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Short Note

Histological and Histochemical Studies of the Post-ovulatory Follicles during the Annual Reproductive Cycle Including Nesting Cycle of a Wild Avian Species, the Pied Myna, *Sturnus contra contra* L.

Satish Kumar Gupta and Biswa Ranjan Maiti*

Histophysiology Laboratory, Department of Zoology, University of Calcutta (Ballygunge Campus), 35 Ballygunge Circular Road, Calcutta - 700 019, India

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Satish Kumar Gupta and Biswa Ranjan Maiti (1996) Histological and histochemical studies of the post-ovulatory follicles during the annual reproductive cycle including nesting cycle of a wild avian species, the pied myna, *Sturnus contra contra* L. *Zoological Studies* **35**(4): 292-295. The post-ovulatory follicles were studied during the annual reproductive cycle and nesting cycle of the pied myna, *Sturnus contra contra*. These follicles were observed during the breeding phase (May) of the reproductive cycle. Histologically, they were found to regress in 4 stages. Stages I and II were found in the egg-laying period, whereas Stages III and IV were observed in the incubation period of the nesting cycle. The follicles were drastically reduced in the nestling period. 3β-HSDH activity, present in both granulosa and theca interna of the follicles, was intense during the initial regression Stage (I) and decreased subsequently (Stages II, III and IV). On the other hand, sudanophilic lipid reaction, localized in both layers of the follicles, was weak in Stage I but increased in subsequent Stages (II, III and IV). The findings are briefly discussed.

Key words: Post-ovulatory follicle, 3β-HSDH, Lipid, Pied Myna.

Post-ovulatory follicles are known to regress rapidly in birds (Davis 1942, Dominic 1960, Payne 1966, Erpino 1969, Kern 1972, Pal 1976), but they may be retained for a longer period in some avain species: 6 months in the ring-necked pheasant, *Phasianus colchicus* (Kabat et al. 1948, Meyer et al. 1955), and even 1 year in a sea-bird, *Fulmaris glacialis* (Wynne-Edwards 1939). Histologically, post-ovulatory follicles can be recognized till the 25th day after ovulation in the black birds, *Agelaius tricolor* and *A. phoeniceus* (Payne 1966), but in the black-billed magpie, these follicles cannot be distinguished with certainty beyond 5 days after ovulation (Erpino 1969). Post-ovulatory follicles that persist in the incubation period or later in the white-crowned sparrow, *Zonotrichia leucophrys gambelli*, are only large solid spindles of collagen (Kern 1972).

The avian post-ovulatory follicle plays some physiological role, though it does not produce a typical corpus luteum like that of mammals (Van Tienhoven 1961, Lofts and Murton 1973, Saidapur 1982). In contrast, Dominic (1960) and Payne (1966) believed them to be non-secretory, but Guraya and Chalana (1975) described the formation of a corpus luteum, similar cytologically and histochemically to that of mammals, in the house-sparrow. Post-ovulatory follicles in fowl are reported to produce steroid hormones since they possess cells which show ultrastructural features characteristic of steroid-producing cells (Floquet and Grignon 1964, Aitken 1966, Guzsal 1966, Wyburn et al. 1966), have intense 3β-HSDH activity (Chieffi and Botte 1965, Armstrong et al. 1977), and are able to synthesize steroid-hormones in vitro (Senior and Furr 1975, Dick et al. 1978, Huang and Nalbandov 1979, Huang et al. 1979). Results from in vitro studies are, however, controversial about the exact nature of the hormone(s) secreted by these follicles.

Post-ovulatory follicles during the annual reproductive cycle and nesting cycle (nest-building, egg-laying, etc.) have not been studied adequately in wild birds. Additionally, the steroidogenic activity of these follicles during the above cycles has not been reported for any wild avian species so far. Thus, in the current article, these problems have been resolved in a wild avian species, the pied myna.

Materials and Methods—Adult female specimens of Indian pied myna, *Sturnus contra contra* L., were collected from local natural populations during the annual reproductive cycle, and were also captured from their natural breeding grounds during different periods of the nesting cycle (early nest-building, late nest-building, egg-laying, incubation, and nestling).

*To whom all correspondence and reprint requests should be addressed.

Birds were killed by cervical dislocation and ovaries were quickly dissected out, fixed in Bouin's fluid and processed routinely for paraffin microtomy. Serial sections (6 μm) of the ovary were cut and stained by Masson's trichrome method for histological study.

The ovary was halved and 1 half was used for studying sudanophilic lipids (calcium-formol fixed frozen sections) (Pearse 1968) and the other half for 3β-hydroxysteroid dehydrogenase (3β-HSDH) activity (fresh-frozen cryocut sections) (Pearse 1972). Dehydroepiandrosterone (DHEA) was used as substrate. Control sections were simultaneously incubated in a medium lacking DHEA. The above cytochemical studies were carried out in serial sections of the ovary. Both cytochemical tests were used for evaluation of steroidogenic activity of the ovarian post-ovulatory follicles (Saidapur 1982).

