

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 embryotropic. 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*.

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Die anatomie en histologie van die ovaria van *C. superciliosus* en *C. dorsalis* word beskryf. Die ovaria is omvorm tot trofiese organe aangesien embryo's van beide spesies intrafollikulêr ontwikkel en van moederlike voedselsekresies afhanklik is tydens hul ontwikkeling. Die follikulêre selle van die twee spesies het 'n sekretoriese funksie, en is verdik in gebiede wat embriotroof produseer. In ander gebiede is die follikulêre selle afgeplat om sodoende noue kontak tussen die embryos en die moederlike bloedsisteem, wat onder die epiteel voorkom, te bewerkstellig. In *C. superciliosus* is embrioniese epidermale makroriwe teenaan gebiede van follikulêre hipertrofie geleë om 'n pseudoplasenta te vorm. 'n Soortgelyke situasie kom nie by *C. dorsalis* voor nie.

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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 0,25 to 8,48 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

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of the gravid gonads, which are ordinarily not freely penetrable by paraffin wax particularly in larger specimens. This also

necessitated sectioning at 10 μ m. Sections were subsequently stained with azocarmine and Mallory's triple stain.

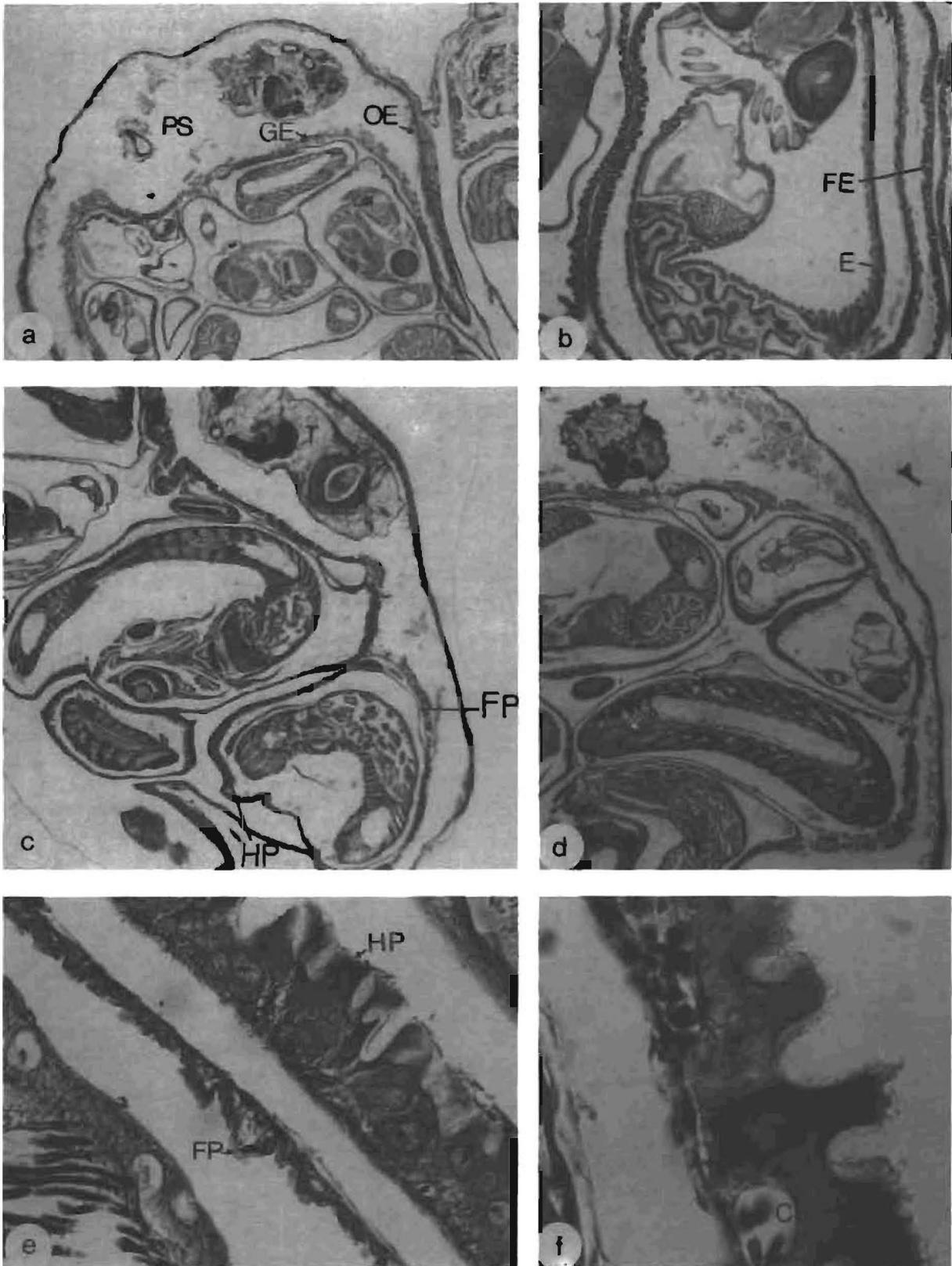


Figure 1 (a) Cross section through the ovary of *C. superciliosus* showing that the germinal epithelium is continuous with the epithelium lining the ovisac. GE – germinal epithelium; OE – ovisac epithelium; PE – periovarian space; T – trematode parasite ($\times 9,84$). (b) Cross section through an embryo of *C. superciliosus* showing its position within the follicle. Note the folding of the embryonic epidermis. FE – follicular epithelium; E – embryonic epidermis ($\times 20,5$). (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 ($\times 9,84$). (d) Cross section through the ovary of *C. superciliosus* showing superembryonation. Note that the thin and thick sections of the follicular wall can be clearly distinguished in the smaller follicles ($\times 9,84$). (e) Cross section through two follicular epithelia of *C. superciliosus* showing hypertrophied follicular cells and the folded thinner section of a follicular epithelium ($\times 131,2$). (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 ($\times 328$).

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 μ m. Staining was carried out in azo-carmin 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 dropules (Figure 2d). These dropules probably contain the lipids and proteins which form a substantial portion of nutrients present in the embryotrophe 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). Embryotrophe 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 *Anableps* (Knight, *et al.* 1985) the follicular cells do not interdigitate with embryonic trophoderm macro-

