Verhandlungen der Gesellschaft für Ichthyologie Band 5, 2006, 7-11

The envelope of fully grown, unfertilised oocytes in *Heterandria formosa* (Poeciliidae) and *Xenotoca eiseni* (Goodeidae)

Die Hülle reifer, unbefruchteter Oocyten von *Heterandria formosa* (Poeciliidae) und *Xenotoca eiseni* (Goodeidae)

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Summary: When mechanically separated from the ovary, unfertilised mature oocytes of virgins of the poeciliid fish *Heterandria formosa* were always covered with cellular debris, mainly of the follicle epithelium, whereas those of the goodeid fish *Xenotoca eiseni* could be isolated without any contamination. Therefore, the oocytes of *X. eiseni* appear more suitable for *in vitro*-fertilisation attempts. Electron microscopical studies showed that in *H. formosa* the envelope of these oocytes did not contain radial canals; in *X. eiseni* results were less clear as some egg envelopes showed obliterated radial canals, whereas others did not.

Key words: envelope of oocytes, isolation of oocytes, radial canals, follicle epithelium, viviparity

Zusammenfassung: Bei dem Versuch, reife, unbefruchtete Oocyten aus dem Ovar zu isolieren, zeigte sich, dass bei jungfräulichen *Heterandria formosa* (Poeciliidae) diese stets mit Zellen des Follikelepithels und eventuell der Theca bedeckt waren, während bei jungfräulichen *Xenotoca eiseni* (Goodeidae) die Oocyten ohne jede Kontamination aus dem Follikel fallen. Für Versuche zur *in vitro*-Befruchtung scheinen daher die Oocyten von *X. eiseni* besser geeignet zu sein. Nach elektronenmikroskopischen Befunden besitzt die Hülle dieser Oocyten von *H. formosa* keine Radiärkanäle. Für X. *eiseni* waren die Ergebnisse weniger klar; einige Eihüllen zeigten offenbar Radiärkanäle, die verschlossen waren, andere waren jedoch weitgehend homogen.

Schlüsselwörter: Oozytenhülle, Isolierung reifer Oozyten, Radiärkanäle, Follikelepithel, Viviparie

1. Introduction

The mature oocyte of all teleosts is surrounded by an acellular, lamellate and/or fibrous envelope called also chorion, *zona radiata* or *zona pellucida* (see the discussion in Lo Nostro et al. 2003). Deposition takes place between the microvilli of the oocyte creating the typical radiation. In addition, villi of the surrounding follicle cells may penetrate the envelope. The intimate contact between oocyte and follicle epithelium may enhance exchange of substances (for review see Kunz 2004), but may also stabilize the oocyte within the follicle. Depending on the habitat and mode of reproduction the thickness and structure of the envelope varies among teleosts. In viviparous species it is the thinnest and largely lacks lamellae (e.g., Poeciliidae: Jollie and Jollie 1964, Erhardt and Götting 1970,Azevedo 1974, Grove and Wourms 1994; Hemirhamphidae: Flegler 1977; Goodeidae: Riehl and Greven 1993).

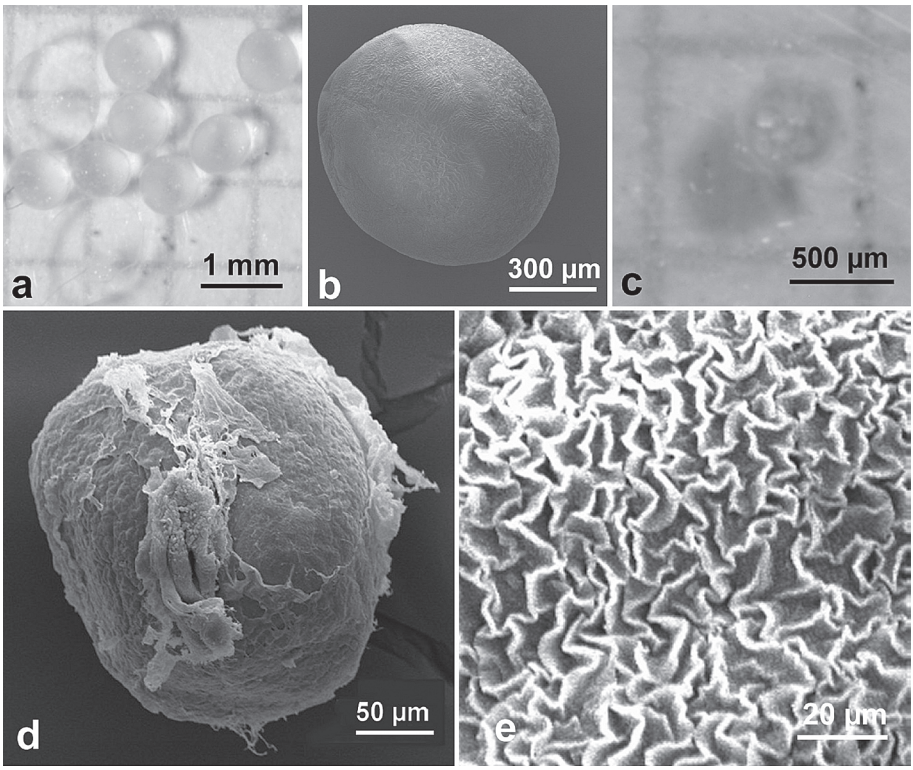
The intimate contact between the follicle epithelium and the oocyte must be loosened at the latest when the mature oocyte leaves the follicle during spawning (oviparous species) or the embryo is released into the ovarian cavity and/or into the environment (viviparous species). In oviparous fish microvilli of the oocyte and the follicle epithelium are withdrawn prior to ovulation and radiation disappears leaving a compact envelope ("cyprinodont type") or radial canals are plugged, but remain visible ("salmonid type"). Sperm entry is achieved by the micropyle (summarized by Kunz 2004). Regarding viviparous fish, the site of sperm entry and structural alterations of the egg envelope before fertilisation are unknown.
To gain single oocytes for fertilisation experiments we mechanically removed mature oocytes from the ovary of virgin females of two livebearers and checked the results by SEM and TEM.

