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Fish anatomy

POST-OVULATORY FOLLICLE (CORPUSLUTEUM) HISTOLOGY OF A SNOWTROUT *SCHIZOTHORAX PLAGIOSTOMUS* (HECKEL) FROM GARHWAL HIMALAYA

HISTOLOGIA PĘCHERZYKA JAJNIKOWEGO (CORPUSLUTEUM) TROCI, *SCHIZOTHORAX PLAGIOSTOMUS* (HECKEL) Z REJONU GARHWAL (HIMALAJE)

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The evolution and involution of post-ovulatory follicle (POF) has been studied under five stages. The granulosa cells become proliferated, hypertrophied, differentiated forming the luteal cells and accumulated in the centre of the POF. The POF is surrounded by highly vascular thical elements which remain separate from the granulosa luteal cell mass and have their own entity.

INTRODUCTION

Teleosts in general exhibit a definite reproductive periodicity by passing through the periods of multiplication, growth, differentiation maturity, depletion and rest. During the ovarian cyclicity, the oocyte as well as ovarian development progressively take place. However, the process of degeneration (atresia) is also a consistent feature of taleost ovary (Agarwal, 1988). It is two types: the pre-ovulatory follicular stresia (prior to ovulation) derived from the resorption of previtellogenics and vitellogenic yolky oocytes. This has been extensively studied in *S. plagiostomus* (Agarwal and Singh, 1990) and in other teleosts (Guraya et al, 1975). The other type of atresia occurs in the ruptured follicle left after ovulation. They become atretic forming the corpus luteum of ovulation or post-ovulatory atretic follicle (Browning, 1973; Saidapur, 1978). Contradictory views have been explained in regard to the origin and function of luteal cells in the post-ovulatory atretic follicle in fishes (Browning, 1973; Nicholls and Maple, 1972; Guraya and Kaur, 1979). Hence the present study was made to understand the origin and function of luteal cells as well as the other associated histological changes occuring during the formation and involution of post-ovulatory follicle (POF) in the ovary of an oviparous snowtrout *S. plagiostomus.*

