Formation and structure of egg envelopes in Russian sturgeon *Acipenser gueldenstaedtii* (Acipenseriformes: Acipenseridae)

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The covering of the eggs in Russian sturgeon *Acipenser gueldenstaedtii* consists of three envelopes (the vitelline envelope, chorion and extrachorion) and is equipped with multiple micropyles. The most proximal to the oocyte is the vitelline envelope that consists of four layers of filamentous and trabecular material. The structural components of this envelope are synthesized by the oocyte (primary envelope). The chorion encloses the vitelline envelope. The extrachorion covers the external surface of the egg. Examination of the arrangement of layers that comprise the egg envelopes together with the ultrastructure of follicular cells revealed that the chorion and extrachorion are secondary envelopes. They are secreted by follicular cells and are built of homogeneous material. During formation of egg envelopes, the follicular cells gradually diversify into three morphologically different populations: 1) cells covering the animal oocyte region (cuboid), (2) main body cells (cylindrical) and (3) micropylar cells. The apical surfaces of follicular cells from the first two populations form processes that remain connected with the oocyte plasma membrane by means of gap junctions. Micropylar cells are located at the animal region of the oocyte. Their apical parts bear projections that form a barrier to the deposition of materials for egg envelopes, resulting in the formation of the micropylar canal.

Key words: Chondrostei; follicular cells; micropyle; ultrastructure.

INTRODUCTION

The eggs of fishes are covered by two or three envelopes (Dumont & Brummet, 1980; Le Menn & Pelissero, 1991; Riehl & Patzner, 1998; Quagio-Grassiottto & Guimarães, 2003). The most proximal to the oocyte is the vitelline envelope. The chorion encloses the vitelline envelope. A jelly envelope is located on the surface of the chorion in eggs of some fishes. It has adhesive properties and fastens the laid eggs to plants or stones in water (Cherr & Clark, 1985; Riehl & Patzner, 1998; Quagio-Grassiottto & Guimarães, 2003). A similar sticky envelope surrounds the eggs of some aquatic insects and is often referred to as an extrachorion (Rościszewska, 1995; Gaino *et al*., 2008). The proteins of fish egg envelopes (zona radiata proteins, zrp) are synthesized in the liver and are sequestered from the plasma (Arukwe & Goksøyr,
2003). During their formation in ovarian follicles, the egg envelopes develop numerous canals. Due to this process, either all the envelopes or only the vitelline envelope are referred to as zona radiata (Le Menn & Pelissero, 1991; Riehl, 1999; Debus et al., 2008). The main functions of egg envelopes include the fixation of a deposited egg to the substratum, sperm attraction, prevention of polyspermy and antibacterial and mechanical protection. For the developing embryo, the egg envelopes enable gas exchange, excretion and transport of nutrients from the external environment (Riehl, 1999). In sea urchin, the substances released from egg envelopes increase sperm motility and enable the release of Ca ions from the oocyte (Epel, 1977).

Fish eggs have a single micropyle, a canal through the egg envelopes with an outer opening on the egg surface and an inner opening near the surface of the oocyte plasma membrane (oolemma). The micropyle allows the passage of sperm to the oocyte. It has been shown in trout Salmo trutta L. and zebrafish Danio rerio (Hamilton) that the presence of a single micropyle deters polyspermy (Ginsburg, 1961; Gupta et al., 2008; Marlow & Mullins, 2008). In D. rerio, the proper formation of a single micropyle is controlled by at least two genes: magellan (Gupta et al., 2008) and bucky ball (Marlow & Mullins, 2008). In mutant D. rerio eggs, up to 12 micropyles are scattered on the surface of the egg and are functional (causing polyspermy) (Marlow & Mullins, 2008). The micropylar canal is formed by a large cell of the follicular epithelium that surrounds the developing oocyte, termed the micropylar cell, plug cell or Zapfenzelle (Riehl, 1999; Kunz, 2004). The micropylar cell is equipped with a single projection in its apical part that is attached to the surface of the animal pole of the oocyte by means of desmosomes. The projection comprises numerous microtubules and forms a barrier to the deposition of egg envelopes, which results in the formation of a micropylar canal (Hart, 1990). The same has been observed in numerous insect groups (Yamauchi & Yoshitake, 1984; Zarani & Margaritis, 1985, 1991; Wenzel et al., 1990; Kubrakiewicz et al., 2005).

