XXVII International Symposium Molecular and Physiological Aspects of Regulatory Processes in the Organism

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16.00 Closing remarks
prof. dr hab. Henryk Lach, dr hab. Elżbieta Kołaczkowska, prof. UJ

Lectures/Invited speakers

Effects of nitrophenol exposure on chicken ovarian steroidogenesis

Andrzej Sechman

Department of Animal Physiology and Endocrinology, University of Agriculture in Krakow, Poland

Nitrophenols (NPs), common environmental contaminants which are either precursors or derivatives of a several number of pollutants, exert serious effects on animal and human health. Although previous studies indicate that NPs belong to endocrine disrupting chemicals, knowledge concerning influence of NPs on ovarian function is limited. We investigated the *in vitro* and *in vivo* effects of two selected NPs, i.e. 4-nitrophenol (PNP) and 3-methyl-4-nitrophenol (PNMC) on ovarian steroidogenesis using the chicken ovary as a model. In both types of experiments, white (1-4 mm) and yellowish (4-8 mm) prehierarchical follicles as well as theca and granulosa layers of preovulatory follicles (F3-F1; 20-36 mm) were isolated 2 h after ovulation from the hen ovary. Following *in vitro* (6 or 24 h incubation) or *in vivo* (6-day i.m. administration) exposure to PNP or PNMC, ovarian steroids (progesterone, testosterone and estradiol) in incubating medium or blood plasma, and mRNA expression of steroidogenic proteins (*STAR*, *HSD3B* and *CYP19A1*) and hormonal receptors (*LHR*, *ESR1* and *ESR2*) in collected tissues were determined. Results obtained showed that PNP and PNMC decreased secretion of sex steroids from ovarian follicles and significantly affected mRNA expression of all investigated genes in their tissues. These studies revealed that: (i) NPs exert inhibitory effects on steroidogenesis process in the chicken ovarian follicles, (ii) the effects of PNs are associated with down-regulation of steroidogenic proteins as well as LH and estrogen receptor transcription. We suggest that anti-steroidogenic actions of NPs may impair the selection of prehierarchical follicles to preovulatory hierarchy and disturb their growth and maturation.

The study was supported by grant of National Science Centre, Poland: UMO-2014/15/B/NZ9/01986.

Human TRAIL-producing *Lactococcus lactis* bacteria for potential immunotherapy of colon cancer

Jarek Baran

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One of the leading problems in the current treatment of colon cancer is resistance of the tumor cells to chemotherapy. TRAIL is a natural protein that effectively kills many types of tumor cells and potentially may act synergistically with some chemotherapeutics. However, the biological half-life of TRAIL in mammalian organism is very short, significantly affecting its therapeutic effectiveness. The aim of the study was to investigate, if non-pathogenic *Lactococcus lactis* bacteria can be used as a safe carrier of the TRAIL protein, enabling both, the control of TRAIL secretion over a period of time and elimination of tumor cells *in vitro* and *in vivo*. The use of lactic acid bacteria as delivery system for TRAIL might enable production of TRAIL locally in the tumor site for a longer period of time, while chemotherapeutics might restore sensitivity to TRAIL-induced apoptosis, acting together in tumor elimination.

Acknowledgments: This study was supported by the National Science Centre in Poland (Grant no. UMO2014/15/B/NZ5/03484) and the EU Horizon 2020 Framework Programme (H2020-MSCA-RISE-2017 no.777682 “CANCER”).

Nutritional, metabolic and pharmacological aspects of polyunsaturated fatty acids in inflammatory processes

Joanna Gdula-Argasińska[1], Anna Zając[1], Katarzyna Sroczyńska[1], Karoline Bartusek[2], Tadeusz Librowski[1]

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Essential Fatty Acids (EFA) include linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3), which produce respectively,arachidonic acid (AA, 20:4 n-6) and n-3 acids, i.e., eicosapentaenoic (EPA, 20:5 n-3) and docosahexaenoic acids (DHA, 22:6 n-3). PUFA of n-6 family are predominant in the typical Western diet, and therefore a general deficiency of n-3 acids is noted, which can cause an excessive release of pro-inflammatory metabolites, mainly arachidonic acid derivatives. Too high ratio of n-6 to n-3 acids provided with the diet is a risk factor for many diseases, including those of an inflammation nature.

Reports of recent years clearly indicate that n-3 FA and their derivatives are essential for proper growth and development, act in an immunomodulating, anti-inflammatory, anti-arteriosclerosis, anticancer manner and are resolvents of inflammation. Enzymatic and non-enzymatic reactions involving PUFA occur under physiological and pathological conditions and resulted a lipid mediators biosynthesis. Lipid mediators, i.e. eicosanoids or endocannabinoids are pharmacologically active but constitute yet not fully known group of compounds.

Lipid mediators are endogenous factors determining the kinetics of inflammation, and also its resolution and in the organism can play both pro- and anti-inflammatory function. Eicosanoids are necessary for an effective inflammation process in the organism, and also for its resolution. The synthesis of eicosanoids during the inflammatory process is significantly enhanced. These compounds when synthetized in excess affect the development of pathological conditions (chronic inflammation), therefore, enzymatic pathways leading to the formation of pro - inflammatory lipid mediators are the starting point for the uptake of many drugs. We will present the latest data on the role of fatty acids in physiological and pathological states.

Oral Presentations

In vivo neutrophil extracellular trap (NET) formation in obese mice during systemic inflammation – role of leptin and CXCR2 receptor

Iwona Cichoń, Weronika Ortmann, Elżbieta Kołaczkowska

Department of Experimental Hematology, Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland

While negative effects of obesity on health status are well known, its effects on sepsis outcome seem to be more obscure. In line with the latter, several studies indicated improved survival of sepsis by moderately obese patients. Importantly, obesity is accompanied with higher leptin levels which correlate with neutrophil infiltration and increased neutrophil elastase (NE) expression. Since, neutrophil migration is regulated by CXCR2 receptor and NE of neutrophil origin is an integral part of neutrophil extracellular traps (NETs), the aim of this study was to determine NET formation in septic obese and lean mice, and subsequently to assess the impact of leptin and CXCR2 on NET release. Genetic (ob/ob mice) and diet-induced obesity (60% fat diet) murine models were examined. Prior to lipopolisacharide sepsis induction, some mice were treated with recombinant leptin or antibodies neutralizing leptin, or with CXCR2 antagonist. Intravital imaging of mouse liver was performed using spinning disk confocal microscope. The obtained results show that neutrophils from obese mice form substantially less NETs when compared to lean controls. Interestingly, recombinant leptin intensifies NET casting only in lean mice with no changes in neutrophil counts. In turn, neutralization of leptin has no impact on NET formation neither in lean nor obese individuals, however, it decreases neutrophil numbers in lean mice. Furthermore, blocking CXCR2 receptor attenuates NET release in both study groups. In conclusion, the process of NET formation during sepsis depends on the immuno-metabolic state of affected individuals.

Study supported by National Science Centre of Poland, grant No. 2018/29/B/NZ6/00713.

Endocrine-disrupting chemicals present in follicular fluid increased IGF1 secretion by ovarian granulosa cell tumor via estrogen receptor (ERα) signaling pathway

Justyna Gogola, Marta Hoffmann, Anna Ptak

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More and more studies show that humans are exposed to persistent organic pollutants. It has been demonstrated that endocrine-disrupting chemicals (EDCs) such as perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), 2,2-dichlorodiphenyldichloroethylene (p,p'-DDE), hexachlorobenzene (HCB) and polychlorinated biphenyl 153 (PCB153) are accumulated in ovarian follicular fluid (FF) in women. Because these compounds accumulate in FF, they may have direct effects on the granulosa cells lining the fluid-filled antrum of ovarian follicles. EDCs can act as agonists or antagonists for hormone receptors and may activate pathways involved in the progression of hormone-related cancers, in which ovarian granulosa tumors (GCTs) are included. The research aimed to indicate the effect of EDCs mixtures on insulin-like growth factor 1 (IGF1) secretion by granulosa cell tumor represented by KGN cells. IGF1 is a hormone crucially involved in the physiology of cell proliferation, and its expression is regulated by estrogen in several reproductive organs including the uterus and ovary.

In this study KGN cells were cultured using a three-dimensional conditions (3D) to reflect tumor microenvironment. Spheroids were cultured in DMEM/F12 medium containing 10% FBS with the mixture of the test compounds containing: PFOA (2 ng/ml), PFOS (8 ng/ml), HCB (50 pg/ml), p,p'-DDE (1 ng/ml), and PCB153 (100 pg/ml). Secretion of IGF1 was determined by Human IGF1 ELISA Kit according to the manufacturer's instructions. The expression of IGF1 was evaluated by real-time PCR and confirmed by western blot. siRNAs were introduced into KGN spheroids using DharmFECT 3 transfection reagents and targeting human *ESR1,* according to the manufacturer's instructions. Statistical analysis was performed using one-way ANOVA (Tukey's test, $P<0.05$).

Firstly, in this study, we have found that all individual test compounds significantly increased IGF1 expression and secretion in KGN cells. However, humans are frequently exposed to mixtures of these chemicals, and the biologic effects of the possible mixtures require elucidation. Interestingly, a mixture of the five compounds also significantly stimulated the IGF1 expression; however, the observed effect was lower than predicted. Furthermore, our studies indicated that EDCs mixtures act via estrogen receptor (ERα) signaling pathway. To sum up, we have indicated that EDCs mixtures found in follicular fluid increasing IGF1 secretion via ERα signaling pathway contributed to AGCT progression.

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Shedding light on the evolution of chemical communication in chelonians

Alejandro Ibáñez[1], Albert Martínez-Silvestre[2], Emilia Rydzy[1], Uwe Fritz[3], Maciej Pabijan[1]

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Chemical communication though pheromones is involved in many activities such as searching for potential partners. However, how chemical signaling pathways have evolved remain unknown for most organisms. Given their high ability to recognize scents, turtles (i.e. chelonians) are very suitable models to test hypotheses on the evolution and function of chemical communication. In addition turtles possess mental glands (MG) that produce secretions that may be involved in communication, particularly during courtship and breeding. Here we aimed to reconstruct the evolutionary history of MGs in turtles. We examined specimens from museums and live collections to reassess the occurrence of MGs in extant Testudinoidea (the only group of turtles having MGs), creating a comprehensive dataset spanning most turtle species. MGs show different degrees of development, being large and obvious in some taxa, reduced (likely vestigial) to absent in others. The most parsimonious reconstruction suggested a single origin for MGs in the most recent common ancestor of Testudinoidea, and multiple losses of this trait in each major lineage, particularly in strictly terrestrial genera or species, suggesting the importance of other channels of communication in some clades. In addition, we examined the histology of MGs in a subset of species, and identified chemicals present in the lipid fraction of one of the species. Our results, together with the available information on gland histology in the literature, show a similar structure in MGs among species, supporting a single origin of this gland in turtles.