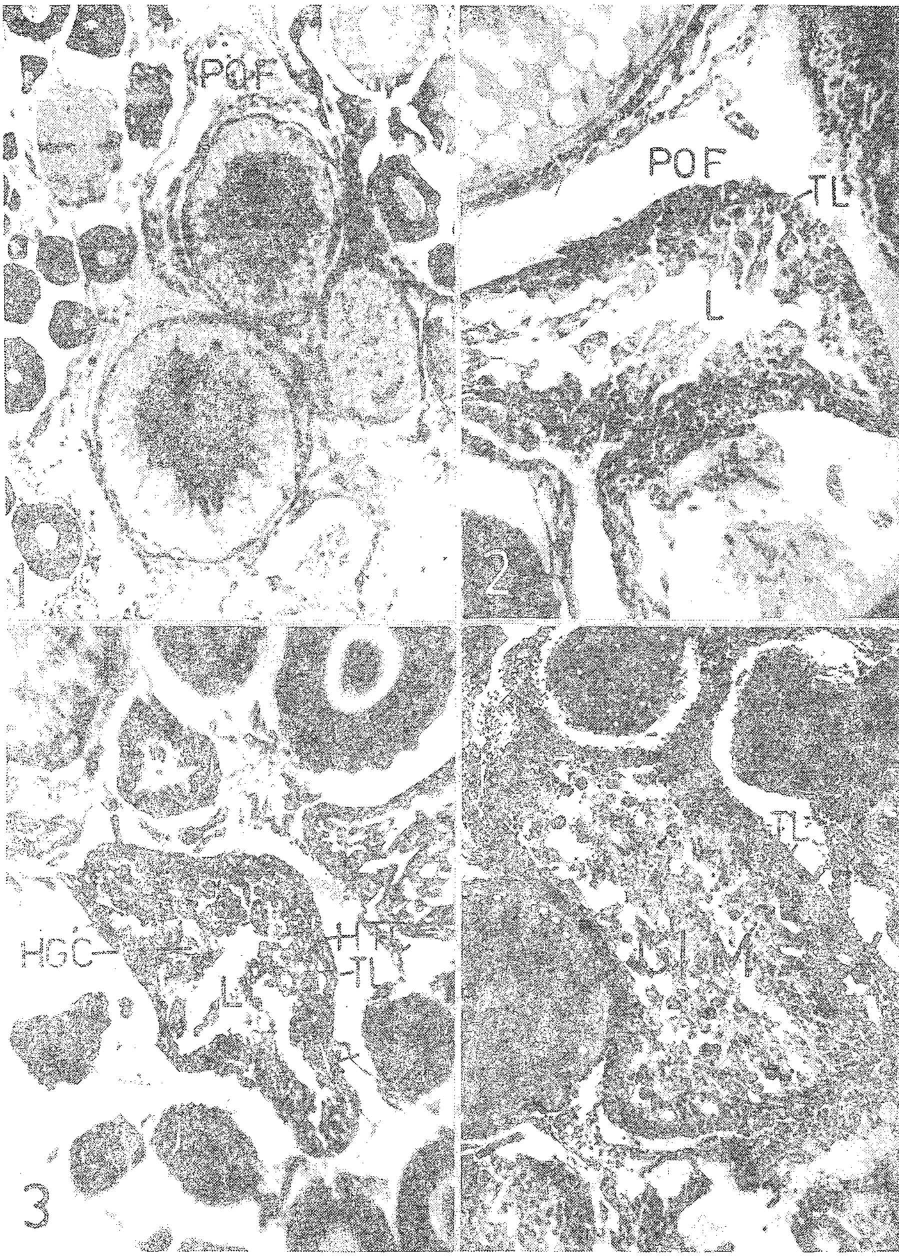

Fig. 1. Portion of the spent ovary showing post ovulatory follicle. Small developing follicles and few mature oocytes are also seen. x 50

Fig. 2. Post ovulatory follicle (stage I) showing large lumen, columnar granulosa cell layer and hypertrophied thecal gland cells in the thecal layer. Not the broken layer of granulosa and thecal cells through which the egg ovulates. x 100

Fig. 3. Post ovulatory follicle (stage II) showing reduced lumen. The hypertrophied granulosa cells projecting into the lumen. The space between thecal layer and follicular epithelium can be clearly seen (arrow). x 100

Fig. 4. Post ovulatory follicle (stage III) illustrating the granulosa luteal cell mass obliterating the lumen of the follicle. The space between thecal layer and follicular epithelium is more pronounced (arrow). x 100

ABBREVIATIONS: BC: Blood cell. DGLC: Generated granulosa luteal cells. GLM: Granulosa luteal cell mass. HGC: Hypertrophied granulosa cell: HTC: Hypertrophied thecal cell. L: Lumen. PN: Pycnotic nuclei. POF: Post ovulatory follicle. TL: Thecal layer

MATERIAL AND METHODS

The ovary samples of the present investigation were taken from the fish *Schizothorax plagiostomus* during the spawning period. This is an annual breeder and spawns from late August to November in the river Alaknanda (Singh and Agarwal, 1986). The first sample of the ovary was taken from the freshly captured fish just after spawning (Ovulation). The next samples were taken on each alternate days from different females in order to follow fully reorganization of spent ovary. After washing off the blood in saline solution, the cut pieces of fresh ovary were immediately fixed in alcoholic Boin's (AB) and neutral formalin (NF). After proper washing (in 70% alcohol for AB and distilled water for NF) dehydration, clearing, and embedding, 8 μ thick sections were cut and stained with Iron Haematoxylin and Delafield' Haematoxylin and subjected to microscopic study.

