POSTERS

Morphology of the prostate gland of *Helix pomatia* L. (Mollusca, Gastropoda, Pulmonata, Stylommatophora)

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Pulmonata are hermaphrodites. In the reproductive system those gastropods one can distinguish common organs (hermaphroditic gland, hermaphroditic duct) and separate ones: female (oviduct, vagina) and male (spermiduct, vas deferens, penis). Different of accessory glands are connected with all the parts of this system. In the male part, the biggest one is the prostate gland, which opens into the lumen of the spermiduct. This report present the morphology of the prostate of *Helix pomatia* in the light and electron microscopes.

The prostate gland is built of numerous tubules. In the epithelium of the tubules non-secretory and secretory cells are found. Non-secretory cells are covered with microvilli and cilia. Cytoplasm is of medium electron density and with a numerous of mitochondria. The endoplasmic reticulum and the Golgi apparatus are poorly developed. Intercellular spaces are closed with gap junctions and septate junctions.

Secretory cells of the prostate have a well-developed endoplasmic reticulum and well-developed Golgi apparatus. Nucleus is located in the basal part of the cell. The apical part of the cell contain vacuoles filled with a flocculent electron material. The histochemical reaction (PAS) has shown this material to consist primarily of polysaccharides.

The presence of polysaccharides clearly indicates that the prostate gland of *Helix pomatia* takes part in the process of spermatophore formation. According to some authors (Lind, 1973; Zubiaga et al., 1989), polysaccharides in snails form the spermatophoric wall and are a component of the seminal fluid in which the spermatozoa are suspended.

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REFERENCES:

LIND H. 1973. The functional significance of the spermatophore and the fate of spermatozoa in the genital tract of *Helix pomatia (Gastropoda, Stylomatophora)*. *J Zool London* 169: 39–64.

ZUBIAGA A, MOYA J, GOMEZ BJ, and ANGULO E. 1989. Ultrastructures studies on the spermoviduct of the *Arion subfuscus* (Drapanaud, 1805) *(Gastropoda, Pulmonata)*. *Biol Struct Morphogenesis* 2: 124–135.

Number of primary spermatocytes in testicular cysts of water frogs *Rana ridibunda* and *Rana lessonae* (Amphibia, Anura)

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Amphibian testis is composed of convoluted seminiferous tubules, which are separated from each other by interstitial tissue and loose connective tissue. Spermatogenesis occurs inside additional compartments of seminiferous tubules formed by a number of spherical vesicles known as cysts. Walls of cysts are composed of one or few Sertoli cells. Cysts are closed until germ cells achieve a stage of elongated spermatids. Then cysts open and differentiating spermatozoa gain contact with the lumen of the seminiferous tubule. During spermatogenesis, the primary spermatogonium inside a cyst starts to multiply, thereby giving rise to a few generations (clones) of secondary spermatogonia, which enter meiosis. The aim of this study was to estimate the number of primary spermatocytes in two parental species (*R. ridibunda* and *R. lessonae*) of a natural hybridogenetic hybrid, *R. esculenta*. We used testes of 4 adult *R. lessonae* (30 cysts), 6 adult (38 cysts) and 2 juvenile (19 cysts) *R. ridibunda*. Primary spermatocytes at bouquet stages (leptotene/zygotene) were counted on series sections stained with iron haematoxylin. The values per cyst in adults of both species ranges from 8.99 to 127.62 (mean 50.7), which is the result of 4–7 mitotic cycles. The mean values for juvenile cysts were lower (14.85 – 60.91, mean 31.1, *i.e.*, 4–6 cycles per cyst). In both juvenile and adult testes the number of spermatocytes per cyst was lower than the expected theoretical value 2^n. The values in adults were: $2^5 > 2^6$ (42%), $2^6 > 2^7$ (32%), and $2^4 > 2^5$ (26%) , whereas in juveniles were lower: $2^4 > 2^5$ (58%), $2^5 > 2^6$ (31%), and lower than 2^4 (11%). The results indicate that meiosis in amphibians is preceded by 4–7 synchronic mitotic cycles, as is the rule for other vertebrates. The number of spermatocytes unequal to 2^n indicates that some cells degenerate and/or some cycles can be asynchronous.

The development of tarsal bones in human embryos

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The tarsal bones together with metatarsal and phalanges form a terminal part of the lower limb which is called autopodium. Present study was made to correlate the development of the tarsal bones with embryonic staging.

Sixty two embryos from the Collection of the Department of Anatomy were used for study. Embryos were embedded in *toto* in paraplast and sectioned serially in sagittal, frontal and horizontal planes. Sections were stained according to routine methods and with silver salts.

The lower limb bud appears in the middle of fifth week (stage 13) at the somatic levels s. 25–29.

At 39 days (stage 16) the foot plate is visible. The skeletal elements of the foot are mesenchymal and by the end of the 6th week (stage 17) digital prolongations are seen.

Condensations of the tarsal bones are first noted at the beginning of the 7th week (stage 18). Within a few days (stage 19) the tarsal bones begin to chondrify. At the same stage chondrification of metatarsals is observed. Between the tibia and fibula and the talus is condensed mesenchyme which forms primordium of the talocrural joint.

At the end of 7th week (stage 20) the cellular condensation on the posterior end of the calcaneus forming primordium of the tuber calcanei is visible. At stage 21 (8th week) the sustentaculum tali develops and it chondrifies by the end of the embryonic period. At this stage the main parts of the talus are distinguishable and they begin to chondrify.

By the end of embryonic period all metatarsal bones and phalanges are cartilaginous.

Metabolism of trehalose during *Ascaris suum* (Nematoda) embryogenesis

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Besides glycogen, trehalose (-D-glukopiranozylo-1,1--D-glukopiranozyd) is the major reserve sugar in *Ascaris* eggs. Trehalose in nematodes has important physiological functions as it forms the energetic reserve, it is a "circulating" sugar and it protects cellular structures of embryos and mature forms during environmental stress allowing closing of the life cycle. It also participates in the mechanism of hatching the larvae from eggs (Behm, 1997; Pellerone et al., 2003). In this study the content of trehalose and activity of the enzyme hydrolyzing it – trehalase during embryogenesis of *A. suum* were investigated.

We observed in our investigates that increase in trehalase activity during the process of *Ascaris* embryogenesis always precedes the processes involving high energy output, such as blastulation and gastrulation and ecdysis of L_1 larvae. The activity level of the enzyme is the measure of trehalose utilization rate by the developing embryo, and trehalose content may be considered of rate of cellular metabolism during embryonic development of the *A. suum* eggs. A high content of the sugar after reaching the stage of blastula and during gastrulation are consistent with the knowledge that this disaccharide fulfills the function of a substance protecting the tissues of still poorly developed embryo against harmful environmental effects. Comparing the changes in concentrations of glycogen and triacyloglycerols during embryogenesis of *A. suum* it can be assumed that the level of trehalose in the eggs of the parasite is maintained owing to gradual utilization of the above compounds. It should be stressed that the concentration of trehalose is twice higher in the invasive larvae than in the zygote. That is linked to the role of trehalose during hatching of the larvae and the necessity to protect the larvae during the time of waiting for the suitable host.

REFERENCES:

BEHM CA. 1997. The role of trehalose in the physiology of nematodes. *Int J Parasitol* 27: 215–229.

PELLERONE FI, ARCHER SK, BEHM CA, GRANT W, LACEY MJ, and SOMERVILLE AC. 2003. Trehalose metabolism genes in *Caenorabditis elegans* and filarial nematodes. *Int J Parasitol* 33: 1195–1206.

Embryonic development of the nematode *Contracaecum rudolphii* at different temperatures

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The definitive hosts of the nematode *Contracaecum rudolphii* are piscivorous birds; in Europe, these primarily include the cormorants (*Phalacrocorax carbo, Ph. aristotelis*) and the mergansers (*Mergus merganser* and *M. serrator*). The prevalence of the nematode infestation in cormorants is very high and depends on the host's age; it may be as high as 100% in the adult cormorants.

The present study was aimed at following temperature effects on the embryonic development of the parasite. Adult nematodes were dissected from the digestive system of cormorants living at Katy Rybackie. Using a dissection needle, nematode eggs were isolated from the terminal part of the uterus of nematode females and placed in the physiological salt solution. The egg suspension was divided into 3 parts and the parts were placed at different temperatures (4°C, 15°C and 23°C). Egg development in all the samples was checked daily. Some nematodes were, prior to egg extraction, placed in a freezer and kept at -17°C for 2 months. After thawing, the eggs were isolated and treated as the eggs from non-frozen females.

The eggs of *C. rudolphii*, isolated from the uterus, are spherical, 51–54 μm in diameter, and surrounded by a double, transparent proteinaceous membrane. No egg development was observed on the culture start; about 2% of the eggs showed blastomere stage 2 only. On day 2, all the eggs in the sample kept at 23°C showed the blastula stage. On day 3, the late gastrula stage was recorded, whereas on day 4 most of the eggs contained larvae. The eggs kept at 15°C developed at a much slower rate; the developmental stages appeared about 5 days later, compared to the 23°C sample. The eggs kept at 4°C failed to developed. After 5 weeks, they were transferred to 23°C and were observed to develop at a rate similar to that shown by the eggs kept at that temperature directly after isolation from the uterus. Those eggs isolated from the females that had been kept for 2 months at -17°C failed to develop at all the experimental temperatures.

The temperature sensitivity of the *C. rudolphii* eggs is most probably a result of a lack of a chitinous envelope.

Growth of the oocytes of the river lamprey (*Lampetra fluviatilis* L.) during spawning run

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Although biology of the river lamprey is well known, not much information is available on the development of their gonads during the spawning run. The aim of the study was to describe the growth of oocytes of the river lamprey during the spawning run into one of the rivers in north-western Poland. The measurements were performed in the months of the spawning run, from October to May.

The average length and mass of the females studied was 36.7 cm and 110.9 g. In subsequent months of migration, with approaching mating season, the average length and mass of females decreased. At the beginning of the anadromous run, the oocytes were ellipsoidal with nucleus in the polar position. With approaching mating season, the diameter of the long and short axes of oocytes and thickness of the layer of the follicle cells increased. The thickness of the chorion increased, and the yolk platelets enlarged. At the animal pole an elevation of theca over the chorion was observed.

Intercellular junctions in the male gonad of *Saduria entomon* L. (Crustacea, Isopoda)

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Intercellular junctions in the male gonad of isopod, *Saduria entomon*, have been studied by transmission electron microscopy. Electron dense tracers lanthanum nitrate and horseradish peroxidase have been used to investigate the significance of these junctions in permeability barrier.

In isopods each of the paired testes consists of three testicular tubules. The testicular tubules are composed of somatic Sertoli cells, that adjoin and build a tubule wall, and germ cells at various stages of maturation. The germ cells at the same stage of spermatogenesis occupy specific areas in the testicular tubule. In *S. entomon* there are two types of Sertoli cells. Sertoli A cells occupy the part of the tubule containing spermatogonia and primary spermatocytes and their processes associate with these germ cells only. Sertoli B cells compose most of the testicular tubule wall and resemble a columnar epithelium. They send long processes into the tubule lumen and connect with maturing spermatids.

Very little is known of the intercellular junctions in the crustacean testis, but do not know anything at all about that in the testis of isopods. In *S. entomon* both between Sertoli A cells processes and between Sertoli A cells processes and germ cells (spermatogonia or primary spermatocytes) numerous junctional complexes are aligned. Move of the tracers is visibly stopped along these junctions. They seem to be similar to tight junctions of vertebrates. Short gap junctions and desmosomes are also occurred, but they are random.

In basal area of columnar Sertoli B cells the long gap junctions are located. Above them long smooth septate junctions extend between adjacent Sertoli B cells. In this area penetration of the tracers from the basal lamina is abruptly stopped. In apical area the gap junctions are located again and intermediate junctions occur next to the tubule lumen. Between adjoining Sertoli B cells processes the short gap and septate junctions are present. Between these processes and maturing spermatids form non-typical septate junctions, in which an electron dense flocculent or amorphous material in intercellular space is visible. The tight junctions are not found between either Sertoli B cells or Sertoli B cells processes and spermatids.

Successful cryopreservation of porcine embryos using a minimum volume of vitrification solution

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Recent research on cryoconservation of pig embryos has focused mainly on their vitrification. However, no satisfactory method of freezing or vitrification of pig embryos has been developed so far. Our previous experiment with vitrification of porcine embryos showed that significantly higher full developmental capacity was observed for vitrified blastocysts transferred into the oviduct versus blastocysts transferred into the uterus. A plastic straw with a standard volume of solution was used for vitrification.

The aim of this study was to determine if developmental capacity of vitrified pig blastocysts could be improved by a minimum volume of vitrification solution.

