# CHAPTER 1

## Histoenzymological observations of 3β-HSDH activity in the gonadal and extra-gonadal tissues of seasonally reproducing birds.

In seasonally reproducing birds, fluctuations in the external environment are known to trigger off various physiological changes especially those related to reproduction in birds (Lofts and Murton, 1973; Murton and Westwood, 1977; Immelman, 1971; Phillips *et al.*, 1985; Dawson, 1999). The cascade of changes observed in relation to breeding include the external morphology like the plumage colour transformation, alterations in the call/song, vigorous and aggressive territorial behaviour and gearing up for the successful completion of the forthcoming breeding phase. Seasonal breeders show periodical gonadal recrudescence prior to breeding, and regression during post-breeding period (Thapaliyal, 1978). Hence, seasonally breeding species of birds are an excellent model to study the changing pattern of gonadal functions at different periods/phases of the reproductive cycle. Such cyclic changes are marked by significant histophysiological alterations related with gametogenic and steroidogenic processes associated with breeding. Gonads, the organs concerned with gametogenesis as well as steroidogenesis, producing ebb and flow of the steroid hormones during the varied stages of reproductive cycle which influence not only secondary reproductive organs, but also the histophysiology of non-reproductive tissues (Nalbandov, 1970).

Ovaries and testes are the most investigated tissues especially in domesticated species like hens (Woods and Domm, 1966; Bell and Freeman, 1971; Tingari, 1973; Armstrong, 1982, 1985; Hertlendy and Asem, 1984; Asem and Hertlendy, 1986; Marrone and Sebring, 1989), quails (Sayler *et al.*, 1970; Asem *et al.*, 1985; Artoni *et al.*, 1997, 1999;) and some wild species of birds like eider (Gorman, 1974), Mynas (Gupta and Maiti, 1986; Ambadkar and Padate, 1993, 1995), pigeons (Ambadkar and Kotak, 1976; Bhujle *et al.*, 1979; Patel and Ramachandran, 1988), Crow Pheasant (Bhujle and Nadkarni, 1976) and kingfisher (Bhujle and Nadkarni, 1978). Of recent, with the functioning of ovaries and testes, attention has also been directed to the functioning of extra-gonadal tissues like liver, intestine, kidney *etc.* in relation to reproduction. Some of the 3β- and 17β- hydroxysteroid dehydrogenases have been documented in the liver of domestic fowl (Nishinaka *et al.*, 1991; Yammamuro *et al.*, 1994), rat, hamster, rabbit (Mason *et al.*, 1997), monkey (Martel *et al.*, 1994), human being (Lang *et al.*, 1986; Pirog and Collins, 1999), in the intestine of fishes (Harpaz and Uni, 1999) and rats (Farthing *et al.*, 1982) and in both liver and intestine of green frog (Picariello *et al.*, 1982; Di Fiore *et al.*, 1998; Paolucci *et al.*, 1999; Belvedere *et al.*, 2001) and in the kidney of white breasted water-hen and stork billed kingfisher (Bhujle and Nadkarni, 1975, 1978), pigeon (Ambadkar and Kotak, 1978) and chick (Elaroussi *et al.*, 1993).

Presence of progesterone receptors has been reported in the chicken intestinal mesothelium and smooth muscles (Salomaa *et al.*, 1989). Further, high levels of progesterone concentration have been observed in the diencephalon and cerebrum of the quail brain with lowest values in the mesencephalon with presence of high level of 3β-hydroxysteroid dehydrogenase in the cerebrum and low level in the mesencephalon, indicating region-dependent biosynthesis of

progesterone (Ukena *et al.*, 1999). Expression of 3β-HSDH-isomerase has also been reported in the female rat pituitary suggesting steroid handling capacity of this gland, which is affected by ovarian endocrine function (Vidal *et al.*, 2000). In the light of the above information, it can be said that these tissues have possibly some role in the metabolism of steroid hormones. In the present study 3β-HSDH, one of the key enzymes in the sex-steroid metabolism has been investigated histoenzymologically through the reproductive cycles in the gonads and extra-gonadal tissues *i.e.*, liver, intestine and kidneys of two seasonally breeding bird species *viz.* - Bank Myna, *Acridotheres ginginianus* and Brahminy Myna, *Sturnus pagodarum*. Significant variations in steroid dehydrogenase activities in extra-gonadal tissues during different phases of reproductive cycle are expected to provide some evidence about their role in metabolism of sex steroids.

## Results :

Pre-breeding Season :

In the beginning of reproductive cycle *i.e.*, pre-breeding phase, high 3β-HSDH activity was observed in the thecal layer of the ovary whereas, the granulosa layer and the interstitial cells of small developing and pre-ovulatory follicles showed moderate activities in the Bank Mynas [Plate I (a)] as well as Brahminy Mynas [Plate I (e)] [Table: 1]. Corresponding to this, the extra-ovarian tissues also showed varying 3β-HSDH activities. The liver of Brahminy Myna females showed moderate localization of 3β-HSDH, [Plate I (f)], whereas in the Bank Mynas female, the activity was mild [Plate I (b)] [Table: 1]. In the intestine of both the Mynas, moderate 3β-HSDH activity was observed in the epithelium of villi, whereas the interstitial glands and the corium of villi showed mild activities during pre-

breeding season [Plate I (c, g)] [Table: 1]. Only in Brahminy Mynas, muscularis externa and tunica propria showed mild activity. Further in all the three lobes of kidney *i.e.*, anterior, middle and posterior lobes, moderate activities of 3β-HSDH were observed in the nephric tubules while little activity was observed in the glomeruli of both the species of female Mynas [Plate I (d, h)] [Table: 1].

In the testes, [Plate II (a, e)] little activity was observed in the seminiferous tubules and the interstitial cells in both the species of Mynas. The hepatocytes of male Brahminy Mynas showed moderate 3β-HSDH activity [Plate II (f)] and that of male Bank Mynas showed mild 3β-HSDH activity [Plate II (b)] [Table: 2]. The intestinal 3β-HSDH activities were similar in the male Mynas as in the female Mynas *i.e.,* moderate enzyme activity in the villar cells and mild activity in the intestinal gland cells and the corium of villi [Plate II (c, g). In the nephric tubules of the male Mynas, 3β-HSDH activity was little and it was mild in the glomeruli [Plate II (d, h)][Table: 2].

