General Considerations

It is well known that sex-steroids play an important role in the functioning of breeding machinery (Clinton and Haines, 2001). The activities of various enzymes involved in the interconversions of steroid metabolites in the steroidogenic pathway, *i.e.,* steroid dehydrogenases, indicate the involvement of the tissues/organs in the metabolism of steroids. As far as steroidogenesis is concerned till recently only the gonads were given attention, however, of recent attention has been diverted to the role of extra-gonadal tissues in the metabolism of steroid hormones. In the present study in relation to a range of hydroxysteroid dehydrogenases involved in steroidogenesis/steroid metabolism the role of three extra-gonadal tissues, *viz.* liver- the main site for all intermediary metabolism; intestine- the site of entero-hepatic circulation, absorption and elimination of cholesterol and other steroid metabolites with bile and finally the kidneys- the organ for excretion of various metabolites are investigated along with the gonads. Since the past couple of decades several studies have thrown some light on the role of peripheral tissues like liver (Akao *et al.,* 1990; Nishinaka *et al.*, 1991; Blomquist, 1995; Anderson, 1995; Mason *et al.*, 1997; Di Fiore *et al.*, 1998; Paolucci *et al.*, 1999; Assisi *et al.*, 2000; Stupans *et al.*, 2000; Belvedere *et al.*, 2001), intestine (Farthing *et al.,* 1982; Anderson, 1995; Belvedere *et al.*, 2001), kidney (Bhujle and Nadkarni, 1975; Blomquist, 1995; Rogerson *et al.*, 1995; Mason *et al.*, 1997; Quinkler *et al.*, 1999), and brain (Mensah-Nayagan *et al.,* 1996; Ukena *et al.*, 1999; Vidal *et al.*, 2000; Guerriero and Ciarcia, 2001) in the metabolism of steroids. Immunohistochemically in the cardiovascular tissues together with the ventricle, atrium, aortic arch, abdominal aorta, and inferior venacava have shown positive reactions for 3β-hydroxysteroid dehydrogenase, (3β-HSDH), suggesting that cardiocytes may synthesize the steroidogenic 3β-HSDH enzyme (Oh *et al.*, 1998). In view of the available literature, in the present study, possibility of involvement of extra-gonadal tissues having an important role in steroid metabolism is the prime objective.

Majority of temperate and sub-tropical birds show seasonal patterns of breeding, which involves a cascade of cyclic histo-physiological changes influenced by the external environment. Proximate and ultimate factors like changes in day length, environmental temperature, lunar changes, tidal cycles and rainfall cycles serve as a source of predictive information in influencing the annual breeding cycle of birds (Murton and Westwood, 1977). These environmental signals provide the appropriate stimuli for the initiation of reproductive cycle through release of hormones and then continuation of the cyclic pattern of breeding. Seasonal changes in the levels of sex-steroid hormones result in a succession of changes in the steroid-dependent behaviours like courtship, copulation, parental care, territorial defense and aggression, with pronounced seasonal fluctuations in occurrence that are linked directly to seasonal rhythms in reproductive functions (Dawson, 1999).

Certain rhythmicity is maintained in the gonadal development with marked changes in the external appearances as well as behavioral patterns of the breeding birds. 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 show distinct pre-breeding, breeding, post-breeding and non-breeding phases of the reproductive cycle which provide an excellent opportunity for comparative study. Hence, these commonly available bird species, belonging to Family Sturnidae were selected for the present study.

Among the hydroxysteroid dehydrogenases (HSDHs) one of the cardinal enzymes involved in the metabolism of steroid hormones, is the Δ^5-3β-HDSH (Baillie *et al.,* 1966; Marrone and Hertlendy, 1983; Wiebie *et al.*, 1990), fluctuating intensities of which signifies circulating levels of sex hormones; 17β-HSDH yet another pivotal enzyme that catalyzes oxidation or reduction at position 17 of the steroid molecule (Adamski and Jacob, 2001; Baker, 2001) and 3α-HSDH, also an important multifunctional oxidoreductase capable of metabolizing steroid hormones (Stolz *et al.*, 1991; Degtiar *et al.*, 2001), were studied histochemically. With reference to the information available, the present study was conducted in relation to the HSDH activities in the gonads and the extra-gonadal tissues like liver, intestine and kidney of the said bird species and the cyclic variations in the distributions patterns of steroid dehydrogenases: 3β-Hydroxysteroid dehydrogenase (HSDH) (Chapter 1), 17β-Hydroxysteroid dehydrogenase (HSDH) (Chapter 2) and 3α-Hydroxysteroid dehydrogenase (HSDH)(Chapter 3) along with lipid accumulation (Chapter 5) have been corroborated. Along with this, the hormonal titers (plasma testosterone and plasma progesterone levels) in the birds studied are also measured which are a sure indicative of the categorization of the reproductive state (Chapter 4). Male and female birds of both Bank Mynas and Brahminy Mynas were procured from a local bird supplier and kept in the Department Aviary till they were sacrificed eventually. Food and water were provided *ad libitum.* After collecting blood samples the birds were decapitated, the tissues were removed and the processing of tissues was carried out as specified in Materials and Methods. These studies were carried out for a period of three years commencing from 1997.

