Ovarian adaptations in the viviparous teleosts Clinus superciliosus and Clinus dorsalis (Perciformes: Clinidae)

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The anatomy and histology of the ovaries of C. superciliosus and C. dorsalis are described. The ovaries are extensively modified as trophic organs since embryos of both species develop intrafollicularly and are dependent on maternal nutrient secretions during development. The follicular epithelia of the two species are modified to perform a secretory function and follicular cells are hypertrophied in areas producing embryotrope. In non-hypertrophied areas the follicular cells remain thin to bring about close contact between the embryos and the maternal vascular system underlying the epithelium. In C. superciliosus embryonic epidermal macroridges are closely aligned to areas of follicular hypertrophy thus forming a pseudoplacenta. A similar arrangement is not found in C. dorsalis.


Die anatomie en histologie van die ovaria van C. superciliosus en C. dorsalis word beskryf. Die ovaria is omvorm tot trofiese organe aangesien embrios van beide spesies intrafollikular ontwikkel en van moederlike voedelseksriesies afhanklik is tydens hul ontwikkeling. Die folliculêre selle van die twee spesies het 'n sekretoriese funksie, en is verdik in gebiede wat embryotroof produseer. In ander gebiede is die folliculêre selle afgeplaat om sodoende noue kontakt tussen die embrios en die moederlike bloedsisteem, wat onder die epitheel voorkom, te bewerkstellig. In C. superciliosus is embryoniese epidermale makroriwwe teenaan gebiede van folliculêre hipertrofie geleë om 'n pseudoplasenta te vorm. 'n Soortgelyke situasie kom nie by C. dorsalis voor nie.


The genus Clinus comprises eighteen species, all of which are live bearing and occur intertidally along the South African coast. Clinus superciliosus represents one of the largest species in the genus and Veith (1979a,b, 1980) found that the developing embryos of this species absorbed nutrients through their extensively modified epidermis and gut. Clinus dorsalis is the smallest species of the genus and embryos of this species likewise absorb nutrients through a modified epidermis and gut. The embryonic structure and mode of nutrient absorption, however, differ markedly from those of C. superciliosus (Cornish & Veith 1986).

As is the case in the Poeciliidae and the Anablepidae (Turner 1938, 1940a,b; Knight, Lombardi, Wourms & Burns 1985) gestation in the Clinidae takes place intrafollicularly and in cases where ovoviviparity has evolved towards viviparity, the ovary has become modified so as to supply nutrients to the developing young. In ovoviviparous species such as Sebastodis paucispinis, (Moser 1967) and Sebastodis oblongus (Fujita 1985), two scorpaenid fishes, the ovary has not developed structures which actively supply nutrients to developing young. Extensive ovarian modifications are, however, found in species where a pseudo-placental arrangement is found as in embiotocid fishes (Webb & Brett 1972a,b) where ovarian nutrient secreting tissue penetrates the gill clefts of developing embryos. Most authors agree that the ovaries of viviparous fishes adopt a secretory function whereby the embryos are nourished (Turner 1933, 1936, 1938, 1940; Igarashi 1961, 1962; Lagios 1965; Wiebe 1968; Mendoza 1956, 1972; Veith 1979b).

Embryonic survival in C. superciliosus and C. dorsalis is high and considering that up to 450 embryos develop simultaneously in the ovary of C. superciliosus (Veith 1979a), the nutrient and oxygen supply to developing embryos must be extremely efficient and should be reflected in ovarian structure. The present study entails a histological examination of ovarian structure and association with embryonic tissues in the two clinid species.

Methods

C. superciliosus

The reproductive tracts of five gravid females, with body masses varying from 17,2 g to 68,2 g were excised and fixed in 10% buffered formaldehyde. Gonad mass varied from 17,2 g to 68,2 g. After fixation the gonads were dehydrated in alcohol and infiltrated with methyl benzoate celloidin. The clearing agent used was benzene and Altmans wax was used for imbedding. The gonads were then serially sectioned at 10 µm. The above procedure was used in view of the sponginess...
of the gravid gonads, which are ordinarily not freely penetrable by paraffin wax particularly in larger specimens. This also necessitated sectioning at 10 μm. Sections were subsequently stained with azocarmine and Mallory’s triple stain.