Eggs of Acipenseriformes (sturgeons and paddlefishes) are sticky, smooth and equipped with so-called multiple micropyles, i.e. numerous micropylar openings located in a small area at the animal region of the egg (Cherr & Clark, 1982, 1985; Dettlaff et al., 1993; Linhart & Kudo 1997; Debus et al., 2008). In Acipenseridae, the number of micropylar openings varies not only among species but also among different females of the same species [white sturgeon Acipenser transmontanus Richardson: average seven micropylar openings (Cherr & Clark, 1982); shortnose sturgeon Acipenser brevirostrum Lesueur: several (Flynn & Benfey, 2007); beluga Huso huso (L.): up to 52 micropyles (Ginsburg, 1972)]. In egg envelopes of Russian sturgeon Acipenser gueldenstaedtii Brandt & Ratzeberg, 30 or more micropylar openings have been reported (Dettlaff et al., 1993). The presence of multiple micropyles in this group of fishes is not a developmental defect; sturgeons have developed effective mechanisms that prevent polyspermy. Insemination and gamete interactions have been examined in A. transmontanus (Cherr & Clark, 1985) and in Siberian sturgeon Acipenser baerii Brandt (Psenicka et al., 2010). In A. transmontanus, after the eggs are laid in fresh water, a soluble factor (66 KD glycoprotein) that induces the acrosome reaction in homologous sperm is released from the egg envelopes. During insemination, spermatozoa enter each micropylar canal, but only one reaches and contacts the oolemma. This induces an extremely fast cortical reaction that is quick enough to block supernumerary sperm from penetrating the oolemma. The cortical
reaction is connected with the exocytosis of cortical alveoli (cortical granules) content, which is released into and generates the perivitelline space. This space expands from the site of sperm entry and eventually encloses the entire egg. The cortical alveoli content seals the inner micropylar openings and prevents the access of sperm (Cherr & Clark, 1985). In a fertilized micropyle, the apex of a fertilization cone blocks the other sperm (Psenicka et al., 2010).

The formation of multiple micropyles is interesting. It is commonly accepted that during oocyte development the follicular cells and oocyte interact. This has been thoroughly investigated in numerous insects, including the model organism genus Drosophila (Ogorzałek, 2007; Jaglarz et al., 2008; Wu et al., 2008). As a result of these interactions, the follicular cells diversify, leading to the formation of morphologically different populations of cells. These populations are responsible for the formation of chorion specializations including the formation of micropyle. During formation of ovarian follicles in insects, the differentiating follicular cells may divide incompletely and remain connected by intercellular bridges (Woodruff & Tilney, 1998). On the basis of this knowledge, it is tempting to hypothesize that multiple micropyle in sturgeons is formed by numerous micropylar cells and that at least some of these cells remain connected by such bridges during oocyte development and formation of egg envelopes. This hypothesis is supported by scanning electron microscopy observations of egg envelopes of acipenserid eggs (Cherr & Clark, 1982; Dettlaff et al., 1993). In this paper, the ultrastructure of egg envelopes and their formation in A. gueldenstaedtii were studied. The diversification of follicular cells is also described.

MATERIALS AND METHODS

The ovary of the fish was obtained from a specimen bred in an artificial pond (Department of Ichthyobiology and Fisheries, Experimental Unit at Mydlniki, Agricultural University, Kraków, Poland). For light (LM) and transmission electron microscopy (TEM), the ovary was cut into pieces and samples were fixed in ice-cold 2.5% glutaraldehyde (Sigma, www.sigmaaldrich.com) in 0.1 M phosphate buffer (pH 7.4). Following several hours of fixation, the ovarian follicles in various stages of egg envelope formation were isolated from the ovary. According to the classification of Hurvitz et al. (2007), white (with diameters >250 μm), yellow and grey oocytes were isolated. Later, these oocytes were rinsed and post-fixed in 1% osmium tetroxide in the same buffer, dehydrated in a series of ethanol and acetone and embedded in Epon 812 (Agar; www.agarscientific.com). Semithin sections (0.7 μm) were stained with 1% osmium tetroxide in the same buffer, dehydrated in a series of ethanol and acetone and embedded in Epon 812 (Agar; www.agarscientific.com). Semithin sections (0.7 μm) were stained with 1% methylene blue in 1% borax and photographed under a Jenalumar (Zeiss; www.micro-shop.zeis.com) LM. Ultrathin sections (90 nm) were contrasted with uranyl acetate and lead citrate, and analysed in a Jeol 100SX (www.jeol.com) TEM, at 80 kV.