As is known, in the seasonally reproducing bird species, the gonads are significantly active during the breeding season. The ovaries and testes start developing at the advent of pre-breeding phase of the reproductive cycle. Histoenzymologically, high intensities of 3β-HDSH (Table: 1 & 2) and 17β-HSDH (Table: 3 & 4) activities in the ovaries and testes of Bank Mynas and Brahminy Mynas, are an indicative of initiation of biosynthesis of steroid hormones for the forth-coming breeding season. During breeding season the theca layers showed steroid synthesizing activities in the females whereas the granulosa layer of both the Mynas gain importance steroidogenically during the post-breeding season (Table: 1). Granulosa layer is the eminent site for progesterone production, which in turn influences the parental behaviour of the female birds. 17β-HSDH enzyme activity was more in the granulosa layer during all the four phases of the reproductive cycle in the Bank Myna the colonial hole nester, whereas this activity was constant in the ovaries of Brahminy Myna, the individual hole nester (Table: 3). noted?

Increased 3β-HDSH and17β-HSDH enzyme activities in the seminiferous tubules of testes of both the Mynas but lower than that in ~~of~~ the females during the breeding and post-breeding phases of the reproductive cycle (Table: 2&4) indicate partial involvement of males but to a lower magnitude than the females. On the contrary, high 3β-HDSH and 17β-HSDH enzyme activity in the Leydig cells of testes in both the Mynas during the post breeding season are related to the synthesis of hormones during this period for post-hatching care taking behaviour by the male birds (Table: 2&4). Gradual depletion of the enzyme activities in the gonads of the both the sexes of Mynas as the non-breeding season approaches are indications of regressive phase of the reproductive cycle.

The moderate 3α-HSDH activities in the granulosa layer and the interstitial cells in the pre-breeding ovaries of Bank Myna and Brahminy Myna indicate the initiation of the oxidoreductive processes of the steroid hormones. The high 3α-HSDH activity in the granulosa layer during the breeding phase in Bank Mynas and moderate activity in the Brahminy Mynas can be concluded as species-specific differences. Though both the species belong to same family, *i.e.*, Sturnidae, Bank Myna, *Acridotheres ginginianus*, is a colonial hole nester whereas, Brahminy Myna, *Sturnus pagodarum*, is an individual hole nester. The interaction between behaviour, hormones and self-perception of one's place in the social hierarchy is complex and subtle for some species of birds (Lofts and Murton. 1973; Murton and Westwood, 1977). Social behaviour involves, interactions between individuals in which one or more of the animals benefits from the interaction. Moreover, seasonal changes in reproductive hormones, result in a cascade of seasonal changes in the steroid dependent behaviours. In the post-breeding phase and non-breeding phase the interstitial cells showed high 3α-HSDH stating the fact that the interstitial cells are the site for androgen metabolism and oxidoreductive processes of steroid hormones during these phases. The granulosa layer and the theca layer showed mild and little activities respectively suggesting a decline in the steroid hormone biosynthesis and their interconversions.

In the male Mynas, moderate 3α-HSDH activity was observed in the seminiferous tubules during all the four phases of the reproductive cycle. Only the interstitial cells of Leydig showed variations in both the male Mynas [Table: 6]. These results indicate that the multifunctional oxidoreductase enzyme 3α-HSDH, metabolizes the steroid hormones at a moderate rate during the pre-breeding phase, which decreases in the breeding phase and post-breeding phases and this activity of oxidoreduction again increases during the non-breeding phase in both the male individuals of Mynas.

