells to two TORCH viruses: Herpes Simplex Virus 2 (HSV2) and Coxsackie B5 virus (COXB5). We found dramatic cytopathic effects after the infection and disruption of normal pTBK1 local-

ization, as already reported for ZIKV. Notably, pTBK1 disruption may represent a molecular event shared by different TORCH viruses at the base of microcephaly etiogenesis. Then, we focused on another player involved in neural stem cell proliferation and self-renewal, *FOXG1* gene, coding for a forebrain transcription factor and mutations of which are also associated with severe developmental disorders, including microcephaly[2]. Specifically, we investigated ZIKV impact on FOXG1 protein. We found that, following infection, FOXG1 nuclear localization is disrupted in human neural progenitor cells. Collectively, our data suggest new potential targets of congenital microcephaly.

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A MODIFIED *XENOPUS LAEVIS* APPROACH (R-FETAX) AS ALTERNATIVE TO TEST THE FOETAL VALPROATE SPECTRUM DISORDER (FVSD)

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Valproic acid (VPA) is an effective broad-spectrum antiepileptic drug recently also repositioned for new therapeutic purposes. VPA exposure during gestation has been related to the Foetal Valproate Spectrum Disorder (FVSD) in humans at therapeutic maternal plasma concentrations (280-700 M). FVSD characteristics include facial features and defects (including cleft lip/palate), neural tube defects, heart defects, genital and skeletal defects as well as developmental delays and neurological deficits. We propose an alternative totally animal-free method (R-FETAX) for FVSD evaluation: embryos were obtained by not-hormonally induced natural mating, exposed to 0-500-750-1500 M VPA (sodium salt) during the whole test period (Nieuwkoop and Faber stages NF 8-46), during the phylotypic morphogenetic window (NF 13-26) or during the key neurodevelopmental window (NF 37-46). Tadpoles were evaluated at the end of the test for morphological parameters and double stained for cartilage and Ca-rich tissues. A new R-FETAX behavioural test (swimming test) is proposed to evaluate VPA-induced neurodevelopmental disorders: tadpoles transferred into a circular arena on an under-illuminated stereomicroscope and 3'' videos were taken using a digital camera and swimming activity subsequently analysed. Concentration- related embryo-lethal effects were observed after the exposure during the entire test period; concentration-related specific teratogenic effects (craniofacial defects, neural tube defects) were observed in tadpoles exposed to VPA at NF 13-26 stages; neurobehavioral deficits were described in samples exposed at NF stages 37-46. The selection of windows of exposure allowed to minimize the embryo lethal effects and to evaluate complex pictures of the FVSD. We suggest the use of the proposed protocol as an alternative fully animal-free test in order to evaluate complex developmental spectrum disorders induced by chemicals.

HEMOCYTE DEPLETION AFFECTS CEPHALIC TENTACLE REGENERATION IN THE APPLE SNAIL *POMACEA CANALICULATA*

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Many studies have described how adult regeneration unfolds in invertebrate and vertebrate models,[1] nonetheless important gaps remain. The freshwater snail *Pomacea canaliculata* is capable of adult sensory organ regeneration.[2,3] We focused on the cephalic tentacle, which is an important sensory organ for food search, co-specific recognition, and orienting.[4] Histological studies focusing on the early cephalic tentacle regeneration, have demonstrated that wound closure and blastema formation took place within the first 24 h post amputation.[3] During this early phase, several immune-related cells, *i.e.* hemocytes, were retrieved in the blastema. Through a new protocol of computer-assisted image analysis, we could quantify a phagocytic hemocyte subpopulation in the blastema. The presence of hemocytes in the blastema was further confirmed by qPCR and fluorescent *in situ* hybridization (FISH) targeting the hemocyte marker *Pc-hemocyanin*. We then assessed whether hemocyte depletion could affect tentacle regeneration in *P. canaliculata*. Flow cytometry analysis confirmed that the injection of the phagocyte-specific drug Clophosome®[5] (45 µg/g snail) could transiently remove circulating hemocytes. Consistently, histological experiments demonstrated that few hemocytes were present in the early regenerating tentacles of Clophosome®-injected snails. To further investigate the role for hemocytes in tentacle regeneration, we combined FISH and qPCR experiments searching the blastema for the expression of hemocyte-derived cell-proliferation markers, *e.g. Pc*-Allograft Inflammatory Factor-1, *Pc*-RUNT domain containing protein (*Pc*-RUNT) and *Pc*-transglutaminase 2A (*Pc*-TGA2). A significant increase in the expression of *Pc-runt* and *Pc-tga2*, in concomitance with hemocyte accumulation during early blastema formation was found. This nurtured the hypothesis that immune-related components may play a pivotal role in adult regeneration of *P. canaliculata*.

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AMPHIOXUS NEUROGLIA: MOLECULAR CHARACTERIZATION AND EVIDENCE FOR EARLY COMPARTMENTALIZATION OF THE DEVELOPING NERVE CORD

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Glial cells play important roles in the development and homeostasis of metazoan nervous systems. However, while their involvement in the development and function in the central nervous system of vertebrates is increasingly well understood, much less is known about invertebrate glia and the evolutionary history of glial cells in metazoans. An investigation into amphioxus glia provides a window on the role of glial cells development and function at the transition between invertebrates and vertebrates, as this organism is the best living proxy for the last common ancestor of all chordates. We report our findings[1] on amphioxus glia as characterized by molecular probes correlated with anatomical data at the TEM level. The results show amphioxus glial lineages express genes typical of vertebrate astroglia and radial glia and segregate early in development, forming what appears to be a spatially separated cell proliferation zone positioned laterally, between the dorsal and ventral zones of neural cell proliferation. Our study provides strong evidence for the presence of vertebrate-type glial cells in amphioxus.

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WIF1 MEDIATED WNT SIGNALING IN HABENULAR NEURON DIFFERENTIATION AND AXONAL TARGETING

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Bilateral clusters of habenular neurons in the forebrain of vertebrates relay cognitive information into the interpeduncular nucleus and the median raphe in the ventral mid- and hindbrain, respectively. This neurotransmitter system has been implicated in behaviours from fear and social behaviour to reward responses and addiction. It is also linked to pathophysiological syndromes such as depression, autism and schizophrenia[1]. Our studies in zebrafish have revealed that the Wnt/beta-catenin signalling pathway gene Tcf7l2 is pivotal for the establishment of habenular neuron diversity[2]. We now find that premature activation of Wnt signalling delays habenular neuron differentiation, severely perturbs correct habenular neuron identities and abrogates the laterotopic segregation of habenular efferent axons in the IPN target. Our gene expression and functional analysis provide strong evidence that the secreted tumour suppressor Wnt inhibitory factor 1 (Wif1) is mediating the temporal control of Wnt signalling[3]. Once initiated, *Wif1* expression in turn depends on Wnt signalling itself similar to findings in cancer cells *in vitro* suggesting a conserved mechanism underlying different processes[4]. This knowledge enables us now to generate fish with defined aberrations in the habenulae for analysing the impact on behavior. Indeed, Tcf7l2 and Wif1, have been linked to schizophrenia and autism paving the path for further exploring the link between molecule, neural circuit and pathophysiological syndrome[5,6]. We apply *in vivo* HTS to identify candidate therapeutic compounds 1) impacting habenula development and habenular neuron differentiation and 2) having an ameliorating effect on habenular neuron malformation and malfunction.

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FUNCTIONAL INVESTIGATIONS OF MATRIX METALLOPROTEINASES IN BREAST CANCER: FOCUS ON MMP9 AND MMP2

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Breast cancer (BC) represents the most common type and the leading cause of death of cancer among females. The etiology of BC is almost complex, involving several genetic and epigenetic changes. BC is a heterogeneous disease with several subtypes of different molecular alterations, as well as clinical behavior.[1] Despite the achieved improvements, the prognosis of BC patients is still poor predictable and the identification of more reliable biomarkers is necessary to explore. Matrix metalloproteinases (MMPs) are proteolytic enzymes involved in ECM remodeling. Depending on the context, MMPs can promote or suppress several biological functions by regulating the cell surface growth factor "shedding".[2] Increased MMPs expression, promotes hallmarks of tumor progression including angiogenesis, invasion, and metastasis, and correlated with shortened survival.[3] Nevertheless, the collective role and the possible coordination of MMP members in BC are poorly investigated. Here we performed a multi-omics analysis of MMP expression in BC using data mining and experimental investigations. Several databases were used to deeply mine different expressions between tumor and normal tissues, the genetic and epigenetic alterations, the prognostic value as well as the inter-relationships with Tumor Immune Infiltrating Cells (TIICs). A special focus was deserved to MMP2 and MMP9: their activity levels was detected by zymography in a cohort of breast cancer samples including tissues and the corresponding sera. Our findings suggested that MMPs could have a high potency as targeted in breast cancer and might serve as novel biomarkers. However, further studies are needed to explore the detailed biological functions and molecular mechanisms of MMPs in BC, also in consideration of their expression and different regulation in several tissues.