Blastocysts were collected from superovulated pigs by uterus flushing with supplemented PBS at 30°C. Embryos were then transferred into PBS supplemented with 20% of fetal calf serum (FCS). Vitrification was performed in standard plastic straws (0.25 mL volume) (group A) or in open pulled straws with a minimal volume (about 20 μl) (group B) of vitrification solution, containing 40% of ethylene glycol, 18% of ficoll and 0.3 M of sucrose. After equilibration embryos in vitrification solution were placed in straws and plunged in liquid nitrogen. Samples were stored in liquid nitrogen for 1 to 3 months. Thawing was performed in 0.5 M sucrose. Next embryos were transferred into PBS supplemented with FCS and rinsed twice. Survival of vitrified embryos was assessed after culture in NCSU-23 medium or after surgical transfer into the oviduct of synchronized recipients.

After culture the survival rate of vitrified blastocysts in group B was slightly higher (15.4%) than that of group A (12.0%). Blastocysts vitrified in group A (232) were transferred to 12 recipients and blastocysts vitrified in group B (117) were transferred to 5 recipients. Pregnancy rate for embryos vitrified in group B was higher (60%) than that of group A (41.7%).

The results of our experiment suggest that porcine embryos vitrified with a minimal volume of vitrification solution have a higher capacity to survive the vitrification process than embryos vitrified in standard straws.

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Localization of estrogen receptors α and β in the reproductive tissues of the stallion and of the cryptorchid

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During the past decade, several studies have clearly demonstrated an essential role for estrogen receptors in regulating reproductive function in the male [for review, see Hess, 2003; Paziewska-Hejmej and Bilinska, 2003; Saunders, 2005]. However, the distribution of these receptors in the equine male reproductive system has not been examined yet. Therefore, this study was designed (1) to characterize localization of ERα and ERβ in the testis, epididymis and prostate of the stallion, and (2) to examine the possible redistribution of estrogen receptors in the reproductive organs in naturally occurring bilateral cryptorchidism in the horse.

Immunohistochemistry was performed on paraffin-embedded tissue sections using a mouse monoclonal antibody against human ERα and a rabbit polyclonal antibody against rat ERβ.

In the testis of the stallion, ERα immunoexpression was confined to the nuclei of Leydig cells. In the epithelial cells of the caput and corpus epididymis, strong nuclear staining was observed, whereas in the cauda weak staining was found. Positive nuclear immunostaining for ERα was also detected in the prostatic glandular epithelium. In the cryptorchid, cytoplasmic localization of ERα was observed in the same types of cells.

In stallion's testis, nuclear staining for ERβ was found in Leydig, Sertoli and germ cells. ERβ was strongly expressed in the nuclei of epithelial cells of the caput and cauda epididymis but weakly expressed in the epithelium of the corpus. The glandular epithelium of the prostate was immunopositive for ERβ. In the cryptorchid, the distribution pattern for ERβ in the testis, epididymis and prostate was cytoplasmic.

Taken together, it appears that ERα and ERβ are ubiquitously distributed in the reproductive tract of the normal horse as well as of the cryptorchid. However, cytoplasmic localization of estrogen receptors in the cryptorchid may indicate not functionally active receptors and hence impaired estrogen action in such a male.

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REFERENCES:

HESS RA. 2003. Estrogen in the adult male reproductive tract: a review. *Reprod Biol Endocrinol* 1: 52.

PAZIEWSKA-HEJMEJ A, and BILINSKA B. 2003. Estrogeny i ich rola w regulacji spermatogenezy. *Post Biol Kom* 30: 461–482.

SAUNDERS PT. 2005. Does estrogen receptor β play a significant role in human reproduction? *Trends Endocrinol Metab* 16: 222–227.

Disturbances in embryological processes in *Conyza canadensis* L. (Asteraceae) in the polluted environment

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The studies concern the processes in reproductive organs of *Conyza canadensis* growing in two contaminated sites: (1) slopes of the post-flotation waste reservoir Zelazny Most in the Legnica-Głogów Copper Basin – the site polluted with Cu, Pb , Cd, Mg and Zn; (2) spoil heaps of the Mining and Smelting Works "Orzeł Biały" Ltd. in Bytom, strongly contaminated with Zn and Pb. The control plants grew on non-polluted soil in Krempna near Jasło.

Conyza canadensis having wild ecological amplitude and entering in natural spontaneous succession on polluted sites was expected to be well adapted to harsh environmental conditions.

Micro- and megagametophyte development and embryo and endosperm formation were analyzed in anthers of 70 capitula and 750 ovules in material from polluted sites and in 45 capitula, 400 ovules from the control plants.

Cytological disturbances in microsporogenesis were observed only exceptionally and the regular course of pollen development was confirmed by Alexander test and acetocarmine test (viability of pollen ranging from 90% to 93%).

In comparison with the control material detailed embryological analysis showed that the frequency of ovules with typical and viable embryo sac, typical embryo and endosperm development was reduced in specimens from both polluted sites.

The most notable embryological irregularity which occurred in 10% of inflorescences of plants growing in polluted wastes in Bytom was lack of stability of correlation between the start of embryo and endosperm development. In *C. canadensis* these processes are preceded by double fertilization and embryo sacs with several cells in endosperm accompanied by zygote/two-celled proembryo were typical both in the control and in the major part of material from polluted sites. However, in 10% of capitula, some embryos started their development independently of endosperm. They attained advanced globular stage, whereas secondary nucleus was still undivided; unfortunately it was not possible to distinguish the level of its ploidy. In the same capitula other embryos at torpedo-shaped stage were accompanied by multicellular endosperm. There are two possible interpretations of atypical secondary nucleus behavior: a distinct retardation of its division or lack of fusion with the sperm cell. Young proembryos could receive nourishment by the haustorial antipodal cells penetrating the surrounding tissue, however, further growth and differentiation of the embryos require endosperm formation.

Lms, a new muscle identity gene required for lateral transverse muscle development in *Drosophila* embryos

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Myogenesis involves a series of different processes including specification of mesoderm lineages, determination of muscle identity, differentiation, and patterning. Each of 30 *Drosophila* larval somatic muscles is a single, multinucleated fiber with an individual shape, insertion site and innervation. Muscle fiber diversity is manifested by differences in the patterns of gene expression. Muscle marker genes can be expressed in individual muscle fiber as even-skipped and ladybird or in groups by combinatorial cod manner. The four lateral transversal muscles (LT1–LT4) are thought to be specified by the combinatorial activity of the *Kruppel* (*Kr), apterus* (*ap*), and *muscle specific homeobox* (*msh*) genes whilst the activity of the *ladybird* (lb) genes is required for proper formation of the segmental border muscle (SBM) in the same lateral area. We report here expression pattern of a new gene (CG13424), we named ***lateral muscle specific*** (*lms*) and its possible function in myogenesis. It encodes a product with potential transcription factor activity. The sequence alignment indicated that *lms* is ortologue of the vertebrate *Vax-1* .*Vax-2* genes. Moreover the homeobox sequence of *lms* is highly homologous to the *Drosophila NK7.1* and *Tlx3, Hmx1* homeobox genes. *Lms* starts to be expressed in early stage 11 in somatic LT muscle precursors and is seen until stage 16 in these muscles. We detect also expression of this gene later in developing central nerves system (CNS). Knock down of lms induced by ds RNA injection into MHC GFP embryos leads to the partial loss of LT muscles, indicating that *lms* plays a key role in LT muscle identity. This suggests it represents a new component of the combinatorial genetic code specifying lateral muscles.

The dorsal notch in 8 month age papillon

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The morphology of foramen magnum is important in clinical causes and comparative reaserches. The malformations of foramen magnum and the occipital bone morphology lead to neurological symptoms as convulsions, losses of consciousness, ataxias and many others. The pathological shape of foramen magnum is element of Arnold-Chiari syndrom, described also in dogs (Parker and Park, 1974a; Parker and Park, 1974b; Churcher and Child, 2000). The morphology of occipital bone with incomplete ossification and foramen magnum with dorsal notch is described in this paper. The foramen magnum in 10 month papillon was described. The foramen magnum had a hexagonal shape with a significant dorsal notch in squamous part of the occipital bone. The dorsal notch was located in saggital plane and was symetrical. The shape of the dorsal notch was rectangular. The total height of foramen magnum was 14,97 mm. the width 9,69 mm. Foramen magnum index was 162,54. The height of dorsal notch was 5,28 mm and was rectangular. Watson at al. (1989) and Simoens at al. (1994) on the base of aquired results put thesis, dorsal notch is not a patological formation but the morphological variation. Onar at al. (1997) in sheperd dogs and Chrószcz at al. (2006) in american staffordshire terriers not found the similar creations.

REFERENCES:

CHRÓSZCZ A, JANECZEK M, WOJNAR M, and POSPIESZNY N. Morphological analysis and morphometry of the foramen magnum *(foramen magnum)* of the american staffordshire breed newborns. *Medycyna Wet* (in press).

CHURCHER RK, and CHILD G. 2000. Chiari 1/syringomyelia complex on a King Charles Spaniel. *Aus Vet J* 78: 92–95.

ONAR V, MUTUS R, and KAHVECIOGLU KO. 1997. Morphometric analysis of the foramen magnum in German Shepherd dogs (Alsatians). *Ann Anat* 179: 563–568.

PARKER A J, and PARK RD. 1974a Occipital dysplasia in the dog. *J Am Hosp Assoc* 10: 520–525.

PARKER AJ, and PARK RD. 1974b. Unusual deformity of the occipital bone in a dog (case report). *Vet Med Small Anim Clin* 69: 440–441.

SIMOENS P, POELS P, and Lauwrens H. 1994. Morphometric analysis of the foramen magnum in Pekingese dogs. *Am J Vet Res* 55: 34–39.

WATSON AG, de LAHUNTA A, EVANS HE. 1989. Dorsal notch of the foramen magnum due to incomplete ossification of supraoccipital bone in the dogs. *J Small Anim Pract* 30: 666–673.

The morphology of the alimentary canal of the juvenile racer goby *Neogobius gymnotrachelus* Kessler, 1956

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The racer goby is a Ponto-Caspian fish which is expanding their range upstream and has recently invaded rivers in Poland. The aim of the study was to analyse the morphology of the alimentary canal of racer goby fry. It will be more detailed study of the development and structure of the digestive system of this species.

10 individuals specimens taken from the lower Vistula River were studied. Their total body length (L_s) ranged from 20.0 mm to 38.8 mm. The paraffin method was used to prepare the serial histological specimens. 5 μm thick paraffin sections were stained with H+E.

The alimentary canal consists of the oesophagus and the anterior and posterior intestines. Between the oesophagus and the intestine is the oeso-intestinal valve, which is the equivalent to the pyloric valve of fish which have a stomach (Morrison and Wright, 1999). The intestine forms two loops. The intestinal bulb, often found in stomachless fish (Sinha, 1976; Morrison and Wright, 1999) is absent. The oesophagus constitutes the shortest part of the alimentary canal (8,7% of the total lenght) and the anterior intestine is the longest – 81,7%. There is no ileo-rectal valve between the anterior and posterior intestines. It is found in the fish with stomach (Morrison and Wright, 1999).

The configuration of the mucosa, which can be seen in sections cut tangentially to the intestinal wall, shows that mucosal folds vary in appearance. In the oesophagus there are mainly longitudinal folds, in both intestines they form a mesh consists of zigzag folds, with a more regular oval shape to the posterior intestine. The transverse sections show that the folds vary in hight. They are higher in the posterior intestine.

REFERENCES:

MORRISON CM. WRIGHT JR 1999. A study of the histology of the digestive tract of the Nile tilapia. *Journal of Fish Biology* 54: 597–606.

SINHA GM. 1976. Comparative morphology, anatomy and histology of the alimentary canal of an Indian Freshwater major carp, *Labeo Calabus* (Hamilton) during the different life-history stages in relation to food and feding habits. *Anat Anz Bd* 139: 348–362.

The histological structure of the alimentary canal wall of the juvenile racer goby *Neogobius gymnotrachelus* Kessler, 1956

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The aim of the study was to analyse the histological structure of the wall of the alimentary canal of racer goby fry. 10 individuals specimens taken from the lower Vistula River with total body length (L_s) ranged from 20.0 mm to 38.8 mm were studied. The paraffin embedded histological preparations were stained with H+E.

The alimentary canal is divided into: the oesophagus, the oeso-intestinal valve and the anterior and posterior intestines. The wall of the alimentary canal consists of three layers: the mucosa composed of epithelium and the lamina propria; the muscularis and tunica serosa. In the oesophagus there is a stratified cuboidal epihelium in which Goblet cells and taste buds are present. The tunica muscularis of the oesophagus consists of two layers of striated muscles, an inner longitudinal and outer circular as in other species of fish (Morrison and Wright, 1999). The striated muscles fibres of the oesophagus are gradually replaced by smooth muscles in the oeso-intestinal valve and tunica muscularis became composed of the inner circular and the outer longitudinal layer. In this part of the alimentary canal the epithelium of the oesophagus changes into to the columnar epithelium. The oeso-intestinal valve with the mixed epithelia and the muscle fibres is recorded in *Catla catla* (Kapoor, 1958). The lamina propria of the wall of the alimentary canal composes of loose connective tissue fibres with numerous fibroblasts. The muscularis is developed best in the middle of the oeso-intestinal valve. The thickest lamina propria is in the oesophagus and in the valve. In the lamina propria of the valve there are the alveolar serous glands which are bunched and with gland ducts opening on the surface between the folds. The presence of Goblet cells in the intestine epithelium was not confirmed using this staining method. The abundance of goblet cells in the anterior and posterior segment have been deserved in the alimentary canal of the fish (Al-Hussaini, 1949 in Kapoor, 1958), especially in the fry (Sinha, 1976).