In relation to the gonads, the varying HSDH activities in the extra-gonadal tissues during the four phases of the reproductive cycles denote probable involvement of the liver, intestine and kidneys in steroid metabolism. With reference to 3β-HDSH activities it can be said that in the female Mynas, among the three extra-gonadal tissues, in Brahminy Mynas, the intestine is actively involved in steroid metabolism during all the three initial phases of the reproductive cycle *i.e.*, pre-breeding, breeding and post-breeding phases, along with liver and kidneys only during pre-breeding and breeding phases. Whereas in Bank Myna females, intestine is the major extra-gonadal tissue involved with little contribution from liver and kidneys during the non-breeding phases. Involvement of nephric tubules in Bank Myna was probably restricted to the pre-breeding phases only. In the male birds, the intestine is involved in steroid metabolism all through the reproductive cycles (Table: 1&2) and hepatic and nephric tubules gradually withdraw from the process of metabolism of steroid through breeding and post-breeding phases. Further higher 3β-HSDH in the nephric tissues during the non-breeding season is probably required to metabolize rise in corticosteroids in response to lower ambient temperatures of this period.

However with reference to 17β-HSDH, the intestine seems to be moderately functional throughout the reproductive cycle in oxidoreductive reactions of steroids in the Bank Mynas whereas in the Brahminy Mynas, the involvement of intestine was comparatively higher indicating species-specific differences but a continued persistent role in steroid metabolism (Table: 3&4). 7 different types of 17β-HSDHs have been reported in various tissues (Labrie *et al.*, 1997; 2000), which provide the target cells with means of precisely controlling the intracellular concentration of each sex-steroids according to local needs. With reference to 17β-HSDH, the liver and kidney seems to be steroidogenically involved during the pre-breeding and breeding phases in both the male and female Mynas. Some indirect evidences on the involvement of hepatic tissues in non-avian species may be sighted here. Estrogen binding molecules have been reported in the cytosol and nuclear extracts of hepatocytes in the green frog, *Rana esculenta*, by Picariello *et al.*, (1982) and Paulocci and Botte, (1988), which indicate their role in the yolk protein synthesis. More recently, in the females of the same species, *Rana esculenta*, the presence of testosterone receptors in liver have been demonstrated. Here, the uptake of plasma testosterone is reported to induce hepatic aromatase system under influence of androgen receptors for local conversion of testosterone to estradiol, a putative testosterone used as an additional estrogen supplier which inturn induces vitellogenin synthesis (Di Fiore *et al.*, 1998; Assissi *et al.*, 2000). Further in the mammalian liver, testosterone is converted to two 17-keto-compounds, androsterone and etiocholanolone, which are in turn conjugated to either glucuronic acid or sulfate to yield a water-soluble form amenable to urinary excretion (Norman and Litwack, 1997).

Of the four phases of reproductive cycle, during the post-breeding months conspicuous increase in the 17β-HDSH activity (Table: 1,2,3&4) was noted in the hepatocytes, which could be at contributed to the continued circulation and decreased hormonal uptake by target organ accompanied with accelerated elimination of metabolites of various sex hormones *via* the liver. However, gradual withdrawal of 3β-HDSH in the hepatic and nephric tissues along with the intestine was observed from post-breeding season to the non-breeding season in female Mynas (Table: 1). Here it can be said that whatever basal levels of sex-steroids are found may probably come from the involuting ovaries or the adrenocortical tissue and metabolized slowly in these tissues. In the male birds, kidneys were prominently involved in steroid metabolism during post-breeding season probably due to the some amount of circulating androgen levels again due to lower uptake by target tissue during this phase (Table: 2). Discernible species-specific differences in the nephric tubules between the two species of Mynas *i.e.*, high 17β-HSDH activity in the initial phases of reproductive cycle (pre-breeding, breeding and post-breeding season) in Bank Mynas and high activity in the non-breeding phases in Brahminy Mynas indicate perceptible involvement of nephric tubules in steroid metabolism in both sexes of Mynas (Table: 3&4). The above discussion indicates that, along with the gonads, intestine, liver and kidneys are also involved in the steroid metabolism.