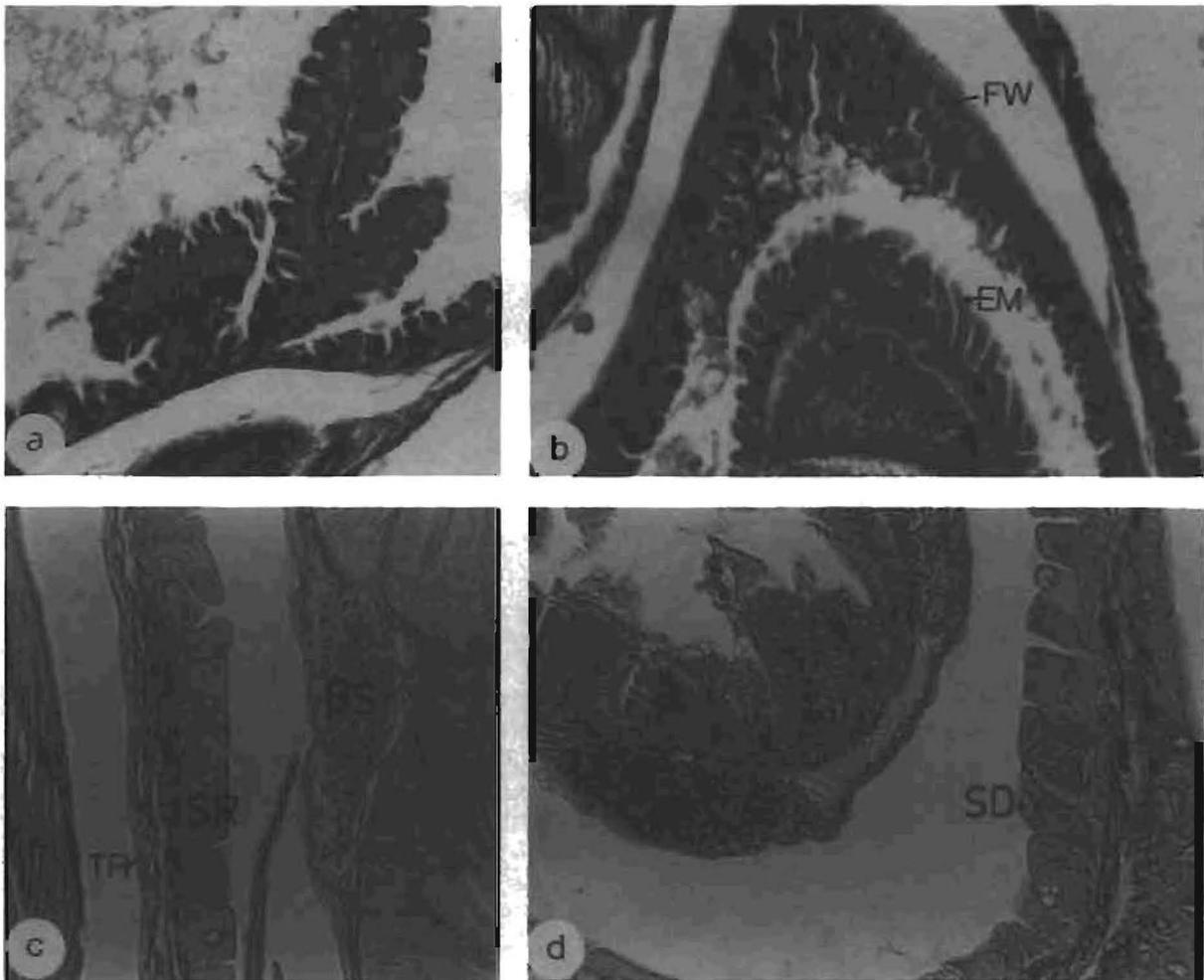


Figure 2 Cross section through the hypertrophied portion of the follicular wall of *C. superciliosus* showing a secondary involution ($\times 51,66$). (b) Cross section through a follicle of *C. superciliosus* showing the hypertrophied follicular epithelium forming a pseudo-placenta in conjunction with embryonic macroridges. EM – embryonic macroridges; FW – follicular wall ($\times 51,66$). (c) Cross section through the ovary of *C. dorsalis* showing the secretory and thin regions of the follicular epithelia. SR – secretory region; TR – thin region; BS – blood sinus ($\times 82$). (d) Cross section through the ovary of *C. dorsalis*, showing secretory dropules in the secretory region of the follicular epithelium. SD – secretory dropule ($\times 131,2$).

ridges and the term pseudoplacenta 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 dropules 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 periovarian space and can be elicited by movements of juveniles within the periovarian 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 periovarian space where they elicit contractions of the ovisac musculature.

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References

- CORNISH, D.A. 1983. Reproductive biology and viviparity in the teleost *Clinus dorsalis* (Perciformes: Clinidae). M.Sc. dissertation, Zoology Department, University of Stellenbosch.
- CORNISH, D.A. & VEITH, W.J. 1986. Embryonic adaptations and nutrition in the viviparous teleost *Clinus dorsalis*. (Perciformes: Clinidae) *S. Afr. J. Zool.* 21: 79–84.
- FUJITA, S. 1958. On the egg development and larval stages of a viviparous scorpaenid fish, *Sebastes oblongus* Günther. *Bull. Jap. Soc. Sci. Fish.*, 24(6 & 7): 475–479.

