ORAL PRESENTATION

Regulation of MPF activity in fertilized maturing mouse oocytes

Anna Ajduk[1,2], Maria A. Ciemerych[1], Karl Swann[2], and Marek Maleszewski[1]

[1]*Department of Embryology, Institute of Zoology, Warsaw University, Miecznikowa 1, 02-096 Warsaw, Poland*
[2]*Department of Obstetrics and Gynaecology, Wales College of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK, *e-mail: aajduk@biol.uw.edu.pl*

MPF (***M**-phase **p**romoting **f**actor*) is an essential regulator of meiotic division in oocytes. It consists of catalytic subunit CDK1 / $p34^{cdc2}$ (***c**yclin-**d**ependent **k**inase1*) and regulatory subunit – cyclin B. During meiotic maturation of the mouse oocyte MPF activity increases, as oocytes progress towards metaphase of the first meiotic division (metaphase I). Next MPF activity is reduced at metaphase I/anaphaseI transition and rises again in the metaphase of the second meiotic division (metaphase II) (Verlhac et al., 1994). After fertilization of metaphase II oocyte, MPF activity is diminished in process involving calcium-dependent proteolysis of cyclin B (Nixon et al., 2002; Verlhac et al., 1994).

Spermatozoa introduced into maturing oocytes trigger transient calcium increases in ooplasm, which are similar to these observed after fertilization of mature, metaphase II oocytes, but less numerous (Carroll et al., 1994). We showed that polispermic (about 4-5 spermatozoa per an oocyte) fertilization of maturing oocytes 2h after resumption of meiosis is accompanied by downregulation of MPF activity. One and 2 hours after this precocious fertilization we observed 1,5-fold decrease in MPF activity. However it is not caused by proteolysis of cyclin B, since fertilization of maturing oocytes did not affected the cyclin B level. Moreover, the decrease in MPF activity is not dependent on increase of the concentration of Ca^{2+}. In experiments in which we preincubated maturing oocytes in Ca^{2+}-chelator, BAPTA prior to fertilization, MPF activity was also diminished.

There are two main conclusions from our data. Firstly, a signal transduction pathway, which acts between the activating factor delivered by spermatozoon, and cyclin B proteolysis does not function in maturing oocytes. Secondly, there must be other mechanism that enables a decrease in MPF activity in fertilized maturing oocytes. It might involve changes in the phosphorylation of CDK1, a catalytic subunit of MPF. Further experiments are planned in order to elucidate this problem.

REFERENCES:

CARROLL J, SWANN K, WHITTINGHAM D, WHITAKER M. 1994. Spatiotemporal dynamics of intracellular $[Ca^{2+}]i$ oscillations during the growth and meiotic maturation of mouse oocytes. *Development* 120: 3507–3517.

NIXON VL, LEVASSEUR M, MACDOUGALL A, JONES KT. 2002. Ca^{2+} oscillations promote APC/C-dependent cyclin B1 degradation during metaphase arrest and completion of meiosis in fertilized mouse eggs. *Curr Biol* 12: 746–750.

VERLHAC MH, KUBIAK JZ, CLARKE HJ, MARO B. 1994. Microtubule and chromatin behavior follow MAP kinase activity during meiosis in mouse oocytes. *Development* 120: 1017–1025.

Formation of a pool of diplotene oocytes in juvenile ovaries of *Rana temporaria* (Amphibia, Anura)

Renata Augustynska[1], Jerzy Ihnatowicz[2], and Maria Ogielska[1]

[1]*Department of Biology and Conservation of Vertebrates, Zoological Institute, Wrocław University, Sienkiewicza 21, 50-335 Wrocław, e-mail: ogielska@biol.uni.wroc.pl*
[2] *Institute of Electronics, Silesian University of Technology, Akademicka 16, 44-100 Gliwice, Poland*

Oocytes produced by a female amphibian are descendants of a species-specific number of primordial germ cells (about 60 in *Rana temporaria*). A still open question concerns time and mode of formation of several generations of oocytes produced by a female during her whole life span. Two hypotheses are discussed: 1) the most common that a new portion of oocytes is generated each year after spawning from rudimental patches of primary oogonia, which multiply and enter meiosis, and 2) that all oocytes are produced during juvenile period and then are recruited from the pool after each breeding season (mammalian model). Our former results were devoted to early juvenile period (before I hibernation) and indicated that the mean number of diplotene oocytes per female was 6960. This number is equal to not more than 3 spawnings but is not sufficient for the rest of sexual activity (Kotusz et al., 2002). The aim of the present study was to estimate the number of oocytes during the second and third year of juvenile period. We analyzed ovaries of 67 individuals (1- and 2-year-old frogs). The age was estmated by skeletochronology. Ovaries were grupped into 4 groups according to oocyte size. The number of oocytes was estimated by sterelogy on serial sections of a piece of an ovary, and then extrapolated to both ovaries. Our results indicate that the number of oocytes initially increases, reaches the maximum (mean 54 480), and then rapidly decreases (mean 23 440). The decreasing number of ooctes is accompanied by massive atresia. The final number of oocytes is sufficient for 12 spawnings, which is much beyond the longevity of this species. In conclusion we can say that the number of diplotene oocytes in a female amphibian is established during juvenile period in fashion, which strongly resembles the mammalian model.

REFERENCES:

KOTUSZ A, IHNATOWICZ J, OGIELSKA M 2002. Estimation of diplotene oocyte number in juvenile ovaries of the grass frog, *Rana temporaria* (Amphibia, Anura) by stereology. XXV Embryological Conference, Wrocław. *Zool Pol* 47/Suppl: 31–33.

Asymmetric distribution of organelles in the oocytes of an African tiger beetle

Agnieszka Baster and Mariusz K. Jaglarz

Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Cracow, Poland, e-mail: jagla@zuk.iz.uj.edu.pl

It is well established that the framework for embryonic development is created during oogenesis as oocytes are loaded with a variety of organelles, nutrient materials and maternally derived developmental information. It appears that the spatially and temporally regulated localization of these cytoplasmic components in discrete oocyte regions is a prerequisite for a proper course of early embryogenesis. In insect oocytes, the organelles and other cytoplasmic constituents are usually evenly distributed within the ooplasm. Local asymmetries in the ooplasmic architecture have only been described in a handful of insect species (Bradley et al., 2001; Jaglarz et al., 2003).

Here we report an early asymmetry in the distribution of organelles in the previtellogenic oocytes of an African tiger beetle (Coleoptera-Adephaga, Cicindelidae). The ovary in this species is of the meroistic-polytrophic type. We have followed the events leading to the formation of the organelle aggregate within the ooplasm and the ultimate fate of its components. Ultrastructural studies have demonstrated that the aggregate consists of mitochondria, Golgi complexes, vesicular structures and accumulations of electron-dense granulo-fibrillar material. These organelles originate in nurse cells and are transported *via* intercellular bridges and the nutritive appendix into the oocyte. Within the ooplasm, some of these organelles accumulate in the vicinity of the oocyte nucleus, forming an aggregate resembling *Xenopus* mitochondrial cloud or Balbiani body. Subsequently, this aggregation of organelles disperses and some of its components migrate towards the posterior pole of the oocyte, where they appear to participate in the formation of the pole plasm or ooso.

ACKNOWLEDGEMENTS: We are indebted to Prof. Szczepan Bilinski for providing specimens for this study.

REFERENCES:

BRADLEY JT, KLOC M, WOLFE KG, ESTRIDGE BH, and BILINSKI SM. 2000. Balbiani bodies in cricket oocytes: Development, ultrastructure, and presence of localized RNAs. *Differentiation* 67: 117–127.

JAGLARZ MK, NOWAK Z, and BILINSKI SM. 2003. The Balbiani body and generation of early asymmetry in the oocyte of a tiger beetle. *Differentiation* 71: 142–151.

Clone and cloning in amphi- and apomictic Angiospermae

Romana Czapik

Jagellonian University, Institute of Botany, Department of Plant Cytology and Embryology, Grodzka 52, 31-044 Cracow, Poland, e-mail: ubczapik@ cyf-kr.edu.pl

The terms clone and cloning, referring mostly to humans and animals, involve many scientific and ethical problems and are popular in discussions in which a clone means an exact genetic copy of a molecule, cell, plant, animal or a human. In botany the question has a specific theoretical meaning in relation to apomixis. Some authors claim that the progeny of an apomict forms a clone. One may ask whether that opinion connected with a problem of the relation between vegetative and apomictic reproduction is justified.

The first who coined the term "clone" was Webber, (1903). His definiton is relevant to the vegetative reproduction *in vivo* and means "a group of plants that are propagated by the use of any forms of vegetative parts such as bulbs, tubers, cuttings, grafts, etc. and which are simply parts of the same individual seedling."

In the course of the years the Webber's definition has been changed and the stress has been put on the asexual not purely vegetative reproduction. For instance:

- "Clone – a collective name for all the plants asexually reproduced from a common ancestor" (Knight, 1948).
- "Clone, a group of organisms descended by mitosis from a common ancestor, i.e. multiplied by asexual reproduction. Webber (1903)" (Darlington and Mather, 1949)
- "Clone (Webber, 1903) – a population of cells or organisms derived from a single cell or a common ancestor by mitoses. The mode of reproduction giving rise to a clone is asexual. A clone is not necessarily homogenous and therefore the terms clone or cloned should not be used to indicate homogeneity in a population." (Rieger et al., 1976).