Breeding Season :

Both the Mynas, Bank and Brahminy, are observed nesting during the months of May, June and July, with two broods in and around Vadodara. With the advancement of breeding season, a little decrease from high to moderate 3β-HSDH activities in the theca layer of developing follicles was noted whereas in the granulosa layer, the activity was maintained at moderate level [Plate III (a, e)]. This difference was also noted in the large and small ovulatory follicles of female individuals of both the species. The stromal gland cells were weakly stained with 3β-HSDH. During this period, the intensity in the interstitial cells also decreased to little activity in the females of both the species [Table: 1]. Among the extra-gonadal tissues, persistent moderate 3β-HSDH activity was observed in the liver during the

breeding months in the Brahminy Myna females [Plate III (f)] whereas weaker activity was observed in the Bank Myna females [Plate III (b)] [Table: 1]. In the intestine, the epithelium of villi had moderate 3β-HSDH activity and the enzyme localization was mild in the intestinal glands and corium of villi which was alike that of the pre-breeding season [Plate III (c, g)]. In the kidneys of both the female Mynas, when compared to the pre-breeding phase, there was a transitional change in the localization of 3β-HSDH activities. Nephric tubules had little activity and glomeruli showed mild 3β-HSDH localization in the kidneys of female Mynas [Plate III (d, h)] [Table: 1].

Compared to the previous season during this season, the 3β-HSDH activities in the seminiferous tubules increased from little to moderate whereas in the interstitial cells of Leydig, it decreased from little to mild [Plate IV (a, e)] [Table: 2] in testes of both the Bank Myna and Brahminy Myna. Moderate 3β-HSDH activities were persistent in the hepatocytes of Brahminy Myna males [Plate IV (f)] whereas in the Bank Mynas males little activity was noted [Plate IV (b)] (Table: 2). In the intestine, 3β-HSDH activity was similar to that observed during the pre-breeding phase, with moderate activity in the epithelium of villi and mild activity in the intestinal glands and corium of villi. This was similar to that observed in female Mynas [Plate III (c, g); Plate IV (c, g)] [Table: 2]. The enzyme activity in the kidneys of male Mynas during this phase of the reproductive cycle was similar to that observed in the female birds [Plate IV (d, h)] [Table: 2].

Post-Breeding Season :

The post-breeding ovary of the Bank Mynas exhibited a noticeable shift in the 3β-HSDH activities of the ovarian components [Table: 1]. Here, in the thecal layer, the enzyme activity decreased from moderate level of breeding phase to little activity in the post-

breeding season, whereas in the granulosa layer at the same time the activity increased significantly from moderate to intense level [Plate V (a)]. Though similar trend is also observed in the Brahminy Mynas, shift from breeding to post-breeding is not as noteworthy as that in Bank Mynas. In this species, the granulosa layer showed moderate activity as compared to the no activity in the thecal layer during the post-breeding phase [Plate V (e)]. Small ovulatory follicles were scattered all over the ovary and the number of atretic follicles had increased along with the post-ovulatory follicles. Among the extra-gonadal tissues, in the hepatocytes of the Brahminy Myna females, the 3β-HSDH activity dropped to mild [Plate V (f)], whereas it was maintained to little level in the Bank Myna females [Plate V (b)] [Table: 1]. During the post-breeding phase moderate 3β-HSDH activity was noted in the epithelium of villi in both the species of Mynas [Plate V (c, g)] [Table: 1]. However, the intestinal glands showed variations in both the species. In the female Bank Mynas, it was mild, whereas in the female Brahminy Mynas, it was little (Table 1). The nephric tubules and the glomeruli in the kidneys showed little and mild 3β-HSDH activities respectively in the females of both the species studied [Plate V (d, h)] [Table: 1].

Concurrently in the testes of the male Bank Mynas and Brahminy Mynas, there was a moderate increase in the 3β-HSDH activity in the interstitial cells of Leydig, whereas in the seminiferous tubules the activity reduced to mild from moderate activity of the breeding season [Plate VI (a, e)] [Table: 2]. In the male Brahminy Mynas, the 3β-HSDH activity in the hepatocytes was maintained at moderate level and in the Bank Mynas at little level [Plate VI (b, f)] [Table: 2]. The 3β-HSDH activities were moderate in the epithelium of villi as well as the intestinal glands in the male birds of both the Mynas [Plate VI (c, g)] during the post-breeding season [Table: 2]. Compared to the females,

the nephric tubules of the male birds showed moderate 3β-HSDH activity and the glomeruli showed little activity [Plate VI (d, h)] [Table: 2]. A significant observation in the intestine of Brahminy Mynas was the presence of mild 3β-HSDH activity in the muscularis externa and tunica propria during all the three phases of breeding cycles except the non-breeding phase in the females, but in the males this mild activity was observed during the non-breeding phase also. In both sexes of Bank Mynas, mild 3β-HSDH activity was observed in the muscularis mucosa during the post-breeding and non-breeding months [Table: 1 & 2].

Non-Breeding Season :

In the non-breeding phase the 3β-HSDH activity was moderate in the thecal layer of the ovarian follicles of Bank Mynas [Plate VII (a)], whereas, in the Brahminy Mynas [Plate VII (e)] [Table: 2] there was no activity in this layer as was in the post-breeding phase. Granulosa layer showed no activity in the follicles of Brahminy Mynas [Plate VII (e)] but had little 3β-HSDH activity in the Bank Mynas [Plate VII (a)]. The small ovulatory follicles had increased numerously in the ovaries of both the Mynas. During this period, hepatic 3β-HSDH activity was reduced to mild level in the Bank Myna females [Plate VII (f)] and it was maintained at mild level in the Brahminy Myna females [Plate VII (b)] [Table: 1]. The epithelium of villi also showed little activity as compared to the other phases of reproductive cycle in both the species of female Mynas [Plate VII (c, g)] [Table: 1] The Bank Myna females had mild 3β-HSDH activity in the muscularis externa, tunica propria and little activity in the intestinal glands. The non-breeding months were indicated by a decline in the localization of 3β-HSDH in the kidneys too, *i.e.*, from little to mild intensity in the

nephric tubules and nil activity in the glomeruli of female individuals of both the species [Plate VII (d, h)] [Table: 1].

