

POSTERS

Comparative scanning studies on cockerell (*Gallus domesticus*) spermatozoa with the use of SEM and AFM method

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Fresh cockerell sperm after double rinsing in the diluter was fixed according to standard procedures in 2.5% glutaraldehyde in PBS buffer, pH 7.4. Following dehydration in a graded ethanol series the sperm was prepared for SEM and AFM studies on cover glasses and

mica. Photographs were taken with scanning microscope TESLA BS 300 and with atomic force microscope LFM - 3. The micrographs show morphological differentiation of the surface of the spermatozoa depending on the preparation method applied.

Influence of caffeine on follicular development in Balb/c mice

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Fecundability is defined as ability to achieve a conception or recognized pregnancy. It can be also determined as the time to pregnancy (TTP). Impairment of fecundability may be a sensitive marker of environmental impact on reproductive health.

The process of ovarian follicle development involves coordination of growth and differentiation of the oocyte and somatic cells. The bidirectional communication between oocytes and granulosa cells maintained via cytoplasmic processes and contact between granulosa cells, resulting from the presence of adhesion-type junctions, is important for development of the follicles.

Reproductive function of the follicles is very sensitive to many environmental factors, which may impair their normal development and increase atresia. Molecular mechanism underlying this process is apoptosis. Follicular degeneration is accompanied by ultrastructural morphological changes in oocytes and granulosa cells.

The aim of this study was to characterize the morphological structure of the ovaries and granulosa cells after applying caffeine. The mouse ovaries were obtained from Balb/c mice. Adult female mice were divided into the three groups and injected subcutaneously three days a week for four weeks with caffeine solutions: 50 mg/kg, 100mg/kg and 150 mg/kg, respectively. The mice from control group were injected with 0,9% saline solution

alone. The animals were killed by dislocation of the spinal cord and then ovaries were collected and prepared for light and electron microscope examination.

The cumulus – oocyte complex of the follicles were examined in the study. In follicles from control group this complex was characterized by a compact region of stratified cuboidal granulosa cells that were separated from the oocyte by a zona pellucida. There were many striations in the zona pellucida due to the cytoplasmic processes. In the third group we observed dramatic changes in the complex. It was less compact; the number of the cells in the cumulus was reduced and dissociated. Granulosa cells of the cumulus exhibited only short and thick processes and the number of them was significantly reduced.

We observed specific histological changes in the granulosa cells of follicles in advanced stages of atresia: the presence of pyknotic nuclei; massive reduction in the number of granulosa cells; high density of cytoplasm.

Morphological signs of atresia were revealed in oocytes: mitochondria were dispersed in cytoplasm; reduced number of cytoplasmic processes; increased number of lipid droplets; vesicular bodies and varying sizes of cytoplasmic vacuoles.

The molecular mechanism injuring follicular cells by caffeine remains unresolved.

Analysis of fixing methods for chosen organs of the embryo of leopard gecko *Eublepharis macularius* Blyth (Reptilia, Sauria, Gekkota, Eublepharidae) with the use of electron microscopy

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A great variety of methods for preserving animal tissues and organs are available, however no fully satisfactory procedure of reptile organ fixing has as yet been found. The aim of the experiment was to find the best method for fixing neural tube, liver and heart of the embryo of leopard gecko (*Eublepharis macularius* Blyth) with the use of electron microscopy.

In the experimental stage fertilized eggs of leopard gecko were used and the chosen organs i.e. neural tube, liver and heart were extracted from them. The obtained material was placed in four different fixing solutions and subjected to two fixing times i.e. 4 h in 4°C and one week at room temperature. The fixed material was exposed to a variety of alcohols and acetone. Then it was immersed in a mixture of epons and subsequently contrasted by using techniques routinely applied in electron microscopy. From our experiment it results that the best fixing solution for

all examined organs is the mixture of 3.5% glutaraldehyde with 3% dextran in a cacodylic buffe with the osmotic pressure of 0.08M. In the case of this mixture the time and the temperature of the fixing solution (4 h in 4°C and 1 week in room temperature) do not seem to affect the quality of the fixing process. The mixture of 3.5% glutaraldehyde with 3% dextran in a cacodylic buffe with the osmotic pressure of 0.125M seems equally good, however the best results were obtained after fixing the material for 4 h in 4°C. The remaining fixing solutions used in the experiment showed significant changes in the fixed material and therefore were not taken into consideration.

It can be concluded that the reason for the worse quality of the material kept in fixing solution for 1 week at room temperature in comparison with the material kept for a shorter period of time for 4h in 4°C is the temperature of the fixing solution rather than the time of fixing.

Proliferation and differentiation of in vitro isolated and cultured endosperm of selected species of Angiospermae

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The in vitro competency of isolated endosperm of selected important crop plants was assessed for proliferation and differentiation on culture media with the plant growth regulators. Mature endosperm were excised from seeds of kiwifruit (*Actinidia deliciosa* cv. Hayward) and eight cultivars of winter, spring and durum wheat (*Triticum aestivum* and *T. durum*). Unmature endosperm of *Triticum* was isolated after 8 and 12 days after pollination (DAP). The basal medium consisting of MS (Murashige and Skoog, 1962) salts and vitamins was supplemented with 3 or 8% (w/v) sucrose and different concentrations of auxins (2,4-D, dicamba), cytokinins (kinetine, thidiazuron – TDZ) and steroid (homobrassinolide – HBL). Culture were incubated in the dark or exposed to a 16 h photoperiod provided by cool-white fluorescent tubes (60-90 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The material for sectioning (fresh isolated and cultured endosperm) was prepared by embedding tissues in Technovit 7100 (Heraeus Kulzer). Mature endosperm and endosperm isolated 12 DAP of wheat were not taken the development. After one week of the culture, tissue proliferation of endosperm of spring wheat cv. Tonacja

isolated 8 DAP was observed. Endosperm developed in the dark, on medium supplemented with 8% sucrose, 2,4-D and TDZ. In kiwifruit the long-term culture of endosperm-derived callus and organogenesis induction were described previously (Góralski et al., 2005; Popielarska et al., 2006). At present work influences of new media with another plant growth regulators, which are involved in the control of plant embryogenesis (dicamba, HBL) were tested.

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Ultrastructure of the hepatopancreas epithelium in *Porcellio scaber* Latr. (Crustacea, Isopoda)

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The aim of our studies was to describe the ultrastructure and regeneration of the hepatopancreas epithelium in *Porcellio scaber*. The hepatopancreas of *Porcellio scaber* consists of two pairs of blind-ended tubules, which opens into the foregut. This structure is homologous to midguts of other animals. Each tubule is composed of an epithelium which is made up of two cell types. The larger, usually binucleate and poliploid cells are called B and the smaller S cells. No regenerative cells were seen among differentiated cells in any of preparations examined. The B-cells are characterized by the presence of well developed microvilli, active Golgi complexes, extensive arrays of rough endoplasmic reticulum, numerous mitochondria and a lot of lipid droplets. This ultrastructural features suggest the role of the B-cells in secretion of digestive enzymes and absorption of nutrients. The main characteristic of the S-cells is a paucity of Golgi complexes and an extensive endoplasmic reticu-

lum, as well as presence of electron dense granules.

Both types of cells are connected by two kinds of intercellular junctions. The first one is a new type of junction, whose structure is not well known yet, although we know, that they do not contain the actine filaments. Second type of connection is gap junction.

The cells, which made up an epithelium of hepatopancreas differ in size. At the tip of this organ there are undifferentiated, small, mononucleate cells that lack microvilli. It is supposed that they serve as a source of regenerative cells that replace used up cells of the main epithelium. As the cells pass to the further part of the epithelium they enlarge, become binucleate, poliploidal and their adluminal surfaces become covered by microvilli. Similar sequences of changes one can see in differentiating hepatopancreas epithelium in juvenile specimens.

The morphology of caecum in sheep in perinatal period

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The accessible literature lacks of wider studies on morphology of sheep's caecum in perinatal period. The investigations were carried out on 18 sheep fetuses from perinatal period. The morphology of caecum was described. The precise location of the organ was carrier out with topographical anatomy methods; sceletotopy, syntptpy and holotopy. During nerve preparation the methods based on acetic acid and ethanol solution, due to make them more visible. The investigated organ begins on the level of the first or the second lumbar vertebra (L1 – L2) and its top reaches the level of the first or the second sacral vertebra (S1-S3) The measurements of caecum were carried out in three dimensions. The ostium ileocaecocolicum was also measured and the volume of caecum was estimated (ca. 2,73 ml). The caecum in investigated sheep's fetuses, lies on the right side of 1/3 upper part of abdomen. Cranially the body of caecum passes into ansa proximalis coli which has got similar

diameter, caudally apex caeci reaches the pelvic cavity, ending beyond the caudal extremity of the left kidney. Dorsally it neighbors with ansa proximalis et distalis coli and medially with ansa spiralis coli and ileum, which joins with caecum by plica ileocaecalis. The caecal lymph node (limphonodus caecalis) is located dorsally and caudal to caecum and ileum, in neighborhood of ostium ileocaecocolicum. The blind intestine takes the cylindrical form, it is arch-like flexed dorsally and medially, with the rounded top. The mentioned intestine has smooth walls and on the level of the ileo-caeco-colic opening it passes into the first section of colon with the same diameter. The caecal lymph node in investigated sheeps showed considerable differences in its shape, from oval and bilaterally flatted, ovoid to spherical. This lymphnode provides lymph drainage from the investigated part of the alimentary tract.

The morphology and development of tunica muscularis in domestic pig's stomach in prenatal period

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The bibliography lacks of wider information about pig stomach development. The investigations were carried out on 138 domestic pig fetuses from 35–114 day of prenatal life, including 68 males and 70 females. Whole investigated material was genetic equal, known origin (including the horn of uterus), sex and age. The number of examples had allowed the statistical analysis and the acquisition of statistical important findings. The population profile method (Bożiłow and Sawicki, 1980), dividing the approachable material to six groups, using method introduced by Marrable (1971). The acquired results of microscopic observations were produced as photos. The histometric measurements of tunica muscularis were produced as tables and statistically analysed. It was proved, the sex of foetuses and their location in the horn of uterus has no significant influence on any investigated value related to the stomach musculature development.

The highest growth rate of the majority parametres occurred in 5th and 6th group. Both layers of tunica muscularis was microscopically visible in 60th day of pregnancy. Their thickness decreases rapidly in 60th day of prenatal life, due to their into two layer division. The architecture of tunica muscularis in perinatal period is similar to observations carried out postnatally (Nickel et al., 2004).

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Microscopic structure of swine fetus aorta in relation to their biomechanical properties in prenatal period

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During prenatal period arteries, in consideration of increased peripheral resistance, gradual development of arteries wall take place. This is mainly fibrous elements in big arteries what result in rearrangement of their run and number what allows to adapt to changing conditions.

We have examined 5 aorta from fetuses of pig of both sexes from one litter. Samples of aorta from abdominal part 5 cm long were transported in 0,75% NaCl solution in reduced temperature conditions and next submitted biomechanical and histological examinations in as short as possible time after samples collection. In biomechanical test, stretching in one axis were performed both longitudinal and cross to the aorta wall.

For histological examinations material was taken before and shortly after biomechanical test of samples stretching and fixed in 4% of buffered formalin. After washing in fresh water material was dehydrated in alcohol and embedded in paraffin. Slices 5 µm thick were stained with hematoxylin and eosin and according to van Gieson method for elastic fibers. The durability of aorta wall on stretching were designated. In histological examination, changes in aorta layers as result of stretching are described. Observed changes were highest in tunica intima and media and are connected with rearrangement of collagen and elastic fibers.

Histological structure of pig skin in relation to biomechanical properties in prenatal period

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During prenatal period in skin gradual development of specific, typical for region structures arisen from ectoderm and mesoderm take place. Increase of collagen and elastic fibers and their relationships have direct influence on examined sample to resist on load bearing. We have examined 13 fetuses of pig (*Sus scrofa f. domestica*) of both sexes from two families from one husbandry farm. Animals were kept in that same zoohygienical environments, fed with that same food so similar homogeneous material was obtained and reliable examinations could be performed. Material was transported in 0,75% NaCl solution in reduced temperature conditions and next submitted biomechanical and histological examinations in as short as possible time after samples collection. Stripes of skin 5×50 mm were taken from following regions: dorsal and lateral of neck (*regines colli dor-*

salis et lateralis), between shoulder blades region (*regio interscapularis*), from dorsal part of chest (*regio vertebralis thoracis*) and cranial and medial part of abdomen (*regiones abdominis cranialis et media*).

For histological examinations material was taken before and shortly after biomechanical test of samples elongation in one axis and fixed in 4% of buffered formalin. After washing in fresh water material was dehydrated in alcohol and embedded in paraffin. Slices 5 µm thick were stained with hematoxylin and eosin and according to van Gieson method for elastic fibers. Differences in stress resistance of skin samples were observed. It was caused by alternate number and thickness of collagen fibers and their meshwork structure. After extension test collagen fibers permanently change their dimensional position.