REFERENCES:

KAPOOR BG. 1958. The anatomy and histology of the digestive tract of a cyprinoid fish, *Catla catla* (Hamilton). *Annali del Museo civico di storia naturale Giacomo Doria* 70: 100–115.

MORRISON CM, WRIGHT JR. 1999. A study of the histology of the digestive tract of the Nile tilapia. *Journal of Fish Biology* 54: 597–606.

SINHA GM. 1976. Comparative morphology, anatomy and histology of the alimentary canal of an Indian Freshwater major carp, *Labeo Calbasu* (Hamilton) during the different life-history stages in relation to food and feeding habits. *Anat Anz Bd* 139: 348–362.

Formation and structure of yolk nuclei in the oocytes of some spider species

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In oocytes of many animal species ooplasmic components (mitochondria, dictyosomes, ER and RNP) are asymmetrically distributed and form different specific structures. Previous observations showed that in some spiders accumulations of organelles within the ooplasm take form one of two distinct types of structure: 1) yolk nucleus or 2) nuage material accumulation. Formation and structure of yolk nucleus was investigated in spiders representing of 3 families: Clubionidae (*Clubiona* sp.), Lycosidae (*Pardosa* sp., *Pirata* sp.) and Thomisidae (*Xysticus* sp., *Ozyptila* sp.). In all five species the stages of yolk nuclei formation are very similar. Since the very beginning of previtellogenic growth, cytoplasmic components show asymmetrical distribution. Initially, a cap-shaped accumulation of organelles appears at one pole of the oocyte nucleus and subsequently transforms into a single spherical structure in a juxtanuclear position, referred to as yolk nucleus. Primarily, yolk nucleus consists of a core and a cortex. In subsequent stages of previtellogenesis, the structure of yolk nucleus becomes more complex. It increases in size and pushes aside the oocyte nucleus to the cell periphery, while its components become arranged into clear concentric layers whose number increases. Ultrastructural observations showed that yolk nuclei consist mostly of mitochondria, dictyosomes, endoplasmic reticulum and electron dense material. Detailed analyses showed that participation of particular yolk nuclei components varies slightly among the studied spiders. Histochemical methods revealed different DNA and RNA contents within their yolk nuclei. On the other hand, actin filaments were found to be stable and characteristic cytoskeleton elements for yolk nuclei in all investigated spiders. During formation of yolk nucleus, bundles of actin filaments aggregate on its surface and form a kind of cage surrounding its central part until the early stages of vitellogenesis. With the onset of vitellogenesis, lipid droplets aggregate at the surface of yolk nuclei and gradually fill its inner parts. During vitellogenesis yolk nucleus becomes more compact and shows differences in composition in studied species. In all cases yolk nuclei remain their central position within the ooplasm till the end of oogenesis. In comparison with accumulation of organelles present in other animal species yolk nuclei show some structural similarities. However, the role of yolk nucleus still remains unclear and needs further investigations.

Reproduction ability of tetraploid *Cobitis* (Pisces, Cobitidae) males

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The spined loach *Cobitis taenia* L. appears in diploid populations and mixed diploid-poliploid populations. In the most *Cobitis* mixed populations allotriploid females dominated, co-existed with *C. taenia* or *C. elongatoides* and few tetraploid males and females (Boron, 2003). Triploid females gynogenetically produce triploid eggs, stimulated by the sperm from *Cobitis* males. Some of these eggs are fertilized, which leads to production of bisexual tetraploids (Saat, 1991).

Males of *C. taenia* (2n=48) from a diploid population in Lake Klawój (46 individuals) and from a mixed *Cobitis* population in the Bug River (7 individuals), and three tetraploid males (4n=98) from a mixed population were examined. All the fish were analyzed karyologically. Testes were fixed in buffered formalin. Histological sections were hematoxylin and eosin stained.

Tubules with cysts of testes of *C. taenia* from both populations filled with germ cells at various developmental stages were observed. Among fishes from Lake Klawój sperm maturation in portions simultaneously with portion spawning of *C. taenia* females (Juchno, 2004) was found.

Testes of loach *C. taenia* from a mixed population in the Bug River over the entire reproductive season were filled with spermatozoa. Sperm maturation in portions was not observed. It seems to be connected with a few diploids males in this population. So, a continuous process of spermatogenesis in their testes is required.

In testes of all tetraploid *Cobitis* males only cells characteristics of early stages of spermatogenesis were observed, without spermatids and spermatozoa. In addition, the histological sections of the testis of male captured at August, revealed fragments with connective tissue between germ cells. The participation of tetraploid, infertile *Cobitis* males in the process of reproduction in investigated mixed population remains controversial. The results obtained so far, revealed that even unless fertile the sperm of tetraploid males may induce gynogenesis in *Cobitis* triploid females.

REFERENCES:

BORON A. 2003. Karyotypes and cytogenetic diversity of the genus *Cobitis* (Pisces, Cobitidae) in Poland: a review. Cytogenetic evidence for a hybrid origin of some *Cobitis* triploids. *Folia Biol* 51: 49–54.

JUCHNO D. 2004. Rozwój gonad i gametogeneza kozy *Cobitis taenia* (L.) i naturalnych poliploidalnych *Cobitis* (Pisces, Cobitidae). Ph.D. disseration, University of Warmia and Mazury, Olsztyn.

SAAT TV. 1991. Reproduction of the diploid and polyploid loaches (*Cobitis*, Teleostei). Oocyte maturation and fertilization in the triploid form. *Ontogenez* 22: 533–541.

Ultrastructure of the Japanese quail (*Coturnix coturnix*) and chicken (*Gallus domesticus*) frozen and thawed spermatozoa

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Changes in the ultrastructure of the Japanese quail (*Coturnix coturnix*) and chicken (*Gallus domesticus*) spermatozoa before and after freezing were examined under the light (LM), scanning (SEM) and transmission (TEM) electron microscopes. The following samples were obtained for study: fresh spermatozoa; semen samples diluted with Lake's diluting medium (Lake et al., 1968) containing 6 % dimethylacetamide (DMA); frozen and thawed spermatozoa as in artificial insemination or thawed with freeze-substitution method. Freeze-substitution are know from light microscopy and have been used in electron microscopy (Zalokar, 1966). Our results indicate that, the frozen and thawed semen disrupted many of cells and resulted different micro- and ultrastructural damage. The defects mainly included loose jointing of the head, middle piece and flagellum, structural alterations of the nucleus and changes both in the structure of the mitochondria and in their arrangement. No big differences were noted between fresh spermatozoa and suspended in medium containing 6 % dimethylacetamide.

REFERENCES:

LAKE PE, SMITH W, and YOUNG D. 1968. The ultrastructure of the ejaculated fowl spermatozoon. *Poultry Science* 53: 834–837.

ZALOKAR M. 1966. A simple freeze-substitution method for electron microscopy. *J Ultrastruct Res* 15: 469–479.

Histological analysis of the lacrimal gland in pig's foetuses

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The lacrimal gland belongs to the accessory organs of eye. It is located in the dorso-lateral angle of orbital cavity. The histological investigations were carried out on pig's foetuses coming from the 63rd, 94th and 112th day of gestation. The lacrimal gland was stained with H-E method. The slides were examined in the light microscope with 5×, 10×, 40× and 100× multiplication. The photos were taken with microscope OLYMPUS PX 41.

The investigation proved the lacrimal gland is surrounded by the connective tissue with the great number of fibroblasts and lymphocytes, which produced and divided the parenchyma of gland to lobes. The lacrimal gland lobes consisting of 10–16 secretory sectisus in 64th day of gestation. In 94th day of prenatal life they are built by 3–8 lobules and the number of lobules increased to 8–22 in 112th day of pregnancy, which are built with the simple cuboids epithelium with spherical nucleuses.

Histological analysis of the upper et lower eyelid in pig's foetuses

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The upper and lower eyelid belong to the accessory organs of eye. The histological investigations were carried out on foetuses coming from the 27th, 35th, 63rd, 94th and 112th day of pregnancy. All material was stained with H-E method. The slides were examinated in the light microscope with 5×, 10×, 40× and 100× multiplication. The photos were taken with microscope OLYMPUS PX 41.

The results proved, the eyelid primordia are occurred in 27th day of gestation. In 35th day of pregnancy the anterior surface of eyelid was covered with the stratified sqamosus epithelium consisting of 3–5 cell layers. The posterior surface of eyelid was covered with double epithelium. The margins of both eyelids are connected with the palpebral raphe. The tarsal gland are developed from ectodermal epithelium. The anterior surface of eyelid was covered with the stratified squamosus epithelium consisting of 4–5 layers in 63rd day of prenatal life. The posterior surface of eyelid was covered with stratified prismatic epithelium consisting of 2–3 layers. The tarsal gland developed their excretory ducts. In 94th day of pregnancy the anterior surface of eyelid was covered with the stratified squamosus epithelium. The eyelashes primordia and eyelash glands were occurred. The stroma of eyelid produced the eyelid tarsal cartilage. The development of above mentioned structures was continued until the end of pregnancy.

Tissue distribution of estrogen receptors alpha (ERα) and beta (ERβ) in the pig embryo

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Estrogens play a crucial role in female sexual development. During development, estrogen may have direct effects on cells that express estrogen receptors, therefore determining when ERs are present during development would indicate at which developmental stage(s) fetal tissues are potentially sensitive to estrogens. The ERα and ERβ are hormone-activated transcription factors that regulate the expression of specific genes by binding to steroid-responsive elements. The tissue distribution of both ERs mRNA in human midgestational fetus has been analyzed by semiquantitative PCR. It was shown that the expression profile of the two ERs is different, with ERβ being expressed in a variety of tissues during human development (Branderberger et al., 1997). Our previous study on the porcine ovary of estrous cycle showed differential distribution of both receptors (Słomczynska and Wozniak, 2001). It could be assumed that the presence of both receptors and their ratio during fetal development may be one of the main factors that influence embryonic development. Therefore, the aim of the present study was to investigate estrogen receptors expression in the developing porcine fetuses.

The localization of ERs was conducted on embryos obtained in different days of gestation: 18, 22, 32, 40, 50, 70, 90 *post coitum* (p.c.). The visualization of both proteins was conducted in various fetal organs such lung, kidney, intestine and gonads. The sections were exposed to monoclonal (ERα) and polyclonal (ERβ) antibody and color reaction was developed using DAB system.

Our preliminary data show the coexpression of both ERs in the reproductive tract of the porcine fetus as well as in other tissues which are not believed to be estrogen-dependent. The intensity of immunoreaction was analyzed on each section. The most noticeable changes in the expression of ERα and ERβ pattern were observed in the developing gonads.

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REFERENCES:

BRANDENBERGER AW, TEE MK, LEE JY, CHAO V, and JAFFE RB. 1997. Tissue distribution of estrogen receptors alpha and beta mRNA in the midgestational human fetus. *J Clin Endocrinol Metab* 82: 3509–3512.

SŁOMCZYNSKA M, and WOZNIAK J. 2001. Differential distribution of estrogen receptor-β and estrogen receptor-α in the porcine ovary. *Exp Clin Endocrinol Diabetes* 109: 238–244.

Localization of LKB1/PAR4 protein in maturing mouse oocytes and in parthenogenetic embryos

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During meiotic maturation the mouse oocytes undergo two asymmetric divisions, which lead to the formation of large secondary oocytes and two small polar bodies. The mouse oocyte is polarized cell, but molecular mechanisms responsible for establishment of this polarity are still unknown. Recent works suggest that microtubules and conserved PAR proteins play important role in cell polarity in many cell types. It was shown that the mouse homologues of *C. elegans* PAR3 and PAR6 proteins localize on microtubules of migrating meiotic spindle in maturing oocytes (Duncan et al., 2005; Vinot et al., 2004).

Here we showed that LKB1 kinase, a mouse homologue of *Caenorhabditis elegans* PAR4 protein, associates with microtubules of metaphase I and metaphase II meiotic spindles (Szczepanska and Maleszewski, 2005). We also investigated the localization of LKB1 protein during the first mitotic division of parthenogenetic embryos. We demonstrated that the localization of LKB1 kinase in mouse embryos differs from those observed in maturing oocytes. We also showed that the localization of the LKB1 kinase in cybryds obtained after fusion of 1/8 blastomeres and cytoplasts from GV oocytes depends on the manner of division. When cybryds divided asymmetrically a localization pattern of LKB1 kinase resembled these observed in maturing oocytes. When cybryds divided symmetrically localization of LKB1 protein was similar to the localization of this protein in parthenogenetic embryos.