A significant change was observed in the testes of both the Mynas. The interstitial cells showed no activity and the cellular layers of the seminiferous tubules had mild 3β-HSDH localization [Plate VIII (a, e)][Table: 2]. In the Bank Myna males, the 3β-HSDH activity in the hepatocytes was little [Plate VIII (b)] whereas in the Brahminy Myna males it was mild [Plate VIII (f)] [Table: 2]. Moderate 3β-HSDH localization was observed in the epithelium of villi of the male Mynas [Plate VIII (c, g)] [Table: 2]. The other areas of the intestinal tissue *i.e.*, the tunica propria, intestinal glands and the corium of villi showed mild 3β-HSDH activities in both the species of male Mynas. In the kidneys of male birds, there was a significant increase in the 3β-HSDH activities of the nephric tubules *i.e.*, it was high in the nephric tubules and little in the glomeruli of both the species [PlateVIII (d, h)(Table: 2).

## Discussion :

Traditionally, mainly based on mammalian physiology, it has been thought that the chief sources of sex steroid hormones are the gonads and to a certain extent the adrenal cortex. However, during last five decades or so information has been accumulating on possibilities of involvement of extra-gonadal and non-adrenal tissues in steroid metabolism (Cameron, 1964). Another point to be mentioned here is that, there appear to be notable variations in the patterns of metabolism of mammals and birds. There exists enough literature on the role of liver as far as handling of steroid metabolites is concerned in various groups of vertebrates (Picariello *et al.*, 1982; Nishinaka *et al.*, 1991; Rogerson *et al.*, 1995; Di Fiore *et al.*, 1998; Paolucci *et al.*, 1999 and Belvedere *et al.*, 2001). Similarly, there are reports on the capacity of

nephric tubules in the metabolic handling of steroid hormones (Bhujle and Nadkarni, 1975; Elaroussi *et al.*, 1993; Rogerson *et al.*, 1995). Here, a point worth considering pertains to highly evolved water conservation mechanisms in birds, which would have bearings on the patterns of excretion of steroid metabolites. Further, there exist some reports on the involvement of intestinal epithelium in the process of handling intraluminal steroid metabolites (Salomaa *et al.*, 1989: Belvedere *et al.*, 2001). Here, it is possible that liver and intestine may be intimately connected with the enterohepatic circulation of steroid metabolites.

Taking into consideration these aspects of the role of the extra-gonadal tissues *viz.*, liver, intestine and kidney in steroid metabolism, it was thought desirable to further substantiate or otherwise the reports referred to, incase of two seasonally breeding bird species during their reproductive cycles in the light of histoenzymological observations carried out on patterns of localization of three important steroid dehydrogenases.

The present chapter would deal with the variations in intensities and the localization of 3β-HSDH enzyme activity. It is known since long that this enzyme activity is an important one in the metabolic conversions of pregnenolone to progesterone in $\Delta^4$ -3-ketosteroid pathway as well as dehydroepiandrosterone to androstenedione in the $\Delta^5$ -3-beta hydroxysteroid pathway (Baillie *et al.*, 1966; Huang and Nalbandov, 1979; Marrone and Hertelendy, 1983; Porter *et al.*, 1989 and Wiebie *et al.*, 1990) (Flow Chart: 1). With respect to histochemical localization of 3β-HSDH, intense or strong activity indicates that the sex steroid hormone biosynthesis is progressing at an accelerated rate (Baillie *et al.*, 1966).

In the present study, an attempt is being made to find out the relationship of extra-gonadal tissues to that of the gonads with respect

to reproductive cycles in two species of Mynas. In the present chapter, 3β-HSDH activity has been investigated simultaneously in gonadal and extra-gonadal tissues like liver, intestine and kidney. The activities in the gonads were taken as the stages of breeding cycles and at the same state, the changes in extra-gonadal tissues were evaluated [Table: 1 & 2]. For the sake of convenience of discussion, observations will be discussed together in the three tissues selected *vis-à-vis* the gonads, first in the female individuals of the two species and then in the male individuals, noting the significant similarities and differences so as to understand the steroid metabolism in a better light.

From the observations tabulated in Table: 1 regarding the 3β-enzyme activity, it can be seen that among the three extra-gonadal tissues the intestine was observed to be actively involved in steroid metabolism during all the four phases of the reproductive cycle, except the non-breeding phase in the both Bank Myna [Plate I, III, V, VII (c)] and Brahminy Myna [Plate I, III, V, VII (g)] females. In the Brahminy Myna females, the liver also seems to be active in steroid metabolism along with intestine and kidney in the pre-breeding [Plate I (f, g, h)] and breeding phases [Plate III (f, g, h)] (Table 1). In the Bank Mynas, the intestine is the major extra-gonadal site of steroid metabolism with a little contribution from liver and kidneys during post-breeding and non-breeding phases. Nephric tubules of the kidneys played a significant role in the metabolic activity of hormones only during the pre-breeding phase. During the other phases, the kidney seems to play a minor role in both the female Mynas. From the observations in Table 1, it becomes clear that there is a gradual withdrawal of hepatic and nephric tissues in the process of metabolism of steroids through breeding and post-breeding phases. However, during the non-breeding season, the intestinal involvement is also reduced. Whatever basal levels of sex steroids are necessary probably come from the involuting

ovarian tissue, and probably from the heightened adreno-nephric tissue during this particular phase of the reproductive cycle.

Observations in the Bank Myna males, when assessed comparatively, indicated a stronger and uniform pattern of steroid metabolic activity in the epithelium of intestinal villi during all the four phases of the reproductive cycle [Plate II, IV, VI, VIII (c)] (Table: 2). From this it is apparent that the intestinal epithelium in the male Bank Mynas is intimately associated with steroid metabolism throughout the year. Besides its involvement during the breeding season, its role during non-breeding phase appears to be associated with metabolic handling of a certain basal level of circulating progesterone throughout the year (Chapter 4). Liver and Kidneys are secondary in importance as they were involved in steroid metabolism during the pre-breeding [Plate II (b, d)] and breeding [Plate IV (b, d)] phases as observed. Apparently, this may be the mechanism to deal with rising levels of circulating gonadal steroids. In the post-breeding [Plate VI (b, d)] and non-breeding [Plate VIII (b, d)] months, however, the kidneys probably play a prominent role than liver in the Bank Myna males. These observations indicate that the role of nephric tubules gains prominence as far as the metabolic conversions of steroids are concerned. The intestinal epithelial enzyme activity remaining unchanged and that of hepatocytes going down is probably an indication of catabolic role of the nephric tubules during these two phases of the reproductive cycle. This may be, as stated in previous case, related more with circulating progesterone levels. The enhanced involvement of 3β-HSDH activities in the kidneys of the males during the non-breeding season, when the environmental temperature is lowered, is probably required to metabolize rise in corticosteroids in response to lower temperatures (Hagen-Jean, 1975). Brahminy Myna males showed significant enzyme activity in the liver during the first three phases [Plate II, IV, VI (f)] as