RESULTS

In the A. gueldenstaedtii ovary each individual ovarian follicle consisted of a spherical oocyte surrounded by follicular cells. In this study, six consecutive stages (1, 2, 3, 3/4, 4 and 5) of egg envelope formation were distinguished according to the size of the ovarian follicles and examined.
THE EGG ENVELOPE

Stage 1 of egg envelope formation (ovarian follicles with diameters c. 400 μm)

The egg envelopes are secreted to the space enclosed between the oocyte and apical parts of follicular cells [Fig. 1(a)]. This space is referred to as perioocytic space. In this stage, two envelopes cover the oocyte surface; these are (1) the vitelline envelope and (2) the chorion [Fig. 1(b)]. The vitelline envelope is secreted by the oocyte and is composed of filaments. The filaments are arranged parallel to the surface of the oocyte and located between the oocyte microvilli [Fig. 1(b)]. The chorion is a single layer of homogeneous material that covers the vitelline envelope and adheres to the apical surfaces of follicular cells [Fig. 1(b)]. The chorion is secreted by follicular cells [see vacuoles with homogeneous material in the cytoplasm of the follicular cell in Fig. 2(f),(i)].

The lipid body composed of lipid droplets is located in the central parts of the oocyte (not shown). It stays in this position during all stages described below. The oocyte plasma membrane forms numerous microvilli oriented towards the apical surfaces of follicular cells [Fig. 1(b)]. In some places, microvilli gather into groups that are directed towards intercellular spaces between follicular cells [Fig. 1(b)]. In further stages (2 to 5), microvilli penetrate the egg envelopes [Figs 1(f), 2(b),(d),(e) and 3(e),(f)]. The apical surfaces of follicular cells form processes oriented towards the oolemma [Figs 1(b), 2(a),(c),(d),(g)–(i) and 3(a),(b)]. The bases of these processes are thicker than in the oocyte microvilli. As the deposition of egg envelopes proceeds, the processes lengthen, go through the whole thickness of the egg envelopes but remain connected with the oolemma [Figs 2(d),(e) and 3(a)].

Stage 2 of egg envelope formation (ovarian follicles with diameters c. 1100 μm)

During this stage, an additional layer of vitelline envelope is secreted and deposited on the oocyte surface. It forms the internal layer of the vitelline envelope [Fig. 1(c)]. On the surface of the chorion, the outermost envelope of the egg, i.e. the extrachorion, is deposited [Fig. 1(c),(d)]. As a result, the covering of the egg is composed of four layers: two internal layers that constitute the vitelline envelope [Fig. 1(c),(d),(f)], the chorion layer proximal to the vitelline envelope [Fig. 1(c),(d),(f)], and the most external layer, the extrachorion [Fig. 1(c),(d)]. The material for the vitelline envelope is enclosed in numerous vacuoles located in the peripheral ooplasm [Fig. 1(e)]. The inner-most layer of the vitelline envelope, secreted during this stage, is composed of filaments that are thin and short. This layer will be referred to as the filamentous layer [Fig. 1(f)]. The outermost layer of the vitelline envelope (t1) is composed of trabecules [Fig. 1(f)]. At the border between filamentous and trabecular layers, fibrils are located [Fig. 1(f)]. Fibrils are formed of filaments that are arranged more tightly than in the filamentous layer. As the deposition of new filaments proceeds, these fibrils form trabecules that build the t1 layer [Fig. 1(f)]. The t1 layer of the vitelline envelope is covered by a layer of chorion [Fig. 1(f)]. In sturgeons, this envelope is referred to as the alveolar chorion (Debus et al., 2008). The vitelline envelope and alveolar chorion are perforated by numerous canals containing the processes of follicular cells and oocyte microvilli [Fig. 1(f)]. The external surface of the alveolar chorion (directed towards the follicular cells) is folded. The extrachorion is located in depressions between folds [Fig. 1(c),(d)]. Its external surface is smooth.
Fig. 1. (a), (b) Stage 1 of egg envelope formation. (c)–(f) Stage 2 of egg envelope formation. (a) O, Oocyte; FC, follicular cells; b1, basal lamina; perioocytic space. (b) Vitelline envelope composed of filaments; ch, chorion; O, oocyte; mv, oocyte microvilli; FC, follicular cells; follicular cells processes; r, cytoskeleton; <> desmosome; m, mitochondrium; b1, basal lamina. (c) Vitelline envelope: the internal layer of the vitelline envelope; ch, chorion; ex, extrachorion; FC, follicular cells; O, oocyte; 1d, lipid droplets. (d) Vitelline envelope; ch, chorion; ex, extrachorion; FC, follicular cells; dividing follicular cell; O, oocyte. (e) Vacuoles containing material for the vitelline envelope in the peripheral ooplasm; er, endoplasmic reticulum; m, mitochondria; 1d, lipid droplets. (f) Vitelline envelope: FL, the filamentous layer; t, trabecules; t1 (zre), trabecular layer t1 = zona radiata externa (Debus et al., 2008); fibrils; ch, alveolar chorion; the canals; O, oocyte; mv, oocyte microvilli.
In subsequent stages of egg envelope formation, the structures of the chorion and extrachorion do not change [Figs 2(a),(c),(g),(h) and 3(b)–(d)].