Therefore, we suggest that LKB1 protein like other two PAR proteins may play role in the establishment of the oocyte polarity.

REFERENCES:

DUNCAN FE, MOSS SB, SCHULTZ RM, and WILLIAMS CJ. 2005. PAR-3 defines a central subdomain of the cortical actin cap in mouse eggs. *Developmental Biology* 280: 38–47.

SZCZEPANSKA K, AND MALESZEWSKI M. 2005. LKB1/PAR4 protein is asymmetrically localized in mouse oocytes and associates with meiotic spindle. *Gene Expression Patterns* 6: 86–93.

VINOT S, LE T, MARO B, and LOUVET-VALLEE S. 2004. Two PAR6 proteins become asymmetrically localized during establishment of polarity in mouse oocytes. *Current Biology* 14: 520–525.

Mice with *mosaic* mutation; morphological and immunohistochemical study of the testis

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The *mosaic* mutation (*Atp7a*$^{mo\text{-}ms}$) in mice is associated with abnormal copper metabolism that affects reproductive system. This mutation is lethal, the majority of *mosaic* males die, only 4% survive to sexual maturity and sometimes are fertile (Styrna, 1977).

Aromatase is a terminal enzyme which transforms irreversibly androgens into estrogens. In the light of recent data estrogens are absolutely required for normal fertility in the male (for reviews, Carreau et al., 1999; Hess, 2003).

The aim of the present study was to show morphology and the expression of aromatase in the *mosaic* mouse testes.

The testicular sections from both, control and *mosaic* males, were routinely stained for morphology and immunohistochemistry according to the method described in detail (Gancarczyk et al., 2004).

Morphological analysis of testes of *mosaic* revealed the presence of many degenerating seminiferous tubules besides normal-looking ones in comparison with those of the controls. Moreover, in mutant testes increased number of apoptotic germ cells was found. In the interstitial tissue empty spaces, indicating lack of Leydig cells were observed. In testicular sections of control mice weak or moderate immunoreactivity for aromatase was detected in Leydig, Sertoli, and germ cells, whereas in testicular cells of *mosaic* mice stronger intensity of immunostaining was observed, especially in spermatocytes and spermatids. Immunopositive staining for aromatase was also found in cultured Leydig cells derived either from control or mutant males. In Western-blot one major band of 55 kDa was identified, representing aromatase protein. In mutant males, testosterone level was significantly lower, whereas that of estradiol was higher in comparison with their respective levels in control males.

Disrupted morphology and function of *mosaic* mouse testes can be an effect of abnormal copper metabolism in these males. It is possible that higher level of endogenous estrogen can affect adversely the morphology of mutant testes.

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REFERENCES:

STYRNA J. 1997. Analysis of causes of lethality in mice with the Ms (Mosaic) gene. *Genet Polonica* 18: 61–79.

CARREAU S, GENISSEL C, BILINSKA B, and LEVALLET J. 1999. Sources of oestrogen in the testis and reproductive tract of the male. *Int J Androl* 22: 211–223.

HESS RA. 2003. Estrogen in the adult male reproductive tract: a review. *Reprod Biol Endocrinol* 1: 52.

GANCARCZYK M, PAZIEWSKA-HEJMEJ A, CARREAU S, TABAROWSKI Z, and BILINSKA B. 2004. Dose- and photoperiod- dependent effects of 17β-estradiol and the anti-estrogen ICI 182,780 on testicular structure, acceleration of spermatogenesis, and aromatase immunoexpression in immature bank voles. *Acta Histochem* 106: 269–278.

Detection of caspase-9 and caspase-3 activity and phosphatidylserine location in human spermatozoa stimulated by EDS and TPEN

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Mechanism of spermatozoa death has not been clearly identified. Both necrosis and apoptosis are discussed. Recently the biochemical apoptotic markers (characteristic for somatic cells) were observed in human spermatozoa.

The aim of the study was estimation of the presence of active form of caspase-3 and caspase-9 as well as phosphatidylserine (PS) translocation in human spermatozoa stimulated by proapoptoic factors.

The material consisted of human semen obtained from 42 normozoospermic men. The spermatozoa were incubated with ethane dimethane sulfonate (EDS; 2 and 5 mM) and the metal ion chelator NNN(N(-tetrakis-(2-pyridylmethyl)ethylendiamine (TPEN; 2 and 5 nM). The PS translocation (Annexin-V-FLUOS), active caspase-3 (FITC-DEVD-FMK) and active caspase-9 (Red-Lehd-FMK) were investigated in spermatozoa incubated for 1.5, 3 and 24 hours. The spermatozoa vitality was examined with propidium iodide (PI). Not less than 500 spermatozoa were analysed for each case. Observation was made under confocal microscope LSM 510 (Zeiss).

Three spermatozoa subpopulations were observed: 1) vital spermatozoa without PS translocation (AN-V negative/ PI negative), 2) vital spermatozoa with PS translocation (AN-V positive/ PI- negative) and 3) death spermatozoa with PS translocation (AN-V positive/ PI positive). The PS translocation was found mainly in the midpiece. In the control group the percentage of AN-V positive/ PI negative spermatozoa measured immediately after ejaculation was 1.0 $\pm$ 0.3%, while it was 1.4 $\pm$ 1.5%, 1.4 $\pm$ 1.3 and 0.4 $\pm$ 0.5% after 1, 5, 3 and 24h respectively. In the control group the percentage of AN-V negative/ PI negative spermatozoa was 56.5 $\pm$ 8% after 24h and the percentage of AN-V positive/ PI positive spermatozoa was 42.8 $\pm$ 7% after 24 h. EDS and TPEN did not significantly influenced the percentage of spermatozoa subpopulations ($p>0.05$). Active caspase-3 and caspase-9 spermatozoa were observed in midpiece of spermatozoa after ejaculation. EDS but not TPEN stimulation increased the percentage of vital and mobile spermatozoa presenting active form of caspase-3 and caspase-9.

The data suggest that the apoptotic process can be detected in human spermatozoa. Further studies can reveal whether spermatozoa apoptosis follow the same mechanism as somatic cells apoptosis and whether this process constitutes a dominant form of spermatozoa death.

The actin filaments of the embryo-suspensor in *Sedum acre* L.

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Differentiation of the suspensor in *Sedum acre* L. was studied in comparison with the development of embryo-proper. The zygote divides into the apical cell and the basal cell (BC), which becomes the basal cell of the suspensor. The BC produces haustorial branches invading the surrounding integumentary cells, sometimes up to the epidermis. The fully differentiated suspensor is composed of a large, pear-shaped BC and a few chalazal cells. The actin cytoskeleton was investigated by immunohistochemistry and epifluorescence microscopy.

Ovules were excised from ovaries in various developmental stages and immediately fixed. After fixation the plant material was embedded in Steedman's Wax. Immunohistochemical assays were performed on sections using monoclonal antibody (Mab): mouse anti-actin. Antigen-Mab complexes were localized by a secondary, Alexa 488-conjugated anti-mouse, antibody. The actin filaments were also stained by rhodamine-phalloidin.

During the early phase of BC development, the microfilaments were oriented longitudinally to the long axis of the cell. F-actin was observed to concentrate near the micropylar apex of the BC. A delicate network of actin filaments was found in the micropylar haustorium. The fully differentiated and the final phase of suspensor development were similar to each other in relation to distribution of microfilaments. At these both stages, actin filaments formed a prominent network. The microfilaments could also be localized in the cortical region of the cell and they had a netlike distribution. In addition, actin was observed around the large nucleus. A numerous microfilaments were noted in the cytoplasmic layer adjacent to the wall, separating the BC from the first layer of the chalazal suspensor cells. In the micropylar haustorium, the localization of microfilament cytoskeleton was similar to the previous stage of development of suspensor. BC. At all stages of suspensor development in the embryo-proper the fluorescence of actin was weak.

These investigations reveal new details of the microfilament cytoskeleton in the developing suspensor.

Ethylene may also play a role as a plant sex hormone in algae

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Studies carried out using a three-zonal model and exogenously applied ACC (1-aminocyclopropane-1-carboxylic acid) the key by-product in the ethylene synthesis pathway revealed that ethylene, a gaseous plant hormone played an important role in GA_3-induced male sex determination in *Anemia phyllitidis* gametophytes. Moreover, analyses of the extracts from gametophytes of this fern carried out by capillary electrophoresis showed the presence of ACC and revealed changes of its level during GA_3-induced antheridia formation.

The wide spectrum of studies performed on exogenously applied and on endogenously occuring gibberellic acid showed that this hormone participated in the control of differrentiation of antheridia and thalli in *Chara tomentosa* and *Chara vulagaris* algae. The results of these studies indicate that gibberellic acid in *Chara* species plays a similar role as a male sex hormone to that in *Anemia phyllitidis*. This fact inspired us to study the involvement of ethylene in the control of *Chara vulgaris* morphogenesis. To explained this problem the capillary electrophoresis was used to answer the questions: (1) does *Chara vulgaris* thalli containe ACC and (2) does ACC synthesis may be under control of AOA (aminooxyacetic acid; inhibitor of ACC synthesis). Preliminary results concerning the above problem indicate that ethylene may play a similar role in sex determination in *C. vulgaris* and *A. phyllitidis.*

Unio tumidus Philipsson, 1788 (Bivalvia: Unionidae) gonads development with the presence of *Rhodeus sericeus* Pallas, 1776 (Pisces: Cyprinidae) larvae

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The research of West Pomeranian Region malacofauna started by our team concentrates mainly on the alien species (Domagała et al., 2003; Domagała et al., 2004; Łabecka et al., 2005). However, the studies on the native mollusks are still continued (Łabecka and Migdalska, 2004). The distribution of ostracophilic fish is strictly connected with Unionidae mussels presence. *Rhodeus sericeus* is the only european fish, in which eggs' fertilization and early development take place in mussels' gill cavity. In Europe, bitterlings spawn between April and August, with a peak in spawning occurring in May (Zhul'kov and Nikiforov, 1988; Douglas, 2003).

The sample of *Unio tumidus* was collected in spring 2004 during scuba-diving in Rybacki Channel (Miedzyodrze area). In the inner and outer gills of some mussels *Rhodeus sericeus* larvae with yolk sack were found.

The mollusks were fixed in formalin and their gonads were embedded in paraffin. Serial sections (5 μm thick) were cut by a rotation microtome and then stained with hematoxylin. The slides were examined under light microscope coupled with the computer image analysis system. The development of mussels' gonads was investigated during an incubation of bitterling's larvae.

REFERENCES:

DOMAGAŁA J, MIGDALSKA B, ŁABECKA AM, and PILECKA-RAPACZ M. 2003. *Anodonta woodiana* (Lea, 1834) in the Western Pomerania. [In Polish, English abstract]. *Zeszyty Naukowe Uniwersytetu Szczecinskiego. Acta Biologica* 10: 199–202.

DOMAGAŁA J, ŁABECKA AM, PILECKA-RAPACZ M, and MIGDALSKA B. 2004. *Corbicula fluminea* (O. F. Müller, 1774) (Bivalvia: Corbiculidae) – a species new to the Polish malacofauna. *Folia Malacologica* 12(3): 145–148.

DOUGLAS A. 2003. Population ecology and behaviour of juvenile bitterling *(Rhodeus sericeus*, Pallas). Ph.D. Thesis. Queen Mary College University of London.

ŁABECKA AM, and MIGDALSKA B. 2004. Shell variation in *Unio tumidus* Phillipson, 1788 from the warm canal of the power plant Dolna Odra. In: Pokryszko B. The 20-th Polish Malacological Seminar. *Folia Malacologica* 12(2): 83–106.

ŁABECKA AM, DOMAGAŁA J, and PILECKA-RAPACZ M. 2005. First record of *Corbicula fluminalis* (O.F. Müller, 1774) (Bivalvia: Corbiculidae) – in Poland. *Folia Malacologica* 13(1): 25–27.

ZHUL'KOV AI, and NIKIFOROV SN. 1988. Some data on the morphology and biology of the bitterling *Rhodeus sericeus* of the Tym' River, Sakhalin. *Vopr Ikhtiol* 28: 149–153.

The effects of daidzein injection on histological changes in the mouse testis

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There is a lot of evidence suggesting that phytoestrogens, which can be found in some plants, may influence human and animal reproduction. It is possible, because these substances, e.g. genistein and daidzein, possess estrogen- like biological activity. The aim of this study was to investigate the effects of daidzein injection on histology and function of mouse testis.

