A COMPARATIVE HISTOENZYMOLOGICAL STUDY OF SOME TISSUES WITH REFERENCE TO REPRODUCTIVE CYCLES IN TWO SPECIES OF BIRDS

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Summary

Seasonally breeding animals detect and respond to cyclic environmental cues that accurately signal well in advance, the variations of seasonal periodicities, which then favours successful reproduction (Lofts and Murton, 1973; Dawson 1999). Hormonal mechanisms regulate and synchronize all aspects of reproduction, from the maturation of sperms and eggs to the expression of species-specific behavioral patterns that are employed to defend a territory, court a female and ensure the fertilization of eggs, incubation of egg and care of hatchlings. Gonads are the major sites for sex-steroid hormone production. Testosterone or Androgens (male hormones) and Progesterone and Estradiol (essential female hormones) are released from the gonads into the blood stream for further eliciting reproductive functions (Nalbandov, 1970; Paster, 1991).

Certain rhythmicity is maintained in the development of gonads and marked changes in the external appearance as well as behavioral patterns of the bird. Such periodicities in the annual reproductive cycle have been observed in two seasonally reproducing bird species: Bank Myna (*Acridotheres ginginianus*) and Brahminy Myna (*Sturnus*

pagodarum). They have distinct pre-breeding, breeding, post-breeding and non-breeding phases of the reproductive cycle. Hence, these commonly available bird species, which exhibit distinctive physiological patterns of seasonal breeders, were selected for the present study.

Organs other than the gonads and adrenals are recently being recognized as steroid metabolizing sites, *viz.*, liver, intestine kidney etc., (Farhting *et al.*, 1982; Andersson, 1995; Andersson *et al.*, 1995;Ghraf *et al.*, 1995; Lateef *et al.*, 1997;Stupans *et al.*, 2000). Convincing evidences of the role of liver, intestine and kidney as important sites for steroid metabolic pathways (Ambadkar and Kotak, 1978; Bhujle and Nadkarni, 1975, 1978; Farthing *et al.*, 1982; Antoun *et al.*, 1985 Nishinaka *et al.*, 1991 Elaroussi *et al.*, 1993. Martel *et al.*, 1994; Yammamuro *et al.*, 1994; Andersson, 1995; Andersson *et al.*, 1995; Lateef *et al.*, 1997 Harpaz and Uni, 1999) in addition to gonads are progressive signs in this field of research.

As a sequel to the above thoughts, histoenzymological alterations of steroid dehydrogenases were carried out in the liver, intestine and kidneys to establish the probability of the role of extra-gonadal tissues along with the gonads in steroid metabolism in the two seasonally breeding species of Mynas.

Intensities of the localization patterns of three steroid dehydrogenases - 3β-hydroxysteroid dehydrogenase (3β-HSDH),

17β-hydroxysteroid dehydrogenase (17β-HSDH) and 3α - hydroxysteroid dehydrogenase (3α-HSDH) are known to be indicative of various levels of steroid hormone biosynthesis/metabolism (Baillie *et al.*, 1966).

As discussed in Chapter 1, the intestinal epithelium with moderate 3β-HSDH activity in both the female and male Mynas seems to be actively involved in steroid metabolism during all the four phases of the reproductive cycle. The liver also seems to be active in steroid metabolism along with intestine and kidney during the pre-breeding and breeding phases, which withdraws later on through the post-breeding and non-breeding phases in the female Mynas. However, during the post-breeding and non-breeding months the kidneys showed increased 3β-HSDH indicating a prominent role than liver in the Bank Mynas males. The nephric tubules gain prominence as far as the metabolic conversions of steroids are concerned during this period. Species-specific differences with comparatively higher level of steroid metabolism in the gonads, as well as related organs in relation to the reproductive activities in both the sexes of Brahminy Mynas as compared to the Bank Mynas were observed.