The effect of medium aeration on the embryological development of *Contracecum rudolphii* Hartwich, 1964

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The definitive hosts of the nematode *Contracecum rudolphii* in Poland are mainly the cormorants (*Phalacrocorax carbo*). These birds are very attractive definitive hosts of many parasites because of their big mobility among the water areas on different continents. The parasites' eggs together with the cormorant's excrements get directly into the water where they go through the further development stages. The purpose of this research was to investigate the influence of water aeration on the further embryonic development of the examined parasite.

Nematode eggs were isolated from the terminal part of the adult females' uterus and placed in the tap water. The egg suspension was divided into 4 parts, the first 2 parts were placed on the Petri dishes and the remaining ones were placed in the high beakers. Within each group half of the culture was aerated several times a day using sampling pipette, whereas the rest was not. All the cultures were kept at the room temperature (17–21°C). The microscope examination of the eggs development in both samples was conducted each day.

On the day the culture was established there were around 2% of the eggs in blastomer stage 2. The larvae stage was obtained only by the eggs kept in aerated cultures, both in the thin (Petri dishes) and thick (beakers) water layers. In the cultures placed on the Petri dishes the larvae stage was reached by 80% of eggs in the 7th day of incubation, whereas for the culture kept in beakers this result amounts to 60%. The eggs placed in the thin water layer which was not aerated were developing very slowly. During the 7 days only 7% of the eggs achieved the larvae stage. The eggs placed in the high not aerated water layer did not reach the larvae stadium during the whole experiment period.

The conducted experiment may support the aerobic metabolism of the *C. rudolphii* embryos. The nematodes eggs which together with the cormorants excrements get directly into the water fall down quickly on the bottom of the water reservoirs. The most optimal natural conditions for the *C. rudolphii* eggs development are the shore water zones of the water reservoirs where the water oxygenation is the greatest.

Histology of the mature gonads of the sea trout (*Salmo trutta m. trutta* L.) and brown trout (*Salmo trutta m. fario* L.)

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The histology of the mature spawn gonads has been studied on the material collected from the male sea trout migrating for spawn from the sea to the river of the mean body size 56.6 cm (SD=5.6) and from brown trout living in the stream of the mean body size 24.1 cm (SD=4.7). The gonads were collected during the spawning season and fixed in Bouin's fluid.

The gonads were compared as to the developmental stage. The cross-sections of the testes and tubules were

analysed under an optical microscope in histological sections taken from three parts of the testes: anterior, intermediate and posterior. The elements analysed included: the cross-section area of the gonad and tubules, wall height of the tubules and the diameter of the vacuoles occurring in the tubule wall. Also the number of the tubules was counted and the number of the tubules per the surface area of the gonad was determined.

Cytogenetical and histological analysis of *Arabidopsis* somatic embryos

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Somatic embryogenesis is a regeneration system leading to form bipolar structures from somatic cells resembling zygotic embryos. This regeneration system for *Arabidopsis* became an attractive model for detailed analyses of cellular and genetic basis of somatic embryogenesis (Ikeda-Iwai et al., 2002; Gaj et al., 2005). However, some differences at the morphological, histological and cytogenetical level between zygotic and somatic embryos of diploid plant of *Arabidopsis thaliana* ecotype were revealed. First stages of the zygotic and somatic embryos were very similar to each other. When the primordia of cotyledons appeared, about 80% of all somatic embryos exhibited alternation in size and morphology involving mainly cotyledons. Another interesting difference was the occurrence of regular vascular system in somatic embryos during early embryogenesis. This was

contrary to zygotic embryos, in which vascular system in the form of procambial strands was present during whole embryogenesis. The regenerated plants characterized by the diploid and tetraploid genome. Among diploid as well as tetraploid regenerants were observed plants with highly endoreduplicated cells in leaves, in some cases achieved 128C DNA level.

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Ovary structure and oogenesis in *Limnodrillus* (Oligochaeta, Tubificidae)

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Among annelids the ovary organization and oogenesis are well-known in Polychaeta. In Clitellata only some groups as Enchytreidae, Lumbricidae, Glossiphoniidae, Piscicolidae, Erpobdellidae and Hirudiniformes have been studied. Especially ovary architecture and oogenesis are poorly known in non-leech Clitellata (Oligochaeta sensu stricto). The formation and structure of germline clones in non-leech Clitellata is also unknown. According to some authors (Jamieson, 2006), in contrast to leeches, in Oligochaeta sensu stricto, except *Dendrobaena veneta* (Siekierska, 2003), there are no nurse cells supporting growing oocytes; the germ cell clones, if they form, break down during early oogenesis.

Here we present our preliminary studies on ovary organization and oogenesis in *Limnodrillus* sp. (Clitellata, Tubificidae). Using light and electron microscopy we have shown that in general the ovary structure in this species resembles the ovary cords (ovary strings) described from leech ovaries. However, in *Limnodrillus* sp. in contrast to leeches, the germ cells do not develop within the coelomic sacs; they are surrounded only by a thin layer of somatic cells. The ovary (ovary cord) in *Limnodrillus* sp. consists of numerous groups of germ cells connected to a common cytoplasmic mass (cytophore) via cytoplasmic bridges. Like in other Oligochaeta, each germ cell has also only one bridge

connecting it to the cytophore. Similarly to ovary cords in Erpobdellidae and Hirudiniformes the ovary in *Limnodrillus* is polarized and, at least 3 zones can there be discerned. At the top of the ovary there are numerous oogonia and small cysts of undifferentiated germ cells. The middle part of the cord is occupied by differentiating germ cells, i.e. by numerous nurse cells and few oocytes. In 3rd zone growing oocytes undergo previtellogenesis and gradually protrude into coelom; in this zone the cytophore reaches its maximal dimension. Vitellogenic and ripe oocytes float freely in the coelom. In *Limnodrillus* ovary apart from germ cells there are also somatic cells. These flattened cells surround the entire ovary from the outside and also enter among cysts of germ cells.

Our results show that ovaries in *Limnodrillus* are more similar to leech ovary cords than to other ovaries in Oligochaeta sensu stricto described so far.

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Microsporidia infecting tissues of *Isohypsibius granulifer* Thulin, 1928 (Tardigrada: Eutardigrada)

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The Microsporidia are obligatory intercellular parasites found in a wide range of vertebrate and invertebrate hosts. They are especially common in insects, crustaceans and fishes (Larsson, 1999). These parasites are characterized by considerable diversity of structure, however they are unified by: 1) sporoplast that is uni- or binucleate; 2) uninuclear spores; 3) an extrusion apparatus in the spores includes polar filament; 4) lack of mitochondria and flagellum.

Microsporidium infecting *Isohypsibius granulifer* belongs to the family Unicarionidae. Following developmental stages have been found: meront, sporont, sporoblast and spore. All stages, except early meronts, are sur-

rounded by a sporophorous vacuole where the sporonts developed into sporoblasts. Mature spore are ellipsoidal. The polar filament is deposited in a single coil of 6–8 turns. The species of this Microsporidium were found in different tissues of *I. granulifer* (midgut epithelium, body wall, body cavity cells, oocytes, trophocytes, and gonad wall). Despite of the fact that Microsporidia infected oocytes and trophocytes it is difficult to prove maternal transmission.

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Cryopreservation of in vivo matured pig oocytes by OPS vitrification

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The sensitivity of mammalian ova to cryopreservation is species specific. It is known that cryotolerance of pig oocytes and embryos is very low. This might be due to the higher lipid content in cytoplasm of porcine ova compared to other mammalian embryos. The development of delipitation technique has provided the first evidence that intracellular lipids are linked to hypothermic sensitivity (Nagashima et al., 1996). Recent research on cryopreservation of pig oocytes and embryos has focused mainly on their vitrification (Gajda and Smorağ, 2003). It was demonstrated that the open pulled straw (OPS) vitrification method has been successfully applied to the cryoconservation of matured bovine oocytes (Vajta et al., 1998). The objective of the experiment was to examine the effect of OPS vitrification on morphology and survival ability of in vivo matured pig oocytes. Oocytes at metaphase II derived from superovulated gilts were divided into three groups: untreated (control), exposed for 1, 2 or 5 min to vitrification solution (VS), or cryopreserved by OPS vitrification. After exposition to VS approx. 80% of oocytes were evaluated as morphologically normal. Parthenogenetic embryos developed to morula at rates between 40 to 45 %, compared to 60% in control. After vitrification approx. 70% of the oocytes were morphologically normal. The other 30% had dark

contracted cytoplasm, vacuolization or cracked zona pellucida. After transfer of 45 vitrified oocytes to 2 recipients no pregnancies were obtained. Our preliminary results demonstrated that exposure to VS undependently of exposure time decreases parthenogenetic development of porcine matured oocytes. A relatively high proportion of morphologically normal vitrified oocytes noticed in our experiment suggests that OPS vitrification procedure should be considered as a method for cryoconservation of porcine matured oocytes. Further studies to evaluate fertilization and in vivo development of vitrified oocytes are required.

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Structural changes in the male gonads of *Danio rerio* (Teleostei: Cyprinidae) during reproductive cycle

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The structure of the *Danio rerio* testis and maturity stages were investigated. The structure of the testis corresponded to the anastomosing tubular type with unrestricted spermatogonia. During the reproductive cycle eight reproductive classes were described based on histological characteristics and stereological analysis. Stages I – III corresponded to the regressed class, which was characterized by presence of spermatogonia associated with Sertoli cells and small, scattered spermatocyst containing spermatocytes and early spermatids. Residual sperm were present in the lumen of tubules. During stages IV – VI (maturation class) the germinal epithelium was continuous and active spermatogenesis occurred throughout the testis. Spermatids and spermatozoa were more abundant in the tubules than in the regressed class. The increase in volume of spermatocysts were also observed. They mainly contained spermatocytes and spermatids. During spermiation (stages VII – VIII) a large amount of spermatozoa were observed in the lumina of tubules and sperm duct. Small germinal cysts containing spermatogonia and sper-

matides were present in the walls of tubules. As spermatogenesis progressed, an elongation of the testicular tubules took place. The changes in relative volumes of developing spermatocysts between the first and the next spawning were counted with the use of stereologic methods. Kruskal-Wallis non-parametric ANOVA test was used to test for differences in germ cells and interstitial tissue values between reproductive classes. Results were considered significant at $p < 0,05$. there was significant difference ($p < 0,05$) in cysts of germ cells (spermatogonia A, spermatogonia B, spermatocytes, spermatides and spermatozoa) between reproductive classes (regressed, maturation and spermiation class). The volume of cysts containing spermatogonia A, spermatogonia B and spermatocytes was decreasing during spermatogenesis. In the same time volume of cysts with spermatides and spermatozoa was increasing considerably. The aim of these study was to describe changes in the testicular germinal epithelium during reproductive cycle in *Danio rerio*.

The SEM study on brush cells on surface of tongue in cat's embryos

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Brush cells are cells defined as tuft cells also or fil-brillo-vesicular cells and their occurrence was described often in epithelia covered mucosa of particular segments of the digestive or respiratory systems (Iseki, 1991; Luciano et al., 2003). The brush cells characterize numerous cytoplasmatic projections like stereocilia on apical surface and microfilaments, numerous membrane-bound vesicles or granules in the cytoplasm. How the authors of earlier reports show brush cells probably fulfil chemoreceptory function. Sofar the presence of the brush cells in embryonic epithelia in esophagus, trachea and gallbladder (Raymond et al., 1991).

The method of scanning the microscopy of electron in our investigations of surface of the tongue at the cat's embryos in first part of the gestation (CRL for 2.5 cm. to 5.5 cm) was used.

The results of SEM observation in the embryos at about 20 day p.c. showed only the regular surface of the embryonic mucosal epithelium with a polygonal, flat superficial cells covered with grain-like microvilli. The

first occurrence of the brush cells was noticed in next studied stage about 25–30 day of development. These cells were placed on the surface of the root of the tongue. The single brush cells were observed among elongated primordia of conical papillae, but in flat part of the root adjacent to throat, these cells was present in clusters. The diameter of the brush cells the measured on SEM figures is about 10–20 μm . Every brush cell possesses numerous, long on 30 μm , cytoplasmatic cilia, reminding brush border.

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Gonadal development of allotriploid *Cobitis* females (Pisces, Cobitidae)

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The spined loach *Cobitis taenia* L. creates exclusively diploid and mixed diploid-polyploid populations. Allotriploid females, coexisting with their parental species, viz. *C. taenia* and/or *C. elongatoides* and a few tetraploids (males and females), dominate in most of mixed populations (Boroń, 2003). This dominance may be connected with unisexual/ gynogenetic reproduction (Saat, 1991), elongated spawning period or laying bigger eggs by triploids in comparison with diploids (Juchno et al., 2007).

Gonad development of 16 individuals of gynogenetic offspring produced by experimentally induced gynogenesis using the sperm from *C. taenia* (T) and *Carassius auratus gibelio* (C) males and 12 allotriploid *Cobitis* females collected from mixed population (Bug River) was investigated by gonad histology (light microscopy). All the individuals were verified karyologically as possessing $3n = 74$ chromosomes.

Germ cells have been recognized at 60 days post-hatch specimens with the body length 22.2 and 39.4 mm respectively in C and T offspring. Gonads of the C offspring contained germ cells at the chromatin-nucleolar stage and perinucleolar oocytes surrounded by a layer of

somatic cells. In the gonads of T offspring only germ cells at the chromatin-nucleolar stage have been observed. Therefore the gonads analyzed were presumptive ovaries.