RESULTS

With the advancement of maturity the previtellogenic oocytes become vitellogenic with the deposition of yolk and the follicle wall seems more developed with clear differentiation into theca, follicular epithelium and zone pellucida. After attaining full maturity ovulation takes place. The theca and granulosa cell layers are left behind after the discharge of ova and the structure formed is now referred to as the post-ovulatory follicle or discharge follicle or ovulation scar or corpus luteum of ovulation. The ovary of spawning and spent phase shows a number of postovulatory follicles (POF) in various stages of their development and degeneration alongwith some ripe unspawned yolky oocytes and immature oocytes (fig. 1). In the beginning, POF shows the broken layer of granulosa and thecal cells through which the egg ovulates by leaving a large lumen previously occupied by the egg (Fig. 2). The POF exhibits several morphological changes during its formation and regression which are described in a series of stages.

STAGE – I. The POF of this stage has a large lumen surrounded by an outer thecal and an inner follicular epithelium. The follicular epithelium becomes thickened and folded (Fig. 2). The lumen is gradually reduced in size due to the shrinkage of follicle.

The granulosa cells of post-ovulatory follicles become hypertrophied with their cytoplasm being homogenously eosinophilic. The cells of the hypertophied granulosa are columnar in shaps with spherical nuclei at their bases. The thecal layer has some spaces which contain several blood cells and some deeply eosinophilic cells. It thickens due to the shrinkage of follicle. Thecal cells vary in shape and size, being either round or rectangular or elongated. (Fig. 2).

STAGE – II. In this stage, reduction in the size od POF takes place. The follicular epithelium at certain places projects into the lumen forming villus-like projections (Fig. 3). It loses its uniformity as it is single layered at certain places while at others it seems multilayered. The nucleoli of hypertrophied granulosa cells with homogenously eosinophilic cytoplasm are round or oval. The spâce between the thecal layer and follicular epithelium is still there and the thecal layer becomes more thick wavy and vascularised (Fig. 3).

STAGE – III. The lumen of the POF is now very much reduced due to its continuous shrinkage. The hypertrophied granulosa cells obliterate the cavity of POF, where they form the granulosa luteal cell mass (GLM) (Fig. 4). The chromatin material of these cells lies adjacent to the nuclear membrane. Some of the nuclei become pycnotic as they are stained dark with haematoxylin. The cytoplasm of hypertrophied thecal gland cells becomes vacuolated. Blood cells are still present in the thecal layer (fig. 5).

STAGE – IV. The size of POF is greatly decreased. The granulosa cells are randomly arranged and later on get separated from each other. The number of pycnotic nuclei are further increased and almost all the nuclei become pycnotic (Fig. 6). The thecal layer shows reduction in vascularization and becomes more fibrous (Fig. 6).