This study was supported by NCN grant No. 2017/25/B/NZ8/01498.

Removal of extracellular proteins by immune cells during resolution of inflammation visualized by intravital microscopy

Michał Santocki, Elżbieta Kołaczkowska

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Intravital (in vivo) microscopy is a revolutionary imaging technique that allows to observe dynamic processes in living animals in real time. Thanks to microsurgical tissue preparation and use of target-specific monoclonal antibodies, it is possible to follow the cells of interest and watch their interactions with other cells and tissues in their natural environment. We studied such processes during resolution stage of inflammation, which is an important step of immune response that allows to quench leukocyte activity. The precise resolution of inflammation often determines its outcome. In this study we followed resolution of lipopolysaccharide-induced sepsis in C57BL/6J mice, concentrating on kinetics of removal of extracellular proteins deposited in vasculature during the immune response. We aimed to verify their clearance and identify cells involved. By using spinning-disk confocal microscopy we focused on the engulfment of neutrophil elastase (NE) by immune cells in the sinusoids of the inflamed liver. Using advanced image analysis software (IMARIS) we recreated the 3D structure of the liver and cells present therein from a series of optical scans (*z-stacks*) of the imaged organ. At first, we verified specificity of our approach using proper isotype controls and Fc block antibodies. Then, we were able to identify the NE engulfing cells (phagocytes) and measure the volume of intracellular elastase (MeasurementPRO). Having established that, we can now focus on identification of receptors involved in this process. Altogether, this new approach allowed to create the basis for further research on the removal of various molecules and structures from blood and/or endothelium.

Study supported by National Science Centre of Poland, grant No. 2018/29/B/NZ6/00713.

Pxt1 expression is regulated by miRNA

Igor Tomczyk[1], Bernadetta Pawlicka[1], Wioleta Sieńko[1], Jasmin Wiench[1], Karina Kaczmarek[2], Paweł Grzmil[1]

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Mouse *Pxt1* (*Peroxisomal, testis specific 1*) belongs to a group of proapoptotic genes. Protein encoded by this gene contains a functional BH3-like domain, characteristic for death agonists. It's expression is specific to primary spermatocytes. Bioinformatic analysis revealed 10 candidate miRNA particles that can have putative binding capacity to *Pxt1* mRNA. Testis expression of all miRNAs was confirmed *in vivo*. To assess, whether candidate miRNA particles have influence on *Pxt1* gene expression we used reporter assay based on luciferase activity. We have amplified 3'UTR part of *Pxt1* gene, which was then inserted into plasmid containing Firefly luciferase open reading frame and Renilla luciferase gene for normalization. Using this construct we have transfected MC3T3-E1 mouse bone marrow cell line, in which we have shown expression of miRNA-125a and miRNA-495. The luciferase reporter assay showed that in those cells, luciferase activity was reduced in comparison to control, which indicates miRNAs regulation of *Pxt1* 3'UTR. MiRNA-125a is known to be involved in wide range of functions, one of them is related with apoptosis control. Thus, we demonstrated that *Px1* expression is indeed regulated by miRNA.

This work was supported by polish National Science Centre grant no 2015/19/B/NZ4/00576.

Poster Presentations

Poster Presentation # 1

Optimization of method for RNA isolation from chicken embryo bones suitable for gene expression

Piotr A. Antos

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Extraction appropriate quantity of high quality RNA is an essential step in analyzing gene expression. The RNA isolation is particularly challenging in bone, because of low numbers of cells embedded within mineralized tissue. Current methods of RNA isolation from bone consist of multiple steps in which tissue is frozen in liquid nitrogen and turned into powder using mortar and pestle. Then, powdered bone is transferred to phenol-guanidinium reagent for RNA extraction. Because of multiple steps in that procedure there is a possibility of crosscontamination and sample loss. Therefore, the aim of the carried study was to optimize simple step RNA extraction method from chicken embryo skeletal system using *stay*RNA™ (A&A Biotechnology), phenol-guanidinium based TRIzol® Reagent (Molecular Research Center, Inc.) and Ultra-Turrax homogenizer (IKA-Labortechnik).

Fertilized hen eggs of Hy-Line Brown strain were incubated at standard conditions for 14 days (t=37.8°C, RH=55%, *Brinsea Ova-Easy Advance* incubator). The eggs were candled on day 5 of the incubation to eliminate dead embryos. To day 14 of embryonic development (E14) embryos were decapitated and femur, tibia, fibula, metatarsal bones and sternum were collected and kept frozen in *stay*RNA until RNA isolation. The purity of the RNA samples was checked spectrophotometrically by measuring the optical densities at 260 and 280 nm and evaluating the ratio 260/280 nm. The results showed that the method can be optimized to obtain high quantity and quality RNA from most collected tissues.

This research was financed by the Ministry of Science and Higher Education of the Republic of Poland.

Poster Presentation # 2

In vitro response of human ovarian granulosa cells (HGL5) and human ovarian carcinoma cells (OVCAR-3) to papaverine hydrochloride and quercetin treatment

Simona Baldovská[1], Klaudia Jaszcza[2*], Krystyna Pierzchała-Koziec[2], Adriana Kolesárová[1]

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Nowadays, thousands of studies are focused on natural substances and their effect on reproductive functions. The papaverine and quercetin have antibacterial, antioxidant, anti-inflammatory, vasodilatory, antiarrhythmic properties and play an important role in decreasing the risk of chronical diseases. They are food supplements and are generally recognized as safe. Researches are aimed to improve reproductive health and treatment of different types of cancer. Ovarian cancer causes the highest mortality rates worldwide. Thus, the aim of this study was to examine the *in vitro* effect of alkaloid (*Papaverine hydrochloride)* and polyphenol (*Quercetin)* at 2.5, 5.0 and 10.0μg/ml concentrations on the viability of human ovarian carcinoma (OVCAR-3) and healthy human ovarian granulosa (HGL5) cells and secretion of 17β-estradiol by HGL5 cells during 24h of cultivation. The metabolic activity was assessed by Alamarblue^{TM} viability assay, the release of 17β-estradiol was estimated by ELISA method. Papaverine caused a significant decrease ($P \leq 0.05$) of the number of viable cancer cells OVCAR-3 in a dose-dependent manner and slightly increased HGL5 cell viability. Interestingly, this alkaloid did not change the 17β-estradiol secretion by the HGL5 cells. On the other hand, quercetin in the highest concentration (10 μg/ml) negatively affected the viability of ovarian non-cancer cells HGL5 ($P \leq 0.001$), as well as ovarian cancer cells ($P \leq 0.01$). Quercetin caused the tendency to increase secretion of 17β-estradiol, except for the lowest concentration. In conclusion, the study suggested the possibility of papaverine to decrease the viability of human ovarian cancer cells *in vitro*, however, further research is required.

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Poster Presentation # 3

Synergistic induction of apoptosis in human leukemia cells by plant polyphenols and mafosfamide

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The ability to induce regulated death in pathological cells is important in assessing the anticancer potential of the tested agents. Epigallocatechin-3-gallate (EGCG) and resveratrol (RES) are natural polyphenolic compounds of plant origin. Mafosfamide (MAF) represents a new generation of oxazaphosphorines. The relationship between anticancer activity and triggering apoptotic cell death by the combined action of these chemical compounds is still an open issue. Thus, the aim of the present study was to compare the extent of apoptosis - induction in MOLT-4 and ML-1 cells by EGCG, RES, and MAF, when applied alone and in combinations. The study was conducted using flow cytometric assays with annexin V-FITC/propidium iodide, caspase-3/7, tetramethylrhodamine ethyl ester, and APO-BrdU test. Application of the polyphenols and mafosfamide resulted in disorders in asymmetry of membrane lipids and cell membrane integrity, caspase-3/7 activation, dissipation of mitochondrial membrane potential, and DNA fragmentation. The greater degree of apoptosis was found in the case of the combined action of the polyphenols with mafosfamide than when the tested agents were applied alone. The combined action of EGCG with MAF was more effective than that of RES with MAF in triggering apoptotic cell death. MOLT-4 cells were more sensitive than ML-1 cells to apoptosis - induction. The synergistic effects of EGCG, RES, and MAF on induction of apoptotic death in the human leukemia cells, were shown. The combined action of the plant polyphenols and mafosfamide isa promising direction in the development of new therapeutic strategies.

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Poster Presentation # 4

VASPIN in Polycystic Ovary Syndrome (PCOS) – plasma concentration, protein expression and immunolocalization in adipose tissue and ovaries in rat model of PCOS

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Polycystic ovary syndrome (PCOS) is an exceedingly complex endocrine, reproductive and metabolic disorder of a diverse and still unclear pathogenesis. Recent studies have focused on the role of obesity, or more specifically the role of adipose derived hormones – adipokines in PCOS. The aim of the study was measurement vaspin levels in plasma, adipose tissue and ovaries in rat model of PCOS. Vaspin (Visceral adipose tissue-derived serine protease inhibitor) is a novel adipocytokine identified in obese diabetic rats, where it improves glucose tolerance and insulin sensitivity.

Twenty four Wistar rats were divided into two groups, control (n=16) that received vehicle only (2% DMSO in rapeseed oil) and treatment groups (n=8) administered nonsteroidal inhibitor of aromatase - letrozole (1 mg/kg) per 21 days. During this period, vaginal smears were collected daily for estrus cycle determination (control: proestrus or diestrus). On the day subsequent to last letrozole dose administration, rats were killed and samples (plasma, ovaries and adipose tissue) were collected.

In letrozole- induced PCOS rats we observed elevated levels of androgens and high incidence of ovarian cyst. Plasma level of vaspin was significantly higher in letrozole-induced rats but no change during estrous cycle. Moreover, we noted that both vaspin and its receptor GRP78 were higher in adipose tissue and lower in ovaries in treatment group.

We found a strong connection between vaspin and PCOS physiopathology; higher levels of vaspin in plasma in treatment group is probably by vaspin production by adipose tissue, not ovaries.