The smallest juveniles of 43.3 mm, identified as triploid females, from a diploid-polyploid population possessed gonads filled with densely packed and well-differentiated perinucleolar oocytes. Females reached sexual maturity during the second year of life at body size of 59.7 mm.

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Structure and phytochemistry of the fruit of *Hacquetia epipactis* (Scop.) DC. (Saniculoideae, Apiaceae)

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Hacquetia epipactis (Scop.) DC. is the only species of the genus *Hacquetia* Neckar, growing sporadically in South Poland, Czech Republic e.g. in Stramberk, and in Slovakia near Ladon Hora. In Poland, this plant is rare and seriously endangered in several natural stands, mainly in the area of the Upper Silesia. Moreover, this species is very difficult to reproduce in laboratory and garden conditions.

Although *Hacquetia* has been included in molecular phylogenetic studies on Apiaceae family, using the chloroplast DNA rpl16 intron sequence and ITS sequences, there is almost no information about this species in the carpological literature. So present studies were undertaken to investigate the micromorphology, anatomy and volatile terpenoid compounds of mature fruits of *H. epipactis* with the aim of providing new diagnostic features for characterizing of this species. Fruits at different developmental stages were collected from plant natural populations around Cieszyn and Raciborz. Our study revealed that the mature fruit is a schizocarp consisting of two one-seeded mericarps. The seed is anatropous and enclosed within indehiscent pericarp. SEM analysis of the fruit surfaces demonstrated the existence

of micromorphological diversity of epicarp cells. The fruit surface patterns were uniform within *Hacquetia* species both at an inter- and intra-population level. The anatomical study of the fruits showed that the development of the seed and its embryo as well as the synthesis of reserve materials follow before the final pericarp structure in an immature fruit is developed. This may be used practically in germination physiology. Besides *Hacquetia* fruit contains biologically active secondary products, and it is also important from a pharmacognostical point of view. Bartnik et al. (2003) analysed mature fruits for the presence of phenolic acids. These compounds may possess pharmacological and biological activities. In conclusion, fruit structural characters may provide criteria useful for delimitation of this species, and also for the identification of isolated fruits.

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Poly(A) RNA as a new component of Cajal bodies in microsporocytes of larch

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Nucleus of eukaryotic cells contains dynamic structures called nuclear bodies, among them there is a group connected with RNA metabolism. To this category belong Cajal bodies. Research at electron microscopy reveal that Cajal bodies are built of coiled fibers and therefore are often called "coiled bodies". We made investigations during first meiotic division in larch. Larch microsporocytes are characterized by high degree of anthers developmental synchronization and long, about 6 month, duration (Smoliński et al., 2007). Our investigations showed that in the microsporocyte of larch poly(A) RNA accumulate inside spherical structures of diameter 1–5 µm. Their shape, number and often spatial associations with nucleolus resemble Cajal bodies. Using double labeling technique we showed that likewise at Cajal bodies (Smoliński et al., 2003), nuclear bodies containing poly(A) RNA pos-

sess splicing factors such as U snRNA – both mature and immature (TMG, U2 snRNA) and Sm proteins. This is first report revealing presence of poly(A) RNA within Cajal bodies. Significance of this new component of Cajal bodies with reference to dramatic metabolic changes which occur during microspirogenesis in Larch (Smoliński et al., 2007) will be discussed.

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The ultrastructure of the chicken embryonal cornea and lens after caffeine administration

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Caffeine is one of the most often consumed psychoactive chemicals. It has been known for several years that high doses of caffeine affect negatively fertility of women and laboratory animals. Caffeine consumption is associated with the intrauterine growth retardation, low birth weight, but it doesn't cause preterm birth. During the prenatal period it can also damage the normal development of many organs. It can cause delayed neural tube closure, craniofacial and limb malformations, cleft palate. So far few studies have suggested some destructive effects of caffeine on some structures of the developing eyes, especially on the cornea and lens. Excessive gestational caffeine intake has been shown histopathologically to have some teratogenic effects on newborn rat cornea and cataractogenic effects on developing crystalline lenses (Evereklioglu, 2003, 2004).

The aim of this study is to show the ultrastructural changes in the developing cornea and the lens after administration of caffeine to the chicken embryos. The experimental materials were chicken embryos from eggs incubated at 37–38°C, and 50–60% relative humidity. The eggs were divided randomly into 2 groups. In the 48th hour of the incubation (12 stages of development according to Hamburger-Hamilton) Ringer's solution has been administered to the 10 eggs of the control group. To the 20 eggs of the experimental group a teratogenic dose

of caffeine has been used – 3,5mg/egg into the amniotic sac. After closing the window in the eggshell with glass plates and paraffin, the eggs were put back to the electric cabinet. On the 10th day (36 stages of the development according to Hamburger-Hamilton) and on the 19th day of the incubation (45 stages of the development according to Hamburger-Hamilton) the corneas and the lenses were taken to conduct the morphological analysis in the electron microscope.

The results of the study show a teratogenic effects of caffeine on the developing cornea. Caffeine affects the collagen fibers of the Bowman's membrane and stromal fibers. The corneal fibers were irregularly oriented. However, it wasn't observed any morphological changes in the lens after administration of the caffeine.

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Developmental biology of *Arabidopsis thaliana* At1g67580 mutant

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Cyclin-dependent protein kinases (CDK) are serin/threonin kinases which play a major role in cell cycle control in all eukaryotic organisms. Many genes encoding putative cell cycle regulators have been identified in *Arabidopsis* genome, but the biological function of their products remains largely unknown. The gene *At1g67580*, located on the first chromosome, encodes a putative CDK (predicted molecular weight 85.2 kDa) containing the PLTSLRE motif in its amino acid sequence and expressing significant similarity to animal and human PITSLRE kinases – p34^{cdc2} family members. In animals and humans PITSLRE kinases have been shown to play a role in RNA processing, regulation of transcription, apoptosis induction, oncogenesis and dopamine/glutamate signaling in the nervous system (Knockaert et al., 2002; Trembley et al., 2004).

For a study of the biological function of PLTSLRE kinase in plants, a homozygotic *Arabidopsis* knock-out line was selected, using an insertionally mutagenized seed population generated by the Salk Institute Genomic Analysis Laboratory (Alonso et al., 2003). The line contained a T-DNA insertion in the first exon of *At1g67580*. No expression product of this gene was detected in the selected line by RT-PCR versus the genetic background (ca. 3000 bp in Columbia-0 ecotype). To analyze the influence of the *At1g67580* mutation on developmental processes in *A. thaliana*, wild type and homozygous mutant life cycles were compared. Seed size and germination rate, seedling and hypocotyl length, shoot and root meristem formation, leaf venation, pollen viability,

flower and fruit characters, embryo and endosperm development and chromosome number were assessed. Leaf venation, flower structure, pollen size and viability did not differ between the wild genotype and mutant. Several qualitative characters differed significantly between the Columbia wild type and *At1g67580* mutant. Seeds of the mutant were smaller, with average length 385 μm (416 μm in wild type) and average frequency of seed germination on all media used (soil, agar, MS) decreased to 76% in the mutant (93% in wild type). Seedling hypocotyls after culture on agar and MS (in light and in dark) were evidently shorter in the mutant: e.g., on MS in light 3.72 mm, in wild type 6.14 mm. Disturbed mitoses were observed in root meristematic cells of the mutant, leading to aneuploid and polyploid chromosome numbers counted in 70% of seedlings. Some developmental disturbances yielding altered seedling phenotypes resembling the well known *fass* *Arabidopsis* mutant were observed in 6% of the seedlings. Mutation also affected the embryo and endosperm. Abnormal proembryos with nuclei conspicuously differing in size and with disturbed organization of the suspensor indicate the role of PLTSLRE kinase in the control of early stages of plant embryogenesis.

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Sperm quality parameters and testis histology of p53 mutant mice

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The most important protein responsible for genomic integrity and cell cycle control is tumor suppressor protein, Trp53 (p53). This small protein (53kDa) is coded by p53 gene called 'the guardian of the genome'. p53 binds to the specific DNA sequences and regulates the expression of about 60 genes. TRP53 gene mutations are present in 50% of all human neoplasm.

In this work we used male mice from 129/Sv and C57BL/6J strains and also male mice with disrupted *Trp53* gene on a mixed (C57BL/6Jx129/Sv) genetic background. Because the role of the *p53* gene in spermatogenesis is not clear we simply compared sperm head abnormality level in mutant (p53^{-/-}) and control (p53^{+/+}) C57BL/6Jx129/Sv males with that in males from 129/Sv and C57BL/6J strains. Sperm head morphology analysis showed that control males on the mixed C57BL/6Jx129/Sv genetic background have better sperm quality than males from both 129/Sv and C57BL/6J strains. It seems that the influence of the genetic background on sperm quality is much stronger than of the p53 protein status.

We also analyzed testis histology in the individuals of above strains. Histological analysis of the C57BL/6J male testis revealed lack of changes in tissue organization. Male testis histology from 129/Sv strain is characterized by increased gaps between tubules and tubules' diameters. Testis weight of 129/Sv males is higher than of C57BL/6J males. Histological image of mutant males (p53^{-/-}) on a mixed genetic background showed disorders in the general structure of this organ. We observed big gaps between tubules, lack of light on sections through sperm producing tubules and also discontinuous tubules' membrane. These changes in histological image of mutant testis are caused by non functional p53 protein.

The deletion in the long arm of the Y chromosome correlates with flat type sperm head morphology of about 30% spermatozoa in B10.BR-Ydel strain. Our further investigation will be focused on the observation of gametes quality in males possessing both p53 knockout and the deleted Y chromosome. This animal model would help to understand if the p53-dependent apoptotic pathway is turned on by the absence of genes localized in the chromosome Y deleted region.

Universal pattern of calreticulin distribution in pollen-pistil interaction in *Petunia hybrida* and *Haemanthus albiflos*

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Calreticulin (CRT) is a highly conserved and ubiquitously expressed Ca²⁺ binding/buffering protein that is involved in regulation intracellular Ca²⁺ homeostasis and chaperoning in the endoplasmic reticulum (ER). Although plant CRT has been suggested to have similar molecular structure and functional properties as its animal homologue, the current knowledge about expression, physiological role, and subcellular distribution of CRT isoforms in plants is still poorly understood. However, the possible role of that protein in pollen-pistil interaction has been already postulated (Lenartowska et al., 2002). In this study, the distribution of CRT in germinating pollen and growing pollen tubes in *Petunia hybrida* wet and *Haemanthus albiflos* dry stigmas was analyzed. Immunocytochemical studies were shown the

similar pattern of CRT localization in anatomically different types of unpollinated and pollinated stigmas. In both species CRT was localized preferentially in germinating pollen and growing pollen tubes in vivo. In the somatic cells of unpollinated stigmas the level of CRT was lower and no significant changes of labeling in these cells were observed after pollination. The obtained results suggest that CRT could be involved in first step of pollen-pistil interaction in vivo.

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Marsupial larvae of *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionoida: Unionidae)

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The Unionoida have three types of larvae: lasidia, haustoria and glochidia. The species present in the malacofauna in Poland produce only glochidia in their life cycle. They are the parasite developmental stages attacking mainly the fish skin, fins and gills. The adult bivalves incubate these stages in their marsupia prior to the larvae discharge into the water.

Sinanodonta woodiana comes from the basins of the Amur and Yangtze Rivers. This species appeared in Poland in the heated post-cooling waters in the beginning

of 1980s. The offspring of this bivalve is kept in the outer demibranchs. The glochidia have two-valve shells, each equipped in a sharp hook covered with a number of teeth. The parameters of the shell have been measured under a light microscope Nikon Eclipse 80i in configuration with a digital camera and image analysing program NIS Elements BR 2.30.

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Effect of stress conditions of calamine waste heaps on reproductive processes of *Lotus corniculatus* L.

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The southern Poland is the most industrialized region as nearly all the mineral resources of the country are located there. Zinc, lead and silver ores situated in the vicinity of Olkusz have been exploited since Middle Ages. As a result of mining activity spoils of different ages became a permanent part of the landscape in this region. Mine spoils provide extreme habitat conditions, therefore plant vegetation, especially on the youngest heaps, is poor.

The studies concerned the processes in reproductive organs of *Lotus corniculatus* from two contaminated sites of that region: the old Bolesław heap more than 100 years old, and a younger one, 10–20 years old, where only a few specimens were recorded. Population from the site with soils non-enriched in heavy metals was recognized as a control.

Abnormalities and degenerative processes were observed in flowers of plants from both populations occurring on heaps. They included necroses of the whole anthers or disturbances in microsporogenesis, degeneration of pollen grains as well as precocious necroses of tapetum. These processes affected 52% of the studied flowers from the old spoil and 70% of flowers from the

younger one. Pollen viability estimated during two seasons (2005, 2006) varied from 61% to 95% in plants from the old spoil and from 58% to 88% in plants from the recent spoil (in control plants 95%).

Developmental disturbances and necrotic processes occurred also in ca 47% of ovules from plants growing in both sites at stages of female gametophyte development. Embryo viability estimated by tetrazolium histochemical test was reduced to 84%. Average number of seeds found in pods constituted 20% of formed ovules in population from the old heap, 17% in plants from the recent heap, and 42% in non-contaminated site.