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ENVIRONMENTALLY RELEVANT CONCENTRATIONS OF TRICLOCARBAN AFFECT MORPHOLOGICAL TRAITS AND MELANOGENESIS IN ZEBRAFISH LARVAE

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Human activity is responsible for producing several chemical compounds, which contaminate the aquatic environment and adversely influence the survival of aquatic species and indirectly human health. Triclocarban (TCC) belongs to the category of emerging pollutants and its presence in aquatic environment is justified by its wide use as antimicrobial agent in personal care products[1]. The concern about this chemical is due to the risk of persistence in water and soils[2] and its endocrine-disrupting effects[3]. The present study evaluated the developmental toxicity of TCC in zebrafish early-life stages starting with the assessment of acute toxicity and then focusing on the integrative analyses of the observed phenotype on zebrafish development. For this purpose, lethal and sublethal alterations of zebrafish embryos were investigated by the Fish Embryo Acute Toxicity Tests (FET tests). Subsequently, two concentrations of TCC were used to investigate the morphometric features and defects in larvae developmental pigmentation: an environmentally relevant (5 µg/L) and toxicological (50 µg/L), derived from the No Observed Effect Concentration (NOEC) value concentration. Furthermore, the expression levels of a key transcription factor for melanocyte differentiation and melanin syntheses, such as *mitfa* (microphthalmia-associated transcription factor) and *tyr* (tyrosinase) and its activity, were evaluated.
The results showed that TCC can alter larvae phenotype and influence melanogenesis process and eyes size, prompting us to further investigate on a possible correlation with its thyroid-disrupting effects[4] and eyes development.

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ALEXANDER DISEASE MODELING IN ZEBRAFISH: AN *IN VIVO* SYSTEM SUITABLE TO PERFORM DRUG SCREENING

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Alexander disease (AxD) is a rare astrogliopathy caused by heterozygous mutations in the glial fibrillary acid protein (GFAP) gene[1], encoding the glial intermediate filament, that make the protein prone to form aggregates that, together with HSP27, aB-crystallin, ubiquitin and proteasome form Rosenthal fibers, which are cytotoxic. Both *in vitro* models of AxD and *in vivo* transgenic mice models of AxD, suffer from limitations in studying this disease. Zebrafish is commonly adopted for studying nervous system development and neurodegenerative diseases. The aim of this study has been the production of a zebrafish model for AxD, based on Tol2 transposon approach, to have a system more complex than cell cultures and more reliable than mice models. Zebrafish embryos were microinjected with pTol2-GFAP WT-GFP and pTol- GFAP(R239C)-GFP plasmids encoding WT or mutant GFAP fused to GFP, whose expression in glial cells was driven by the promoter of the zebrafish *gfap* gene. We confirmed the glial localization of aggregates by immunofluorescence and TEM, more frequent in cells expressing mutant than WT GFAP. Our results showed the positive effects of both ceftriaxone treatments[3] and sHSPs stimulation on mutant embryos p.R239C in terms of GFAP aggregates reduction. Moreover, by Microelectrode array platform we observed a significant decrease in the head network burst duration and rate of GFAP R239C compared with control. Overall, we propose zebrafish as a powerful model for both the study of the molecular pathogenesis and for drug screenings for AxD.

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NEUROBEHAVIOURAL, HISTOLOGICAL, PHYSIOLOGICAL AND GENE EXPRESSION ALTERATIONS IN ZEBRAFISH BRAIN EXPOSED TO ALUMINIUM

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Aluminium (Al), an extremely widespread but non-essential metal, is considered dangerous for both the environment and human health[1]. It is also indicated as a possible etiological factor in neurodegenerative diseases[2], although its neurotoxic role is still not completely understood. In this study, zebrafish was used as a model organism, being useful for both ecotoxicology studies and for exploring neurodegenerative diseases[3]. Adult zebrafish were exposed to 11 mg/L Al and the swimming ability and the behavioural responses were assessed at 10, 15 and 20 days of exposure. These parameters were correlated with the amount of Al within brain, activated antioxidant-defences and changes in metabolism and neurotransmission. Furthermore, its neurotoxic role was further investigated by evaluating induced neurodegeneration and gene expression of markers involved in the parkinsonism. Behavioural and locomotory responses suggested an increase in the anxiety, especially in animals exposed to Al for 15

days, when the neurodegeneration and accumulation of Al in the brain were also evident. The activity of antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase and metallothioneins levels increased after short-term exposures (10-15 days) and tended to decrease or stabilize over longer times (20 days), however, the reactive oxygen species increased in a time-dependent trend. Finally, the expression of genes linked to Parkinsonism was also influenced by exposure to the metal, with an evident greater impact after short periods of exposure. Overall, the results contribute to understand the neurotoxic mechanisms activated by Al highlighting correlations between behavioural disorders, oxidative state and neurodegenerative processes.

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THE NEWT *CYNOPS ORIENTALIS*, A SPECIES WITH A GIANT GENOME: FOCUS ON TRANSPOSABLE ELEMENT ACTIVITY AND GENES INVOLVED IN THEIR SILENCING

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Genome size varies considerably across eukaryotes and it is not correlated neither with the number of genes, nor with the morpho-functional complexity of a species. Besides having a significant impact on the number and the size of introns, as well as on the placement of regulatory regions, genome size is profoundly influenced by the relative abundance and activity of transposable elements (TEs). Caudata is an order of amphibians with great variation in genome size, which can reach enormous dimensions in salamanders. In this work, we analysed the activity of TEs in the transcriptomes obtained from female and male gonads of the Chinese fire-bellied newt, *Cynops orientalis*, a species with a genome about 12-fold larger than the human genome. We also compared these data with genomes of two basal sarcopterygians, coelacanth and lungfish. In the newt our findings highlighted a major impact of non-LTR retroelements and a greater total TE activity compared to the lungfish *Protopterus annectens*, an organism also characterized by a giant genome. This difference in TE activity might be due to the presence of young copies in newt in agreement also with the increase in the genome size, an event that occurred independently and later than lungfish. Moreover, the activity of 33 target genes encoding proteins involved in the TE host silencing mechanisms, such as *Ago/Piwi* and *NuRD* complex, was evaluated and compared between the three species analysed. These data revealed high transcriptional levels of the target genes in both newt and lungfish and confirmed the activity of NuRD complex genes in adults. Our results confirmed that the gigantism of the newt genomes may be attributed to the activity and accumulation of TEs.

TRANSPOSABLE ELEMENTS AND GENES INVOLVED IN THEIR SILENCING MECHANISMS IN DIADROMOUS FISH SPECIES

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Transposable elements (TEs) represent a considerable fraction of eukaryotic genomes and they might contribute to genome size, chromosomal rearrangements, and to the generation of new coding genes or regulatory elements. An increasing number of works have reported a link between the genomic abundance of TEs and the adaptation to specific environmental conditions. Diadromy represents a fascinating feature of fish, protagonists of migratory routes between marine and freshwater for reproduction. In this work, we investigated the available genomes of catadromous and anadromous ray-finned species. The relative contribution of different TE types of these species showed clear differences between catadromous and anadromous fish regarding SINE retroelements. Statistical analyses support a correlation between a higher SINE retroelement content observed in catadromous and their migratory behavior. The activity of TEs and genes involved in their silencing mechanisms (NuRD complex) was evaluated in available transcriptomic data of *Anguilla marmorata* (catadromous species) and *Onchorhynchus keta* (anadromous species) after treatments at different salinity. The anadromous species showed no differences between tested conditions, in the case of *A. marmorata* the transcriptional activity of TEs and NuRD complex genes decreased at higher salinity values. The findings obtained in *Anguilla* might be related to the substantial environmental changes faced by this species during its migratory route differently from *O. keta*.

ANTEDON MEDITERRANEA AS NOVEL MODEL ORGANISM IN EVODEVO

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Despite their key phylogenetic position within the echinoderm phylum[1,2], crinoids have been scarcely considered in both developmental and evolutionary studies. Indeed, these animals are usually common in deep water[3,4] and difficult to maintain in aquaria. *Antedon mediterranea* is a stalkless crinoid widely distributed in the Mediterranean and Aegean Sea. It is usually found between 15 and 80 m depth, but in areas rich in particle suspension and water currents it can migrate to the water surface. This behaviour greatly facilitates animal collection, allowing us to exploit this species to study the crinoid life cycle[5]. Particularly, crinoid neural development has always been elusive. Our understanding of crinoid nervous system development is mainly based on morphological analyses[6] while molecular approaches have been scarcely applied. In the present work, we optimized our previous techniques and protocols[5] to provide a comprehensive description of neural organization of *A. mediterranea* developmental stages. In the swimming doliolaria larva, we

observed a basiepithelial nerve plexus with different neural populations, including a serotoninergic apical organ and an anterior cluster of GABAergic neurons. The post-metamorphic nervous system differentiates early, being already present in the cystidean larva. In the juvenile phase, the pentacrinoid, analyses showed the presence of a cholinergic endoneural nervous system while the ectoneural plexus appeared more composite, displaying different neural populations. Overall, our results provide precious data about crinoid neuroanatomy and set the stage for future investigations that will allow to complete the intriguing puzzle of crinoid evo-devo neurobiology.

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EX VIVO CULTURE OF HYPERTROPHIC HEART OF ZEBRAFISH WOULD BE A POWERFUL TRANSLATIONAL MODEL TO TEST DRUGS

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Cardiac hypertrophy is the most frequent cause of sudden death in humans from heart failure and stroke. It causes an increase in the size of the heart, due both to the sarcomeric reorganization in the cardiomyocytes and to an alteration of the embryonic cardiac gene program.[1] Alterations in the expression of micro-RNA have been highlighted in conjunction with the pathology both in humans and in mouse and fish experimental models.[2-5] Furthermore, most of the genes involved in cardiovascular reorganization processes are conserved in all vertebrates and are activated during the induction of hypertrophy in both mouse and fish models.[1,6] The model of *ex vivo* cardiac hypertrophy induced by phenylephrine (PE) in zebrafish was used as a comparison to evaluate the use of chemotherapy drugs such as blebbistatin (BL). This drug has the ability to inhibit some calcium-dependent signalling pathways activated by PE. The experiments were performed on *ex vivo* culture samples treated with PE simultaneously, before or after that with BL. Gene expression analyzes conducted in qRT-PCR or immunohistochemistry of the expression of micro-RNAs or embryonic genes confirmed both the hypertrophic action of PE and the antagonistic action of BL. These data comfort us in the belief that the possibility of having an *ex vivo* research model, which can be easily manipulated and analyzed, will make it possible to use it to obtain knowledge on the transduction pathways activated by blebbistatin and the possible use of this drug in the treatment of cardiac hypertrophy in humans.