**Stage 3 of egg envelope formation (ovarian follicles with diameters c. 1400 μm)**

The secretion of the vitelline envelope is continued during this stage [Fig. 2(a),(b)]. Newly secreted material is built of short filaments that are deposited on the surface of the oocyte between its microvilli [Fig. 2(b)]. As the deposition of the vitelline envelope proceeds, some filaments become attached to their neighbours and form fibrils and eventually trabecules. As a result, the second trabecular layer (t2) of the vitelline envelope develops on the surface of the oocyte [Fig. 2(a)]. This layer consists of trabecules that are located underneath the layer deposited in the previous stage on the oocyte surface and built of thin and short filaments. These filaments are densely packed [homogeneous layer, Fig. 2(a),(b)]. Numerous vesicles of endoplasmic reticulum (ER) and mitochondria are located in the cortical ooplasm [Fig. 2(b)]. This compartment contains pigment granules (not shown) and variously shaped yolk platelets that harbour prismatic inclusions embedded in an amorphous electron-dense substance [Fig. 2(b)].

**Stage 3–4 of egg envelope formation (ovarian follicles with diameters c. 1500 μm)**

The vitelline envelope is composed of four layers [Fig. 2(c)]. Newly deposited filaments form a filamentous layer that adheres to the surface of the oocyte [Fig. 2(c)]. As the deposition of filaments proceeds, those deposited earlier become attached to the t2 trabecular layer. As a result, the filamentous layer of the vitelline envelope remains thin [Fig. 2(c)] and the t2 layer of the vitelline envelope significantly thickens in comparison with the previous stage [compare Fig. 2(a),(c)].

**Stage 4 of egg envelope formation (ovarian follicles with diameters c. 1800 μm)**

The egg covering is composed of three envelopes: (1) a vitelline envelope built of four layers of filamentous and trabecular material [Fig. 2(g),(h)]; (2) a chorion, the alveolar layer composed of homogeneous material [Fig. 2(g),(h)]; (3) an extrachorion, a homogeneous layer [Fig. 2(g)–(i)]. During this stage, the layer of the vitelline envelope deposited on the oocyte surface during stage 2 and situated between trabecular layers t1 and t2 [Fig. 2(g),(h)] acquires an homogeneous appearance. This is due to the reduction of space between filaments (not shown). The filamentous layer is the inner-most layer of the vitelline envelope [Fig. 3(a)].