- IGARASHI, T. 1961. Histological and cytological changes in the ovary of a viviparous teleost *Neoditrema ransonneti* Steindachner during gestation. *Bull. Fac. Fish. Hokkaido Univ.* 12: 181–188.
- IGARASHI, T. 1962. Morphological changes of the embryo of a viviparous teleost, *Neoditrema ransonneti* during gestation. *Bull. Fac. Fish. Hokkaido Univ.* 13: 47–52.
- KNIGHT, F.M., LOMBARDI, J., WOURMS, J.P. & BURNS, J.R. 1985. Follicular placenta and embryonic growth of the viviparous four-eyed fish (*Anableps*). *J. Morph.* 185: 131–142.
- LAGIOS, M.D. 1965. Seasonal changes in the cytology of the adenohypophysis, testes and ovaries of the black surperch, *Embiotoca jacksoni*, a viviparous percomorph fish. *Gen. Comp. Endocrinol.*, 5: 207–221.
- LAMBERT, J.G.D. 1966. Location of hormone production in the ovary of the guppy, *Poecilia reticulata*. *Experientia* 22: 476.
- MENDOZA, G. 1956. Adaptations during gestation in the viviparous cyprinodont teleost, *Hubbsina turneri*. *J. Morph.* 99: 73–96.
- MENDOZA, G. 1972. The fine structure of an absorptive epithelium in a viviparous teleost. *J. Morph.* 136: 109–115.
- MOSER, H.G. 1967. Seasonal histological changes in the gonads of *Sebastes paucispinis* Ayers, an ovoviviparous teleost (family Scorpaenidae). *J. Morph.* 123: 329–353.
- STOLK, A. 1951a. Histo-endocrinological analysis of gestation phenomena in the cyprinodont *Lebistes reticulatus* Peters. I. Thyroid activity during pregnancy. *Koninkl. Ned. Akad. Wetenschap., Proc. C54*: 550–557.
- STOLK, A. 1951b. Histo-endocrinological analysis of gestation phenomena in the cyprinodont *Lebistes reticulatus* Peters. II. The corpus luteum, cycle during pregnancy. *Koninkl. Ned. Akad. Wetenschap., Proc. C54*: 558–565.
- STOLK, A. 1951c. Histo-endocrinological analysis of gestation phenomena in the cyprinodont *Lebistes reticulatus* Peters. III. Changes in the pituitary gland during pregnancy. *Koninkl. Ned. Akad. Wetenschap., Proc. C54*: 566–573.
- STOLK, A. 1951d. Histo-endocrinological analysis of gestation phenomena in the cyprinodont *Lebistes reticulatus* Peters. IV. The oocyte cycle during pregnancy. *Koninkl. Ned. Akad. Wetenschap., Proc. C54*: 574–578.
- TURNER C.L. 1933. Viviparity superimposed upon ovo-viviparity in the Goodeidae, a family of cyprinodont teleost fishes of the Mexican Plateau. *J. Morph.* 55: 207–234.
- TURNER, C.L. 1936. The absorptive processes in the embryos of *Parabrotula dentiens*, a viviparous deep-sea brotulid fish. *J. Morph.* 59: 313–321.
- TURNER, C.L. 1938. Adaptations for viviparity in embryos and ovary of *Anableps anableps*. *J. Morph.* 62: 323–349.
- TURNER, C.L. 1940a. Pseudoamnion, pseudochorion and follicular pseudoplacenta in poeciliid fishes. *J. Morph.* 67: 59–79.
- TURNER, C.L. 1940b. Follicular pseudoplacenta and gut modifications in anablepid fishes. *J. Morph.* 67: 91–101.
- VEITH, W.J. 1979a. Reproduction in the live-bearing teleost *Clinus superciliosus*. *S. Afr. J. Zool.* 14(4): 208–211.
- VEITH, W.J. 1979b. The chemical composition of the follicular fluid of the viviparous teleost *Clinus superciliosus*. *Comp. Biochem. Physiol.* 63A: 37–40.
- VEITH, W.J. 1980. Viviparity and embryonic adaptations in the teleost *Clinus superciliosus*. *Can. J. Zool.* 58: 1–12.
- WEBB, P.W. & BRETT, J.R. 1972a. Respiratory adaptations of prenatal young in the ovary of two species of viviparous seaperch, *Rhacochilus vacca* and *Embiotoca lateralis*. *J. Fish. Res. Bd Can.* 29: 1525–1542.
- WEBB, P.W. & BRETT, J.R. 1972b. Oxygen consumption of embryos and parents and oxygen transfer characteristics within the ovary of two species of viviparous seaperch, *Rhacochilus vacca* and *Embiotoca lateralis*. *J. Fish. Res. Bd Can.*, 29: 1543–1553.
- WIEBE, J.P. 1968. The reproductive cycle of the viviparous seaperch, *Cymatogaster aggregata* Gibbons. *Can. J. Zool.* 46: 1221–1234.