The changes of the definition introducing term "asexual reproduction" and the possibility of a clone development from a single cell allowed to speak about cloning in apomixis and *in vitro* cultures. The view that the progeny of an apomict is a clone involves not always accepted opinion that at least some forms of vegetative reproduction belong to apomixis. However, the homogeneity of a clone as well as of a apomictic progeny cannot be expected both in facultative and obligatory apomixis.

Considering every thing, cloning in apomixis is a theoretical and terminological problem waiting for progress of the studies and for a generally accepted classification of apomixis.

REFERENCES:

DARLINGTON CD, and MATHER K. 1949. *The Elements of Genetics*. Allen & Unwin, London.

KNIGHT RL. 1948. *Dictionary of genetics*. Waltham Mass, USA.

RIEGER R, MICHAELIS A, and GREEN MM. 1976. *Glossary of Genetics and Cytogenetics.* 4th edition. Gustav Fischer, Jena.

WEBBER HJ. 1903. New horticultural and agricultural terms, *Science* 19: 501.

Adult muscle precursors/satellite like cells during the development of adult muscles of *Drosophila melanogaster*

Małgorzata Daczewska[1], Teresa Jagla[2], Cedrik Soler[2] and Krzysztof Jagla[2]

[1]Department of General Zoology, Zoological Institute, University of Wrocław, Sienkiewicza 21, 50-335 Wrocław, Poland
[2]INSERM U. 384, 28 Place Henri Dunant, 63000 Clermont Ferrand, France, e-mail: daczew@biol.uni.wroc.pl

During *Drosophila* development, two waves of myogenesis can be distinguished: the early embryonic wave, giving rise to the larval musculature; and the imaginal wave that leads to the formation of muscle of adult fly. *Drosophila* somatic muscles arise from muscle progenitor cells of mesodermal origin, which express a high level of *twist* (*twi*). Individual larval muscle fibres are derived from specialized myoblasts called founder cells. Muscle progenitor cells are thought to divide asymmetrically and produce a pair of sibling cells: either muscle founders or the precursors of adult muscles (aP cells). During the metamorphosis, nearly all larval muscles degenerate and are replaced by a set of adult-specific muscle. Myoblasts (aP cells) that migrate out of the imaginal disc contribute to the formation of these muscles. While most adult muscles in the thorax develop *de novo*, one set of indirect flight muscles, the dorsolongitudinal muscle (DLMs), develops by the fusion of imaginal myoblasts with persisting larval muscles, which serve as a scaffold for assembly of DLMs.

The aim of our study was to analyze when and where aP cells appear during the development and if in adult muscle some aP cells remain as satellite cells. To follow aP cells during the pupal stages and in the adult fly we generated several compound transgenic lines: 1151Gal4; UASGFP, Myosin-tauGFP; 1151GAL4x UASdsRed, Myosin-tauGFP; Twist-lacZ, 1151Gal4; TwistlacZ × UASGFPlns. Our observation indicate that in pupa stages aP cells are usually connected with neurons, and express Twist protein and 1151GFP. During the pupal life the number of aP dramatically decreases and in newborn fly a few aP/satellite like cells remain connected with neurons in thorax, abdomen and legs.

Analysis of NADH-dependent dehydrogenases activity in mouse sperm mitochondria

Aniela Gołas, Paulina Małek, and Józefa Styrna

Department of Genetics and Evolution, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Cracow, Poland, e-mail: aniela@autocom.pl

In our Department we crossed two parental strains of mice – KE and CBA/Kw – that differ in gametes quality (Krzanowska et al., 1995) to obtain recombinant inbred (RI) strains. Thus, the set of RI strains enables to map genes controlling quantitative traits (QT) such us oocyte and sperm quality parameters (Gołas et al., 2001).

The aim of this study was to measure the activity of mouse sperm mitochondrial NADH-dependent dehydrogenases. During the cytochemical reaction NADH-dependent dehydrogenases translocate a hydrogen from exogenous NADH to the artificial acceptor NBT (nitro blue tetrazolium salt). Reduced NBT forms dark blue deposits (diformazans). The intensity of the reaction depends on the activity of the dehydrogenases and can be measured by densitometry (Quantimet 600S, Cambridge, UK) (Piasecka et al., 2001). Four characteristics were estimated:

- integrated optical density (IOD): the sum of the optical densities of pixels along the sperm midpiece
- mean optical density (MOD): the average optical density of the pixels along the midpiece
- the midpiece length
- the area of the midpiece

Our initial study revealed that spermatozoa from KE and CBA/Kw strains have different cytochemical reaction intensity (both MOD and IOD, $p<0.001$ and $p<0.01$ respectively), midpiece length and area ($p<0.02$ and $p<0.001$, respectively). We correlated the strain distribution pattern (SDP) of these four characteristics in 10RI strains with the SDP of microsatellite markers (MapManager QTX software) (Manly et al., 2001) to find the chromosomal regions containing hypothetical genes controlling these parameters.

REFERENCES:

KRZANOWSKA H, STYRNA J, and WABIK-SLIZ B. 1995. Analysis of sperm quality in recombinant inbred mouse strains: correlation of sperm head shape with sperm abnormalities and with the incidence of supplementary spermatozoa in the perivitelline space. *Journal of Reproduction and Fertility* 104: 147–154.

GOŁAS A, GRZMIL P, MULLER CH, and STYRNA J. 2004. Chromosome 7q11 controls sperm beat cross frequency (BCF) in mice. *Folia Biologica (Cracow)* 52: 211–217.

PIASECKA M, WENDA-RÓZEWICKA L, and OGONSKI T. 2001. Computerized analysis of cytochemical reactions for dehydrogenases and oxygraphic studies as methods to evaluate the function of the mitochondrial sheath in rat spermatozoa. *Andrologia* 33: 1–12.

MANLY KF, CUDMORE RH Jr, and MEER JM. 2001. Map Manager QTX, cross-platform software for genetic mapping. *Mammalian Genome* 12: 930–932.

Spermiogenesis in the *Danio rerio* (Teleostei: Cyprinidae). Ultrastructural study

Joanna Huszno

Department of Animal Histology and Embryology, Silesian University, Bankowa 9, 40-007 Katowice, Poland

The ultrastructure of male gonads of *Danio rerio* is similar to that described for other teleostean species. It belongs to tubular, unrestricted type with cystic spermatogenesis.

In the process of spermiogenesis 4 stages were distinguished. At the first stage the spermatid is characterised by round nucleus, enclosed within regular envelope, that has small indentation (the fossa) on one of the poles. In this place the proximal centriole will be localised. In the cytoplasm there are numerous mitochondria gathered in the vicinity of the nucleus. On the pole opposite to the fossa, Golgi Aparatus is located. Beside the above organelles, long cisternae of the endoplasmic reticulum are scattered throughout the cytoplasm. During whole spermatogenesis the germ cells are connected by cytoplasmic bridges and surrounded by the cytoplasm of Sertoli Cells.

In the second stage the spermatids are smaller, have more compact chromatin in their nuclei and are devoid of Golgi Aparatus. The centrioles are located in the fossa, which has become deeper. The distal centriole settle down at an angle of 40 degrees with the proximal centriole. Near to the centrioles, under the nucleus mitochondria are gathered. Close to the emerging flagellum drop of cytoplasm with numerous ribosomes, vacuoles and membranes, is seen.

During the third stage of spermiogenesis, the connection between Sertoli cells and spermatids become weaker and first spermatids are released to the lumen. Each spermatid has still more compact chromatin in its nucleus, it has flagellum connected to the distal centriole and encircled with numerous mitochondria.

In the fourth stage the nuclei have a form of regular spheres with chromatin completely condensed, From the nucleus, the flagellum is attached at 40 degrees to the sperm head diameter. The base of the flagellum has an envelope of mitochondria. The acrosom is absent. The mitochondria are C- shaped and located around the initial segment of the axoneme. Such a structure of spermatozoa is typical for teleostean fish with external fertilization.

Different patterns of myotomal muscle fibre formation in fishes

Agata Kacperczyk[1], Przemysław Pokorny[2], and Małgorzata Daczewska[1]

[1]*Department of General Zoology, Zoological Institute, University of Wrocław, 50-335 Wrocław, Sienkiewicza 21, Poland, e-mail: kacperczyka@biol.uni.wroc.pl*
[2]*Laboratory of Limnology and Fishery, Agricultural University, 51-630 Wrocław, Chełmonskiego 38c, Poland*

Comparative studies on myotomal myogenesis show that in fishes the process does not follow a common developmental pathway.

In a salmonid (*Coregonus lavaretus*) two myotomal cell populations, which start playing their myoblast role at different times, have been distinguished. The first to start differentiation are the cells that build the somite wall. They elongate centripetally and fuse to form multinucleated myotubes. The mononucleate cells, which initially occupied the somite center, remain situated between the myotubes, and continue their division. These mononucleate cells do not fuse with each other and their role consists in fusing with already existing myotubes. This suggests that in *C. lavaretus* multinucleate myotubes, in early myogenesis, are of myotomal/mesodermal origin.