The results of presented study indicate that extreme living conditions occurring in the area of the zinc-lead spoils may have negative influence on embryological processes in plants colonizing these sites. The specimens of *L. corniculatus* have adapted to heavy metal-contaminated soils in Bolesław, even these in the initial stage of colonization of the recent heap; they can survive and propagate, however, their fertility is lower than control plants and adaptation of various specimens in contaminated sites is differentiated.

Histological changes in the mouse testis during LPS-induced inflammation

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There is a considerable body of clinical evidence suggesting that testis function is compromised during illness or infection. In our work we have adopted the experimental model of lipopolysaccharide (LPS)-induced inflammation to investigate the effects of TLR4 stimulation changes on histology and function of testis. We have found that single dose of i.p. LPS (*E. coli* 026:B6) injection induces testitis. During inflammation, Leydig's gland contain increased number of macrophages. This process starts 24 h after LPS treatment and peaks at day 3 post injection when macrophage number doubles when compared to control mice. During inflammation we observed increased number of blood vessels in intertubule space.

Diameter of blood vessels was increased what caused exudate containing red blood cells in intertubule space. Inflammation affects cytoarchitecture of seminiferous epithelium and that process starts 5 days after LPS injection. It causes inhibition of mitosis and meiosis what disturbs spermatogenesis. As a result of inhibited spermatogenesis we found total absence of sperm cells in morphology of seminiferous tubules. We observed maximal changes in morphology of seminiferous epithelium 15 days after LPS injection. At that time we found degeneration of seminiferous epithelium and Sertoli cells. Inflammatory response in testis resolves by day 28 after LPS injection. At that time also fertility is restored.

The effect of interaction between autosomal genes and genes on Y chromosome on sperm quality parameters in mice

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Majority of our knowledge about the function of genes in spermatogenesis has been obtained from the analysis of transgenic and knockout mice. In this study we analyzed six genes coding for histone H1.1 (*H1.1*), transition protein 2 (*Tnp-2*), proacrosin (*Acr*), testicular expressed protein (*Tep22*), cAMP response element binding protein 3-like 4 (*Creb3l4*) and testicular haploid expressed gene (*Theg*).

The linker histone subtype H1.1 belongs to the group of main-type histones and is synthesized in somatic tissues and germ cells during the S-phase of the cell cycle. *Tnp-2* participates in the removal of the nuclear histones and in initial condensation of the spermatid nucleus. Proacrosin is an acrosomal endoprotease synthesized as inactive zymogen and activated after acrosome reaction into acrosin. *Tep22* protein is involved in the acrosome biogenesis and in the function of murine spermatozoa midpiece. *Creb3l4* belongs to a multiprotein family of transcription factors that mediate transcription in response to intracellular signaling. *Theg* protein is specifically expressed in spermatids and regulated by Sertoli cells.

Knockout mice with only one of these genes disrupted are fertile and show no gross defects in spermatogen-

esis (or there is no such data available). To analyze the synergistic effects of these genes in spermatogenesis, two lines of fourfold null mice (*H1.1*^{-/-}/*Tnp-2*^{-/-}/*Acr*^{-/-}/*Tep22*^{-/-} named DKO26 and *H1.1*^{-/-}/*Tnp-2*^{-/-}/*Acr*^{-/-}/*Theg*^{-/-} named DKO20) and one line of fivefold null mice (*H1.1*^{-/-}/*Tnp-2*^{-/-}/*Acr*^{-/-}/*Theg*^{-/-}/*Creb3l4*^{-/-} named DKO24) were studied.

Although all males from these lines are fertile, different abnormalities are prominently manifested. In DKO26 males older than 1 year (average weight 48,7g), motility and vitality of sperms is strongly reduced (16,1% and 9%, respectively); younger males (average weight 35,4g) have better semen quality parameters. In DKO24 males significant reduction of spermatozoa with morphologically normal head was noted (50,4%) in comparison to 80,3% in DKO20 males and 67,2% in DKO26 males. DKO24 males revealed no gross abnormalities of motility (59,8%) and vitality (30,5%) in comparison to these parameters in DKO20 males (44,1% and 18,4%, respectively).

Each of these lines were crossed with B10.BR-Y^{del} lacking 2/3 of Y chromosome's long arm and with B10.BR as a control. It would make us possible to investigate the interaction of these genes with those localized on Y chromosome.

Differentiation of follicular epithelium in polytrophic ovaries of *Pieris napi* (Lepidoptera, Pieridae) – preliminary results

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Paired, polytrophic ovaries of *Pieris napi* are built of seven elongate ovarian tubules (ovarioles). A germarium and vitellarium can be distinguished in each ovariole. In the germarium gonial cells divide, functional egg chambers form and first stages of cluster component differentiation take place. Like in butterflies of the family Pyralidae (Santos and Gregorio, 2006), four zones can be distinguished in the germarium: I – zone of mitotic divisions of gonial cells, II – zone of formation of germ cell clusters, III – zone containing clusters of cystocytes in meiotic prophase, and IV – zone of formation of functional egg chambers. The vitellarium is formed of numerous, linearly arranged egg chambers. These chambers in *Pieris* are built of clusters of eight germ line cells connected by intercellular bridges and surrounded by somatic follicular epithelium. From early stages of differentiation of the germs cells into the oocyte and polyploid trophocytes, in the egg chambers of *Pieris*, four subpopulations of follicular epithelium can be discerned: follicular cells associated with the oocyte surface, cells surrounding the anterior pole of the oocyte, cells extended

on the surface of the trophocytes and cells of interfollicular styli. The population of follicular cells located between the oocyte and the trophocytes is morphologically different from the remaining follicular cells associated with the oocyte surface. They are strongly flattened while the remaining follicular cells surrounding the growing oocyte are high. Initially these cells form a compact, monolayer epithelium. In subsequent stages of oogenesis, as the oocyte volume increases, the follicular epithelium associated with its surface undergoes characteristic transformations. The follicular cells increase their volume and migrate within the epithelial layer to become arranged in rows. The transformation of the oocyte-surrounding follicular epithelium proceeds from the posterior to the anterior part of the oocyte.

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Ultrastructural studies on the transovarial transmission of endosymbiotic yeast-like microorganisms in *Conomelus anceps* (Insecta, Hemiptera, Fulgoromorpha: Delphacidae) and *Metcalfa pruinosa* (Insecta, Hemiptera, Fulgoromorpha: Flatidae)

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The ovaries of *Conomelus anceps* and *Metcalfa pruinosa* are accompanied by large syncytial organs of irregular shape termed mycetomes. The mycetome cytoplasm is tightly packed with endosymbiotic yeast-like microorganisms. These endosymbionts are rod-shaped. They are surrounded by a thick cell wall composed of two distinct layers. The central part of the cell is occupied by a large, spherical nucleus with a single nucleolus. The rest of cytoplasm is filled with ribosomes, mitochondria, rough endoplasmic reticulum.

The yeast-like microorganisms reproduce by budding. These endosymbionts are transovarially transmitted from one generation to the next. The yeasts leave the mycetomes and start to migrate towards the vitellogenic oocytes. They transverse the cells of the ovariole stalk (pedicel) and enter the perivitelline space. Concurrently, at the posterior pole of the oocyte a deep depression is formed. The endosymbionts gather in the oocyte depression and form a characteristic "symbiont ball".

Study of rRNA transcripts distribution against a background of total transcriptional activity of *Hyacinthus orientalis* L. female gametophyte cells before and after fertilization

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The aim of the work was to investigate the distribution and level of rRNA (mature transcripts and ITS1 (*internal transcribed spacer 1*) pre-rRNA) in comparison with total transcriptional activity in the cells of *H. orientalis* embryo sac before and after fertilization.

Mature transcripts of rRNA and ITS1 pre-rRNA were localized using fluorescence in situ hybridization (26S and ITS1 oligo probes). Total transcription was detected with immunofluorescence techniques by incubation of the material with BrU. Semithin sections were analyzed with fluorescence microscope Nikon Eclipse 80i. The results were registered with a Nikon DS-5Mc camera and Lucia General software. The obtained results have shown that cells of unfertilized embryo sac differ in the level of transcription and distribution pattern of rRNA. The egg cell and central cell of embryo sac exhibited very low transcriptional activity, but the level of rRNA in both of these cells was high. The mature rRNA was present especially in the cytoplasm. In the large nucleolus of the egg cell and the central cell the level of 26S RNA was low and transcripts

were localized mainly on its periphery. In the nucleolus high accumulation of ITS1 pre-rRNA was observed. In highly transcriptionally active synergids and antipods, increased levels of 26S rRNA and ITS1 pre-rRNA were also observed. Therefore we suggest that cells of the mature embryo sac, which are the targets for male gametes, although their low transcriptional activity, store rRNA, which could be used after fertilization. In the nucleolus, rRNA is stored mainly as pre-rRNA transcripts and in the cytoplasm as mature ribosomes.

Immediately after fertilization the restart of nuclear transcription occurs in the zygote. In nucleoli low levels of new transcripts of rRNA were detected only around the NOR. At this developmental stage an increased level of 26S rRNA was indicated in the nucleoli and nucleoplasm of the zygote. This in turn, undoubtedly reflects maturation of rRNA, which was stored in the egg nucleolus, and then is transported to the zygote's cytoplasm. In the opposite, the progressing decrease of nascent transcripts and rRNA levels was observed in degenerating antipods and synergid cells.

Test tube pollination of stigmas, ovaries and ovules of *Salix viminalis* with pollen grains of *Alnus*, *Betula* and *Corylus*

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The main aim of our work was to estimate if parthenogenetic process in *Salix viminalis* can be induced by foreign pollen grains of trees species. Programic phase of both components was synchronized by accelerating maturity of female and male flowers. Pollen grains of *Alnus glutinosa*, *Betula verrucosa* and *Corylus avellana* were placed on cultured in vitro whole pistils, opened ovaries or single ovules of *Salix viminalis*. Germination of pollen grains, and pollen tube morphology were evaluated 24–48 hours following self (control) and cross pollination. Pollen tubes were stained

with DAPI. Tips of selfed plants grew faster and were longer than those representing incompatible ones, which were short, twisted and corkscrew-shaped and they were often bursted. Fresh and fixed in AA ovaries were stained with carmine acetate and analyzed under the light microscope till the 10 day following pollination. This procedure enabled to distinguish the development of embryos inside the embryosacs. *Salix viminalis* ovules enlarged very rare (two embryo-like structures were found), only when *Alnus* pollen grains were used to pollination.

The ultrastructure and the formation of the egg capsule in *Isohypsibius granulifer* Thulin, 1928 (Tardigrada: Eutardigrada)

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The egg of *Isohypsibius granulifer* Thulin 1928 (Tardigrada:Eutardigrada), similar to other Tardigrada eggs (Kinchin, 1994; Poprawa, 2005), is covered with egg capsule which is composed of two shells: the thin vitelline envelope and the thick, multilayered chorion.

The process of choriogenesis begins during the middle of vitellogenesis. The oocyte and the gonad wall participate actively in this process. The gonad wall cells synthesize and secrete flocculent middle electron dense material which is deposited on the oocyte surface, forming layers of chorion. Precursors of chorion are also synthesized by the oocyte. The completely developed chorion is consisted of 3 layers (1) the inner, medium electron dense layer; (2) the middle labyrinthine layer; (3) the

outer, medium electron dense layer. After chorion formation the oocyte secretes flocculent middle electron dense material into the space between the oolemma and the chorion. This material is composed of vitelline envelope precursors.

The surface of the egg is smooth and there is no micropylar opening.

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Scanning electron microscope analysis of the distal vagus nerve ganglion (ganglion distale nervi vagi) in newborn calves in standard husbandry

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The aim of this study was to describe structure and the presence of elements in cells of the distal vagus nerve ganglion.

The research was conducted on 12 newborn calves of h-f breed. Animals were fed with fodders of known qualitative-quantitative composition, and kept in the same husbandry conditions. Both distal ganglions of vague nerve were morphologically assessed, their components and derivatives like: laryngeal cranial nerve (n. laryngeus cranialis) the way of heart aorta nerve (n. depressor cordis) leaving. Both ganglions were subject to morphometry, skeletopy, holotopy and synthopy that was of significance while determining the symmetry and asymmetry. Analyzed ganglions were also subject to X-ray microanalysis using LEO V435 microscope. Surface analysis of the whole ganglions and point analysis of single nerve insets were conducted. The material was dusted with gold and analyzed using scanning electron micro-

scope (SEM) LEO V435 with accelerating voltage of 20000 eV.

Analyzed distal left ganglions of vagus nerve were morphometrically bigger than on the right side of preparations. In standardized fodder also certain chemical elements and the whole range of vitamins were found. As a result of conducted qualitative analysis of ganglions using histological methods mentioned above it was demonstrated that the characteristic for left ganglion is higher level of nerve fibers organization. Fibers are tightly stuck creating bunches running to the circumference and also towards spinal cord. They are surrounded by collagen fibers composing perineurium. X-ray analysis showed irregular distribution and occurrence of chemical elements in developing ganglion area. Among trace elements there were aluminum, silicon and molybdenum. However, in point analysis significant higher concentration of silicon was noted.