Concurrently the presence of 17β-HSDH in the intestinal epithelium as discussed in Chapter 2 indicate that the intestine could be an important site of steroid metabolism active almost throughout the

reproductive cycle in both the species of Mynas. Moderate to high levels of 17β-HSDH in hepatocytes during pre-breeding, breeding and non-breeding months in both the male and female Mynas indicate possible involvement of liver in oxidative and reductive reaction of steroid metabolites. There appears to be a marked species-specific difference between the two species in relation to the role of kidneys in both the Mynas. Involvement of cortical tubules in steroid metabolism in both the sexes is probably higher during the first three phases of the reproductive cycle and declines during the non-breeding phase. As opposed to this, the subdued involvement of the nephric epithelial 17β-HSDH activity in Brahminy Mynas during the pre-breeding and breeding phases but noticeably enhanced activity during the post-breeding and non-breeding phases of the reproductive cycle indicate the involvement of nephric tubules in steroid metabolism probably in response to adrenal steroids during cold climatic conditions.

Among the three extra-gonadal tissues under consideration, the intestinal epithelium apparently seems to play an important role in the metabolism of steroid intermediates by 3α-HSDH (Chapter 3) that may escape into the lumen from the liver through bile. The hepatic 3α-HSDH enzyme activity in all the four phases of reproductive cycle, though not very high, probably points to release of the steroid metabolites *via* bile. In this whole process in the aves, the nephric

epithelium also seems to be actively involved but only during pre-breeding phase in female Bank Mynas whereas during other phases, involvement of nephric tubules in steroid metabolism with respect to the presence of 3α-HSDH is subdued. As far as the post-breeding and non-breeding phases are concerned, in female Brahminy Mynas as compared to the Bank Mynas counterparts, the circulating levels of gonadal hormones are lower probably resulting in the gradual withdrawal of participation of hepatocytes as well as the intestinal cells in the steroid metabolism. Strangely enough, higher intensity of 3α-HSDH in the nephric tubules during these two phases apparently indicates a species-specific difference as far as the metabolism of circulating minimal levels of sex steroids is concerned. In the male Mynas of both the species the involvement of nephric epithelium, though less than intestinal epithelium was noteworthy, however, no species-specific difference in the involvement of this tissue was noted in contrast to what has been observed in the female birds.

Varying reproductive behavioural activities are related to varying intensities of male and female sex hormones (Chapter 4). The elevated levels of male hormone testosterone during the breeding season in both female Mynas supports the role of testosterone in females in courtship and nest-building activities (Logan and Wingfield, 1995; Penfold *et al.*, 2000). Similarly in both the species, the minimal

basal level of progesterone in male Mynas as reported in other male birds as well (Johnson, 1986b), can be related to the role of progesterone as a precursor of all the steroid hormones.

Higher lipid accumulation in the female birds as compared to the males (Chapter 5) indicate variations in physiological activities of male and female birds, which are more pronounced in the female birds than in the males for egg production and nurture of young. The lipids synthesized by the liver cells are actively liberated in the circulation and taken up by the gonadal cells in preparation for follicular development as well as gametogenesis during the pre-breeding and breeding phases in both the mynas. Though lipid accumulation was observed in the epithelium of villi, the intestinal glands and the nephric tubules it appears that the localization of lipids in these areas is not directly related to the functional relationship of liver and gonads during different phases of reproductive cycles.

Concurrent morphological variations in the ovaries and testes of the two Mynas have been noted in Chapter 6, which can be related to the variations in the hydroxysteroid dehydrogenase activities during the annual reproductive cycles. However, though variations in HSDHs and lipids accumulation of extra-gonadal tissues are noted in earlier chapters, no histomorphological differences were noted in these tissues over the reproductive cycle. All the extra-gonadal tissues showed a

typical avian pattern and species-specific differences in histoarchitecture could not be found between the two species of Mynas.

