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Histological Study of Testis of Brahminy Myna Under NDL and LDL Conditions

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Abstract

Histological study was done in this experiment which provided information with regard to the structure of testis and its status. Birds were procured from the wild and acclimatized in the out door aviary. The experiment was started on 15 March 2005. Two groups of photosensitive birds (n=6 each) were exposed to short day lengths for 60 days and then one group was transferred to natural day lengths and second group was transferred to long day length for 9 months. In this experiment birds were castrated and their testes were removed every month from the experiment and fixed for histological studies. In all histological studies, we performed Hematoxylin - Eosin double staining of paraffin-embedded tissue sections of 7 micron thickness. Food and water were available *ad libitum*.

Key words- brahminy myna, testis, histology, photoperiod

Introduction

Among vertebrates, birds exhibit pronounced seasonal cycles in various behavioural and physiological functions, and several of them are influenced by annual changes in daylength. Some notable examples of bird species in which photoperiodic control of reproductive cycles has been investigated (Kumar and Tewary, 1983; Tewary and Kumar, 1982; Chandola *et al.,* 1973). An interesting feature of photoperiodism is the development photorefractoriness, which is characterized by spontaneous gonadal regression even though birds are exposed to stimulatory photoperiods. Photorefractoriness is adaptive and an advantageous stage in the annual reproductive cycle since it limits reproduction to the best suited time of the year (Farner and Follett, 1979), and avoids wastage of the reproductive potential (Farner and Lewis, 1971). Recovery from photorefractoriness is accelerated by winter day lengths in the wild and by short day lengths in the laboratory condition (Kumar, 1997).

Material and methods

Histological study was also done in this experiment which provided information with regard to structure of testis and its status. Besides observations on body fattening (change in body mass) and growth and development of testes done periodically, birds were castrated and their testes were removed for histological study. Testes were fixed into (aqueous/alcoholic) Bouin's Fixative for histology. The fixed testes were washed thoroughly in running water and when all the fixative was removed, the testes were transferred to 70% alcohol and were given 3 changes of 2 hours each. They were then dehydrated in 90% alcohol and then absolute alcohol for 4 hours in each. Tissue was cleared in cedar-wood oil, and then embedded in paraffin wax (M.P. 58-62^{0}C). Blocks were prepared by transferring the tissue to the space provided by keeping two 'L' pieces together on a glass plate in such a way that a rectangular space is formed in

between them. When the wax got solidified the L-pieces were removes and the block was trimmed so as to remove extra wax around the tissue. The trimmed block was placed on a block holder, which was fixed on to a microtome (Rotary microtome). The tissue was sectioned at 7µ in thickness. The sections were placed onto a clean slide coated with Meyer's albumin. The slide with sections was heated on a hot plate at 55^0C to spread the sections properly. The sections on the slides were then stained through double staining technique using Harris haemotoxylin (for nuclei) and Eosin (background/cytoplasmic stain). The sections were cleared in xylol and mounted with DPX (1,3 dipropyl xanthine, Thomas Baker Chemicals Limited).

Experiment

This experiment was carried out on adult male brahminy myna. Birds were procured from the wild in the month of February 2005 and acclimatized in the out door aviary. The experiment was started on 15 March 2005. Two groups of photosensitive birds (n=6 each) were exposed to short day lengths for two months and then one group was transferred to natural day lengths and second group was transferred to long day lengths (LDL 15L: 9D; 15 hr light; 9 hr darkness) for 9 months. Birds were castrated and their testes were removed for histological study. Food and water were available ad libitum.

Result

In this experiment birds were castrated and their testes were removed from the experiment and fixed for histological study. Birds were exposed to short day photoperiod (8L:16D) from March to May months. Seminiferous tubules are narrow and lined by a single or double-layered spermatogonial cells. Tunica propria is thin and distinct. Tunica albuginea is fibrous and thick. The intertubular spaces are wide containing interstitial cells. In June month of both group of NDL and LDL, seminiferous tubules are highly stretched due to maximum with increase in population of dividing germinal cells during the breeding phase. Spermatogonia cells are present but sperms are not much active. In July month of both group of NDL and LDL, peak of reproductive activity is attained. Bunches of spermatozoa are present in wide lumen. Intertubular spaces are much reduced, and are confined to only triangular areas. In August (group of NDL) marks beginning of the end of reproductive activity. Regressive changes become much distinct. Tubules are narrow and tunica propria is indistinct. In August (but not in September, group of LDL) bunches of spermatozoa are attached to cells of sertoli in seminiferous tubules. Spermatogenetic activity seems to be maximum in wide lumen. The interstitial tissue between adjacent tubules has become greatly compressed. Intertubular spaces are triangular in shape. In September and October months (group of NDL) cellular debris are present in lumen and fully regressed condition of testis is found. The seminiferous tubules are narrow and lined by one or two layers of inactive spermatogonial cells. Wide intertubular spaces containing number of interstitial cells are present. Tunica propria is thin but indistinct probably due to the presence of interstitial cells. In October month (group of LDL) regressive changes become much distinct. Tubules are narrow and tunica propria is indistinct.