Poster Presentation # 5

Expression and localization of novel adipokines and their receptors in rat testis after short-term treatment with flutamide

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Adipokines, such as chemerin, vaspin and apelin have been described mainly as regulators of female metabolism. We hypothesized that these adipokines may be involved in male steroidogenesis. The aim of this study was to investigate gene expression levels of chemerin and its receptors CCRL2, CMKLR1, GPR1, vaspin and its receptor GRP78, apelin and its receptor APLNR in the testis of adult rat exposed to short-term treatment with anti-androgen flutamide.

Flutamide (50mg/kg bw) was injected into 82-days-old Wistar rats every day in seven doses. Testis and blood samples from 90-day-old control (n=10) and flutamide-treated rats (n=10) were used for all analyses. To determine the expression of adipokines and their receptors three complementary methods (real-time PCR, western blot, immunohistochemistry) were used, whereas hormone levels were determined by ELISA.

Downregulation of chemerin, CMKLR1, vaspin, GRP78, APLNR at the mRNA and protein level was demonstrated after anti-androgen exposure. Apelin and CCRL2 expression increased after flutamide, whereas GPR1 expression was not changed. A p-value from 0.05 to 0.001 was considered statistically significant. Decreased or increased intensity of immunoreactive proteins after flutamide exposure versus control corroborated gene expression results. Vaspin concentration in serum significantly increased in flutamide-treated rats, but there were no statistically significant differences in the concentrations of chemerin and apelin.

The results showed that the expression of apelin, chemerin and vaspin and their receptors in rat testes after experimentally induced androgen withdrawal was altered. Further work will be required to understand the relationship between adipokines and steroidogenesis in flutamide-treated rats.

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Poster Presentation # 6

The impact of methoxychlor exposure on PI3K/Akt pathway in pig uterus

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Methoxychlor (MXC) is an organochlorine pesticide, used worldwide till early 2000s. MXC was banned for its toxicity, bioaccumulation, and endocrine disruption activity. This pesticide with estrogenic/antiestrogenic/ antiandrogenic properties alters the reproductive function and behaviour. Crucial role in cell survival, cell proliferation, growth, metabolism, and angiogenesis plays PI3K/Akt pathway. Abnormal activity of the this pathway can lead to dysfunction in reproductive tract and infertility.

The aim of the study was to examine the effects of exposition to methoxychlor during neonatal period, on *PI3K*/*Akt*/mTOR pathway proteins in neonatal and adult pigs uteri. Neonatal pigs were injected with methoxychlor (MXC-20µg/kg bw,) between days 1 to 10 post partum (n = 8 for each group) or corn oil (control, n=8 for each group). The uteri were collected in the experimental groups from 11-day-old pigs (n=4) or adult pigs (n=4) in their second estrous cycle. Part of uterus from each animal was fixed for immunohistochemistry (IHC) and the other was frozen and used for Western blot analysis (WB). In neonatal and adult uteri the MXC treatments resulted in a significantly higher relative Akt, pAkt, PI3K expression compared to the control group.

Obtained data shows that methoxychlor treatment in neonatal period may cause changes in expression of *PI3K*/*Akt*/mTOR pathway proteins both in neonatal as well as in adult pigs.

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Poster Presentation # 7

Differential Met-enkephalin activity in the brain structures of diurnal and nocturnal animals

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Met-enkephalin, one of the most active opioid, exists in the blood and tissues in two forms-native, five amino acids and as large precursor, called cryptic. It was found that it is involved in the regulation of immune and nervous systems, attenuates stress responses, mood and appetite. However, the question arises whether activity of this enkephalin system depends on the nocturnal or diurnal type of life. Thus, the aim of the study was to compare the degree of synthesis and concentration of native and cryptic Met-enkephalins, in the hypothalamus and pituitary of animals being nocturnal or diurnal.

Experiments were conducted on chicken (diurnal) and rat (nocturnal) kept under different light regime: control group at 12L (7.00a.m.-7.00p.m) and experimental at 24D (darkness for 24 h) with water and food *ad libitum*. Met-enkephalins and mRNA proenkephalin were measured in the hypothalamus and pituitary by RIA and hybridization *in situ*, respectively.

During control conditions, Met-enkephalin concentrations were much higher ($P<0.001$) in the rat hypothalamus (62 times) and pituitary (32 times) than in chicken brain structures. Also, the expression of proenkephalin gene was higher in the rat hypothalamus and pituitary of nocturnal animals. Twenty four hours of darkness caused decrease ($P<0.01$) of both forms of enkephalin in the pituitary of chicken (by 28-32%) and rat (by 73-75%). Unexpectedly, darkness increased the expression of proenkephalin gene in the pituitary of both animal species.

The obtained results showed that activity of enkephalin brain system depends on the animal sensitivity to the light.

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Poster Presentation # 8

Impact of estrogens on regulation of lipid status in testicular steroidogenic cells

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In endocrine cells, steroidogenesis depends upon availability of lipids, however connection between lipid homeostasis and steroidogenesis is not fully understood. Lipophagy, an intracellular process of degradation of lipid droplets, might contribute to lipid availability. Leydig cells of the testis, which synthesize steroid sex hormones, require involvement of diverse specifically-acting steroidogenic enzymes and regulating molecules to maintain lipid homeostasis. The study was undertaken to characterize the effect of GPER (G-protein coupled estrogen receptor – GPER) blockage together with estradiol and BPA treatment and involvement of various types of estrogen receptors on lipid status in Leydig cell. Special emphasis was put on *in vitro* cell function characteristics.

Mouse Leydig cells (MA-10) were treated for 48h with GPER antagonist (G-15; 10 nM), ER antagonists (ICI 182, 780; 10 μM), 17β-estradiol (10 mM), and bisphenol A (10 nM) alone or in combinations.

We found changes in lipid droplet size and distribution among treated cells. Additionally, ultrastructural analysis revealed presence of droplets with double membrane structures and degenerating ones. No changes in ERRs expression were revealed, whereas partial translocation of ERRβ and γ from the cell nucleus to cytoplasm was observed in G-15-treated cells when compared to control. We detected modulated expression of proteins associated with steroidogenesis and lipid metabolism in treated cells.

Obtained results provides new insight into complex and diverse estrogen effects on mouse Leydig cells at various steps of steroid hormone. Lipid homeostasis and metabolism in these cells were affected by endogenous or exogenous estrogens, interactions between receptors (GPER, ER and ERR) and antagonists (G-15 and ICI).

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Poster Presentation # 9

Comparison of the Balbiani body formation during oogenesis in internaly and externaly fertilizing osteoglossomorph species, *Pantodon buchholzi* and *Osteoglossum bicirrhosum* (Teleostei: Osteoglossomorpha: Osteoglossiformes)

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The 272 living species of Osteoglossomorpha exhibit various modes of reproductive biology demonstrated by a great differentiation described in gonad structure and male gametes ultrastructure (e.g. aflagellate and uniflagellate aquasperm and complex introsperm).

Therefore, the aim of our study was to analyze ovaries structure, oogenesis and oocyte development in the internally and externally fertilizing osteoglossomorph representatives, *Pantodon buchholzi* and *Osteoglossum bicirrhosum*, respectively.

Results reveal that ovaries of *P. buchholzi* are paired and contain oocytes in various stages of oogenesis without signs of synchronous development, which was observed in a single ovary of *O. bicirrhosum.* Also, the process of the oocyte differentiation exhibits various arrangement of the Balbiani body (= an aggregate of proteins, germ plasm, mRNA and membrane-bounded organelles, mainly mitochondria, Golgi apparatuses and endoplasmic reticulum), which facilitate the organization of the oocyte cytoplasm. In *P. buchholzi* ooplasm displays three different degrees of electron density; from electron-dense cytoplasm dominating in previtellogenic ovarian follicles through medium-dense to electron-lucent cytoplasm filling vitellogenic oocytes. Within medium dense cytoplasm nuage is arranged into a net structure and keeps elongated mitochondria together forming mitochondrial aggregation. In *O. bicirrhosum* ooplasm shows concentric organization. Nucleus is surrounded by granular cytoplasm containing organelles, mainly mitochondria bond by nuage into mitochondrial nets, whereas homogenous cytoplasm is located peripherally.

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Poster Presentation # 10

Preliminary study of olfactory organs of the African butterflyfish *Pantodon buchholzi* Peters 1887 (Teleostei, Osteoglossomorpha)

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Pantodon buchholzi, a freshwater African fish, belongs to Osteoglossomorpha, a phylogenetically basal group of Teleostei. The foraging mode of this species is uncommon among fish, as individuals jump above the surface of the water to capture prey.

The main aim of this study was to check if the olfactory organ of *P. buchholzi* has evolved adaptations to short ventures out of water. For research we used electron microscopy (TEM, SEM) and immunohistochemistry.

Preliminary observations show typical structure (like most Teleostei) of the olfactory rosette in *P. buchholzi.* The olfactory epithelium on the olfactory lamellae is arranged zonally. The olfactory sensory epithelium is present from the side of the median raphe of the olfactory rosette. Ciliated sensory neurons are most common, but microvillus sensory neurons and single giant cells are also present within the olfactory sensory epithelium. On the posterior wall of the olfactory chamber (under the posterior nostril) there is a cudgel-shaped structure, the wider end of which is located above the posterior olfactory lamellae. Our preliminary conclusions indicate that the structure of the olfactory organ of *P. buchholzi* shows adaptations for protection of olfactory sensory neurons against drying through: (i) the presence of additional structures in the olfactory chamber that probably allow water to be retained during jumping above the water surface; (ii) location of the olfactory lamellae close together; (iii) the presence of numerous mucus cells within the olfactory lamellae epithelium.

Poster Presentation # 11

Evaluation of antinociceptive properties of 1-h-pyrrolo[3,4-c]pyridine-1,3(2h)-dion derivatives in experimental model of pain

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The aim of present study was to examine potential antinociceptive, antiedematous (antiinflammatory) and antiallodynic activities of two 1*H*- pyrrolo[3,4-c]pyridine -1,3(2*H*) – dione derivatives (DSZ-13: 4-butoxy-N-[4-(2-etoxyphenyl)-1-piperazinyl]-methyl/-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dion and DSZ-19: 4-etoxy-N-[4-/2-fluoridephenyl/-1-piperazinyl)-methyl]-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dion) in various experimental models of pain.