Figure 1 (a) Cross section through the ovary of *C. superciliosus* showing that the germinal epithelium is continuous with the epithelium lining the ovisac. GE - germinal epithelium; OE - ovisac epithelium; PE - periovarian space; T - trematode parasite (× 9,84). (b) Cross section through an embryo of *C. superciliosus* showing its position within the follicle. Note the folding of the embryonic epidermis. FE - follicular epithelium; E - embryonic epidermis (× 20,5). (c) Cross section through the ovary of *C. superciliosus* showing follicles with the hypertrophied and non-hypertrophied portions of the follicular epithelium. FP - flattened portion; HP - hypertrophied portion (× 9,84). (d) Cross section through the ovary of *C. superciliosus* showing superfembyronation. Note that the thin and thick sections of the follicular wall can be clearly distinguished in the smaller follicles (× 9,84). (e) Cross section through two follicular epithelia of *C. superciliosus* showing hypertrophied follicular cells and the folded thinner section of a follicular epithelium (× 131,2). (f) Cross section through the hypertrophied portion of the follicular wall of *C. superciliosus* showing the capillaries at the base of the cells. C - capillary (× 328).
C. dorsalis
The six gravid females used in this examination varied in mass from 0.984 g to 1.183 g and the gonad mass ranged from 110 mg to 130 mg. In view of the far smaller gonads in this species than in C. superciliosus a standard histological procedure could be followed.

The gonads were fixed in Bouin’s fixative, dehydrated in alcohol and chloroform was used as a clearing agent. The gonads were then embedded in a Paraplast Plus Phenanthrene mixture (100 g Paraplast Plus to 3 g Phenanthrene) and serially sectioned at 8 µm. Staining was carried out in azocarmine and Mallory’s triple stain.

Measurements of fish lengths are presented as standard length, being the length in mm from the tip of the snout to the base of the tail.

Results
General anatomy
The general anatomy of both C. superciliosus and C. dorsalis is essentially similar. In both species there are two ovaries which are enclosed by a single bilobed ovisac which opens by means of a single genital pore into the cloaca. The posterior portions of the ovaries do not fuse but remain separate structures. Moreover, the left ovary in both C. superciliosus and C. dorsalis is always smaller than the right ovary.

Medially and dorsally to the ovaries there is a tube-like bladder attached to the ovisac which opens posteriorly to the genital pore into the cloaca by means of a short urethra.

The ovisacs of the two species serve as parturition chambers. Embryos breaking out of the follicles end up in the periovarian space, from where they can escape to the exterior. Only a few embryos are expelled at any one time, as both species exhibit superembryonation with as many as 12 broods in the case of C. superciliosus and five in the case of C. dorsalis developing simultaneously in the ovaries (Veith 1979b; Cornish 1983).

Ovarian histology of C. superciliosus
The ovaries of C. superciliosus are hollow structures into which the follicles, containing embryos in various stages of development, project. The two ovaries are attached to the mid-dorsal line by means of a vascular mesenterium. The germinal epithelium is continuous, medially, with the epithelium lining the ovisac (Figure 1a) but posteriorly and anteriorly the two ovaries project freely into the lumen of the ovisac.

The embryos of C. superciliosus remain in their follicles throughout the gestation period and nutrients required for growth and metabolic processes are supplied by the follicular epithelium, portions of which perform a secretory function. In follicles containing small embryos (up to 10 mm standard length) portions of the follicular wall are hypertrophied and probably actively secrete nutrients. The apical regions of the follicular cells bud off and nutrients are thus released by apocrine secretion.

Only portions of the follicular wall are hypertrophied (Figures 1c & d) and the remainder consists of flatter epithelium which is mostly folded to accommodate the follicular capillaries found under the follicular epithelium (Figures 1b, e & f). The surface area of the glandular region of the follicular epithelium is increased by secondary involutions which are frequently found in C. superciliosus (Figure 2a).

The glandular region of the follicular epithelium frequently enters into a pseudo-placental arrangement with the epidermal ridges of the embryos. In these pseudo-placental arrangements it is found that it is not only the follicular wall which is hypertrophied, but the macroridges of the embryonic epidermis are also larger than elsewhere on the embryo (Figure 2b).

With the increase in embryo and follicle size the proportion of hypertrophied epithelial area is reduced relative to the thin area.

Ovarian arterial system
The ovaries of C. superciliosus are supplied with oxygenated blood via left and right ovarian arteries which arise from the coeliaco-mesenteric artery. Posteriorly the ovary receives the posterior ovarian artery, which arises directly from the dorsal aorta. There thus exists an arterial loop similar to the one described for Sebastodis paucispinis (Moser 1967).