compared to Bank Mynas. Therefore, it appears that, along with the intestine, the liver also has an important role in the steroid metabolism during the pre-breeding, breeding and post-breeding phases of Brahminy Mynas. The presence of progesterone receptors in the liver of green frog, *Rana esculenta*, has been reported (Picariello *et al.*, 1982; Paolucci *et al.*, 1999,) stating its down regulation by estradiol and/or progesterone with differences in immunological and biochemical characteristics. Moreover, experiments showing transformation of [4-14C] pregnenolone into progesterone in the liver and into 17α-hydroxypregnenolone in the intestine postulate that both liver and intestine in green frog, *Rana esculenta*, can be independent sources of hormonally active steroids in both males and females (Belvedere *et al.*, 2001). In the non-breeding phase, kidney seems to have taken over the role of handling the metabolic activities of the hormones in the male Brahminy Mynas perhaps in a similar way as described for male Bank Mynas.

When 3β-HSDH localization in the hepatocytes of Bank Mynas and Brahminy Mynas were compared, a marked difference was observed. The enzyme activity was more in both the sexes of Brahminy Mynas than in the Bank Mynas (Table: 1&2). This can be related to species-specific differences, *i.e.*, Brahminy Mynas being a solitary hole nester requires to put in more efforts in the nest building and territorial activities than the Bank Mynas, which is a colonial hole nester. Hence, there is 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. The statements made presently do find enough support in the works cited in the following reports. Liver, though is intimately associated with the digestive and metabolic activities of vertebrates, is known to be the main center for the production and distribution of intermediary metabolites (Popper and

Schaffner, 1957). As mentioned earlier, it also has an important role in steroid hormone catabolism where steroids are converted to metabolically inactive forms (Cameron, 1964). The presence of 3β-HSDH, a component of 17β-dehydrogenases has been indicated in the chicken liver by Thin Layer Chromatography, High Pressure Liquid Chromatography and Gas Chromatography (Nishinaka *et al.*, 1991). Further Rogerson *et al.*, (1995) have characterized 3 isoforms of 3β-HSDH/$\Delta^{5-4}$ isomerases in the male liver, kidney and adrenal of hamster, but were unable to document the function of the same in these tissues. 3β-HSDH in the liver could be the component of 17β-HSDH another enzyme involved in biosynthesis of steroid hormones as described by Nishinaka *et al.*, (1991).

Steady levels of 3β-HSDH activity in the intestinal epithelium and its gradual rise in the nephric tubules in the males of both the species during the non-breeding season stands out in contrast with those of the females in both the species. On the basis of the available observations, this situation cannot be explained properly, and therefore it certainly needs additional work to come to a satisfactory explanation. However it is worth pointing out here the previous findings on increased levels of circulating cholesterols in males as well as females of both the species, but, on a comparative basis, slightly lower levels of circulating cholesterol in male birds of both the species (Ambadkar and Padate, 1993, 1995). This may indicate its conversion of cholesterol to progesterone by the nephric tubules in males only to a certain extent. This can probably explain the possible nephric contribution to maintenance of circulating basal level of this hormone in the male birds even during non-breeding season, supplementing the continued role of intestinal epithelium in this direction. From extension of this line of thought, in case of female birds of both the species, it is apparent from the observations given in Table: 1 that all the three extragonadal

tissues show reduction in their contribution towards synthesis of progesterone (basal levels). This finds support in increased circulating cholesterol levels, higher in the females than in the male birds of both the species of Mynas as mentioned earlier. It is therefore a noticeably different pattern of hormonal variations as far as the sexes are concerned. It should however be added here that in case of the females the basal circulating progesterone level is probably solely maintained by the remnants of theca tissue/interstitial cells of the involuting ovaries. Partial supplementation by the adreno-nephric tissue to the progesterone pool, in both the sexes of both species is also a possibility, as this phase of the reproductive cycle occurs during the colder months of the year when the corticosteroids normally goes up (Hagen-Jean, 1975). Of the two species of Mynas, observed in laboratory and in the field by the author, both sexes of Brahminy Mynas exhibit stronger aggressive and territorial behaviour. The present observations on the 3β-HSDH activities support this contention to a good extent. From the above findings, it can be stated that proper functioning of reproductive mechanisms also requires the simultaneous proper functioning of the extra-gonadal tissues during the reproductive cycle of seasonally reproducing birds.

## Abbreviations :

Tl = Theca Layer

G = Granulosa

Ic = Interstitial Cells

S = Stroma

ST = Seminiferous Tubule

M.Externa = Muscularis externa

T.Propria= Tunica Propria

Int. Glands=Intestinal Glands

Epi. Of Villi= Epithelium of Villi

Cor. Of Villi= Corium of Villi

Nephric T= Nephric Tubules

Glom=Glomeruli

Pr-Br = Pre-Breeding

Br = Breeding

Po-Br = Post-Breeding

Non-Br = Non-breeding

## Activity Pattern :

- – : No activity
- ± : Mild activity
- + : Little activity
- ++ : Moderate activity
- +++ : High activity
- +++++ : Intense-activity

Table 1: 3β-HSDH activities in the ovaries, liver, intestine and kidneys of Bank Myna, *Acridotheres ginginianus* and Brahminy Myna, *Sturnus pagodarum*

| | Bank Myna | | | | Brahminy Myna | | | |
|---|---|---|---|---|---|---|---|---|
| BreedingPhases | Pr-Br | Br | Po-Br | Non-Br | Pr-Br | Br | Po-Br | Non-Br |
| Tissues | Ovary | | | | | | | |
| Tl | +++ | ++ | + | ++ | +++ | ++ | - | - |
| G | ++ | ++ | +++++ | + | ++ | ++ | ++ | - |
| Ic | ++ | + | + | + | ++ | + | ++ | + |
| S | ± | ± | ± | - | + | + | ± | ± |
| | Liver | | | | | | | |
| Hepatocytes | ± | + | + | ± | ++ | ++ | ± | ± |
| | Intestine | | | | | | | |
| M.ext. | - | - | ± | ± | ± | ± | ± | - |
| T.Prop | - | - | - | ± | ± | ± | ± | ± |
| Int.Gl. | ± | ± | ± | + | ± | ± | + | ± |
| Epi. Villi | ++ | ++ | ++ | + | ++ | ++ | ++ | + |
| Cor. villi | ± | ± | ± | ± | ± | ± | ± | ± |
| | Kidney | | | | | | | |
| Medulla | - | - | - | - | - | - | - | - |
| Neph.Tub | ++ | + | + | ± | ++ | + | + | ± |
| Glom | + | ± | ± | - | + | ± | ± | - |