In an esocid (*Esox lucius*) and a percid (*Pterophyllum scalare*) at early stages of myogenesis, multinucleate myotubes form as a result of myoblast fusion (of myotomal origin). The myotomes are composed of homogenous population of myotomal cells. During the later stages of myogenesis, mesenchymal cells participate in muscle growth. Thus muscle fibres in these species are of mesodermal – mesenchymal origin.

In *Neoceratodus forsteri* (Dipnoi: Ceratodontidae), primary myoblasts of myotomal/mesodermal origin fuse to form multinucleated muscle lamellae. Then, the lamellae are converted into muscle fibres. At later developmental stages, myotomes are invaded by mesenchymal cells. These cells are thought to be involved in hyperplastic growth in this species.

Behaviour of myogenic cells in myogenesis of chordate skeletal muscles

Leokadia Kiełb

Department of General Zoology, Zoological Institute, University of Wrocław, Sienkiewicza 21, 50-335 Wrocław, Poland

Myogenic cells of chordate skeletal muscles are of mesodermal origin. Their source is somites. Somite myogenic cells differentiate in situ or leave the somite and migrate to pre-determined places, e.g. bird limb bud or ventral side of amphibian tadpole. Myogenic cells migrating from the bird dermatomyotome contain regulatory protein Pax3. Besides, cells migrating to myotomes have been found in many fish and amphibian species. Their origin is unclear. Presumptive myogenic cells of the somite in Amniota receive signals emitted by axial organs (notochord, neural tube), but mesenchymal cells migrating to limb buds are influenced by somatopleura. Cells differentiating into musculus rectus abdominis in the tadpole are probably also under the effect of somatopleura, whereas cells that have migrated into the myotomes are most probably influenced by myotubes. Migrating myogenic cells, irrespective of their ultimate location, differentiate according to a similar model. Following active proloferation, they withdraw from the cell cycle at G1 (G0) phase, and fuse into multinucleate myotubes. Contrary to migrating myogenic cells,cells differentiating in situ in the myotome follow a variety of patterns.

The induction process is best known in Amniota. Diffusion proteins Wnt and Shh, emitted from axial organs, activate genes of regulatory proteins. Differentiation of muscle cells itself occurs only in tunicate tail, under the effect of determinants. The determinants are present only in certain ooplasm fields. Accumulation of transcript of the gene coding for regulatory proteins has been recently detected in these fields.

Regulatory proteins of MyoD family are present only in myogenic cells of chordate skeletal muscles. The family includes four proteins: MyoD, myogenin, MRF4, myf-5. With other proteins, MEF2 and E-12, they activate structural muscle genes. MyoD and myf-5 determine and activate myogenic cells. The remaining proteins appear at later stages of myogenesis. Only in mammals myogenesis is initiated by myf-5.

Chordate myogenic cells show a very diverse range of behaviours during myotomal myogenesis. The fact is associated with the chordate phylogeny.

Transcription activity of germ line cells and follicular epithelium differentiation in Nematocera

Marta Mazurkiewicz and Janusz Kubrakiewicz

Department of General Zoology, Zoological Institute, Wrocław Uniwersity, Sienkiewicza 21, 50-335 Wrocław, Poland, e-mail: marcysia@biol.uni.wroc.pl

Massive production of ribosomes is one of the major events during the egg cell formation. Although different strategies can be employed to enable the intense ribosome synthesis during oogenesis, all of them seem to depend on the increase in the number of transcriptionally active ribosomal genes (= rDNA). In meroistic ovaries the number of ribosomal genes is significantly magnified in the polyploid genome of the nurse cells. There are also some well documented examples of selective replication of rDNA within the oocyte nucleus, a process known as extrachromosomal amplification. Comparative analysis of the ovary structure and function among nematoceran flies indicates that while in some groups the ribosome synthesis is entirely taken over by the highly polyploid nurse cells (Psychodomorpha), in the others it depends predominantly on the high level of rDNA amplification in the oocyte nucleus (Polyneura, Ptychopteridae). In the latter case the transcriptional activity of the nurse cells is conspicuously limited. Interestingly, in the representatives of Culicomorpha (e.g. Chironomidae, Chaoboridae), both, highly polyploid nurse cells and transcriptionally active oocyte nucleus containing amplified copies of ribosomal genes seem to contribute to the total ribosome production.

The engagement of the nurse cells in the synthetic activity noticeably conditions their behavior during oogenesis. Active, polyploid nurse cells function till the final stages of oogenesis, while those with limited activity are eliminated earlier.

The major dipteran lineages may significantly differ in the mode of follicular cells' differentiation. For instance, in contrast to the situation found in true flies (Brachycera), in which follicular cells undertake even long distance migrations within the egg chamber, in nematoceran ovaries the follicular cells do not migrate at all. Although this "rule of immobility" seems to be obeyed throughout Nematocera, particular subgroups may exhibit different temporal sequence of the events during the differentiation of their follicular cells. Our comparative studies suggest that the sequence of events during the follicular cells' differentiation in Nematocera is, to some extent, influenced by the the behavior of the nurse cells.

Flow cytometric analysis of DNA content in the embryo, endosperm and young seedlings of *Rudbeckia bicolor* Nutt.

Krystyna Musiał[1], Maria Koscinska-Pajak[1], and Elwira Sliwinska[2]

[1] Department of Plant Cytology and Embryology, Jagiellonian University, Grodzka 52, 31-044 Cracow, Poland, e-mail: k.musial@iphils.uj.edu.pl
[2] Department of Genetics and Plant Breeding, University of Technology and Agriculture, Kaliskiego 7, 85-789 Bydgoszcz, Poland

Hemigamy is a type of fertilization in which a sperm nucleus entered the egg cell, but does not fuse with its nucleus. The egg and sperm nuclei divide independently giving rise to a mosaic embryo. The occurrence of this process was described in *Rudbeckia bicolor* Nutt. embryo sacs.

In the studied material a sperm was often noticed inside an egg cell cytoplasm, but the fusion of those nuclei as well as their divisions were not observed. However, no distinct differences in size of nuclei of embryo cells were found.

The cytological analysis confirmed the karyological diversity in young seedlings; euploid (2n = 38, 57, 76) and aneuploid (2n = 48, 64, 66, 68) chromosome numbers occurred in root tips. The metaphase plates with haploid chromosome number were not stated. Therefore, the flow cytometry was applied to estimation of the DNA content in the cells of embryo, endosperm and young seedlings. Flow cytometric analysis showed the 2C and 5C DNA amount in seeds whereas 2C (14,5 pg) in cells of shoot apex, cotyledons and first leaves derived from seedlings. These results have revealed that in studied material embryo and endosperm developed after double ferilization and that the embryos do not show mosaic structure.

The results of DNA content measurement suggest that the hemigamy is not a common process within species of *Rudbeckia bicolor.* Therefore it seems that the occurrence of hemigamy is presumable limited to the selected cultivars of this plant. The karyological diversity observed in root tips of seedlings is most probably not connected with the hemigamic embryos origin as it was suggested in previous study.

Organization of the hermaphroditic gonad and transovarial transmission of endosymbiotic bacteria in *Icerya purchasi* (Insecta, Hemiptera, Coccinea: Monophlebidae)

Sylwia Niznik and Teresa Szklarzewicz

Department of Systematic Zoology and Zoogeography, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Cracow, Poland, e-mail: nsylvia@poczta.fm

The cottony cushion scale, *Icerya purchasi* is a functional hermaphrodite. The paired gonads of the first instar are filled with numerous germ cells. In this stage of development, both male part as well as female part of the gonad look similar. In the second instar, the germ cells are segregated into two groups: large, diploid cells and small, haploid ones. The diploid cells develop into the female gametes, whereas smaller cells develop into the male gametes. The last generation of the oogonial cells termed cystoblasts undergoes 3 cycles of incomplete mitotic divisions resulting in the formation of a cluster of 8 cells termed cystocytes. Each cluster of cystocytes forms a rosette, in the centre of which a polyfusome is present. In the close vicinity of the rosettes, numerous large cells termed bacteriocytes occur. Each bacteriocyte is surrounded with small epithelial cells. Both bacteriocytes as well as epithelial cells are packed with endosymbiotic microorganisms. In bacteriocytes large pleomorphic bacteria occur, whereas in epithelial cells small rod-shaped microorganisms are present. The rod-shaped bacteria are less numerous than pleomorphic ones. In the third instar, the female part of the gonad is composed of young ovarioles, while the male part consists of binucleate spermatids. The gonad of the adult hermaphrodite is composed of numerous, short telotrophic ovarioles and a long testicular tubule. Each ovariole consists of a trophic chamber (tropharium) and vitellarium. The tropharium encloses 7 trophocytes (nurse cells). In the vitellarium a single oocyte develops. The centre of the tropharium, termed a trophic core, is devoid of cells and is filled with microtubules, ribosomes and mitochondria. Both trophocytes and oocytes are connected to the core: trophocytes by broad cytoplasmic processes, oocytes my means of nutritive cords. The trophocyte cytoplasm is tightly packed with numerous rod-shaped bacteria which through the nutrive cord migrate into the oocyte. The oocytes in the older ovarioles are invaded by pleomorphic microorganisms. Both the ovarioles and the testicular tubule are connected to the common duct that is filled with numerous bundles of sperm cells.