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ALKYPHENOL EXPOSURE ALTERS *PODARCIS SICULUS* SPERMATOGENESIS

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Nonylphenol (NP) and Octylphenol (OP) are persistent and non-biodegradable environmental contaminant classified as endocrine disruptor chemicals (EDCs)[1]. These compounds are widely used as several industrial applications and present estrogen-like properties which have extensively been studied in aquatic organisms[2,3]. However, the dangerous effects of EDCs have been conducted also on the terrestrial vertebrate as the reptile *Podarcis siculus.* In particular in the *P. siculus* lizard has been demonstrated that the exposure to NP can alter the function of adrenal gland[4]. The present study aimed to verify the interference of these compounds alone and in mixture on the reproductive cycle of the male *Podarcis siculus* focusing mainly on the steroidogenesis process. More recently, we have demonstrated in *P. siculus* testis, the distribution in both somatic and germ cells of all steroidogenic enzymes such as 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β- hydroxysteroid dehydrogenase (17β-HSD) and P450 aromatase[5]. In this work, male lizards have been treated with different injections of both NP and OP alone and in mixture and evaluation has been carried out using histological approach. Obtained results show that both substances are able to alter both testis histology and localization of key steroidogenic enzymes such as 3β-HSD, 17β-HSD and P450 aromatase. Moreover, OP exerts a preponderant negative effect and the P450 aromatase represents the major target of both chemicals.

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POLYCHLORINATED BIPHENYLS EFFECTS ON THYROID GLAND OF *PODARCIS SICULUS* LIZARD

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Polychlorinated biphenyls (PCBs) are organochlorine compounds with persistent and bioaccumulative properties that can accumulate in soil, plants and animals so entering in the food chain[1]. They are also classified as "Endocrine Disruptor Chemicals" (EDCs) because they can interact with several functions of endocrine system. PCB congeners are similar in thyroid hormone (TH) structure and are able to interact with TH receptors (TR) leading to destruction of the normal thyroid homeostasis[2,3]. Our study was designed to evaluate the effects of PCBs in lizards living on PCBs-contaminated soil. The soil for the terraria was taken from three areas with different concentrations of PCBs from the Bagnoli brownfield area situated into the western part of the city of Naples (Campania region), Southern Italy. The concentrations of ∑PCB in the soil were 2.55 mg/kg (low-dose: Group A), 4.31 mg/kg (medium-dose: Group B) and 7.60 mg/kg (high-dose: Group C). After 120 days, blood samples were collected to perform hormonal dosages and thyroid gland was removed and weighed for histopathological analysis. We have demonstrated that PCB-polluted soil induced a dose- and time-dependent reduction of lizard weight and a 40% of mortality in group C. Moreover, PCB pollution induced a strong increase of TRH plasma levels but a dose-dependent decrease of TSH, T3 and T4 plasma levels. Hormonal reduction was also confirmed by histological feature showing a strong decrease of thyroid epithelium height. Altogether, our results suggest that PCB pollution in the soil was able to negative affect functionality of thyroid gland with a persistent inhibition pituitary-thyroid gland.

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VANADIUM INDUCES CALCIUM DEPLETION AND CELL SELECTIVE APOPTOSIS DURING DEVELOPMENT OF SEA URCHIN EMBRYOS

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Vanadium (V) is a metal widely distributed in soil, water and air. It has recently received growing interest because its compounds are often used in different applications, from industry to medicine.[1] Here, using atomic absorption spectrometry, we demonstrate the predisposition of V to accumulate directly into embryonic cells, interfering with Ca uptake. At the morphological level, we observed dose- and time-dependent effects on phenotypes and on skeletal malformations. At the molecular level, V-exposed embryos showed the activation of the cellular stress response, inducing Hsp 60 and Hsp 70 synthesis and the activation of autophagy and apoptosis. The Hsps-mediated stress response to V appeared to counteract the damage induced by low (50 nM and 100 nM) and intermediate (500 nM and 1 μM) concentrations, while high cytotoxic doses (500 μM and 1 mM) induced more marked cell death mechanisms starting at 24 h of development, when the control embryos reached the gastrula stage.[2] Only few cells showed nuclei with apoptotic DNA fragmentation, particularly in the ectodermal layer. Mesodermal and endodermal cells did not appear to be involved in this process of selective apoptosis.[3] Microscopic fluorescence inspections indicated that primary mesenchyme cells (PMCs) were not involved in apoptotic processes; therefore, their inability to carry on the skeletogenesis could be due to the Ca depletion. These results allow us to elect the sea urchin embryo as a suitable experimental model for studying the metal-correlated cellular/molecular responses.[4,5]

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VANADIUM PERTURBS THE FERTILIZATION OUTCOME AND THE METALLOPROTEINASE ACTIVITY IN SEA URCHIN EMBRYOS

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Metal toxicology represents a current major topic due to the dispersion of these elements in the environment. Metals are released from both natural sources and industrial activities. Some of them have also a clinical interest due to their application as metallodrugs (*i.e.*, Pt, Cu, Au, Ru, and Y) or in medical diagnosis (Gd).[1,2] Recently, V derivatives are considered as potential therapeutic factors in some diseases (*e.g.*, obesity, diabetes, cancer, neurodegenerative and heart disorders). As a consequence, pharmaceutical residues could represent emerging pollutants of aquatic environments, as wastewater treatment plants do not sufficiently remove these compounds[3]. Embryonic models represent an adequate system for testing metal toxicity as they are sensitive to these elements. Here, we analysed the effects of different V concentrations, from very cytotoxic (1mM) to environmentally relevant doses (50nM), using two approaches: the fertilization test (FT) and the metalloproteinase (MMPs) activity.[4] We observed that V affected, in a dose-dependent manner, the percentage of fertilization and increased abnormalities regarding the egg and/or the fertilization membrane morphology. MMPs could represent another marker of V toxicity since it generates a cellular imbalance of metal ions. This would disturb the catalytic mechanism of these enzymes as they require ions as cofactors. Therefore, their dysfunction could represent a biomarker of metal-induced damage. We observed a total of 9 MMPs. Those with high molecular weight (from 309 to 59kDa) seemed to be mainly induced by elevated V concentrations (1mM, 500μM and 100μM). Conversely, low molecular weight MMPs (from 34 to 22kDa) appeared to be completely inhibited by these high V doses. On the other hand, lower V concentrations seemed to be more tolerated as there were no significant differences compared to control. In conclusion, FT and MMP activity could represent a reliable method to test V toxicity, using the sea urchin as a sensitive model system.

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CADMIUM EFFECTS ON GLYCAN CONTENT IN *DANIO RERIO* GUT MUCOSA

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Cadmium is a widespread environmental contaminant extremely toxic for plants and animals. By inducing oxidative damage[1], it interferes with behavior and tissue organization[2]. In the gut, it also compromises the microbiota thus reducing mucosal efficiency as barrier and causing inflammation and metabolic disorders[3]. Toxicity is particularly severe in aquatic organisms exposed from ovo to death via multiple routes, skin, gut and gills in particular[4]. In the present work we studied the effects of waterborne cadmium chloride (25 and 100 µM, 30 days) on gut mucosa of *Danio rerio*. Particular attention was dedicated to changes in glycan residues distribution. Glycans in fact play fundamental roles in cell-cell interaction and signaling, and in gut mucus properties, contributing diverse biologic properties to the mucosa. WGA, PNA and RCA fluorescent lectins[5] were used to highlight interferences in N-acetyl-glucosamine, N-acetyl-galactosamine and galactose distribution while mucosal defense response was determined by analyzing metallothionein expression via immunocytochemistry. Results demonstrate that cadmium induces extensive seric infiltration in the villi but not blood stasis or hemorrhage. Cadmium modifies the presence and/or distribution of glycans in enterocytes brush border and cytoplasm and in the goblet cells cytoplasm. The effects are dose and site dependent the anterior gut being more markedly influenced than the mid gut. Metallothionein, concentrated in the apical cytoplasm of enterocytes, markedly reduces at the higher dose. Results suggest a significant interference of cadmium with mucosal efficiency.

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WDR62 MUTATIONS HAMPERS GOLGI TO CENTROSOMES TRANSLOCATION RESULTING IN NEURAL PROGENITOR PROLIFERATION DEFECTS

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Microcephaly is a heterogeneous and incurable disorder and some of the involved genes control crucial aspects of neural development. Mutations in *WDR62* cause the second most common form of autosomal recessive primary microcephaly (MCPH2) suggesting that it acts as a critical hub of human cerebral development. WDR62 is a centrosome-associated protein, involved in symmetric versus asymmetric cell division choice from neural stem cells during corticogenesis. Intriguingly, *WDR62,* together with other genes involved in microcephaly onset, has been also ascribed to the "Golgipathies" group[1]. While the functions of some of these proteins has been broadly investigated[2], little is known about WDR62 role and localization kinetics at Golgi apparatus (GA). To address this issue in a neural development scenario, we availed of human induced pluripotent stem cells (hiPSCs) to recapitulate *in vitro* the main hallmarks of human ontogenesis, including neocortex development and its pathological alterations. Starting from iPSCs derived from a microcephalic patient carrying a novel *WDR62* mutation[3] and from isogenic corrected lines, we differentiated iPSC-derived neuroepithelial stem (NES) cells. NES cells are able to arrange themselves in a rosette-like shape establishing an apico-basal polarity that recapitulates the typical neural tube organization. In addition, they are long-term self-renewing and tripotent, representing an amenable model of the founder population of the developing neocortex. Since *WDR62* mutations may primarily affect neural progenitor pool expansion and given the importance of GA during mitosis[1], we focused on NES cell proliferation and on WDR62-GA relation. Our results highlight different aspects of cell cycle progression impairment, suggest mutant WDR62 association to GA, and a potential GA-centrosome translocation mechanism.