Differentiation of the human myocardium (trabeculation and compaction) during early intrauterine period

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Ventricular trabeculation and subsequent compaction are important cardiac morphogenetic process and are critical to the formation and function of ventricular wall. Investigations were performed on human embryos (stages from 13 to 23, aged 32 to 56 days) from the Department Collection. All embryos were embedded in toto in paraplast and serial section were made in horizontal, frontal, saggital planes. The thickness of the myocardium was measured with aid of a Leica Image Processing System. In embryos at stage 13 (32 days) the myocardium is about 2–3 cells thick.

In stage 14 (33 days) the outer compact and inner trabeculated myocardium is observed. The compact layer is initially only a few cells thick. The interventricular septum is the first structure that shows a compact myocardial morphology. Later the free ventricular wall begins to develop a compact myocardial layer. This process starts in the interventricular septum, expands earlier to the left ventricular free wall and finally continues also to the right ventricular wall. At stage 16 trabeculated myocardium could be subdivided into outer part adjacent to the

compact layers and central part. The outer basal layer could be distinguished from the inner luminal by short and fine trabeculae with round intertrabecular spaces. In embryo at stage 16 a compact layer is slightly more developed in the left ventricle, than in the right one.

At the beginning of the 6th week the trabeculation continues to increase and a fine trabecular network starts to occupy almost entirely both ventricular cavities. The trabeculae were oriented perpendicular to the outer compact layer in frontal plane. The intertrabecular spaces were small and round, and their pattern was similar in both right and left ventricle. The right ventricles show more trabeculations than the left one, because the compact myocardium has not been fully developed yet.

At the beginning of the 8th week the compact layer has thickened and the three major components: outer longitudinal, middle circular, and inner longitudinal are easily discernible. The outer layer was common for the both ventricles, the middle formed the main part of the interventricular septum, and inner layer takes part in formation the papillary muscles.

The influence of 5-Azacytidine on female gametophytes development in unpollinated ovaries of *Arabidopsis thaliana* and *Brassica napus*, cultured in vitro

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DNA methylation has been implicated in many aspects of the control of gene expression in both animals and plants (Finnegan et al., 1998); it certainly plays an important role in endosperm development. Although hypomethylation alone did not promote fertilization-independent endosperm proliferation in wild-type with *FIS* gene expression, a combination of maternal genome hypomethylation and loss of *FIE* function appear to promote autonomous endosperm formation (Vinkenoog and Scott, 2001; Li et al., 2002). Autonomous endosperm development, induced in culture condition resembled the AE in *fie* mutants: it was free nuclear and not differentiated into specific regions. Sporadically, a cluster of nuclei in the centre of the female gametophyte was observed, but the structure did not resemble a typical model of chalazal endosperm. To improve AE quality induced in wild type of *Arabidopsis* (Rojek et al., 2005; Kapusta et al., 2007) and *Brassica napus* (Rojek et al., 2002) we combined in vitro condition with 5-AzaC treatment. 5-Azacytidine (5-azaC), an inhibitor of CG and methylation induces hypomethylation in plants and animals. Treatment of plant and animal cells with

5-azaC results in the demethylation of DNA directly by incorporation of the analogue in place of cytosine during DNA replication and indirectly by inhibition of the action of the methyltransferase enzyme (Burn et al., 1993). We suggest that culture condition and demethylation caused 5-azaC may give the same effect as combination of *fie* mutation and hypomethylation, and lead to complete autonomous endosperm development in the absence of fertilization.

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The role of cell death in the midgut epithelium of *Filientomon takanawanum* (Protura)

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When the midgut epithelium cells are infected by viruses or parasites in insects, the cell death is the mechanism which eliminates the harmful effect (Vaidyanathan and Scott, 2006; Rost-Roszkowska and Poprawa, 2007). Autophagy is the process which leads to the disintegration of cytoplasmic components, while apoptosis enables removal of cells from tissues. As a result of these processes, the homeostasis is maintained (Tettamanti et al., 2007).

Midgut epithelium of *Filientomon takanawanum* is composed of epithelial cells and regenerative cells which are singly distributed at the anterior and posterior ends of the midgut epithelium. In about 80% of adult specimens examined, midgut epithelial cells are infected by pathogens as well as fat body and gonads. Two major processes - autophagy and apoptosis - are involved in the removal of pathogens from midgut epithelial cells. First autophagy is activated, and numerous autophagosomes and autolysosomes are formed. Cisterns of endoplasmic reticulum surround pathogens and completely enclose them, forming autophagosomes. Some of epithelial cells

proceed in apoptosis, to become electron dense and suffer from shrinkage. Then being fragmented into apoptotic bodies, they are finally phagocytosed by adjacent epithelial cells. Apoptosis and autophagy which are probably two kinds of protection against the pathogens in the midgut epithelium of *F. takanawanum*, were analyzed with transmission electron microscopy.

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Degeneration of the midgut epithelium of *Lepismachilis notata* (Insecta, Archeognatha)

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During necrosis the cell is swelling, its membranes break and all organelles are shifted into the extracellular space. Although necrosis is suggested to be a kind of a programmed cell death - all necrotic changes probably induce pathological events (Proskuryakov et al., 2003). Insect midgut epithelium fulfilling functions of digestion, secretion and absorption also proceeds degeneration (Rost, 2006; Rost-Roszkowska, 2008).

Midgut epithelium of *Lepismachilis notata* is composed of epithelial cells and regenerative cell groups. In adult specimens autophagy is observed in all epithelial cells. In such a way single organelles are disintegrated. When too many autophagosomes are observed in epithelial cytoplasm, necrotic processes are involved in midgut epithelium degeneration. *L. notata* is the specimen which molts during the entire lifespan. Necrosis proceeds more intensively just before molting. Two modes of necrosis of epithelial cells are observed.

When the cell proceeds typical necrosis with strong vacuolization of cytoplasm, it is completely separated from the basal lamina and removed into the midgut lumen. In some of the epithelial cells their apical mem-

brane forms small bleb into the midgut lumen, which breaks and forms small canal used for shifting some organelles into the midgut lumen. In such a way only single autophagosomes, multivesicular bodies and structures which resemble urospherites are removed into the midgut lumen. The cytoplasm of that cell does not proceed vacuolization and does not differ from epithelial cells. It is not separated from the basal lamina, small canal of apical membrane is closed and the cell still has the epithelial character.

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Does the midgut epithelium of *Allacma fusca* (Insecta, Collembola, Symphypleona) proceed regeneration?

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In the majority of insect species the midgut epithelium is formed by columnar cells having epithelial character and regenerative cells. Regenerative cells form groups called regenerative nests or crypts, or they might be located singly among epithelial cells (Rost, 2006). They are regarded as stem cells. However, in some cases, the lack of regenerative cells in the midgut epithelium has been described (Krzysztofowicz et al., 1973; Rost-Roszkowska, 2007). In these cases the process of regeneration seems to be problematic. *Allacma fusca* belongs to the primitive wingless insect group Collembola (Symphypleona). Its midgut epithelium is only composed of the columnar cells of epithelial character. No regenerative cells are observed. Between each molting period degeneration of epithelial cells, which goes under necrosis or apoptosis, is common. Midgut epithelial cells do not possess the abilities to proliferate. It is probable that in larval stages they are capable to divide, but it might be lost in the adult specimens. The growth of the midgut epithelium of analyzed species proceeds only due to the extension of existing epithelial cells. The midgut epithelium undergoes only differentiation and at the end of animal life it degenerates. According to the suggestion that Collembola is the

group, which developed irrespective of insects (Nardi et al. 2003), the similarity of *Allacma fusca* midgut epithelium and hepatopancreas of Isopoda (Crustacea) appears (Rost-Roszkowska and Undrul, 2008). The process of the differentiation of the midgut epithelium of *Allacma fusca* was observed with the use of transmission electron microscopy.

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The ultrastructure of endocrine cells in the larval midgut epithelium of *Spodoptera exiqua* (Insecta, Lepidoptera)

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The insects midgut epithelium is composed of columnar digestive cells and regenerative cells. In addition, single endocrine cells and goblets cells, arranged between digestive cells are found in the midgut epithelium of Lepidoptera, Ephemeroptera, Trichoptera, and Plecoptera (Montuenga et al., 1989; Rost-Roszkowska, 2008). Endocrine cells are responsible for the regulation of synthesis and secretion of enzymes.

The material for study originated from the laboratory culture of *Spodoptera exiqua* (Lepidoptera). Midgut of 1st to 5th larval stages was prepared to the analysis with the use of transmission electron microscopy.

Among epithelial cells in the midgut epithelium of *Spodoptera exiqua* single distributed endocrine cells are observed. They are mainly accumulated in the neighborhood of regenerative cells. Endocrine cells are of the

"open" type, that means that their apical membranes, which form microvilli, contact with the midgut lumen. Two kinds of "open" endocrine cells are observed: with numerous granules of glycogen and with large electron lucent vacuoles. Their cytoplasm is electron lucent and is rich in dictyosomes and cisterns of SER.

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The role of stem cells in midgut growth in *Epilachna* cf. *nylanderi* (Insecta, Coccinellidae)

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Growth of the midgut in adult insects proceeds due to enrichment of already existing cells, total replacement of cells by new populations, or the proliferation and differentiation of the regenerative cells, which play the role of the stem cells (Evangelista and Leite, 2003; Rost-Roszkowska, 2008; Rost-Roszkowska and Undrul, 2008).

The process of the midgut stem cells' differentiation was analyzed with the use of transmission electron microscope. Midgut epithelium of young *Epilachna nylanderi* beetles is composed of typical epithelial columnar cells and single regenerative cells located between a series of columnar cells. Initially, not many organelles can be seen in cytoplasm of the regenerative cells, mainly single mitochondria, free ribosomes and SER cisterns. These cells start to divide intensively, and soon after groups of regenerative cells appear. Then some regenerative cells of each regenerative group start to differentiate. An increase in number of organelles such as dictyosomes, cisterns of RER and electron dense bodies gradually are observed in their cytoplasm. Next intercellular junctions between differentiating and epithelial

cells appear. Newly formed epithelial cells separate degenerated cells from the basal lamina, and extracellular vacuoles with electron dense content appear between them. Regionalization in organelles' distribution appears when new epithelial cell gets the contact with the midgut lumen. In each of the regenerative group some cells do not differentiate and they fulfill the role of stem cells, which according to degenerative processes, will proliferate intensively.

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Germ cell degeneration (intrafollicular atresia) in amphibian ovary

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Physiological cell death is widely known in a variety of tissues and organs. Cell death in ovaries was formerly described as atresia, and this term is still in use in fishes and amphibians. Atresia is a specific kind of physiological intraovarian degeneration that affects germ cells at all stages of oogenesis, but especially in vitellogenic diplotene oocytes. We studied morphology of germ cell degeneration and changes in follicular cells of atretic ovarian vesicles in three European frog species *Rana temporaria*, *R. lessonae*, and *R. ridibunda*. We identified three types of atresia. In type I the oocyte is digested by its own follicle cells, which proliferate, hypertrophy, and become phagocytic. Connective tissue of the ovarian theca does not invade the follicle, but become thicker as a result of the follicle shrinkage. The follicular cells eventually disappear, and only remnants of the theca are still recognizable as a group of cells that accumulate black

pigment. Type I is subdivided into four stages: A – the germinal vesicle shrinks, nucleoli fuse, oocytes envelope interrupts at several sites, and follicular cells hypertrophy; B – after disintegration of the oocyte envelope, the follicular cells multiply and invade the vesicle, thus becoming phagocytic; the nucleus is not visible; C – the entire vesicle is filled by phagocytic and degenerating follicle cells; D – degenerating phagocytic cells accumulate black pigment. Type II is rare and resembles cytolysis of the oocyte; its cytoplasm is spread in the space between adjacent vesicles. In type III the ooplasm and germinal vesicle shrink and become denser, but the follicle cells do not invade the follicle, and finally the vesicle collapsed. Type I is characteristic of vitellogenic oocytes, whereas types II and III were observed mostly in pre-vitellogenic oocytes.

Development of ovaries of water frogs during juvenile period

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The frog *Rana esculenta* is a hybrid (RL) between *R. lessonae* (LL) and *R. ridibunda* (RR) that reproduces by hybridogenesis when one of the parental sets (L or R) of chromosomes is eliminated from the germ line before meiosis prolongs gametogenesis (Ogielska, 1995). We compared the rate of ovarian development in the parental species and in the hybrid. Ovaries in species follow differentiation characteristic of amphibians (Ogielska and Kotusz, 2004) and after first hibernation are composed of previtellogenic diplotene oocytes belonging to class IA and IB. At the same time primary oogonia become restricted to "germ patches" and the number of nests of early oocytes decreases. After second hibernation rate of ovary development starts differentiating in the species: in *R. lessonae* is faster and oocytes of class I-V are present, whereas in *R. ridibunda* is slower and oocytes of class I-III are present. This indicates that *R. lessonae* is sexually mature when two years old and *R. ridibunda* – when three years old.