The 3α-HSDH enzyme, an important multifunctional oxidoreductase system, capable of metabolizing steroid hormones, was more prevalent in the intestinal glands during the three initial phases of the reproductive cycle in both the mynas. The varying intensities of 3α-HSDH in the liver and intestine during all the four phases of reproductive cycle in both the sexes of Mynas possibly suggest the role of 3α-HSDH as a shuttle in bile transport across the hepatocytes and also in elimination of androgens by catabolic processes. Kidneys are efficient in metabolically handling steroid hormones during post-breeding and non-breeding season in Brahminy Myna females whereas in the Bank Myna females the steroid metabolizing potential was more in the post-breeding phase. During the other phases, (pre-breeding and breeding) the intestine and liver takes over the function of steroid metabolism. In the male birds, the involvement of kidney was higher during breeding season with additional input from hepatocytes and intestine. This could be related to high corticosteroid circulation and its catabolic activities. This species-specific variation has to be more clearly understood in case of seasonally reproducing birds.

Higher levels of enzyme activities, which in turn indicates high steroid metabolism, have been observed in the gonads as well as extra-gonadal tissues of Brahminy Mynas as compared to the Bank Mynas. This can be attributed to their nesting habits. Brahminy Myna being a solitary hole nester requires more efforts for nest-building, territorial establishment as well as post-hatching activities and other related activities and hence the hormonal upsurge as compared to the Bank Myna, which is a colonial hole nester. As was observed in the substantiated reports of the present study (Chapter 4), the higher levels of plasma testosterone in the male Mynas during the reproductive cycles was more prominent in Brahminy Myna males than in the Bank Myna males during pre-breeding and post-breeding phases but plasma testosterone were at peak levels in the Bank Myna during breeding season was noted. Nevertheless, in the female birds when compared to the male birds, low levels of circulating testosterone were measured and this higher levels during breeding season could also be contributing to the reproductive activities like courtship, nest-building and aggressive territorial behaviours. The female sex hormone progesterone, which can be associated with courtship, incubation and parental care behaviour in the female birds, was evidently high in both the Mynas during the breeding season. Progesterone is the main precursor from which all other steroids are synthesized. Some amount of basal plasma progesterone was also observed in the male Mynas all throughout the reproductive cycle.

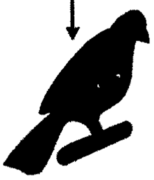
It is known that lipids form the major energy supplement for the wide-ranging activities. High localization of lipids was observed in the liver of female birds rather than in the males during breeding phases. The females have probably increased energy demands for activities like courtship, nest-building, egg laying and the associated activities. The liver lipids are mobilized to ovaries for egg-yolk synthesis during breeding season, which in the later part of post-breeding and the non-breeding phase gets accumulated. These increased lipids in liver could be the lipid-rich cellular debris and non-utilized lipids during these periods of the reproductive cycle. The concurrent high levels of lipids in the ovaries are the lipids taken up from the liver for follicular development during breeding months which during the terminating phases of the breeding cycle are metabolically unutilized leading to their accumulation. The other two extra-gonadal tissues, intestine and kidneys, showed consistent amount of lipid localization. Here it could be suggested that they support energy requirements locally for dietary transport or urinary excretion respectively, rather than for any direct relation to the reproductive biology of birds. The role of gastric cyto-protection by the phospholipids cannot be ruled out as observed in rat gastro-duodenal and mucosal cells (Kao and Lichtenberger, 1990; 1991; Schmitz and Muller, 1991).

Finally, the gonadal weights and histological variation were also studied in all phases in both the species of Mynas. The increase in the ovarian weights with development of follicles and in testes with development of seminiferous tubules during the breeding season and then gradual decrease as the regressive phases started were also noted (Chapter 6). Right testis was always heavier than the left testis. Histological observations have shown characteristic avian features of the developing ovaries and testes and this is correlated with the steroidogenic patterns of variations during all the four phases of reproductive cycle (Chapter 6). Liver, intestine and kidneys as expected did not show any morphological changes all throughout the breeding cycle though they are also significantly involved in steroid metabolism.

By and large, the outcome in the current studies puts forward an evident and suggestive relationship between the gonadal and extra-gonadal tissues in two seasonally reproducing species of birds. That the liver, intestine and kidneys have a probable role in steroid metabolism along with the gonads is certainly an explicit aspect in the field of reproductive biology.

HYPOTHESIS OF THE PROBABLE INVOLVEMENT OF EXTRA GONADAL TISSUES IN STEROID METABOLISM IN AVES

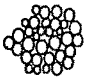
Proximate & Ultimate Factors



Hypothalamic GnRH

Pituitary (FSH and LH)

Ovaries



Progesterone, Estradiol

Testes

Testosterone

Extra gonadal tissues involved (Unknown Mechanism)