Discussion

Brahminy myna is a strongly photosensitive species. When studied in nature that the annual cycles of body mass and of testes in brahminy myna correspond to annual variations in day length, similar to a number of temperate and tropical/subtropical species (Kumar and Kumar, 1993; Deviche and Small, 2001). In this experiment the response under natural and long

photoperiods of the brahminy myna can easily and reliably be mimicked under artificial day lengths. These observations clearly mean that myna use the length of daily photoperiod as source of temporal information for regulating their seasonal cycles. If this were the case, exposure of birds to non-stimulatory photoperiods in which birds will never undergo gonadal development would also ensure that they never become photorefractory.

This experiment provides evidence that artificial long day lengths (15L:8D) reproduce a photoperiodic response that is normally seen under increasing NDL, indicating that the day length regulates gonadal cycle and associated events in the brahminy myna. This suggests that under long day lengths induction of a photoperiodic response was faster (Kumar and Kumar, 1991), but the short day length (8L:16D) was not inductive to brahminy myna. In July month of both group of NDL and LDL, peak of reproductive activity is attained. Bunches of spermatozoa are present in wide lumen. Intertubular spaces are much reduced, and are confined to only triangular areas.

Thus, the brahminy myna's photoperiodic response system is selectively responsive to day lengths, and undergoes a photorefractory period indicating the end of the breeding season. Photoperiod-dependent regulation of seasonality has been reported in many species (Gwinner *et al*., 1988). Several of these species undergo spontaneous gonadal collapse under continuous long photoperiods. In this experiment in the month of August (group of NDL) marks beginning of the end of reproductive activity. Regressive changes become much distinct. But in LDL group regressive changes become much distinct in October month. Whether refractoriness developed is of 'absolute' or 'relative' type needs to be clarified (Robinson and Follett, 1982). In some species, however, long photoperiods (16 h or longer light per day) can keep gonads active for at least a year (Lewis *et al*., 1974). Interesting responses are seen in the blackheaded munia, which shows testicular development both under short and long photoperiods (Pandha and Thapliyal, 1969). Bhatt *et al*. 1986 suggest that photoperiod acts only as synchronizer of the endogenous circannual rhythm of breeding cycle of the spotted munia. All these suggest a divergent photoperiodic strategy, which may have evolved over time.

It is concluded that brahminy myna is a photoperiodic bird and show distinct seasonality in gonadal development. The seasonality in body mass was less dramatic, however, it appears that the day length is involved in regulation of seasonal cycle

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PLATE I

Photomicrographs of the transverse section of testis of brahminy myna (*Sturnus pagodarum*) to examine the effect of photoperiod under natural and long day length:

Figure 1-2. Section of testis of brahminy myna in April and May month and exposed to 8L photoperiod. Tunica albuginea is fibrous and thick. Seminiferous tubules are narrow, lined by the germinal epithelium consisting of developing germ cells and nongerminal sustentacular or sertoli cells and spermatogonia start dividing mitotically. The intertubular spaces are wide enough containing interstitial cells. Tunica propria is thin and distinct.

Figure 3. Section of testis in June month and exposed to NDL. Seminiferous tubules are highly stretched due to increase in population of dividing germinal cells during the breeding phase. Spermatogonia cells are present but sperms are not much active. Lumen is wide. Due to a great tubular expansion, intertubular spaces are much reduced.

Figure 4. Section of testis in June month and exposed to LDL, c.f. figure 3.

Figure 5. Section of testis in July month and exposed to NDL. Mature sperms are present in seminiferous tubules. Spermatogenetic activity seems to be maximum in wide lumen. The interstitial tissue between adjacent tubules has become greatly compressed.

Figure 6. Section of testis in July month and exposed to LDL, c.f. figure 5.

PLATE II

Photomicrographs of the transverse section of testis of brahminy myna (*Sturnus pagodarum*) to examine the effect of photoperiod under natural and long day length:

Figure 7. Section of testis in August month and exposed to NDL, marks beginning of the end of reproductive activity. Regressive changes become distinct. Tubules are narrow and tunica propria is indistinct.