Hybrids do not follow the above scheme and we elaborated specific staging for *esculenta* ovaries (ESC 1 – ESC 6). In ESC 1 only proliferating and degenerating primary oogonia are observed. In ESC 2, some early meiotic oocytes and single diplotene oocytes appear; however

most of them degenerate. In ESC 3 oogonia still proliferate, but numerous nests of meiocytes and diplotene class 0, IA and few IB, IC are present. In ESC 4 the pool of class I oocytes is established, proliferation of primary oogonia decreases, and single oocytes of class II – IV appear. Cortex of ESC 5 and ESC 6 is composed mainly of oocytes class IB and IC; classes III – IV are also present, being more numerous in ESC 6.

The variety of ovarian types in *R. esculenta* makes difficult to define the age of sexual maturation. 59% of females reproduce when 3 years old, 19% are sexually mature but of low fecundity, and 22% will reproduce one year later.

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Histological studies of the retina differentiation in grass snake *Natrix natrix* L.* during embryogenesis

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During embryogenesis most of the retinal cells derive from a simple pseudostratified neuroepithelium, whose cells will then divide to generate seven different cell types. We have analyzed retinal morphogenesis with the use of histological methods. The embryos of *Natrix* were isolated at regular intervals, starting at eggs lying and finishing at hatching of the first individuals. The age of embryos was calculated using the table of species development (Rupik, 2002). Our studies indicated, that at the developmental stage III the prospective retina was making contact with the presumptive retinal pigment epithelium (pRPE), but most of the retinal anlage was composed of a pseudo-stratified columnar epithelium with no apparent morphological signs of differentiated neurons. At the developmental stage VI the inner plexiform layer (IPL) and the outer plexiform layer (OPL) was appearing, but the appearance of the OPL occurred shortly after. In this stage the typical multilayered structure of the retina was observed in the central retina, but, in regions where no obvious OPL was apparent, the retina only consisted of an inner ganglion cell layer (GCL) and an outer neuroblastic layer (NbL). At the developmental stage VII the two plexiform layers were better established and clearly observable in the central and mid-peripheral regions of the retina. Both the GCL and the inner nuclear layer (INL) were many cells thick. At

the developmental stage IX, the entire retina showed both plexiform layers that progressively became thickened except at the extreme peripheral margin. The GCL and the INL formed a multilayered sheet of cells. At the developmental stage XI a thinning of the GCL and the INL was observed. However, there was an increase in the thickness of the plexiform layers. At the developmental stage XII, the retina has attained a mature appearance and arrangement.

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Programmed cell death during retinal development in grass snake *Natrix natrix* L.* (Lepidosauria, Serpentes) embryos

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Regulation of proper cell number in tissues depends upon a balance between cell proliferation and cell death. The physiological cell death is termed apoptosis. Most cells that die by apoptosis exhibit a characteristic set of molecular, biochemical and structural changes. Physiological cell death is identified morphologically by an initial hypercondensation of chromatin and nuclear shrinkage. Within the apoptosis pathway, caspases play a crucial role. Caspases are important mediators of neuronal apoptosis. They play a pivotal role in development of the nervous system. The physiological cell death is an important process in development and takes place in many regions of the central nervous system, including the visual system. The temporal and spatial localization of dying cells in developing retina of *Natrix* embryos were studied with the use of immunocytochemical method. The paraffin sections of the embryonic retina were analyzed in embryos from eggs lying to the hatching. The age of embryos was calculated using the table of species development (Rupik, 2002). Dying retinal cells were identified by the TUNEL methods, which specifically labels fragmented DNA and Caspase-3 expression. Our findings indicate that dying cells are located in the ganglion cell layer (GCL), the inner nuclear layer (INL) and the outer nuclear layer (ONL). Retinal apoptosis in *Natrix* embryos start very early, in the developmental stage V, but dying cells are located initially in INL and ONL layer in the central part of retina, then they spread to the GCL layer. At the developmental stage X to XII the

number of dying cells in GCL increased. Before hatching TUNEL positive reaction was observed in inner plexiform layer (IPL). In the course of retina development, we did not observe co-localization of fragmented DNA and Caspase-3 expression. The pattern of cell death of developing retina of *Natrix* embryos is similar that in turtle, bird and mammals (Morcillo et al., 2004; Gallardo et al., 2005).

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Expression of the pigment-dispersing factor (PDF) in developing retina of the grass snake *Natrix natrix* L.* (Lepidosauria, Serpentes) embryos. Immunocytochemical studies

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Pigment Dispersing Hormone (PDH) was first characterized by Ferlund (1976) from the eyestalks of *Pandalus borealis*. This octadecapeptide was first named Distal Retinal Pigment Hormone (DRPH) and later one always referred to as PDH. This peptide triggers light-adaptational screening pigment movements in the eye of but also pigment dispersion in chromatophores of crustaceans. The sequence of PDH is: Asn-Ser-Gly-Met-Ile-Asn-Ser-Ile-Leu-Gly-Ile-Pro-Arg-Val-Met-Thr-Glu-Ala-NH₂. PDH exist in two isoforms (α , β) and until now was characterized in 14 crustacean species (Ranga Rao, 2001). The related Pigment Dispersing Factor (PDF) appear to serve as a transmitter of circadian signals in the regulation of biological rhythms in insects (Jackson et al., 2001). The crustacean PDHs and insect PDFs are members of the PDH/PDF peptide family and share some characteristic features.

The temporal and spatial expression of the PDF in developing retina of *Natrix* embryos were studied with the use of DAB immunocytochemical method. The paraffin sections of the embryonic retina were analyzed in embryos from eggs laid to hatched animals. The age of embryos was calculated using the table of species development (Rupik, 2002). Our findings indicate that PDF not express in the developing retina until to developmental stage VI. The PDF expression was found in the some cells in ganglion cells layer (GCL) and in the many-cells of the neuroblastic layer (NBL) at the developmental stage VII. From the developmental stage IX to XI PDF

was also expressed in cells of the inner nuclear layer (INL). At the developmental stage XII to hatching, we noted expression of the PDF in GCL, INL and in the outer nuclear layer (ONL). This is the first evidence on the presence of PDF in the retinal cells of this species. No reports are known showing this factor in the case of reptiles.

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The differentiation of the skin pigmentation in the grass snake *Natrix natrix* L.* (Lepidosauria, Serpentes) embryos. Morphological and histological studies

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The epidermal melanocytes are predominantly responsible for the prehatching pigmentation pattern (Alibardi and Thompson, 2003), but the dermal melanophores are involved in the young and adult skin pattern (Spearman and Riley, 1969). Morphological observation indicated that at the developmental stage IX dorsal scales on trunk contained small quantities of brownish-grey pigment, which formed a fine irregular pattern of the dorsal skin, but on ventral body wall, gastrostages primordia were not yet pigmented. At the developmental stage X along the borderline between the head and the trunk, pigment accumulated in a pattern of symmetrical crescents steel grey in colour. The pigment accumulated in delicate dorsal scales forms three parallel lines running to the base of the tail but ventral side of the embryo is still without pigmentation. At the XII developmental stage the pigmentation of head and body wall is species specific. Pigment cells in *Natrix* embryos were detected in paraffin sections with Bodian's silvering method. At the developmental stage VII on the dorsal side, between the cells of germinal layer, pigment cells appear for the first time. At the developmental stage IX between the cells of differentiating epidermis within the scales numerous, branching pigment cells are observed; the pigment cells are less numerous within the gastrostages. Using the immunocytochemical method we also investigated temporal pattern of Pigment Dispersing

Hormon (PDH) expression. Our finding indicated that this hormone appeared the first time at the X developmental stage. However PDH concentration in the dermal pigment cells is larger than in the epidermal pigment cells. This is the first evidence on the presence of PDH in the skin of this species. No reports are known showing this factor in the case of reptiles.

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Ultrastructural studies of the thyrocytes changes and shedding complex differentiation in grass snake *Natrix natrix* L*. (Lepidosauria, Serpentes) during embryogenesis

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The sloughing cycle in reptile is under thyroid secretion control. The ultrastructural study shows that thyroid primordium contained small follicles without lumen or with small irregular lumen at the stage VI (the age of embryos was calculated using the table of species development (Rupik, 2002). The cells were poor in organelles. At the stage, VII to XII thyroid follicles consisted of two types of epithelial cells (dark and light). Epithelial cells varied in shape from columnar to flat. The luminal surface of thyrocytes showed increased size and number of microvilli. Proliferation of rough endoplasmic reticulum was seen with significantly dilated cisterns containing low electron density material. The Golgi complex was well developed. In the middle of embryogenesis the follicular epithelium cells frequently showed apocrine secretion into follicular lumens. These ultrastructural changes of thyroid cells coincide with the changes in the skin of *Natrix*, such as α and β keratin layers formation. In the developmental stage X we may distinguish respective epidermal layers. The innermost layer of epidermis is the stratum germinativum. Above this single layer are the single mesos layer and then thick β layer (or α and β layers), thin oberhäuten layer, and the periderm. These layers correspond to the distribution of two different types of keratin. On the outer surface of scales and gastrosteges the β layer is very much thicker than the α layer,

but on the inner surface of the scale and in the hinge region the layer is predominant. In the stage XI, epidermis forms the first embryonic shedding complex and at the end of the developmental stage XII periderm layer begins to detach. These findings indicate that initial shedding complex is formed under the increasing activation of thyrocytes. Our findings indicate that in the early stages of integument differentiation thyroid activity is high. During the period of shedding complex formation, thyrocytes are hypoactive, but after shedding the activity of follicular cells increased.

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Structural and ultrastructural studies of the notochord differentiation in zebrafish *Danio rerio* (Cyprinidae)

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The notochord is essential to the normal vertebrate development providing both mechanical and signalling functions, but *Danio rerio* (Cyprinidae) is a favorite model organism for embryological studies. This popular aquarium fish has a rapid embryonic development, transparent embryos, and the large number of offspring. The developing notochord was investigated from the end of segmentation period (24 hpf) to the early larval period (144 hpf) with routine light and electron microscopic methods. The developmental stages of zebrafish embryos were calculated using the table of species development (Kimmel et al., 1995). Morphological studies indicated that in the course of the notochord primordium differentiation, their cells changed their shape. We also noted that from the pharyngula period to the hatching period the shape and length of the notochord primordium was changed. The stretching and shape changing of the notochord primordium is a result of cells intercalation. Ultrastructural studies shown that in the segmentation period the immature cells of the notochord primordium are in rather loose contact and contain accumulations of glycogen. At the pharyngula period the cells become much more closely associated, the surface

of contact being usually thrown into slight waves or bumps and caveolae are present in all cells of the notochord. In the hatching period the medulla of the notochord consists of vacuolated cells connected by desmosomes. Between desmosomes, there are numerous caveolae. The vacuolated cells of notochord primordium contain giant mitochondria, well-developed rough endoplasmic reticulum Golgi apparatus and a dense cytoplasmic network of intermediate filaments surrounding the vacuoles. The high concentration of caveolae forms in the large vacuolated cells of the notochord at the hatching period to the early larva period. At the early larva period the notochord were constricted with a fibrous sheath, which surrounds a single layer of 'epithelial' cells and a central core of large vacuolated cells. The epithelial and vacuolated notochordal cells are all linked by numerous desmosomes, which are the predominant type of intercellular junction in the notochord.

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Changes in primary and secondary spermatocytes in seminal vesicles in the earthworm *Dendrobaena veneta* (Rosa) after 10 days of cadmium exposure

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The previous study (Siekierska and Brzozowa, 2006) indicated that cadmium in soil affected the normal course of spermatogenesis in the earthworm *Dendrobaena veneta*. Cadmium caused distinct degenerative changes in primary and secondary spermatocytes after 20 days of exposure. It seemed to be interesting to check whether cadmium affects the early stages of spermatogenesis in seminal vesicles in the earthworms after 10 days of exposure. Thus, the aim of this study was to determine what changes in primary and secondary spermatocytes occurred in earthworms *D. veneta* maintained in soil with CdCl₂ at concentrations: 10 and 50 mg Cd kg⁻¹ for 10 days and whether those changes were dose-dependent. It was also intended to compare these results with previous ones obtained after maintaining earthworms in Cd-contaminated soils for 20 days. Electron microscopy examinations revealed that cadmium at both concentrations induced distinct degenerative changes in primary and secondary spermatocytes. In comparison to the controls the cytoplasm of primary spermatocytes contained shrunk dictyosomes of Golgi complexes accompanied by vacuoles. Mitochondria were shrunk and contained large vacuoles. In earthworms exposed to 50 mg

Cd kg⁻¹ of wet soil cell nuclei contained very large, abnormal nucleoli. In secondary spermatocytes degenerative changes took place in Golgi complexes where many large vacuoles were detected. The cytoplasm was vacuolised and contained shrunk mitochondria with lamellar inclusions and electron translucent areas. The nuclear sheath was undulated and ruptured in many places. Swelling of spaces between the outer and inner nuclear membranes were also visible. Cadmium occurrence in habitat caused distinct degenerative changes in primary and secondary spermatocytes and affected the early stages of spermatogenesis. Those changes were dose- and time-dependent. More advanced changes took place in earthworms exposed to 50 mg Cd kg⁻¹ for 10 days. After 20 days of experiment degenerative changes were more profound.