ACKNOWLEDGEMENTS: This work was supported by KBN grant 2 P04C 004 27.

Testis structure, spermatogenesis and spermatozoon ultrastructure in a four-eyed fish *Anableps anableps* L. 1758 (Teleostei: Atherinomorpha: Cyprinodontiformes: Anablepidae)

Anna Pecio[1], John R. Burns[2], and Harry J. Grier[3]

[1]Department of Comparative Anatomy, Institute of Zoology, Jagiellonian University, Cracow, Poland
[2]Department of Biological Sciences, George Washington University, Washington, DC, USA
[3]Fish and Wildlife Research Institute, St. Petersburg, Florida, USA, e-mail: pecio@zuk.iz.uj.edu.pl, jrburns@gwu.edu, Harry.Grier@MyFWC.com

The testis of *Anableps anableps* is classified as a restricted lobular testis type or atherinomorph testis type (Parenti and Grier, 2004) because of the distribution of spermatogonia at the distal ends of the lobules, which lack lumina. Sertoli cells form the spermatocysts in which developing sperm mature synchronously. Spermatocysts have a nearly linear arrangement within the lobules in which they move centrally toward the vasa efferentia, where free spermatozoa are released.

In early spermatids, the process of spermiogenesis begins with formation of the flagellum and migration of mitochondria along and around the developing cytoplasmic canal, lying obliquely to the nucleus. A prominent Golgi apparatus is found in the vicinity of the centriolar complex. Subsequently, elongation of the nucleus occurs alongside the basal body and elongating flagellum. A shallow fossa develops on the flagellar side of the nucleus while chromatin condensation begins at its periphery. Rotation of the nucleus occurs next, causing the flagellar axis to become perpendicular to the base of nucleus.

During spermiogenesis, a variable arrangement of the spermatids within a spermatocyst was observed. Early spermatids, which are often connected by intercellular bridges, were randomly distributed throughout the cysts. However, during nuclear elongation and rotation, the later spermatids have their heads oriented towards one pole of the cyst and the flagella at the opposite. After nuclear rotation, all spermatids become oriented radially, with their heads embedded in the cytoplasm of the Sertoli cells and their flagella projecting into the center of the cyst.

In the mature spermatozoon, the nucleus has compact chromatin and forms an elongate cone with a deep implantation fossa containing both centrioles in perpendicular arrangement. The distal centriole, which forms the basal body, is very long and possesses an amorphous electron dense structure on one side. The midpiece is comprised of a long mitochondrial sheath containing many separate mitochondria with longitudinal cristae. They lie in a cytoplasmic sleeve in longitudinal rows. In a cross section of the mitochondrial sleeve, two to four C-shaped mitochondria, surrounding the flagellum, are located along the cytoplasmic canal. The 9+2 flagellum has two lateral fins present only at the posterior part in the cytoplasmic canal and just beyond; fins are absent along the rest of flagellum.

The results of this study are discussed in view of Sertoli cells-germ cells relationships, adaptations to internal fertilization, and the phylogenetic relationships between Anablepidae and the closely related Poeciliidae.

ACKNOWLEDGEMENTS: This work was supported by the research grant BW/IZ/28B/2004.

Fusomorfogenesis in *Utricularia intermedia* Hayne

Bartosz J. Płachno[1*], Piotr Swiatek[2], and Andrzej Jankun[1]

*[1]Department of Plant Cytology and Embryology, Jagiellonian University, Grodzka 52, 31- 044 Cracow, Poland, *e-mail: bartek78pl@poczta.onet.pl*
[2]Department of Animal Histology and Embryology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland, e-mail: swiatek@us.edu.pl

In *Utricularia intermedia* during endosperm development micropylar and chalazal endospermal haustoria are formed. The micropylar haustorium has direct contact with the nutritive tissue of the placenta. The walls of the nutritive tissue cells, which are near the haustorium, are digested and, finally, cytoplasmic domains of these cells are fused. Then, in the wall of micropylar haustorium the openings are formed and in consequence the cytoplasmic domain of haustorium merges with earlier fused cytoplasmic domains of placental nutritive tissue cells. During this procces in the fused cytoplasm the strong activity of Golgi apparatus and ER are observed. At last, the giant heterokaryotic syncytium forms which posses two large amoeboidal-shaped, polyploid nuclei (endosperm origin) and many small placental nutritive tissue origin nuclei.

The formation of this kind of organ, a syncytium which consists of both somatic and generative components, is unique in plant kingdom and only restricted to some *Utricularia* species.

Analysis of lipids in pig embryos produced *in vivo* and *in vitro*

Marek Romek[1], Barbara Gajda[2], Ewa Krzysztofowicz[1], and Zdzisław Smorag[2]

[1]*Department of Cytology and Histology, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Cracow, Poland*
[2]*Department of Animal Reproduction Biotechnology, National Research Institute of Animal Production, 32-083 Balice / Cracow, Poland*

It is assumed that the lipid content decreases as the pig embryo reaches more advanced stages of development, but so far no data are available concerning the level and type of lipids in pig embryos. On the other hand, several experiments demonstrated the low cryotolerance of pig embryos. This sensitivity decreases as the development of the embryo progresses and is accompanied by lipid reduction. Thus, the objective of this study was to determine qualitatively and quantitatively the lipid content of pig embryos from the zygote stage to the stage of blastocyst produced *in vivo* and *in vitro* .

The experiment was carried out on pig zygotes to blastocyst stage embryos produced *in vivo*, and 2-cell to blastocyst stage embryos produced *in vitro*.

For quantitative measurements, all embryos were fixed in glutaraldehyde and osmium tetroxide and subsequently embedded in epoxy resin. Then they were cut into semi-thin sections and stained with Azure II and Methylene blue. For stereological analysis serial sectioning and Cavalieri and point counting methods were used. For each stage of development volume of embryo V(e), volume of lipid droplets V(fat) and volume density of lipid droplets Vv(fat,c) were estimated. To analyse the type of lipid droplets in the embryo cytoplasm, histochemical methods with nile blue sulfate, osmium tetroxide, oil red O and light microscopy were used.

For embryos produced *in vivo* the mean values of V(e) significantly increased from zygote (946×10^3 μm^3) to blastocyst (1780×10^3 μm^3). At the same time the volume of lipid droplets V(fat) significantly decreased from zygote (243×10^3 μm^3) to blastocyst (156×10^3 μm^3). The difference between zygote and blastocyst in the volume of lipid droplets was accompanied by a statistical difference in Vv(fat,c). For embryos produced *in vitro* the time courses of V(e), V(fat) Vv(fat,c) were similar to those found in the embryo *in vivo*. In lipid droplets of embryos produced both *in vivo* and *in vitro* we detected unsaturated hydrophobic lipids, free fatty acids and phospholipids.

In conclusion, the lipid content of pig embryos produced both *in vivo* and *in vitro* decreased from the zygote to the blastocyst stage.

ACKNOWLEDGEMENTS: Research was funded by the State Committee for Scientific Research (Project No. 2 P06D 003 26).

Regeneration of the midgut epithelium in *Podura aquatica* L. (Insecta, Collembola, Arthropleona)

Magdalena M. Rost

Silesian University, Department of Animal Histology and Embryology, Bankowa 9, 40-007 Katowice, e-mail: mrost@us.edu.pl

The midgut epithelium of Podura aquatica L. (Insecta, Collembola, Arthropleona) is formed by the epithelial and regenerative cells. The epithelial cells show distinct regionalisation in the organelles' distribution. The ultrastructure of the basal, perinuclear and apical regions of the epithelial cells is described. Regenerative cells, which do not form regenerative groups, are arranged between epithelial cells. Their mitotic divisions were not observed.

The midgut epithelium is removed together with each molting. As the shedding of the old epithelium approaches, the number of structures, which resemble urospherites, in the cytoplasm of the midgut epithelial cells significantly increases. Small vesicles and vacuoles appear and increase in number. A decrease in the amount of the rough and smooth endoplasmic reticulum, dictyosomes, mitochondria and free ribosomes is observed. These changes in the ultrastructure of epithelial cells initially are observed only in individual cells but they spread through the entire epithelium. They are a morphological depiction of cellular degeneration.

After degeneration of the entire midgut epithelium, a new epithelium is formed from the regenerative cells. Some of the regenerative cells undergo transformation into epithelial cells. Initially, the apical cell membranes do not come into contact with the midgut lumen, hence microvilli are still not present on their surface. This newly formed epithelium takes position just between the basal lamina and the degenerated epithelium. Finally the latter is completely detached into the midgut lumen. The apical cell membranes of the new epithelium, reaching the midgut lumen, start to form microvilli.