For this purpose, the hot plate test, the formalin test, the carrageenan model and oxaliplatin – induced allodynia test were performed. In the hot plate test, none of the tested compounds were active. In the formalin model of tonic pain, DSZ-13 (2,5-20 mg/kg) and DSZ-19 (2,5-20 mg/kg) revealed dose dependent antinociceptive activity in both phases. To determine the plausible mechanism in tonic pain model, pretreatment with naloxone failed to affect antinociceptive activity of the both compounds, but pretreatment with caffeine, DPCPX reversed the antinociceptive effect of DSZ-13 (5 mg/kg) and DSZ-19 (5 mg/kg). Similar to ketoprofen, DSZ-13 (20 mg/kg) and DSZ-19 (20 mg/kg) presented antiedematous (antiinflammatory) and antihiperalgesic activity. Furthermore, both compounds (5 and 10 mg/kg) demonstrated a significant antiallodynic activity in the oxaliplatin – induced neuropathic pain model. Moreover, at active doses, no neurotoxic effect was observed in the rotarod test.

The study was supported by statutory grants: K/ZDS/006/237 and N42/DBS/000049.

Poster Presentation # 12

The analysis of genes and proteins involved in iron metabolism of neonatal liver – study based on *Hmox1* knockout mice

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In neonatal period liver plays a key role in systemic iron metabolism - it storages iron reserves and it is responsible for mammalian blood cells production and degradation. One of the main iron regulators is heme oxygenase 1 (HO1), an enzyme that catalyzes the breakdown of heme released from disrupted erythrocytes. HO1 cleaves the porphyrin ring to release ferrous iron, CO and biliverdin. Although many important aspects of iron metabolism were examined in adult HO1 knockout mice, there is still a very little knowledge about this issue in HO1 knockout neonates and very young individuals.

In present study we investigated iron and iron-related genes and proteins in HO1 knockout and wild-type mice aged from 3 to 11 days postpartum. We analyzed two very important genes responsible for regulation of systemic iron metabolism: ferroportin 1 (Fpn1) which is the only known cellular iron exporter and hepcidin, peptide hormone synthetized in liver and responsible for regulating iron efflux from the cells by suppressing Fpn1 activity. In addition, we analyzed non-heme iron content in neonatal livers and we investigated liver histology in HO1 knockout neonates in comparison to adult HO1 knockout individuals. We observed differences in ferroportin 1 and hepcidin expression between wild-type and knockout neonates in respective age groups, also, we noticed differences in a number of erythroblastic islands and iron deposits. Our results may suggest that in conditions of reduced or absent HO1 activity iron homeostasis is dysregulated, which is particularly unfavorable in developing and growing organisms.

The study was supported by NCN grant No. 2015/19/N/N24/00998.

Poster Presentation # 13

Protective role of metallothionein, lysenine and sodium dismutase in cadmium-exposed *Eisenia fetida*, *Eisenia andrei* and their hybrids

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Eisenia fetida and *Eisenia andrei* are closely related earthworm species, capable of asymmetrical hybridization. Those lumbricid earthworms are ubiquitous and highly resistant to a variety of environmental stressors, such as heavy metals. Differences occur in the immune reaction of those invertebrates and may thus differ in hybrids between both species. This might affect their survival in hostile environments. Identification of the origin of the mitochondrial plasmid and of the nuclear genome in hybrids (either *E. andrei* or *E.fetida*) combined with measures of the gene expression of mitochondrial as well as nuclear genes involved in detoxification processes allow to gain insight in the defense mechanisms. The main aim of the present study was to investigate the cadmium-related defense mechanisms at transcriptomic level in genetically defined *E.fetida*, *E. andrei* and their hybrids exposed for 2 and 7 days to Cd-polluted soil in mesocosms (x=425 ± 45.66 mg/kg). The level of gene expression of glutathione S-transferase (*gst*), lysenin (*lys*), metalothionein (*mt*), superoxide dismutase (*sod*) and phytochelatine synthase (*pcs*) was assessed in extruded coelomocytes, i.e.the immune cells present in earthworm coelomic cavity, and related to the expression of two stable house-keeping genes – actin (*act*) and ribo13 (*r13*). No important differences in *gst* and *pcs* expression were noted. Lysenin expression was down-regulated following the Cd exposure, while the expression of *mt* and *sod* increased after 2 days in *E.fetida* and hybrids and after 7 days in *E.andrei*. The expression of *mt* was slightly higher in hybrids compared to pure species. The study was supported by NCN grant (2016/23/B/NZ8/00748).

Poster Presentation # 14

Taurine and L-arginine mitigate the effect of *in ovo* chromium injection on intestinal proteins in newly hatched chicks

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Chromium supplementation may be involved in the glucose turnover, cholesterol metabolism and weight lost in people. Chromium may affect kidney, liver, gastrointestinal tract and endocrine system. However, it also had risks and its use is somewhat controversial.

Thus, the aim of the study was to examine the taurine and L-arginine mitigation of the chromium effect on the proteins release and concentration in the animal intestine.

Experiments were carried out on newly hatched chicks which were supplemented *in ovo* by 1.5 or 15 µg of chromium on 17th day of incubation. Fragments of intestine were immediately directed to the *in vitro* secretion of proteins and to the measurement of their concentrations. Taurine and L-arginine (89.5 µg/ml medium) were added to the culture medium for 30 min. Proteins level was measured in the tissue and culture medium by the Lowry method.

Supplementation of chromium significantly increased the protein concentration in the intestine tissue ($P<0.05$) but did not affected the basal *in vitro* secretion of proteins. Interestingly, addition of taurine and L-arginine significantly increased ($P<0.05$) the protein secretion from the tissue taken from chromium treated chicks. Unexpectedly, the lower dose of chromium had stronger effects on the proteins release and concentration.

The obtained results clearly showed the impact of chromium on the protein synthesis in the chicks intestine. This model proved also the positive effect of commercial supplement containing taurine and arginine on the concentration and *in vitro* release of protein from the intestine.

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Poster Presentation # 15

Gellan gum properties and their influence on viability and migration of tooth-derived stem cells

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Gellan gum (GG) is a water soluble exopolysaccharide synthesized by bacterial genus *Sphingomonas*. GG is promising compound in the development of films, scaffolds and medical devices for regenerative medicine. We used adherent periodontal ligament stem cells (PDLSCs) to verify applicability of GG for tooth-derived stem cell growth and migration. PDLSC viability was determined using MTS assay in the presence of native GG (0.75%-1.50% (w/o)) as well as 1.25% GG cross-linked with Ca^{2+} in relation to GG untreated cells (Control). Then, the cells were seeded either onto 1.25% GG surface or plastic surface but close to a droplet of GG. Both, adhesion and migration of the cells were studied using phase-contrast microscopy. Cell viability decreased in the presence of increasing concentrations of GG. Significantly reduced proliferation of PDLSCs was observed in 1.25% GG cross-linked with Ca^{2+} when compared to Control. However, no changes in cell morphology appeared in all experimental conditions. It suggests that degradation products of GG might not be neutral for stem cells and chemical modification of GG-based polymers should omit Ca^{2+} cross-linking. The anionic properties of GG inhibited cell adhesion to its surface, however, PDLSCs did not reveal apoptosis even after 48 hours of culture in suspension. Further, GG has sufficiently impaired PDLSCs migration in the proximity of the GG droplet for the first two days of culture. Antiadhesive properties of GG might be successfully employed in regeneration where development of connective tissue has to be avoided. The study was supported by M-ERA.NET2 grant “Pelargodont” (NCN 2016/22/Z/ST5/00962).

Poster Presentation # 16

Expression of phoenixin-14 (PXN) and its receptor GPR173 in the adipose tissue and ovaries in letrozole induced rat model of polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a complex and heterogeneous endocrine disorder, generally exhibiting the characteristic features of hyperandrogenemia, insulin resistance (IR) and obesity. Some research has revealed that, while the underlying mechanisms are not yet clear, an interaction of environment and hormones may contribute to the pathology of PCOS. Phoenixin-14 (PNX) is a newly discovered peptide produced by proteolytic cleavage of the small integral membrane protein 20. PNX was characterized as a reproductive peptide which regulates pituitary gonadotropin secretion as well as GnRH expression in rats.

The aim of the study was to examine protein expression of PNX and its receptor GPR173 in adipose tissue and ovaries of rat model of PCOS.

Twenty four Wistar rats were divided into two groups, control (n=16) that received vehicle only (2% DMSO in rapeseed oil) and treatment groups (n=8) administered nonsteroidal inhibitor of aromatase - letrozole (1 mg/kg) per 21 days. During this period, vaginal smears were collected daily for estrus cycle determination. On the day subsequent to last letrozole dose administration, rats were killed and samples (ovaries and adipose tissue) were collected. Protein expression of PNX and GPR173 were measurement by Western Blot.

We observed that PNX expression was higher both in the adipose tissue and ovaries in letrozole treatment rats; however expression of GPR173 was lower in the ovaries in treatment group.

These data indicated that the increase in PNX may contribute to the mechanism governing PCOS, and might provide a new potential target for therapies aimed at treating PCOS.

Poster Presentation # 17

Symbiotic associates of planthoppers from the family Issidae (Hemiptera, Fulgoromorpha)

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The symbiotic systems of three planthopper species (*Issus coleoptratus*, *Zopherisca tendinosa*, *Scorlupella discolor*) from the family Issidae were examined by means of microscopic and molecular methods. Planthoppers, like other plant sap-sucking hemipterans, live in mutualistic relationships with microorganisms which supplement their unbalanced diet in essential amino acids. Our histological, ultrastructural and molecular analyses revealed that *S. discolor* is host to two bacterial symbionts: *Sulcia* (Bacteroidetes) and *Vidania*, whereas in *I. coleoptratus* and *Z. tendinosa*, apart from these microorganisms, the bacterium *Sodalis* is present. The symbiotic bacteria are harbored in separate bacteriocytes which are integrated into large elongated organs termed bacteriomes. The bacteriomes are localized in the close vicinity to the ovaries. Additionally, bacterium *Vidania* occurs in bacteriocytes occupying the lumen of the hindgut. All the symbionts are transovarially transmitted from one generation to the next i.e. through the infection of female germ cells. In reproductive females, the symbionts leave the bacteriocytes and begin to invade the follicular cells which surround the posterior pole of the terminal oocyte. Bacteria temporarily accumulate in the cytoplasm of the follicular cells. Next, symbionts leave the follicular cells and gather in the space between the oocyte surface and follicular epithelium where they form a structure termed a “symbiont ball”.