2. Material and methods

We used virgins of the goodeid *Xenotoca eiseni* and the poeciliid *Heterandria formosa* older than one year (old virgins) and younger than one year (young virgins).
The ovaries were excised and torn by fine forceps. Large and putative mature oocytes (diameter approx. 750 µm in *X. eiseni* and 500 µm in *H. formosa*) were fixed in 2% glutaraldehyde in 0.1 mol/l cacodylate buffer, ph 7.3, either dehydrated and critical point dried (for Scanning Electron Microscopy; SEM) or postfixed with osmiumtetroxide (1%) plus 1.5% hexacyanoferrate, dehydrated and embedded in Spurr's (Spurr 1969) resin (for Transmission Electron Microscopy, TEM). SEM-preparations were sputtered with gold and viewed in a Leitz-AMR 1000; ultrathin sections were stained with lead citrate (Venable and Coggeshall 1965) and viewed in a Zeiss EM 9S.

3. Results and discussion

When tearing the ovaries of *X. eiseni* the largest oocytes readily felt out in young and old virgins (fig. 1a). In the latter some abnormal eggs differing in size and shape could be seen (fig. 1a). Isolation of large oocytes in *H. formosa* was more difficult and did not succeed satisfactorily (figs. 1c, d).
Under the SEM isolated oocytes of *X. eiseni* showed a relatively smooth surface in low power micrographs (fig. 1b). Higher magnification revealed a wrinkled surface that may be caused at least in part by the preparation. Oocytes of *H. formosa* were always covered with cellular debris (fig. 1d).
Ultrathin sections revealed that in old and young virgins of *H. formosa* a thin acellular envelope measuring approximately 2 µm in thickness surrounds the large oocytes. The envelope did not show radial canals; it was covered by the follicle epithelium and partly by theca cells (figs. 2a, b). Typically, the developing egg envelope in both species is penetrated mainly by oocyte microvilli as seen under TEM (see Riehl and Greven 1991, 1993).
Homogeneous thin envelopes, in which radial canals have disappeared, were found in late embryogenesis of *H. formosa* (Grove and Wourms 1994), the guppy *Poecilia reticulata* (Jollie and Jollie 1964), and the hemirhamphid *Dermogenys pusillus* (Flegler 1977). In the guppy microvilli of the oocyte and macrovilli of the follicle epithelium degenerate before fertilisation and mature oocytes have only few radial canals, if any (see Jollie and Jollie 1964; Greven et al., unpublished).
In *X. eiseni* the envelope showed only a small fuzzy covering; cellular debris, e.g. from the follicle epithelium, was absent. However, comparing old and young virgins, we gained some puzzling results. In the old virgins the envelope of the smallest egg that felt out revealed some radial canals obviously obliterated by an electron dense material (fig. 2c), whereas the envelope of the young



Figs. 1a-e: Oocytes isolated from the ovary of mature virgin females. **a**, **b**, **e** *Xenotoca eiseni*; note the large abnormal oocytes in a; the relative smooth surface of the oocyte in b, and its wrinkled surface in e. **c**, **d** *Heterandria formosa*; note the cellular debris covering the egg in d. SEM-micrographs (b, d, e).

Abb. 1a-e: Isolierte Oocyten von adulten jungfräulichen Weibchen. **a**, **b**, **c** *Xenotoca eiseni*; man beachte die großen abnormen Oocyten in a, die relativ glatte Oberfläche der Oocyte in b, und ihre gefaltete Oberfläche in e. **c**, **d** *Heterandria formosa*; man beachte die zellulären Rückstände auf der Oberfläche des Eies in d. REM-Aufnahmen (b, d, e).