Figure 8. Section of testis in August month and exposed to LDL, bunches of spermatozoa are attached to cells of Sertoli. Spermatogenetic activity seems to be maximum in wide lumen. The interstitial tissue between adjacent tubules has become greatly compressed.

Figure 9&11. Section of testis in September and October months and exposed to NDL, cellular debris are present in lumen and fully regressed condition of testis is found. The seminiferous tubules are narrow and lined by one or two layers of inactive spermatogonial cells. Wide intertubular spaces containing number of interstitial cells are present. Tunica propria is thin but indistinct probably due to the presence of interstitial cells.

Figure 10 Section of testis in September month and exposed to LDL, spermatogonia cells are present but sperms are not much active. Lumen is wide. Due to a great tubular expansion, intertubular spaces are much reduced.

Figure 12. Section of testis in October month and exposed to LDL, c.f. figure 7.

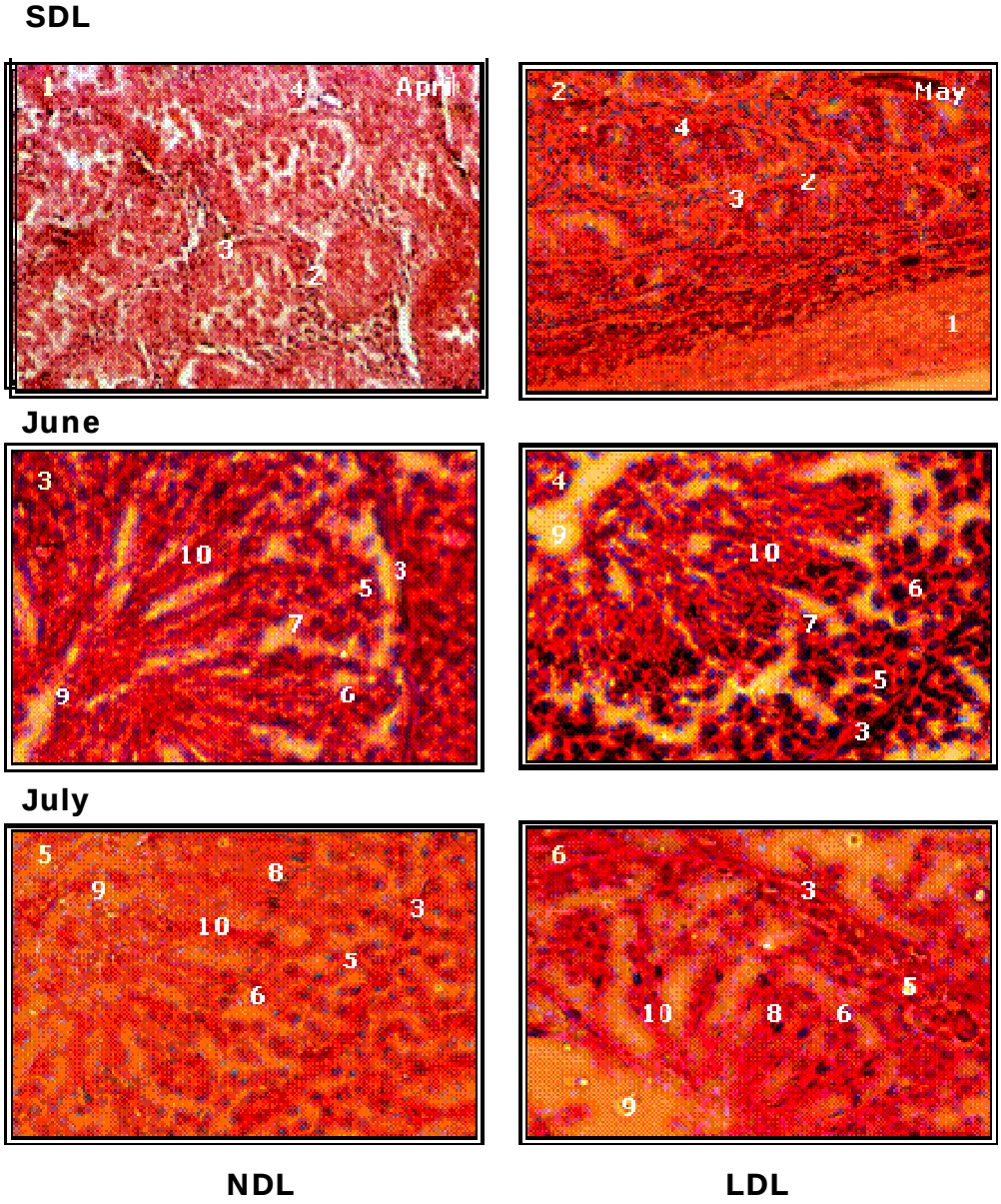
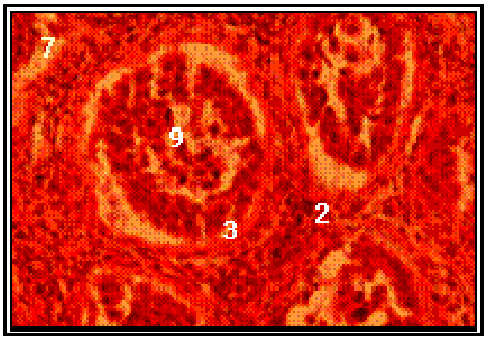