Adult male Balb/c mice were injected every day for 2 weeks intraperitonealy with 0.1 ml of saline containing 5mg of daidzein (these animals were marked as DA I $_{1\text{-}10}$) or 50μg of daidzein (these animals were marked as DA II $_{1\text{-}10}$). The mice from control group were injected intraperitonealy with saline alone. Animals marked as DA I $_{1\text{-}5}$ and DA II $_{1\text{-}5}$ were killed by dislocation of spinal cord one day after last injection and mice marked as DA I $_{6\text{-}10}$ and DA II $_{6\text{-}10}$ were killed 14 days after last injection. Then, the testes and the epididymis were collected and the testes were weighed and prepared for light microscope examination. Daidzein induced significant degeneration of seminiferous epithelium inside tubules of testis. We noticed inhibition of mitosis and meiosis in seminiferous epithelium, decreased number of primary spermatocytes and spermatids inside seminiferous tubules and decreased number of sperm cells both in seminiferous tubules and epididymis duct. Sometimes the stages of the seminiferous cycles were difficult to discriminate. We observed that some seminiferous epithelial cells exfoliate inside tubules. This visible degeneration was more severe in group DA II than DA I. Partial reversion of these changes was visible 14 days after last daidzein injection. In groups DA I $_{1\text{-}5}$ and DA II $_{1\text{-}5}$ testes were smaller than in the control group, but in groups DA I $_{6\text{-}10}$ and DA II $_{6\text{-}10}$ the weight of testes was similar to that found in the control group (but this differencess were not significant). In group DA II $_{1\text{-}10}$ we found more macrophages in Leydig's glands than in the control group.

Preliminary characteristic of centromeric and pericentromeric regions during oogenesis and meiotic maturation of mouse oocytes

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The centromeres are the fragments of DNA that are responsible for proper chromosome segregation during mitosis and meiosis. These domains are required to assemble complexes of kinetochore proteins. The latter are known to participate in such processes as attachment of microtubules and chromosome movement, maintaining cohesion between sister chromatids and arresting the cell cycle progression in case of any problems with spindle attachment (Sullivan et al., 2001; Smith, 2002). Centromeric chromatin is surrounded by pericentromeric heterochromatin. In mitotically dividing somatic cells it has been shown, that both can be characterized by the presence of certain proteins, specific pattern of epigenetic modifications of core histones and transcriptional inactivity (Sullivan and Karpen, 2004). Although recently the localization of centromeres during folliculogenesis of mouse oocytes has been shown (Garagna et al., 2004), the characteristic of these chromatin fragments in meiotic oocytes has not been presented. Here we show the transcriptional activity and the pattern of epigenetic modifications (acetylation, methylation) of histone H3 during oogenesis of the mouse oocytes (from primordial, to fully grown) and during their meiotic maturation. We also demonstrate the localization of heterochromatin protein-1α (HP1α), which is involved in the maintenance of pericentromeric heterochromatin (Maison and Almouzni, 2004). Our results suggests, that in primary and maturing mouse oocytes the centromeric and pericentromeric regions correlate with the areas of highly condensed chromatin, well visible and distinguishable in Hoechst staining. These regions are characterized by low acetylation, but high methylation of histone H3, the presence of HP1α and the lack of transcriptional activity in primary oocytes.

REFERENCES:

GARAGNA S, MERICO V, SEBASTIANO V, MONTI M, ORLANDINI G, GATTI R, SCANDROGLIO R, REDI CA, ZUCCOTTI M. 2004. Three-dimensional localization and dynamics of centromeres in mouse oocytes during folliculogenesis. *J Mol Histol* 35: 631–638.

MAISON C, ALMOUZNI G. 2004. HP1 and the dynamics of heterochromatin maintenance. *Nat Rev Mol Cell Biol* 5: 296–304.

SMITH MM. 2002. Centromeres and variant histones: what, where, when and why? *Curr Opin Cell Biol* 14: 279–285.

SULLIVAN BA, BLOWER MD, KARPEN GH. 2001. Determining centromere identity: cyclical stories and forking paths. *Nat Rev Genet* 2: 584–596.

SULLIVAN BA, KARPEN GH. 2004. Centromeric chromatin exhibits a histone modification pattern that is distinct fromboth euchromatin and heterochromatin. *Nat Struct Mol Biol* 11: 1076–1083.

The effects of exposure of developing *Tegenaria atrica* C.L.Koch embryos to alternating temperatures

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Long-term studies on the effect of supraoptimal temperature and of alternating temperatures on embryos of *Tegenaria atrica* C.L.Koch have demonstrated that it is a factor significantly influencing the embryos' development causing to teratological changes in the external and internal structure of the entire prosoma and in the pre-spinneret and post-spinneret part of the opistosoma (Jacunski, 1969, 1984, 2002a; Jacunski at al., 2004). The mechanism of the formation of development abnormalities in embryos treated alternately with temperatures lower and higher than optimal has been described by Jacunski (2002b).

Recent laboratory research work on the effect of alternating temperatures of 14°C and 32°C (changes every 12 hours for 10 days, i.e. until the moment of germ band segmentation) on developing embryos of *Tegenaria atrica* C.L.Koch has supplied new interesting material for further analysis of the pathological changes in the prosoma and its appendages. That teratogenic factor caused the development of oligomely (unilateral or bilateral reduction of appendage number), schistomely (bifurcation of the whole appendage or only its distal part), heterosymely (accretion of two contiguous appendages), polymely (development of one or more accesory appendages), and complex anomalies. The latter kind of deformity consisted in simultaneous occurence of different types of deformity in one individual (e.g. schistomely, oligomely and heterosymely). Two very rarely found cases of development deformity deserve particular attention. One of them is an uncommon kind of polymely, in which the pedipalpi were multiplied, which resulted in the broadening of the front prosoma and shifting of the mouth opening towards one side. The other case consisted in the shortening of the appendage on the petiolus, which is considered to be an atavistic characteristic (Jacunski, 1984).

REFERENCES:

JACUNSKI L. 1969. The effect of temperature treatment at various stages of embryonic development on the formation of monstrosities in the spider *Tegenaria atrica* C.L.Koch. *Bulletin de L'academie Polonaise des Sciences,* Cl.II 17(9): 555–557.

JACUNSKI L. 1984. Studia nad teratogeneza eksperymentalna u pajaka *Tegenaria atrica* C.L.Koch. UMK Torun.

JACUNSKI L. 2002a. Anomalies of the abdomen in *Tegenaria atrica* C.L.Koch (*Araneae, Agelenidae). Bulletin of the Polish Academy of Sciences, Biological Sciences* 50(3): 189–191.

JACUNSKI L. 2002b. Structural anomalies thermally induced in *Tegenaria atrica* C.L.Koch embryos. *Bulletin of the Polish Academy of Sciences, Biological Sciences* 50(2): 153–157.

JACUNSKI L, NAPIÓRKOWSKA T, TEMPLIN J, and TESZNAR L. 2004. Anomalies in the cephalic part of prosoma in *Tegenaria atrica* C.L.Koch*. Zoologica Poloniae* 49(1–4): 97–110.

Pneumatic spaces of the facial skeleton in human fetuses in computed tomographic study

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The pneumatic bones of the skull located around the nasal cavity and the ear are of great clinical importance. There are many controversies as to the development of the paranasal sinuses and air spaces in human. This was the stimulus for study.

Ten fetuses aged between 19 and 38 weeks, from Collection of Department of Anatomy in Poznan were investigated. Study was made in CT Laboratory, by Picker CT PQ Apparatus. The thickness of layer was 1.5 mm. Images were recorded in magnification film screen technique. Special attention was paid to the frontal and maxillary sinuses and the anterior and posterior ethmoidal cells.

In fetus at 19 weeks narrow space is observed between the squamous and orbital part of the frontal bone. At this time single ethmoidal cells are seen in the ethmoidal labyrinth.

At 24 weeks the slightly calcified middle nasal concha and the middle nasal meatus with excavation in lateral wall of the nasal cavity, corresponding the maxillary sinus are seen. Ethmoidal cells are multiple (5 in number) with partially calcified walls. At this period the communication between the pneumatic spaces of the body of sphenoid and the sphenoethmoid recess are observed.

At 27 weeks the recess of the developing middle nasal meatus passes upwards into oval thin walled space of the developing frontal sinus is seen adjacent to ethmoidal labyrinth.

At 29 weeks excavation in lateral wall of the nasal cavity representing maxillary sinus enlarges.

In fetus of 34 weeks the sizes of the maxillar sinus are 4 mm in antero-posteror diameter and 0.8 mm in transverse diameter.

At 36 weeks within the ethmoidal labyrinth there are 7–8 ethmoidal cells varying in size and communicate with each other.

In fetus at 38 weeks the maxillary sinus has the following measurments 6.0 mm in antero-posterior diameter and 1.1 mm in transverse. It is separated by thin bony wall of the inferior nasal meatus. At this time the ethmoidal labyrinth and the ethmoidal cells as well as the frontal sinus communicate with each other.

REFERENCES:

NEMZEK WR, BRODIE HA, CHONG BW, BABCOOK CJ, HECHT ST, SALAMAT S, ELLIS WG, SEIBERT JA. 1996. Imaging findings of the developing temporal bone in fetal specimens. *Am J Neuroradiol* 17: 1467–1477.

Expression of angiotensin II AT1 and AT2 receptors in fetal mammalian kidney

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The renal renin-angiotensin system (RAS) is known to be up-regulated during mammalian renal development and in the perinatal period. The AT2 receptor is found ubiquitously in fetal tissues and plays a role in cell growth and differentiation. In the fetal kidney, AT2 receptor expression is seen mostly in the mesenchymal cells of differentiating cortex and medulla, which are surrounding glomeruli in the cortex in tubular tissues. The mesenchymal cells undergo apoptosis and are replaced by tubular tissues. Expression of AT2 decreases rapidly after birth while AT1 increases in maturation. We have studied expression of AT1 and AT2 receptors in fetal kidneys of lamb, pig, dog and cat. Our findings suggest that AT1 and AT2 receptors are both involved in the development of the nephron. Moreover, the AT2 expression increases markedly in adult kidney after injury, suggesting its role in repairing mechanisms.

Cytochemical and immunocytochemical identification of histones and protamines during *Chara vulgaris* spermiogenesis

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The formation of mobile male gametes, spermatozoids is accompanied by flagella appearance (the organs which enable free moving in water), replacement of nuclear histones with strong basic proteins – protamines and super condensation of chromatin leading to "sleeping genome".

Spermiogenesis in *Chara* algae which has been divided into 10 stages (sp I–X), is similar to spermiogenesis in animals. There takes place the exchange of histone proteins into protamine-like proteins. Earlier cytochemical studies showed that at sp I–IV stages only histones were present, at sp V–VIII stages – the amount of nuclear protamine-type proteins increased and that of histones decreased while at sp IX–X only protamine-type proteins were present. This was also confirmed with capilar electrophoresis.

In order to localize more precisely both histones and protamines the immunocytochemical studies with the use of anti-protamine antibodies which were obtained from *Chara tomentosa* antheridia and anti-histone H3 antibodies have been carried out.

The use of the anti-protamine antibodies did not reveal a positive reaction at sp I–IV stages while at sp V–X strong antigenic signals were noted. At spV these strong strand-like signals were observed in cytoplasm which might suggest that protamine synthesis took place in ER. At sp VI–VIII the reaction was observed both in the cytoplasm and nucleus. At the last stages (sp IX–X) strong signals were visible at nuclear peripheries and slightly weaker inside the nucleus.

The use of the anti-histone H3 antibodies strongly supported cytochemical results. The antigenic signals strong at the beginning (sp I–IV) gradually became weaker (sp V–VIII) and finally disappeared.

Ultrastructure of the young gonad of the hermaphroditic species *Isohypsibius granulifer* Thulin, 1928 (Tardigrada: Eutardigrada)

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The reproductive system of hermaphroditic *Isohypsibius granulifer* consists of the gonad and the gonoduct that opens into the rectum (Weglarska, 1987). The sac-like gonad lies on the dorsal part of the body and its anterior part is suspended from the dorsal body wall by two ligaments. This gonad consists of three parts: two of them (germarium, vitellarium) belong to the ovary and the third to the testis. In the young gonad (previtellogenesis and early vitellogenesis) the gonad wall is built of one layer of the little flattened cells supported on the basal lamina. The protrusions of these cells enter among the female germ cells. There are no protrusions in the male part of the gonad. The germarium is filled with small, non-differentiated cells (cystoblasts) containing the large nuclei, few mitochondria, single cisterns of RER and ribosomes. The cystoblasts, as a consequence of the incomplete mitotic divisions, are connected by the cytoplasmatic bridges. The second part of the gonad (vitellarium) is large. The female germ cells, localized in the vitellarium, are still connected by the cytoplasmatic bridges. They have similar sizes and shapes. This is difficult to show which cell will be oocyte because during early vitellogenesis all cells in the vitellarium begin to sinthesize the reserve materials. The volume of their cytoplasm gradually increases. The large nucleus with granular nucleolus, numerous mitochondria, cisternae of RER, cisternae of smooth endoplasmic reticulum, a lot of free ribosomes and Golgi complexes and the first small, electron dense spheres, which blend together creating bigger structures, are visible in the cell cytoplasm. Distal part of the gonad is occupied by male germ cells. They have lobular shape and small size. The large nucleus, mitochondria and Golgi complexes are visible in their cytoplasm. The Golgi complex is situated near the nucleus. Completely developed sperms are observed among young male germ cells. Sperm tail has 9 + 2 axoneme. There is no border between female and male germ cells. The two types of cells make direct contact.