Results—Post-ovulatory follicles were previously observed only during the breeding phase (May) of the annual reproductive cycle (Gupta and Maiti 1987). After extrusion of the egg, the normal follicular shape was lost. The follicles appeared collapsed and they are subsequently regressed.

Histology

Four stages of regression could be recognized histologically in these follicles.

Stage I: The follicle at this stage was large in size with very large lumen. The basement membrane was conspicuous. The granulosa became multilayered but mitotic cells were absent (Fig. 1). The theca externa was a thick layer of mostly collagenous materials consisting of epitheloid cells with strands of connective tissue and blood capillaries. Large blood vessels were present at its periphery.

Stage II: The follicles became smaller. The basement membrane between the granulosa and theca interna became very thick, at some places penetrating into the granulosa layer. The granulosa cells showed cytoplasmic vacuolations and pycnotic nuclei. The theca externa contained fewer and smaller blood vessels, and showed an abundance of capillaries.

Stage III: The follicle and its lumen further decreased in size. The basement membrane became disorganized and fragmented and was absent in some regions. Vacuolations were abundant in the granulosa layer and their nuclei were mostly pycnotic. Because of further shrinkage of the follicle, the height of the granulosa layer appeared to have increased. The boundary between the granulosa and the theca interna was not distinct. The capillaries in the theca interna became disorganized.

Stage IV: The follicular size was drastically reduced and the lumen became extremely narrow. The follicle showed a darkly stained, highly compressed theca interna layer followed by degenerated and vacuolated granulosa cells with pycnotic nuclei (Fig. 2). The ruptured site of the follicle became very narrow but was clearly observable. There were greatly reduced blood vessels in the theca externa.

Blood cells were commonly observed in the lumen of the post-ovulatory follicles belonging to different regression stages.

During the nesting cycle, post-ovulatory follicles in the egg-laying period were large (Stages I and II of regression). The size decreased dramatically during the incubation period (Stages III and IV of regression). In the nestling period, they were minute and could not be clearly identified.

Histochemistry

3β-HSDH: 3β-HSDH activity was very strong in the granulosa layer and moderate in the theca interna of the post-ovulatory follicles at Stage I of regression (Fig. 3) and decreased in later Stages (II, III and IV) of regression of the follicles. The reaction was almost absent in the theca externa (Table 1).

Sudanophilic lipids: The granulosa layer contained a small amount of sudanophilic lipids in Stage I of regression and abundant quantities in Stages II, III and IV. The theca interna also showed parallel reactions but the staining intensity was less than that in the granulosa (Table 1). The theca externa contained a few scattered sudanophilic islets.

Discussion—Post-ovulatory follicles in the pied myna were found to regress rapidly as reported in other avian species (cf. Saidapur 1982). They could be identified only until the incubation period, but not in the nestling period. Changes in the histology during regression of the post-ovulatory follicles in the pied myna are also similar to those described previously

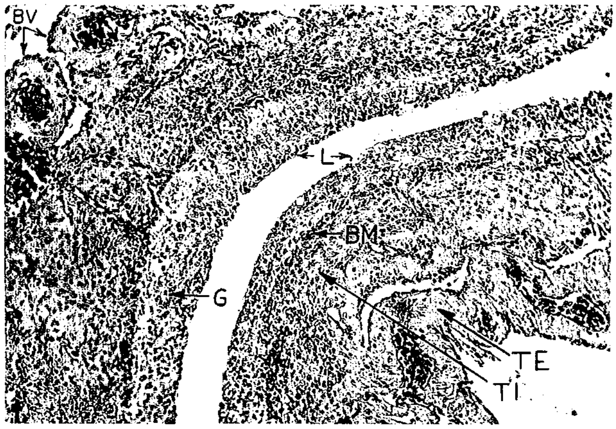

Fig. 1. Post-ovulatory follicle during Stage I of regression in the pied myna ovary with Masson's trichrome stain. Note the presence of a very large lumen (L) lined by multi-layered granulosa (G), a conspicuous basement membrane (BM) and theca interna (TI), as well as a thick theca externa (TE) with large blood vessels (BV).