The epithelium, which is detached into the midgut lumen is finally digested. Regenerative cells play a role of primordial cells (stem cells) during epithelial regeneration (Rost, 2006).

ACKNOWLEDGMENTS: I would like to express my gratitude to Dr. Piotr Swiatek from the Department of Animal Histology and Embryology (Silesian University) for collecting *Podura aquatica*.

REFERENCES:

ROST MM. 2006. Ultrastructural changes in the midgut epithelium in *Podura aquatica* L. (Insecta, Collembola, Arthropleona) during regeneration. *ASD* (in press).

Ultrastructural changes in the midgut epithelium in *Acheta domesticus* (Insecta, Orthoptera, Gryllidae) during regeneration

Magdalena M. Rost

Silesian University, Department of Animal Histology and Embryology, Bankowa 9, 40-007 Katowice, e-mail: mrost@us.edu.pl

Midgut of *Acheta domesticus* is composed of two parts: the anterior midgut, which forms two characteristic large and ovoid digestive ceca, and the posterior midgut which joins the hindgut.

Midgut epithelium is composed of the epithelial cells and regenerative cells groups. Between the regenerative cells groups 12–16 epithelial cells, which are very thin, are observed. The distinct regionalisation in organelles distribution is observed in the cytoplasm of epithelial cells. Few differences are described between epithelial cells of the anterior and posterior midgut. The lipid droplets and glycogen are only in the posterior midgut.

Among the regenerative cells groups mitotic divisions are common, but they mainly affect the regenerative cells situated just beneath the basal lamina. Regenerative cells are rather poor in organelles. Nuclei of epithelial cells in digestive ceca have prominent nucleoli composed of the filamentous part of spherical shape, which is surrounded by the granular part. Such nucleoli are not observed in the regenerative cells of the posterior midgut.

The morphological sign of epithelial cells degeneration is appearing numerous single vesicles, vacuoles, lamellar bodies and multivesicular bodies in their apical regions. The cytoplasm is being electron lucent. The apical membrane forms characteristic club-shaped invagination into the midgut lumen and finally breaks off. All organelles move into the midgut lumen, where they are digested. During that time, external regenerative cells adopt epithelial character and shift the remains of digested cells into the midgut lumen. Degeneration and regeneration proceeds much faster in the posterior midgut and they go under the continous manner.

DAPI staining was used for the detection of mitotic divisions of the regenerative cells. Study was carried using TEM and fluorescent microscope.

Organization of the reproductive system and ultrastructure of the larval ovarioles of australian stoneflies (Plecoptera: Antarctoperlaria)

Elzbieta Rosciszewska

Departament of Systematic Zoology and Zoogeography, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Cracow, Poland, e-mail: rosc@zuk.iz.uj.edu.pl

Male and female reproductive systems organization and ultrastructure of early larval ovarioles of several species representing Plecoptera inhabiting the southern hemisphere, (Suborder: Antarctoperlaria), was studied by means of light and electron microscopes (SEM and TEM). The insects under study* represent two families: Eustheniidae (*Thaumatoperla flaveola*, *T. alpina*, *Eusthenia venosa*, *Cosmioperla kuna*, *C. denise*) and Gripopterygidae (*Trinotoperla nivata*, *T. irrorata*).

The reproductive systems in Plecoptera were described (Zwick, 1973). The present investigation supplements these earlier results by SEM and TEM results.

The male reproductive system of a mature individual of *T. flaveola* consists of a paired, metameric testes with medially fused anterior ends, long sperm ducts, a seminal vesicles, a genital chamber with paired penis.

The mature female reproductive system of *T. flaveola*, consists of: the paired comb-like ovaries; lateral oviducts with anterior and posterior ends medially fused; impaired common oviduct with the spermatheca; vagina; and two genital openings. The ovary organization, in general, corresponds to the mature gonad of Arctoperlaria (Rosciszewska, 2003 as a review). It is composed of numerous panoistic ovarioles. Each ovariole lacks terminal filament; germarium degenerates; vitellarium houses numerous oocytes in consecutive stages of vitellogenesis and choriogenesis; posterior ovariole end is connected to the lateral oviduct by a prominent pedicel. The oviducts in *Thaumatoperla* are large, supported with musculature.

The fusion of ovaries and testes, mentioned above, is unique feature supporting Plecoptera monophyly (Zwick, 2000).

The early-larval ovarioles of the investigated representatives of Antarctoperlaria are composed of a functional germarium and a vitellarium housing previtellogenic ovarian follicles. In Eustheniidae, the germ cell clusters were not observed in the germaria. This is in contrast to all studied Arctoperlaria and to the presently investigated Gripopterygidae. In the mentioned family, unlikely in other Plecoptera studied, ovarioles are surrounded with thick, multileyered envelopes: the interior one, acellular; the middle, formed of cells containing secretory grains; and the external, composed of flattened cells with well developed endoplasmic reticulum filled with a flocculent material.

The phylogenetic aspects of the reported results are discussed.

REFERENCES:

ROSCISZEWSKA E. 2003. Budowa i rozwój jajników oraz choriogeneza i ultrastruktura kapsuł jajowych widelnic (Insecta: Plecoptera). Wydawnictwo Uniwersytetu Jagiellonskiego.

ZWICK P. 1973. Insecta. Plecoptera. *Das Tierreich*, Lief. 94. W. de Gruyter, Berlin.

ZWICK P. 2000. Phylogenetic System and Zoogeography of the Plecoptera. *Annual Review of Entomology* 45: 709–746.

* The examined insects were supplied by courtesy of Professor John Brittain, Oslo University, and Professor Peter Zwick of the Max Planck Istitute, Schlitz.

The ultrastructural studies of the sexually differentiated gonads in the grass snake *Natrix natrix* L.* (Lepidosauria, Serpentes) embryos

Weronika Rupik

Department of Animal Histology and Embryology, Silesian University, Bankowa 9, 40-007 Katowice, Poland, e-mail: rupik@us.edu.pl.

The ultrastructure of sexually differentiated gonads in grass snake (*Natrix natrix* L.) embryos was investigated with the use of electron microscope. The eggs of *Natrix* were incubated in the laboratory in constant temperature at 30°C and 100% relative humidity. Embryos were isolated in regular sequence of time from egg lying till hatching. The age of embryos was calculated using the table of species development (Rupik, 2002). Cells from medullary zone of the testis form the cellular cords, which give rise to seminiferous tubules. Presumptive seminiferous tubules are composed of somatic columnar cells and germ cells. The columnar cells have dark cytoplasm with large nuclei, numerous mitochondria, Golgi apparatus, moderately developed rough endoplasmic reticulum and lipid droplets. Many of columnar cells differentiated into Sertoli cells. The germ cells are larger than columnar cells and have light cytoplasm with large spherical nucleus containing one or two nucleolus. The cytoplasm of germ cells has also small amounts of rough endoplasmic reticulum and mitochondria, which are grouped in a juxtanuclear crescent. Mesenchyme cells between seminiferous tubules differentiated into steroidogenic cell, and some of them degenerated.

Ultrathin sections of female embryonic gonads show that the ovarian epithelium differentiated into follicle-like structures. The structures consist of four cell types: cuboidal small cells, round intermediate cells, pyriform cells and germ cells. Small cells and intermediate cells have mesenchyme origin, but pyriform cells probably originate from germinal cell lineage. The diameter, spherical nucleus with nucleolus and light cytoplasm of pyriform cells are similar to the germinal cells. The follicles-like structures are surrounded by the differentiated thecal envelope, which consists of two parts. External part of this envelope consists of fibroblasts rich in rough endoplasmic reticulum, but internal part consists of steroidogenic cells rich in lipid droplets and smooth endoplasmic reticulum.

REFERENCES:

RUPIK W. 2002. Normal developmental table. In: Early development of adrenal glands in grass snake *Natrix natrix* L. *(Lepidosauria, Serpentes). Adv Anat Embryol Cell Biol* 164: 24–32.

* 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 approvals of Local Ethics Commission (27/05 z 18.05.05) and Polish Ministry of Environment Protection for performing studies on protected species (DOPog-4201-02-94/05/aj). The grass snake *Natrix natrix* L. is not included in Washington Convention of 1973, ratified by Poland in 1991 (Dz.U . nr 27 poz.112).