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APOPTOSIS DETECTION DURING THE REVERSE DEVELOPMENT OF THE IMMORTAL JELLYFISH *TURRITOPSIS DOHRNII* (HYDROZOA, CNIDARIA)

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Turritopsis dohrnii is also known as the immortal jellyfish since, in particular stress conditions or due to senescence, it can revert from medusa stage to the polyp one in an opposite process to the traditional life cycle of cnidarians. This process is called Reverse Development (RD) and it is characterized by a series of ontogenetic events many of which are based on the remodelling of cellular tissues permitting the re-expression of the polyp phenotype. One of the most important processes is apoptosis, or programmed cell death, used by multicellular organisms to dispose functionally spent or damaged cells in a diversity of settings[1,2], but in a controlled manner to prevent those neighbouring structures to remain affected. Apoptosis is characterized by DNA condensation and fragmentation into smaller pieces[1,2,3]. These conditions allow the detection of apoptotic cells through TUNEL assay in combination with confocal microscopy analysis. In this study, we analysed different stages of the RD of *T. dohrnii* (polyp stage, medusa buds, healthy medusa, bubble stage, and pre-cyst stage) by whole mount TUNEL assay. We found that apoptosis is preeminent during the central phases of reverse development, and decreases during the cyst stage. Our findings are in contrast with those reported on *T. nutricula* in which apoptosis increases in cyst stage[4]. These results suggest that in *T. dohrnii* during RD

there is a developmental program that initially reduces all body parts no longer necessary and then reaches a distinct turning point followed by subsequent development of cyst features.

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FUNCTIONAL ANALYSIS OF THE AGE-REGULATED ZINC FINGER FACTOR *ZNF367* IN EMBRYONIC AND ADULT NEUROGENESIS

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Ageing is a time-dependent functional decline affecting living organisms. Despite differences in lifespan, ageing is a universally inevitable gradual process leading to reduced fitness, increased susceptibility to pathologies and increased mortality rate. Along these lines, neural aging may be defined as a progressive loss of central nervous system function that promotes neurodegeneration and impairs neurogenesis. We envisage that genes controlling age-dependent processes act in continuity between development, adulthood, and aging. The starting point of our work is a list of brain age-regulated mRNAs that we have previously obtained by RNA-seq and validated by qPCR and in situ hybridization[1]. Among them, we are currently studying the function of z*nf367* gene, codifying a transcription factor. Functional studies suggested that this gene could be involved in the regulation of embryonic neurogenesis, both in Xenopus and Zebrafish embryos. In particular, *znf367* emerged as a new player in primary neurogenesis regulating neuroblast cell-cycle progression[2]. As the *znf367* mRNA is present in the zebrafish adult brain, especially in the medial subpallium and in the posterior zone of the dorsal telencephalic area, we hypothesized a znf367 role in the maintenance of neurogenic niches not only during embryonic development but also in adulthood. For this reason, we generated a zebrafish mutant line to knock-out *znf367* for unveiling its role in adult neurogenesis. We used CRISPR/CAS9 technology to induce a mutation in the second exon encoding the first zinc finger domain of *znf367*. Preliminary results, obtained by molecular analysis on developing and adult brains, suggested a role of znf367 in controlling embryonic and adult neurogenesis affecting the neuroblast proliferation rate and the p53 expression. Understanding the genetic pathways and molecular mechanisms underlying embryonic and adult neurogenesis may represent the first step in defining interventions that could increase neurogenesis in the aged brain and that could prevent/delay neurodegenerative diseases.

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DETOXIFICATION MECHANISM IN IMMUNOCYTES OF THE COLONIAL ASCIDIAN *BOTRYLLUS SCHLOSSERI* DURING THE BLASTOGENETIC CYCLE

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Botryllus schlosseri is a colonial ascidian easily found in the Lagoon of Venice, that undergoes weekly generation changes or take-overs (TOs).[1] The blastogenetic cycle is defined as the period between two successive TOs. During the TO, lasting 24-36 h, old zooids are progressively resorbed and replaced by their buds that grow to adult size and then open their siphons and start filtering. At TO, a diffuse apoptosis occurs in tissues of adult zooids; circulating phagocytes infiltrate the tissues and clear the effete cells.[2] In this phase, an increased oxygen consumption (respiratory burst) is observed with the consequent production of reactive oxygen species.[3] To protect themselves from oxidative stress, circulating immunocytes have evolved detoxification mechanisms, which involved anti-stress proteins, such as Cu/Zn superoxide dismutase (SOD), glutathione peroxidases (GPx3 and GPx5), γ-glutamyl-cysteine ligase modulatory subunit (GCLM) and glutathione synthase (GS).[4] In this study we analyzed, for the first time in a colonial ascidian, the role of stress granules (SGs) in anti-oxidant responses during TO. SGs are cytoplasmic ribonucleoproteic foci operating in anti-stress protein mRNA preservation and so in the regulation of stress responses.[5] We considered two important protein components of SGs: TIA-1 related nucleolysin (TIAR) and tristetraprolin (TTP). The mRNA transcription levels for TIAR and TTP in the haemolymph of *B. schlosseri* during the blastogenetic cycle were analysed by quantitative Real Time PCR (qRT-PCR) and the location of the transcripts in the haemocytes was studied through *in situ* hybridization (ISH). Our results confirm that immunocytes represent the major detoxification system in ascidians and are active in the transcription of stress-related genes, such as *tiar* and *ttp*, the transcription of which is modulated during the blastogenetic cycle.

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D-ASPARTATE INDUCES SPERMATOCYTE GC-2 CELL PROLIFERATION VIA AMPA RECEPTOR

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Numerous evidences suggest that D Asp plays a key role in vertebrate reproductive processes. In rat testis, D Asp induces testosterone synthesis and upregulates androgen receptor expression throughout glutamate receptors[1,2]. In addition, D-Asp directly promotes spermatogonia proliferation by activating ERK/Aurora B pathway[3]. Further confirmation that D Asp plays

an active role in spermatogenesis is the increase in the expression of prolyl endopeptidase (PREP) and disheveled associated activator of morphogenesis 1 (DAAM1) in the testis of D Asp treated rats[4,5]. These two proteins are involved in cytoskeleton remodeling, which is an integral aspect of spermatogenesis and is therefore essential for male fertility. In this study, a mouse spermatocyte-derived cell line arrested in premeiotic stage (GC-2) was employed to explore a direct effect of D-Asp on molecular pathways involved in spermatocyte proliferation. GC-2 were exposed to 200 M D-Asp for 30 min, 2 h or 5 h. The expression of both AMPAR (GluA1-GluA2/3 subunits) and cell proliferation markers was determined at different incubation times. The results showed that the GluA2/3 subunit was more expressed than GluA1 in the GC 2 cells. At 30 min of incubation, D-Asp treated GC 2 cells showed significantly higher expression levels of GluA2/3; GluA1 expression levels increased at 5 h of incubation. Furthermore, PCNA and P-H3 expressions (markers of DNA synthesis and chromatin condensation, respectively) were enhanced in 30 min D-Asp treated GC 2 cells; the levels of SCP3, a meiotic marker (expressed in pachytene), increased after 2 h of incubation. These results are the first demonstration of a direct effect of D-Asp on spermatocyte meiotic activity. Finally, the increased protein expression levels of GluA1 and GluA2/3 in D-Asp treated GC 2 cells suggest that D-Asp could activate the proliferative pathway via AMPAR.

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HISTOPATHOLOGICAL EFFECTS OF COCAINE ON THE EUROPEAN EEL (*ANGUILLA ANGUILLA*) OVARY

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Cocaine (COC) is an illicit drug widespread in surface waters[1], in concentrations ranging from μg.L^{-1} to ng.L^{-1}. The presence of cocaine in the environment represents a potential risk for aquatic organisms, but the ecological effects are still poorly understood[2]. More recently, in the European eel (*Anguilla anguilla*) it has been demonstrated that cocaine accumulates into tissues generating serious injury in skeletal muscle. It also affects the gill epithelium and increases plasma levels of cortisol and prolactin after exposure to environmental concentrations of COC (20 ng.L^{-1})[3,4]. The aim of this study was to evaluate the influence of environmental relevant concentration of cocaine (20 ng.L^{-1}) on the morphological development of eel ovaries and to immunolocalize enzymes as 3β-HSD, 17β-HSD and P450 aromatase involved in the synthesis of two sex hormones: testosterone and 17β-estradiol. Compared to controls, cocaine-exposed animals showed a smaller follicles area and a higher percentage of connective tissue; the histological analyses showed that the control specimens exhibited numerous full vitellogenic oocytes (fvOo) and early vitellogenic oocytes (evOo), while the exposed animals frequently showed previtellogenic oocytes (pOo); moreover, in the ovary of control animals, a strong presence of 3β-HSD, 17β-HSD and P450 aromatase was observed. Our results show that even a low environmental concentration of cocaine is able to affect the morphology and the enzymatic response of the ovaries of *A. anguilla*. Thus, considering the complex life cycle of the eel, the changes observed in the ovary could threaten the reproduction of the eel and potentially affect the survival of this species.