Table 2: 3β-HSDH activities in the testes, liver, intestine and kidneys of Bank Myna, *Acridotheres ginginianus* and Brahminy Myna, *Sturnus pagodarum*.

| | Bank Myna | | | | Brahminy Myna | | | |
|---|---|---|---|---|---|---|---|---|
| BreedingPhases | Pr-Br | Br | Po-Br | Non-Br | Pr-Br | Br | Po-Br | Non-Br |
| Tissues | Testis | | | | | | | |
| ST | + | ++ | ± | ± | + | ++ | + | ± |
| Ic | + | ± | ++ | - | + | ± | ++ | - |
| | Liver | | | | | | | |
| Hepatocytes | ± | + | + | + | ++ | ++ | ++ | ± |
| | Intestine | | | | | | | |
| M.ext. | - | - | ± | ± | ± | ± | ± | ± |
| T.Prop | - | - | - | ± | ± | ± | ± | - |
| Int.Gl. | ± | ± | ++ | ± | ± | ± | ++ | ± |
| Epi. Villi | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| Cor. villi | ± | ± | ± | ± | ± | ± | ± | ± |
| | Kidney | | | | | | | |
| Medulla | - | - | - | - | - | - | - | ± |
| Neph.Tub. | + | + | ++ | +++ | + | + | ++ | +++ |
| Glom | ± | ± | ± | + | ± | ± | + | + |

PLATE I

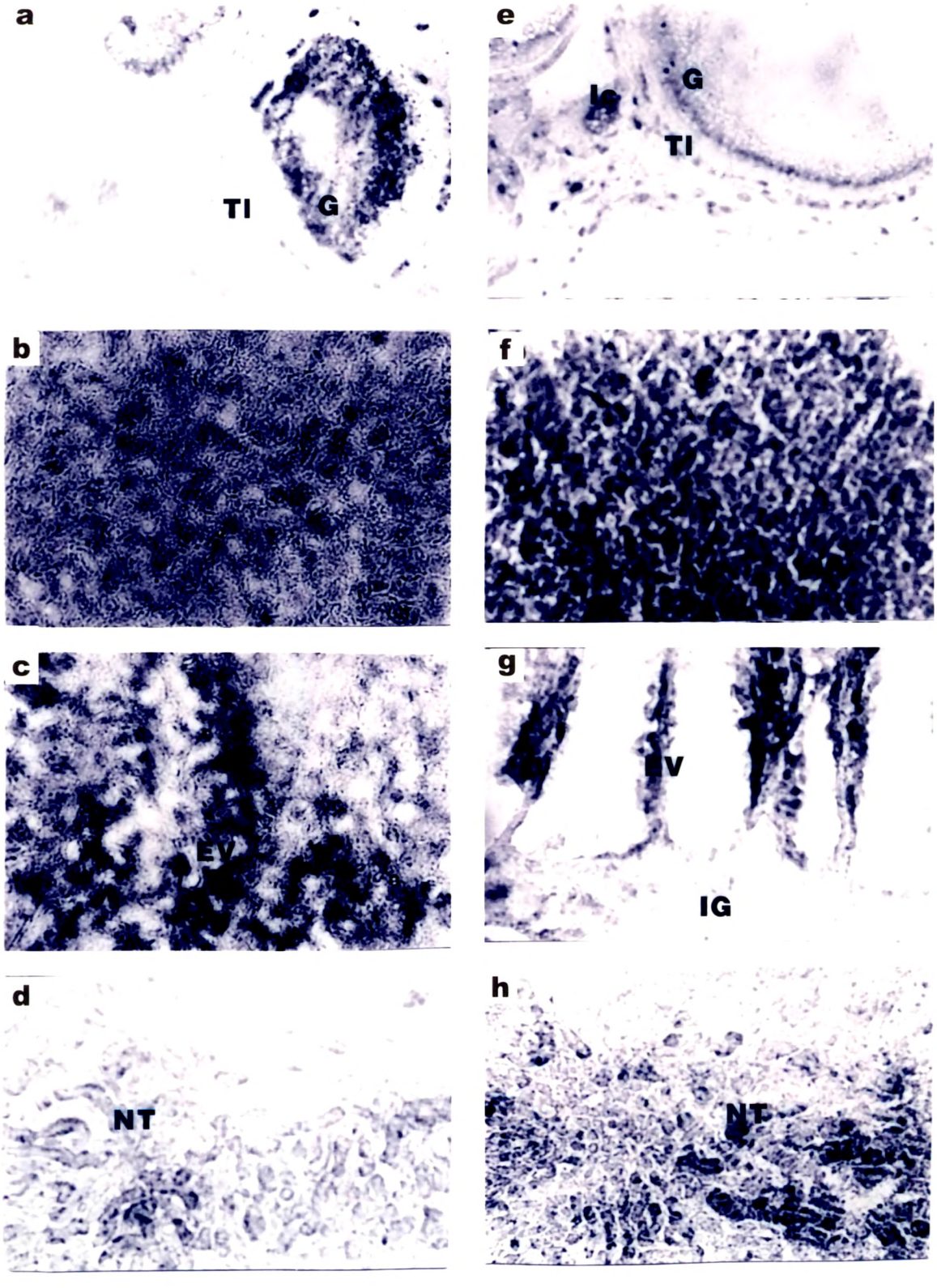
3β-Hydroxysteroid dehydrogenase activities in ovarian and extra ovarian tissues of Bank Myna, *Acridotheres ginginianus*, an Brahminy Myna, *Sturnus pagodarum*.

**- Pre-Breeding phase –**

| **Bank Myna ♀** | | **Brahminy Myna ♀** | |
|---|---|---|---|
| a. Ovary | (500X) | e. Ovary | (500X) |
| b. Liver | (800X) | f. Liver | (800X) |
| c. Intestine | (800X) | g. Intestine | (500X) |
| d. Kidney | (500X) | h. Kidney | (500X) |

Abbreviation :
Tl : Theca Layer ; G : Granulosa ; Ic : Interstitial cells ;
EV : Epithelium of villi ; IG : Intestinal Glands ; NT : Nephric Tissue

# PLATE I



## PLATE II

3β-Hydroxysteroid dehydrogenase activities in testicular and extra-testicular tissues of Bank Myna, *Acridotheres ginginianus*, and Brahminy Myna, *Sturnus pagodarum*.