REFERENCES:

WEGLARSKA B. 1987. Yolk formation in *Isohypsibius* (Eutardigrada). *Zoomorphology* 107: 287–292.

The development of the alveolar part of the mandible in human embryos and fetuses

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The mandible is formed of 6 developmental skeletal units (basilar, alveolar, coronoid, angular, condylar, and mental). Each of these units is influenced by a functional matrix that acts upon the bone. The development of alveolar part is in close relation with the dental lamina and teeth.

The aim of study was to follow the formation of alveolar part in human prenatal development. Study was performed on 15 staged human embryos and 12 fetuses.

The formation of alveolar part of mandible begins in embryos at stage 13 with the thickening of mandibular process epithelium giving rise to the dental lamina. In embryos at stages 15 to 17, the dental lamina deepens into underlying mesenchyme and by the end of 6th week it forms continuous horseshoe shaped plates. At the beginning of 7th week (stage 18) from the primary ossification center the bony trabeculae extend to the body and ramus of mandible. In embryos at stages 19 and 20 secondary dental lamina invaginates between anterior, better developed bony lamina of the future alveolar part and Meckel's cartilage. During the 8th week (stages 21 to 23) developing anterior lamina of alveolar part overgrowths Meckel's cartilage.

In early fetal period all skeletal parts of the mandible unite. In fetuses at 10th week internal lamina of alveolar part develops and mesenchyme surrounding the dental buds forms bony crypts with developing teeth. In fetuses from 13 to 18 weeks alveolar part of mandible is formed by woven bone, whereas in other parts of mandible the initial woven bone formed along Meckel's cartilage is replaced by lamellar one. In fetuses from 19 to 35 weeks the walls of alveolar sockets are formed by bony trabeculae, which begin to form over the buds, closing the alveolar sockets.

Ultrastructural changes in the midgut epithelium in *Lepisma saccharina* L. (Insecta, Zygentoma) during regeneration

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Regeneration is a process of rebuilding damaged or disrupted cells and tissues. The insect's midgut epithelium becomes damaged by passage of the food mass and by secretory processes.

Midgut epithelium of *Lepisma saccharina* is formed by the epithelial and regenerative cells. The latter form regenerative groups which lie on the basal lamina and are squeezed between epithelial cells. 8 to 12 regenerative groups can be seen in a transverse section through the midgut. Each group is composed of 8–14 cells.

In *L. saccharina* the midgut epithelium, after the degeneration, is totally removed into the midgut lumen. Only fragments of membranes and some organelles remain between the regenerative cells groups. The most externally located cells of the regenerative cells group begin to transform into epithelial cells: a distinct arrangement of the organelles in the cytoplasm is observed. The basal parts of these cells widen and start to contact the newly formed epithelial cells from neighboring regenerative groups. As a result the regenerative cells groups are separated from the midgut lumen by the apical parts of the new epithelial cells. Afterwards, succeeding regenerative cells transform into epithelial cells and characteristic extracellular vacuoles appear between them. Membranes of the adjacent cells form microvilli into their lumen. Following the transformation, vacuoles move towards the apical regions of these cells. They finally open into the midgut lumen. The characteristic vacuoles take part in microvilli formation. In this way the midgut epithelial cells are replaced.

Regenerative cells groups form the new epithelium simultaneously in a cyclic manner. They, being responsible for all regenerative mechanisms, fulfill the role of primordial cells of the midgut epithelium (Rost, 2006).

REFERENCES:

ROST MM. 2006. Comparative studies on regeneration of the midgut epithelium in *Lepisma saccharina* L. and *Thermobia domestica* Packard (Insecta; Zygentoma). *Ann Entomol Soc Am* (in press).

Regeneration in the midgut epithelium of *Thermobia domestica* (Packard) (Insecta, Zygentoma)

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Midgut epithelium of *Thermobia domestica* is composed of the epithelial and the regenerative cells (Rost et al., 2005). Regenerative cells form groups, which lie on the basal lamina. Four to 6 regenerative cell groups are observed in transverse sections of the midgut. Each regenerative group consists of 4–8 cells.

The midgut degeneration process is observed only in individual cells. The morphological representation of the beginning of midgut epithelium cell degeneration is the appearance of single vacuoles in the apical region of some of epithelial cells. The number of vacuoles increases. The external cells of the regenerative group begin to acquire an epithelial character; basal membrane forms folds, regional distribution of all organelles appears. The basal membrane of a degenerating cell ruptures and the remaining organelles are moved into the midgut lumen. The apical membranes of the newly formed cells that are in contact with its lumen start to form microvilli. Gap and septate junctions appear. Thus, the degeneration and regeneration processes of the midgut epithelium in adult specimens and larvae of *T. domestica* occur in a continuous manner (Rost, 2006).

REFERENCES:

ROST MM, KUCZERA M, MALINOWSKA J, POLAK M, and SIDOR B. 2005. Midgut epithelium formation in *Thermobia domestica* (Packard) (Insecta, Zygentoma). *Tissue Cell* 37(2): 135–143.

ROST MM. 2006. Comparative studies on regeneration of the midgut epithelium in *Lepisma saccharina* L. and *Thermobia domestica* Packard (Insecta; Zygentoma). *Ann Entomol Soc Am* (in press).

Germ band of *Thermobia domestica* (Packard) (Insecta, Zygentoma) formation

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In eggs of *Thermobia domestica* as a result of cleavage cellular blastoderm is formed (Rost and Poprawa, 2006).

During the second day of embryogenesis the germ band formation proceeds. Cells of the blastoderm, which lie on the postero-lateral region of the egg, begin to change their shapes from flat to cylindrical. They represent the germ band primordium. The rest of blastodermal cells differentiate into serosa.

Among cells of germ band primordium mitotic divisions are observed. Initially mitotic spindles line up perpedicularly to the egg surface. This leads to the formation of the multicellular germ band. It has the shape of a small circular disc. Succeeding mitotic divisions lead to the germ band elongation. It is composed of two head lobes and the proliferating zone and is heart-shaped. Due to mitotic divisions of cells in the proliferating zone, the germ band is elongating. Succeding segments of the head with mouth apparatus appendages, thorax and abdomen are added. Embryo begins to sink into the yolk mass and blastokinesis starts.

During the elongation of the germ band, gastrulation starts. It originates from the midline of the germ band. As a result, on the external surface of the germ band slight primitive groove appears. Two layers of tissue are formed. The inner one forms coelomic sacs.

Study was carried using TEM, SEM and fluorescent microscope.

REFERENCES:

ROST MM, and POPRAWA I. 2006. Cellularization during the development of *Thermobia domestica* (Packard) (Insecta, Zygentoma, Lepismatidae). *Ann Entomol Soc Am* (in press).

Midgut epithelium formation in *Lepisma saccharina* L. (Packard) (Insecta, Zygentoma)

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At the end of the embryogenesis of *Lepisma saccharina*, when stomodaeum and proctodaeum are completely formed, midgut epithelium is absent. It is replaced by the primary midgut, which is yolk mass surrounded by a cell membrane.

The process of midgut epithelium formation begins in the 1st instar larva. Energids (nuclei surrounded by a thin layer of the non-membraned cyoplasm) move towards the yolk periphery. They aggregate just beneath the cell membrane and are gradually enclosed by the outer cell membrane invaginations. Single cells are formed. Succeeding energids join just formed cells and are again wrapped by cell membrane folds. In this way groups of cells, called regenerative cells groups, are formed. Their number gradually increases.

In the 2nd instar larva of *L. saccharina* numerous mitochondria, cisternae of RER and SER, and dictyosomes appear in the cytoplasm of the regenerative cells. The external cells of the regenerative cells groups transform into epithelial cells: the distinct regionalisation in organelles distribution appears. Their basal regions spread towards the next regenerative cells groups. After the transformation of the succeeding regenerative cells, epithelial cells of the neighboring regenerative groups join each other. The epithelium is formed.

At the end of the 2nd instar larval stage, just before molting, the process of degeneration of newly formed epithelium begins. Remains of organelles and basal membrane are observed between the regenerative cells groups. The new epithelium is formed from the regenerative cells groups in the 3rd instar larval stage.

This process is described with the use of the transmission electron microscopy.

Expression of β-catenin and DE-cadherin in the midgut epithelium of *Thermobia domestica* (Packard) (Insecta, Zygentoma)

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The intercellular adhesive molecules are responsible for adhesion of neighbouring cells and for their contact with the extracellular matrix. They decide which cells will contact the neighbour and how the tissue will be organized. Cadherins are intercellular adhesive molecules, whose cytoplasmic domains are bound to catenins joined with the cytoskeletal elements. Extracellular domains conjoin with the same domains of the analogous cadherin. β-catenin and DE-cadherin are also involved in the process of midgut epithelium formation in some insect species (Baumann, 2001; Wang et al., 2004).

Zonula continua (smooth septate junction) is observed as the intercellular junction in apical regions of the midgut epithelium of *Thermobia domestica*. This kind of intercellular junctions is common for insects.

We used antibodies against β-catenin (Sigma-Aldrich), Armadillo (Developmental Studies Hybridoma Bank) and DE-cadherin (Dr. Hiroki Oda, Kyoto University, Japan) for immunostaining. Secondary antibodies were conjugated with FITC.

The expression of β-catenin and Armadillo was observed in the apical regions of epithelial cells. The surprising was that they were also detected around regenerative cells groups, but not between regenerative cells. The expression of DE-cadherin was observed only in the apical regions of epithelial cells, where *zonula continua* junctions are present.

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REFERENCES:

BAUMANN O. 2001. Posterior midgut epithelial cells differ in their organization of the membrane skeleton from other *Drosophila* epithelia. *Exp Cell Res* 270: 176–187.

WANG F, DUMSTREI K, HAAG T, and HARTENSTEIN V. 2004. The role of DE-cadherin during cellularization, germ layer formation and early neurogenesis in the *Drosophila* embryo. *Dev Biol* 270: 350–363.

The morphological phases of the gonads development in the grass snake *Natrix natrix* L.* (Lepidosauria, Serpentes) embryos

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The morphological phases of the development of the gonads in grass snake (*Natrix natrix* L.) embryos were investigated with histological methods. 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). According to light microscope investigation in the course of gonads development there may be distinguished four morphological phases. First phase of the gonad development is a genital ridge. This phase were observed between I and III developmental stages of *Natrix* embryos. The genital ridge lies between the base of the mesonephros and the root on the dorsal mesentery. This ridge is formed by the coelomic epithelium, within which the primordial germ cells become lodged during the course of their migration. Second phase of the gonads development is named undifferentiated gonad. This phase was observed between IV and V developmental stages of *Natrix* embryos. In the undifferentiated gonad the coelomic epithelium starts to proliferate. In this phase gonad's primordia consists of a mass of cells containing: pre-existing mesenchymal cells and primordial germ cells. Third phase of the gonads development the bipotential gonad was observed between VI and VII developmental stages of *Natrix* embryos. In this phase the gonadal primordia are similar in both sexes. PGCs were found both in the medulla and cortex of gonads primordia. Forth phase of the gonads development starts when sexual differentiation becomes visible. This phase was observed between VIII and XII developmental stages of *Natrix* embryos. In embryonic males, cells from medullary zone of the gonads form the cellular cords, which give rise to the seminiferous tubules. In female embryos the embryos the germinal epithelium proliferates and thickens, and the germinal cells located there divide and enter into meiotic prophase.

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. nr 2 poz. 11 z 1984 r., Dz. U. nr 114 poz. 492 z 1991 r.). 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 hsp70-related gene during mouse embryogenesis

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One of the 70-kDa heat shock protein genes is specifically expressed in testis of mature animals. The gene, named *Hsp70 .2* in mouse and *Hst70* in rat, codes for the protein that is associated with synaptonemal complexes, and works as molecular chaperone for cyclin-dependent CDC2 kinase required for completion of the meiotic division. Here we investigated activity of the *Hsp70.2/Hst70* gene promoter during embryogenesis 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. EGFP fluorescence was not observed in preimplantation embryos. RNA template-specific-polymerase chain reaction (RS-PCR) confirmed that *Hsp70.2* mRNA was repressed in unfertilized eggs, 1-cell and blastocyst stages and only slightly induced by zygotic gene activation at 2-cell stage (that was below detection of EGFP in transgenic mice). During postimplantation phase of embryogenesis, the EGFP fluorescence was observed mainly in developing brain and nervous system 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. The transgene was expressed also in developing limb. *Hst70* gene promoter was activated in bone tissue and in myocytes but not in bone marrow cavity and hypertrophic chondrocytes, where intensive apoptotic cell death takes place in developing long bones. We found EGFP fluorescence also in extra-embryonic membranes mainly in the yolk sac and allantois. Our observations showed that spatial and temporal expression of hst70 gene coincide with series of major developmental events, such as extra-embryonic membranes formation, axial rotation, formation of the neural tube (primordium of central nervous system), formation of differentiated somites, extensive remodeling of the heart, development of long bones occurring by means of endochondral ossification, and sensory organ formation. These results indicated also that localization of hst70 gene expression in the developing mouse embryos corresponds with localization of HSC70 expression.