**Stage 5 of egg envelope formation (ovarian follicles with diameters c. 2000 μm)**

During this stage, the composition of the vitelline envelope is the same as in the previous stage [Fig. 3(b)]. The extrachorion is spread over the alveolar chorion [Fig. 3(b)–(d)]. Canals that perforate the alveolar chorion contain the oocyte microvilli and follicular cell processes [Fig. 3(e)]. Canals that perforate the external trabecular layer t1 of the vitelline envelope, however, are filled with a homogenous material, most probably with the chorion [Fig. 3(b),(f)]. The peripheral ooplasm accommodates numerous pigment granules, yolk platelets and irregularly shaped vesicles with granular content, probably the cortical alveoli [Fig. 3(b)]. In the follicular epithelium, micropylar cells are located between follicular cells, at the animal region of the oocyte [Fig. 3(c),(d)].
Fig. 2. (a),(b) and (f) Stage 3 of egg envelope formation. (c)–(e) Stage 3/4 of egg envelope formation. (g)–(i) Stage 4 of egg envelope formation. (a) ve, vitelline envelope; →, the newly formed layer of the vitelline envelope t2 = zona radiata interna (Zri; Debus et al. (2008); △, the homogeneous layer of the vitelline envelope built of densely packed filaments deposited on the oocyte surface during stage 2 of egg envelope formation = epilayer 1 (Debus et al., 2008); ch, alveolar chorion; ex, extrachorion; FC, follicular cells; —,— process of follicular cell, note the cuboid shape of follicular cells; O, oocyte. (b) →, newly deposited filaments; t2(zri), trabecular layer t2; →, the homogeneous layer; t1 (zre), trabecular layer t1; O, oocyte; mv, oocyte microvilli; er, endoplasmic reticulum; yp, yolk platelet; m, mitochondria. (c) ve, Vitelline envelope; two layers are indicated: →, the filamentous layer; →, the homogeneous layer; O, oocyte; ch, alveolar chorion; ex, extrachorion; FC, follicular cells; →, processes of follicular cells. Lipid droplets are located in the follicular cells cytoplasm. (d) →, Follicular cell process; mv, oocyte microvilli; O, oocyte. (e) ○. Plasma membranes of the process of follicular cell and oocyte microvillus connected by a gap junction; mv, oocyte microvilli; O, oocyte. (f) Fragment of the follicular cell cytoplasm. Vacuoles containing material for: →, chorion and extrachorion; ←, cytoskeleton; er, endoplasmic reticulum; m, mitochondria. (g) Animal region of the ovarian follicle: O, oocyte; ve, vitelline envelope; →, the homogeneous layer of the vitelline envelope; ch, alveolar chorion; ex, extrachorion; FC, animal region follicular cells; →, processes of follicular cells. (h) Main body (vegetative) region of the ovarian follicle. ve, vitelline envelope; →, the homogeneous layer of the vitelline envelope; ch, alveolar chorion; ex, extrachorion; FC, main body follicular cells; →, processes of follicular cells. (i) Main body follicular cells. Vacuoles containing material for: →, chorion and extrachorion; ←, cytoskeleton; ○, desmosome; →, process of a cell; N, nucleus; er, endoplasmic reticulum; m, mitochondria; ld, lipid droplets; ex, extrachorion; b1, basal lamina.
Fig. 3. (a) Stage 4 of egg envelope formation. (b)–(h) Stage 5 of egg envelope formation. (a) Layers of the vitelline envelope that are proximal to the oocyte: FL, the filamentous layer; t2 [zona radiata interna (zri)], trabecular layer t2 (zri). process of follicular cell; gap junction; O, oocyte. (b) Main body (vegetative) region of the ovarian follicle: ve, vitelline envelope; , the filamentous layer; t2(zri), trabecular layer t2; , the homogeneous layer; t1(zre), trabecular layer t1. The canal in t1 filled with chorion is encircled by a black line; ch, alveolar chorion; ex, extrachorion; FC, main body follicular cells; process of follicular cell; O, oocyte; cortical alveoli. White dotted line outlines fragment of the ooplasm containing granules of pigment. (c) Fragment of animal region of the ovarian follicle: MC, fragment of micropylar cell; FC, animal region follicular cells; ch, alveolar chorion; ex, extrachorion. (d) Fragment of animal region of the ovarian follicle: projections of micropylar cells; FC, animal region follicular cells; ch, alveolar chorion; ex, extrachorion. (e) Cross-section through the alveolar chorion: canals containing oocyte microvilli and follicular cell processes. (f) Cross-section through the vitelline envelope: canal; ch, chorion; t1(zre), trabecular layer t1. (g) Fragment of micropylar cell cytoplasm: cytoskeleton; vesicles with homogeneous material; N, nucleus; er, endoplasmic reticulum; m, mitochondria; 1d, lipid droplets. (h) Fragment of a cytoplasm in the projection of micropylar cell: vesicle containing homogeneous material; m, mitochondria. The regions of the cytoplasm containing cross-sectioned microtubules are encircled.
FOLLICULAR CELLS