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Poster Presentation # 18

Eisenia andrei, E. fetida and their hybrids as perfect cadmium accumulators

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Composting earthworms from *Eisenia* sp. are very resistant to polluted substratum, including heavy metal contaminations, in comparison with other lumbricid species. Among them, *Eisenia andrei* (Ea) and *E. fetida* (Ef) can develop fertile inter-specific hybrids. The main aim of present investigations was to compare viability and cadmium accumulation in adult earthworms from the pure species *Ea*, two mitochondrial lineages of *Ef* (Ef1 and Ef2) and inter-specific hybrids exposed for one month to commercial soil either unpolluted (control) or spiked with moderate or high concentration of cadmium chloride. Earthworms were individually delimitated by species-specific sequences of mitochondrial COI gene (either 'a' or 'f' or 'f2' for Ea, Ef1, and Ef2, respectively), and diploid nuclear 28S rRNA gene (either 'AA' or 'FF' for pure species or AF/FA for hybrids derived from Ea or Ef ova, respectively). All earthworms survived experimental period in the (almost) cadmium-free control soil and polluted with moderate or very massive cadmium concentration (425 and 835 mg/kg, respectively). During 4-week cadmium exposure, pure species and hybrids have accumulated huge cadmium quantities, up to 377 and 454 micrograms per g of dry body weights, in medium or highly polluted soil, respectively. Reproduction was inhibited in cadmium exposed earthworms, while body weights were decreased only in hybrids. These results show that Ea, Ef and their hybrids are perfect cadmium accumulators that only slightly impairs their functioning. Mechanisms of cadmium accumulation and detoxification in earthworm bodies are worth detailed examination.

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Poster Presentation # 19

Zinc xantonyloxyalcanoic acid derivatives effect on the level of pro-inflammatory proteins in HepG2 cells

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Xanthones are compounds in which the structure is the core of γ-pyrone skeleton condensed with two rings of benzene. Naturally occurring xanthones have a wide spectrum of pharmacological effects, including anti-inflammatory, antioxidant and hepatoprotective. The aim of this study was to evaluate the activity of zinc xantonyloxyalkanoic acid derivatives (compounds MH-106, MH-107 and MH-109) on the level of pro-inflammatory proteins in HepG2 cells, activated with lipopolysaccharide.The conducted research showed that all zinc xanthonyloxalcanoic acid derivatives have antioxidant activity, resulted in an increase of the nuclear factor Nrf2 level in HepG2 cells. The lowest COX-2 expression was demonstrated in hepatocytes incubated with the MH-109 compound, despite the LPS activation of the cells. This may suggests the anti-inflammatory potential of the MH-109 compound. The most preferred compound appears to be MH-109, because at the highest expression for Nrf2, and the lowest value for the expression of the pro-inflammatory proteins COX-2 and the FP receptor in HepG2 cells. It seems advisable to continue further *in vitro* and *in vivo* studies on the pharmacological activity of zinc xanthonyloxalkanoic acid derivatives.

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Poster Presentation # 20

Can *Euphorbia* latex be the scaffold for animal and human cells *in vitro* culture?

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Genus *Euphorbia* L. (family Euphorbiaceae) counts up to more than 5000 species, what makes it fourth-largest genus of flowering plants. *Euphorbia* latex has a wide range of applications in folk medicine as remedy for various diseases for instance: asthma, excrescences, tumors, cancers, warts, leprosy, gonorrhoea, syphilis, as well as sexual impotence. For this reason, latex is an interesting research object in modern medicine and pharmacy in aspect of potential source of secondary metabolites and for therapeutic purposes. Studies reveal that latex extract might be useful in antitumor and antiviral treatments. A few studies revealed that latex can be useful as surface for *in vitro* cell cultures. The main aim of our study was to examine *Euphorbia* latex as potential scaffold for human and animal cells. We also wanted to check, how latex can modulated the activity of testicular steroidogenic Leydig cells. We used three species of *Euphorbia*: *E. leuconeura* Boiss., *E. milii* Des Moul. and *E. trigona* Mill. In the context of above we used Leydig cells isolated from mouse testes and commercially available Leydig cell lines (mouse; MA-10 and human; HLC) to examine morphological and biochemical properties of the cells growing on *Euphorbia* scaffold. For the study both scanning electron microscopy and light microscopy were used. Cell biochemical parameters (expression of protein markers) and secretion of hormones were examined by western blotting and ELISA immunoassay, respectively. We found, that dependently on used latex type and cell culture type (mouse or human, primary or immortal line) Leydig cell morphology including proliferation and apoptosis as well as expression of steroidogenic markers (lutropin receptor, 3β-hydroxysteroid dehydrogenase, insulin-like peptide 3 and transcription factor DAX1) and progesterone or androgen secretion were diversely modulated. These results seem to be important for further detailed studies that can allow for implementation of some improvements to *in vitro* animal cell culture technique.

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Poster Presentation # 21

The effect of berberine hydrochloride on the ultrastructural changes in HepG2 cells

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Berberine, an isoquinoline alkaloid, is widely distributed in plants used in the tradidional Chinese medicine. It displays a wide range of biological activites and the mechanism of action. It has demonstrated a significant antimicrobial activity towards a variety of organisms including bacteria, fungi, protozoans, viruses, chlamydia and helmints. It can also be used as an antidiarrhea, antihypertension, antiarrhythmias and the antiiflamantory agent. Berberine is also demonstrated to possess antitumor activity.

The aim of this study was to investigate the effect of berberine hydrochloride on the ultrastructure changes of HepG2 cells lines (human hepatocellular carcinoma).

The experiment was carried out on HepG2 cell-line cultured in standard culture conditions, in MEM supplemented with a 10% fetal calf serum and with the addition of a mixture of antibiotics. After 48 hours of incubation, the fluid was changed to the culture medium with the addition of the berberine hydrochloride at concentrations of 10 nm, 10 µm, 100 µm. The cells were fixed in 3% glutaraldehyde and in 1% osmium tetroxide according to the modified Marzelli and Glauman's method (1980). Evaluation of ultrastructure was performed using a transmission electron microscope Tesla BS-500 with Frame Transfer-1K-CCD-Camera (TRS, Germany).

Electron microscope observation show changes in rough endoplasmic reticulum (RER) and mitochondria. We observed the increased dilatation of RER and mitochondria and also increased of amount of vacuoles. The ultrastructure changes in the HepG2 cells were dependent on an application doses of berberine hydrochloride.

Poster Presentation # 22

Delta-like 1 and Jagged 1 regulate claudin expression in Sertoli cells

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Delta-like 1 (DLL1) and Jagged 1 (JAG1) are ligands of Notch receptors involved in direct cell-to-cell communication in seminiferous epithelium. However, to date the role of these proteins in the control of Sertoli cell function has not been fully defined. Therefore, our study was aimed to explore the role of DLL1 and JAG1 in the regulation of blood- -testis barrier proteins, claudin-5 and claudin-11 in murine Sertoli cell line, TM4.

First, to examine whether the Notch pathway activation by DLL1 and JAG1 controls the expression of claudins, immobilized ligands were used. Next, γ-secretase inhibitor (DAPT) and recombination signal binding protein (RBP-J) silencing were performed to test the effect of Notch signaling inhibition on claudins expression. Real-time RT-PCR and western blot were employed to determine claudin mRNA and protein expression.

Activation of Notch pathway by JAG1 downregulated claudin-5 ($p < 0.01$; $p < 0.001$), while DLL1 decreased claudin-11 expression ($p < 0.01$; $p < 0.001$). Changes in the expression of both claudins were abolished by DAPT. Claudin-5 and claudin-11 mRNA and protein expressions were upregulated after both DAPT treatment ($p < 0.01$; $p < 0.001$) and RBP-J knockdown ($p < 0.01$; $p < 0.001$).

In summary, our results indicate that the expression of claudin-5 and claudin-11 is under inhibitory influence of JAG1 and DLL1, respectively. Thus, we have identified Notch pathway as a regulator of blood-testis barrier proteins in Sertoli cells.

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Poster Presentation # 23

In vitro antileukemic activity of formamidine derivatives of doxorubicin

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Doxorubicin is one of the anthracycline drugs widely used in anticancer therapy. The clinical efficiency of the drug and restrictions arising from its application are the foundation of the search of its more effective analogs. Structural modifications of doxorubicin are an important way to change its anticancer activity. In the search for new derivatives of the anthracycline, formamidinodoxorubicins containing in the amidine group either a morpholine moiety or a hexamethyleneimine moiety, were synthesized. The present study was undertaken to determine and compare the *in vitro* effects of doxorubicin and its formamidine derivatives on human lymphoblastic and myeloblastic leukemia cells. The study was conducted using the Coulter, spectrophotometry, and flow cytometry methods. It was found that the anthracycline agents triggered regulated cell death and affected the cell-cycle phase distribution. After application of the anthracyclines, the various patterns of temporary changes in the leukemia cell viability, count and volume, disorders in asymmetry of membrane lipids and cell membrane integrity, dissipation of mitochondrial membrane potential, and the cell-cycle disturbance, were observed. The cytotoxic effects of the parent anthracycline and formamidinodoxorubicins on the human leukemia cells depended on the tested agent and its concentration, the time interval after its application, and the cell line used. The structural modifications of doxorubicin were responsible for the different antileukemic activities of the formamidinodoxorubicin compounds. The search for the relationship between the chemical structure and action of the anthracycline agents on pathological hematopoietic cells is essential for the development of novel therapeutic strategies.

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Poster Presentation # 24

Kinetics of microparticle (MP) and neutrophil extracellular trap (NET) secretion during systemic inflammation in mice

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Microparticles (MPs) are structures secreted by various cells including neutrophils and they are found in numerous body fluids. MPs transport bioactive molecules, thus play an important role in intercellular communication. Neutrophils are also known for neutrophil extracellular trap (NET) formation, and NETs can be induced by MPs and what more, MPs can be attached to ejected traps. Therefore, the aim of the study was to investigate the secretion of microparticles and to correlate them with NETs in healthy and septic (endotoxemic - lipopolisaccharide) mice. As MPs are found in numerous body compartments, they were quantitatively estimated in blood (plasma) and peritoneal fluid by Nanoparticle Tracking Analysis (NTA). The results of *ex vivo* studies indicate low amounts of MPs in plasma and peritoneal fluid of healthy mice, and their increased secretion in septic animals. In order to estimate and visualize neutrophil MPs and NETs *in vivo* intravital microscopy (IVM) was used to image liver sinusoids and cremaster muscle vasculature. The *in vivo* studies indicate that the number of neutrophil MPs increases over time during systemic inflammation and reveals positive correlations between ejected NETs and numbers of neutrophils infiltrating the liver. We conclude that secretion of MPs and NETs are highly dynamic processes occurring in different tissue/body compartments during sepsis and they are released in a similar time frame.