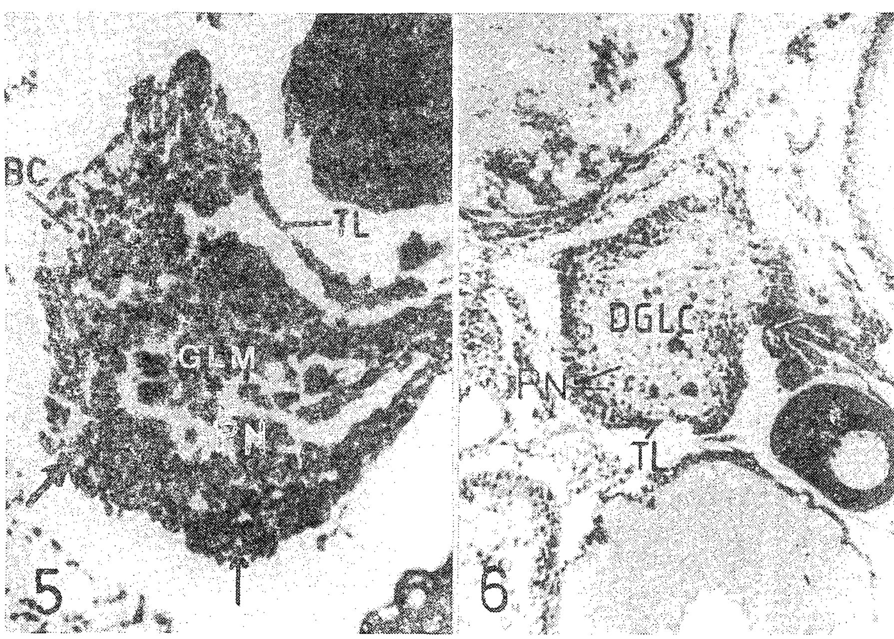

Fig. 5. Post ovulatory follicle in advance phase of stage III showing pycnotic nuclei in the granulosa luteal cell mass. The thecal layer shows the blood cells and vacuolated thecal cells (arrow). x 100
Fig. 6. Post ovulatory follicle of stage IV showing the small mass of degenerated granulosa luteal cell surrounded by thecal layer. x 100

STAGE – V. During this stage the POF seems like a nodule. The residual granulosa luteal cells are engulfed by the thecal layer and hypertrophied gland cells are still present in the thecal layers (Fig. 5).

DISCUSSION

The post ovulatory follicle have been widely described in oviparous teleosts (Lambert and Van Oordt, 1974; Guraya and Kaur, 1979; Goldberg et al 1984), and in other ovuparous vertebrates including amphibians (Guraya, 1968), reptiles and birds (Varma, 1970; Guraya, 1976). Reports are also available on the POF of viviparous vertebrates (Browning, 1973). The POF of *Gobius giuris* (Rajlakshmi, 1966) and *Gillichthys mirabilis* (Vlaming, 1972) do not show hypertrophy and any evidence of a physiological activity. They disintegrate and disappear in the reorganising ovary without forming a *corpus luteum.* Contrary to this, the present study clearly reveals that the follicular epithelium of POF takes part in the formation of granulosa luteal cell mass. The granulosa cells of young POF are hypertrophied later and obliterate the cavity of ruptured follicle leading to the formation of *corpus luteum.* Similar views have also been expressed by Rastogi (1966) and Guraya and Kaur (1979). However Rai (1966) opined that both the thecal and granulosa cells become hypertrophied by forming the vacuolated luteal cell mass in the POF of *Tor (Barbus)* tor. Sanwal and Khanna (1972) regarded the POF in *Channa gachua* as hypertrophied epithelial cells. In S. plagiostomus the epithelial cell mass is entirely formed by outer highly vascular thical tissue and inner granulosa luteal cell mass. The thecal elements have their own entity, remaining separate from the granulosa cells like other oviparous teleosts (Nicholls and Maple, 1972; Guraya and Kaur, 1979).

The POF are short lived structures in oviparous teleosts (Lambert and Van Oordt, 1974; Hunter and Goldberg, 1980). They also exist for a shor time in other oviparous vertebrates including amphibians, reptiles and birds (Guraya, 1976). But in viviparous vertebrates their life is prolonged to a variable degree depending upon the duration of gestation (Browning, 1973). The POF of *S. plagiostomus* (oviparous teleost) is also a short lived structure as its granulosa cells proliferate and differentiate within a few hours after ovulation and soon after begin to degenerate. This is in accordance with Sanwal and Khanna (1972) and Guraya and Kaur (1979) in Indian oviparous teleosts.

The functional significance of POF in fishes is enigmatic and still under considerable discussion (Saidapur, 1978). They have been shown to develop cytological, ultrastructural and histochemical features of well established steroid secreting cells of mammalian corpus luteum (Nagahama et al, 1978). This synthetic ability of POF is likely to have some physiological significance. Such as ovarian maintenance, and/or an involvement in the spawning process. Hunter and Goldberg (1980) and Goldberg et al (1984) established importance of the POF as a means of estimating incidance of spawning in multiple spawning fishes.

REFERENCES

Agarwal N.K., 1988: Studies on the gonadal cycle and spawning ecology of Schizothorax plagiostomus in Garhwall Himalaya. Ph.D. thesis, HNB Garhwal University, Srinagar Garhwal.