From the findings, in the present work it can be stated that proper functioning of gonadal reproductive mechanisms is supported by simultaneous proper functioning of the extra-gonadal tissues over the reproductive cycle of seasonally reproducing birds. By and large, the outcome in the current studies puts forward an explicit and suggestive relationship between the gonadal and extra-gonadal tissues in two seasonally reproducing species of birds. Further research by radiolabelled immunocytometric methods could probably reaffirm or emphasize the exact route/ pathway of the steroid metabolites from the site of production enroute the site of metabolism and finally excretion.

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SUMMARY

In and around Baroda, (Long.73°15'18"N and Lat.22°15'59"E) the Bank Mynas and Brahminy Mynas initiate their breeding cycles with pair-bonding and nest-site selection during the month of April-May. This is followed by the nest building and egg-laying during the month of June-July and a short incubation period of 21 days after which the chicks hatch out during the months of August-September. Both the parents are observed to take care of the young ones with females devoting more time for this activity (Khera and Kalsi, 1980 and personal field observations). It is known that the sex hormones, testosterone, estrogen and progesterone influence all these patterns of reproductive behaviors during the reproductive cycle of birds. Hydroxysteroid dehydrogenases, important enzymes for metabolism of sex-steroids, are expected to be present in the ovaries and testes. In the present study, the role of other non-gonadal tissues like liver, intestine and kidneys of Bank Mynas and Brahminy Mynas in this regard is investigated. These extra-gonadal tissues showed varied intensities of HSDHs during four phases of breeding cycle.

Chapter : 1

The first Chapter deals with 3β-HSDH activities in gonads and extra-gonadal tissues of Bank Myna and Brahminy Myna over the reproductive cycle.

Strong 3β-HSDH activities in the ovaries and testes during the pre-breeding and breeding months indicate increased turnover of progesterone and androstenedione, which could then be converted to

these tissues in steroid metabolism. These tissues may be the sites for steroid metabolic interconversions before they are recycled all the way through entero-hepatic circulation *via* the intestine or excreted through kidney by means of water-soluble pathway into urine. Thus, the varying 3β-HSDH localizations indicate that when the hormone synthesis is high during the breeding season in the gonads and the circulating levels of hormones increases in the body, the non-reproductive tissues like liver, intestine and kidneys may have some role to play. During the non-breeding months when almost no steroid biosynthesis is taking place, circulation of hormones decreases and involvement of the said tissue also decreases. This needs further investigation by radioimmuno-histochemical methods.

Chapter: 2

This chapter deals with 17β-HSDH activities in the same tissues all through the reproductive cycle of Bank Mynas and Brahminy Mynas.

In the pre-breeding, breeding and post-breeding phases of the reproductive cycle of both the Mynas, the 17β-HSDH activity was observed to be intense and high in the granulosa cells and thecal cells respectively indicating elevated estrogen/androgen synthesis in the ovary. Similar trends were also observed in the testes of both the Mynas, in the peripheral cells of the seminiferous tubules and the interstitial cells of Leydig. These increased levels imply active stages of breeding behaviour in the Mynas. The presence of elevated levels of 17β-HSDH in the extra-gonadal tissues during the pre-breeding, breeding and post-breeding phases in different components of ovaries and testes as well as extra-gonadal tissues relates to the indirect role of extra-gonadal tissues during the active reproductive phases in the Mynas. The liver and kidneys are the major sites for oxidation,

reduction or biotransformation of the sex steroid metabolites. The intestine contributes to the metabolic recycling and elimination of the androgen/estrogen metabolites *via* the enterohepatic circulation or elimination of the same through the cloaca with fecal matter. In conclusion, while some questions remains to be answered, the results presented here establishes that there is some correlation of the gonadal steroidogenic activity with the extra-gonadal tissues in the reproductive cycle of the Mynas.

Chapter : 3

This chapter deals with activity patterns of 3α-HSDH, one of the oxido-reductive enzymes of steroidogenic pathway in the gonadal and extra-gonadal tissues of the Mynas.