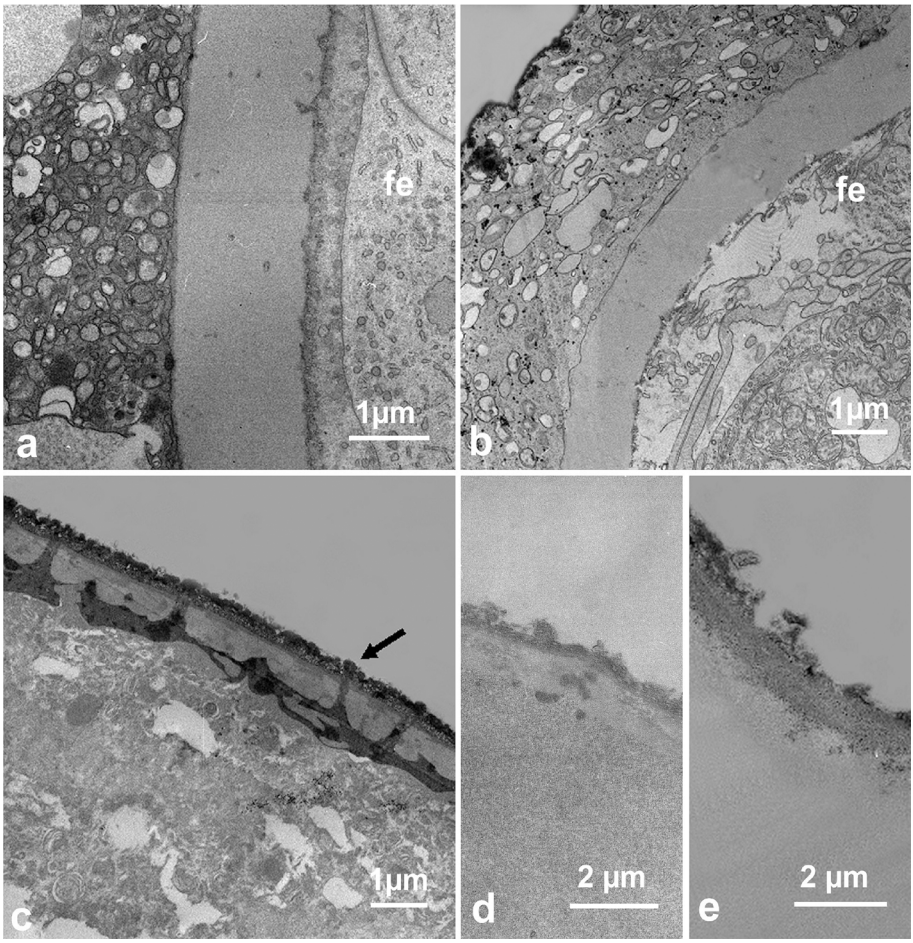
virgins had a fibrous appearance obviously without radial canals (fig. 2d). The envelope of the abnormal, perhaps degenerating egg looked similarly (fig. 2e).

4. Conclusions

The envelope of mature oocytes of the two viviparous teleosts examined undergoes remarkable changes prior to fertilisation. These changes resemble the pattern known from oviparous species, which lead to an envelope that either completely lacks radial canals or whose radial canals remain visible, but are closed by electron dense material.

At least in the few poeciliids and in the single hemirhamphid so far studied, later developmental stages are covered by a homogeneous envelope similar to the envelope, which covers the putative fertilisable oocyte described herein; the former is, however, remarkably thinner. The situation of the examined goodeid species seems less clear. However, we suggest presence of plugged radial canals in mature unfertilised oocytes.

To attempt *in vitro*-fertilization and to study the site of sperm entry into the egg of vi-



Figs. 2a-e: TEM micrographs of the envelope of isolated oocytes. **a**, **b** *Heterandria formosa*; homogeneous envelope of oocytes in an old (a) and in a young (b) virgin covered by the follicle epithelium (fe). **c**, **d**, **e** *Xenotoca eiseni*; envelope of an mature egg in an old (c) virgin showing obliterated radial canals, in a young virgin (d), and in a degenerating egg (e); in d and e the envelope appears fibrous lacking radial canals. Note the fuzzy covering in c (arrow).

Abb. 2a-e: TEM-Aufnahmen der Hülle isolierter reifer Oocyten. **a**, **b** *Heterandria formosa*; homogene Oozytenhülle eines alten (a) und eines jungen (b) jungfräulichen Weibchens. Die Eier sind vom Follikelepithel (fe) bedeckt. **c**, **d**, **e** *Xenotoca eiseni*; Eihülle eines alten (c) jungfräulichen Weibchens mit verschlossenen Radiärkanälen, eines jungen jungfräulichen Weibchens (d) und eines degenerierten Eies (e); in d und e erscheint die Hülle fibrös; sie besitzt keine Radiärkanäle. Man beachte die Auflagerung in c (Pfeil).

viparous fishes, goodeids appear to be more suitable than poeciliids due to the easy separation of mature eggs in the former.

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