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Poster Presentation # 25

BAG6 protects mouse male germ cells from PXT1 induced apoptosis

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For the proper male gametes production two opposite processes have to act in a balanced manner. On the one hand germ cells proliferation is required to provide adequate number of sperm and, on the other hand the elimination of damaged germ cells, usually by apoptosis, guaranties the quality of produced sperm. Peroxisomal, testis specific 1 (*Pxt1*) gene encodes for a mouse male germ cell specific protein. Testis specific overexpression of *Pxt1* resulted in induction of male germ cells' apoptosis mainly in primary spermatocytes, finally leading to male infertility in transgenic mouse model. We have also demonstrated that PXT1 interacts with BCL2-associated athanogene 6 (BAG6, also known as BAT3) and that this interaction protects cells from PXT1-induced apoptosis in *in vitro* experiments. In the *Bag6*$^{-/-}$ knockout mice massive apoptosis of primary spermatocytes was observed. Here we demonstrate that in the testis of *Bag6*$^{-/-}$ mice the *Pxt1* expression is elevated reaching the expression level observed in *Pxt1*- overexpressing transgenic males. We therefore generated a double knockout mouse model with the disruption of both *Bag6* and *Pxt1* genes. The analysis of testes of homozygous double mutants revealed normal spermatogenesis. Therefore we concluded that the main reason of infertility of *Bag6* knockout males is the proapoptotic action of PXT1. Thus, we present the model how BAG6-PXT1 interplay controls proper spermatogenesis in mice.

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Poster Presentation # 26

Catecholamines depletion affected opioid-like peptides in the hypothalamo-pituitary-adrenal axis in chicken

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Catecholamines interact with endogenous opioid peptides during immediate stress responses, however estimation of this effect is difficult due to spontaneous noradrenaline release. Thus, chemical sympathectomy by guanethidine is often used in animal models. Guanethidine is transported across the sympathetic nerve membrane by the same mechanism that transports norepinephrine itself, it is concentrated in transmitter vesicles, where it replaces norepinephrine. This leads to a gradual depletion of norepinephrine stores in the nerve endings. The aim of the study was to estimate the activity of enkephalin-like peptides in the hypothalamo-pituitary-adrenal (HPA) axis in control and stressed chicken in the absence of catecholamines.

Young chickens (n=24) were divided into 4 groups: control (C); stressed by 30 min of restraint (R); treated by guanethidine (Gua); stressed and treated with guanethidine (R+Gua). Met-enkephalin was measured in the hypothalamus, pituitary and adrenal by RIA method. The catecholamines level was measured by RIA method in the blood. Prolonged guanethidine injections (50 mg/kg b.w.) dramatically decreased the plasma levels of adrenaline and noradrenaline ($P<0.001$). Restraint increased the concentration of opioid in all tested tissues ($P<0.05$). Guanethidine caused much higher increase of Met-enkephalin concentration in the hypothalamus and pituitary without affecting the opioid level in the adrenal. Unexpectedly, guanethidine potentiated the stress effect on the opioid level in the pituitary but significantly attenuated it in the hypothalamus and adrenal.

The obtained results suggest close relationship of catecholamine and opioid systems in the regulation of HPA activity during physiological and stressful conditions.

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Poster Presentation # 27

Effect of $1,25(OH)_2D_3$ on steroids secretion *in vitro* by porcine ovarian follicles

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Vitamin D_3 (VD) is well-known as a major regulator of calcium-phosphorus homeostasis. However, growing evidence highlights its crucial role in the regulation of reproductive processes. It was shown that human and animal ovary is an extrarenal site of VD metabolism, and VD deficiency is observed in ovarian pathologies. Thus, the particular attention is directed towards the local action of VD within the ovary. The aim of the study was to examine the concentration of active VD ($1,25(OH)_2D_3$) in follicular fluid (FF) and the effect of $1,25(OH)_2D_3$ on steroids secretion *in vitro* by porcine ovarian follicles. Ovaries were harvested from mature pigs at a local abattoir and small (SF), medium (MF) and large (LF) antral follicles were isolated. FF was collected from examined follicles (n=20/each class) and $1,25(OH)_2D_3$ level was analyzed. SF (n=5), MF (n=5) and LF (n=5) were incubated with 1, 10, 50 and 100 ng/ml of $1,25(OH)_2D_3$ to determine the influence on progesterone (P4), testosterone (T) and estradiol (E2) release *in vitro*. Data were analyzed using nonparametric Kruskal-Wallis test. $1,25(OH)_2D_3$ concentration was the greatest ($p<0.05$) in FF of MF, lower in LF and the lowest in SF. Analysis of $1,25(OH)_2D_3$ effect on steroids secretion revealed: 1/ increased P4 release by SF and MF ($p<0.05$) following 10 and 50 ng/ml; 2/ increased E2 release by SF ($p<0.05$) following 10, 50 and 100 ng/ml, and MF ($p<0.05$) following 1, 10, 50 and 100 ng/ml. Concluding, VD is a regulator of steroidogenic function of ovarian follicles in pigs. Supported by 217-DZ12.

Poster Presentation # 28

Lack of activity of heme oxygenase 1 (HO1) lead to delay in kidney development and resulted in kidney disorders in *Hmox1 -/-* knockout mice

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Heme oxygenase 1 (HO1) encoded by the *Hmox1* gene, play a particularly important role in systemic and cellular iron metabolism, microelement essential for the course of many metabolic processes in the organism. In mammals iron homeostasis is based on the absorption of iron in the intestine, its storage in the liver, the use of erythroblasts to form hemoglobin and iron recirculation by phagocytosis of old erythrocytes by spleen and liver macrophages. Detoxification of heme released from Hb in the cellular compartment involves the activity of HO1, a key enzyme in heme catabolism that cleaves the porphyrin ring, to release ferrous iron, CO and biliverdin. The lack of activity of this enzyme leads to disorders of iron metabolism and iron deposition in cervical organs such as the spleen, liver and kidney, anaemia and growth disorders, which contributes to premature death. Our results showed that HO-1 has an important effect on the normal kidney development in the newborn mice. In the *Hmox1* knockout mice (*Hmox1-/-*) changes in the kidney structure and a delay in the rate of development of this organ were found, also histochemical analysis showed the presence of iron deposits in the kidneys of the 2-week-old *Hmox1-/-* individuals. In the adult *Hmox1-/-* we found progressive and increased with the age, iron accumulation in the epithelial cells of the renal tubules. It resulted with severe kidney damage and lead to pathological changes in the renal corpuscle and renal tubules. However we noticed that type of kidney damage is sex-dependent. In *Hmox1-/-* females, pathological changes are usually limited renal tubules, while in *Hmox1-/-* males, both renal corpuscle and renal tubules are strongly damaged.

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Poster Presentation # 29

Molecular dynamics of proteins from the lysenin family with a map of post-translational modifications

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Lysenin is a pore-forming protein originally discovered in lumbricid earthworm *Eisenia* sp. Apart from lysenin, few related proteins were discovered (Lysenin Related Proteins). They were named LPR-1, LRP-2 and LRP-3. To date, these homologues have not been compared using molecular dynamics approach. In this work, we decided to create models LRP-1, LRP-2 and LRP-3 using the lysenin matrix, with atomic resolution. After the simulation, the places of registered post-translational modifications were analyzed. It turned out that the proteins have highly similar II-nd and III-rd order structures. The models differ in the distribution of surface electrostatic potentials and the fluctuation of the carbon chain, which correlates with the increase in evolutionary distance. Modifications include both N and C terminal domains, including surface and core residues. The modifications do not include pore-forming tongue but only parts of the chain above the target biological membrane. We predict the existence of post-translational modifications islands in analyzed proteins. Phosphorylation dominates the surface amino acids of receptor domains that recognize the target lipid membranes. Deamidations and oxidations are more abundant, and occur at various depths. The number of currently registered sites for modification of proteins from the lysenin family is higher in *E.andrei* than in *E.fetida*.
The study was supported by NCN grant No 2016/23/B/NZ8/00748 (BP).

Poster Presentation # 30

NLX-240, a functionally selective 5-HT1A receptor biased agonist, does not affect intermediate-term memory in mice

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As the population ages, the number of people suffering from memory disorders increases. Scientists are still searching for more effective treatments. Currently the focus of interest is upon the serotonergic system and biased agonism. Biased agonists preferentially activate receptor subpopulations and specific signaling pathway, not affecting or even blocking another response.

The aim of our study was to test the influence of four functionally selective 5-HT1A agonists (NLX-219, NLX-225, NLX-240 and NLX-249) on intermediate-term memory using novel object recognition test in mice.

We used adult male Albino-Swiss mice (CD-1). After administration with studied compounds or saline (p.o.) we placed mice separately in experimental cages containing two identical objects. Mice were left there until the total time of the exploration of two objects was 20s, but no longer than 10 min. After 4h we placed mice again in the cages, except that one object was switched for a new one. Mice were left in the cages until the total time of the exploration of two objects was 20s, but no longer than 10 min as previously. In this case we measured the novel object exploration time. All experimental procedures were approved by the I Local Ethics Committee for Experiments on Animals of the Jagiellonian University in Krakow, Poland.

We proved that only NLX-240 at doses 4 and 16 mg/kg did not interfere the memory formation in mice – they spent more time exploring the new object than the familiar one. We observed intermediate-term memory impairment when we used other compounds.

Poster Presentation # 31

Ovarian follicular cells of viviparous insect, *Hemimerus talpoides* are involved in the nourishment of embryo during initial stages of embryogenesis

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Hemimeridae is a family of the highly specialized, epizoic and viviparous earwigs. Their embryos develop inside terminal ovarian follicles (also termed embryonic follicles) and rely solely on nutrients transferred from mother's tissues. This extreme reproduction mode is referred to as the intraovarian matrotrophy. We performed ultrastructural and histochemical studies of the initial stage of *Hemimerus talpoides* development. Specimens of this species were collected from the fur of Gambian pouched rats trapped in Lajuma Research Centre (Sautpansberg Mountain Range, Republic of South Africa). Our results show that the follicular cells surrounding fully grown oocyte of *H. talpoides* do not degenerate after initiation of embryogenesis but transform and gradually form the multi-layered wall of the incubation chamber in which the embryo develops. In addition, we show that amniotic cells of the early embryo remain in direct contact with transformed follicular cells. In the region of contact, short outgrowths of the amniotic cells associate with irregular surface specializations of the transformed follicular cells. Based on these findings, we suggest that extended "postfertilization" activity of hemimerid follicular cells represents an adaptation to viviparity and matrotrophic nourishment of the embryo in this earwig taxon. The study was funded by a research grant OPUS 11 (UMO-2016/21/B/NZ8/00560) from the National Science Centre, Poland.