The elevated activities of 3α-HSDH in the breeding gonads of both the Mynas are an indicative of intra-gonadal metabolism of steroids in the gonads itself at the site of synthesis with simultaneous biosynthesis and release in to circulating blood. The varying intensities of 3α-HSDH in the liver and simultaneously in the intestine during all the four phases of the reproductive cycles in the male and female Mynas possibly suggest the role of liver as a shuttle in the transport of bile acids and steroid hormones across the hepatocytes and also its involvement in recycling or elimination of androgens/ estrogens by catabolic or anabolic processes via the entero-hepatic pathway. Varied 3α-HSDH activities in the nephric tubules indicate species-specific differences in the involvement of kidney in the metabolic handling of circulating sex steroids during different phases of the reproductive cycle.

Chapter : 4

Varying reproductive behavioural activities are related to varying intensities of male and female sex hormones, testosterone, estrogen and progesterone in both the sexes of birds. In this Chapter, the elevated levels of male hormone testosterone during the breeding season as in the female birds supports the role of testosterone in courtship and nest-building activities by female birds. The minimal basal level of progesterone in male as reported in other birds as well can be related to the role of progesterone as a precursor of all the steroid hormones.

Chapter : 5

The lipid accumulation in extra-gonadal tissues of Bank Mynas and Brahminy Mynas all through the reproductive cycle were comparatively higher in the females than in the males, probably because females have high energy requirements for added contribution in various activities like nest-building, egg-laying, taking care of the young ones and other breeding activities. Gonads and liver are found to be inversely involved during various phases of the reproductive cycle. The other extra-gonadal tissues, intestine (epithelium of villi and intestinal glands) and kidney (nephric tubules) are involved in lipid absorption/storage for energy utilization but they are not directly related to the functional relationship of gonads and liver during different phases of the reproductive cycle.

Chapter : 6

In order to validate the cyclic changes observed in earlier chapters during the reproductive cycle of two species of Mynas *i.e.*, Bank Myna and Brahminy Myna, histological changes and cytometric

analysis of the testes and ovaries were carried out. Histomorphological changes were observed in the ovaries and testes of both Bank Myna and Brahminy Myna during the Pre-breeding, Breeding, Post-breeding and Non-Breeding phases of the reproductive cycle. However, though variations in HSDHs of extra-gonadal tissues are noted in earlier chapters, no histomorphological differences were noted over the reproductive cycle. All the extra-gonadal tissues showed a typical avian pattern and not much species-specific differences could be found between the two species of Mynas.

By way of summarizing, in general, the observations mentioned regarding the possible involvement of the three extra-gonadal tissues with reference to the distribution patterns of three main hydroxysteroid dehydrogenases the following few points may be mentioned here:

(1) As is known since long, the liver has been known to be intimately associated with biological inactivation of steroid hormones. Of recent enough literature has accumulated on several other aspects of hepatic functions influencing the steroid metabolism. Further, liver under the influence of these same hormones exhibits significant alterations in its own physiology. So in the present context it could be generalized on the basis of observations recorded here that the hepatic tissue, in case of both sexes in both the species of Mynas, does participate in the overall metabolism of sex steroids but to varying degrees during different phases of the reproductive cycle.

(2) To a more or less similar extent, the nephric epithelial cells are also involved in handling steroids and its metabolites.

(3) However, the participation in the steroid metabolism by the intestinal epithelial elements appears to be of greater importance. Therefore, it would not be out of place, to suggest that the avian

intestinal epithelial cells along with nephric tubules need to be studied in an extensive manner.

Here a word of caution may be added that the tinctorial intensities observable through the Histoenzymological procedures may vary according to the simultaneous high or low distribution of lipids in the tissues concerned as well as their phases of reproductive cycle. Keeping this view in mind proper caution has been exerted in interpreting the observations reported here.