CHAPTER 4

Testosterone and Progesterone levels in Bank Myna and Brahminy Myna over the reproductive cycles.

Endocrine studies have greatly expanded our understanding of hormonal interactions with social and ecological signals that affect the timing, sequence and frequency of the reproductive activities (Morton *et al.*, 1990). Hormones are highly potent biomolecules that exhibit tissue specific activity and regulation. Thus, a valuable source of information regarding the relationship between hormones and sexual behaviour lies in understanding the development of sex-specific and species- specific differences and similarities (Balthazard *et al.*, 1983).

It is well known that in males testosterone is required for normal sexual behaviour. In birds, testosterone induces courtship, establishment of territory, nest-building and other related activities (Dawson, 1983; Delvillie *et al.*, 1984; Hau *et al.*, 2000; Soma *et al.*, 2000; Rieters *et al.*, 2002). Secondary sexual characteristics that may differentiate the male from female, like size of the comb, plumage and bill colour, structure of feathers, vocalizations and behaviour especially during the breeding season are all under the influence of testosterone (Welty and Baptista, 1990; Johnson, 1986b; Etches, 1996). Moreover, testosterone is also known to affect both signal development and basal metabolic rate in the House Sparrow (*Passer domesticus*) before the onset of the breeding season and prior to autumn moult (Buchanan *et al.*, 2001). Further, in Baya weaver, *Ploceus phillipinus*, testosterone

Further, in females, progesterone contributes to changes in the ovarian cycle. Progesterone is produced in the granulosa cells of developing ovaries and is known to be metabolized in liver (Bell and Freeman, 1971; Johnson, 1986a). Decreased levels of progesterone are known to cause follicular atresia, inhibition of ovulation, egg formation and also induce moulting in birds (Johnson, 1986a). Evidently, progesterone has an indispensable role in reproduction. The relationship of testosterone and progesterone levels with breeding activities maybe related in the males as well as females as demonstrated in the Northern Mocking birds, *Mimus polyglottis* (Logan and Wingfield, 1995). Stuab and Dè Beer, (1997), have reviewed the role of androgens in female vertebrates and investigations on its role has been suggested as a promising approach to learn about androgen functions in females.

To evaluate and further substantiate the findings in earlier chapters and to understand the sex steroid fluctuations in Bank Myna and Brahminy Myna, two hormones, testosterone and progesterone were estimated in blood plasma over the reproductive cycles. Present study was conducted simultaneously with histochemical studies during four phases of the reproductive cycles using Enzyme Linked Immunosorbant Assay (ELISA) technique.

Results:

Testosterone levels : *(Table : 7) (Graph : 1a & b)*

The mean testosterone level in the female birds of both the species of Mynas during the pre-breeding season was 0.23 ng/ml of blood plasma. These levels rose to 1.5 ng/ml in both the Mynas during the breeding season. When both the species entered the post-breeding

season, a sharp decrease to 0.3 ng/ml of blood plasma was noted which was maintained till the non-breeding season ended.

In the Bank Myna males, compared to female birds, higher levels *i.e.*, 0.65ng/ml plasma testosterone was present during the pre-breeding season, which increased significantly to 2.08 ng/ml of plasma during the breeding season. Thereafter a steady decrease to 0.55 ng/ml in the post-breeding phase and to 0.33 ng/ml during the non-breeding phase was noted. In the other species, the Brahminy Myna also, similar pattern of variation was seen except that during the breeding season the plasma testosterone levels were comparatively lower and during pre-breeding and post-breeding seasons they were higher than those noted for Bank Myna. In this species, plasma testosterone levels were 0.93 ng/ml during the pre-breeding phase, which rose to 1.5 ng/ml during the breeding season and decreased gradually to 0.98 ng/ml during the post-breeding season and the again decreased to 0.38 ng/ml in the non-breeding season. The increase during the breeding season was of lower amplitude in the later species.

Progesterone levels : *(Table : 8) (Graph : 2a & b)*

Progesterone, one of the female sex hormones was high only during breeding phase in females of both the species of Mynas, which was 1ng/ml of blood plasma. During the other phases of reproduction *i.e.*, pre-breeding, post-breeding and non-breeding phases, it was almost same at 0.1ng/ml of blood plasma in both the species of Mynas. No prominent species-specific difference was noted in the female birds.