Expression of the testis-specific 70 kDa heat shock protein (HST70) during mouse embryogenesis

Weronika Rupik[1], Ewa Małusecka[2], and Wiesława Widłak[2]

[1]*Department of Histology and Embryology, Silesian University, Bankowa 9, 40-007 Katowice, Poland*
[2]*Department of Tumor Biology, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland, e-mail: wwidlak@io.gliwice.pl*

Heat shock proteins (Hsps) are typically overexpressed after various stress conditions. However, some of them are developmentally regulated. The *Hst70* gene, which is specifically expressed in pachytene spermatocytes, encodes for the protein associated with synaptonemal complexes and is required for completion of the meiotic division. We have investigated the localization of the HST70 protein during early stages of embryogenesis by immunohistochemistry. The distribution of endogenous HST70 was compared with an activity of the *Hst70* gene promoter. This was assessed using transgenic mice expressing EGFP as a reporter gene (to obtain position-independent expression of the transgene a DNaseI-hypersensitive site 5'HS4 from the chicken β-globin gene was used as an insulator). We found the correlation between activity of the *Hst70* promoter (visible as EGFP fluorescence) and synthesis of HST70 protein. In the early stages of the mouse development (E8.5 - E9.5), when morphogenesis starts, a strong EGFP fluorescence was observed mainly in somites and developing nervous system, especially in optic vesicle. We noted the HST70 immunostaining in some cells of neural tube and neural crest, optic vesicle and somites. In older embryos (E11.5 – E15.5) EGFP fluorescence was found in a coat of the spinal cord, an anterior and posterior roots of the spinal cord, intervertebral ganglions, a cerebellum, a wall of the ventricles of the brain, arachnoid mater, a median eminence, a pituitary and eye cornea. However, we were not able to detect HST70 protein in such older embryos, even in total embryo's extracts analyzed by Western-blot analysis. We cannot exclude that distribution of EGFP fluorescence in older embryos may only reflects distribution of cells expressing the *Hst70* gene in early stage of development. In the stage E8.5–E9, embryonic cells have similar developmental potential to differentiated cancer cells. Both are highly proliferate and poorly differentiate. Presumably it is possible that in the course of normal embryonic development specific for spermatogenese Hst70 protein serves similar function to other members of Hsp70 family proteins, which promote cell growth and survival in the course of tumor genesis.

The assessment of epididymal cat (*Felis catus* L.) semen using flow cytometry method and computer-assisted sperm analysis (CASA)

Marta Siemieniuch and Andrzej Dubiel

Faculty of Veterinary Medicine, Department and Clinic of Reproduction, Ruminant Diseases and Animal Health Protection Agricultural University of Wrocław, Plac Grunwaldzki 49, 50-366 Wrocław, Poland, e-mail: msiemieniuch@interia.pl

The research was carried out on 22 epididymal sperm samples from clinically healthy male cats aged between 1–2 years using standard orchiectomy procedures.

The aim of this study was to estimate the value of epididymal sperm taking into account the percentage of live, early-apoptotic and late-apoptotic cells using flow cytometry techniques and the evaluation of sperm properties in CASA examination. Applied methods belong to the most modern techniques in andrological laboratory and allowing the prediction the fertilizing potential of semen better than subjective, traditional methods (Garner and Johnson, 1995; Pena et al., 2003; Verstegen et al., 2002). The fact of the high percentage of malformed spermatozoa in cat semen is well known and new methods allowing the assessment the fertilizing potential cat sperm are needed (Siemieniuch et al., 2002).

The percent motility (MOT) and progressive motility (PMOT) were determined using computer-assisted semen analysis and demonstrated 51.5 ± 21.8% and 26.2 ± 14.5%, respectively. The average path velocity (VAP), curvilinear velocity (VCL), straight line velocity (VSL) and amplitude of lateral head displacement (ALH) was 104.8 ± 27.5, 84.2 ± 23.2, 188.7 ± 41.7 and 7.5 ± 3.1, respectively.

The obtained data revealed 71.3 ± 8.8% live cells flushed from epididymes using LIVE/DEAD® Sperm Viability Kit (Molecular Probes). The application of the Apoptosis detection kit® (Oncogene) demonstrated that percentage of early-apoptotic and late-apoptotic in epididymal sperm was insignificant and included 0.8 ± 0.6% and 1.1 ± 0.6%, respectively.

Obtained score revealed that fresh epididymal spermatozoa have satisfying properties for preservation and application in artificial insemination techniques.

REFERENCES:

GARNER DL, and JOHNSON LA. 1995. Viability assessment of mammalian sperm using SYBR-14 and propidium iodide. *Biol Reprod* 53: 276–284.

PENA FJ, JOHANNISSON A, WALLGREN M, and RODRGUEZ-MARTNEZ H. 2003. Assessment of fresh and frozen-thawed boar semen using an Annexin-V assay: a new method of evaluating sperm membrane integrity. *Theriogenology* 60: 677–689.

SIEMIENIUCH M, DUBIEL A, BIELAS W, BŁASZKOWSKA M, and WŁODARCZYK M. 2005. Konserwacja nasienia kota domowego w rozrzedzalnikach płynnych. *Medycyna Weterynaryjna* 61: 317–320.

VERSTEGEN JP, IGUER-OUADA M, and ONCLIN K. 2002. Computer assisted semen analysis in andrology research and veterinary practice. *Theriogenology* 57: 149–179.

Metal-tolerant genotype of *Viola tricolor* L. occurs on calamine spoil heap in Bolesław in the vicinity of Olkusz (S. Poland)

Aneta Siuta[1], Marta Libik[2], Grazyna Szarek-Łukaszewska[3], Robert Konieczny[1], and Elzbieta Kuta[1]

[1]*Department of Plant Cytology and Embryology, Jagiellonian University, Cracow, Poland, e-mail: e.kuta@iphils.uj.edu.pl, a.siuta@iphils.uj.edu.pl*
[2]*Institute of Plant Physiology, Polish Academy of Science, Cracow, Poland*
[3]*Institute of Botany, Polish Academy of Science, Cracow, Poland*

Viola tricolor L. is a common species on hilly pastures and banks, invading waste places and fallow ground, throughout Europe and Asia. In Poland it is spread all over the country. Large population of this pansy exists on calamine spoil heap in Bolesław near Olkusz as an effect of natural succession, forming with other species vegetation of calamine heap. Harsh environmental conditions (strong insolation, water deficit, elevated heavy metal concentration) enhance rapid plants adaptation and underlie the microevolutionary processes. Only selected genotypes are capable to survive and thus to create new ecotypes.

The aim of our study was to determine (1) the level of stress conditions, measured as heavy metal concentration in plant organs, (2) plant physiological response to stress, (3) influence of stress condition on reproductive processes.

The soil from calamine spoil heap was rich in zinc (1%), lead (0.1%), and cadmium (0.001%). The mean concentration of zinc in above grounds parts (0.20%) was higher than in roots (0.17%). Flowers were not devoid of heavy metals; their content exceeded the norm. Histochemical localization, using diphenylthiocarbazone confirmed the presence of metals in different parts of roots and shoots.

Heavy metals evoke oxidative stress in plant cells and tissues, generating Reactive Oxygen Intermediates (ROIs) eg., O_2^-, HO_2^-, H_2O_2 and others, understood as a overproduction. Roots and leaves of *V. tricolor* from calamine heap contained elevated amount of hydrogen peroxide (H_2O_2) (270 µmol/g in f.w., 11,000 µmol/g in f.w., respectively) in comparison with control plants.

Stressful conditions disturbed reproductive processes of *V. tricolor*, affecting an early and later stages of anther and ovule development. Irregularities in microsporogenesis resulted in formation of abnormal tetrads and polyads. As a consequence, pollen viability was reduced to ~73% and pollen grains conspicuously differed in size. Megasporogenesis was also disturbed. Additionally, process of cell degeneration, starting from an early stage of ovule development, resulted in degeneration of several ovules per ovary. Embryo viability assessed by tetrazolium test was reduced to 61%. Immunodetection of arabinogalactan proteins and pectins, using monoclonal antibodies and other histochemical techniques for pectin exudates, callose deposition identification are in progress.

Abundant occurrence of *V. tricolor* on calamine waste heap indicates that, despite of observed disturbances in sexual reproduction, the genotype developed strategy to survive in harsh environment, although is still evolving.

Ultrastructural studies of the embryonic development of the integument in the grass snake *Natrix natrix* L.* (Lepidosauria, Serpents)

Elwira Swadzba

Department of Animal Histology and Embryology, Silesian University, Bankowa 9, 40-007 Katowice, Poland, e-mail: eswadzba@us.edu.pl

The embryonic development of snake's skin has not been studied up till now. The development of integument in grass snake (*Natrix natrix* L.) was examined with the use of electron microscope. The studies based on material from our collection of embryos of the grass snake. The eggs of *Natrix* were incubated in the laboratory in constant temperature at 30°C and 100% relative humidity. Embryos were isolated in regular sequence of time from egg lying till hatching. The age of embryos was calculated using the table of species development (Rupik, 2002). Columnar cells are attached to the basal membrane by hemidesmosomes. The cytoplasm of the columnar cells is transparent for electrons, contains bundles of intermediate filaments and glycogen accumulations. Among columnar cells there are isolated pigment cells. Processes of the pigment cells are visible among the most of the epithelium layers. The cytoplasm of pigment cells contains many vesicles filled granules of pigment, mitochondria and smooth endoplasmic reticulum. The cells lying above the columnar layer are flat and contain mitochondria and rough endoplasmic reticulum. The flat cells are joined to the columnar layer by desmosomes. The desmosomes are also visible between laterals surface of the flat and columnar cells.

REFERENCES:

RUPIK W. 2002. Normal developmental table. In: Early development of adrenal glands in grass snake *Natrix natrix* L. (Lepidosauria, Serpentes). *Adv Anat Embryol Cell Biol* 164: 24–32.