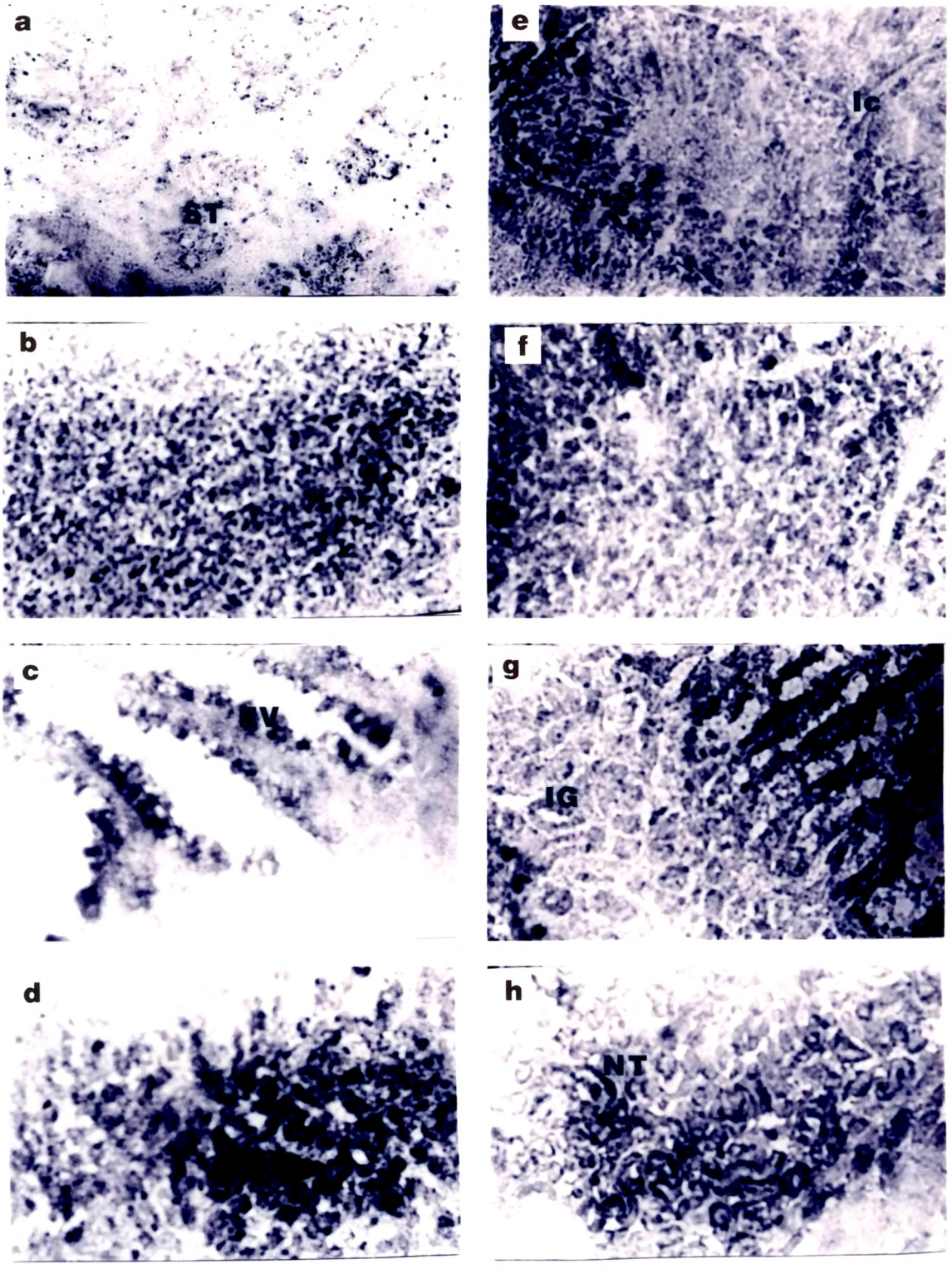
**- Pre-Breeding phase –**

| **Bank Myna ♂** | | **Brahminy Myna ♂** | |
|---|---|---|---|
| a. Testis | (500X) | e. Testis | (500X) |
| b. Liver | (800 X) | f. Liver | (800X) |
| c. Intestine | (800 X) | g. Intestine | (500X) |
| d. Kidney | (500 X) | h. Kidney | (500X) |

Abbreviation :

ST : Seminiferous Tubule ; Ic : Interstitial cells ; EV : Epithelium of villi ; IG : Intestinal Glands ; NT : Nephric Tissue

# PLATE II



## PLATE III

3β-Hydroxysteroid dehydrogenase activities in ovarian and extra ovarian tissues of Bank Myna, *Acridotheres ginginianus*, and Brahminy Myna, *Sturnus pagodarum*.

**- Breeding phase –**

| **Bank Myna ♀** | | **Brahminy Myna ♀** | |
|---|---|---|---|
| a. Ovary | (500X) | e. Ovary | (500X) |
| b. Liver | (800X) | f. Liver | (800X) |
| c. Intestine | (800X) | g. Intestine | (500X) |
| d. Kidney | (500X) | h. Kidney | (500X) |

Abbreviation :
Tl : Theca Layer ; G : Granulosa ; EV : Epithelium of villi ;
NT : Nephric Tissue