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DIGITAL 3D RECONSTRUCTION OF THE MOUSE OVARY

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In the adult mouse ovary, folliculogenesis progresses from the primordial type 1 (T1) to the fully-grown T8 follicle[1], and it is regulated by a continuous exchange of information between follicles and the surrounding vasculature meshwork. To date, few studies attempted to investigate the ovary's physiology while maintaining its 3D organisation, *i.e.*, the dynamic histo-functional environment in which the follicle grows and acquires its developmental potential. A 3D reconstruction of the ovary would further our understanding of folliculogenesis dynamics inside the intact organ, and, when combined with functional markers, would help to reveal the flow of molecular information that contributes to its biological function[2]. Micro-Computed Tomography (microCT) is an X-ray imaging combining a high spatial resolution (down to ~1μm) with the production of an isotropic 3D organ reconstruction[3]. Here, we describe a method that allowed the 3D identification, mapping and counting of follicles from the secondary T4 (53.2±12.7 μm in diameter) to the fully-grown antral T8 (321.0±21.3 μm), together with *corpora lutea*[4]. MicroCT brought up the main follicle's compartments, such as granulosa and cumulus cells layers, antrum, zona pellucida, and the oocyte with its nucleus. Also, this approach allowed the visualisation of the main vasculature, from the largest vessels at the ovarian hilum site (~150 μm size) to smaller (~35 μm). The results showed that the eight ovarian sectors, virtually segmented along the dorsal-ventral axis, houses an equal number of each follicle type, suggesting a homogeneously distributed follicle recruitment and a subsequent growth within the same region. This topographic 3D reconstruction of the ovary could contribute our understanding of folliculogenesis dynamics not only under normal conditions, but also during ageing, in the presence of pathologies or after hormones administration.

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DELORAZEPAM IMPAIRS THE EMBRYONIC DEVELOPMENT OF *XENOPUS LAEVIS*

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Benzodiazepines, used for the treatment of sleep disorders, anxiety and epilepsy, represent an important class of emerging pollutants[1]. As occurring for most pharmaceutical residues, they are released into the wastewater but not degraded during sewage treatment [2] therefore accumulating in effluent waters at concentrations ranging from μg/L to ng/L[3]. Resulting environmental concentrations may come close to human therapeutic plasma concentrations[4]. Bioaccumulation is already reported in fish [5] and small crustaceans [6] as significant effects on behavior, gene expression and enzymes activity. Environmental and human health protection prompt for a more accurate estimate of the impact of this drug on non-target aquatic organisms and, in particular, on early developmental stages. Therefore, in this study we investigated the effects of the benzodiazepine Delorazepam on *Xenopus laevis* embryos. Environmental (1 μg/L)[7] and 5 and 10 times higher (5 and 10 μg/L) concentrations were tested. Preliminary trials indicate that the drug reduces vitality (decreased heart rate and motility), induces marked cephalic and abdominal edema and causes morphological alterations in the gut. At the molecular level, altered expression of developmental genes is observed together with the production of inflammatory molecules. The resulting stress condition significantly impairs embryos development and threatens their survival. Similar effects should be expected also in embryos belonging to other aquatic species though so far, they have not been considered target for benzodiazepines.

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USE OF PROBIOTIC TO MITIGATE THE REPRODUCTIVE DISORDERS CAUSED BY BPA USING ZEBRAFISH AS MODEL

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Several studies documented the ability of Bisphenol A (BPA) to interfere with the hypothalamus-hypophysis-gonadal axis[1], leading to alteration of male and female gametogenesis and reproductive impairment[2]. Since several studies reported that Probiotic administration ameliorates reproductive health[3,4], the aim of the present study was to investigate if a mix of probiotic (SLAB51) could mitigate the detrimental effects induced by chronic exposure to BPA in *Danio rerio* at reproductive level. Adult male and female fish were treated for 28 days, as follow: (C) commercial diet; (BPA) 10 g/l of BPA; (P) 10^9 CFU of SLAB51; (BPA+P) 10^9 CFU of SLAB51 and 10 g/l of BPA. Testis histology showed that P administration increased both spermatogonia and spermatozoa abundance respect to C. BPA alone induced a decrease of spermatogonia, but not of spermatozoa respect to C; when co-administered with P, an increase of spermatogonia was seen, suggesting the role of probiotic in counteract BPA negative effects. Regarding spermatozoa, only in P fish a significant increase was observed confirming the positive role of the bacteria, but no differences were observed among C and BPA and BPA+P fish. Ovarian histology showed an increase of class III follicles in BPA and in P ovaries, respect to C. All treatments did not affect class I-II and IV follicles frequency. Only in BPA+P group the number of class I-II and IV follicle was lower respect to either BPA or P groups. The transcription of *gdf-9* and *ccn1* and of genes codifying for membrane hormone receptors (*fshr, lhcgr, pgrmc1* and *pgrmc2*), conducted on class III and IV follicle evidenced the ability of BPA to alter oocyte maturation process. Molecular studies in testis are still in progress. The results so far obtained evidenced the ability of SLAB 51 to mitigate the gonadal toxicity[2] of BPA in a gender specific manner.

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ALUMINIUM CHLORIDE INDUCES ALTERATIONS IN THE MUCINS SECRETED BY THE TERRESTRIAL SNAIL, *EOBANIA VERMICULATA*

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Aluminium, widely present in daily life[1], is reported to be linked to several neurological disorders[2]. We evaluated the effects of $AlCl_3$ on the terrestrial snail *Eobania vermiculata,* an excellent bioindicator of soil pollutants[3,4]. Histochemical and lectin histochemical techniques were used to investigate glycopattern variation in the mucins secreted by the pedal glandular system, involved in several functions, such as adhesion, locomotion, and protection. Three groups of adult snails (mean shell size = 2.5 cm; mean weight = 4.6 g) were fed daily with 4 g of lettuce soaked in a $AlCl_3$ water solution at three different concentrations (0, 50, and 200 μM). After 30 days, animals were sacrificed, Bouin-fixed and embedded in paraffin. Sections, 6- m thick, were analysed by histochemical methods (PAS, AB pH 2.5, HID-AB pH 2.5) and lectin binding experiments (PNA, SBA, WGA, LTA, UEA-I, AAA, SNA, MAA II, ConA). Controls were positive to PAS, AB pH 2.5, and with HID stained mostly brown, indicating the presence of carboxylated and sulphated glycans. Positivity was reduced in the treated snails, where the secretion was concentrated in the gland ducts rather than in the adenomeres. ConA (linking to mannosylated and/or glycosylated residuals) bound strongly to all the samples both in the dorsal area and the sole. SBA and WGA (linking to galactosaminylated and glycosaminylated/sialylated residuals, respectively) increased their binding in

the dorsal area of the foot of treatments. AAA, linking to fucosylated residuals, decreased its binding in the treatments. In the sole, SBA-binding decreased in the treatments. In conclusion, the $AlCl_3$ treatment affects the quali-quantitative expression of glycans in the foot. Physiopathological implications of changes will be investigated in further research.

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ALTERED GLYCOSILATION IN GASTRIC MUCINS SECRETED BY AQUAPORIN-4-DEFICIENT MICE.

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Aquaporines are important for water transport in the gastrointestinal tract[1]. Changes in their expression and/or localization can result in a number of disorders and can be used as therapeutic targets[2]. Aquaporin-4 (AQP4) is expressed predominantly on the basolateral membrane of the parietal cells in the fundi of the gastric glands of the murine stomach[3]. In AQP4-deficient knockout mice the absence of aquaporin leads to a reduction in mucosal secretion[1]. We evaluated whether the lack of AQP4 also induces changes in the glycopatterns of gastric mucins, as detected by histochemical, lectin histochemical and immunohistochemical techniques. Wild type (WT) and AQP4-deficient knockout mice (KO) were fed a standard diet *ad libitum* for 25 weeks, then they were sacrificed, and samples of stomach were taken and processed routinely for buffered-formalin fixation and paraffin embedding. Sections, 6- m thick, were analysed by histochemical methods (PAS, AB pH 2.5, HID-AB pH 2.5) and lectin binding experiments (PNA, DBA, SBA, WGA, UEA-I, AAA, ConA). In respect to WT, KO mice showed a reduction in the thickness of the mucus layer, as well weaker PAS and AB pH 2.5 positivity, indicating a general decrease in mucin secretion. Lectin binding experiments revealed a lower affinity in KO for SBA and PNA, suggesting a reduction of galactosyl/galactosaminylated residuals in the oligosaccharidic chains of mucins. The distribution of ConA-binding residuals (mannose and/or galactose) changed from predominantly apical in WT to widespread at the perinuclear level in KO. These alterations could lead to more complex pathological conditions. Future studies will be needed to understand the pathophysiological implications of these findings.