Poster Presentation # 32

Effect of vaspin on progesterone and human chorionic gonadotropin secretion by human trophoblast BeWo cells. Preliminary studies.

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Introduction. Vaspin is an adipocytokine, which has been originally isolated from the visceral adipose tissue in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, an animal model of obesity with type 2 diabetes. This protein is known to have an insulin-sensitizing effect and serum concentrations of vaspin correlates with an obesity. Last data documented expression of vaspin in the ovaries and placenta cells, suggesting its role in female reproduction. However, the effect of vaspin on placenta endocrinology is yet not well known. Here, we examined action of vaspin on progesterone (P4) and human chorionic gonadotropin (hCG) secretion by human placental syncytiotrophoblast BeWo cells.
Material and methods. BeWo cells (4×10^3 cells per well on 96-well plate) were cultured with increasing concentrations of vaspin (0.01; 0.10; 1.00; 10.00; 100.00 ng/mL) for 24, 48 and 72 hours. Secretion of the hormones into the culture medium were analyzed by using ELISA kits (EIA-1561 for P4, EIA-1469 for hCG), whereas statistical analysis was performed using Kruskal-Wallis test.
Results. We observed that vaspin upregulated secretion of both hormones in time- and dose-dependent manner: differences in secretion were detectable after 48 and 72 hours and the strongest effect was observed in cells treated with 0.10 and 1.00 ng/mL of vaspin.
Conclusion. Vaspin by increasing P4 and hCG secretion is a new regulator of placental function, contributing directly to maintenance of the myometrium quiescence or suppressing the maternal immunology. However, our preliminary studies should be confirmed to molecular mechanism of vaspin action in the placenta cells.

Poster Presentation # 33

Interactions of eicosapentaenoic acid and docosahexaenoic acid in RAW 264.7 macrophages activated by benzo(a)pyrene

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Many diseases are caused by environmental and occupational exposure to chemicals, which is one of the major public health problems. Polycyclic aromatic hydrocarbons (PAHs) are a large family of toxic compounds which the main sources are combustion of organic materials, diesel exhaust fumes and industrial waste. Exposure to these compounds results in a diverse molecular response in the body, i.e. oxidative stress, enzyme activation, oxidation and / or signal transduction. The macrophage phenotype depends on signals received from the environment and polarization of cells and can be modified e.g. by supplementation with fatty acids. Activation of macrophages results among others, in the synthesis of eicosanoids and other lipid mediators.

The aim of the study was to determine the interaction of polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in mouse macrophages RAW 264.7 exposed to benzo(a)pyrene (BaP).

The effects of EPA (40 µmol) and DHA (40 µmol) and / or BaP (1 µmol) in RAW 264.7 cells on the level of cyclooxygenase-2 (COX-2), prostaglandin E2 synthase (cPGES), S-glutathione transferase (GSTM1) and the receptor for aromatic hydrocarbons (AHR) was analyzed by Western blot. Additionally, isoprostane levels were determined using the 8-Isoprostane and Prostaglandin F2α EIA kit tests.

The presence of PGF2α and 8-isoPGF2α was proved in RAW 264.7 cells activated by BaP. Supplementation with 40 µmol EPA and DHA and incubation with BaP resulted in a statistically significant reduction COX-2, cPGES and AHR pro-inflammatory proteins level compared to macrophages activated with BaP. Incubation of cells with EPA and DHA also showed an increase in GSTM1 levels, which may be related to the anti-oxidative properties of EPA and DHA in macrophages exposed to BaP.

The obtained results clearly indicate that EPA and DHA have anti-oxidative, anti-inflammatory and extinguishing inflammation properties, which can significantly contribute to reducing the negative effects caused by benzo(a)pyrene in macrophages.

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Poster Presentation # 34

In look for the source of the MUG fluorophore – a molecular fingerprint of *Eisenia andrei* earthworms

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Eisenia andrei earthworms are popular model organisms used in biomedical and bioremediation studies. During electric current stimulation, they expel coelomic fluid, which contains a variety of vital components. These components include two fluorophores: Riboflavin (vitamin B2) and MUG. Riboflavin, stored mostly in eleocytes, is essential for maintaining various biological processes. Since animals, including *E. andrei*, are deprived of riboflavin biosynthesis machinery, they rely on other sources, such as a plant based diet or intestinal microflora. In contrast to riboflavin, the knowledge of the MUG fluorophore is limited. The exact function, place of storage, and source of this *E. andrei* specific fluorophore is not revealed. It is believed, that one of the potential sources of the MUG in *E. andrei* is an intestinal microflora. The main purpose of this work was to investigate how seven day food deprivation and antibacterial treatment influence riboflavin and MUG regeneration in expelled *E. andrei* earthworms. The one week antibiotic treatment inhibited worm body weight gain and had a statistically significant effect on MUG regeneration, but an insignificant effect on riboflavin restoration. These results suggest, that intestinal microflora plays an important role as a potential source of the MUG fluorophore. The conducted analysis of the fluorophores content were measured in extruded coelomic fluid. The fluid was obtained with two different methods: „freezing" and „triton". The efficiency of both methods was compared and also presented.

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Poster Presentation # 35

Effect of stress on glutamic acid *in vivo* release from rabbit adrenal gland

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It is known that the hormonal activity of the adrenal glands is associated with the synthesis and release glucocorticoid into the circulatory system, but also catecholamines. All hormones are released in increased amounts in situations of disturbed homeostasis, e.g. under stress. Glutamic acid (GLU) is the main stimulant mediator in the nervous system, and its receptors are present in the adrenal glands and can modulate the hormonal activity of the adrenal glands.

The aim of the study was to determine the effect of stress on the amount of glutamic acid (GLU) concentration in adrenal gland tissue. In addition, specific agonist and antagonist of glutaminergic receptors have been used.

The experiment was carried out on 12 weeks old, 32 Popielno White female rabbits divided into groups: Gr. 1 – control, Gr. 2 – stress, Gr. 3 – GLU (30μM/kg b.w.), Gr. 4 - stress + GLU, Gr. 5 – GLU agonist (30μM/kg b.w.), Gr. 6 - stress + GLU agonist, Gr. 7 - GLU antagonist (30μM/kg b.w.), Gr. 8 - stress + GLU antagonist.

The stress factor was induced by hanging the rabbit for 30 minutes. The method of suspension was to prevent contact between the fore and hind legs with the ground. All of the above-mentioned compounds were administered intraperitoneally (*i.p.*) in a volume of 2.0-2.5 ml, 0.9% NaCl. After 30 min animals from all groups were killed and the adrenal glands obtained from them were frozen and on the day of the analysis were homogenized. In the supernatant the GLU concentration was determined by ELISA, converting the result to mg adrenal tissue.

The results of the experiment indicate that the action of the stress factor did not change the amount of GLU from rabbit adrenal tissue, while the addition of GLU to incubation caused a significant decrease in the secretion of this neurotransmitter. When the selected GLU antagonist and agonist was used, the adrenal response in GLU secretion was significantly greater, but no clear differences were found in the amount of this neurotransmitter secreted into the incubation medium. This indicates an unexplained mechanism of this neurotransmitter action in the adrenal glands and perhaps the lack of some of its many receptors in the adrenal glands, which prompts further research on this topic.

Poster Presentation # 36

Ultrastructural changes of CHO-K1 cells exposed to fexofenadine hydrochloride

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H1 antihistamines, including fexofenadine, are recommended to treat the most common allergic diseases (especially seasonal allergic rhinitis). Research to date focuses on the mechanisms associated with the inhibitory effect of the drug on the release of non-histamine pro-inflammatory mediators, i.e. LTC4, LTD4, LTE4 and ICAM-1, VCAM-1 or RANTES. The aim of this study was to evaluate the effect of fexofenadine on sub-microscopic changes of CHO-K1 cells, with particular emphasis on the lysosomal compartment and mitochondria, organelles associated with cell processes that are critical to the proper functioning of the cell.

Cells were maintained under standard culture conditions on DMEM medium (GIBCO, USA) and exposed to fexofenadine hydrochloride (Sigma-Aldrich, USA) for 48 hours at concentrations of 15 - 125 µM. After 24h of incubation, the cells were prepared for observation in a transmission electron microscope (TECNAI G2 SPIRIT FEI COMPANY).

The study results suggest that fexofenadine has numerous effects on the morphological profile of CHO-K1 cells. Typical features of the autophagy process have been demonstrated, such as an increase in the number of primary lysosomes and autophagic vacuoles, as well as swollen Golgi apparatus with numerous vesicles and strongly expanded channels of endoplasmic reticulum. Moreover, as a consequence of the action of high concentrations of fexofenadine, changes typical for apoptosis were observed, including both in the cell nucleus (change of shape, localized condensation of chromatin, pycnotic nucleus), as well as in mitochondria, whose profile clearly showed signs of damage. The obtained changes indicate the multidirectional mechanism of action of fexofenadine.

Poster Presentation # 37

Changes of HeLa cells exposed to physcion

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Anthraquinones are a group of compounds based on the anthracene skeleton found in plants such as rhubarb, aloe and legumes. They include emodine, aloe-emodine, chrysophanol, or physcion, which is the object of the current study. In addition to antibacterial, antifungal and antioxidant properties, it also has potential anti-cancer properties. The latter is particularly important, hence a lot of research is being conducted to understand the mechanisms of anthraquinones action in various cancer cell lines.

In the current study, cervical cancer cells (HeLa lines) maintained in standard culture conditions on DMEM medium (GIBCO, USA) were exposed to physcion (1,8-Dihydroxy-3-methoxy-6-methylanthraquinone) (Sigma-Aldrich, USA) in the concentration range 80-300 μM for 48 hours. The apoptotic changes in cells was analyzed using FITC/annexin V staining and propidium iodide using a FACSCanto flow cytometer (BD Biosciences, USA). The nuclei morphology of 4',6-diamidino-2-phenylindole-labeled cell (DAPI) were analyzed using a Nikon TiE *epi*-fluorescent microscope (Nikon Instruments).