# PLATE **III**

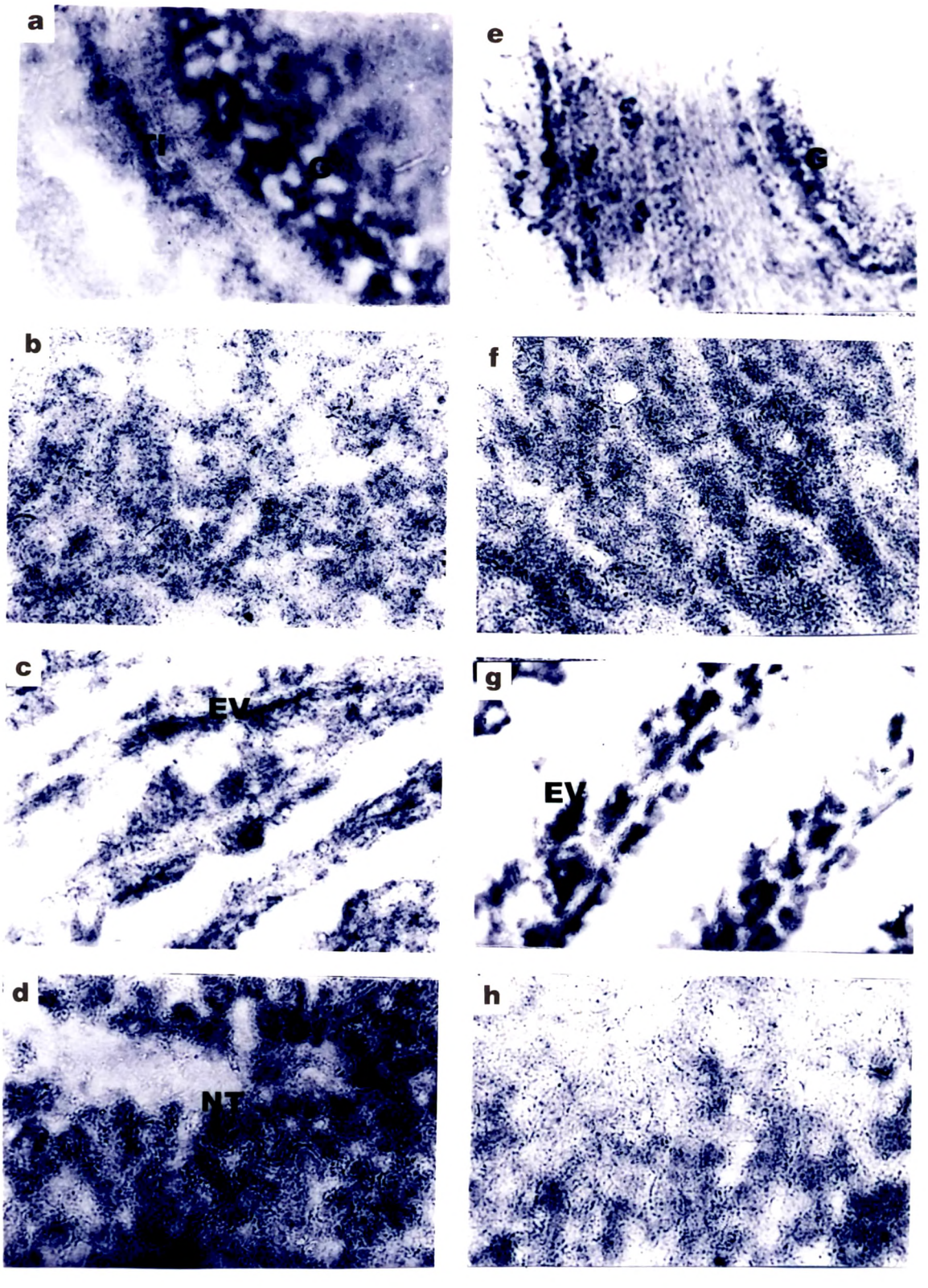


PLATE IV

3β-Hydroxysteroid dehydrogenase activities in testicular and extra-testicular tissues of Bank Myna, *Acridotheres ginginianus*, and Brahminy Myna, *Sturnus pagodarum*.

**- Breeding phase –**

| **Bank Myna ♂** | | **Brahminy Myna ♂** | |
|---|---|---|---|
| a. Testis | (500X) | e. Testis | (500X) |
| b. Liver | (800X) | f. Liver | (800X) |
| c. Intestine | (800X) | g. Intestine | (500X) |
| d. Kidney | (500X) | h. Kidney | (500X) |

Abbreviation :

ST : Seminiferous Tubule ; Ic : Interstitial cells ; EV : Epithelium of villi ; CV : Corium of Villi ; NT : Nephric Tissue ; M : Medulla