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THE FLAME RETARDANT TRIS(1-CHLORO-2-PROPYL)PHOSPHATE (TCPP) AFFECTS THE DEVELOPMENT OF TWO COGENERIC SPECIES OF ASCIDIANS WITH DIFFERENT SEVERITY

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Tris(1-chloro-2-propyl)phosphate (TCPP) is the organophosphorus flame retardant (OPFR) with the highest production volume in Europe[1]. It is considered ubiquitous in the environment[2], but its toxicity was mostly assessed *in vitro* or in vertebrates[2,3], leaving a big gap of knowledge about its adverse effects on invertebrates. Among them, aquatic invertebrates are particularly threatened by pollutants as the most sensitive phases of their lifecycles, *i.e.* fertilization and embryonic development, occur in water column, directly in contact with any contaminants. In the present study, we investigated TCPP effects on fertilization and embryogenesis of two cogeneric ascidian species *Ciona intestinalis* and *Ciona robusta*. TCPP exposure did not markedly interfere with ascidian fertilization, but it affected embryos development and survival in both species. Noteworthy, the calculated median effective concentration (EC_{50}) resulted very different between the two species, as *C. robusta* displayed higher susceptibility. Even if *C. robusta* and *C. intestinalis* appear morphologically similar, they are highly genetically divergent[4] and these molecular differences could also comprise their abilities in pollutants detoxification. During ascidians development, TCPP caused malformations on larvae tail suggesting that this molecule could affect myogenesis as already reported for other OPFRs[3,5,6]. Moreover, impairment of neural fibers arrangement was observed. Overall, our results suggested the presence of species-specific differences in embryonic sensitiveness to contaminants, pointing out the importance of evaluating chemicals teratogenic profile in different species to have a reliable framework of the effects on a community.

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EXPRESSION PATTERN OF VASA, PIWI, AND TDRKH PROTEINS IN MALE GERM CELLS OF THE TELEOST FISH *POECILIA RETICULATA*

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Spermatogenesis is the process that leads to the differentiation of mature male gametes. Spermatogenesis begins in the testis with the mitotic proliferation of diploid spermatogonia, proceeds through meiosis, and is concluded with spermiogenesis, process in

which haploid spermatids differentiate into spermatozoa[1]. Vasa, PIWI and TDRKH are proteins typically expressed in germ granules of germ cells: they are conserved across metazoans and are involved both in germline specification and in germ cell differentiation[2,3]. The role of these germline markers during spermatogenesis is not completely understood, but mutational experiments demonstrated that they are essential for the correct development of male germline. Thus, the investigation of their expression during spermatogenesis can help us understanding their role in germ cell differentiation. Interestingly, their pattern of expression is not always conserved across species and they are not always expressed in all stages of germ cell specification[4]. In this way, their expression and distribution pattern are usually peculiar, allowing the identification of specific stages of germ cell development. So far, the studies in this regard are limited to a few species with few molecular markers. We investigated the expression of Vasa, PIWI and TDRKH proteins in male gonads of the fish *Poecilia reticulata*. More in detail, we performed immunohistochemistry and immunofluorescence assays to analyze the expression pattern of the three germline markers during the different stages of spermatogenesis. A histological study was also conducted to better understand the structural organization of *P. reticulata* male gonad. Vasa localization allowed to trace germ cells inside testis, and the similar distribution of PIWI and TDRKH suggested, also for the latter, a role in the piRNA pathway. We believe that this work may help increasing our knowledge on germ cell development.

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EFFECTS OF EDC MIXTURE ON HUMAN PROSTATE CELLS

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Numerous environmental pollutants, named Endocrine Disrupting Chemicals (EDCs), have gained attention as potentially injurious to animal and human health due to their ability to interfere with the endocrine system[1-4]. EDCs are detected in different environmental matrices; they can bioaccumulate in adipose tissue and biomagnificate in food chain. So, main exposure to EDCs occurs through food intake. In the present work, we evaluated the effect of mixture of EDCs, such as phthalates and alkylphenol, and endogenous sexual hormones as estradiol and testosterone on LnCaP and PNT1a cell lines. The first data showed the preponderant effect of Nonyphenol (NP) in all mixtures on PNT1a cell line, whereas Dibutylphtalate (DBP) overrided the NP effect on LnCaP cell line. There was an increase cellular viability in LnCaP treated with NP and 17 β-estradiol, indicating the possible synergistic effect between the compounds. On the contrary, DBP induced a decrease of cell vitality. These effects were mediated by estrogen receptor pathways, mainly ER . In conclusion, we have pointed attention on dangerousness of the mixtures able to induce a strong imbalance of prostate cell physiology.

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MOLECULAR CHARACTERIZATION AND IMMUNOLOCALIZATION OF TDRD7 IN *POECILIA RETICULATA* (ACTINOPTERYGII, CYPRINODONTIFORMES)

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In Metazoa, pluricellularity led to the evolution of a totipotent cellular lineage involved in the genetic inheritance across generations: the germline. The patterns of differentiation of such lineage differ widely throughout species, however, some features are considered nearly ubiquitous, like the expression of some specific genetic determinants[1]. Another shared characteristic of the germline is the presence of supermolecular cytoplasmic structures (collectively called germ plasm-related structures) at some point during its differentiation. Between species, these structures are found at different levels of organization, at different stages of germ cell differentiation, and different molecular mechanisms are involved in their assembly. TDRD7 is one of the proteins that have been associated to the proper assembly of germ plasm-related structures in different species: spanning from *Drosophila melanogaster* germline perinuclear nuage[2], to *Danio rerio* germ cell granular structures[3], to mammal male germline chromatoid bodies[4]. In the multiple combination of the domains TUDOR (RNA-binding domain common in germline determinants) and LOTUS (a protein-protein interaction domain found in some germ plasm assemblers) within TDRD7 could lie the molecular mechanisms involved in the ribonucleoproteic granules assembly. In the present study, we used 93 RefSeq metazoan proteomes to investigate presence and molecular evolution of TDRD7 homologues in the animal phylogenetic tree. We confirmed the nearly ubiquitous distribution of such protein in animals and observed diverse patterns of domain acquisition and loss in different lineages. We then focused on characterizing with immunofluorescence assays the localization of the protein in gonads of the non-model fish *Poecilia reticulata*. We could identify different stages of germ cell differentiation of both sexes, and we observed both a sex-specific and a stage-specific pattern of TDRD7 expression.

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M2 MUSCARINIC RECEPTOR ACTIVATION HELPS THE MAINTENANCE OF HUMAN SCHWANN-LIKE ADIPOSE-DERIVED STEM CELL PHENOTYPE: IMPLICATION IN PERIPHERAL NERVE REGENERATION

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Schwann cells (SCs) play a central role in the response to axon injury but there are several restrictions hindering their clinical application[1]. Adipose-derived stem cells (ASCs) present good properties for peripheral nerve regeneration and when exposed to specific growth factors *in vitro*, they can acquire a SC-like phenotype (dASCs)[2]. Unfortunately, ASC differentiation protocol is a constant chemical stimulation and after growth factor withdrawal, dASCs revert their morphology and gene expression towards ASC phenotype. M2 muscarinic receptors are potential pharmacological targets and are expressed in rat and human SCs[3,4] and dASCs[5,6], with roles in the regulation of cell growth, neurotrophic properties and differentiation. Here we present the role of M2 receptor in controlling human dASC differentiation. M2 stimulation, using the preferential agonist Arecaidine Propargyl Ester (APE), is able to decrease dASC cell growth, enhancing the differentiation phenotype. Moreover, in absence of growth factors but with M2 receptor selective stimulation, human dASCs do not revert towards undifferentiated ASCs but maintain a spindle-shaped morphology and SC-like marker expression. These data are the first evidence that human dASCs are cholinoceptive and M2 selective activation contributes to dASC terminal differentiation.

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BUTYRATE EFFECTS ON LIVER MITOCHONDRIAL COMPARTMENT IN INSULIN-RESISTANT OBESE MICE: AN ULTRASTRUCTURAL AND STEREOLOGICAL STUDY

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Fatty liver, mitochondrial dysfunction and oxidative stress represent pathophysiological features of insulin resistance (IR) and obesity. Butyrate, a short-chain fatty acid product in the large intestine by gut microbiota fermentation and its synthetic more palatable derivative, the N-(1-carbamoyl-2-phenyl-ethyl) butyramide (FBA) have been demonstrated to be protective against diet-induced insulin resistance and fatty liver[1]. Mitochondria were identified as the main target of the beneficial effect of both compounds[2]. We comparatively evaluated the effects of sodium butyrate and FBA on liver lipid content and mitochondrial compartment in a mice model of obesity and IR, by using transmission electron microscopy and the point-sampling technique of classic stereology to measure mitochondrial density and lipid volume density. Four experimental groups were considered: standard diet (STD)-fed, high-fat diet (HFD)-fed, HFD-fed treated with butyrate or FBA animals. In HFD-fed mice, the lipids are more abundant and larger than in STD-, butyrate- and FBA-treated mice; stereology investigations revealed that lipid density was significantly decreased in the butyrate and even more in FBA groups compared with HFD mice. In the liver of the HFD mice, mitochondrial dumbbell-shaped and fission pictures are evident, while fusion events are recognizable in butyrate-treated mice; giant and elongated mitochondria, resulting from fusion, are recognizable in the FBA group. Mitochondrial area and volume density were significantly lower in the HFD group compared with the other groups, probably associated to the increased lipid compartment. Our results confirm the association between HFD-induced hepatocellular lipids storage and alterations in the mitochondrial compartment[3], furthermore demonstrating a restoring activity of butyrate and FBA.