Ultrastructural studies of ovary structure and transovarial transmission of endosymbiotic microorganisms in *Palaeococcus fuscipennis* (Burmeister) (Insecta, Hemiptera, Coccinea: Monophlebidae)

Teresa Szklarzewicz, Katarzyna Kedra, and Sylwia Niznik

Department of Systematic Zoology and Zoogeography, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Cracow, Poland, e-mail: szkla@zuk.iz.uj.edu.pl

Ovaries of *Palaeococcus fuscipennis* are composed of about 100 short telotrophic ovarioles that develop asynchronously. Each ovariole is subdivided into an apical tropharium (trophic chamber) and posterior vitellarium. The terminal filament is absent. The ovariole encloses 8 interconnected germ cells that belong to one cluster. In the tropharium 7 trophocytes (nurse cells) are present. In the vitellarium a single oocyte encompassed by a follicular epithelium develops. The follicular cells do not undergo diversification into subpopulations. The ovaries are accompanied by large organs termed bacteriomes which comprise several giant cells termed bacteriocytes. Each bacteriocyte is surrounded by a layer of flattened epithelial cells. The bacteriocyte cytoplasm is filled with pleomorphic bacteria, whereas in the epithelial cells small coccoid microorganisms are present. The coccoid bacteria are less numerous than pleomorphic microorganisms. In the older females both types of microorganisms leave the bacteriocyte and enter the cytoplasm of follicular cells surrounding the posterior pole of the oocyte. Next, the bacteria undergo exocytosis into the perivitelline space. At the time the bacteria migrate into the perivitelline space, the posterior pole of the oocyte is not covered with egg envelopes. The lack of egg coverings around the posterior pole of the oocyte facilitates entry of microorganisms to the perivitelline space. Till the end of oocyte growth the bacteria do not enter the ooplasm.

AKNOWLEDGEMENT: This work was supported by research grant BW/IZ/23b/2004.

* 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 approvals of Local Ethics Commission (27/05 z 18.05.05) and Polish Ministry of Environment Protection for performing studies on protected species (DOPog-4201-02-94/05/aj). The grass snake *Natrix natrix* L. is not included in Washington Convention of 1973, ratified by Poland in 1991 (Dz.U. nr 27 poz.112).

Mammalian spermatozoa chemotactic bahaviour induced by cytokines and growth factors

Leopold Sliwa

Department of Human Developmental Biology, Collegium Medicun, Jagiellonian University, Kopernika 7, 31-034 Cracow, Poland, e-mail: leosliwa@cm-uj.krakow.pl

An open question in mammalian fertilization is whether the egg communicates within spermatozoa before actual contact between them. *In vitro* studies have demonstrated that follicular fluid attract spermatozoa by chemotaxis. It is not clear which of the chemical substances present in the follicular fluid are chemoattractant for the sperm cells. The present study was designed to evaluate possible, direct effects of growth factors and interleukins presence in environment on spermatozoa chemotactically behavior.

In an *in vitro* model, the number of spermatozoa migrating to medium containing IGF-I (Insulin-like Growth Factor) in concentrations 100 ng/ml and 500 ng/ml were significantly higher than the number migrating to control. This effect was observed after 3h and 5h of incubation. The presence of IGF-II did not cause any statistically visible changes on the migration of spermatozoa. Effect of combination of IGF-I and IGF-II was similar to influence of purely IGF-I.

The number of spermatozoa migrating to medium containing TGFα (Transforming Growth Factor) in concentration 500 ng/ml were significantly higher than the number migrating to control. This effect was observed after 3h and 5h of incubation. The presence of TGFβ in comcentration 100 ng/ml and 500 ng/ml in incubation medium did not cause changes on the migration of spermatozoa. Effect of combination of both cytokines was similar to purely TGFα.

The number of spermatozoa migrating to wells containing PAF (Platelet Activating Factor) in concentrations 50 nM and 200 nM were significantly higher than the number observed in control. This effect was observed after 3h and 5h of incubation. The presence of 10 nM of PAF in incubation fluid did not cause any statistically visible changes on the bahaviour of spermatozoa.

Differences in the chemotactic migration of mouse spermatozoa to wells containing interleukins (IL 6 and IL 8) were observed only after 5h of incubation. Statistically significant changes in spermatozoa behavior occurred in interleukins concnentartions: IL 6; 60 pg/ml and IL 8; 1000 pg/ml.

Described effects of spermatozoa migration vas interpreted as chemotaxis but not chemokinesis induced by influence of growth factors and interleukins on cell membrane receptors. It is suggested that investigated substance contained in follicular fluid plays a direct important role in the process of mammalian fertilization.

Sperm route across vector tissue in piscicolid leeches (Hirudinea, Piscicolidae)

Piotr Swiatek[1], Jerzy Klag[1], and Aleksander Bielecki[2]

[1]*Department of Animal Histology and Embryology, Silesian University, Bankowa 9, 40-007 Katowice, Poland, e-mail: swiatek@us.edu.pl, klag@us.edu.pl*
[2]*Department of Zoology, University of Warmia and Mazury, Oczapowskiego 5, 10-967 Olsztyn-Kortowo, Poland, e-mail: alekb@uwm.edu.pl*

In piscicolid leeches (Hirudinea, Rhynchobdellida, Piscicolidae) hypodermic insemination occurs. The spermatophore is implanted in a specialised region of leech body (i.e. in the copulatory area). Just beneath the copulatory area, there is a specialised connective tissue, the so-called vector tissue, which is considered to guide sperm towards the ovaries.

In this study, the authors using light, fluorescence and TEM, have studied the vector tissue in the four species from genus *Piscicola* (*P. geometra*, *P. margaritae*, *P. pawlowskii* and *P. pojmanskae*). The research was performed in young (virgin) leeches, in specimens following copulation during the period of sperm transfer and after the sperm passage, when the spermatozoa are already inside the ovaries.

The vector tissue is composed of a ventral mass of cells located directly beneath the copulatory area, and two thin strands extending from the ventral mass towards the ovaries. Four cell types form the vector tissue, namely: granular cells, plasmatic cells, vesicular envelope cells and flat envelope cells. The envelope of this tissue is made up of a thick basal lamina, and two types of cells: the vesicular and flat envelope cells, which are embedded within the lamina. The rest of the tissue is formed from: granular and plasmatic cells. Both of these cell types have prominent cytoplasmic projections, filled with a filamentous material. However, only granular cells have numerous small electron-dense granules in their cytoplasm.

Immediately after implantation of the spermatophore into the copulatory area the sperm passes through the vector tissue within free spaces between the granular and plasmatic cells. During the passage of sperm, the projections of the granular and plasmatic cells change the form into parallel folds and as a result wide intercellular spaces are formed. The projections protruding into the intercellular spaces are especially long and complicated in *Piscicola geometra*.

Moreover, we revealed that the characteristic vector tissue cells occur also within the ovary wall and inside the ovary lumen. This supports earlier data, which postulated that the vector tissue appears to be an outgrowth of the ovary wall (Brumpt, 1900).

ACKNOWLEDGEMENT: This work was supported by a research grant from the State Committee for Scientific Research (KBN, no. 2P04C 004 28).

REFERENCES:

BRUMPT E. 1900. Reproduction des Hirudinées. *Mem Soc Zool Fr* 13: 286–430.

The cytoskeleton of endosperm chalazal haustorium of *Rhinanthus serotinus*

Joanna Swierczynska[1], Józef Bednara[2], and Jerzy Bohdanowicz[1]

[1]*Department of Genetics and Cytology, University of Gdansk, Kładki 24, 80-822 Gdansk, Poland, e-mail: joannasw@biotech.univ.gda.pl,*

[2]*Department of Plant Anatomy and Cytology, Maria-Curie Skłodowska University, Akademicka 19, 20-033 Lublin, Poland*

During the development of endosperm in *Rhinanthus serotinus* (Schnheit) Oborny (Scrophulariaceae) two types of haustoria are formed: micropylar and chalazal. After the first division of the primary endosperm nucleus, two cells are formed: the initial chalazal haustorium cell and micropylar cell. The micropylar cell divides several times and develops into the endosperm proper and the micropylar haustorium, whereas the initial chalazal haustorium cell forms the chalazal haustorium (CH). As a result of endoreduplication the CH nuclei gradually grow to a considerable size, and the amount of nuclear DNA also increases. The nuclei attain a maximum ploidy level of 2×768C. Changes in the cytoskeleton formation during development of highly polyploid chalazal haustorium cell in *R. serotinus* were studied using a modified indirect immunofluorescence staining and electron microscopy. Actin filaments were also stained by rhodamine-phalloidin. We focused our studies on the distribution of microtubules (MT) and microfilaments (MF) in different parts of haustorium cell and in endosperm proper cells, including cytoplasm surrounding the cellular organelles. At the initial stage of CH development microtubules congregate around the nuclei and many microtubules radiate from the nuclear surface. The ultrastructural observation confirms that where microtubules attach to the nuclear envelope; it appears more electron dens. Fine tubulin bundles are present in haustorium cytoplasm, some of which display clear longitudinal orientation. A dense prominent tubulin and actin network reveals at the chalazal end of the haustorium cell. Actin filaments don't form a distinct array around the nuclei. At the next stage the haustorium cell enlarges, crushes the surrounding tissue and adheres to the endosperm only at the proximal pole, while the distal pole penetrates the ovular tissue. A large vacuole, traversed with several cytoplasmic strands, occupies the major part of the haustorium. In these cytoplasmic strands many actin and tubulin filaments arrange with the long axis of haustorium cell. Organelles, especially long profiles of RER and vesicles, are abundant. Numerous vesicles associate with microfilaments bundles. A delicate meshwork of cross-linked microtubules occurs in the cytoplasmic strands which adjacent to the cell wall. The microtubules again form extremely abundant and distinct array around the nuclei and some actin material is also present around the large haustorium nuclei. A transfer wall with ramifying ingrowths forms at the chalazal end of the CH cell. A large amount of actin filaments and tubulin bundles concentrate near the transfer wall and appear as an intricate network. At the finale phase of CH development the cytoskeletal elements disorganize, some disrupted microtubules aggregate around nuclei and in chalazal apex of haustorium cell. In endosperm proper cells F-actin shows an extensive and intricate cortical network. Abundant microtubular cytoskeleton occurs in endosperm proper cells and forms typical configurations for mitotically active cells: the microtubular system in the cortical cytoplasm and at the nuclear surface, preprophase band of microtubules, metaphase spindle, anaphase, telophase and phragmoplasts. These observations reveal new information of morphology and structure of cytoskeleton in the developing endosperm haustoria.