Spermatogenesis in an earthworm *Dendrobaena veneta* (Rosa)

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Spermatogenesis in an earthworm *Dendrobaena veneta* was studied with the use of light and electron microscopes. *D. veneta* had two pairs of small testes lying on both sides of ventral parts in 10 and 11 segments and connected to the septa. Testes contained spermatogonia which divided mitotically. Divisions were synchronous and resulted in the development of groups of cells interconnected by cytoplasmic bridges formed clusters (morula). The last mitotic division of spermatogonia gave primary spermatocytes which were released from testes into seminal vesicles where spermatogenesis continued. In seminal vesicles the next spermatogenic stages were observed: clusters of primary and secondary spermatocytes, spermatids and spermatozoa. Germ cells in a cluster were conected by cytoplasmic bridges to the central acellular mass – cytophore. The primary spermatocyte clusters possessed only small cytophores, in secondary spermatocytes clusters cytophores enlarged and in spermatid morulae reached its maximal size and became irregular in shape, sometimes elongated. These cytophores were similar to elongated projections of nurse cells in *D. veneta* ovary. The cytoplasmic mass of cytophores included cell organelles as mitochondria, lysosomal bodies, ribosomes and endoplasmic reticulum formed circular patterns. *D. veneta* had three pairs of seminal vesicles in 9, 11 and 12 segment of the body. The inside of seminal vesicles was formed of somatic tissue not of celomic fluid as in the others Annelids.

Embryological features of *Armeria maritima* (Mill.) Willd. s.l. with special reference to calamine population

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The studies were carried out on *Armeria maritima* (Mill.) Willd. s.l. (Plumbaginaceae), a member of calamine flora adapted to high levels of heavy metals in the soil. The studied specimens grew on spoil heap in Bolesław near Olkusz, rich in Zn, Pb, Cd and Fe.

The aim of the studies was to determine male and female gametophyte development and embryo formation in stress conditions of the polluted site as well as in order to elucidate low seed production in the waste population.

Some embryological features of *A. maritima* are distinctive in the family Plumbaginaceae, e.g. circinotropous ovule, Fritillaria type of embryo sac development exhibiting the phenomenon referred to the "Carano-Bambacioni effect". The embryogeny corresponds to Solanad type; the massive suspensor becomes cordate and closely surrounds the tip of the radicle. In microsporogenesis cytokinesis following meiosis is simultaneous and the pollen is shed at the three-celled stage. In *A. maritima* two different pollen and stigma types occur and pollen incompatibility reaction manifests itself when pollen tube growth is arrested in the style. Self-incompatibility after open pollination affected ca 40% of studied flowers; necrosis of unfertilized embryo sacs resulted in low seed set. These observations indicate that the studied population consists of numerous incompatible clones originating from vegetative reproduction.

On the other hand, some developmental disturbances and embryological irregularities observed before pollination in 16% of studied flower buds (e.g. necrosis of whole flower buds or ovules, disturbances in embryo sac development and in microsporogenesis) are interpreted as result of unfavorable environmental factors in the polluted site.

Expression of pluripotency and differentiation markers in mouse and bank vole embryonic cells

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Pluripotent embryonic stem cells (ES cells) can be derived from inner cell mass (ICM) of preimplantation embryos at the blastocyst stage (reviewed in Smith, 2001). Isolation and long-term *in vitro* culture of ES cells derived from species other than human and mouse, including bank vole (*Clethrionomys glareolus*), turned out to be extremely difficult.

A number of unique proteins characteristic for pluripotent and differentiating cells have been identified in the preimplantation mouse embryo. Factors that maintain pluripotency are expressed in the ICM of the blastocyst. Down-regulation of the expression of these proteins is related to the loss of the self-renewal capacity of embryonic cells and to their differentiation into variety of cell types. The objective of this study was to investigate the potency of bank vole embryonic cells to sustain their pluripotent character during *in vitro* culture and to determine the optimal conditions for the derivation of bank vole ES cells.

We compared the presence of specific pluripotency and differentiation markers during *in vitro* culture of blastocysts and ICM outgrowths obtained from bank vole, and from two mouse hybrids [F1(C57Bl/6xCBA/H) and F1(C57Bl/6x129/Sv)]. Expression of Oct-4 and SSEA-1 (markers of pluripotent, undifferentiated cells), GATA-4 (primitive endoderm marker) and Cdx-2 (trophectoderm marker) was examined in blastocysts and 2-, 3- and 4-day-old ICM outgrowths. We found that markers of pluripotent cells, i.e. transcription factor Oct-4 and cell-surface marker SSEA-1, were expressed longer during *in vitro* culture of embryos originated from F1(C57Bl/6x129/Sv) than from F1(C57Bl/6xCBA/H) mouse hybrids. At the 4th day of culture approximately 70% of F1(C57Bl/6x129/Sv) outgrowths and 50% of F1(C57Bl/6xCBA/H) outgrowths contained cells expressing these pluripotency markers. These observations are in agreement with the published data showing that the efficiency of ES cells derivation is greatly strain dependent. During *in vitro* culture of bank vole embryos the expression of pluripotency markers was rapidly down-regulated already in 2-day-old ICM outgrowths. It was correlated with the enhanced expression of GATA-4 and Cdx-2 thus indicating differentiation towards primitive endoderm and trophectoderm, respectively.

In conclusion, the observed differences in expression of pluripotency markers between different mouse strains and between the mouse and the bank vole suggest that genetic background and the species specific features may influence the success rate of the derivation of ES cells.

REFERENCES:

SMITH AG. 2001. Embryo-derived stem cells: of mice and men. *Annu Rev Cell Dev Biol* 17: 435–462.

Structure of ovaries of *Stomaphis quercus* (Insecta, Hemiptera, Aphidinea: Lachnidae) and *Phylloxera* sp. (Insecta, Hemiptera, Aphidinea: Phylloxeridae)

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Stomaphis quercus (Aphidinea: Lachnidae) has 5 generations each year. The first four generations are viviparous, the last generation is oviparous. All generations of *Phylloxera* sp. (Aphidinea: Phylloxeridae) are oviparous.

Ovaries of aphids are composed of telotrophic ovarioles that are surrounded by a basal lamina and inner epithelial sheath. Ovaries of *Phylloxera* sp. comprise 1–4 ovarioles, oviparous generation of *Stomaphis quercus* – 5 ovarioles, viviparous generations of *Stomaphis quercus* – 6–8 ovarioles. The individual ovariole is subdivided into a teminal filament, tropharium (trophic chamber), vitellarium and ovariole stalk (pedicel). Terminal filaments are inconspicuous and combine forming a suspensory ligament that join the ovary to the lobe of the fat body. The tropharia enclose nurse cells (trophocytes) and early previtellogenic oocytes termed arrested oocytes. The vitellaria contain 1–2 linearly arranged developing oocytes that are surrounded by a follicular epithelium. Follicular cells do not undergo diversification into subpopulations. The central part of the tropharium, termed a trophic core, is free of cells and is connected both with trophocytes and oocytes. The trophocytes are joined to the core by cytoplasmic processes, whereas oocytes communicate with the core via nutritive cords. In the ovarioles of *Phylloxera* 29–53 germ cells are present. The ovarioles of viviparous generations of *Stomaphis* contain 16–30 germ cells, oviparous generation – 46–63 germ cells. The comparison of generations of *Stomaphis* revealed that viviparous females have lower number of trophocytes and lower level of polyploidization than the oviparous females.

Ovaries of all generations of *Stomaphis* are accompanied by large organs termed bacteriomes that consist of numerous cells termed bacteriocytes. The bacteriocyte cytoplasm is filled with endosymbiotic microorganisms which are transovarially transmitted into the next generation. In contrast to other aphids, in the body of *Phylloxera* endosymbionts do not occur.

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Structure and development of the ovary of *Steingelia gorodetskia* (Insecta, Hemiptera, Coccinea: Steingeliidae)

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Ovaries of the third larval (last) instar of *Steingelia gorodetskia* consist of numerous spindle-shaped ovarioles that are devoid of terminal filaments and radially extend from the apex of lateral oviduct. Each ovariole is surrounded by an inner epithelial sheath and contains one cluster of interconnected germ cells. Larval ovarioles are differentiated into an apical tropharium and posterior vitellarium. The main part of the ovariole is occupied by the tropharium that is filled with large, polyploid trophocytes. The nuclei of trophocytes are lobated. In the centre of the tropharium a trophic core is present. The trophic core is connected via broad processes both with trophocytes and oocytes. The trophic core and processes are tightly packed with microtubules. In the basal part of the vitellarium several oocytes occur. The oocytes possess small, spherical nuclei. In germ cells, in somatic cells and in the trophic core numerous, small, rod-shaped endosymbiotic bacteria exist. The ovarioles of an adult female are elongated. Their long, tube-shaped tropharia comprise trophocytes and previtellogenic oocytes that are capable of further development. Contrary to the situation described in remaining scale insects, vitellaria of *Steingelia* comprise 2–4 linearly arranged developing oocytes at consecutive stages of oogenesis. Previtellogenic and vitellogenic oocytes are connected to the trophic core by means of long extensions termed nutritive cords. At the beginning of choriogenesis nutritive cords degenerate. Vitellarial oocytes are surrounded by a follicular epithelium. Follicular cells are not diversified into subpopulations. Somatic cells of the ovariole, trophocytes, oocytes and nutritive cords contain endosymbiotic bacteria. These microorganisms are transovarially transmitted from one insect generation to the next one.

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Ovary cord structure in *Hirudo medicinalis* (Hirudinea, Hirudinidae) ovary

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The female reproductive system of *Hirudo medicinalis* is composed of paired ovaries, oviducts and glands engaged in cocoon formation. Each ovary consists of an outer envelope (ovisac) and one or two ovary cords (ovary strings) containing germ line cells. The ovary cords are bathed in hemocoelomic fluid in which numerous coelomocytes can be observed. The ovisac (ovary envelope) in fact is the coelomic sac and it comprises two fenestrated epithelia separated by connective tissue and muscle cells. The ovary cords are solid, unbranched and very convoluted. The proximal end of the ovary string is club-shaped, the rest is cylindrical. The distal end of ovary string contains mainly degenerating cells. The cord is enveloped by a layer of elongated cells. Similar cells occur inside the cord. The club-shaped end of ovary string contains oogonia and small clusters (clones) of germ cells. The cells in clusters are connected by stable intercellular bridges not to each other, but to central anucleate cytoplasmic mass termed cytophore. The cells in these clones are morphologically identical, i.e. no differentiation into oocytes or nurse cells can be observed. The main part of ovary cord is composed of germ cell clones. Here, the germ cells are pear-shaped with narrow end connected to the cytophore. In clusters some cells become oocytes, they gather nutrients, detach from the cytophore, gradually protrude into the ovary lumen and eventually float freely in hemocoelomic fluid. The remaining cells in clones (nurse cells) degenerate. The detailed analysis of ultrastructure of germ cells and studies on oogenesis are still in preparation.

Embryonic development of *Tegenaria atrica* C.L.Koch

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Differences in spider embryogeny concern mainly the development of the opisthosomal tagma. In primitive *Liphistiidae*, numerous *Mygalomorphae* and lower *Entelegynae* at that time forms a comparatively long opisthosoma, with two distinguishable parts. The front segments of the opisthosoma are broad and spread over the surface of the vitellus, while the terminal ones form the postabdomen, curled up to the dorsal side, which is made up of much slenderer metameres. In progressive spiders, where higher *Entelegynae* belong, the opisthosma at the time of embryogeny is oval in shape and broad-spread over the surface of the vitellus. After reversions its terminal segments expand on the dorsal side of egg (Ivanov, 1965).

The second type of development is characteristic of *Tegenaria atrica,* which belongs to progressive spiders. The embryogenesis of *Tegenaria atrica* C.L.Koch resembles that of other spiders of that group, such as *Torania variata* Poc. (Ehn, 1963) or *Pirata piraticus* (Pen Yu et al., 2001). Three main phases can be distinguished in the embryonic development of *Tegenaria atrica* C.L.Koch (Jacunski and Wisniewski, 1997). The first one includes the initial divisions of the egg nucleus, the formation of the early and late blastoderm and its contraction until the formation of the germ disc. The second one includes the germ disc stage, the primitive plate stage, the "cumulus primitivus" stage and the germ band stage together with its metamerism. The third phase of embryogenesis includes the development of appendage buds, the reversion stage, the stage of appendage extension and that of preparation for hatching. The particular stages of embryogeny were observed by immersing the developing eggs in paraffin oil, which made their membranes transparent, after which photographic documentation of the particular embryonic stages was carried out.

REFERENCES:

EHN A. 1963. The embryonic of the spider *Torania variata* Poc. (*Sparassidae*). *Zoologiska Bidrag Fran Uppsala* 36: 37–54.