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GEBR-7B COUNTERACTS THE EPITHELIAL-TO-MESENCHYMAL TRANSITION BY MODULATING TRANSCRIPTION OF THE IGF2/H19 CLUSTER IN HCC CELL LINES

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The epithelial-to-mesenchymal transition (EMT) is associated with metastasis and chemoresistance in many types of cancers including hepatocellular carcinoma (HCC). In a few tumours, a connection between EMT activation and disruption of the cAMP pathway has been found. Moreover, overexpression of phosphodiesterase 4D (PDE4D) seems to be involved in this process. Our previous study[1] showed that PDE4D is overexpressed in HCC cell lines and tissues, and its depletion/inhibition reduced the growth of HCC cells by causing apoptosis and interfering with the expression of various cell cycle effectors and other pro-oncogenic genes, such as the insulin growth factor 2 (IGF2) gene. However, regulation of the PDE4D-IGF2 network and its role in the EMT remain to be explored. IGF2 is an imprinted gene whose transcription is regulated in a cluster with the H19 gene, which produces a non-coding RNA. After PDE4D silencing/inhibition we found a significant downregulation of IGF2 gene and protein expression in HCC, and in this tumour IGF2 protein expression positively correlated with cell proliferation, migration and invasion. Here, we also show that selective pharmacological inhibition of PDE4D, using Gebr-7b, induces cell-cell adhesion proteins, such as E-cadherin, and decreases mesenchymal markers, including Snail and Twist, in Western Blot experiments. Gebr-7b

treatment also significantly reduced HCC cell migration, as revealed by Incucyte® Scratch Wound Assays. In addition, Gebr-7b treatment induces deregulation of the IGF2/H19 cluster. It is conceivable that this PDE4D-dependent modulation of the IGF2/H19 cluster could be crucial in control of the EMT in HCC. These preliminary data suggest that targeting of PDE4D may reverse the EMT, thus preventing metastatic dissemination of HCC by acting on the IGF2/H19 cluster.

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CELL PROLIFERATION INCREASE INDUCED BY PROTEIN SYNTHESIS UPREGULATION ARRESTS NEURONAL *DIFFERENTIATION IN DROSOPHILA* MELANOGASTER NERVOUS SYSTEM

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The tissue homeostasis in the development and adult organs is fine maintained by the balance between cell proliferation and cell differentiation[1]. In *Drosophila melanogaster*, an aberrant overground of a specific tissue due to genetic mutations or diseases, such tumors, reduces the growth of others organs[2]. Our preliminary results report that the up-regulation of global translation in eye imaginal discs increases the proliferation of neuronal undifferentiated cells to reduce or set back the differentiation in photoreceptors, the cells organized in ommatidia which compose the adult eyes. By the *ey-gal4>uas* system, we up-regulated the ribosomal scaffold protein, RACK1[3], in eye imaginal discs and observed by immunofluorescence studies an increase of global protein synthesis and the phosphorylation of H3 histone, used as proliferation index. Moreover, the cell morphology, visualized by phalloidine-staining was also altered by the RACK1 up-regulation. These larval defects reduced the size of eye adult when compared to control animals. The up-regulation of RACK1 by *ppk-gal4>uas* method in C4da neurons localized in the peripheric nervous system reduced the dendritic arborization and the translation of specific mRNA, Mical, required for neuronal differentiation[4]. Thus, these results indicate that the modulation of global translation and the translation of specific mRNA hold the balance of power in cell proliferation and differentiation.

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EFFECTS OF FATTY ACID AMIDE HYDROLASE INHIBITION ON THE PROLIFERATION OF NEURAL STEM CULTURES DERIVED FROM THE MURINE DEVELOPING CORTEX

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Fatty acid amide hydrolase (FAAH) is an integral membrane serine hydrolase, highly expressed in the brain and upregulated in several neurological conditions[1]. FAAH catalyzes the degradation of acylethanolamides (NAEs), like palmitoylethanolamide (PEA), Oleoylethanolamide (OEA) and Anandamide (AEA), one of the most characterized endocannabinoids. In neurons, endocannabinoids inhibit neurotransmitter release from presynaptic elements. Furthermore, a functional endocannabinoid system is present in neural stem/progenitor cells (NSPCs) in the embryonic cortex[2], suggesting a role in neurogenesis. We studied the effects of FAAH inhibition on NSPC cultures derived from the murine cerebral cortex at embryonic day 13.5, when the peak in neurogenesis occurs[3]. We employed this *in vitro* system to dissect the mechanisms of FAAH function in NSPCs, which is difficult to do in the complex *in vivo* brain environment, using the previously characterized irreversible FAAH inhibitor PF3845. Four days after seeding with different doses of PF3845, the culture growth of NSPCs was significantly reduced compared to controls in a dose-dependent manner. A time-course of three days treatment showed a dose-dependent increase in trypan blue-positive cells in the treated cultures with a peak at 24h. The cell cycle analysis of cultures treated with PF3845 1 M by flow cytometry showed an increase of 55% in the sub-G1 fraction (apoptotic cell fraction) and an increase of 5% in G0/G1 fraction at the expense of S and G2/M fractions, as confirmed by Ki67 immunostaining. Gene expression analysis revealed an increase in GADD45, p21 and BAX and a decrease in cdk4 and ARPC5 consistently with the reduced growth phenotype.

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STUDY OF BONE DEVELOPMENT MODULATION BY TWO PROBIOTIC SPECIES USING SP7: GFP AND COL10A1A: COL10A1A- GFP ZEBRAFISH TG LINES

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Many probiotic bacterial species, including *Bacillus subtilis*[1] and *Lactococcus lactis*[2], are documented producers of various menaquinone (vitamin K2) forms. Menaquinones are considered to have an important role in bone health since vitamin K is the enzyme co-factor for catalysing the carboxylation of glutamate

(Gla) residues in many Gla proteins including osteocalcin[3]. Menaquinones also have a role in maintaining bone strength by regulating the bone mineral density via balancing osteoblast-osteoclast activity[4]. We analysed the modulatory effects of the two selected probiotic bacteria in the early skeletal development of zebrafish using two transgenic lines expressing fluorescent proteins under transcriptional control of the regulatory regions for i) the osteoblast marker sp7 (Sp7 transcription factor) gene and ii) its downstream target gene col10a1a, encoding the osteoblast- and hypertrophic chondrocyte-specific collagen type X alpha 1a chain[5]. Zebrafish, particularly transgenic lines, are an ideal model for studies on skeletogenesis due to its fast development and low pigmentation at larval stages. In the current study, the extent of skeletal development was analysed in transgenic lines of zebrafish larvae treated with probiotics from 5DPF to 10DPF with additional live Alizarin Red staining for the visualisation of bone calcification. Quantification of the fluorescence in the images using ImageJ showed the difference in the expression of the two marker genes studied between the treated and untreated larvae. This gave further evidence to the role of selected probiotic strains in modulating the ossification process during zebrafish development.

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SLC6A1 KNOCKOUT ZEBRAFISH MODEL: AN INNOVATIVE TOOL TO UNVEIL THE PATHOGENETIC MECHANISMS OF THE MYOCLONIC-ASTATIC EPILEPSY

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Epilepsy is one of the most common worldwide neurological disorders with a high incidence in childhood. Up to 30% of the cases have genetic etiology. In particular, mutations of genes involved in the inhibitory gamma-aminobutyric acid (GABA)-ergic signalling have been identified in patients affected by forms of myoclonic-astatic epilepsy (MAE)[1]. The MAE syndrome is characterized by an early childhood onset together with behavioural disorders as well as the high daily frequency of seizures[2-3]. Despite the progress so far, the pathogenetic mechanism of MAE is still not fully understood. Since zebrafish (*Danio rerio*) has recently emerged as an amenable vertebrate genetic model for *in vivo* analysis of epilepsy-related mutations[4], a slc6a1 heterozygous knockout zebrafish mutant line was generated, in our lab, by CRISPR/Cas9 gene-editing technique in order to achieve a deeper comprehension of the mechanism underlying this particular form of MAE. Over the analysed developmental stages, no changes in both growth and survival rate were observed while the whole mount in-situ hybridization revealed a remarkable alteration of slac6a1 expression in mutant larvae with respect to the controls. Considering that many epilepsy models in zebrafish are characterized by seizure-like behaviour, locomotor analysis was also performed. Interestingly, mutants resulted characterized by an altered locomotor activity following dark- light stimulus. In conclusion, our findings point out that this genetic mutant zebrafish may provide a promising model to study *in vivo* the neurological effects associated with alterations of the slc6a1 gene since it *i)* can reproduce the genetic condition observed in SLC6A1 patients (50% reduction of wild-type protein), *ii)* shows locomotor hyperactivity in different developmental stages.

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STEM CELLS CONTRIBUTION TO THE ASEXUAL REPRODUCTION IN THE COLONIAL TUNICATE *BOTRYLLUS SCHLOSSERI*

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Among chordates, tunicates, the sister group of vertebrates, possess the most astonishing regenerative abilities. The colonial tunicate *Botryllus schlosseri* forms thousands of individuals (zooids) by budding from pluripotent cells of the body wall, but it can also regenerate the whole colony starting from circulating stem cells (SC) when all zooids are removed. It is not clear if the bud rudiment can form all the tissues of newly developing zooid, or if circulating SC participate to organogenesis. SC home into niches of adult zooids, where they can proliferate. Niches are transient, since adult zooids cyclically are resorbed and substituted by their growing buds. During this phase, SC leave the original niches to colonize those of the new adults. Since their precise morphological characterization is still missing, in this work we characterized the SC niches by means of whole mount observations, histology and 3D reconstructions. We identified candidate SC and verified that they undergo mitosis and differentiation in the subendostyle niche. Moreover, we developed a method to verify the candidate SC contribution to bud organogenesis. By isolating a bud, so that it could not receive SC from parent niches, we labelled circulating cells and daily monitored the bud *in vivo* and by confocal microscopy. We found that labelled candidate SC were able to localize in tunic, gonad niche, body wall, nervous system rudiment and in some epithelia, therefore undergoing mesenchymal-epithelial transition. Dilution of the dye in labelled cells suggested proliferation in homing tissues. Candidate SC were also found in the forming niches. Control buds, totally isolated from the circulation, failed to develop, whereas a few blood ampullae with their hemocytes were sufficient to guarantee bud development. Candidate SC sorted by FACS from dissociated colonies, labelled and injected into compatible colonies confirmed these results. In conclusion, we carefully described stem cell niches and evidenced where and how candidate SC contribute to bud organogenesis.