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Cadmium effect on the cytophores structure in seminal vesicles in the earthworm *Dendrobaena veneta* (Rosa)

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Spermatogenesis in the earthworm *Dendrobaena veneta* occurs in clusters of cells called morulae. Germ cells in a cluster are connected by cytoplasmic bridges to the central, acellular mass called cytophore, until they differentiate into mature spermatozoa. This mass is formed by a progressive accumulation of cytoplasm and organelles during germ cells divisions. The cytophore takes part in supporting and synchronization of germ cells development, production of energy through the large number of mitochondria, selective intake of organelles discarded during spermiogenesis, synthesis of materials connected to the sperm morphogenesis (precursors of manchette microtubules and of sperm tails) and in energy production (glycogen of sperm tail). Cadmium affects spermatogenesis and induce structural changes in germ cells. However it has not been explained whether cadmium may induce changes in cytophores. Thus, the aim of this study was to determine what changes in cytophores structure occurred after cadmium exposure and whether those changes are dose-dependent. The objective was also to check if there is any correlation between the changes in cytophores and in germ cells. The cytophore structure was tested in *Dendrobaena veneta* earthworms exposed to cadmium

at concentrations 10 mg and 50 mg Cd kg⁻¹ of wet soil for 20 days and in controls.

In control animals cytophore began to differentiate in clusters of spermatogonia. During the later stages of spermatogenesis the cytophore greatly enlarged. In clusters of spermatids cytophore reached its maximal size and became irregular in shape. The cytoplasmic mass of cytophores included cell organelles as mitochondria, lysosomal bodies, multivesicular bodies, ribosomes, Golgi complexes and endoplasmic reticulum formed circular patterns.

In both experimental doses cadmium caused distinct degenerative changes in cytophores structure. In comparison to the controls the cytophores were strongly vacuolised. The vacuoles were membrane bound and their origin could not be established. Very advanced degenerative alterations occurred in endoplasmic reticulum where large vacuoles were detected.

Those changes were more advanced in earthworms exposed to 50 mg Cd kg⁻¹ of wet soil. Degenerative changes in cytophores were observed in those clusters where germ cells were also affected. Cadmium disturbed the synthesis of glycogen in cytophores, which resulted in lower content of glycogen in spermatids.

Testis structure in the earthworm *Dendrobaena veneta* (Rosa)

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Testis structure in the earthworm *Dendrobaena veneta* (Oligochaeta, Haplotaxida, Lumbricidae) was studied with the use of light and electron microscopes. For light microscopic examinations body segments containing testes (between segment 8 and 12) were isolated and fixed in Bouin's solution and then paraffin embedded. For electron microscopic examinations the testes were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 7 days and then postfixed in 1% OsO₄. The testes were then alcohol- acetone dehydrated and embedded in Epon.

In the earthworm *Dendrobaena veneta* two pairs of testes lay on both sides of ventral parts in segments 10 and 11. The testes were small and flattened. The proximal end of the testis was attached to the intersegmental septum via a ligament whereas the distal end freely

extended into the coelom in close proximity to the seminal vesicles.

Light and electron microscope studies showed that germ cells within testes were arranged in a typical pattern. In the proximal part of the testis protogonia were observed. The central part of testis was occupied by spermatogonia which divided mitotically. Divisions were synchronous and resulted in the development of groups of cells interconnected by cytoplasmic bridges formed clusters (morula). The last mitotic division of spermatogonia gave primary spermatocytes which were observed in the distal part of the testes. Clusters of primary spermatocytes were released from testes into seminal vesicles. Among the germ cells poorly differentiated somatic cells were observed. They made also the wall of the testes.

Ontogeny of the expression of some catecholamine synthesising enzymes in the porcine female hypothalamic suprachiasmatic and supraoptic nuclei

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In this study, hypothalami of porcine foetuses at the age of 112 days $n=4$, 84 days $n=8$ and 70 days $n=9$, and of pigs at the age of 1 day $n=4$, 10 weeks $n=7$ and 8 months $n=3$ were used. The cryostat sections of the tissues were stained with single- and double-labelling immunohistochemistry. Antibodies raised in mice (TH) or rabbits (D β H, PNMT) were applied. Omission, replacement and preabsorption tests proved the specificity of the immunostainings. The preparations were examined with a fluorescent microscope. The suprachiasmatic nucleus (SCHN) of 70 and 84 days old foetuses contained no immunoreactivity for the substances studied. In 112 days old foetuses, only single TH-IR nerve fibres and perikarya were found. In 1 day old piglets, additionally few TH/D β H-IR nerve cell bodies were observed. SCHN of 10 weeks old pigs contained a moderate number of TH-IR, and single TH/D β H-IR nerve fibres and neurones. In SCHN of sexually mature pigs, numerous TH-IR nerve fibres and moderate numbers of perikarya, some of them stained also for D β H, were observed. In the supraoptic

nucleus (SON) of 10 weeks old foetuses, moderate numbers of TH- and in some cases D β H-IR nerve fibres and perikarya were found. In SON of 12 weeks old foetuses, axons and perikarya were more numerous. In SON of 16 weeks old foetuses, increased number of TH-IR nerve fibres and perikarya was found. Part of them expressed D β H but solely D β H-IR axons and cell bodies were also encountered. Part of the fibres formed "basket like a structures" surrounding nerve cell bodies. In SON of 1 day old piglets, less numerous, only TH-IR and a few only D β H-IR nerve fibres were found. Observed neurones were mainly TH-IR but some of them displayed also immunoreactivity to D β H. Single perikarya stained only for D β H. In 10 weeks old pigs, nerve fibres and cell bodies were more numerous than those found in the previous animal groups. In SON of the sexually mature pigs, numerous TH D β H-IR but also some PNMT-IR nerve fibres were determined. TH-IR nerve cell bodies were very numerous; some of them expressed also D β H. Single neurones were only D β H-IR.

The influence of natural dye, curcumin on divisions of preimplantation murine embryos

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Curcumin (diferuloyl methane) is a naturally occurring yellow pigment derived from the rhizome of *Curcuma longa*. Curcumin exhibits a variety of pharmacological effects including anti-inflammatory, anti-infectious and anti-cancer activities. The massive exposure of populations worldwide to curcumin, and its many uses, have led to studies aimed at elucidating the mechanism of its activities. Curcumin has been shown inhibit proliferation and/or induce cell death (apoptosis) in *in vitro* experiments. Moreover, it is believed that curcumin exerts anti-proliferative and pro-apoptotic activities selectively on cancer cells. However, so far published data concerning curcumin's influence on normal cells didn't confirm this thesis. Accordingly, the aim of our study was to elucidate how curcumin affects divisions and survival of murine embryos *in vitro*. Two-cell embryos were isolated and treated for 24h with curcumin at different concentration. The number of divided blastomeres in each embryo were counted. In almost all (96%) non-treated embryos blastomeres divided one or two times. Curcumin seriously affected cleavage of the blastomeres and the effect was dose dependent. Namely,

10 μ M curcumin only slightly affected blastomeres divisions as more than 70% were divided. Upon 30 μ M curcumin treatment less than 60% blastomeres proceeded divisions, but no 8-cell embryos were found. The highest (50 μ M) concentration of curcumin allowed to divide only 16% of blastomeres. To elucidate the mechanism of curcumin action on the embryos, we performed chromosome and mitotic spindle analysis by using confocal microscope. In embryos treated with 10 μ M curcumin no meaningful aberrations was observed; the proper chromosome alignments and mitotic spindle assembly in dividing cells were observed. Whereas, 30 μ M curcumin induced visible structural changes of the mitotic spindle, however all chromosomes were aligned in a metaphase plate suggesting the lack of mitotic spindle depolarization. Upon 50 μ M curcumin treatment, chromosomes formation occurred but mitotic spindle was not observed, presumably leading to rapid cell death. Altogether, our results showed that the mechanisms of curcumin's action on murine embryos was strongly depended on dye concentration and that curcumin caused changes in the structure of mitotic spindle.

Immunocytochemical studies of the keratinization in the epidermis of grass snake *Natrix natrix* L.* (Lepidosauria, Serpentes) during embryogenesis

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Reptiles are terrestrial animals, which have become completely independent from aquatic environment; their skin is dry and impermeable to water. All reptilian species have the characteristic scaled skin with a more or less keratinized epidermis. This kind of integument protects the organism from mechanical damages and dehydration. Epidermis of adult squamates consists of six layers, built from different types of cells: 1. Oberhäuten, 2. β cells (so-called β -keratin layer), 3 intermediary cells (so-called mesos layer), 4. α cells (α -keratin layer), 5. lacunar cells (layer of cells undergoing lyses and forming a fissure detaching molt), 6. clear cells (so-called clear layer) (Maderson et al., 1998). The eggs of embryos were incubated at 30°C with relative humidity 100%. The embryos for examination were isolated at regular intervals, starting with egg laying and finishing when the first individuals hatched. The age of embryos was calculated using the table of species development (Rupik, 2002). The small pieces of skin were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde 1:1 in 0,1 M phosphate buffer, pH 7, 4 in 4°C, dehydrated and embedded in LR White embedding medium. The semi thin sections were immunostained with use of specific anti- α and anti- β keratins antibodies. Our finding indicated that keratins were located in the epidermis of grass snake during final three developmental stages (X–XII). The characteristic for lepidosauria, keratinaceous material deposition starts at the developmental stage X. In

this stage, clearly differentiated layers of cells are observed in the first time. At the developmental stage X β -keratins are observed in Mesos and β layers, but at the developmental stage XI in Oberhäuten, β and α layer. At the developmental stage XII, it is observed in a shedding complex only. We have not observed β -keratin material in a hinge region. The immunocytochemical results coincide with the electron microscopic results.

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*All specimens used in experiment were captured according to Polish legal regulations concerned with wild species protection (Dz.U. nr 2 poz. 11 z 1984 r., Dz.U. nr 114 poz. 492 z 1991r.). Department of Histology and Embryology obtained approval of Polish Ministry of Environment Protection and Forestry for performing studies on protected species (DOPog-4201-02-94/05/aj). The grass snake *Natrix natrix* L. is not included in Washington Convention of 1973, ratified by Poland in 1991 (Dz.U. nr 27 poz.112).

Androgen receptor and 3 β -hydroxysteroid dehydrogenase distribution in periovulatory cumuli oophori of the rat

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In response to the LH surge, the preovulatory follicle stops producing estradiol and starts to synthesize progesterone, an obligatory hormone for the stigma formation and the release of a mature cumulus oophorus complex (COC) into the oviduct to be fertilized. Progesterone, present in the oviductal fluid is an important steroid hormone that effects the epithelial cell function, stimulates acrosome reaction in spermatozoa and modulates the rate of an early embryo transport to the uterus. One of the objectives of the present study was to establish, to what extent COCs of the rat can contribute to the progesterone content in the ampullary fluid. The least known function of steroid hormones is that exerted by androgens. Of interest to the present study was also, whether the periovulatory COCs express androgen receptors (AR). To summarise our studies we also provided our earlier results on apoptosis of postovulatory COCs.

Female Wistar rats, exhibiting a regular 4 day-oestrus cycle, were used in this study. Some of them were killed on the day of pro-oestrus, while the others on Day 1 of pregnancy. Ovaries and oviducts were excised and subjected to a routine histological procedure. On paraplasm sections immunolocalisation of 3 β -hydroxysteroid dehydrogenase (3 β -HSD) and (AR) was investigated by an

immunohistological method, while apoptotic cells were identified with Tunel reaction.

Preovulatory follicles showed a negative 3 β -HSD immunolabelling. Ovulated COCs, present in the ampullas, demonstrated varying intensity of cytoplasmic 3 β -HSD immunoreaction. A very strong immunolabelling was found in the peripheral granulosa cells, and a weaker one in the cells lying in the vicinity of oocytes. The ampullary cumulus granulosa cells exhibited a relatively strong immunolabelling for AR, similar to that found in the preovulatory follicles. Our results indicated also that some of the postovulatory cumulus granulosa cells started to undergo apoptosis. Apoptotic cells were not numerous and generally limited to the peripheral parts of COCs.

Obtained results suggest that the ampullary COCs produce significant amount of progesterone. Our results suggest also that androgens, acting via receptor mediated pathway, regulate the pre-, and postovulatory cumulus granulosa cell function.

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Expression changes of estrogen receptors α and β in testis and epididymis of male mice under the influence of diet rich in phytoestrogens

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Phytoestrogens are substances which are similar in their structure to estradiol. They occur in many plants, e.g. in soya. Consumption of food containing these substances increased in the last years, because of their beneficial influence on human health, for example on cardiovascular system. There is evidence however, that phytoestrogens can disturb male hormonal balance and, in this way, reduce male fertility. The aim of our study was to measure the level of expression of estrogen receptors type α and β in testis and epididymis of male mice after feeding them special diet. Three groups of adult mice

were fed the animal chow that differed in content of phytoestrogens. 8, 16 and 24 weeks after feeding a part of animals was killed from each group. The immunohistochemical method was used to measure the level of receptors expression in testis and epididymis. We noticed that the level of receptors expression, mainly type β after 24 weeks of experiment, decreased. We observed these changes in Sertoli cells and cells of corpus and cauda of epididymal epithelium. We conclude, that phytoestrogens influence the expression of estrogen receptors β in testis and epididymis.