In the male birds, a basal level of plasma progesterone was noted at 0.1ng/ml all through out the reproductive cycle in both the species of Mynas.

Discussion :

In seasonally breeding species of birds, the changes in the external environmental factors like light, rain, *etc.* are known to trigger the cyclic reproductive activities (Murton and Westwood, 1977; Immelman, 1971; Phillips *et al.*, 1985). The hormonal interplay and its influence on reproductive mechanism can be better understood by studying cyclic alterations in the levels of sex hormones over the reproductive cycle. In the present study two sex hormones were evaluated to support the findings in the activities of various hydroxysteroid dehydrogenases as discussed in earlier chapters.

Some amount of testosterone circulates in the avian blood all throughout the reproductive cycle (Johnson, 1986b). In the female Mynas low levels of circulating testosterone were measured during the pre-breeding, post-breeding and non-breeding phases (Table: 7)(Graph: 1a). The higher testosterone levels in blood plasma of females in the breeding season only could be related to influence of this hormone in various breeding activities. Logan and Winfield (1995) have related increased testosterone levels in female Mocking Birds, *Mimus polyglottis*, with courtship behaviour. However, Potter and Cockerem (1992) have reported a low constant level of testosterone in female North Island Brown Kiwi, (*Apteryx australis mantelli*), throughout the year. In the present study, 17β-HSDH, one of the key enzymes involved in testosterone metabolism remained at a constant level in almost all tissues studied in both the female individuals of Mynas during the reproductive cycle but was comparatively higher in the breeding season (Chapter 2).

Higher levels of plasma testosterone in male Mynas during pre-breeding as compared to the non-breeding phase of reproductive cycle indicate the initiation of breeding machinery (Table: 7). During this

period, there was a moderate localization of 17β-HSDH, in the testicular and extra-testicular tissues (Chapter 2). With advancement to the breeding season, the testosterone levels increased significantly in the male mynas which again is reflected by increase in the 17β-HSDH enzyme activity in the seminiferous tubules, hepatocytes, the epithelium of villi and nephric tubules (Chapter 2). Elevated plasma testosterone levels during breeding season in the male birds have been shown in Starlings (Dawson, 1983) in Mocking birds (Logan and Wingfield, 1995), Japanese Common Pheasant (Sakai and Ishi, 1986), Rose-ringed Parakeet (Krishnaprasadan *et al.*, 1988), Zebra finches (Adkins-Regan *et al.*, 1990), Ostriches (Degan *et al.*, 1994), Northern Pintaii, (Penfold *et al.*, 2000), White winged Crossbill (Deviche and Sharp, 2001), Korean ring necked Pheasant (Kim and Yang, 2001) and in the Ring Dove (Fusani *et al.*, 2001a). In the male White Crowned Sparrows peak testosterone levels occur during egg-laying (Morton *et al.*, 1990) territorial aggression and reproductive behaviour in Japanese Quail (Schliger and Callard, 1990; Wingfield *et al.*, 2001). But in the short-day seasonally breeding bird, the Emu (*Dromaius novaehollandiae*), the highest testicular testosterone concentrations were observed in the pre-breeding months and lowest in the non-breeding months, while the values during the breeding season were intermediate (Malecki *et. al.*, 1998). These reports compiles that the peak testosterone levels occurs during various stages of breeding activities in different species of birds.

When the plasma testosterone levels in the males of the two Mynas are compared, a prominent increase during the breeding season and a decrease in the post breeding season is noted in the Bank Myna, the colonial hole nester (Table: 7) (Graph: 1b). However, in the Brahminy Myna males, the individual hole nester, this increase during the breeding season and then decrease during the non-breeding season

was gradual. This species-specific difference can be explained as, later species being an individual hole nester has to put in more aggressive efforts for territory establishment as well as nest guarding for a longer period.