# PLATE **IV**

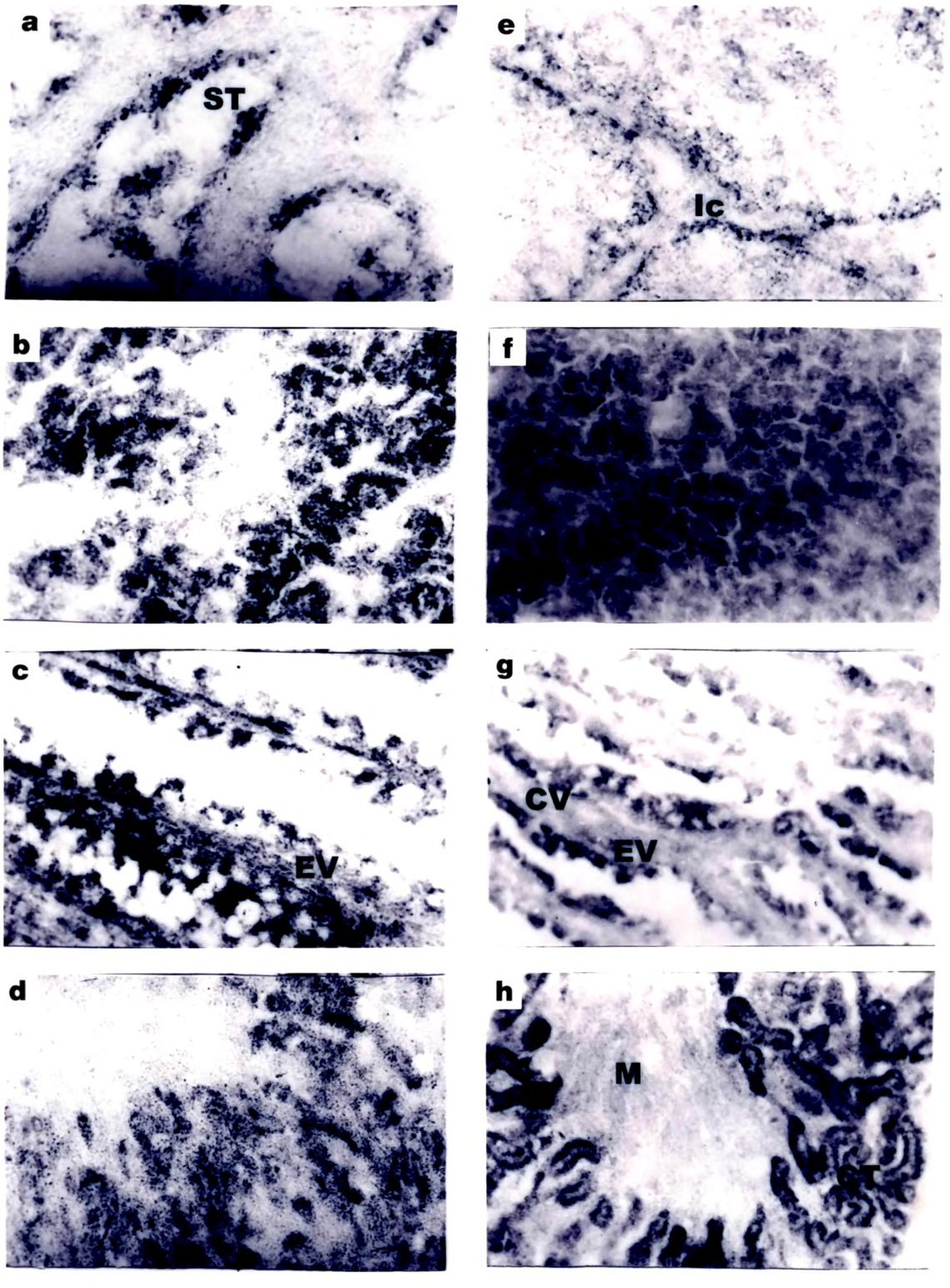


PLATE V

3β-Hydroxysteroid dehydrogenase activities in ovarian and extra-ovarian tissues of Bank Myna, *Acridotheres ginginianus*, and Brahminy Myna, *Sturnus pagodarum*.

**- Post-Breeding phase –**

| **Bank Myna ♀** | | **Brahminy Myna ♀** | |
|---|---|---|---|
| a. Ovary | (500X) | e. Ovary | (500X) |
| b. Liver | (800X) | f. Liver | (800X) |
| c. Intestine | (800X) | g. Intestine | (500X) |
| d. Kidney | (500X) | h. Kidney | (500X) |

Abbreviation :
S : Stroma; EV : Epithelium of villi ; NT : Nephric Tissue

# PLATE V

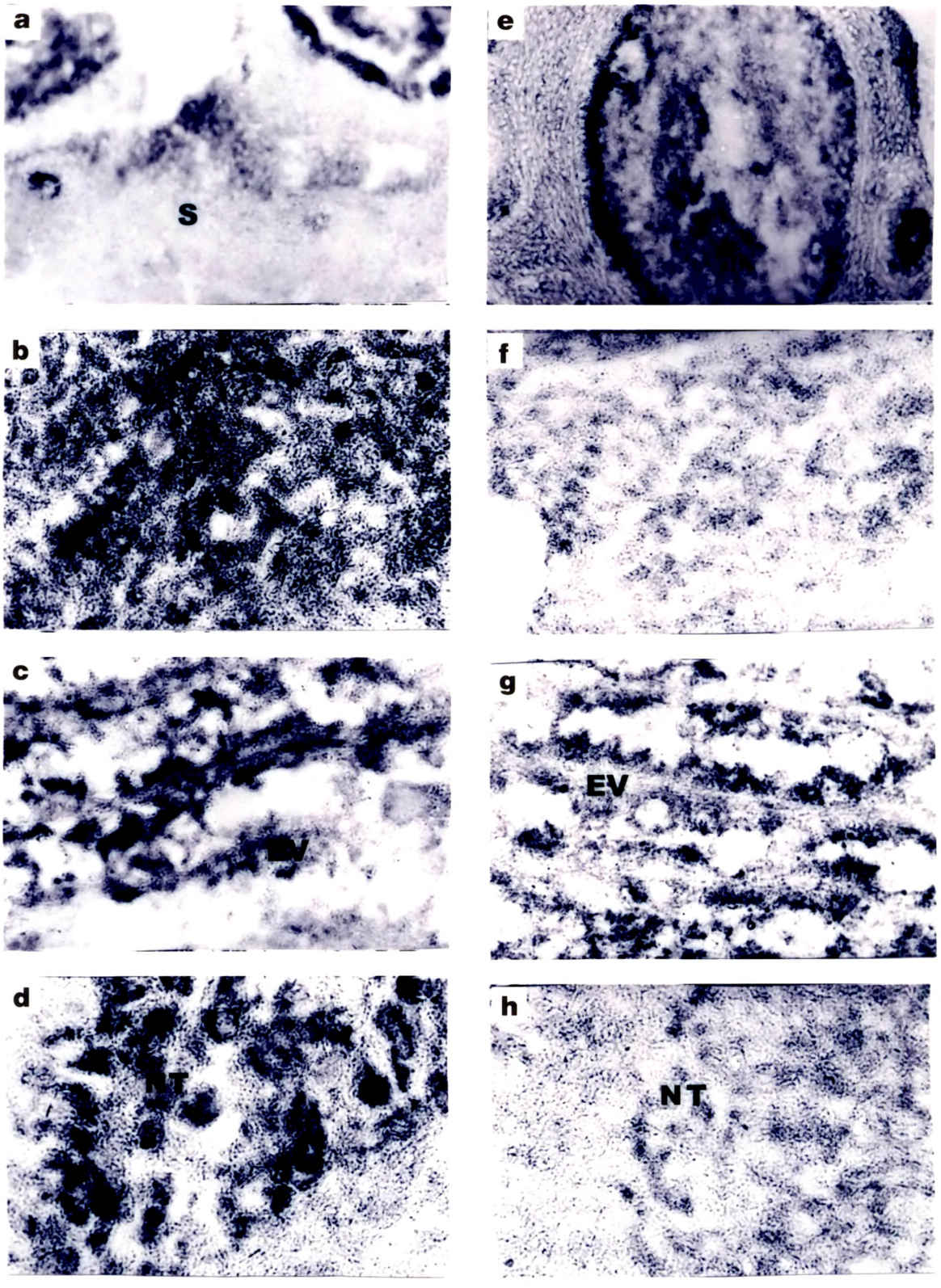


PLATE VI

3β-Hydroxysteroid dehydrogenase activities in testicular and extra-testicular tissues of Bank Myna, *Acridotheres ginginianus*, and Brahminy Myna, *Sturnus pagodarum*.

**- Post-Breeding phase –**

| **Bank Myna ♂** | | **Brahminy Myna ♂** | |
|---|---|---|---|
| a. Testis | (500X) | e. Testis | (500X) |
| b. Liver | (800X) | f. Liver | (800X) |
| c. