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PLENARY LECTURES

SEM study of the morphogenesis of lingual papillae in mammals in the prenatal period

Hanna Jackowiak

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The numerous morphological studies conducted on the mammals tongue have reported the wide variety of microstructure and distribution of the gustatory and mechanical papillae on the dorsal surface of this organ. This diversity is related to systematic position and feeding behavior of the species. In such kind of studies the scanning electron microscopy (SEM) is very useful tool which allows to show the three-dimensional and not only topographic profile of the tongue mucosa, as well as the microstructure of the epithelium and connective tissue. So far, SEM studies on the development of the tongue were conducted in the rodents, lagomorphs, carnivores and in the human.

The tongue develops from several swellings on the floor of the pharynx. In the early embryos the flat surface of tongue is covered by undifferentiated simply epithelium, which change slowly into stratified one. The observations in carnivores and human have revealed that on the surface of the epithelium the highly specialized brush cells appear additionally.

The development of lingual papillae is under control of several growth factors and as the first ones the gustatory papillae develop. The distinct symptom of fungiform, vallate and foliate papillae development is the formation of structures called prepapillary placodes. The placodes are epithelial structures which have often different spatial distribution as similarly to adult individuals. Moreover, the number of placodes is species specific and can be the same during the development as in adult animals or, in some cases, the placodes grow in number by division or develops de novo.

During the next period of prenatal development, beneath the gustatory placodes, the fibrillar connective tissue cores with capillary loops appear. The fungiform papillae project over the surface of the tongue, but the vallate or foliate papillae are formed by epithelial ingrowths. The taste buds in the gustatory papillae are recognized in the epithelium after formation of connective tissue cores.

The development of primordia of the mechanical papillae starts after formation of gustatory papillae or just before the birth. The pattern of development of the conical and filiform papillae, and keratinization of their epithelium is different in particular parts of the tongue and is continued in perinatal period. The interesting event during development of the tongue in carnivores is formation of marginal papillae on its apex, which papillae undergo degeneration after the birth.

Sempervivum embryogenesis: the development and ultrastructure of the suspensor

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The embryogenesis in *Sempervivum* (Crassulaceae) was investigated using cytochemical methods, light and electron microscopy. After the first division of the zygote, two cells of unequal size are formed: the large basal cell (BC) and the smaller apical one. The BC undergoes no division, becomes much enlarged, and gives off haustorial branches invading the micropyle and adjacent tissues.

The mature suspensor consists of a large, pear-shaped BC and a few chalazal cells. The nucleus has a folded surface and a few nucleoli. The micropylar part of the wall is covered with wall ingrowths typical for a transfer cells. The ingrowths may also partially cover the lateral wall. The dense cytoplasm filling the basal cell is rich in ribosomes, vacuoles, rough endoplasmic reticulum, dictyosomes, lipid droplets, and contains also mitochondria, microbodies and plastids.

The apical cell develops into the embryo-proper and chalazal suspensor. In ultrastructure the chalazal suspensor cells and the embryo-proper are similar.

Investigations of cytochemistry and ultrastructure of the suspensor in *Sempervivum* at various stages of the development of the embryo-proper, suggest the basal cell functions as a synthetically active transfer cell absorbing nutrients from maternal tissues, metabolizing them and translocating through the chalazal suspensor cells to the growing embryo-proper.

Dynamics of RNA polymerase II in female gametophyte cells of *Hyacinthus orientalis* L. before and after fertilization

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The aim of the work was to investigate the distribution and level of the different forms of RNA polymerase II in the cells of *H. orientalis* L. embryo sac before and after fertilization.

Polymerase II RNA was detected by the immunofluorescence methods using antibodies: (1) 4H8 – anty-total pool of RNA pol II, (2) H14 – anty-initiation, hypophosphorylated form of RNA pol II (RNA pol IIA), (3) H5 – anty-elongation, hyperphosphorylated form of RNA pol II (RNA pol IIO).

In mature, unfertilized embryo sac in the cells, which are the targets for male gametes, the level of the total pool of RNA pol II and also initiation and elongation forms was low. In both the synergides, which attracts pollen tubes and antipodes, which perform nutritious functions, the levels of the investigated molecules were higher.

After fertilization, in the zygote and in the arising endosperm cells the level of the total pool pol II RNA and also initiation and elongation forms intensively increased. In the degenerated synergides and antypodies, which performed its biological functions, dramatic decrease the level of the total pool of RNA pol II and both forms of the enzyme was observed.

The obtained results were analyzed in comparison with previously known dynamics of the total transcriptional activity (Pięciński et al., 2008a) and the level of the Poly(A) RNA (Pięciński et al., 2008b) in the cells of *H. orientalis* L. embryo sac. We confirm that the levels of the investigated molecules in those cells both before and after fertilization were positive correlated with its total transcriptional activity and with the level of the mature mRNA transcripts.

ACKNOWLEDGMENTS: This work was supported by the grant of Polish Ministry of Science and Higher Education no. N N303 290434.

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ORAL PRESENTATIONS

Phylogenetic assessment of the leeches (Clitellata, Hirudinida) using features of reproductive system and molecular data

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The studies concerning phylogenetic relationships of type species for higher taxonomic units of true leeches (Hirudinida, Euhirudinea, Glossiphoniidae: *Glossiphonia complanata, Alboglossiphonia heteroclita*; Piscicolidae: *Piscicola geometra, Cystobranchus respirans*; Erpobdelliformes: *Erpobdella octoculata*; Hirudiniformes: *Haemopis sanguisuga, Hirudo medicinalis*) were conduced using characters of reproductive system and mitochondrial and nuclear genes sequences. *Dendrobaena veneta* was chosen as an ancestor. Parsimony analyses were performed using PAUP* 4.06b10 (Swofford, 2000). The hypothetic parsimonious cladogram shows Hirudiniformes as monophyletic group, which contains Hirudinidae and Haemopidae shown as sister groups. Moreover Glossiphoniidae and Piscicolidae appeared not to be sister taxa. These facts support the data received by other researchers on morphological and molecular level (e.g. Siddall and Burreson, 1998; Trontelj et al., 1999; Utevsky and Trontelj, 2004). Additionally our analyses show that Erpobdelliformes are sister either to Glossiphoniidae (results of analyses based on characters of reproductive system only) or to Hirudiniformes and Piscicolidae (combined data sets). To better understand the phylogenetic relations between main leech groups including additive features (for example leech body form, new external and internal morphological characters, molecular and other available data) is needed.

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Development and ultrastructure of helobial endosperm in *Butomus umbellatus* L.

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In double fertilization, one of the two sperm cells from the pollen tube fuses with the egg to produce the zygote and the other sperm fuses with the central cell to produce the primary endosperm cell. Three types of endosperm development are recognized in Angiosperms: nuclear, cellular and helobial. In *Butomus umbellatus* L. the helobial type have been observed. The initial division of primary endosperm cell, in this pattern of development, is asymmetric and accompanied by cytokinesis.

The smaller one of daughter cells (called chalazal chamber) remains uninucleate while the larger (micropylar chamber) undergoes a series of free nuclear divisions before cellularizing. The divisions lead to the rise of the multinucleate syncytium with nuclei arranged along wall which contacts with nucellar cells, nearby embryo and chalazal chamber. Cytoplasm of micropylar chamber is mostly vacuolized and in comparison with chalazal chamber contains less organelles. Cellularization of the micropylar chamber is initiated at the micropylar end near the embryo and proceeds chalazally and centripetally. The syncytium is divided into compartments (alveoli) by anticlinal cell walls extending centripetally around all nucleus, with their open ends towards the micropylar chamber interior. Periclinal divisions in alveoli cut off an outer layer of cells and displace the alveoli inward. Finally cells fill nearly all micropylar chamber.

The fully differentiated chalazal chamber is a huge cell, filled with differently sized vacuoles and dense cytoplasm with numerous organelles, particularly rough endoplasmic reticulum (RER), mitochondria, dictyosomes and plastids. Chalazal wall of the chamber, bordering gradually degenerating nucellar cells, bears numerous ingrowths, which form an extensive plasma membrane-lined labyrinth. In the vicinity of the labyrinth abundant organelles (especially mitochondria, RER and dictyosomes) have been constantly found. The nucleus of the chalazal chamber is huge, irregularly shaped, polyploid and contains one or a few nucleoli. The results suggest the chalazal chamber of the helobial endosperm in *B. umbellatus* plays an important role in the development of young seed transferring nutrients from chalazal nucellar cells to micropylar chamber.

Germ-line cysts with a central cytoplasmic mass are formed during early oogenesis in Lumbriculidae (Annelida, Clitellata).

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Lumbriculidae are microdrile oligochaetes common in fresh-water environments, including streams, lakes, marshes, wells and ground-water. These animals possess some characteristic features which allow place them among the most archaic species of the Oligochaeta (Hrabě, 1983), however, according to actual views on phylogenetic relationship on Clitellata, lumbriculids are considered as a sister group of leeches (Hirudinea) (Siddal et al., 2001). Knowledge about oogenesis and ovary organization in Oligochaeta is still incomplete, especially nothing is known about Lumbriculidae.

From our studies on *Lumbriculus variegatus* and *Stylodrilus* spp., we know that, their ovaries are formed from numerous cysts of germ-line cells surrounded with somatic (follicular) cells. The architecture of germ-line cyst is very characteristic; each germ cell is connected via only one cytoplasmic bridge to a central and anuclear cytoplamic mass, a cytophore. Initially all cells within a cyst enter meiosis and then their fate is diversified. Only a few (one?) cell within a given cyst continue meiosis and become oocyte(s), the rest of cells transform into nurse cells. Together with the diversification of cell fates, the cytophore grows and it becomes large and extensive.

Germ-line cysts of the same architecture as found in Lumbriculidae are known from the leech ovaries (Świątek, 2008). We suggest that the common ancestor of Lumbriculidae and Hirudinea also possessed ovaries equipped with germ-line cysts with central cytoplasmic mass.

ACKNOWLEDGMENTS: This work was supported by research grant: N N303 423636 from Ministry of Science and Informatization.

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Early differentiation of testes in water frogs (Amphibia, Anura)

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Testes were studied in tadpoles and juvenile *Pelophylax* (formerly *Rana*) lessonae and *P. ridibundus*. We present ultrastructure, histology and anatomy of testes of tadpoles at Gosner (1960) stages 28 – 46, *i.e.*, from premetamorphosis to metamorphic climax.

A testis develops from the metameric gonadal anlage; the most anterior part looses its germinal function and differentiates into fat body, the rest of the anterior part transforms into gonad proper, and the posterior part becomes a thread-like process that finally disappears. The gonad proper starts to enlarge. Its cortical part is devoid of primary spermatogonia that migrate into the medulla. Cells of the external epithelium proliferate into the medulla and give rise to Sertoli cells, seminiferous tubules, and *rete tesis*. Leydig cells and *interstitium* are descendants of mesenchymal cells that invade early gonad *via* mesorchium. Primary spermatogonia proliferate and are located in presumptive seminiferous tubules (Ogielska and Bartmańska, 1999; 2009).

In this study we focused on changes of the posterior part of a gonadal anlage because this process has not been described in detail so far. The distal part retains its metamery longer that the proper gonad. Until stage 32 the "knots" of the medulla contain proliferating somatic cells and primary spermatogonia that start degenerating. Nuclei of the degenerating spermatogonia contain blocks of dense heterochromatin; apoptotic bodies are also observed. After degeneration of spermatogonia the knots are composed of somatic cells and the entire distal part start thinning and shortening. After metamorphosis is completed (stage 46) the somatic cells also degenerate and remnants of the distal part disappear.

Rate of differentiation and development of the gonads differ. As a rule the left gonad is more advanced and bigger, and degeneration of its distal part is faster. This difference in size is retained also in adults.

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Early mouse oocytes contain Balbiani body and show transient polarity

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Mammalian oocytes are traditionally view as spherical cells with organelles evenly distributed around the nucleus or germinal vesicle and with no obvious polarity. Our ultrastructural analysis has revealed that oocytes in mouse neonatal germline cysts contain collection of organelles resembling the Balbiani body or mitochondrial cloud characteristic of *Xenopus* oocytes (Heasman et al., 1984; Kloc et al., 2004). In the mouse early oocytes, the Balbiani body is asymmetrically adjacent to one pole of the germinal vesicle and consists of multiple Golgi complexes surrounded peripherically by numerous mitochondria and cisternae of rough endoplasmic reticulum. The Golgi complexes are arranged around a pair of centrioles and the area surrounding the centrioles contains an aggregation of electron-dense material. Immunogold labeling with PCM1 antibody demonstrated that this material is enriched with PCM1 proteins and corresponds to pericentriolar material. Careful analysis of serial sections showed that the pair of centrioles is invariably located in the immediate vicinity of the rim of the cytoplasmic bridges connecting early oocytes. Electron microscopy analysis of P0 oocytes also showed the presence of aggregates of vesicles filled with electron-dense material pinching off from the trans-face of the Golgi complexes. By examining ovaries in different developmental stages, we found that the Balbiani body forms in oogonial cells in mouse embryonic ovaries, persists in oocytes in neonatal ovaries, and disperses in stage P4 oocytes. Using three-dimensional reconstruction of serial semithin and ultrathin sections of mouse neonatal germline cysts, we demonstrated that early mouse oocytes are both asymmetrical and transiently polar. We suggest that the transient polarity of the mouse oocytes is imposed by the position of the centrioles at the cytoplasmic bridges.

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Yolk nucleus is a site of lipid droplet formation in spider oocytes

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Oocytes (future egg cells) of various animal groups often contain complex organelle assemblages (Balbiani bodies, yolk nuclei). The molecular composition and function of Balbiani bodies, such as those found in the oocytes of *Xenopus laevis*, have been recently recognized. In contrast, the functional significance of more complex and highly ordered yolk nuclei has not been elucidated to date. In this report we describe the structure, cytochemical content and evolution of the yolk nucleus in the oocytes of a common spider, *Clubiona* sp. We show that the yolk nucleus is a spherical, rather compact and persistent cytoplasmic accumulation of several different organelles. It consists predominantly of a highly elaborate cytoskeletal scaffold of condensed filamentous actin and a dense meshwork of intermediate-sized filaments (IFs). Preliminary immunological analyses imply that the IFs consist of a vimentin-like protein. The yolk nucleus also comprises cisterns of endoplasmic reticulum, mitochondria, lipid droplets and other organelles. Nascent lipid droplets are regularly found in the cortical regions of the yolk nucleus in association with the endoplasmic reticulum. Single lipid droplets become surrounded by filamentous cages formed by IFs. Structural and cytochemical analyses indicate that yolk nuclei in spider oocytes should not be considered as homologous to Balbiani bodies. Coexistence of the forming lipid droplets with the endoplasmic reticulum in the cortical zone of the yolk nucleus and their later investment by intermediate-sized filamentous cages suggest that the yolk nucleus is the birth place of lipid droplets.

Intracellular organization of splicing machinery in diplotene microsporocytes of European larch

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During prophase I meiotic division cells of generative lines are transcriptional active. Prophase RNA synthesis is well known in animal cells. Knowledge of this process in plants is still fragmentary. Even less is known organization of splicing system in this cells. The aim of this study was to know the intracellular organization of the splicing system against a background of transcriptional activity of European larch microsporocytes (*Larix decidua* Mill.).

Studies have shown that, during meiotic prophase microsporocytes of larch are transcriptionaly active. The highest level of RNA synthesis occurs in diplotene, which is the longest stage of prophase. Therefore, this stage was chosen as a model for detailed analysis of the relationship between the transcription and organization of splicing system in plant cells. The level of transcription in diplotene microsporocytes was not constant. The investigations have demonstrated that in diplotene microsporocytes of larch there are 5 periods of increased transcriptional activity. To analyze intracellular organization of splicing system in microsporocytes of larch was studied distribution and level of snRNAs and Sm proteins. It was found that during the diplotene occurred 4 periods of increase the level of these molecules. Each of these periods preceded transcriptional activity of microsporocytes. Increase of splicing factor's level was connected with the change in their distribution, both in the nucleus and cytoplasm of microsporocytes. Cyclically repeated changes in the level and intracellular distribution of splicing elements were the basis for creating a model of the snRNP biogenesis cycle in diplotene microsporocytes of larch. According to the proposed model, each cycle start with the synthesis of new snRNAs in the nucleus and the Sm proteins in the cytoplasm. After the synthesis of nascent transcripts snRNAs are exported to the cytoplasm, where, together with Sm proteins accumulate in clusters, called cytoplasmic bodies. Within this bodies probably take place a cytoplasmic phase of snRNP maturation. After maturation, snRNP complexes reimport to nucleoplasm. Increasing levels of Sm-snRNP in nucleoplasm may be a factor leading to the formation of Cajal bodies, which held its last modification. snRNP molecules prepared in that way may participate in the process of splicing.

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Olfactory organs of male and female rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) depending on the reproductive season

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It is known from the literature that well developed olfactory organs in salmonids are an important factor allowing for finding the spawning location (Halama, 1982). Studies were carried out on females and males of the rainbow trout from a fish farm in the non-reproductive season and during the spawning time. Observations were carried out using routine scanning electron microscopy methods, while the measurement of the olfactory area was performed using Jakubowski's method (Jakubowski and Kunysz, 1979). The aim of the present study was to reveal possible differences in the structure of olfactory rosettes in both males and females between the breeding and non-reproductive season.

The location and structure of olfactory organs of the rainbow trout is typical, as in the majority of bony fish species. The lining of the olfactory cavity floor forms an oval olfactory rosette. No differences in the structure of the olfactory cavity, olfactory rosette as well as distribution and general structure of the olfactory sensory epithelium between fish of both sexes have been found. In both female and male individuals of the studied species secondary folds on olfactory lamellae have been found. However, no sensory olfactory epithelium was present there, what confirms data obtained by Halama (1982). In female individuals the number of lamellae forming the olfactory rosette was somewhat higher during the spawning season than in the other month. However, in male individuals the number of lamellae did not differ. Moreover there were no important differences in the area of olfactory lamellae between the spawning season and the rest of the year.

Observations carried out in SEM indicate, that the number of olfactory cells with flagella during the spawning season is higher than in the remaining part of the year. This allows us to postulate that the spawning season may have an influence on the rainbow trout's olfactory capability. However, this requires further studies optimally performed on wild-caught individuals.

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Floral structure and pollen morphology of two zinc violets (*Viola lutea* ssp. *calaminaria* and *V. lutea* ssp. *westfalica*) indicate their taxonomic affinity to putative ancestor *Viola lutea*

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The two endemic zinc violets (Melanium section) of Central Europe have locally restricted distribution. The yellow zinc violet (*V. lutea* ssp. *calaminaria*) grows on heavy metal heaps (Zn and Pb contaminated soil) between Aachen, Germany and Liège, Belgium. The blue zinc violet (*V. lutea* ssp. *westfalica*) is only found on a medieval Cu-Pb ditch in Blankenrode close to Paderborn, Eastern Westfalia, Germany. The taxonomic affinity of both zinc violets is not yet clear. Macromorphological and chromosome data suggested *V. lutea* and *V. tricolor* as putative ancestors of zinc violets. Earlier investigators (see Nauenburg, 1986) regarded the yellow zinc violet as own species *V. calaminaria* and the blue one as *Viola guestphalica*. Molecular phylogeny based on ITS (ITS1-5.8S rDNA-ITS2 and 18S-r DNA) showed that both zinc violets are closely related to *V. lutea* but not to *V. tricolor* (Hildebrandt et al., 2006).

Since so far published morphological criteria and the molecular data did not match, a more detailed micromorphological characterization of both zinc violets and their potential relatives is necessary.

The present study was therefore undertaken to characterize the female (shape of style and stigma, types of hairs and papillae), male (hairs on anthers, nectaries) reproductive organs, corolla petals including epidermal cell shape, and pollen grains of two zinc violets and their putative ancestors *V. lutea* and *V. tricolor* in detail by scanning electron microscopy (SEM). The images show an unambiguous relatedness of the zinc violets to *V. lutea* and thus corroborate the molecular data.

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Male reproductive system and spermatogenesis in oribatid mite: *Hermannia gibba* (Acari: Oribatida)

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The paired testes of male of *Hermannia gibba* (CL Koch, 1839) consist of germinal and glandular parts that are connected by transversal testicular bridge (Alberti and Coons, 1999). The short seminal ducts join and as a single part enter into the progenital chamber. The numerous spermatocysts where spermatogenesis takes place fill the germinal part. A single, large somatic cell surrounds all cysts. Each spermatocyst contains germ cells at the same developmental stage. Spermatogonia incompletely divide and form a cluster of spermatocytes, each cyst contains few to several such clusters. The chromatin in spermatocyte nucleus condenses and forms electron-dense ring. This ring opens in the next stage and its free ends fold toward inside part of the structure. Next the chromatin forms a comb-like arrangement: on the inner side threads of chromatin appear. The cytoplasm at this stage contains a few small mitochondria and small, electron-dense bodies. In the next stage of spermatid maturation the threads become denser and the whole nucleus forms an electron-opaque, disc-shaped structure. The redundant cytoplasm is removed; in the rest of the cytoplasm a few mitochondrial derivatives and so called bowl bodies are located. The densely packed mature spermatozoa are released to the light of the testis' glandular part by the cyst's wall bursting. The glandular part consists of the thick walls with gland cells and the canal where the secretion and spermatozoa are released. It this part the fragments of spermatophores are being formed.

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Teacher's knowledge of teratogenic effect of ethyl alcohol

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Foetal Alcohol Spectrum Disorders (FASD) includes the health problems caused by prenatal exposition of the foetus to alcohol. The knowledge about the risk of FASD is still not that common among the society (Czech, 2004). The knowledge among teachers – professionals involved in the education and development of children, also those affected by FASD – on the issue of FASD has been assessed (Sioda, 2009).

A fifteen questions survey concerning "Pregnancy and alcohol" has been developed. A hundred survey forms together with demographics were received back. The analysis of the assembled data revealed that teachers have a wide range of up to date knowledge about this problem (Abel, 1998)

Pupils with FASD cause a lot of educational and behavioural problems. Thus it has been concluded that knowledge alone is insufficient for teachers to cope effectively with these difficulties. Further research will focus on the appropriate practical skills and attitude towards pupils.

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Polyploid synergids in *Allium angulosum* L.

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The establishment of untypical egg apparatus has been observed during the differentiation of the embryo sac elements in *Allium angulosum* L. (2n = 16). The embryo sac is formed according to the bisporic 8-nucleate Allium type of development. Three nuclei on the chalazal end degenerate without forming antipodal cells. The polar nuclei are located in the middle of the sac. The egg apparatus at the micropylar end consist of the egg cell and two synergids. At the time of the egg apparatus organization both of the synergids start to increase their size but one of them becomes definitely larger. It may rich to one-third the length of the embryo sac. Both larger and smaller synergid undergo a few cycles of nuclear DNA endoreduplication. This process leads to the high ploidy level of nuclei (128C in bigger, and 8C in smaller). Polyploidyzations of the nucleus of the larger synergid is accompanied with the formation of the giant chromosomes. As the result of that process nucleus is located in the middle part of the cell and the vacuole vanes at the chalazal end. The smaller synergid exhibits the similar changes in the organelle's polarization. At the extreme micropylar pole of the both synergids the wall is forming the structure known as filiform apparatus. From the filiform apparatus toward the base of the synergids the wall gradually thins. The ultrastructural and cytochemical study show the presence of many mitochondria and dictyosomes, extensive endoplasmatic reticulum, ribosomes and plastids with starch in the cytoplasm of both the cells. This indicates that the considered synergids are highly active cells. The smaller synergid degenerates during fertilization. The bigger synergid retains its physiologicall activity up to stage of globular embryo. At the further stage of embryo development the persistent synergid degenerates.

All the above-mentioned observation, namely, (i) the differentiation of synergids and (ii) their structure, as well as (iii) the long time of life of the persistent one, may suggest that both cells could play an important role in the nutrition of the embryo sac and the developing embryo.

Changes in the somatic cells structure within the seminal vesicles in the earthworm *Dendrobaena veneta* (Annelida, Clitellata) after cadmium intoxication

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Cadmium occurrence in habitat adversely affects reproductive processes, both oogenesis and spermatogenesis in an earthworm Dendrobaena veneta. Spermatogenesis is continued within seminal vesicles where the subsequent spermatogenetic stages are observed: clusters of primary and secondary spermatocytes, spermatids and spermatozoa. The inside of seminal vesicles is formed by somatic tissue not by celomic fluid. Different cell types can be identified in the somatic tissue: numerous elongated stroma cells with processes that vary in length and surround the clusters of germ cells and diverse coelomocytes – amebocytes, granulocytes and cells with bacterioid crystals (Siekierska and Majchrzyk, 2008). The aim of this study was to determine whether Cd present in soil may induce structural and functional changes in those cells and whether the changes were time- and dose dependent.

The ultrastructure of somatic cells and activity of acid phosphatase were tested in *D. veneta* earthworms exposed to cadmium at concentrations 10 and 50 mg Cd kg^{-1} of wet soil for 10 and 20 days and in the controls.

Light and electron microscopy studies revealed that cadmium in both concentrations caused distinct degenerative changes in germ and somatic cells within seminal vesicles. Those changes occurred after 10 days and became more serious after 20 days. In comparison to the controls after cadmium intoxication, the number of germ cells clusters decreased but the amount of somatic tissue cells increased. In stroma cells cytoplasm the number of lysosomes augmented. In amebocytes and granulocytes cytoplasm the number of lysosomes, enlarged endoplasmic reticulum cisternae and dense vesicles increased. Both, in stroma cells and coelomocytes the activity of acid phosphatase increased. The role of coelomocytes in the intact animals is to eliminate degenerated cytophores and irregularly structured spermatozoa.

In earthworms living in cadmium contaminated soil these cells probably perform protective role to germ cells. It may indicate that after cadmium intoxication some spermatids and spermatozoa were regularly structured (Brzozowa, 2008).

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Differentiation of follicular cells in polytrophic ovaries of Pieridae and Nymphalidae (Insecta: Lepidoptera)

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Polytrophic ovaries of *Pieris napi* (Pieridae) and *Melitaea athalia* (Nymphalidae) are paired and consist of 4 elongated ovarian tubes (ovarioles). Three distinct regions can be recognized along the anterior-posterior axis of each ovariole i.e. terminal filament, germarium and vitellarium. Germarium contains gonial cells, germ cell clusters and undifferentiated somatic prefollicular cells. Vitellarium, a major part of the ovariole, is a chain of several dozens of egg chambers in progressively advanced stages of oogenesis (from early previtellogenesis to advanced choriogenesis). Each egg chamber is composed of a cluster of interconnected germ cells enveloped by a simple epithelium of somatic follicular cells (FCs). Germ cells within the egg chamber are diversified into a posteriorly located oocyte (OO) and 7 polyploid nurse cells (NCs). In the early previtellogenic egg chambers most of the FCs cover the germ cells laterally, while only a few can be found internally at the border of the neighboring egg chambers. In the subsequent stages of egg chamber development the FCs become divided into a few distinct subgroups. The FCs that associate with the lateral surface of the OO are initially cuboidal but soon become columnar (main body FCs). Centripetal FCs can be found at the OO/NC interface, while yet another group of FCs become squamous as they stretch over the surface of the NCs. The number of FCs progressively increases. The FCs located at the border of the adjacent egg chambers form prominent tubular stalks. In the advanced previtellogenesis/vitellogenesis the oocyte becomes entirely invested by the main body and centripetal FCs. Initially main body FCs closely associate in a hexagonal fashion. With the vitellogenic growth of the oocyte they undergo coordinated shape changes and rearrangements to form the longitudinal rows over the surface of the oocyte. These rearrangements advance in anterior-posterior direction to a different extent depending on the species. In *Melitaea athalia* rows of main body FCs cover only the anterior half of the oocyte while in *Pieris napi* they expand over the whole oocyte surface. In the late phases of oogenesis the NCs degenerate, while some of the most centrally located centripetal FCs form characteristic petal-like rosette assemblage. These cells contribute to the formation of a micropyle.

Ultrastructure, distribution and transovarial transmission of endosymbiotic microorganisms in aphids (Insecta, Hemiptera, Aphidinea)

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Aphids, like other plant sap-sucking hemipterans, commonly harbor mutualistic endosymbiotic microorganisms (Buchner, 1965). Endosymbionts live inside large cells termed bacteriocytes which are located in the close neighborhood of ovaries. Usually, bacteriocytes are grouped into huge organs called bacteriomes. Bacteriocytes may contain primary (P) or secondary (S) endosymbionts. P-endosymbionts are nessesary for the survival of the aphid (i.e. for their growth and reproduction). The presence of P-endosymbionts in the aphid body is connected with the restricted diet which is rich in carbohydrates but deficient in amino acids. Numerous studies (e.g. Douglas, 1998) have shown that P-endosymbionts are responsible for synthesis of missing substances. In the body of advanced aphids (families Aphididae, Drepanosiphidae, Thelaxidae, Anoeciidae, Lachnidae, Pemphigidae, Mindaridae) commonly occur bacteria belonging to the species *Buchnera aphdicola*. Besides *Buchnera aphidicola* aphids harbor one or two species of S-endosymbionts. Function of S-endosymbionts remains still unclear. In contrast to advanced aphids, members of the primitive family Adelgidae harbor morphologically distinct rod-shaped bacteria of unknown systematic position (Szklarzewicz et al., 2000). In the family Phylloxeridae endosymbiotic microorganisms do not occur (Szklarzewicz et al., 2009). The endosymbionts are transmitted from one aphid generation to the next transovarially. The beginning of the migration of bacteria is correlated with the development of germ cells. In oviparous generation bacteria invade choriogenic oocytes. The endosymbionts migrate between neighboring follicular cells or transverse their cytoplasm. Then they enter the perivitelline space and gather in the cytoplasm of the posterior pole of the oocyte. The egg envelopes covering oocytes posterior pole are produced when the migration of endosymbionts is completed. In viviparous generations endosymbionts infest young embryos.

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Do embryological characters could help in hybrid form identification in *Viola* L.?

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Family Violaceae is reach in metalliferous species occurring exclusively on contaminated with heavy metals sites and taxa forming metalliferous and non-metalliferous populations. In Southern Poland where mining (Zn/Pb) activity is still on, heaps are colonized by *V. tricolor* (Melanium section) and *Viola reichenbachiana* and *Viola riviniana* (Viola Section). Abiotic factors such as extreme edaphic conditions inhabited by metalliferous plants can act as strong selective pressure that may induce genetic variability or even plant speciation (Bone and Farres, 2001). There is also a theory that disturbed sites are colonized by hybrids rather than by parental species as hybrids are better suited to a new formed micronisches than parental species (Neuffer et al., 1999).

In the present studies selected populations of *V. riviniana*, *V. reichenbachiana* and individuals exhibiting intermediate characters between two species (perhaps putative hybrids) from Southern Poland were analyzed. Approximately 30 individuals from three contaminated (elevated concentration of Zn, Pb, Cd) (Chrzanów Warpie, Chrzanów Borowiec, Chrzanów Sośnica) and two non-contaminated populations (Skała Kmity and Modlnica near Kraków) were collected for morphological character analysis, meiosis type (regular, disturbed), pollen viability and heteromorphism, female gametophyte, embryo and endosperm development. As morphological analysis based on 31 quantitative and 26 qualitative characters did not allow to recognize hybrids due to low morphological differences between parental species, other characters were analyzed. The most informative were pollen heteromorphism and pollen diameter but not pollen viability. Differences in pollen size found in analyzed plants from contaminated sites Chrzanów Borowiec and Chrzanów Sośnica, ranged from 40 to76 μm and 27–38 μm, respectively indicated (non-directly) that meiosis was disturbed. This was supported by direct analysis of microsporogenesis. Disturbed meiosis, leading to formation of non-balanced microspores confirms hybrid origin of analyzed specimens. Further embryological studies and molecular analyses are needed to support hybrid origin of individuals colonizing contaminated sites.

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Ultrastructure of *Alisma plantago-aquatica* L. nectaries

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Nectaries are specialized tissues that secrete a sugary solution involved in interactions with animals. There are various types of nectary, situated anywhere in the flower and in widely different parts of plants, with different origins and types of organization. In *Alisma plantago-aquatica*, where the ultrastructure of the nectary was studied when flowers where ready to pollination, the carpels itself are involved in the secretion process *via* epidermal secreting cells. Nectar-secreting structures located in the epidermis are relatively common in flowering plants. There are several ways in which nectar can be exuded through epidermal cells. In *A. plantago-aquatica* the outer cell walls have large ingrowths like transfer cells that facilitate secretion. The nectar exudes from cells of secretory epithelium and accumulates in the subcuticular space formed by separation of the cuticle from the epidermis; later the cuticle is probably cracking and nectar may forms continuous layer on the nectary surface. On the top of the exuding, when flower is ready to pollination, nectar forms drops at the bases of the sides of the carpels. In some places the cuticle is covered by electron-dense substance with unknown origin and functions. Beneath the epidermis there is a tissue composed of a few layers of more vacuolated cells which perform an auxiliary functions. The ultrastructure of epidermal secreting cells is different in comparison with these subsidiary cells. Cytoplasm of the secretory cells is much denser and less vacuolated; furthermore it contains numerous mitochondria, rough endoplasmic reticulum and large quantity of dictyosomes, which are probably involved in nectar production and transport. Plastids have no starch grains; probably to produce excretion epidermic cells use sugars from subepidermal cells, which provide substrates through a large number of the plasmodesmata. Moreover, plastids of the secretory epithelium contain electron-dense bodies – probably aggregates of phytoferritin. Nuclei of the epithelium are situated near inner walls enlarged in comparison with deeper situated cells and probably polyploid.

Modifications for insemination in the male reproductive system in Gladulocaudinae and Stevardiinae (Teleostei: Characiformes: Characidae)

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During the evolution of teleost fishes lasted above 250 mln years in species belonging of unrelated taxa has arisen insemination, which offered protection of the gamete from the harmful environment, increasing the probability of fertilization, permition the temporal and spatial separation of mating and oviposition and allow for sperm competition Independent evolution of insemination is associated with different morphological modifications of fins or genital papilla functioning as the intromittent organs, of the testis structure connected with formation of sperm bundles, minimizing the lost of sperm during the transfer and sperm modification (elongation of the nucleus, enlargement of the midpiece and presence of accessory microtubules).

In about 60 species of Gladulocaudinae and Stevardiinae the testis is divided into spermatogenic part, producing successive generation of sperm cells, and aspermatogenic part, serving as sperm storage organ, containing free spermatozoa (*Corynopoma, Iotabrycon, Chrysobrycon*), spermatozoa aggregating into spermatozeugmata and formed two different types of packets (*Glandulocauda, Mimagoniates, Lophiobrycon*) or complete one-sided spermatozeugmata, formed during spermiogenesis (*Xenurobrycon*, *Tyttocharax*, *Scopaeocharax*) (Pecio and Rafiński, 1994; Pecio et al., 2005; 2007). The most common spermatozoa modification in all species is the elongation of the nucleus, laying laterally to flagellum, location of the centriols on the opposite pole to the enlarged mitochondrial region, presence of long (*Chrysobrycon, Corynopoma*) or reduced cytoplasmic sleeve and microtubules alongside the nucleus in species forming the spermatozeugmata (*Glandulocauda, Mimagoniates, Tyttocharax, Scopaeocharax*).

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Embryology of Lentibulariaceae in evolutionary point of view

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Lentibulariaceae is the largest family among carnivorous plants, which displaying not only unusual morphology and anatomy but also specific changes in the genome and ultra small genomes. Comparison of the morphology and detailed anatomy of *Pinguicula* ovules, female gametophytes, embryos and endosperm with the genera *Genlisea* and *Utricularia* may shed new light on the evolution of Lentibulariaceae.

We find that *Genlisea* ovules retain characters in common with *Pinguicula*: ovules with free funiculus, ES remaining in the ovule. These characters were inherited from a common ancestor. In ovules of subgenus *Genlisea* the micropyle tends to be closer to the funiculus and an unusual jacket-like nutritive tissue of integumental origin is formed.

The most specialized ovules in Lentibulariaceae evolved in the genus *Utricularia*; also this genus has the most specialized embryo. We present hypothesis how these embryos without organs may evolve, and why some of *Utricularia* possess viviparous embryos.

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"Curriculum vitae" of trophocytes during oogenesis in *Isohypsibius granulifer* (Tardigrada: Eutardigrada).

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The hermaphroditic gonad of *Isohypsibius granulifer* consists of three parts: germarium, vitellarium and the distal part filled with male germ cells (Węglarska, 1987). In this species, similar to other Eutardigrada (Węglarska, 1987; Poprawa, 2005), the oocyte is accompanied by trophocytes. During previtellogenesis, the cells localized in the vitellarium are connected with cytoplasmatic bridges and during early vitellogenesis they begin to synthesize reserve materials. It is difficult to say which cell will differentiate into oocyte until the beginning of a middle vitellogenesis. During this stage several cells grow intensively and become oocytes. The remaining cells fulfill a role of nurse cells (trophocytes). They synthesize reserve materials and transport them to the oocytes through cytoplasmatic bridges. Trophocytes are connected with oocytes till late choriogenesis. At the end of choriogenesis the cytoplasmatic bridges between oocytes and trophocytes disappear, while those between trophocytes are still observed. Trophocytes begin to degenerate in an apoptotic manner: the cytoplasm shrinks and becomes electron dense, nucleus achieves a lobular shape, mitochondria undergo transformation, and eventually the entire cell proceeds fragmentation.

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Changes in extracellular matrix composition during the progamic phase in *Larix decidua* Mill.

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Role of extracellular matrix (ECM) components during interaction between male gametophyte and pistil in angiosperm plant has been studied for many years. The obtained results indicate that pollination induces changes in composition of ECM in the tissue of pistil, and components such as pectins and arabinogalactan proteins (AGPs) take part in the formation of optimal environment for pollen tube growth. So far this process has not yet been known in gymnosperm plants.

In *L. decidua* the place of pollination is stigmatic tip of the ovule, from which pollen grains are transfered to micopylar canal. The environment of the pollen tube growth in the direction of archegonium is ECM of nucellus.

The aim of this work was to investigate the localization of pectins and AGPs in the ovule of *Larix decidua* Mill. during progamic phase. Studies were carried out using immunofluorescence techniques. In the present research the pattern of distribution of lowly, highly and Ca^{2+}-associated pectins recognized respectively by JIM5, JIM7 and 2F4 and four carbohydrate epitopes of AGPs recognized by LM2, JIM4, JIM8 and JIM13 monoclonal antibodies was analyzed.

Before pollination in the stigmatic tip of the larch ovule, both lowly and highly esterified pectins were localized. Once pollen was on the stigmatic tip, in the place of its adhesion accumulation of Ca^{2+}-associated pectins recognized occurred. Presence of the pollen grains in the micropylar canal initiated there accumulation of Ca^{2+}-associated pectins and deesterification of pectins in walls of nucellus cells. When pollen grains were situated in the micropylar canal in cytoplasm of nucellus cells AGPs epitopes recognized by LM2, JIM8 and JIM13 were observed. During germination of pollen tubes changes in the composition of ECM of nucellus cells occur. Characteristic accumulation of both lowly esterified and Ca^{2+}-associated pectins was visible above the neck cells of the archegonium, i.e. in the place, which must be found by the pollen tube while walls of the nucellus cells were devoid of these molecules. At this stage of development LM2, JIM4, JIM8 and JIM13 epitopes have been observed in the extracellular matrix, what is probably a sign of the preparation of this tissue to interactions with the growing pollen tube.

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Apoptosis in the midgut epithelia of insects

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Insect midgut epithelium is composed mainly of the epithelial and the regenerative cells. The first type of cells is responsible for food digestion through the production and excretion of enzymes and the absorption of the digests. The regenerative cells replace epithelial cells when they are lost by degeneration or aging processes. Apoptosis and necrosis are processes which enable the persistence of homeostasis in multicellular organisms due to the regulation of the cell number. Thus both apoptosis and necrosis are responsible for cell elimination, however, they differ in their initiation, course and changes that they cause. Necrosis is an incidental and passive cell death caused by disruptive external factors, while apoptosis is recognized as an actively regulated physiological process, which enables removal of useless or unexploited cells (Guimarães and Linden, 2004).

We have analyzed and compared the course of apoptosis in the midgut epithelium of some primitive hexapodan species: *Filientomon takanawanum* (Protura), *Allacma fusca* (Collembola, Symphypleona), *Lepismachilis notata* and *Machilis hrabei* (Archaeognatha), *Atelura formicaria* (Zygentoma, Ateluridae) and *Nicoletia phytophila* (Zygentoma, Nicoletiidae). The apoptosis might affect individual midgut cells or their entire groups. The density of cytoplasm changes, mitochondria transform, nucleus takes a lobular shape and eventually is fragmented. The apoptotic cell is discharged into the midgut lumen where it is digested. The apoptosis differs somewhat between species mentioned above (Rost-Roszkowska, 2008; Rost-Roszkowska et al., 2010a; 2010b).

The transitions of degenerating cells have been described at the ultrastructural level. Immunostaining methods were conducted in order to indicate the early stages of apoptosis.

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Egg capsules organization and choriogenesis in the euholognathan stoneflies *Nemoura cinerea* (Nemouridae) and *Leuctra nigra* (Leuctridae)

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The order Plecoptera (stoneflies) consists of two suborders: Arctoperlaria and Antarctoperlaria, inhabiting respectively northern and southern hemisphere. Arctoperlaria is subdivided into two groups: Euholognatha (studied in this paper) and Systellognatha (Zwick, 2000).

The structure of egg capsules (eggshells) of Systellognatha is known (hard, thick, multilayered and regionally differentiated; Rościszewska, 2003). The results of the present paper confirm our preliminary studies (Rościszewska, 1996; Poprawa et al., 2002) and reveal that euholognathan egg capsules are soft, thin, simple organized and with poor regional differentiation.

The data obtained on choriogenesis in the ovary of the studied insects point out to some activity of an oocyte in eggshell secretion, i.e. both primary and secondary eggshells occur. This primary feature supports the notation that Plecoptera represent the most primitive insects among Neoptera.

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How the epithelium on the tongue of the domestic goose can change during embryonic development? LM, SEM and TEM study

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The aim of the research was to describe the changes in the ultrastructure of the epithelium on the body and lingual prominence of the tongue in the domestic goose from 9th to 26th day of incubation.

Our study indicated that morphological changes of the epithelium on the goose tongue occur in two different ways. The first characterized the epithelium on the body and the second the epithelium on the lingual prominence. Generally, during the development of the lingual epithelium we distinguished three stages: embryonic, transformation and adult epithelium.

The embryonic stages of the epithelium of the body last from 9th to 18th day of incubation. The epithelium consists of few cell layers with flat superficial cells. There is no sign of the presence of the specific cell junctions but cell membranes of neighboring cells are placed in a close proximity to each other. On the lingual prominence this stage lasts longer until 22nd day of incubation. On 9th day the epithelium is multilayered with rounded superficial cells and at 16th day specific structure called "furrows" appeared in the epithelium.

The transformation stage starts at 19th/20th day on the body of the tongue. We can distinguish three layers in the epithelium: basal, intermediate and superficial. The keratins bundles and desmosomes develop in the intermediate layer. The important traits of the epithelium on the body is occurrence of giant granules called "periderm granules" in the superficial layer from 20th to 23rd day of incubation. The reorganization of the lingual prominence epithelium starts later at 23rd/24th day when furrows disappeared and three layers developed in the epithelium.

The third stage – the adult epithelium starts at the 23rd/24th day on the body and at the 25th/26th day on the lingual prominence. In this stage "the periderm granules" of the body epithelium disappeared and the epithelium underwent parakeratinzed processes. This process has not been observed on the lingual prominence.

Proceeded study revealed significant morphological differences in the epithelium of the body and lingual prominence during incubation and provided determination time tables for three stages of the epithelium morphogenesis in the tongue of domestic goose.

Ovary structure and development in primitive scale insects, *Puto albicans* (Coccinea: Putoidae) and *Steatococcus* sp. (Coccinea: Monophlebidae)

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The female genital system of the last larval instar of *Steatococcus* sp. and *Puto albicans* consists of paired, spindle-shaped ovaries. The ovaries are connected to the lateral oviducts that join forming a common oviduct. The ovary interior is tightly packed with numerous (about 200) rosette-shaped clusters of cystocytes. The clusters of *Puto albicans* contain about 40 cystocytes, clusters of *Steatococcus* sp. – 8 cystocytes. At the end of the last larval instar, the clusters of cystocytes start to protrude from the ovary interior into the body cavity forming young, spherical ovarioles. The ovarioles are radially arranged around the anterior part of the lateral oviduct. The ovarioles are devoid of terminal filaments. During further development, the cystocytes differentiate into trophocytes (nurse cells) and oocytes. In ovarioles of *Steatococcus* sp. 7 trophocytes and single oocyte arise, in ovarioles of *Puto albicans* – from 23 to 43 trophocytes and 2 or 3 oocytes. Thus, the total number of germ cells per ovariole in *Puto albicans* is variable and larger than in *Steatococcus* sp. The trophocytes occupy the anterior region of the ovariole, the oocytes its basal part. During "larval-adult" transformation ovarioles undergo elongation and differentiation into tropharia (trophic chambers) and vitellaria. The tropharia of *Steatococcus* sp. comprise trophocytes, whereas in tropharia of *Puto albicans* apart from trophocytes 1 or 2 early previtellogenic oocytes (termed arrested oocytes) occur. The centre of the tropharium is occupied by a common cytoplasmic area (termed trophic core) which is connected both with trophocytes and oocytes. Trophocytes are connected with the trophic core via broad cytoplasmic processes, the oocytes by means of nutritive cords. The trophocyte nuclei are large, lobated with giant nucleoli. The trophocyte cytoplasm is filled with numerous ribosomes. In the vitellaria of both examined species single oocytes develop. The developing oocytes are encompassed by a single-layered follicular epithelium that does not undergo diversification into distinct subpopulations. The ovaries of both examined species are accompanied by large cells (termed bacteriocytes). The bacteriocyte cytoplasm is tightly packed with endosymbiotic microorganisms. The endosymbionts are transovarially transmitted from one generation to the next.

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

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The cytoskeleton plays a crucial role in many cellular processes, like cellular signaling, organelle motility and also subcellular compartmentation during plant growth and development. The presence and changes in the cytoskeleton formation during development of embryo-suspensor in *Gagea lutea* was studied by employing indirect immunofluorescence labelling of microtubules (Mt) and microfilaments (Mf) (Świerczyńska and Bohdanowicz, 2003; Bohdanowicz et al., 2005). The zygote divides once, giving rise to a smaller apical cell and a larger basal cell (BC). The basal cell undergoes no further division, becomes much enlarged, and forms the basal suspensor cell. As a result of endoreduplication the BC nucleus gradually grows to a considerable size, and attains a maximum ploidy level of 128C. The suspensor of this species is a convenient model to study tubulin and actin cytoskeleton of highly polyploidy plant cells. During the early phase of development of basal suspensor cell, the microtubules and microfilaments were found to localize from micropylar to chalazal apex of the cell. Accumulations of numerous Mt bundles around the nucleus were observed. These tubulin filaments were congregate near the nucleus envelope and numerous bundles of microtubules radiating from the nucleus surface. At this time actin filaments were formed a delicate network in the cortical cytoplasm of the BC. In the fully differentiated basal cell, the microtubules and microfilaments were formed a dens prominent network composed of numerous cross-linked filaments. In the distal region of the suspensor basal cell, a distinct tubulin and actin skeleton with numerous filaments were observed in the cytoplasmic layer adjacent to the wall, separating the BC from the first layer of the chalazal suspensor cells. At the late phase of BC development, when the embryo matures, the tubulin and actin network was disorganized. At all stages of basal suspensor cell differentiation in embryo-proper cells an abundant cortical network of actin and tubulin cytoskeleton was visualized.

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Changes of the structure of the lingual papillae of the tongue during the cat prenatal development

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The aim of the research was to describe the changes of the lingual papillae of the tongue in several stages of the cat embryos. Investigations were conducted on tongues between 30 and 60 day pc. Age of the fetuses was determined according to growth curve of the domestic cat (Evans and Sack, 1973). Tissues from apex, body and root of the tongue immediately after dissection were fixed in 10% neutral formalin. Paraplast sections were stained with topographic and histochemical methods. The part of samples was processed for SEM observations. All of the tongues were elongated with rounded apex. The length of tongues was from 5 mm to 1.9 cm. At about the 30^{th}/32^{th} day of gestation the dorsal surface of the apex of the tongue is relatively flat. However in anterior part of the body of the tongue primordia of the giant filiform papillae were visible. In several places on the apex of the tongue spherical thickened areas of cells of the epithelium were visible and probably from these structures primordia of the fungiform papillae will develop. In the posterior part of the tongue, near its root, 2–3 rounded thickening of the epithelium was found, which distribution was similar to the vallate papillae.

In embryos about 40^{th} day of gestation on the dorsal surface of the tongue the primordia of the lingual papillae are enlarged. The epithelium of the primordia is changed from embryonic to multilayered. From about 45^{th} day of gestation in the epithelium of the primordia of the giant filiform papillae and elongated papillae corneous layer was visible.

Also in this period, 45^{th} day p.c. primordia of the fungiform papillae on the apex and body of the tongue and circumvallate papillae on the root of the tongue have the taste buds.

On about $50/52^{th}$ day of gestation giant filiform papillae are well-developed and they resemble the structure of giant filiform papillae on the tongue of the adult cat. Up to the end of the developmental period, at about 60^{th} day p.c. all types of papillae of the tongue are already formed with exception of small filiform papillae on the apex of the tongue which still have the character of primordia.

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Differentiation of the gonads in teleost fish, round goby *Neogobius melanostomus* in Baltic Sea

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The goals of this study were (1) to describe the gonad structure of round goby at the size range from 20 to 180 mm, (2) to determine type of sex differentiation and (3) fish size when process of gonads' maturation is terminated. We examined 30 fish at 20–56 mm of total body length (TL) and body mass ranging from 67.6 to 1677.3 mg, and 5 fish of 91.3–178.9 mm TL and 10–47.3 g of body mass. Fish were caught during winter in 2006 and the period from June 2007 to February 2008 in Puck Bay (Poland). Gonads were preserved in Bouin's fixative, and followed with paraffin embedding and H+E staining for light microscopy analysis.

There were 23 females, 10 males and 2 unidentified juveniles among studied individuals. The ovary of the smallest female (21 mm TL) had oocytes in the first chromatin nucleolus phase. The gonad of the largest female (91.3 mm TL) had oocytes in two different phases, the first stage (chromatin nucleolus phase, perinucleous phase) and cortical alveolus stage. The male at 41 mm TL had testicular tubules with no lumen and with visible primary spermatogonia. The male at 178.9 mm TL had the seminal tubules filled with spermatogonia and spermatocytes in I and II stages. Light microscopy method did not allow to distinguish germinal crests or ridges in the smallest studied individual (TL=20 mm), which was exceptionally cut with the entire trunk.

The obtained preliminary results allow us to conclude that the type of the gonad development in round goby represents 'differentiated gonochorism', when ovarian and testicular differentiation proceeds directly from the undifferentiated gonads. However, to confirm our conclusions further studies of an earlier life stage (less than 20 mm TL) are needed. The histological results are consistent with morphological observations of the genital papilla. Our studies contradict results of previous ecological findings on the sex ratio in natural populations of round goby, suggesting that males outnumber females. However, more individuals should be studied with histological methods and such analyses are continued.

Ultrastructure of the female germline stem cells and their niches in earwigs

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The ovaries of investigated earwig species are paired and composed of 20 – 40 short ovarioles of the meroistic-polytrophic type. As in other insects, each ovariole has three easily recognizable elements: the anterior terminal filament, germarium and vitellarium. The terminal filaments are composed of small, flattened, disc-shaped somatic cells oriented perpendicularly to the long axis of an ovariole. The most posterior cell of the terminal filament (*i.e.* the basal cell of the terminal filament) is morphologically different from the remaining terminal filament cells. Interestingly, between the neighboring terminal filament cells aggregates of tiny vesicles are encountered. The diameters of these vesicles and their localization between adjacent plasma membranes suggest that they might be argosomes and/or exosomes – the vesicular structures implicated in signal transduction in many tissues (Greco et al., 2007). The most anterior part of the germarium is filled with the female germline stem cells (GSCs). The GSCs are surrounded by somatic escort cells. These flattened cells are equipped with long, thin processes that envelope individual germ cells and/or penetrate between them. The processes of escort cells are branched and frequently intertwine with each other. Some of these processes send out filiform extensions that morphologically resemble cytonemes – fragile, filopodial projections connecting neighboring cells and involved in intercellular communication and long-range signaling (Ramirez-Weber and Kornberg, 1999).

Above observations suggest that in earwig ovaries, like in many other tissues, the stem cells function in characteristic microenvironments known as niches that consist of somatic cells that control stem cell self-renewal, proliferation and differentiation (reviewed in Morrison and Spradling, 2008). It is shown here, that in earwigs, the GSC niche is structurally simple and consists of "canonical" terminal filament cells, single basal cell of the terminal filament and several uniform escort cells. The signaling between individual cells of the niche might be mediated by unusual organelles, like argosomes and cytonemes.

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The nests of synchronous oocytes in the ovaries of the North American paddlefish (*Polyodon spathula*) and the Russian sturgeon (*Acipenser gueldenstaedtii*) (Chondrostei, Acipenseriformes)

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The ovaries of the investigated species have been obtained from the specimens reared under artificial conditions. They contain the germinal epithelium. Within this epithelium the ovarian nests, the ovarian follicles, the adipose tissue and the blood vessels are encountered.

In the investigated specimens two types of nests have been found: (1) the nests of synchronous oocytes (in the sturgeon and paddlefish); (2) the nests of asynchronous oocytes (in the paddlefish).

These nests comprise numerous oocytes and somatic prefollicular cells. The prefollicular cells lie on a basement membrane. The external walls of the nests are built of thaecal cells and blood vessels. The thaecal cells are situated on a basement membrane. All the oocytes in the nests are in the prophase of the first meiotic division (diplotene) and represent earlier stages of previtellogenesis than oocytes at the pre-stage 1. In the asynchronous nests of the paddlefish the primordial germ cells are also encountered.

The oocytes in the synchronous nests are not interconnected by intercellular bridges. Their nuclei (germinal vesicles) contain karyosome and multiple nucleoli. In the germinal vesicles of older oocytes the Cajal bodies have also been noticed. The oocyte cytoplasm (ooplasm) contains: mitochondria with well-developed and distorted cristae, the vesicles and cisternae of endoplasmic reticulum, dictyosomes, lipid droplets, microtubules, microfilaments and nuage material. Three morphological types of nuage have been distinguished due to its structure: (1) a translucent body, (2) the aggregations of fine-granular material (located in the vicinity of the endoplasmic reticulum and dispersed in the ooplasm), (3) the mitochondrial cement (located in the close vicinity of the mitochondria) and sponge-like nuage accumulations (dispersed in the ooplasm).

Nuage (2) and (3) together with the other organelles found in the ooplasm constitute the Balbiani body that is located in the vicinity of the germinal vesicle. The function of the translucent body remains unclear. It is always spherical, located in the vicinity of the nuclear envelope and stays in the contact with the vesicles of the endoplasmic reticulum. The fluorescent staining of sections with a mixture of DAPI and propidium iodide have revealed that the translucent body does not contain nucleic acids.

POSTERS

Localization of N-cadherin during folliculogenesis in Balb/c mice

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Caffeine (1,3,7-trimethylxanthine) is naturally occurring compound in food. The main sources of caffeine are coffee, tea, soft drinks, cocoa and chocolate. There are also a lot of medications which contain caffeine (Nawrot and Feeley, 2003).

During folliculogenesis exists a bidirectional communication between oocytes and granulosa cells (GCs). Viability of GCs is essential for ovarian function (Albertini and Barret, 2003). Contact between GCs is due to the presence of adhesion-type junctions resulting from haemophilic binding of N-cadherin (Gumbiner, 2000).

The aim of this study was to characterize the morphological structure of the preantral follicles after applying caffeine. The changes in localization and expression of N-cadherin in adherens junctions between follicular cells were investigated.

For the study females of Balb/c mice were used. The mouse ovaries were obtained from 35 animals. Adult female mice were divided into the three groups which were injected subcutaneously three days a week for four weeks with caffeine solutions: 100 mg/kg, 150 mg/kg, 300 mg/kg. The mice from control group were injected with 0.9% saline solution alone. Ovaries were collected and prepared for light microscope examination and for immunohistochemical studies. Histochemical localization of N-cadherin in follicular cells was performed by using ABC method. In morphometric analysis ImageJ v. 1.38d. software was used.

In the sections of follicular cells brown membranes of granulosa cells were seen. Immunopositive areas were localized in each experimental group and control group within the adhesion-type junctions. Additionally, reaction was seen between ovarian surface epithelial cells. There were no reaction in theca of preantral folliculi. Statistical important differences in ICH_{area} index were indicated in the first group. These observations determined the ability of N-cadherin to play an anti-apoptotic function as Makrigiannakis and Coutifaris (1999) were first suggested. Additionally lower doses of caffeine may be more impairment then highest.

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Apical cell in arhynchobdellid leech ovaries. An ultrastructural study

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In arhynchobdellid leeches (Hirudiniformes and Erpobdelliformes) inside the ovisacs germ-line cysts together with somatic cells form structures termed ovary cords. The ovary cords are long and convoluted (hirudiniform leeches) or short and rod-shaped (erpobdelliform leeches), and in each case they are polarized. One end of the cord (club-like in hirudiniform or conical in erpobdelliform leeches) contains one, huge somatic cell termed apical cell, which is in direct contact with oogonia and cell groups forming germ-line cysts.

Apical cell was found and described at the ultrastructural level in all studied so far arhynchobdellid leeches i.e. *Hirudo medicinalis, H. troctina, Haemopis sanguisuga, Limnatis nilotica* (Hirudiniformes) and *Erpobdella octoculata* (Erpobdelliformes). This cell bears several characteristic features: (1) it is the largest cell in the apical part of the ovary cord; (2) it has many long cytoplasmic projections penetrating the space between neighboring cells; (3) its nucleus has a layer of well developed nuclear lamina; (4) its cytoplasm is loaded with a huge amount of mitochondria, small vesicles of ER, Golgi complexes and electron-dense membrane-bound vacuoles – those organelles are distributed mainly within the perinuclear cytoplasm; (5) a thick layer of peripheral cytoplasm is loaded with numerous bundles of cytoskeletal elements, interpreted as intermediate filaments; (6) it is connected to neighboring cells via hemidesmosomes. We suggest that the apical cell may form a niche for maintaining the germ-line stem cells.

Comparative study on the biology of *Aricia agestis* and *Aricia artaxerxes* (Lepidoptera)

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Life history of *Aricia agestis* (Den. and Schiff) and *Aricia artaxerxes* (F.) is poorly known. Distribution area of *Aricia artaxerxes* in Europe is dispersed and several subspecies are recognized (Buszko and Masłowski, 2008). The species is univoltine and its larval host plants are *Geranium sanguineum* L. and *Helianthemum nummularium* L. The adults visit flowers of *Lotus corniculatus* L., *Achillea millafolium* L. and *G. sanguineum* L. Hibernation takes place in L2 larval instar.

Aricia agestis occurs almost everywhere in Europe except the northern areas (Great Britain, Ireland and Fennoscandia) (Lafranchis, 2007). It develops up to three generations a year. The larvae feed on *G. sanguineum* L. and *G. pusillum* L., *G. pratense* L., *Erodium cicutarium* L. and *H. nummularium*. The adults often visit flowers of *L. corniculatus* L., *Berteroa incana* L. and *Dianthus carthusianorum* L. Like in case of *Aricia artaxerxes* the hibernation is attributed to second larval instar (L2).

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Apoptosis in the midgut epithelium of *Scolopendra cingulata* (Myriapoda, Chilopoda)

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Apoptosis has been described as a wide-spread phenomenon in insects midgut epithelium (Park et al., 2009; Rost-Roszkowska et al., 2010), while it has not been described for Myriapoda.

Midgut epithelium of *Scolopendra cingulata* (Myriapoda, Chilopoda) is composed of three types of cells: columnar digestive cells, endocrine cells with numerous electron dense granules and small regenerative cells. In several digestive cells the autophagy has been observed. Cisterns of RER surround organelles and enclose them in autophagosomes. Eventually autolysosomes and residual bodies are formed. When many autophagosomes and autolysosomes are present in the cytoplasm, apoptosis in activated. The cytoplasm becomes electron dense due to intensive shrinkage of apoptotic cell. The nucleus achieves a lobular shape and proceeds fragmentation. Apoptotic cell gradually loses contact with adjacent epithelial cells and with the basal lamina. Eventually it is discharged into the midgut lumen when it is disintegrated.

The process of apoptosis has been described with the use of transmission electron microscope. Immunostaining methods were conducted in order to indicate the presence of caspase 3, which is one of the key effectors of apoptosis (anti-caspase 3 antibody), and to detect DNA fragmentation that results from apoptotic signaling cascades (TUNEL assay).

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Localization of arabinogalactan proteins during development of gametophytes in *Bellis perennis* L.

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Arabinogalactan proteins (AGPs) are a class of highly glycosylated plant proteoglycans localized mainly on the surface of the plasma membrane and in the extracellular matrix. Expression of particular AGPs epitopes is spatially and temporally regulated in developmental processes.

Immunolocalization of AGPs epitopes recognized by JIM 13, JIM 15 and MAC 207 monoclonal antibodies (mAbs) was performed in developing male and female generative organs of *Bellis perennis*. The monoclonal antibodies were obtained from Complex Carbohydrate Research Center at The University of Georgia, USA. Secondary anti-rat FITC- conjugated antibodie was used and results of immunolabelling were observed under a fluorescence microscope.

AGPs epitopes recognized by JIM 13 and JIM 15 mAbs are specific for both male and female generative cells, while those recognized by MAC 207 Mab are spread in different somatic tissues. During anther development at the pre-meiotic stage, the epitopes recognized by JIM 13 and JIM 15 were undetectable. Both kinds of epitopes appeared first in the thin cell walls surrounding microsporocytes and lasted till the end of meiosis. They were not visible in a newly formed cell walls during cytokinesis. The epitopes recognized by JIM 13 were additionally detectable in the protoplasts of tapetal cells, microsporocytes and microspores. After meiosis, the epitopes were no longer present in anthers. The epitope recognized by MAC 207 mAb in pre-meiotic anthers and during meiosis randomly scattered in different somatic cells, but after cytokinesis they became visible in the protoplasts of immature pollen and were still present in mature pollen grains.

At early stages of ovule development, the epitopes recognized by JIM 13 and JIM 15 were undetectable. They appeared at the end of meiosis in the cell walls of tetrads of megaspores and were markedly visible in the cell walls of the functional megaspore and the embryo sac. In mature ovules, the epitopes were distributed in the cell walls of the embryo sac, egg apparatus, antipodal cells and in the cells of the inner integument. The MAC 207-recognized epitope was uniformly distributed in the somatic cells of the ovules, but was undetectable in the female gametophyte.

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Analysis of pollen tubes growth after self- and cross-pollination in four cultivars of *Lachenalia*

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Lachenalia Jacq. f. ex Murray, belonging to the Hyacinthaceae family, is a bulbous plant endemic to South Africa and Namibia (Kleynhans, 2007). As a pot plant, it is a relatively new product on the international flower market. The aim of the present study was to examine the level of incompatibility after self- and cross-pollination using four cultivars of *Lachenalia*, i.e. 'Ronina', 'Namakwa', 'Rupert' and 'Rosabeth', differing in the time of blooming, flower colour, and leaf morphology. Plants were hand pollinated three days after anthesis. Cross-pollinated flowers were emasculated one or two days before dehiscence. Five plants were self-pollinated per cultivar. Cross-pollination of each cultivar was performed with pollen of two other cultivars (five plants were pollinated per combination). Pollen tubes were stained with aniline blue and their growth was analyzed under fluorescence microscope. Numerous germinating pollen tubes were observed, both on self- and cross-pollinated pistils, for the three of four cultivars. Two days after pollination some pollen tubes reached the style, while others grew up to the ovary. Abnormalities in the pollen tube development were observed in both types of pollination, including swollen and winding tubes, thickened pollen tube tips, and extensive callose deposition in plugs. It was a likely reason for the poor seed yield. Presented data can facilitate breeding programs of new *Lachenalia* varieties.

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Larval stages of *Cacopsylla melanoneura* (Förster, 1848) (Hemiptera: Psylloidea)

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Jumping plant- lice or psyllids (Psylloidea) comprise a group of around 2500 species of small plant-sap-feeding insects allied to the aphids and whiteflies.

The psyllid life cycle typically comprises of an egg state, five larval instars and a sexually reproducing adult stage, with males and females usually showing only moderate deviation from a 1:1 sex ratio at emergence. The immature stages exhibit both morphological and behavioral adaptations to resist desiccation, and the life-cycle is often highly synchronized with host- plant phenology. Furthermore, closely related species usually occur on closely related host-plants. The larval morphology reflects, to a certain extent, the biology: free- living larva tend to be elongate with long limbs, pit-gall inducers are oval or circular and flattened dorsally, and closed-gall inhabitants are weakly sclerotized and "inflated". Generally, three types of nymphs were formerly recognized: psylline-type – does not have the forewing-pad produced anterior into a prominent humeral lobe and the apical extremity projects prominently from the contour of the body; triozine-type – the anteriorly produced humeral lobe to the forewing-pad is present and the margins of the pads are confluent with the body outline; and pauropsylline-type (aphalarine-type) – humeral lobe is present but not produced anterior and the forewing-pad is parallel to the body contour.

Cacopsylla melanoneura (Förster, 1848) is a small psyllids which feeds exclusively on *Crataegus oxyacantha*. Nymphs are free-living, psylline-type. The last instar larva of *C. melanoneura* has dorsal surface of a body and wing-pads with simple setae, which are unique characters and specific shape of circumanal pore rings. *C. melanoneura* is univoltine and this is one of very few species of psyllids overwinter as adults. They disperse onto conifers and move back onto *Crataegus oxyacantha* to mate and oviposit in the spring, usually prior to bud burst. It is not known whether overwintering adults feed on shelter plants, though a consideration on their moisture requirements would suggest they do. Hatching of eggs in the spring occurs at or about bud burst and nymphs move directly onto the flush of new foliage.

Development of the digestive tract in stage III larvae of *Contracaecum rudolphii* Hartwich, 1964 (Nematoda: Anisakidae)

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The structure of the digestive tract of the genus Contracaecum is a highly important in taxonomy. In adult individuals of *Contracaecum rudolphii* Hartwich, 1964, the ratio of the length of anterior intestinal caecum to the length of the posterior ventricular appendix accounts for 3:1 (Baruš et al., 1978). Rudiments of both outgrowths are visible as early as in the first larval stage. In the second stage, the ventricular appendix is already clearly visible in larvae hatching from egg thecas, whereas the presumptive intestinal caecum may be distinguished in the form of a few cells (Bartlett, 1996).

The objective of this study was to conduct observations of the development of the digestive system in the third stage larvae.

The larvae were isolated from experimentally-infected goldfish (*Carassius auratus*). Standard parasitological autopsies of the fish were carried out each week. Organs which the larvae were detected in were digested with a 1% solution of pepsin (pH 2). Measurements and photos of the larvae were taken under an Olympus microscope with the aid of a computer software for image analysis Multiscan v.4.2.

In the larvae isolated in the first two weeks of the study, particular elements of the gastrointestinal tract (esophagus, stomach, and intestine) and ventricular appendix were clearly visible. In contrast, the intestinal caecum was poorly distinguished. In the larvae isolated after three weeks, the length of the intestinal caecum reached 40, and that of the ventricular appendix reached 142.56 m. After 5 weeks, the respective values accounted for 125.21 and 261.42 m. In the larvae isolated in successive weeks, the length of the intestinal caecum was observed to increase considerably faster than that of the ventricular appendix.

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Reactive Oxygen Species (ROS) and mitochondrial superoxide dismutase (Mn SOD) in germ and somatic cells of the ovary in the earthworm *Dendrobaena veneta* (Annelida, Clitellata)

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The line of germ cells is said to be potentially immortal otherwise than somatic cells which build tissues, fulfill their destiny and die. We don't know much about mechanisms that enable germ line to avoid ageing.

According to free radical theory of ageing (Harman, 1956) oxidative stress is considered as a major factor in the ageing process. Mitochondria responsible for providing energy also generate most of the reactive oxygen species present in cells. The number of mitochondria increases rapidly in germ cells during oogenesis but there is no literature data whether this increase is related to ROS accumulation. There is also no knowledge about the level of protection from ROS in germ cells during oogenesis.

The first aim of this study was to examine whether free radicals accumulate in female germ cells at subsequent stages of oogenesis in comparison with somatic cells of the ovary in the earthworm. The second goal was to verify antiROS protection in germ and somatic cells of examined ovary (detecting mitochondrial superoxide dismutase). The most explicit presence of ROS was noted in somatic cells surrounding the biggest oocyte at the end of ovary while there was the lowest level of Mn SOD. The weakest signal from free radicals was in vitellogenic oocytes which had the greatest level of mitochondrial SOD. Trophocytes had average levels of ROS and Mn SOD. Among prooocytes there were cells with stronger and weaker signals from free radicals with no such difference in low SOD activity.

Obtained results revealed that somatic cells were the most exposed for degradation from free radicals. Vitellogenic oocytes exhibited the most advanced protection from cytotoxic active oxygen, and were producing a great amount of protective enzyme. There was no proof that trophocytes help protect oocytes from ROS accumulation. It seemed possible that different signals from prooocytes were the effect of their advance in differentiation into trophocytes and oocytes.

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Stereological study of germ and somatic cells of the ovary in the earthworm *Dendrobaena veneta* (Annelida, Clitellata)

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The structure of the ovary and the process of oogenesis in *Dendrobaena veneta* (Annelida, Clitellata) was already studied and completed (Siekierska, 2003). Germ cells observed in the ovary of the earthworm showed a great diversity in the size, the number, the structure and the spatial arrangement of cell organelles at subsequent stages of oogenesis.

These study was to complete previous research concerning mitochondrial activity in germ and somatic line of the ovary of *D. veneta* (Faron and Bernaś, 2008), which revealed decrease of mitochondrial activity during oogenesis.

Stereological analysis of ultrastructure of germ line (oogonia, prooocytes, trophocytes, vitellogenic oocytes) and also somatic cells of the earthworm's ovary was made to verify differences in organelles' volume density in particular stages of oogenesis and in these two cell lines. The results were analyzed using Kruscal- Wallis non- parametric ANOVA test and considered significant at $p<0,05$. Present study confirmed that relative volume of mitochondria in germ line was higher than in somatic cells of the ovary and that there was considerable increase in mitochondria volume density during oogenesis, beginning from oogonia, prooocytes, trophocytes until vitellogenic oocytes. In oogona volume density of mitochondria grew over twice and in prooocytes three times in comparison with somatic cells. The value for trophocytes was two times higher and for vitellogenic oocytes three times higher than the one for oogonia.

There was no correlation between increase of volume density of mitochondria and higher metabolic rate (increase in activity of mitochondria) in germ cells during oogenesis in the earthworm's ovary.

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Organization of ovaries in *Branchiobdella pentodonta* and *B. parasitica* (Clitellata, Branchiobdellidae) is similar to ovary cords found in true leeches (Clitellata, Euhirudinea)

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Branchiobdellidans are small (usually less than 1cm long) ectoparasites or ectocomensals on freshwater crayfish. According to recent molecular studies they share a common ancestor with true leeches (Euchirudinea) (Siddal et al., 2001). The ovary organization and the course of oogenesis in representatives of this group is poorly known, only light microscopy studies based on paraffin sections were done (D'Angelo, 1965) and using TEM only some details of vitellogenesis were described (Bondi and Facchini, 1972).

From the studies on several families of true leeches (Świątek, 2008) we know that in these animals during early oogenesis germ-line cysts develop. The architecture of such cysts is very characteristic: each germ cell in a cyst has only one cytoplasmic bridge connecting it to a central cytoplasmic mass, a cytophore. Our results show that the germ-line cyst of the same architecture as in true leeches are formed during early oogenesis in two studied branchiobdellids. Moreover, the structure of the ovaries in the species studied resembles the ovary cords known form ovaries of true leeches. The obtained results suggest that the common ancestor of Branchiobdellidae and Euhirudinea also had cord-like ovaries containing germ-cell cysts.

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Karyotype structure and chromatin arrangement in the bivalent-forming *Rhoeo spathacea* var. *concolor*

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Cytogenetic study was performed to reveal the chromosome and nuclear structure in *Rhoeo spathacea* var. *concolor* (2n=12). Cytological examinations uncovered the regular formation of six bivalents and a high percentage of fertile pollen (ca. 90%). Chromosome arm measurements and fluorescence *in situ* hybridization (FISH) revealed that one of the Renner complexes – β complex (chromosomes: 2, 4, 6, 8, 10, 12) was in double and it did not differ markedly from the β complex present in the ring forming *Rhoeo* (Golczyk et al., 2005). It concerned also interstitially located 5S rDNA loci on chromosome arms 8B and 4b. The latter may indicate that the *concolor* variety is a segregant derived from permanent translocation heterozygote (PTH) due to an occasional breaking down of the genetic system eliminating homozygotes. This may be corroborated by the sporadic occurrence of the *concolor* variety in nature and its specific course of meiotic prophase. It was found here that bivalent-forming *Rhoeo* shared essentially the same untypical meiotic scheme with its PTH relatives. From leptotene till pachytene pericentromeres aggregated to form one or several collective chromocenters. The latter prevailed also in all the analyzed somatic tissues, thus being a standard cytological condition. This in turn suggests that the direct link between ring formation and centromere aggregation postulated by some authors (Coleman 1941) does not hold true in *Rhoeo*. The obtained results were discussed in the light of the existing data on PTH organisms and their close bivalent-forming relatives (Golczyk et al., 2008; Rauwolf et al., 2008).

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Interstitial telomeric sequences in *Rhoeo spathacea*. Implications for the evolution of permanent translocation heterozygosity

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A preliminary, FISH-aimed cytogenetic study was performed in the two ring-forming *Rhoeo* varieties: *Rhoeo spathacea* and its variegated form – *R. spathacea* var. *variegata*. A new technique – ND-FISH (nondenaturating fluorescent *in situ* hybridization) described by us recently (Cuadrado et al., 2009) and chromosome identification system developed by Golczyk et al. (2005) were used to investigate the chromosomal organization of interstitial telomeric DNA (TTTAGGG) clusters in mitosis and meiosis. The new technique gave more intense and sharper hybridization fluorescence than conventional FISH and proved to be fully reliable in detecting telomeric DNAs. Based on detailed mapping of interstitial telomeric clusters and their behaviour during meiotic prophase, we make a suggestion that extensive inversions may have preceded translocations and influenced recombination. The possible meaning of our findings for a general view on the evolution of permanent translocation heterozygosity and its fundamental features (for a review see Rauwolf et al., 2008) was discussed.

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NADH-dependant diaphorase activity in single mitochondria of the sperm midpiece – gene mapping study

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NADH diaphorase (dehydrogenase) is an enzyme that belongs to the family of oxidoreductases. It is associated with complex 1 of mitochondrial electron transport chain within the inner mitochondrial membrane and catalyzes the transfer of electrons from NADH to coenzyme Q. This enzyme plays an essential role in mitochondria activity and its activity may be crucial for sperm quality parameters, especially motility.

We performed cytochemical test to evaluate oxidoreductive capability of the sperm mitochondria NADH-dependant diaphorase. During NADH-dependent NBT assay, exogenous NADH is used as a donor of hydrogen, which is translocated by active NADH-dependent dehydrogenase (diaphorase) to the artificial acceptor, NBT (nitro blue tetrazolium; yellow). This leads to the production of reduced tetrazolium salt deposits (diformazan; dark blue). After the NBT assay was conducted in male mice sperm suspension, smears were analyzed for homogenity of the cytochemical reaction. Homogenous, intensive reaction along the sperm midipiece proves equal activity of the NADH-depentant diaphorase in the mitochondria. In case of uneven cytochemical reaction we can assume that in some mitochondria NADH-depentant diaphorase activity is disrupted or incorrect.

Results obtained for the two inbred strains of mice, KE and CBA/Kw, and for 10 recombinant inbred (RI) strains allowed us to map genes controlling diaphorase activity in single mitochondria of the sperm midpiece. Our study showed that diaphorase NADH-dependant activity is regulated by the genes located on the chromosome 11 and 19. Chromosomal region 11q20-11q24 contains three genes (*Adam19, Vdac1* and *Olfr*), and region 19q33 contains four genes (*Uhrf2, Papss2, Atad1* and *Ch25h*) that we postulate to be candidate genes for controlling diaphorase activity in single sperm mitochondria.

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Ultrastructure and immunocytochemistry of dimorphic sperm cells in pollen tubes of *Gagea lutea* L. (Ker-Gawl)

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Dimorphic sperm cells (SCs) and their role in double fertilization is still a great deal of interest. Only one ultrastructural study of *Plumbago zeylanica* (Russel, 1985) showed clearly preferential character of this process. *Gagea lutea* is a proper model plant to such observations, divided SCs show unique difference in size. Microfilaments and microtubules play a crucial role in growth of pollen tube and movement of cells and cytoplasm, while γ-tubulin is responsible for acentrosomal nucleation and organizes microtubule arrays (Wasteneys and Yang, 2004). The aim of observations was to reveal ultrastructure of male germ unit and organization of microtubules and actin filaments at its surface. Pollen tubes of *Gagea lutea* were grown *in vitro* on Read's medium (according to Zhang et al., 1995), first divisions of generative cells (GC) were observed after 8 hours of culture. The ultrastructure of SCs and their association with the vegetative cell (VC) nucleus in pollen tube were observed with electron microscope. The cytoplasm of both SCs included mitochondria, endoplasmic reticulum, dictyosomes, ribosomes and small vacuoles. No plastids were observed. The visualization of the cytoskeleton was conducted by indirect immunofluorescent microscopy. Localization of α- and γ-tubulin; and α-tubulin and F-actin were detected simultaneously using monoclonal antibodies. The observation concentrated on cytoskeleton at the surfaces of GC, VC and SCs. Thick bunches of F-actin were particularly observed near GC and SCs. Dense α-tubulin bundles were especially detected on the surfaces of GC and SCs, while γ-tubulin is mainly visualized on the surface of VC in elongating pollen tube. Additional staining was carried out using $DiOC_6(3)$ and Rhodamine 123 to show numerous mitochondrial nucleoids which are characteristic to GC and SCs and not observed in VC.

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The organization of cytoskeleton in root cells of *Pisum sativum* after protein kinase and protein phosphatase inhibitors treatments

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Plant cytoskeleton plays an active role in many intracellular processes connected to cell shape, movement of organelles and cell division. It consists of two major networks of protein polymers, microfilaments (MFs) and microtubules (MTs). During the cell cycle arrays of MFs and MTs undergo dramatic architectural changes, specifically at the entry into and exit from mitosis (Petrasek and Schwarzerova, 2009). To better understand the changes that occur in cytoskeleton during cell cycle, we studied the effects of protein kinase (staurosporine (ST), olomoucine (OM)) and protein phosphatase (NSC) inhibitors on cytoskeletal components in primary roots of *Pisum sativum*. The visualization of the cytoskeleton was conducted by indirect immunofluorescent microscopy using monoclonal tubulin and actin antibodies. Here, we present results indicating that application of protein kinase and phosphatase inhibitors induced considerably changes in the cytoskeleton. Treatment of cells with ST, OM, NSC and mixture of both inhibitors (ST/NSC; OM/NSC) resulted in morphological alterations and reorganization of actin filaments. Fluorescence labeling showed that staurosporine caused the depolymerization of the actin filaments, whereas microtubular cytoskeleton remained intact. The microfilaments disruption induced by ST was distinguishable from the actin reorganization induced by exposure to the OM, NSC and mixture OM/NSC, ST/NSC. Besides, our studies indicate that protein kinase and phosphatase inhibitors induced changes in the distribution of the microtubular cytoskeleton in all phases of the cell cycle. These results suggested that protein kinases and phosphatases participate in cytoskeleton remodeling in primary root cells of *Pisum sativum*.

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Embryonic development of gynogens of triploid *Cobitis* females (Teleostei, Cobitidae)

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The spined loach *Cobitis taenia* L. in Europe commonly appears in mixed diploid-poliploid populations which are dominated by allotriploid gynogenetic *Cobitis* females (Boroń, 2003). This dominance is connected with their unisexual reproduction (Saat, 1991), longer spawning period and bigger size of eggs in comparison with diploids (Juchno et al., 2007). Development of triploid eggs produced by triploid *Cobitis* females is stimulated by the sperm of *Cobitis* taxa or other related species (Saat, 1991).

Experimental crosses were made between five triploid females of *Cobitis* and males of the spined loach *C. taenia* and the crucian carp *C. carassius*. Spermatozoa were inactivated by UV irradiation. The number of chromosomes of triploids and gynogens was established by their karyotyping.

The eggs were spherical and transparent with a bright yellow yolk and no oil globule. First cleavage began after 1 hour and 30 minutes and then the next cleavages were appeared after c. 20 minutes. Gastrulating took place in 12 hours and hatching was observed 3 days after fertilization. Then in one day external gill filaments, pectoral fins and working heart were visible.

UV-irradiated spermatozoa of both species used in this study stimulated the gynogenetic development of oocytes of triploid *Cobitis* females. In total, 42% and 52% of eggs fertilized with spermatozoa of *C. taenia* and *C. carassius* respectively, were developed into hatchlings. However later, respectively 79% and 40% of them attained the age of 18-days.

Gynogens of *C. taenia* were characterized by a lower mortality and lower number of developmental abnormalities in comparison with those of the crucian carp.

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Micromorphological and histochemical studies of the flower structure of *Epipactis palustris* (L.) Crantz

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Epipactis palustris (L.) Crantz is relatively rare and endangered species in Poland (V endanger category in *Red List of Plants and Fungi in Poland*, 2006), but its flower structure is weakly examined. The lip consists of two movably joined parts. The basal part (hypochil) is concave with two lateral lobes. Midlobe (epichil) is arcuate, roundish, separated from the triangular lateral lobes with broad isthmus. Fleshy, vivid yellow to orange in front, white or pink at the back callus lies at the base of the midlobe.

The elongated hypochil cells are covered with wrinkled cuticle. Remnants of secretions and areas with obviously distended cuticle occur at the lip base. The adaxial surface of entire, glabrous lateral lobes is wrinkled, covered with parallel furrows. In the cross sections of lip callus and adjacent areas residues of protein-polysaccharide secretions are visible. The epichil cells are covered by wrinkled cuticle. The margins are strongly undulate and dentate, curved upward near the midlobe apex. There are no noticeable secretions on the epichil surface.

The dorsal sepal is broadly and distinctly keeled abaxially. On the abaxial surface, densely covered with bicellular elliptical and unbranched multicellular hairs, numerous stomata are visible. The adaxial surface is covered with wrinkled cells and sparse, bicellular, ellipsoidal hairs at the base. On the abaxial side of the lateral sepals, stomata and hairs, especially near the base and along the median nerve, are present. The stomata occur also adaxially. Cross sections of all tepals show raphides and starch grains inside some cells. The abaxial surface of tepals is covered with groups of cells, raised above epidermis surface, with stomata at the top of each group.

According to Szlachetko and Skakuj (1996) flowers of *E. palustris* lack nectar although other species of *Epipactis* Zinn. produce nectar. Secretions observed at the base of the lip and around lip callus may probably be traces of nectar. The role of structures observed at tepals is still unknown.

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Ovary structure in viviparous and oviparous generations of the aphid *Glyphina betulae* (Insecta, Hemiptera, Aphidinea: Thelaxidae)

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Glyphina betulae is a representative of the advanced aphid family, Thelaxidae. The ovaries of the viviparous generations of *G. betuale* are composed of five telotrophic ovarioles, whereas ovaries of oviparous generations contain four ovarioles. The individual ovariole is subdivided into four well-defined regions: a terminal filament, trophic chamber (tropharium), vitellarium and ovariole stalk (pedicel) that joins the ovariole to the lateral oviduct. The ovariole is covered with an inner epithelial sheath that is composed of a monolayer of flattened cells equipped with long processes. All germ cells (oocytes and trophocytes) constituting the ovariole are interconnected and form one cluster. The clusters in the ovarioles of viviparous generations contain 32 germ cells (8 oocytes and 24 trophocytes), whereas in oviparous generations the number of germ cells is not fixed and ranges from 55 to 90 (7–9 oocytes and 47–81 trophocytes). The tropharia comprise individual trophocytes and arrested oocytes. The centre of the tropharium is occupied by a cell-free region, termed a trophic core, which is connected both with trophocytes (by broad cytoplasmic processes) and oocytes (by nutritive cords). The trophic core, nutritive cords and processes of trophocytes are filled with microtubules. The vitellaria of oviparous females contain single oocytes which develop through three stages: previtellogenesis, vitellogenesis and choriogenesis. During vitellogenesis oocytes accumulate reserve substances (yolk granules and lipid droplets). During choriogenesis they become covered with egg envelopes. The vitellaria of viviparous females consist of several oocytes which develop until previtellogenesis. In contrast to oocytes of oviparous generation, the oocytes of viviparous generations do not accumulate yolk and do not become covered with eggshells. In their cytoplasm only lipid droplets are accumulated. Soon after previtellogenesis, their nuclei undergo mitotic divisions to form the embryo.

Both in viviparous as well as in oviparous females, in the close neighborhood of the ovaries large organs termed bacteriomes occur. Each bacteriome consists of huge cells termed bacteriocytes which harbor endosymbiotic bacteria. In *Glyphina betulae* two kinds of bacteriocytes are present: more numerous bacteriocytes containing large spherical bacteria *Buchnera aphidicola* and less numerous bacteriocytes containing small coccoid bacteria. Both these endosymbionts are transmitted from one generation to the next transovarially.

Influence of the Y chromosome deletion in male mice on their daughters' cumulus oophorus resistance

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It was proved, that female mice differ in sensitivity of their egg investments to enzymatic digestion and that this feature is inherited from the paternal strain. This phenomenon was explained by different imprinting of genetic information, which females obtain from their fathers. Then it was found, that cumulus cells surrounding oocytes of B10.BR-Y^{del} females (sired by males with the large deletion in the Y chromosome) disperse in hyaluronidase solution significantly more slowly than those, which surround eggs of B10.BR females. Deletion in the Y chromosome abolishes the influence of paternal genomic imprinting characteristic for B10.BR strain what implies, that in the deleted region there are some genes involved in establishment of imprinting pattern in male germ cells.

In the present study we tested how susceptible to hyaluronidase digestion are cumulus-oocyte complexes of females from consomic strains: DBA, DBA-Y^{BR}, DBA-Y^{del} and CBA, CBA-Y^{BR}, CBA-Y^{del}. The mean time of cumulus cells dispersal was definitely more rapid for females sired by males with the Y chromosome derived from B10.BR strain (Y^{BR}), than for females sired by males with Y^{del}. The results obtained for backcross lines of mice differ clearly from these obtained for pure DBA and CBA strains. It allows to conclude, that the Y chromosome of fathers has a significant influence on their daughters' cumulus oophorus resistance and that this influence is stronger, than the influence of the genetic background.

As we measured, the content of hyaluronan in the cumulus-oocyt complexes of B10.BR and B10.BR-Y^{del} females is equal. Addition of EDTA to the solution of hyaluronidase abolishes completely the difference in cumulus cells dispersal between oocytes of the two investigated groups of mice. Hence we presume, that molecules mediating divalent cation-dependent adhesion, such as integrins, may by involved in the increased resistance of B10.BR-Y^{del} cumulus oophorus extracellular matrix.

TEM observations revealed that cumulus cells surrounding ovulated oocytes of both B10.BR and B10.BR-Y^{del} females have similar ultrastructure reflecting their steroidogenic activity. We noticed additionally, that cells accompanying oocytes of B10.BR-Y^{del} females more often stay tightly attached to zona pellucida projecting expansions in its direction.

Does spindle assembly checkpoint in mouse oocytes deteriorate with maternal age?

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Incorrect chromosome separation at meiosis is an important source of human embryonic aneuploidy leading to spontaneous miscarriages or birth defects. The spindle assembly checkpoint (SAC) is a mechanism which monitors the orientation of chromosomes and blocks anaphase until they are correctly positioned, thus reducing the risk of production of aneuploid cells.

The mice from LT/Sv strain suffer from heritable defect of female meiosis manifested by arresting the progression of meiosis at the first meiotic metaphase stage (MI arrest) instead of completing division. We have recently shown, that MI arrest is related to abnormally prolonged activity of SAC in LT/Sv oocytes. Therefore, LT/Sv strain of mice is an excellent model to study SAC during mammalian female meiosis.

Fertilization of LT/Sv oocytes arrested at the MI stage give rise to triploid embryos. Interestingly, the proportion of triploid embryos produced by LT/Sv mice decreases with the maternal age. We have checked whether such decrease in embryonic triploidy reflects the decline in the frequency of MI-arrest. Indeed, the amount of LT/Sv oocytes arresting at MI decreased from 77% in 2-months-old females to 35% in 11-months-old females. Such age-dependent reduction in MI arrest of LT/Sv oocytes is unclear. Perhaps the primary defect responsible for abnormally prolonged activity of SAC in LT/Sv mice disappears with age. Alternatively, the primary defect persists, however SAC efficiency deteriorates with age resulting with higher number of oocytes escaping SAC control and thus completing first meiotic division. The latter explanation is especially interesting since the incidence of aneuploidy in humans drastically increase with maternal age.

Using oocytes from wild type mice (outbred strain) we currently test the hypothesis that SAC efficiency in oocytes decreases with age. We use small concentration of spindle poison in the culture of oocytes to compare the frequency of MI arrest (induced by SAC in response to abnormal spindle structure) in oocytes from young and old females. The results will be presented.

Changes in thickness of each layer of developing chicken cornea after administration of caffeine

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So far only few studies suggest destructive effects of caffeine on some structures of an eye development, especially on a cornea (Everekliogl u, 2003).

The aim of the work is presentation of changes in thickness of each layer of a developing cornea that came into being under an influence of caffeine which was given to chicken embryos.

Research materials were 31 chicken embryos from laid eggs that had been incubated in electric cabinet, at temperature of 37°C–38°C and 50–60% humidity. In the study 60 laid eggs were used. Eggs were divided into two groups: control group (n=30) in which Ringer liquid was given, and the experimental group (n=30) treated with the teratogenic dose of caffeine-3,5mg/egg (Bruvere et al., 1983). In 36 hour of incubation (9/10th stage of development according to Hamburger-Hamilton) (Hamburger and Hamilton, 1951) solutions were given with cannula through the hole in the egg shell directly onto the amnios membrane. After closing the hole with paraffin eggs were put back to the electric cabinet. In tenth and nineteenth day of incubation (36th and 45th stage of development according to Hamburger-Hamilton) corneas were taken for morphometric and morphological analysis.

In experimental groups have been observed reduction of cornea thickness, thickening of corneal epithelium and corneal endothelium as well as Bowman's and Descemet's membranes and decreasing of thickness of stroma in comparison to the control group. All these symptoms confirm the teratogenic effect of caffeine on the developing cornea.

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Seed sexing revealed female bias in two *Rumex* species

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Sex-ratio bias in seeds of dioecious *Rumex* species with sex chromosomes is an interesting and still unsettled issue. To resolve gender among seeds of *R. acetosa* and *R. thyrsiflorus* (two species with an XX/XY_1Y_2 sex chromosome system), we applied a PCR-based method involving DNA markers located on male sex chromosomes. Two different pairs of primers were tested for this purpose: RAY-f + RAY-r (Korpelainen, 2002), specific for the *RAYSI* sequence located on two male sex chromosomes, and UGR08-f + UGR08-r (Mariotti et al., 2009), specific for the *RAYSII* sequence located on the Y_1 chromosome.

Both analyzed species showed a female-biased primary sex ratio (χ^2 test, $P<0.01$), a result consistent with the view that the sex ratio is aberrant in *Rumex* seeds (Rychlewski and Zarzycki, 1986). The seed sex ratios differed between two species, with substantially greater female bias in *R. acetosa* (1:1.78) than in *R. thyrsiflorus* (1:1.44). The differences in primary sex ratio between populations of the same species were small. The mechanism of the sex ratio bias is very difficult to establish, and can result from different mechanisms which are not well documented, both prezygotic (male pollen mortality, certation) and postzygotic (abortion of male embryos).

We confirmed the usefulness of the RAY-f and RAY-r primers (producing ~930 bp DNA fragments in males) for sexing seeds of *R. acetosa*, and showed that the same primers are also effective for determining gender in *R. thyrsiflorus*. However, the disadvantage of this method was the production of DNA fragments of similar length from some female templates. Our experiment suggests that UGR08-F and UGR08-R primers, producing DNA fragments (~700 bp) exclusively in male plants, may be more useful for molecular sexing of *Rumex* seeds.

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Presence of syncytial follicles in mouse ovary with the deletion of Nobox gene

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The crucial role in the early folliculogenesis play transcription factor encoded by Nobox (newborn ovary homeobox-encoding gene). Expression of this gene is exclusively in female germ cells cysts and primordial and growing oocytes (Suzumori et al., 2002). Previous studies showed that Nobox deficiency in mice and mutation of Nobox homeodomain in women resulted in rapid loss of postnatal oocytes and premature ovarian failure (POF) (Rajkovic et al., 2004). The aim of that work was to establish the role of deletion Nobox gene on process of formation primordial follicle in mice ovaries. The ultrastructural analysis of developing embryonic ovaries from $Nobox^{+/-}$ and $Nobox^{-/-}$ mice clearly showed that primordial follicle deficiency in $Nobox^{-/-}$ mice originates in the inability of somatic cells to invade and break the cyst and to separate and surround individual oocytes. This resulted in the incapability of the formation of primordial ovarian follicles, which were replaced by the complex syncytial follicles containing unseparated oocytes. This is probably caused by faulty signaling between somatic cells and germ line component of the fetal ovaries. In addition, we observed extremely unusual and abnormal presence of adherence junctions between unseparated oocytes within the syncytial follicles. It presumably indicates that faulty communication between germ and somatic cells involves or results in abnormalities in cell adhesion program.

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Ultrastructure of primordial ovarian follicles in the bat, *Carollia perspicillata*

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Seba's short tail bat, *Carollia perspicillata*, is a common and widespread bat from South and Central America which has been proven to be a valuable laboratory model. Here we describe the ultrastructure of fetal ovaries (stages: late 14 and 15) of this species. The ovaries were dissected from the fetuses, fixed, dehydrated and embedded according to standard protocols. Embedded material was cut into semi-thin (1μm) or thin sections and analyzed in a light (Leica DMR) or electron (JEOL 100SX) microscope.

Our analyses showed that even in the youngest analyzed stage (late 14), the ovaries contained primordial follicles only. The cysts (clusters) of germ-line cells were absent. This suggests that the cysts had been split into individual oocytes during earlier developmental stages. Each primordial follicle consisted of a post-pachytene oocyte surrounded by somatic cells and/or their extensions. Oocyte cytoplasm contained free ribosomes, mitochondria, Golgi complexes and secretory vesicles. Analysis of serial sections revealed that the latter were preferentially gathered in one region of the oocyte, suggesting that the early oocytes of *C. perspicillata*, as those of mice (Kloc et al., 2008), are asymmetrical or even polar.

In addition to "orthodox" organelles, each oocyte of the investigated species contained single, lens-shaped aggregate of endoplasmic reticulum (ER) cisternae. Between ER elements, prominent accumulations of electron-transparent material were often encountered. It is worthy to note here that the oocytes as well as early embryos of another bat species, *Macrotus californicus*, contain large paracrystalline bodies (Bleier, 1975). Neither biochemical composition nor the formation of these bodies had been analyzed and therefore they remain completely unknown. Morphological criteria, e.g. size, shape and localization of ER aggregates and paracrystalline bodies, suggest that these two structures are functionally related, and that the accumulations of electron-transparent material present within ER aggregates might represent initial stages of paracrystalline bodies formation.

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Induction of somatic embryogenesis in cacti *Copiapoa tenuissima* Ritt *forma monstruosa*

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Copiapoa tenuissima Ritt *f. monstruosa* is a spontaneous cacti mutant from *Copiapoa tenuissima* Ritt from Chile. *Monstruosa* form has nearly black epidermis and it has no thorns in areoles, and it is very rare and attractive for collectors (Dornig, 1976; Graham, 1998).

There was investigated the induction of direct somatic embryogenesis of *Copiapoa tenuissima* Ritt *f. monstruosa* depending on the explant position on the donor plants.

The initial explants (mammillae with areoles) were taken from donor plants from the collection of Licznerski (Jarużyn Kolonia near Bydgoszcz). The explants were taken from three levels of the main shoot of donor plans: distal, central, and proximal and from young auxiliary shoots. They were sterilized with 70% ethanol for 1–2 s and then with 0.79% hypochloride solution for 15 min, followed by three rinses with distilled sterilized water. There were used 100 explants depending on the level of donor plans and the medium. Explants were cultured on modified Murashige and Skoog (1962) medium with additional 330 mg·dm^{-3} $CaCl_2 \cdot 6H_2O$, 13.9 mg·dm^{-3} $FeSO_4 \cdot 7H_2O$ and 20.6 mg·dm^{-3} $Na_2EDTA \cdot 2H_2O$. The medium contained 3% sucrose, it was solidified with 1.2% PURIFIED LAB-AGAR (Biocorp), pH was 5.7 prior to sterilization.

The explants were cultured on the modified medium with 2 mg·dm^{-3} auxin 2.4-D (2.4-dichlorophenoxyacetic acid) or MS medium without growth regulators (as control). The cultures were kept in a growth room at 24 ± 2°C and exposed to 16 h photoperiod. Daylight was by maintained using Philips TLD54/36 W lamps with the photon flux density of 38.1 mol m^{-2}s^{-1}. After 8 weeks of culture, the explants were examined under the stereomicrocope.

The induction of somatic embryogenesis in cacti *Copiapoa tenuissima* Ritt *f. monstruosa* was obtained only when the media were supplemented with auxin 2.4-D. However, most explants regenerated somatic embryos derived from the distal and central level of main shoots of donor plants and from young auxiliary shoots (to 0.26 per one inoculated explant), yet from the proximal part of the main shoot of cacti the number of explants which regenerated somatic embryos was low (0.02 per inoculated explant) and did not differ from the control.

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Histological analysis of gonads of *Anodonta anatina* (Linnaeus, 1758) (Mollusca: Bivalvia: Unionidae) infected with *Rhipidocotyle campanula* (Dujardin, 1845) (Platyhelminthes: Trematoda: Bucephalidae)

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Freshwater mussels play an important role in a life cycle of some digenetic trematodes as their first intermediate hosts. Larvae of the trematode *Rhipidocotyle campanula* (Dujardin, 1845) develop in the mussel *Anodonta anatina* (Linnaeus, 1758) causing damage to gonads, hepatopancreas, mantle, kidney, foot, or to gills of their host.

Our work aimed at histological investigation of mussels' gonads infected with the larvae of *R. campanula*. In September 2008, two hundred A. anatina individuals were collected from Lake Gant (53°42.5' N; 21°14.2' E; Masurian Lake District, Poland) by scuba diving. Mussels were dissected, and fresh smears from reproductive organs were used to detect oocytes, spermatozoa, spermatid morulae and parasites in gonads. Reproductive glands infected with trematodes were fixed in 6% formalin to prepare histological slides. Tissues were dehydrated in a graded series of ethyl alcohol, cleared in chloroform, and finally embedded in paraffin. 5-μm thick cross-sections were cut with a rotating microtome, stained progressively by Ehrlich hematoxylin and eosin, and examined under a light microscope.

23.5% of the mussels had their gonads infested with *R. campanula*. Sporocysts and cercariae found in follicles were at different developmental stages, which indicate that reproduction of parasites was asynchronous. Infected mussels had degenerated oocytes, damaged gonad tissues, and, in the most extreme cases, they were totally castrated. Five females infected with *R. campanula* were incubating embryos and larvae (glochidia).

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Calreticulin localization in pollen tubes of *Petunia hybrida* Hort

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The aim of the study was to investigate the distribution of calcium binding protein calreticulin (CRT) in *Petunia hybrida* pollen tubes growing *in vitro*. For the first time, the protein was localized in these plant cells with the using of pre-embedding immuno-fluorescent technique. High CRT levels were detected both in germinating pollen as well as in growing pollen tubes. In germinating pollen the strongest signals detected were associated with the aperture regions. In elongated pollen tubes CRT was found to be highly abundant in the sub-apex zone and in the peripheral cytoplasm of the shank region. Ultrastructural observations of *in vitro* cultivated cells on the electron microscopy level have revealed the presence of smooth and rough endoplasmic reticulum (sER/rER) in the regions where CRT was found. Our previous studies showed that ER is the place of CRT mRNA localization in *Petunia hybrida* pollen tubes growing *in vivo* (Lenartowska et al., 2001). Moreover, CRT transcripts were enriched at the apertures and periphery of the *Haemanthus albiflos* tubes growing *in vitro* (Lenartowska et al., 2009). Therefore, in the light of our previous and present results, we suggest that rER could be the sites of CRT synthesis during pollen germination and tube elongation.

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The comparison of the development Cichlidaes from Lakes Tanganyika and Malawi on the example of *Labeotropheus trewavasae* and *Ctenochromis horei*

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There is a need to protect the endemic species of fishes which live in the Great Lakes of Africa. The study of the early ontogenesis at African Cichlids was aimed to examine the course of development from the fertilization to the stage of small juveniles. The results of study could help to develop methods which would allow us to protect these fish with using of ex situ conditions. *Labeotropheus trewavasae* and *Ctenochromis horei* are two species which live in Lake Malawi and Lake Tanganyika. They are mouth-breeding fishes which females incubate the fertilized eggs in the oral cavity.

The embryos were taken from the oral cavity of the female and kept in laboratory conditions in the especially constructed incubator and in the water at temperature at 26.55°C and pH 8.26. Measurements of eggs, embryos and juveniles were made with using of a stereoscopic microscope equipped with micrometric scale.

The entire period of development was divided into two stages: the embryonic and larval stage. In the embryonic stage, the following features were observed: *longitudo ovum, latitudo ovum, longitudo super vitellus, latitudo super vitellus*, and *diameter oculi, longitudo corporis* and the number of heart beats per minute in one individual. During the larva and juvenile stages the following parameters were measured: *longitudo praedorsale, longitudo capitis lateralis, diameter oculi, longitudo praeanalis, longitudo corporis, longitudo totalis, altitudo corporis maxima* and *longitudo et latitudo vesica vitellus*. The observation and obtained data let us to determine the beginning of organogenesis, the moment when first color cells and first body muscle cramps appear, as well as when the heart started to work and the moment of fins appearance.

The preliminary results have shown that the rate of development of both species is very similar in the conditions provided for incubation.

Olfactory organs structure and changes occurring on the surface of the olfactory epithelium in *Cyprinus carpio* L. (Cyprinidae) during ontogenesis

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The presented studies were carried out on Cyprinus carpio, species important in both the economy and fish farming. Studies were performed on six specimens, two from each age class: 1, 2 and 3 years old with total body length (TL) ranging from 13,2 to 41 cm. Observations were carried out using routine scanning electron microscopy methods while the measurement of the olfactory area was done using the method described by Jakubowski (Jakubowski and Kunysz, 1979).

It was found that carp olfactory organ structure was typical, common for the majority of teleost fish: paired olfactory chambers localized in the preorbital part of the head, where the epithelium of the floor forms an oval olfactory rosette. The sensory olfactory epithelium is located on the lamellae of the rosette. It was demonstrated that as the individual grows the number of lamellae in the rosette also increases, from 20 (TL 13,2 cm) to 44 (TL 41 cm). According to Burne's (1909) classification that takes into account the number of lamellae in a rosette, carp can be ascribed to the mediosmatic fish group. The highest increase in both sensory area size and the number of lamellae occurs between the second and third year of life, with TL 24,8 cm: 124,18 mm^2 (30 lamellae), as compared with TL 41 cm: 439,42 mm^2 (44 lamellae). This quick increase in the olfactory area size in that very period is probably connected with the attainment of sexual maturity. The increase in the number of the olfactory lamellae in the rosette during ontogenesis is a common phenomenon among fish, however there are species such as *Salmo gairdneri* (Halama, 1982), where the number of lamellae is established as early as from the first year of life.

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Behaviour of the anther's tapetum in *Taraxacum atricapillum* sect. *Borea* from Poland

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Although pollen production is widespread among asexual dandelions, within the *Taraxacum* genus some species are male sterile. As the production of pollen is considered to be energy-consuming, male sterile dandelions have reallocated their resources into seed production. As a result they produce more capitula than pollen producing taxa (Meirmans et al., 2006). The data obtained so far revealed that the male sterility found in dandelions is of nuclear-cytoplasmic nature (Małecka, 1971; Van der Hulst et al., 2004; Meirmans et al., 2006).

Strongly disturbed meiosis is the consequence of triploid chromosome number of *Taraxacum atricapillum* (2n=3x=24). Moreover the embryological analysis of *T. atricapillum* revealed that investigated plants produced male sterile capitula. In some flower buds we observed the pattern of tapetum behaviour described as characteristic for cytoplasmic male-sterility. The abnormal vacuolization of uninucleate tapetal cells started in the stage of archesporial cells in anthers. Together with the progressing meiosis in PMCs, some cell walls in tapetum disintegrate, finally binucleate cells can be observed. In such anthers degeneration of microsporocytes took place at the II telophase.

In the other flower buds the course of anther's tapetum development was typical for Asteraceae. Mitotic activity of tapetum cells, at first uninucleate, increased during the second meiotic division in PMC's. Because of inhibited cytokinesis tapetal cells became multinucleate with no evidence of precocious abortion. In these anthers microsporocytes did not degenerate before the end of meiosis, but due to disturbances in the process, dyads, triads and other configuration developed instead of regular tetrads. In the analysed material degenerated microspores were observed most frequently. Nonetheless, viable microspores, varying in size, were present in some loculi.

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Lactuca sativa L. – a convenient object for an efficient and rapid sexual reproduction

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In *Lactuca sativa* L. pollen grains start to germinate on stigma about 15 minutes following pollination, about 40 minutes later, pollen tubes enter into the embryo sacs and fertilization probably occurs at this moment. Five days later, heart stage and even cotyledonary embryos are formed. Mature seeds ready to germinate can be obtained 15 days after pollination (Jones, 1927). Thus the whole process of sexual reproduction in *Lactuca sativa* L. which proceeds in such a short time constitue a convenient object for analizing all events connected with the formation of new progeny. The culture *in vitro* ovaries 40 minutes after pollination enabled to obtain fully formed embryos and later plants. More details will be presented concerning the embryogenesis in the *in vitro* cultured ovaries.

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Diversity of gonadal morphogenesis in anuran amphibians

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Traditional view shows that in gonads differentiating into testis germ cells enter the medulla, whereas in a future ovary, the germ cells remain stationary in their initial cortical position. However, this model of gonad differentiation seems oversimplified. Though undifferentiated gonads consist of the cortex and sterile medulla in various anurans, gonodogenesis in *Bombina* deviates from the model. Accelerated sexual differentiation in *Bombina* results in an early rearangement of the gonad and lack of cortico-medullar division within it. Our survey of distinct anurans shows that gonadal differentiation begins at dissimilar stages of undifferentiated gonad. The earliest sexual differentiation, seen in *Bombina*, preceeds the medulla formation and results in early dispersion of germ cells within the testis as well as in the lack of a sterile medulla in testis. Even more accelerated sexual differentiation is typical for green frogs, but here undifferentiated gonads develop enough rapidly, which allows to form cortex and sterile medulla. In other frogs, tree-frogs and toads the period of undifferentiated gonad lasts for relatively the longest lime (even to 42 Gosner stage) due to delayed sex determination. Such retarded differentiation allows somatic cells to form distinct sterile medulla completely separated from the cortex by two basal lamianae and stromal space. The testicular differentiation requires a significant rearrangement since germ cells must translocate from the cortex to medulla. Such changes are absent in the early testis in *Bombina* due to the lack of cortico-medullar subdivision. In *Bufo bufo* it was observed that not all germ cells translocate to the medulla during the testis differentiation and the germ cells remaining in the cortex enter meiosis, which indicates that the localization of germ cells within the anuran gonads determines their sex. The comparison of gonadal differentiation shows that the diversity of gonadal development in anurans results from dissimilarities in somatic cell proliferation, their translocation and changes in basal lamiana distribution. Moreover, heterochronic shifts in sex differentiation exert strong influence on the structure of larval gonad.

In vitro cultured endosperm of selected monocots, dicots, autotrophic and semi-parasitic species

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Endosperm is distinctive tissue in its origin, development and ploidy. The response of endosperm of selected crop and herb plants on *in vitro* culture conditions were examined. Mature endosperm tissues were excised from seeds of kiwifruit (*Actinidia deliciosa* cv. Hayward) and mistletoe (*Viscum album*). The experiments concerning monocots taxa were conducted on immature endosperm of seven cultivars of winter, spring and durum wheat (*Triticum aestivum* and *T. durum*) and winter triticale (*Triticosecale*).

The basal medium (liquid or solidified with 8% agar) consisting of MS salts and vitamins was supplemented with 3% sucrose, auxins (2,4-D, IAA), cytokinins (kinetine, thidiazuron) and ascorbic acid. The material for sectioning (freshly isolated and cultured endosperm) was prepared by embedding tissues in Technovit 7100 and stained with toluidine blue or auramine O.

In kiwifruit the callus and organogenesis induction were described previously (Popielarska et al., 2006; Popielarska-Konieczna et al., 2008). In present work the localization of cutine on callus and meristematic protuberances were analyzed. Cutine was detected on compact callus domain and young shoot buds. Weak fluorescence of auramine O was observed on the surface of embryo-like structure and non-morphogenic callus composed of loosely attached cells.

Studies on proliferation of isolated endosperm of selected cereals: hexaploid *T. aestivum* and tetraploid *T. durum* have been reported recently (Popielarska-Konieczna et al., 2009). The present experiments were conducted on co-culture with kiwifruit endosperm-derived callus. Additionally ovules of non tested yet hybrid species triticale was taken as a source of explants.

Preliminary studies on isolated endosperm of semi-parasitic mistletoe were done. We noted that dark conditions and addition of ascorbic acid for maintaining explants viability and increasing of cells are necessary.

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Revealing of calreticulin at the key stage V of *Chara vulgaris* spermiogenesis using immunogold technique

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Apical parts of *Chara vulgaris* thalli were fixed for electron microscopy analyses. Antheridia were taken from three stages: early (IV sp), middle (V sp) and late spermiogenesis (VIII sp) and ultrathin sections from selected material were prepared. Immunogold reactions were made using Rabbit polyclonal antibody anti-calreticulin (405–417) (Calbiochem) recognizing human 63kDa calreticulin.

Weak immunosignals both in the cytoplasm and in the nucleus at the early stage of spermiogenesis were observed. Immunogold technique revealed gold grains generally in the nucleus, in ER vesicles and in thin and swollen ER cisternae as well as in Golgi apparatus at the V key stage for whole process of maturing spermatozoids. In nuclei immunolabeling could be seen either in peripheral parts with condensed chromatin and in inner parts with noncondensed chromatin, just like protamine-type proteins are localized. Labeling was also observed in nuclear envelope adjacent to condensed chromatin and connected with swollen RER cisternae filled with protamine-type proteins (Kwiatkowska and Popłońska, 2002; Popłońska et al., 2009). In extensive ER system stronger reaction was revealed in subdomain of cisternae than in vesicles. Single gold grains were identified in poorly developed cisternae ER in reduced cytoplasm at the late VIII stage of spermiogenesis. In hook-shaped nucleus the fibrilar chromatin was completely devoided of gold grains, however few immunosignals in spaces between fibrils with lower electron-density were detected.

Current studies revealed time and place of calreticulin occurrence in *C. vulgaris* spermiogenesis. This protein was observed during replacement of nucleohistones into protamine-type proteins (Kwiatkowska and Popłońska, 2002; Popłońska et al., 2007; 2009). Calreticulin clearly appears in the same places of the cell where protamine-type proteins were identified i.e. in the nucleus and in extensive RER (Popłońska et al., 2009).

These observations suggest that calreticulin could participate in transport of newly synthesized protamine-type proteins into *Chara* spermatid nuclei.

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The testis ultrastructure in *Xerobiotus xerophilus* (Tardigrada, Eutardigrada)

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The reproductive system of *Xerobiotus xerophilus*, similarly to other Eutardigrada (Dewel et al., 1993), is composed of single testis and the paired sperm ducts which open into the rectum. The sac-like testis is located in the dorsal part of the body and is attached to the dorsal body wall by two ligaments. The testis wall consists of a single layer of slightly flattened cells supported by a basal lamina. The gonad is filled with male reproductive cells and it does not show any regionalization, e.g. spermatocytes are found together with young and late spermatids and spermatozoa. Spermatocytes as well as spermatids are connected by the cytoplasmatic bridges and they form clusters. These bridges persist up to an advanced stages of maturation and help coordinate cell development in a cluster. The testicular spermatozoon of *X. xerophilus* possesses a head with acrosome and helicoidal nucleus, a midpiece and a tail with terminal tuft. The tail has a typical '9+2' axoneme, but its caudal part loses this organization.

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The role of midgut epithelium in oogenesis in *Isohypsibius granulifer* (Tardigrada, Eutardigrada)

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Midgut epithelium of *Isohypsibius granulifer* (Eutardigrada) is composed of columnar digestive cells. At its anterior end the group of cells with the cytoplasm which differs from the cytoplasm of digestive cells, has been observed. They might respond to crescent-like cells (midgut regenerative cells) described for some tardigradan species (Bertolani, 1970; Ząbczyk, 2000). Their mitotic divisions have not been observed. The ultrastructure of midgut digestive cells in relation to oogenesis (previtellogenesis, vitellogenesis, choriogenesis) has been analyzed with the use of transmission electron microscope. Therefore oogenesis has been divided into 5 stages (A – E).

During oogenesis in the midgut epithelium cells the gradual accumulation of glycogen granules, lipid droplets and structures of the varying electron density appears. The increasing number of organelles which are responsible for intensive synthesis of lipids, proteins and saccharides such as cisterns of RER and SER, Golgi complexes has been observed during vitellogenesis and choriogenesis. At the end of oogenesis also autophagy intensifies, what might be caused by the great amount of reserve material. Midgut epithelium of *Isohypsibius granilfer* takes part in synthesis of yolk precursors.

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Apoptosis, autophagy and necrosis in the midgut epithelium of *Acheta domesticus* (Insecta, Orthoptera, Gryllidae)

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Midgut of *Acheta domesticus* L. (Insecta, Orthoptera, Gryllidae) is composed of the anterior midgut with blind, sacklike caeca and posterior midgut which takes the shape of the long tube. In the midgut epithelium two kinds of cells are distinguished: columnar digestive and regenerative cells. The latter form regenerative crypts (Rost-Roszkowska, 2008).

Autophagy has been observed as the common process in midgut epithelial cells of analyzed species. Cisterns of endoplasmic reticulum start enclosing cytoplasm with organelles and subsequently autophagosomes are formed. Organelles proceed gradual digestion and residual bodies are formed. In several cells of the midgut epithelium which possess numerous autophagosomes, alterations in electron density of the cytoplasm appear. Apoptosis begins. The cell shrinks causing the appearance of distinct extracellular spaces. The nucleus changes its shape into lobular, the chromatin becomes condensed near the nuclear envelope. The cell gradually loses contact with the adjacent midgut epithelial cells. Finally the apoptotic cell is separated from the basal lamina and is discharged into the midgut lumen, where it disintegrates. No differences during apoptosis between anterior and posterior midguts were observed.

Necrosis is more extensive in the posterior midgut, where the entire groups of digestive cells degenerate. During necrosis the cytoplasm of midgut epithelium cell becomes electron lucent and the number of organelles decreases gradually. Apical membrane breaks and all organelles are discharged into the midgut lumen.

The transitions of degenerating cells have been described at the ultrastructural level. Immunostaining methods were conducted in order to indicate the early stages of apoptosis.

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Fine structure of the midgut epithelium of *Lithobius forficatus* (Myriapoda, Chilopoda) with special reference to its degeneration

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In the midgut epithelium of *Lithobius forficatus* (Myriapoda, Chilopoda) two types of epithelial cells have been observed: cells with the cytoplasm rich in large electron dense granules (proteins) and cells, which possess numerous lipid droplets. The cytoplasm of both types of epithelial cells shows distinct regionalization in organelles distribution, therefore basal, perinuclear and apical regions are distinguished. Additionally small cells with the cytoplasm poor in organelles have been described. Some of them possess the cytoplasm rich in numerous electron dense structures. They are probably regenerative cells, which would be able to differentiate into digestive cells. Their mitotic divisions have not been observed.

Midgut epithelium of analyzed species degenerates in two manners: necrotic and apoptotic. During necrosis the cell swells, the number of organelles decreases. Eventually the apical membrane breaks and organelles are discharged into the midgut lumen. Apoptosis seems to be a common process in the midgut epithelium of analyzed species. The cell death of the midgut epithelial cells has been described at the ultrastructural level.

Cell death of the midgut epithelium cells in *Archispirostreptus gigas* (Myriapoda, Diplopoda)

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Degeneration of the midgut epithelium has been described as a wide-spread phenomenon in insects (Park et al., 2009; Rost-Roszkowska et al., 2010), while information connected with myriapods midgut epithelium is rather poor.

Midgut epithelium of *Archispirostreptus gigas* (Myriapoda, Diplopoda) possesses columnar digestive cells which cytoplasm shows characteristic regionalization in organelles distribution. Just beneath the apical membrane, which forms microvilli, a thick cortical layer with filaments appears. According to midgut functions connected with digestion, secretion and absorption, the midgut epithelium of analyzed species degenerates. Two processes of cell death have been observed: apoptosis and necrosis. However just before the cell activates one of those processes, autophagy is used to get rid of organelles. When in the cytoplasm numerous autophagosomes and autolysosomes accumulate, apoptosis or necrosis is activated.

The process of apoptosis has been described with the use of transmission electron microscope. Immunostaining methods were conducted in order to indicate the presence of early stages of the programmed cells death (caspase 3 antibody, Annexin V-FITC, TUNEL assay).

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The expression of mammalian homologue of K1/K10 cytokeratins in differentiating epidermis of grass snake *Natrix natrix* L.* (Lepidosauria, Serpentes) embryos

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Many studies indicated that reptilian epidermis produces two major groups of keratins named alpha and beta. Alpha keratins are very important for epidermal stretching and together with lipid granules act as the principal barrier against water-loss. The alpha keratin of reptiles may be related to the soft alpha keratins of mammals and amphibians. The snake embryos were isolated at regular intervals, starting with the eggs laying till hatching. The age of embryos was calculated using the table of species development (Rupik 2002). The small pieces of skin in Karnovsky fixative were fixed, dehydrated, embedded in LRWhite medium and immunostained with anti-K1/K10 cytokeratins antibody. The K1/K10 antibody stained heavily the beta layer of the epidermis covering outer surfaces of gastrosteges at the beginning of the developmental stage XI but the beta layer in epidermis covering outer surface just at the end of developmental stage XI. The alpha keratinization of Oberhäutchen layers in epidermis covering outer surfaces of gastrosteges and scales begin just before the fusion with beta layers at the developmental stage XI and XII. It is possible that the homologues of mammalian K1 and K10 keratins in the Oberhäutchen and beta layers of grass snake epidermis initially established specific scaffolding for latest beta keratin deposition. After fusion of Oberhäutchen and beta layers, weaker K1/K10 immunolabeling suggests that in this period alpha keratins are masked by beta-keratins, modified or degraded. The Oberhäutchen layer in epidermis covering inner surface of gastrosteges was stained with K1/K10 antibody at the end of developmental stage XI but the Oberhäutchen in the epidermis covering inner surfaces of scales and hinge regions between both scale and gastrosteges did not show K1/K10 immunolabeling until hatching. The K1/K10 antibody does not stain the alpha and mesos layers just until hatching. We suppose that differentiation of these layers begins just after first postnatal sloughing.

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*All specimens used in experiment were captured according to Polish legal regulations concerned with wild species protection (Dz.U. nr2 poz. 11 z 1984r., 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 (ref. No: DOPog-4201-02-94/05/aj, DKFOP-ogiz-4200/II-6/587/08/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).

A comparative studies of scales and gastrosteges formation in grass snake *Natrix natrix* L.* (Lepidosauria, Serpentes) embryos

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Snakes, like other reptilian species have the characteristic scaled skin, which is one of the main features distinguishing them from the other amniotes, birds and mammals (Swadźba et al., 2009). Scales covering of the dorsal site on of the body in snake are called the dorsal scales or scales but the enlarged scales on the belly of the snake are called ventral scales or gastrosteges. Each scale and gastrostege has two surfaces: outer and inner. The skin from the inner surface hinges back and forms a free area, which overlaps the base of the next scale, which emerges below this scale. Scales and gastrosteges protect the body of the snake and aid it in locomotion. In our studies, we have compared differentiation of scales and gastrosteges in grass snake *Natrix natrix* L. embryos. The embryos for examination were isolated at regular intervals, starting with the eggs lying till 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, dehydrated, embedded and contrasted with using routine electron microscopic methods. Our findings indicated that, gastrosteges formation (developmental stage V) precedes formation of the scales (developmental stage VI). The gastrosteges appear to be more developed in shape and their stratification than scales. The differentiation of epidermis on the outer surfaces of scale and gastrosteges appear earlier than on their inner surfaces. The epidermis covering hinge region is not fully differentiated until hatching. These observations indicated that scalation and stratification begins on the ventral body surface, and spreads dorsally.

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Germline stem cells and possibility of neo-oogenesis in ovary in the adult mice

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The determined number of primordial follicles can be found in adult mammalian ovaries. Each follicle contains the cell, which is called the oocyte. The oocytes are arrested in prophase of the first meiotic division and they are responsible for the female fertility. Only in some mammals, e.g. in prosimians, there is a possibility to create in ovaries new generations of oogonia. But there are some evidences suggesting that the permanent presence of primordial germ cells in mouse and human ovaries and early phases of oogenesis (neo-oogenesis) during postnatal life are possible (Johnson et al., 2004; Bukovsky et al., 2004). The aim of this study was to validate the hypothesis of the neo-oogenesis in adult mice ovaries.

Obtained during the section ovarian tissue was stained with hematoxylin and eosin and of the activity of alkaline phosphatase.

The cells, which features were typical for primordial germ cells, were presented in ovarian epithelium. These cells were bigger than other epithelial cells, irregular in shape and displayed alkaline phosphatase activity. The few of them were under mitosis divisions, others start to enlarge and left the epithelium, what suggests their transformation into oogonia. These ameboid in shape cells, which possessed alkaline phosphatase activity, were presented in the most external layer of ovarian cortex and they were in direct contact with cowering epithelium. It was noticed, that these cells were able to migrate to more external cortical layer, where they began prophase of the first meiotic division. Around some of them the granulosa cells formed the primordial follicles. These observations indicate, a limited possibility, that the pregerminal cells may be produced in adult mice ovary and they might be able to multiply and transform into oogonia and oocytes, which are able to regenerate primordial follicles. The genesis and meaning of this process is not clear. This phenomenon might be not significant for female fertility, because of its uniqueness.

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Caffeine influence on transcriptional activity of corneal cells during chicken development

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The aim of this study was to demonstrate the influence of caffeine, administrated during formation of chicken embryos cornea, on transcriptional activity of cells of anterior and posterior epithelial cells and matrix fibroblasts, determined on the basis of quantitative analysis of argyrophylic proteins (AgNOR).

The experimental material was a 120 chick embryos from eggs of hens nesting Ross 308 breed, incubated at 37–38°C and humidity 50–60%. Breeding eggs were randomly divided into two groups. First group was the control group. In the 36th hour of incubation (9/10 stage of development according to Hamburger-Hamilton) (Hamburger and Hamilton, 1951) to 100 eggs for this group Ringer's solution was given. For another 200 eggs, which were in the experimental group, a single dose of caffeine – 3.5 mg/egg (Bruvere et al., 1983) was given. In the tenth (E10) and the nineteenth day (E19) of incubation (36 and 45 stages of development according to Hamburger-Hamilton) corneas were collected for analysis. Argyrophylic proteins were silver stained, according to Ploton's method (Ploton et al., 1986). Quantitative analysis was made using an image analysis system Image J v. 1.34s. The number of silver clusters per nucleus, and their area were measured. Based on obtained measurements, AgNOR content coefficient was calculated. It characterizes transcriptional cell activity.

Compared with the control group, statistically significant increase in the coefficient of AgNOR content and AgNOR mean area in anterior epithelial cells of E10 experimental group and in matrix fibroblasts of E19 was observed. Decrease in the value of those parameters was observed in the posterior corneal epithelial cells; however, statistically significant differences exist only in the E10 group.

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Differentiation of the gonads in teleost fish racer goby *Neogobius gymnotrachelus* in reservoir of the lower Vistula River

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The goals of this study were (1) to describe the gonad structure of racer goby from the size range 23 to 79 mm of total body length (TL), (2) to determine type of sex differentiation and (3) to determine fish size when process of gonads' maturation is terminated. We examined gonads of 88 fish from 23.6–79 mm of TL and body mass ranging from 0.148 g to 5.1 g. Fish were caught in August 2006 (14 individuals), August 2008 (53) and October 2008 (21) from the Włocławek Reservoir of the Vistula River (Poland). Gonads were preserved in Bouin's fixative, and followed with embedding in paraffin and H+E staining for light microscopy analysis.

There were 48 females and 40 males among studied individuals. The ovary of the smallest female (23.6 mm TL) had oocytes in the first chromatin nucleolus phase and perinucleous phase. The gonad of the largest female (73.6 mm TL) had oocytes in the following three stages: the primary growth stage, the cortical alveolus stage and the vitellogenesis stage. The smallest male of 32 mm TL had testicular tubules with distinct lumen filled with primary spermatogonia. The male of 78.4 mm TL had the seminal tubules with spermatogonia and spermatocytes in stages I and II and spermatids as well, which indicates sexual maturity in males. We did not find the undifferentiated gonads among studied individuals. Preliminary histological analysis showed that the type of gonad development in racer goby represents 'differentiated gonochorism', when ovarian and testicular differentiation proceeds directly from the undifferentiated gonads. However, to confirm our conclusions the studies of an earlier life stage and at body length below 23 mm TL are needed. The histological results are consistent with morphological observations of the genital papillas. More individuals should be studied with histological methods and such analyses are in progress.

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Disturbances of microsporogenesis in obligatory apomict *Chondrilla brevirostris* L. (*Asteraceae*)

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The process of microsporogenesis was investigated in triploid representative of Asteraceae, *Chondrilla brevirostris* L. (2n=3x=15). This species is an obligatory apomict with diplosporous embryo sacs of Taraxacum type in which the embryo and endosperm developed autonomously without fertilization.

Embryological investigations, using a DAPI staining, showed disturbances in the conjugation of chromosomes during I prophase of meiosis resulted in the production of high number of univalents, some bivalents and rare trivalents. The presence of univalents located away from the equatorial plane was the consequence of disturbed I meiotic metaphase. In addition lagging uni-, bi- and trivalents situated between cell poles were observed during anaphase I. Disturbances in course of microsporogenesis were manifested in formation of dyads, triads and tetrads contain nuclei differing in size. Moreover, cytokinesis disturbances led to the formation of multinucleate postmeiotic cells and narrow "dumb-bell-shaped" pollen grains with three of four nuclei.

Cytochemical methods, used for the first time with the reference to apomictic plant, showed that the cytoskeletal configuration of microsprogenesis is similar to those described in amphimictic plants, however some differences were observed. In a few hundred analysed meiocytes, aggregation of nucleoids of organelles around the nucleus, as well as plates of plastids and mitochondria, were not formed at telophase I and II. Unlike in amphimicts, poorly developed microtubules of phragmoplast were observed. Such irregularities might have been responsible for the lower vitality of pollen grains which diameter ranged from 13 to 44.99 μm.

In vitro response of sunflower immature zygotic embryos to ethylene inhibitors

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The effect of inhibitor of ethylene biosynthesis (aminoethoxyvinylglycine; AVG) and inhibitor of ethylene signaling (silver nitrate; $AgNO_3$) on somatic embryo and adventitious shoot formation from immature zygotic embryos of *Helianthus annuus* L. was studied. The inhibitors, AVG at 5 μM or $AgNO_3$ at 5 μM and 10 μM were added to the media along with 6,6 μM of cytokinin (6-benzylaminopurine; BAP) and 87 mM (JMS media) or 350 mM (JME media) sucrose. Culture on JMS medium without inhibitors resulted in adventitious shoot formation only, while the explants maintained on JME-free of inhibitors produced shoots along with somatic embryos (Jeanin et al., 1995). During incubation on JME supplemented with AVG (5 μM) and JME containing $AgNO_3$ at two concentrations tested (5 vM and 10 vM) the explants produced higher number of shoots and somatic embryos when compared with media without of inhibitors. On the other hand, application of AVG (5 μM) and $AgNO_3$ (5 μM and 10 vM) together with standard sucrose concentration gave rise to shoots and embryo like structures concomitantly on the same explant. Regenerated shoots, somatic embryos and embryo like structures rooted well when subcultured onto MS media containing IAA (19 μM) and next transferred to soil. Histological analysis and scanning electron microscopic (SEM) observations showed the embryogenic character of embryo like structures occurring on the JMS media with AVG and $AgNO_3$ addition. These structures were similar to zygotic embryos at globular or heart stadium. Moreover, histological observations showed mostly direct (without callus formation) organogenesis and somatic embryogenesis observed on immature zygotic embryos of sunflower under applied conditions.

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Structure of the ovaries and early stages of oogenesis in the parasitic earwig, *Arixenia esau* (Dermaptera: Arixenina)

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The order Dermaptera includes – in addition to well known and common earwigs (Dermaptera: Forficulina) – two small, enigmatic suborders of tropical insects which are ectoparasitic and live on mammals. These are Hemimerina associated with African rats and Arixenina living on Asian bats. Since the representatives of these two groups are strongly adapted for ectoparasitic life conditions they differ markedly from the remaining "orthodox" earwigs. Moreover, the systematic position of these two peculiar orders is still unknown and under the debate. Here we present, results of preliminary histological studies of the ovary structure and early stages of oogenesis in the representative of Arixenina, *Arixenia esau*. The specimens were collected in Deer Cave, Mulu National Park, Sarawak (by PK), inhabited by colonies of *Cheiromeles torquatus* bats.

The ovaries of *Arixenia* are paired and composed of several ovarioles of the meroistic-polytrophic type. Our histological studies have shown that the anterior part of each "mature" ovariole (i.e. the germarium) is filled with numerous 2-cell cysts (clusters) of germline cells. Surprisingly, the growth of neighboring cysts is not synchronous. As a result young cysts were observed next to already differentiated ones. These observations suggest that in *Arixenia*, the neighboring cysts are formed independently, i.e. from different cystoblasts; similarly as it was described in derived earwigs (for further details see Tworzydło et al., 2009). In the posterior part of the germarium, 2-cell cysts differentiate and become invested with somatic cells. Resulting ovarian follicles consist of an oocyte and single, presumably polyploid nurse cell, and are surrounded by one cell thick follicular epithelium. The nuclei of the nurse cells are roughly spherical and contain numerous chromatin aggregations as well as nucleoli. In contrast, the nurse cell nuclei of derived earwigs (forficuloids) are always markedly irregular or even "ameboidal" (Tworzydło and Biliński, 2008).

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The influence of topoisomerase II inhibitor – etoposide on chromatin remodeling during *Chara vulgaris* spermiogenesis

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DNA topoisomerase II plays essential role in animal spermiogenesis. Changes of structure chromatin of spermatid are connected with appearance of transient DNA breaks in certain moment of spermiogenesis since a significant number of nucleosomal DNA supercoils is eliminated from mature spermatids. These breaks appear in whole population of elongated rat and chicken spermatids in mid-spermiogenesis when packing DNA structure, specific for spermatozoids, takes place (Laberge and Boissonneault, 2005). One of the earliest reactions of eukaryotic cells to DNA double-strend breaks in the chromosomal DNA is phosphorylation of the H2A histones, which is very conservative process and is observed in different organisms.

Immunocytochemical studies of *Chara vulgaris* spermatids with the use of antibodies against H2AX histone phosphorylated at serine 139 revealed endogenous double DNA breaks in mid-spermiogenesis (stages V–VII), when chromatin remodeling connected with exchange of nucleohistones into nucleoprotamines takes place (Wojtczak et al., 2008).

Topoisomerase II activity can be inhibited by different inhibitors, e.g. etoposide, suramin. In spermatids *C. vulgaris* after 24-h treatment with etoposide (200 M) a positive weak immunoreaction was observed solely at stage V spermiogenesis in spermatid nucleus.

Comparative percentage analysis of spermatids at all stages of spermiogenesis both in the control and after application of etoposide revealed prolongation early stages: 64/sp-II and 64/sp after 24-h and 48-h treatment with inhibitor, respectively, since an increase in the number of spermatids was observed. At stages IV, V, IX after 24-h and at stages I–IV after 48-h treatment with etoposide a spermatid frequency decreased.

Preliminary ultrastructural research of spermatids showed that etoposide causes disturbances at stages VII–VIII in process of chromatin transformation from somatic to generative form which is characteristic of spermatozoid formation. This will be the subject of further studies.

Above observations confirm earlier hypothesis that topoisomerase II plays significant role also during Chara spermiogenesis, which is similar to mammal spermiogenesis.

ACKNOWLEDGMENTS: This work was supported by the grant 505/390 from University of Łódź.

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Postnatal development of intestinal crypts in the Djungarian hamster

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The aim of the study was an observation of the development of intestinal crypts in the early postnatal period of Djungarian hamster (*Phodopus sungorus*).

To document the regional structural changes in the developing hamsters' small and large intestine tissues from 0-, 3-, 7- and 14-day-old hamsters were analyzed by light microscopy. Segments of duodenum, ileum, jejunum, proximal and distal colon were studied independently at all ages.

The results of the microscopic analysis revealed that the process of crypt formation does not occur synchronously in the various segments of intestine. Well defined short crypts were observed already at birth in the ileum and distal colon, whereas the duodenal, jejunal and proximal colonic crypts appeared during the first 3 postnatal days. At birth, the proximal segments of the small intestine showed cell aggregates developing at the base of the villi, while the proximal colon at all levels of the mucosal folds. These cell aggregates evolved into rudimentary crypts which are fully differentiated by day 14. In the time from birth until age of 14 days the crypts gradually increased in number and depth in all segments of the intestine.

Characteristics of enterocytes in the small intestine of Djungarian hamster during early postnatal development

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The populations of epithelial cells associated with the villi changed during the development of Djungarian hamster (*Phodopus sungorus*).

At birth, enetrocytes had a low columnar shape. In the distal portion of the small intestine they have large supranuclear vacuoles as a result of endocytotic activity during fetal life. Consequently, these enterocytes are higher compared with those in the more proximal segments of the intestine.

During the first 7 days of hamsters' postnatal life, a gradual increase in the height of epithelial cells is observed along the entire length of the small intestine, where it is the most distinct in the distal region. There is also an increase in the degree of vacuolization of enterocytes located in the distal region of the intestine, often causing compression of the cell nucleus. In the second week after birth the enterocytes show further increase in height in the following segments of the intestine, except the distal part of the intestine, where height of enterocytes decreases. This is probably due to the loss of supranuclear vacuoles, which are no longer observed in cells of distal intestine of 14-day-old specimens.

We postulated that in Djungarian hamster between 7 and 14 days after birth vacuolated enterocytes presenting the fetal type of cells are sequentially replaced by the adult population of enterocytes. This indicates that the matured appearance of the enterocytes is observed in the small intestine of two-week-old animals.

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