Agarwal N.K., H.R. Singh, 1990: Pre-ovulatory follicular atresia (Corpora atrectica) in snowtrout Schizothorax plagiostomus (Heckel) of Garhwal Himalaya. J. Anim. Morphol. Physiol.

Browning H.C., 1973: The evolutionary history of the corpus luteum. Biol. Reprod. **8**: 128–157.

de Vlaming V.L., 1972: Reproductive cycling in the estuarine gobiid fish Gillichthys mirabilis. Copeia **2**: 278–291.

Goldberg S.R., V.H. Alarcon, J. Alheit, 1984: Post ovulatory follicle histology of the pacific sardine, Sardinops sagax. from Peru. Fishery Bulletin **82**, **2**: 443–445.

Guraya S.S., Histochemical study of granulosa (follicular cells) in the preovulatory and postovulatory follicles of amphibian ovary. Gen. Comp. Endoorinol. **10**: 138–146.

Guraya S.S., 1976: Recent advances in the morphology, histochemistry and biochemistry of steroid synthesizing cellular sites in non-mammalian vertebrate ovary. Int. Rev. Cytol. **44**: 365–409.

Guraya S.S., S. Kaur, 1979: Morphology of the postovulatory follicle (corpus luteum) of teleost (Cyprinus carpio L.) ovary. Zoologische Beitrage (zool. Beitr) **25**: 381–390.

Guraya S.S., R. Kaur, P.K. Saxena, 1975: Morphology of ovarian changes during the reproductive cycle of the fish Mystus tengara (Ham.). Acta. anat. **91**: 222–260.

Hunter J.R., S.R. Goldberg, 1980: Spawning incidence and batch fecundity in northern anchovy Engraulis mordax. Fish Bull., U.S. **77**: 641–652.

Lambert J.G.D., P.G.W.J. Van Oordt, 1974: Ovarium hormones in teleosts. Sonderdruck aus Fortschritte der Zoologie. **22**: 340–349.

Nagahama Y., K. Chan, W.S. Hoar, 1976: Histochemistry and ultrastructure of pre and post – ovulatory follicles in the ovary of the gold fish Carassius auratus. Can. J. Zool. **54**: 1128–1139.

Nicholls T.J., G. Maple, 1972: Ultrastructural observations on possible sites of steroid biosynthesis in the ovarian follicular epithelium of two species of cichlid fish, Cichlasoma nigrofasciatum and Haplochromis multicolour. Z. Zellforsch. **128**: 317–335.

Rai B.P., 1966: The corpora atretica and so called corpora lutea in the ovary of Tor (Barbus) tor (Ham.). Anat. Anaz. **119**: 459–465.

Rajalakshmi M., 1966: Atresis of oocytes and ruptured follicles in Gobius giuris (Hamilton-Buchanan). Gen. comp. Endocrinol. **6**: 378–385.

Rastogi R.K., 1966: Astudy of the follicular atresia and evaculated follicles in the Indian teleost Xenentodon cancila (Ham.) Acta biol. Hung. **17**: 51–63.

Saidapur S.K., 1978: Follicular atresia in the ovaries of non mammalian vertebrates. Int. Rev. Cytol. **54**: 225–244.

Sanwal R., S.S. Khanna, 1972: Atretic and discharged follicles in a fresh water fish Channa guchua Anat. Anz. **130**: 297–303.

Singh H.R., N.K. Agarwal, 1986: Spawning ecology of snowtrout Schizothorax plagiostomus (Heckel) from garhwal Himalaya. Proc. Natl. Symp. Fish.: 82–85.

Verma S.K., 1970: Morphology of ovarian changes in the garden lizard, Calotes vesicolor. J. Morph. **131**: 195–210.

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STRESZCZENIE

Badano ewolucję i inwolucję pęcherzyka jajnikowego (POF). Ziarenka komórkowe rozmnożyły się i zmieniły tworząc komórki luteinowe zgromadzone w centrum (POF).

POF jest otoczony przez gęsto unaczynione struktury, które pozostają oddzielone od warstwy zewnętrznej komórki luteinowej i tworzą wyodrębnioną całość.

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