Cytometric analysis showed that the increasing concentration of physcion induced an increase in apoptosis (at 300 μM more than 90% of total cell count were dead cells). The cells which were encumbred with the test compound were characterized by changes typical of programmed cell death, i.e. chromatin condensation, change in the shape of the nucleus and its fragmentation, including the formation of apoptotic bodies.

The range of obtained results was depended on the used concentration of anthraquinone. The results indicate that the physcion may have shown a potential anti-tumor activity against cervical cancer cells, and the exact mechanism of its action requires further research.

Poster Presentation # 38

Acrylamide neurotoxicity influences the survival of Drosophila and its circadian clock functioning

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Acrylamide (C_3H_5NO; ACR) is a well-known neurotoxin that can be found at high concentrations in carbohydrate-rich snacks consumed in growing amounts by contemporary humans. In this study we have examined the effect of chronic exposure to small (1, 8 and 10 µg/g) and high (60, 80 and 110 µg/g) doses of ACR (ACR1, ACR8, ACR10, ACR60, ACR80 and ACR110) on the fruit fly, *Drosophila melanogaster*, survival and functioning of its circadian clock. The results have revealed that the chronic dietary intake of ACR changes significantly the flies life span ($Ch^2 = 275.306$, df=4, $p<0.00001$), and influences the circadian pattern of their locomotor activity, which is a direct behavioral readout of the circadian clock functioning.

The ACR10 flies exhibit a prolonged lifespan (which suggests a hormetic response) with respect to control flies (CON), while the lifespan of the remaining ACR groups (ACR60, ACR80 and ACR110) was shorter than CONs and inversely proportional to ACR concentration (which suggests a cumulative effect). Both small and high doses of ACR induce an increase in flies activity (especially the night activity), decrease their morning and evening anticipation and/or change the length of the circadian rhythm period. Being receptive to small doses of ACR, the circadian clock of *Drosophila* appears to be a sensitive model that enables detection of all ACR-derived effects, including the subtle ones.

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Poster Presentation # 39

Morphology of rivaroxaban-induced liver injury

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The exclusion of other causes of liver injury (such as viral hepatitis, AIH, exposure to toxins) and the identification of a pattern of disease manifestation, that is temporarily related to exposure to the suspected drug, allows to make a diagnosis of drug-induced liver injury (DILI). DILI has been reported to occur in 5 - 10% of patients hospitalized for jaundice. The combination of elevations of serum aminotransferases (hepatocellular injury) and serum total bilirubin (jaundice without biliary obstruction) at the same time has been named “Hy’s rule”.

Rivaroxaban is a direct inhibitor of factor Xa that is approved since 2008 for the prevention and treatment of thromboembolic disorder. It is metabolized in the liver by CYP3A4 as well as by CYP-independent mechanisms. Approximately thirty cases of rivaroxaban-induced hepatitis were described in case reports between the years 2008 and 2018. However, mechanisms of rivaroxaban-induced hepatotoxicity are still unknown. Probably, it damages liver in mechanism of an idiosyncratic adverse drug reaction.

The aim of our study was to assess the histological and ultrastructural changes in liver biopsy of 51-year-old female patient who developed liver injury 2 months after starting therapy with rivaroxaban. Light and electron microscopy observation of liver sections revealed portal tracts with mixed inflammatory infiltrates and hepatocytes with features of steatosis. However, most of the changes were in perivenular zone, where necrotic hepatocytes and numerous macrophages loaded with ceroid material were observed. These changes were coincident with higher activity of CYP3A4 and were probably a result of toxicity of drug metabolites.

Poster Presentation # 40

Effect of neonatal exposure to agonists and antagonists of sex steroid receptors on epigenetic regulation of gene expression in corpus luteum of adult pigs

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The various factors and cellular processes are associated with corpus luteum (CL) formation, function and regression, including epigenetic mechanisms such as DNA methylation. This process of gene silencing requires the action of DNA methyltransferases (DNMTs) for the establishment (DNMT3A, DNMT3B) and maintenance (DNMT1) of DNA methylation. Thus, the aim of the study was to determine the effect of neonatal exposure to sex steroid agonists and antagonists on the degree of global DNA methylation and the expression of DNMTs in luteal tissue of adult pigs. Animals were injected with testosterone propionate (androgen activity, 20 mg/kg bw), flutamide (antiandrogen, 50 mg/kg bw), 4-*tert*-octylphenol (estrogen activity, 100 mg/kg bw), ICI 182,780 (antiestrogen, 400 µg/kg bw) or corn oil (controls) between postnatal days 1 and 10 (n = 5/each group). CLs were excised from sexually mature gilts between days 8 and 11 of the estrous cycle and snap-frozen in liquid nitrogen for DNA and protein isolation. Global DNA methylation was determined using MethylFlash™ Global DNA Methylation ELISA Easy Kit. DNMTs protein abundance was assessed by Western blot. Data were analyzed using Mann-Whitney U-test. Only ICI 182,780 significantly increased ($p<0.05$) global percentage of DNA methylation. Moreover, ICI 182,780 increased DNMT1 protein abundance ($p<0.05$) when compared to control. Concluding, neonatal exposure to estrogen antagonists may affect luteal function increasing DNA methylation and DNMT1 expression in CL of adult pigs. The study was supported by NCN grant No. 2015/19/B/NZ9/00420 (KKS) and by the Jagiellonian University, Program No. K/ZDS/008061.

Poster Presentation # 41

MMP-2 and TIMP-2 expression in the chicken ovary during pause in laying induced by fasting

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The study was undertaken to examine the expression of matrix metalloproteinase-2 (MMP-2) and its tissue inhibitor TIMP-2 as well as MMP-2 activity in chicken ovary during a pause in egg laying. The laying hens were fed *ad libitum* (control) or subjected to induced pause in laying by feed deprivation for 5 days (experimental). Birds were sacrificed on day 6 of the experiment and ovarian prehierarchical follicles: white (WF) and yellowish (YF) and three of the largest yellow preovulatory (F3-F1) follicles were isolated. From the F3-F1, the theca (T) and granulosa (G) layers were separated. In the experimental birds separation of the layers was infeasible due to atresia of these follicles. It was found that the relative expression of MMP-2 and TIMP-2 mRNA did not differ between the WF and YF. Within the largest follicles, the lowest expression of MMP-2 was observed in the GF1 and TIMP-2 in GF2. In feed deprived hens, the ovary regression (by 61%) was accompanied by decreased transcript levels of MMP-2 in all follicles and TIMP-2 in the prehierarchical follicles. Elevated expression of TIMP-2 mRNA was found in the TF3–TF1. Both latent and active forms of MMP-2 protein were detected in all tissues. Feed deprivation diminished the abundance of active form of MMP-2 in the WF, TF3, and TF1. The total activity of MMP-2 was lower in the G than in the T layer. During a pause in laying the MMP-2 activity was increased in the YF, and decreased in the TF3-TF1. The results obtained indicate that MMP-2 may not be involved in the regulation of advanced stage of atresia of chicken yellow ovarian follicles. Supported by NCN grant no. UMO-2015/19/B/NZ9/01356.

Poster Presentation # 42

Eicosapentaenoic acid supplementation in A549 cells activated with benzo(α)pyrene

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Exposure to environmental pollution plays an important role in the etiology of inflammation, lung diseases or atherosclerosis. Polycyclic aromatic hydrocarbons (PAHs) are widespread lipophilic impurities present in the air, soil, and also in grilled foods. A549 lung epithelial cells are sensitive to environmental stress, caused by AHR receptor agonists, which results in the activation of pro-inflammatory effects . The process of quenching inflammation is related with lipid mediators, derivatives of polyunsaturated fatty acids.

The aim of the experiments was to determine the pro-or anti-inflammatory effect of eicosapenatenic acid (EPA, C20: 5 n-3) on pro-inflammatory protein levels and the amount of isoprostane in human A549 lung epithelial cells exposed to benzo(α)pyrene (BaP).

The effects of EPA (40 µmol) and/or BaP (1 µmol) in A549 cells on cyclooxygenase-2 (COX-2), prostaglandin E2 synthase (cPGES), S-glutathione transferase (GSTM1) and aromatic hydrocarbon receptor (AHR)) by Western blot. In addition, isoprostane levels were determined using the 8-Isoprostane Express EIA kit test.

In A549 cells supplemented with 40 µmol EPA and incubated with BaP, we observed significant inhibition of COX-2, cPGES and AHR pro-inflammatory proteins was observed compared to BaP activated cells. Overexpression of GSTM1 and decrease in the level of 8-isoPGF2αco have also been shown to be associated with the anti-oxidative properties of EPA in lung epithelial cells exposed to BaP.

The results show that EPA can significantly affect the function of A549 lung epithelial cells and reduce the harmful effects caused by benzo(α)pyrene.

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Poster Presentation # 43

The effect of herbs supplementation on selected biochemical parameters in rabbits blood

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Herbs and their extracts have been used in human and veterinary medicine for a long time but little is still known about their health benefits in rabbits. Thus the present study aimed to examine the influence of the nettle *(Urtica dioica L.)* and fenugreek *(Trigonella foenum-graecum L.)* diet supplementation on the glucose, triacylogliceroles and cholesterol blood level in rabbits. The experiment was performed on Termond White rabbits (18♂, 18♀), housed in a heated hall furnished with water supply (available *ad libitum*), lighting (14L:10D) and exhaust ventilation from the 35th to the 84th day of life. The rabbits were divided into three feeding groups. The standard mixture for the control group included wheat, wheat bran, extracted sunflower seeds, dried alfalfa, roasted soybeans and mineral-vitamin supplement. The experimental groups were fed with a feed containing 2.0% nettle or feed containing 2.0% addition of fenugreek. Blood was taken after decapitation, centrifuged and selected parameters were assayed by commercial kits. It was found that the glucose level was higher after fenugreek. Lowered values of the triacylogliceroles was observed after all served herbs, whereas nettle decreased and fenugreek increased cholesterol plasma level. In addition stronger influence of herbs supplementation was observed in females. The results obtained indicate that herbs supplementation affects the biochemical parameters in blood of rabbits, which may have a significant impact on their health and consequently productivity. This research was financed by the Ministry of Science and Higher Education of the Republic of Poland – SUB215-D204.