Early oogenesis in *Erpobdella* (Oligochaeta, Hirudinida, Erpobdellidae)

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In *Erpobdella* within specialized coelomic sacs (ovisacs) forming ovary wall, 5–7 spindle-shaped ovarian bodies occur. Ovarian bodies are functional units in which oocytes develop. According to Jörgensen (1908), in each fully developed ovarian body 5 zones occur. I zone contains oogonia, in II germ cells are arranged in parallel rows, in III germ cells differentiate into oocytes and nurse cells (trophocytes), in IV zone oocytes grow considerably and detach from the ovarian body, whereas V zone contains degenerating cells only. Each ovarian body is encompassed by somatic cells with "spongy" cytoplasm termed spongiosa. Similarly as in other leeches, in *Erpobdella* during early oogenesis germ line cells form cysts (clones, clusters, nests) of cells interconnected by stable intercellular bridges (ring canals). Between clusters of germ cells numerous smaller and elongated somatic cells occur. The cysts geometry in *Erpobdella* is also similar as in other leeches i.e. each germ cell (cystocyte) has only one ring canal connecting it to common cytoplasmic mass, the cytophore. However, in contrast to other leeches the cytophore in *Erpobdella* is poorly developed, it appears as thin and long cytoplasmic strand stretched out between cystocytes. The number of cystocytes forming clone is still unknown. The formation of clones begins in zone II,

in zone III the cells within clones differentiate into usually one oocyte and several trophocytes. According to older literature (Urbańska-Jasik, 1988) trophocytes forms 2 subpopulations: some cells are connected to the growing oocyte via cytophore and ring canal, the second cells (abortive oocytes) form multinuclear syncytium (follicle) enveloping oocyte. It is worth to add here that such unusual organization of germ line clones, where developing oocytes are enveloped by syncytium made from germ line cells was described only in *Erpobdella*. Our preliminary studies revealed that in zone II some cystocytes are tightly enveloped by elongated somatic cells. We suggest that such somatic cells in still unknown way (cell fusion, endomitotic divisions) form multinuclear syncytium surrounding growing oocytes, whereas the rest of non-surrounded cystocytes become trophocytes.

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The cytoskeleton of the embryo-suspensor in *Gagea lutea* (L.) Ker Gawl.

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The cytological differentiation of the embryo-suspensor in *Gagea lutea* (L.) Ker Gawl. was studied in comparison with the development of embryo-proper. After fertilization, the zygote divides once, giving rise to a smaller apical cell and a larger basal cell (BC). The BC undergoes no further division and becomes the basal cell of the suspensor. The apical cell develops into the embryo-proper and chalazal suspensor. The fully differentiated suspensor consists of a huge basal cell and a few chalazal cells. Ovules were isolated from ovaries in various developmental stages and immediately fixed. After fixation the plant material was embedded in Steedman's wax. Immunofluorescence techniques were performed on sections using monoclonal antibody (Mab): mouse anti-actin. Antigen-Mab complexes were localized by a secondary, Alexa 488-conjugated anti-mouse antibody. The microfilaments were also stained by rhodamine-phalloidin (Świerczyńska and Bohdanowicz, 2003).

At the first stage of development of suspensor BC, the microfilaments were assembled to form a delicate meshwork of actin filaments. Numerous F-actin elements formed irregular bundles distributed in the cortical cytoplasm of the cell. At the next stage of BC development, the

actin cytoskeleton was localized from micropylar to chalazal apex of the cell. The microfilaments were formed an abundant and dens network. At the micropylar pole of the suspensor BC, a high concentration of fine actin bundles were observed. Some of these actin filaments were oriented longitudinally or transversally to the long axis of the cell. A single, huge nucleus of the suspensor BC was located centrally. The actin material appeared also to pass close to the nucleus envelope and some bundles of actin filaments radiated from the nuclear surface. The actin elements were concentrated at the chalazal pole of suspensor BC near the chalazal wall, separating the BC from the other embryo cells. At all stages of suspensor basal cell development in embryo-proper cells an extensive and intricate cortical network of actin cytoskeleton was visualized. These observations suggest that the microfilaments may have an important role in the development of suspensor basal cell in *Gagea lutea* (L.) Ker Gawl.

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Ultrastructure of the germarium in ovary of *Opisthocosmia silvestris* (Dermaptera, Forficulidae)

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The ovaries of *Opisthocosmia silvestris*, as those of other studied forficuloid earwigs, are meroistic-polytrophic and composed of numerous short ovarioles that consist of a terminal filament, germarium and vitellarium. The germaria of adult females are relatively short and comprise individual germ cells (presumably the cystoblasts), germ cell clusters, as well as small somatic prefollicular cells. All germ cell clusters, even the youngest, consist of two cells only that are connected by a single intercellular bridge. Using the TEM techniques we classified the germ cell clusters into 2 developmental categories: clusters in an early stage of differentiation consisting of a prospective oocyte and a pro-trophocyte (1) and differentiated clusters which are composed of an oocyte and a trophocyte (2).

In the early stage of differentiation the cells constituting the cluster are morphologically similar and differ only in the organization of their nuclei. The nucleus of the pro-trophocyte comprises irregular chromatin aggregations, while that of the prospective oocyte is almost translucent and contains postmeiotic chromosomes

only. The cells are connected via intercellular bridge which contains the fusomal material. In their cytoplasm ribosomes, elements of RER, multivesicular bodies and numerous mitochondria are present. The latter are not distributed in the cytoplasm uniformly, but they are concentrated in the certain region of the cell forming a prominent mitochondrial cloud or Balbiani body (Bb). In both cells, the Bb tightly adheres to the nucleus and remains in a direct contact with the fusomal material.

Cells constituting the differentiated clusters are morphologically different. They remain connected by an intercellular bridge which does not contain the fusomal material. The oocyte nucleus is relatively large. It contains chromatin aggregations and a spherical nucleolus. In the ooplasm numerous ribosomes, Golgi complexes and RER elements are present. The trophocyte nucleus envelope is slightly folded and pierced by numerous pore complexes. The cytoplasm of the nurse cell comprises elements of RER, multivesicular bodies and ribosomes. In both cells of the cluster (oocyte and trophocyte) prominent Bb is still present.

Differentiation and diversification of the follicular epithelium in the earwig ovarian follicles

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The ovaries of the studied earwig species (*Forficula auricularia*, *Chelidurella acanthopygia*, *Doru lineare* and *Opisthocosmia silvestris*) are meroistic-polytrophic and composed of numerous short ovarioles that consist of a terminal filament, germarium and vitellarium. The germaria of adult females comprise germ cell clusters, as well as small prefollicular cells. All germ cell clusters consist of two cells that are connected by a single intercellular bridge. In the vitellarium there are usually 2 ovarian follicles only. The individual follicle consists of a synthetically dormant oocyte and a single, polyploid nurse cell and is surrounded by a layer of somatic follicular cells (FCs).

During consecutive stages of oogenesis initially uniform FCs start to diversify into four morphologically and

functionally different subpopulation. Our analyses have shown that in earwigs the diversification of the FCs consists of two phases only. In the first one, the FCs differentiate into uniform cells covering the oocyte-nurse cell complex and cells residing between neighboring ovarian follicles. The latter cells form prominent interfollicular stalks. In the second phase, the FCs remaining in a direct contact with the germ-line cells diversify into three subpopulations: cuboidal cells surrounding the oocyte (1), small, stretched cells covering the nurse cell (2), and cells migrating between the oocyte and the nurse cell (3). Consequently, in earwigs, the final number of FC subpopulations is relatively low. This agrees well with the lack of recognizable regional specializations of the earwig eggshells.

Trial to in vitro induction of androgenesis in *Salix viminalis* anther culture – microspores under stress condition

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Salix viminalis L. plants are economically important trees cultivated for biomass production. Trees are characterized by a long reproductive cycle with several years of juvenile phase and high degree of heterozygosity what causes that the production of dihaploids is important for obtaining homozygous clones. In herbaceous plants extensive studies have been conducted to reach the major aim – cause the haploids production by induction of androgenesis in vitro. In trees the use of anthers cultures remains limited. In presented study of *S. viminalis* the influence of abiotic stress on induction of microspore divisions was investigated.

Anthers with microspores at the uninucleate stage were cultured at $21^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in the dark on solidified MS medium with various concentration and combination of growth regulators. Anthers before transfer on MS medium were influenced on 2,3,4,5,6 or 7 days temperature and mannitol pretreatment. During the pre-culture, anthers were incubated in different temperatures (4°C , 27°C , 32°C) and pretreatment solution based on mannitol, liquid pre-medium called Kyo (Kyo and Harada,

1986) or mannitol dissolved in a pre-medium Kyo. The addition of mannitol solution resulting in a higher osmotic stress and cold or heat shock conditions of pre-culture were considered as factors which may switch the gametophytic pathway into a sporophytic development of the microspores. In our studies the combined treatment of anthers with mannitol dissolved in Kyo medium and 4°C for 7 days caused a formation of multinucleate microspores.

Preliminary ultrastructural observations revealed a strong link between the pre-treatment of mannitol and lack of amyloplasts specialized for starch storage and low number of small vacuoles. The microspores under Kyo pre-treatment contained a large amount of tiny vacuoles.

The cell response to cold and heat shock and mannitol stress is still recorded.

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Development of somatic embryos from suspension culture of *Salix viminalis* L.

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Salix viminalis L. are economically important crops used in bio-energy plantations as a renewable energy source. The production and sustainability of the willow plantation system is based on the ability of trees to resprout after repeated harvesting. The in vitro culture techniques can be utilised for the genetic improvement of basket willow clones to increase biomass productivity.

In present experiments, factors influencing callus initiation from zygotic embryo establishment were studied.

Shoots of selected female and male clones with closed flower buds were collected from the field of the Institute of Plant Genetics, Polish Academy of Sciences in Poznań. Pollinations were performed in semi-vivo conditions on female branches kept standing in the laboratory rooms. Zygotic embryos of 5 clones of *S. viminalis*, 12–15 days after pollinations, were aseptically isolated and cultured on agar nutrient media containing 2,4D [2,4-dichlorophenoxyacetic acid] in combination with various cytokinins BAP [benzyladenine], TDZ [thidiazuron] and

addition of sugar [sucrose, maltose]. The cultures were maintained at continuous light condition ($35 \text{ mmol m}^{-2} \text{ s}^{-1}$, cool white fluorescent light) and in darkness at 24°C and 70–80% relative humidity.

Depending on the cytokinins two types of calluses were formed. On MS medium with TDZ (0.1 mg l^{-1}) and 2,4D (2.0 mg l^{-1}) callus was white, soft with wet-looking surface and composed of long, thin, tubular and highly vacuolated cells. The callus formed on MS medium with BAP (0.1 mg l^{-1}) and 2,4D (1.1 mg l^{-1}) was friable and made up of small compact clumps. The cells were isodiametric and densely cytoplasmic with well stained nucleolus.

The friable callus was embryogenic, and initiated cell-suspension culture. Somatic embryos developed from suspension cells cultured in MS medium supplemented with 2iP [N6-(3-methylbut-2-enyl)adenine (6.0 mg l^{-1})], IAA [indol-3-acetic acid] (0.5 mg l^{-1}) and maltose (10 g l^{-1}). Both, the calluses and somatic embryos were formed in darkness.

Similarities and differences in the development of the human maxilla and mandible

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Skeletogenic cells of the head derive from: 1) paraxial mesoderm, 2) lateral mesoderm, 3) neuronal crest (ectomesenchyme). The face develops from a series of five facial swellings or growth centers, generally termed processes.

Present study was undertaken in order to clarify the similarities and differences during the development of two main bones of the facial skeleton, the maxilla and the mandible.

Investigations were performed on serially sectioned human embryos of development stages 13 to 23 (32 to 56 days) and fetuses aged 9 to 12 weeks.

In embryos at stage 13 (32 days) the mouth is bounded by the frontonasal prominence and the 1st pharyngeal arch. By the end of 5th week (stage 15) the mandibular arch divides into maxillary and mandibular process, forming primordia of the maxilla and the mandible. By stage 17 (6th week) the maxillary processes join the lateral and medial nasal processes to form the upper lip. Ossification of the maxilla begins at stage 20 (7th week) in the place of the future infraorbital foramen.

The mandibular processes form a single structure in embryos of 5th week and the mandible develops lateral to

the cartilaginous skeletal rod which is named mandibular (Meckel) cartilage. Ossification of the mandible precedes ossification of the maxilla and begins 2 days earlier. The primary ossification center appears in the place of the future mental foramen. At the beginning of the fetal period all skeletal units of the mandible are distinguished.

From performed study it may be concluded that during development of investigated bones the similarities and differences may be found.

Similarities: 1) development from the first pharyngeal arch; 2) intramembranous ossification; 3) neural crest origin; 4) alveolar part a teeth; 5) presence of secondary cartilages; 6) ossification centers are located close to nerve trunks; 7) growth according to v principle.

Differences: 1) the mandible is movable bone connected to the skull through the temporomandibular joint; 2) the mandible has prominent muscular processes; 3) the mandible is the second skeletal element to ossify, the clavicle being the first; 4) the mandible is a major determinant in the masticatory function and in facial appearance.