During investigated stages of egg envelope formation the follicular epithelium consists of the main body follicular cells [Figs 1(a),(c),(d), 2(a),(c) and 3(b)] and micropylar cells (located at the animal region of the oocyte) [Fig. 3(c),(d)]. The shape of main body follicular cells changes from squamous [stage 1, Fig. 1(a),(b)], through rectangular in stage 2, when cells are shortened along their basal-apical axes [Fig. 1(c),(d)] to cuboid [stages 3 and 3/4 in Fig. 2(a),(c)]. In stages 4 and 5 cells that cover the animal region of the oocyte remain cuboid [Figs 2(g) and 3(c),(d)], whereas the main body cells acquire a cylindrical shape. The basal-apical axes of these cells are lengthened [Figs 2(h),(i) and 3(b)]. The nuclei of the main body cells contain nucleoli and chromatin that is attached to the nuclear envelope and dispersed in the nucleoplasm [Figs 1(b) and 2(f)]. The cytoplasm comprises ribosomes, ER cisternae and vesicles, vacuoles that contain material for the chorion and extrachorion, mitochondria with several transverse cristae and intramitochondrial granules in a matrix, and lipid droplets [Figs 1(b) and 2(f),(i)]. In the vicinity of nuclei, the cytoplasm contains microtubules and bundles of microfilaments [Figs 1(b) and 2(f),(i)]. Lateral membranes of cells within the epithelium are connected by means of belt desmosomes [Figs 1(b) and 2(i)]. The basal surfaces of cells lie on a basal lamina [Figs 1(a)–(d), 2(a),(c),(g)–(i) and 3(b)–(d)]. During all stages of egg envelope formation, single spherical cells with disintegrated nuclear envelopes and condensed chromatin located in the cell centres are scattered in the area of the follicular epithelium [Fig. 1(d)]. These cells represent various stages of cell division.

Only six micropylar cells in stage 5 of egg envelope formation were obtained for the analysis. Micropylar cells are situated between cuboid cells that cover the animal region of the oocyte [Fig. 3(c),(d)]. They are cylindrical and their cytoplasm is basophilic [Fig. 3(c)]. Micropylar cells are connected with animal region cells by means of desmosomes that clasp their lateral membranes (not shown). The cytoplasm of micropylar cells consists of mitochondria, vesicles of ER, microtubules, bundles of microfilaments, lipid droplets and vesicles containing homogeneous material [Fig. 3(g),(h)]. The apical parts of micropylar cells are devoid of processes; instead, they bear a projection that passes through the extrachorion and chorion towards the surface of the oocyte. Its shape is icicle like [Fig. 3(d)]. Numerous microtubules arranged parallel to the long axis of the projection are located in its cytoplasm [Fig. 3(h)].

DISCUSSION

To date, egg envelopes have been described and compared in several sturgeon species including *A. gueldenstaedtii* (Le Menn & Pelissero, 1991; Debus *et al.*, 2008; Psenicka *et al.*, 2010). This paper provides a more detailed description of the ultrastructure of several consecutive stages of egg envelope formation in this species.

The internal makeup of egg envelopes in *A. gueldenstaedtii* is almost identical to that of *A. baerii* and Persian sturgeon *Acipenser persicus* Borodin and *H. huso* (Le Menn & Pelissero, 1991; Debus *et al.*, 2008). It should be emphasized, however, that in this study the filamentous layer of the vitelline envelope, not found in *A. baerii* and *A. persicus* has been described. In the investigated species, the filamentous layer
is located in the microvilli zone and extra-oocyte matrix (Le Menn & Pelissero, 1991; Debus et al., 2008). It is the most proximal to the oocyte and is always built of filaments. These filaments are secreted by the oocyte (primary envelope). The filaments deposited in stage 1 of egg envelope formation are precursors of fibrils and trabecules, the elements that build the more external layer (t1) of the vitelline envelope in the next stage (2). It is postulated that these filaments aggregate as the oocyte enlarges. The filaments deposited in stage 2 of egg envelope formation do not form fibrils and trabecules. They stay separated and become compact as the oocyte enlarges in stages 3–5 and the deposition of filaments proceeds. As a result, the layer containing these filaments acquires a homogenous appearance. The filaments deposited on the oocyte surface during stages 3–4–5 of egg envelope formation gradually become incorporated into fibrils and trabecules that build the internal (t2) layer of the vitelline envelope.