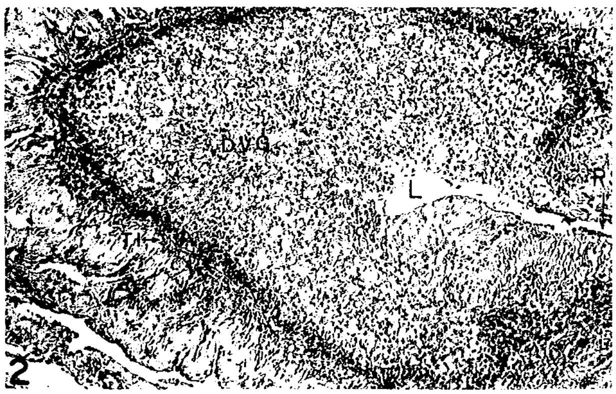

Fig. 2. Post-ovulatory follicle during Stage IV of regression with Masson's trichrome stain. Note the marked reduction in the size of the follicle and that of the central lumen (L). A highly compressed theca interna (TI), and degenerated and vacuolated granulosa (DVG) cells with pycnotic nuclei can also be seen. The ruptured site (RS) is still clearly visible.

Table 1. Histochemical reactions in the post-ovulatory follicles of the ovary of the pied myna

Stage of Regression	3β-Hydroxysteroid dehydrogenase[a]		Sudan black B[a]	
	Theca (interna)	Granulosa	Theca (interna)	Granulosa
I	+ + +	+ + + + +	+ +	+ +
II	+	+ +	+ + +	+ + +
III	+	–	+ + +	+ + + +
IV	–	–	+ + +	+ + + +

[a]Visual rating of intensity of reaction; "–", No action; "+", Very weak; 2 "+", Weak; 3 "+", Moderate; 4 "+", Strong; 5 "+", Very strong.

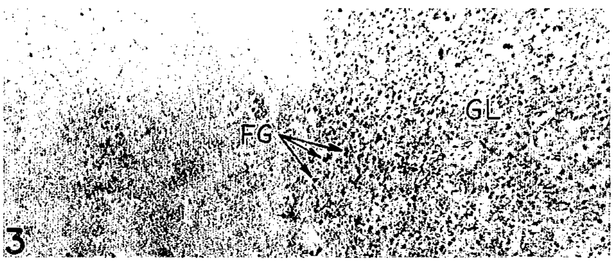

Fig. 3. The 3β-HSDH reaction in Stage I of regression of the post-ovulatory follicle showing intense deposition of formazan granules (FG) in the granulosa layer (GL).

for other birds (Davis 1942, Dominic 1960, Payne 1966, Guraya and Chalana 1975, Pal 1976, Saidapur 1982). However, unlike the earlier reports, the site of rupture in the post-ovulatory follicle was clearly visible even in the last stage of regression in the ovary of this species. The sudanophilic lipid reaction was weak during the early regression phase of the post-ovulatory follicles, but moderate to strong during later phases of these follicles. The 3β-HSDH reaction was intense in the granulosa layer and weak in the theca interna during the early stage of regression of the post-ovulatory follicle. The enzyme activity declined later in both layers. It is known that sudanophilic lipids are depleted during active steroidogenesis and increase during low steroidogenic activity. Furthermore, 3β-hydroxysteroid dehydrogenase activity increases directly with increased steroidogenesis (cf. Findlay 1994). Therefore, the low concentration of sudanophilic lipids with increased 3β-HSDH activity during the early regression phase of the post-ovulatory follicles suggests their strong steroidogenic potentiality. But the reverse correlation of sudanophilic lipids with 3β-HSDH activity during the late regression phases of the post-ovulatory follicles indicates declining steroidogenic function of these follicles in myna ovary. It also indicates that in the pied myna, the post-ovulatory follicles may have an endocrine role but for a brief period only. This is the only report published to date on the subject for any seasonally breeding wild bird. However, in domestic birds a transient endocrine role of the post-ovulatory follicles has been demonstrated from studies of 3β-HSDH activity, ultrastructural features, and in vitro hormone assays (Wyburn et al. 1966, Armstrong et al. 1977, Huang et al. 1979).

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印度野生燕八哥之年生殖週期，包括築巢週期，排卵後濾胞之組織學及組織化學研究

Satis Kumar Gupta[1] and Biswa Ranjan Maiti[1]

本文報告印度野生鳥燕八哥在年週期與築巢週期排卵後濾泡之研究。以生殖週期五月份之交配期之濾胞爲研究對象。以組織學觀察該等濾胞萎縮過程，可分爲四階段。第一及第二階段是在產蛋期，而第三及第四階段是在築巢週期的孵蛋期。在幼雛期間之母鳥，其濾胞大小或數目急遽減少。濾胞顆粒狀層及內莢膜層細胞內之 3-β 羥類固醇去氫酶活性，在萎縮過程之第一階段爲強烈，而在第二、三、及四階段則減弱。另一方面，濾胞之兩層細胞之嗜蘇丹染料反應，在第一階段爲微弱，而二、三、及四階段則增強。

關鍵詞：排卵後濾胞, 3-β 羥類固醇去氫酶, 脂肪, 印度燕八哥。

[1] Histophysiology Laboratory, Department of Zoology, University of Calcutta (Ballygunge Campus), Calcutta, India