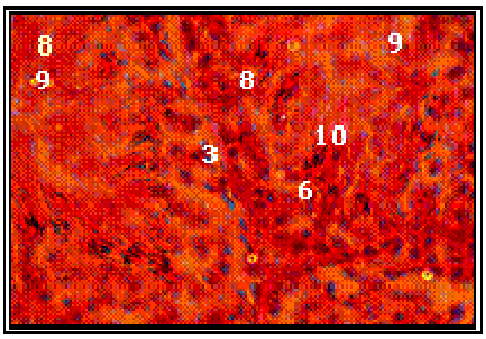
PLATE – I: Photomicrographs of the transverse section of testis of brahminy myna.

1. Tunica albuginea
2. Interstitial cells
3. Seminiferous tubule
4. Spermatogonial cells
5. Spermatocytes
6. Spermatogonia

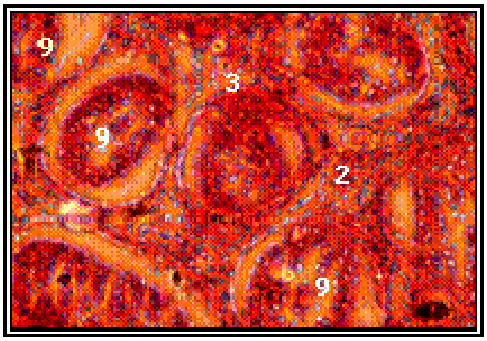
7. Spermatids
8. Sperms
9. Lumen of seminiferous tubules
10. Sertoli cells

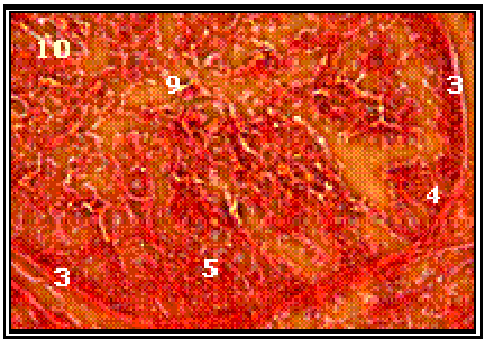
August



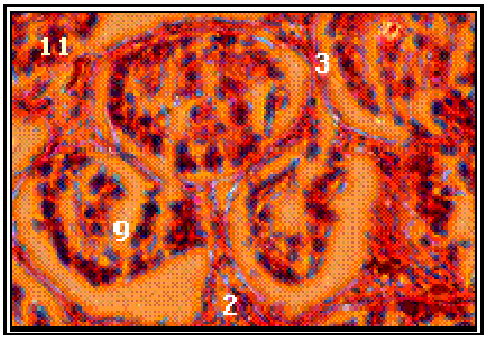


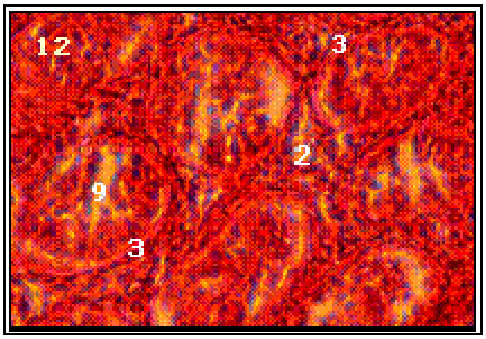
September





October





NDL **LDL**

PLATE – II: Photomicrographs of the transverse section of testis of brahminy myna.

1. Tunica albuginea
2. Interstitial cells
3. Seminiferous tubule
4. Spermatogonial cells
5. Spermatocytes
6. Spermatogonia
7. Spermatids
8. Sperms
9. Lumen of seminiferous tubules Sertoli cell