Terms proposed by Debus et al. (2008) meant to standardize and facilitate the description of the egg envelopes in Acipenseridae have stimulated further work. The classification applied here, based on envelope location on the oocyte surface and egg envelope origin, should be more useful. This type of classification has been successfully used in the description of egg envelopes in several groups of insects (Rościszewska, 1995; Zawadzka et al., 1997; Margaritis & Mazzini, 1998; Jaglarz et al., 2008). The ultrastructure of the oocyte, follicular cells and layers that build the egg envelopes and their arrangement in A. gueldenstaedtii show that the oocyte is covered by three envelopes in stage 5 of egg envelope formation. These envelopes include: (1) a vitelline envelope most proximal to the oocyte. It is the primary envelope and is composed of four layers that are deposited on its surface in different stages of egg envelope formation; (2) a chorion, the external, alveolar secondary envelope that is secreted by follicular cells. It is the most proximal to the external (t1) layer of the vitelline envelope and (3) an extrachorion, the outermost secondary envelope.

The extrachorion (jelly envelope) in the species investigated is derived from follicular cells. The same has been observed in other fishes (Dumont & Brummet, 1980; Quagio-Grassiotto & Guimarães, 2003) and in aquatic insects (Rościszewska, 1995). In insects, this envelope fastens eggs laid in springs and fast-flowing waters to the substratum (Rościszewska, 1996).

The multiple micropyle structure has been described in detail in a related species, A. transmontanus (Cherr & Clark, 1982). In this species, several micropylar openings are located at the animal region of the oocyte. Each opening leads to a micropylar canal that extends across the egg envelopes to the surface of the oolemma. Each micropylar canal is lined by the extrachorion, this lining ends at the border between the alveolar chorion and the vitelline envelope. Micropylar canals taper twice, between the outer opening and the end of the lining of the extrachorion, and between the inner-most layer (=filamentous layer) of the vitelline envelope and the inner opening of the canal. The inner openings of micropylar canals are located within vestibules that are surrounded by spherical perforated areas (the diaphragms) in the filamentous layer of the vitelline envelope (Cherr & Clark, 1982). The oolemma, at the bottom of the micropylar canal, bears an indentation without special structure (Morisawa, 1999). Some micropylar canals are superficially connected by shallow channels running on the surface of the external egg envelope (Cherr & Clark, 1982). Similar channels also exist in A. gueldenstaedtii eggs (Dettlaff et al., 1993). In the
light of these observations, it can be hypothesized that these channels might be formed around intercellular, cytoplasm-filled bridges that connect the micropylar cells. Such bridges have been described by Woodruff & Tilney (1998) in ovarian follicles of *Drosophila*. They are formed between neighbouring follicular cells during their divisions and cluster formation in the germarium. The plasma membranes limiting bridges are lined on the cytoplasmic side by dense material to which the filaments are attached. Such bridges represent the remnants of a spindle that is formed during incomplete cytokinesis (Woodruff & Tilney, 1998). In *A. gueldenstaedtii*, the bridges between micropylar cells that would explain the existence of channels in external egg covering have not been found. Unfortunately, obtaining sections that would allow such observation was impossible. Only recently, Psenicka et al. (2010) have described the micropyle structure in *A. baerii*, but they did not provide information on the interconnections between micropylar canals in this species.

Follicular cells diversified during the investigated stages of oocyte development in *A. gueldenstaedtii*. The diversification, as in other fishes (Francolini et al., 2003; Quagio-Grassiotto & Guimarães, 2003; Santos et al., 2006), includes a change of cell shape. In the investigated species, the shape of main body follicular cells changes from squamous, through rectangular and cuboid in stages 1–3–4 to cuboid (in animal region cells) or cylindrical (in the main body cells) in stages 4–5. It is commonly accepted that all changes in shape during morphogenesis of the follicular epithelium involve the cytoskeleton of cells (Zawadzka et al., 1997; Ogorzałek, 2007; Wu et al., 2008; Jaglarz et al., 2008). In the species investigated, the follicular cells remain connected with the oocyte by means of gap junctions during all stages of egg envelope formation. These junctions are involved in transport of ions and low molecular-mass substances between different cell types (Staehelin, 1974, Wu et al., 2008). Some cells divide within the epithelium. The number of cells grows as the oocyte enlarges due to these divisions. The progeny cells do not extend along the external surface of the egg envelopes. They obtain a defined shape (see above) and maintain it due to a well-developed cytoskeleton. Follicular cells in *A. gueldenstaedtii* ovarian follicles contain lipid droplets in the cytoplasm. Since lipid droplets are found in follicular cells from stage 2 of egg envelope formation, it is postulated that they are synthesized by follicular cells, sequestered from the plasma and not transported to the oocyte for massive uptake.

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