Higher progesterone levels in the female Mynas are associated with various breeding activities like courtship, incubation and brooding. This is reflected by higher levels of plasma progesterone in female individuals of Bank Myna as well as Brahminy Myna during breeding season only (Table: 8) (Graph: 2a). The high levels of plasma progesterone can also be correlated with high 3β-HSDH activity in the granulosa and the thecal cells of developing follicles and the extra-gonadal tissues (Chapter 1), which probably contributes to the rising levels of plasma progesterone. A basal level of progesterone, one of the female sex hormone is known to occur in the male birds (Johnson, 1986a). In the present study also, male birds of both the species showed a constant lower level *i.e.*, 0.1ng/ml of plasma progesterone all throughout the reproductive cycle (Table: 8) (Graph: 2b). The increased levels of progesterone occur during courtship and incubation for replacement broods have been observed in Mocking birds, *Mimus polyglottis,* also (Logan and Wingfiled, 1995).

The current studies thus reveal an interesting fact that testosterone which is considered as a male hormone also influences female breeding activities where as progesterone, a female hormone, as well as precursor of all steroid hormones, is present in male birds at low levels during all the phases of the seasonal reproductive cycle.

Table 7: Variations in the plasma testosterone levels in Bank Myna and Brahminy Myna during the reproductive cycle (ng/ml of blood plasma)

Months	Bank Myna♀	Brahminy Myna♀	Bank Myna♂	Brahminy Myna♂
Pre-Breeding	0.23±0.02*	0.23±0.02*	0.65±0.03***	0.93±0.05@
Breeding	1.58±0.08@	1.50±0.06@	2.08±0.08@	1.50±0.06@
Post-Breeding	0.30±0.04	0.30±0.04	0.55±0.03**	0.98±0.06@
Non-Breeding	0.35±0.03	0.38±0.02	0.33±0.05	0.35±0.02

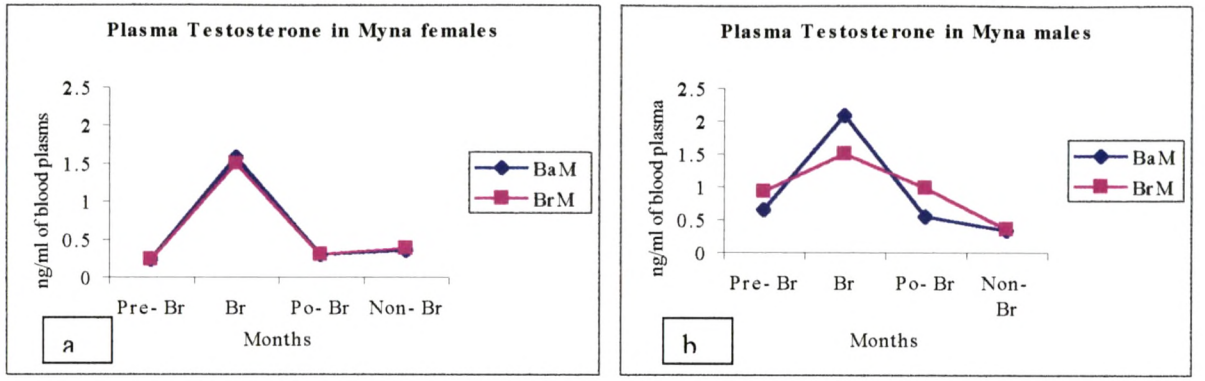
Mean±SD *p<0.02, **p<0.01, ***p<0.001, @ out of range

Table 8: Variations in the plasma progesterone levels in Bank Myna and Brahminy Myna during the reproductive cycle (ng/ml of blood plasma)

Months	Bank Myna♀	Brahminy Myna♀	Bank Myna♂	Brahminy Myna♂
Pre-Breeding	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00
Breeding	1±0.03@	1±0.03@	0.1±0.00	0.09±0.01
Post-Breeding	0.1±0.00	0.09±0.01	0.1±0.00	0.1±0.00
Non-Breeding	0.1±0.00	0.09±0.01	0.1±0.00	0.09±0.01

Mean±SD @ out of range

Graph 1: Variations in the **plasma testosterone** levels in females and males of Bank Myna and Brahminy Myna during the reproductive cycle (ng/ml of blood plasma)



Graph 2: Variations in the **plasma progesterone** levels in females and males of Bank Myna and Brahminy Myna during the reproductive cycle (ng/ml of blood plasma)