The arteries branch freely in the ovarian stroma, and each follicle is supplied by a follicular arteriole, which breaks up into capillaries. These capillaries run parallel to one another in the follicular wall. Most of the embryos receive oxygenated blood from branches of the anterior ovarian arteries and only small portions of the posterior sections of the ovaries receive blood from the smaller ovarian artery.

Ovarian histology of C. dorsalis
The ovary of C. dorsalis is essentially similar to that of C. superciliosus. As is the case in C. superciliosus a portion of the follicular epithelium of C. dorsalis is hypertrophied and probably actively secretes nutrients whilst the remainder remains thin (Figure 2c). No secondary involutions of the hypertrophied area of the follicular epithelium were noted in C. dorsalis but the hypertrophied cells are characterized by secretory dropsules (Figure 2d). These dropsules probably contain the lipids and proteins which form a substantial portion of nutrients present in the embryorrhoea of this species (Cornish 1983).

Discussion
As both C. superciliosus and C. dorsalis exhibit superembryonation, embryos in various stages of development and with varying nutritional demands develop simultaneously in the ovaries of these two species. As functional differentiation of embryonic absorptive structures progresses, there is a shift in embryonic nutrient absorptive sites from the trophoderm to the gut (Veith 1980; Cornish & Veith 1986). Embryorrhoea rich in amino acids and lipids (Veith 1979a; Cornish 1983) is actively secreted by the follicular epithelia of the two species. Veith (1980) showed that tritiated thymidine injected into gravid fish is first concentrated in the follicular epithelium before being absorbed by embryos.

The fact that only portions of the follicular epithelia hypertrophy is of significance to the embryos for a number of reasons: the embryos must obtain both nutrients and oxygen. The oxygen is supplied by the rich capillary network underlying the follicular epithelium and must necessarily diffuse through epithelium in order to reach the embryos. If the entire follicular epithelium were secretory in nature the vascular system would be too far removed for effective oxygen transfer and there is, therefore, a limit to the amount of hypertrophied epithelium directly overlying the capillary network. The total area active in secretion can, however, be increased by secondary involutions of the secretory portion, as these would not overlie the capillary network.

In C. superciliosus the hypertrophied follicular cells are closely aligned to the embryonic trophoderm which also hypertrophies in regions of close contact. Unlike the relationship found in Anaboleps (Knight, et al. 1985) the follicular cells do not interdigitate with embryonic trophoderm macro-
ridges and the term pseudoplaenta rather than placenta is used to describe the structural relationship.

In *C. dorsalis* no pseudo-placental arrangement was apparent but this can possibly be ascribed to differences in the embryonic nutrition of the two species. In *C. dorsalis* embryotrophe lipid concentrations are far higher than in the case of *C. superciliosus* and the embryonic gut of *C. dorsalis* differentiates earlier than that of *C. superciliosus* and is also the principal site of nutrient uptake (Cornish 1983; Cornish & Veith 1986).

The large numbers of secretory droplets in the follicular cells of *C. dorsalis* are probably lipid inclusions, a view supported by the high lipid concentrations in the embryotrophe.

Because superembryonation occurs in both *C. superciliosus* and *C. dorsalis*, a variety of developmental processes occur simultaneously in the ovaries, but the control and synchronization of the processes is unknown. It is difficult to conceive of a cyclic pituitary control as found in *Lebistes* (Stolk 1951a, b, c & d). Gestation is also not maintained by active corpora lutea, as these structures were not found in either of the two species investigated. Corpora lutea do, however, occur in some viviparous fishes (Stolk 1951b; Lambert 1966).

Whether parturition in *C. superciliosus* and *C. dorsalis* takes place by active contractions of the muscular ovisac, or whether it is an embryo-governed process is unknown. As young fish are, however, born in groups, it is likely that contractions of the ovisac musculature are involved in parturition. Such contractions would expel liquid and juvenile fish present in the periovian space and can be elicited by movements of juveniles within the periovian space. It is envisaged that full-term embryos respond to factors such as oxygen stress, rather than hormonal stimuli, break through the follicle wall and end up in the periovian space where they elicit contractions of the ovisac musculature.

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**References**


LAGIOS, M.D. 1965. Seasonal changes in the cytology of the adenohypophysis, testes and ovaries of the black surfperch, Embiotoca jacksoni, a viviparous percomorph fish. Gen. Comp. Endocrinol. 5: 207 – 221.