CADMIUM-INDUCED SPERMATOGENIC TOXICITY IN RAT: MELATONIN AMELIORATIVE EFFECTS ON TESTICULAR CYTOARCHITECTURE AND SPERM QUALITY

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Increasing evidence suggests that, between the environmental pollutants[1], cadmium (Cd) may be directly linked to human male infertility[2] and for this a strong attention is been devoting to its toxicity on testicular physiology also considering its action as endocrine disruptor[3]. For this, research aimed to identify substances that may ameliorate or eliminate Cd toxic effects to be used for new therapeutic approaches is of interest[4,5]. Here we report a study on the effects of melatonin (MLT) in mitigating Cd-induced toxicity on male adult rat testis. Cd-induced oxidative stress and apoptosis of germ and somatic cells, provoked testicular injury, documented by histological alterations, and decreased testosterone level, together with the protein level of steroidogenic enzymes (StAR and 3β-HSD). Importantly, the cytoarchitecture of the blood-testis barrier (BTB) and of germ cells was perturbed, as highlighted by impairment in structural (OCN, VANGL, Cx43, DAAM1 and PREP) and regulative (Src and FAK) protein levels and/or activation. The autophagy activation was highlighted, especially in the Sertoli cells, probably in response to the disorganization of the BTB. This report adds new insights into the mechanism related to the protective role of MLT against Cd-induced toxicity, since it lessened the grade of oxidative damage and apoptosis Cd-induced, with reversal of all the observed changes. In addition, the beneficial effects of MLT alone were evidenced by an increase of SPZ quality, in terms of motility and DNA integrity. The combined results strongly support a role for MLT in improving also human testicular health, not only in men exposed to Cd, but also in those having fertility disorders, to ameliorate SPZ quality and, consequently, reproductive success.

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ERBIUM AFFECTS THE *XENOPUS LAEVIS* DEVELOPMENT

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The use of lanthanides, also known as rare earth elements (REEs), in technological devices and their presence in the e-waste made them a new category of potential emerging contaminants[1,2]. Their toxicological and ecotoxicological effects are still largely unknown. Erbium is one of the most widespread REEs with potential environmental and human health risks associated to its increased release[3]. Reported lanthanides concentrations in the surface water usually vary from 10 ng/LF to 200 ng/L[4]; however, in the very polluted rivers lanthanides concentrations may increase up to 10 μg/L[5]. Only few studies investigated the potential effects of REEs on a long-term basis in freshwater[6,7]. The aim of our study is to examine the influence of Erbium on *Xenopus laevis* embryos, organism never experimented with this lanthanide, to test the eventual adverse effects on developing organisms. We used environmental (1 and 10 μg/L) and twice higher (20 μg/L) concentrations. Our preliminary data indicate that Erbium induces alteration of morphology, with cephalic and abdominal edema and anomalous intestinal winding, ROS production and decreased heart rate, thus suggesting that erbium could be dangerous for *X. laevis* and other non-target species.

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PARACRINE EFFECT OF HUMAN ADIPOSE-DERIVED STEM CELLS IN THE ANGIOGENESIS PROCESS

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Over the last twenty years, significant progresses have been made in the field of regenerative medicine and tissue engineering using bio-compatible scaffolds associated with adult stem cells due to their differentiation potential but, more importantly, to their paracrine effect. Furthermore, angiogenesis is known to be the minimum but necessary condition to promote tissue regeneration. In 2016, Cherubino *et al.*[1] proved that hASCs, seeded onto INTEGRA® Flowable scaffold (FWM) and grafted in mice, were able to induce the vascularization inside the scaffold, though after 30 days hASCs were no longer found. This result confirmed that the presence of the cells is not strictly fundamental for the induction of angiogenesis. In this scenario, the focus of this study has been the *in vivo* evaluation of hASCs paracrine effect on angiogenesis. To achieve this aim, the INTEGRA® Flowable Wound Matrix (FWM) was associated with hASCs, hASC-crude protein extract or hASC-conditioned medium (CM); the four devices were grafted in 7-week-old male athymic BALC-C nude mice. After 30 days, the scaffolds were retrieved and analyzed via optical microscopy and Real Time PCR. The results confirmed the FWM/hASCs as highly efficient system in the induction of angiogenesis; moreover, both the FWM/crude protein extract and FWM/CM exhibited comparable vascularization to that of hASCs, supporting the importance of the paracrine effect in the angiogenic process. For a better characterization, in future experiments, the removed scaffold will be digested with collagenase type II and the recovered cells will be analyzed, by FACS, for the expression of CD31, CD34 and CD105. In conclusion, the results obtained encourage the possibility of successful use of a

cell-free system for regenerative medicine applications, which would ultimately allow overcoming the main problems that occur with the use of stem cells, among those the need for autologous transplant.

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METABOLIC REMODELLING OF THE HEART DURING AGEING: THE ROLE OF HISTONE ACETYLATION

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Cardiac ageing is often associated with a progressive deterioration of the structure and function of the heart[1], as well as a metabolic remodelling within the myocardium. During ageing, the heart shifts from mitochondrial oxidation to anaerobic glycolysis[2]. This process causes an energy deficit that contributes to impairment of cardiac function in the elderly[3]. Preliminary data suggest that the activation of enhancers by H3K27ac promotes an increase in glycolytic pathways in the onset of ageing. To gain insight into how enhancers of glycolytic genes are activated at the onset of ageing, we are now focusing on p300 – a histone acetyltransferase (HAT) required for the activation of enhancers during heart development and for modulating the activity of MEF2C during cardiac hypertrophy[4]. First, we investigated the effect of inhibition of this HAT on cardiac function in mice prior to the onset of ageing: 16-month-old mice were treated with C646, an inhibitor of p300, for 2 months. Echocardiographic analysis carried out every 2 weeks revealed that p300 inhibition improved cardiac function at ageing onset. Metabolic assays for three metabolites of glycolysis (glucocose-6-phosphate, lactate, and pyruvate) suggested that p300 inhibition interferes with the activation of anaerobic glycolysis in mice at the onset of ageing. In support of this, we found that the inhibition of p300 prevented the activation of anaerobic glycolysis in HL1 cells in hypoxia, a condition that induces a shift from aerobic to anaerobic metabolism. Finally, we evaluated whether the inhibition of p300 had an impact on mitochondrial metabolism, through the analysis of mitochondrial ultrastructure in cardiomyocytes of 18-month-old mice that had been treated for 2 months with C646. Transmission electron microscopy revealed that the inhibition of p300 prevented the increase of mitochondrial area occurring in cardiac ageing. These preliminary data suggest a role for p300 in promoting the changes induced by H3K27ac in metabolic remodelling during ageing.

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PPARβ/δ ACTIVATION OVERLOADS PROTEASOME OF OXIDIZED PROTEINS IN PARKINSON'S DISEASE *IN VITRO* AND *IN VIVO* MODELS

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The mechanisms responsible for neurodegeneration in sporadic Parkinson's disease (PD) are still unknown, but oxidative stress, excitotoxicity and neuroinflammation are believed to play pivotal roles in neuronal death. PPARs are a class of transcription factors involved in controlling several metabolic pathways, both physiological and pathological conditions. PPARβ/δ is recognized as an oxidative stress sensor, being activated by the product of lipid peroxidation 4-HNE. We have previously reported that the nuclear receptor PPARβ/δ plays a detrimental role in neurodegeneration. Herein, the biological pathways activated in PD were dissected in an in vitro model of PD along with the effects induced by a specific PPARβ/δ antagonist. This model was implemented with *in vivo* experiments performed on 6-OHDA mouse model administered with a specific PPARβ/δ antagonist upon injury. The biological, behavioral, and morphological data obtained confirmed a detrimental role for PPARβ/δ nuclear receptor in inducing protein oxidation, mitochondrial ROS, and proteasome dysfunction, showing a positive effect of its antagonism.

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DEVELOPMENT AND CHARACTERIZATION OF A DIABETIC RETINOPATHY *IN VITRO* MODEL

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The retinal pigment epithelium (RPE) is a specialized epithelium that forms the outer blood–retinal barrier (BRB), located between the retinal photoreceptors and the choriocapillaris. The tight junctions expressed in this epithelium control fluids and solutes that enter the retina and this sealing function, which is essential for the retinal homeostasis, is damaged in diabetic retinopathy. Diabetic retinopathy is a diabetes complication and the leading cause of visual impairment and blindness in adults.[1] Chronic hyperglycemia plays a crucial role in the damage of the RPE.[2] In this study, it has been characterized a high glucose-injured RPE *in vitro* model using a human RPE cell line (ARPE-19) that is able to form polarized epithelial monolayers on Matrigel coatings.[3] The barrier function of the epithelium has been assessed by transepithelial electrical resistance measure-

ments and immunofluorescence staining for tight junctions. Then, cells were exposed for 72 h to 50 mM D-glucose[4], to induce the high glucose injury. IncuCyte Cytotox assay was performed to evaluate cell death in live cell imaging. The protein levels involved in pro-survival or cell death pathways were analyzed using Western Blotting analysis. Furthermore, dicarbonyl stress compounds and the activity of enzymes that participate to the oxidative state were evaluated by ELISA assays. Finally, a BRB multilayered 3D cellular model has been built-up using HUVECs and ARPE-19[5] in order to evaluate the effects of hyperglycemia. The data obtained dissect at cellular levels the detrimental effects of hyperglicemia on retinal cells.

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