Amplification of ribosomal genes in the ovarian follicles of earwigs (Insecta, Dermaptera, Forficulidae)

Wacław Tworzydło and Szczepan M. Bilinski

Department of Systematic Zoology, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Cracow, e-mail: tworzydlo@interia.pl

The ovaries of studied earwigs from family Forficulidae are meroistic-polytrophic, which means that developing oocytes are accompanied by nurse cells (= trophocytes). The ovaries are composed of numerous short ovarioles that consist of a terminal filament, short germarium and vitellarium. The latter contains only 2 ovarian follicles in subsequent stages of oogenesis. The individual follicle consists of synthetically dormant oocyte and single, highly polyploid nurse cell that are connected by simple intercellular bridge. During previtellogenesis the nurse cell enlarges and becomes highly active. Concurrently its nucleus attains characteristic irregular, "ameboid" shape. In the cytoplasm of vitellogenic nurse cell, numerous mitochondria, elements of RER and symbiotic microorganisms are present. Additionally aggregations of nuage material can be found in the close vicinity of the nuclear envelope.

The nurse cell nuclei of one of the studied species, *Forficula auricularia,* in addition to chromatin aggregations and RNA/AgNOR positive nucleoli contain also single, vacuolated bodies. Performed histochemical tests showed that these bodies are DNA and AgNOR positive. Surprisingly, some of the nucleoli were found in close contact with the surface of the bodies or even inside their vacuoles. Immunostaining with K121antibody against TGM cap, showed that the bodies as well as nucleoli contain snRNAs. In the light of these findings we suggest that the vacuolated bodies represent accumulations of amplified rDNA genes, traditionally termed "extrachromosomal DNA bodies".

Nurse cells of other studies earwigs (*Chelidurella acanthopygia, Doru lineare, Opisthocosmia silvestris*) do not possess equivalent structures which suggests that in these species either amplification of rDNA genes does not take place at all, or, alternatively, amplified rDNA genes become transcriptionally active soon after formation.

During advanced vitellogenesis the molecules synthesized in the nurse cells (various types of RNAs, proteins) are transferred to the ooplasm via the intercellular bridge. During the transfer the nurse cell nucleus is retained in the cell center and does not block the "gateway to the oocyte", i.e. intercellular bridge. We postulate that elongated extensions of nurse cell nucleus are responsible for the maintenance of this position.

ACKNOWLEDGEMENTS: This work was supported by the research grant BW/IZ/26a/2003.

Translocation of phosphatidylserine and acrosome reaction in bull spermatozoa

Jerzy B. Warchoł

Department of Cell Biology, University of Medical Sciences, 60-781 Poznan, Swiecickiego 6, Poland

Translocation of phosphatidylserine from internal into external layer of cellular membrane has been found to be an indicator of apoptosis of somatic cells. Despite all the effort, little information is available on the biological significance of this phenomenon in spermatozoa. Some investigators believe that it indicates an apoptotic process of sperm. On the other hand, this kind of molecular changes may be connected with the process of capacitation or acrosome reaction in spermatozoa.

The aim of this paper was to check the relationship between translocation of phosphatidylserine in sperm cells caused by hydrogen peroxide and the influence of this event on induction of acrosome reaction in these cells.

The experiments were performed on fresh bull sperm cells. Spermatozoa were pre-incubated in medium Ham's F-10 containing H_2O_2 at final concentrations of 20, 40, 80, 160 μM for 15, 30, 60 and 120 min. In the control groups, spermatozoa were incubated with the medium alone. Subsequently, the pre - incubated cells were washed twice and treated with calcium ionophore A23187 (10 μM) to induce acrosome reaction. After 3 hours' incubation with A23187, spermatozoa were stained with FITC-ConA. Additionally, the investigated cells were stained with AnnexinV- FITC, propidium iodide with Hoech 33342 and their mobility was analyzed applying computer technology.

The results indicate that translocation of phosphatydil serine takes place only in the part of spermatozoa. It is located in the plasma membrane of the head, in the middle piece or in both of them. Incubation of these cells in low concentration of H_2O_2 caused obvious increase of percentage of sperm with positive stain of head. In higher concentration of hydrogen peroxide the number of cells with Annexin V positive middle piece was growing. One could notice that pre-incubation of spermatozoa in low concentration H_2O_2 triggered an increase of acrosome reaction of these cells. It correlated with observed translocation of phosphatydilserine in plasma membrane of head. Simultaneously, higher percentage of cells with middle pieces labeled with Annexin V correlates with decreasing number of moving sperm cells. One can conclude that translocation of phosphatydilserine occurs in plasma membrane of spermatozoa in two venues i.e. head and the middle piece. Moreover, these phenomena may play a different role in acrosome reaction and in the movement of these cells.

Comparative study of tongue development in amphibia (Anura and Caudata)

Krystyna Zuwała* and Michał Jakubowski

*Department of Comparative Anatomy, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Cracow, Poland, *e-mail: zuwa@zuk.iz.uj.edu.pl*

The soft (or secondary) muscular tongue present in the vast majority of mature amphibians is the organ that in the evolution of vertebrates appeared for the first time in this very class. Based on the results of our own studies and literature data it was found that during ontogenesis of Anura a single organ called the tongue develops and operates. This is a muscular fold localized in the frontal part of the oral cavity floor forming during the development of tadpoles but fulfilling its functions only in metamorphosed animals. Species of anurans studied by us encompass two groups: having typically formed tongue (*Pelobates fuscus, Hyla arborea, Rana esculenta, Bombina variegata)* and species without discernible typical tongue (*Hymenochirus boettgeri*). The first stage of tongue formation (up to the 39th developmental stage of a tadpole) occurs in all the studied species in a similar manner. In more advanced stages (starting from stage 40/41) in *P.fuscus, H. arborea* and *R. esculenta* the tip of the developing tongue is free while in *B. variegata* it is accreted to the floor of the oral cavity on its whole surface.

In the studied species belonging to Caudata the development of the tongue during ontogenesis occurs in a slightly different way. In the development of *Hynobius retardatus* the terminology of primary tongue (for the larvae) and secondary, or soft muscular tongue (for the metamorphosed animals) had been introduced by Takeuchi et al. (1997). Somewhat later similar process of the formation of the soft tongue was described in *Salamandra salamandra* (Opolka and Clemen, 1998; Zuwała and Jakubowski, 2001) and *Hynobius dunni* (Zuwała, 2005; Zuwała et al., 2002). The anterior part of oropharyngeal lining, supported by the skeleton of the hyoidal apparatus of the larva, is considered as the primary tongue, while the secondary tongue (commonly known as "a tongue"), develop as a muscular fold within oral lining of the "primary tongue".

REFERENCES:

OPOLKA A, and CLEMEN G. 1998. The lingual epithelium of Salamandra salamandra: metamorphic changes during its ontogenesis. *Annales of Anatomy* 180: 537–545.

TAKEUCHI H, IDO S, KAIGAWA Y and NAGAI T. 1997. Taste discs are induced in the lingual epithelium of salamanders during metamorphosis. *Chemical Senses* 22: 535–545.

ZUWAŁA K, and JAKUBOWSKI M. 2001. Two types of taste organs (SEM, TEM) in the development of the spotted salamander *Salamandra salamandra* (L). *Anatomy and Embryology* 204(5): 413–420.

ZUWAŁA K, KATO S, and JAKUBOWSKI M. 2002. Two generations of the tongue and gustatory organs in the development of *Hynobius dunni* Tago. *Journal of Anatomy* 201(1): 91–98.

ZUWAŁA K. 2005. Budowa narzadów smaku w rozwoju osobniczym płazów (Anura, Caudata). Wydawnictwo Uniwersytetu Jagiellonskiego, Rozprawy Habilitacyjne nr 366.