IVANOV AV. 1965. Pauki. Leningrad.

JACUNSKI L, and WISNIEWSKI H. 1996. The influence of temperaturae on the duration of embryonic stages of *Tegenaria atrica* C.L.Koch (*Agelenidae*). *Scientific Papers of Pedagogical University in Bydgoszcz, Natural Studies* 13: 181–203.

PENG YU, HU C, ZHAO JZ, CHEN J. 2001. Changes of the morphological and chemical substances of the egg of *Pirata piraticus* during embryonic development. *Acta Zoologica Sinica* 47(2): 190–195.

The structure of the male reproductive system of selected Hemiptera groups

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The bugs (Hemiptera) are world-wide and numerous (approximately 30 000 species) group of insects reproducted holometabolicly.

Aphids (Aphidoidea) have a special rank among them because of high level of polymorphism and specific mode of reproduction – cyclical parthenogenesis. So far the male reproductive system has been described in about 50 aphid species (Wieczorek and Wojciechowski, 2003).

The great number of testis follicles (7) in aphids is known only in few species; oligomerisation of the number of testis follicles, even to single in each testes, is observed. The number of testis follicles is usually constant on the level of genera The polymerisation of testis follicles which is common among other Hemiptera in aphids is not known. I addition, arranging of the spermatids is disordered. The vasa deferentia run independently on the whole length, the modifications concern mainly connection between the initial parts of these ducts. In comparison with other groups of Hemiptera is characterised by the lack of vesiculae seminalis. Double, ectadenial accessory glands are present in all examined species, in except of Lachnidae (Wojciechowski, 1977) and enter the ejaculatory duct independently and centrally compared to the outlets of vasa deferentia, usually in its apical part. The ejaculatory duct is usually a well defined, in some species is relatively short, sack-like shape and there is within penis.

In Miridae which are the most numerous family within Heteroptera, in contrast, all testis follicles are connected by peritoneal membrane. The number of follicles enclose 1–8 in each testes and is constant on the subfamily level (Akingbohungbe, 1983). Moreover, the whole follicle is divided, according to the development of germinal cells, into three regions. In comparison with aphids the structure of the vasa deferentia in Miridae is more diverse: vesiculae seminalis are always present (as an expansion in different portion of vasa deferentia), in distal part there are seminal reservoires which are connected in vas deferens commune. Likewise, the structure of accessory glands is also more diverse (ectadenial: internal and external lateral glands, mesadenial: dorsal and ventral medial gland). The distal part of the male reproductive system is the ejaculatory bulb. The ectadenial accessory glands always enter the ejaculatory bulb lateral whereas the outlets of vasa deferentia and mesadenial glands frontally (Masner, 1965).

REFERENCES:

AKINGBOHUNGBE AE. 1983. Variation in testis follicle number in the Miridae *(Hemiptera, Heteroptera)* and its relationship to the higher classification of the family. *Ann Entomol Soc Am* 76: 37–43.

MASNER P. 1965. The structure function and the imaginal development of the male inner reproductive organs of *Adelphocoris lineolatus* (Goeze) *(Heteroptera, Miridae)*. *Acta Entomol Bohemoslav* 62(4): 253–276.

WIECZOREK K, and WOJCIECHOWSKI W. 2003. The use of the structure of the male reproductive system of aphids *(Hemiptera, Aphidoidea)* in their phylogeny. *Aphids and other Homopterous Insects* 9: 173–185.

WOJCIECHOWSKI W. 1977. Procesy oligomeryzacji w budowie meskiego układu rozrodczego miodownic (*Homoptera, Lachnidae*). *Acta Biol Univ Sl,* Katowice 3: 140–164.

The prevalence and structure of *manicotto glandularae* in tadpoles of native species of Anura

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The histological structure and development of the precursor of the stomach were described in larvae *Bombina bombina*, *Pelobates fuscus* and *Rana temporaria*. Six individuals of each species in larval stage, marked by Rugh (1951) as IV, X, XVII (prometamorphosis) and XXI (metamorphosis), were studied.

The period of prometamorphosis is charakterized by an intensive development of the stomach precursor. It reached its maximum size in short time before metamorphosis (stage XVII) in all studied anuran species. The largest increase of the length of this section was observed in *B. bombina* (2.5-fold), a little small in *P. fuscus* (2-fold), and the smallest in *R. temporaria* (1.3-fold). In prometamorphic tadpoles the diameter of the stomach precursor was increased by 62% (fire-bellied toad), 50% (garlic toad) and 28% (common grass frog). The increase of the size of the stomach precursor in *P. fuscus* and *B. bombina* was accompanied also by growth of thickness of the wall. It was not stated in *R. temporaria*. In two species, namely in tadpoles of fire-bellied toad and common grass frog, the wall of the stomach precursor made the callosity named *manicotto glandularae*. The lumen of the *manicotto* was lined by simple ciliated epithelium. Their cells had centrally situated an elongated nuclei with many granulations. Under the epithelium was situated a glandular layer, which formed the groups of branching crypts. These crypts communicated with the gut lumen through irregular gaps of epithelium. In the *manicotto*, contary to adult stomach, the spherical nuclei of the gland-like structure were located in the centre of cells. In period of prometamorfosis in *P. fuscus* this layer was about 70%, and in *R. temporaria* 80% of the wall thickness of the *manicotto*. At the begining of metamorphosis (stage XXI) was observed the concentration of the glands and their shape was changed. The groups of glandular cells were partly removed into the gut lumen. In the consequence of these processes in this layer the total thickness of the wall decreased up to 57% in fire-bellied toad and 42% in common grass frog. Below of the degenerating glands appeared the distinct connective tissue and the muscularis, wich were previously poorly developed and consisted of unnumerous cells. The thickness of the wall of the stomach precursor in *P. fuscus* and *R. temporaria* is the biggest in stage XXI. It was the result of increase of thickness of the muscularis (6-fold in *P. fuscus* and 3-fold in *R. temporaria*), decrease of diameter and the length of the stomach precursor which taked place between stages XVII and XXI. Similar transformation of this section was observed in tadpoles *B. bombina*. However, in this case the glands appeared in stage XXI and had a structure as typical stomach glands.

The early development of the human maxilla

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The beginning of the lower part of the face is laid down early in embryonic period and it is formed by the first pharyngeal arch that surrounds the oral pit on both sides of the head.

Study was made in serially sectioned human embryos at developmental stages 13 to 23 and fetuses aged 9 to12 weeks. Embryos and fetuses were from the Collection of the Department of Anatomy in Poznan. All specimens were serially sectioned and stained according to various methods.

In embryos at stage 13 (middle of 5th week) the mouth is visible between the frontonasal prominence and the first pharyngeal arch. By the end of 5th week (stage 15), the mandibular arch divides into maxillary and mandibular process, forming the upper and lower jaws, respectively. The mandibular processes soon merge at the midline to form a single structure. At the same time the nasal placodes develop bilaterally in the lower margin of the frontal process forming the lateral and medial nasal processes which surround the nasal pit. The lateral nasal processes fuse with mandibular processes by stage 17 (6th weeks) and they join the medial nasal processes to form the upper lip and nostrils.

Ossification of the maxilla begins at the end of the 7th week (stage 20) and it is delayed 1 or 2 days as compared to the ossification of the mandible. Along side the lateral cartilage of the nasal capsule appears a fibrocellular tissue. The ossification center is situated above the dental lamina in the place of future canine tooth germ development.

Ossification spreads in five specific directions toward the processes of the maxilla. By the end of embryonic period (stage 22) a secondary cartilage appears as the base of the zygomatic process. In early fetal period all parts of the maxilla are well developed and the bony trabeculae radiate from the center of ossification to processes.

Sturgeons' previtellogenic ovarian follicles – ultrastructural and histological analysis

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According to the majority of recent publications the process of fish oogenesis consists of 3 phases: (1) previtellogenesis that have been arbitrarily subdivided into 3 stages termed "perinucleolar", "lipid" and "cortical alveoli", (2) vitellogenesis and (3) choriogenesis (Ravaglia and Maggese, 2003; Abascal and Medina, 2005).

We examined the structure of previtellogenic ovarian follicles in 3 sturgeon species: the Siberian sturgeon (*Acipenser baeri*), the Russian sturgeon (*Acipenser gueldenstaedtii*) and the American paddlefish (*Polyodon spathula*). The material was obtained by a needle biopsy from immature females. The pieces of ovaries comprised oogonia, early meiotic oocytes and ovarian follicles. Oogonia and early meiotic oocytes occur in nests and are delimited by somatic prefollicular cells. The ovarian follicles are surrounded by a layer of thecal cells and consist of an oocyte surrounded by follicular cells. Based on previous investigations (Kilarski et al., 2004), we classified ovarian follicles into 3 groups: early, mid- and late previtellogenic.

During early previtellogenesis oocytes are relatively small; the germinal vesicles (GV) are spherical and contain single, centrally located nucleoli. The ooplasm contains prominent aggregation of mitochondria, the mitochondrial cloud (MC), located near the GV. Follicular cells are flattened and encompass the oocyte. At this stage the process of an eggshell formation begins and appears to continue throughout the rest of oogenesis. The envelope is deposited between the oolemma and overlying follicular cells.

The midprevitellogenic oocyte is spherical and more voluminous than in the previous stage. GV contains numerous nucleoli located near the nuclear envelope (=perinucleolar stage). The perinuclear ooplasm comprises aggregations of nuage material. The MC enlarges and comes into contact with the nuclear envelope. It consists of variously shaped, often transformed mitochondria, multivesicular bodies and Golgi complexes. Simultaneously, the formation of cytoplasmic lipid droplets begins (=lipid stage).

In late previtellogenic oocyte the membrane-limited, rounded vesicles of variable size are accumulated. These vesicles reside next to the oocyte periphery where they form a single layer (=cortical alveoli stage). GV comprises numerous nucleoli that are distributed unevenly in the nucleoplasm. MC disperses in the ooplasm. Analysis of frozen sections stained with Oil Red O and Sudan (i.e. Black, III) revealed that in sturgeon previtellogenic oocytes the distribution of lipid droplets corresponds to the distribution of mitochondria and other MC components.

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REFERENCES:

ABASCAL FJ, and MEDINA A. 2005. Ultrastructure of oogenesis in bluefin tuna, *Thunnus thynnus*. *Journal of Morphology* 264: 149–160.

KILARSKI W, BILINSKI SM, and KOLMAN R. 2004. Fine structure of sturgeons' previtellogenic oocytes. *Acta Biologica Cracoviensia Series Botanica* 46 Suppl. 1: 37.

RAVAGLIA MA, and MAGGESE MC. 2003. Ovarian follicle ultrastructure in the teleost *Synbranchus marmoratus* (BLOCH, 1795), with special reference to the vitelline envelope development. *Tissue&Cell* 35: 9–17.

Trehalase activity and trehalose level in drone of *Apis mellifera carnica* during their development

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Sugars are known to perform important energy-related functions in insects, an especial role being ascribed to trehalose. Trehalose is one of the major sugars occurring in honeybees. Surprisingly, there is no information concerning the sugar metabolism during the development of bees. In this paper trelalose level and the activity of trehalase, the main enzyme involved in trehalose metabolism, were investigated in drone brood.

Drone larvae were separated according to developmental stage on pre-capping period (with the cell open) including feeding and growing larvae 1–2, 3, 4 and 6-days old (d) and post-capping period (with the cell sealed) including "spining" larval stage (7d, 8d and 9d), prepupae stage (10–11d), pupal stages with white, pale pink, pink and dark eyes, pupae with dark thorax, and newly emerged drone (imago). In the prepared extracts the content of trehalose using high performance liquid chromatography (HPLC), trehalase activity by Dahlqvist (1967) and the protein content by Bradford (1976) were assayed.

There were observed the negative correlations between the level of trehalose and activity of trehalase during drones' development. The concentration of trehalose was low (0.33%) between the 1-st and the 4-th day of larval development. Trehalose was increasing gradually at the sealed stages and it was the highest at the 9-th day (1.8%), than it lowered. There was 0.31% of sugar at newly emerged drones. At the beginning of the larval development (1–2 day) the trehalase activity was hight (317.2 u/mg). Between the 3-th and the 6-th day the enzyme was 50% and 25% less active than at the 1-st day. The activity of trehalase was extremally hight at newly emerged adults (513.1 u/mg). The lowest activity of enzyme was observed at larvae before pupation (at 84.8 u/mg). Obtained results indicated that trehalose was accumulated in the body of drone larvae mainly in the last day before cell sealing, this process last till the time of achieving the prepupae stage. The pupal stages use earlier collected supplies of trehalose. The level of trehalose was lower twice at newly emerged drones than at the pupal stages.

REFERENCES:

BRADFORD J. 1976. A rapid sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein – dye binding. *Anal Biochem* 72: 248–254.

DAHLQVIST A. 1967. Assay of intestinal disaccharidases. *Anal Biochem* 22: 99–107.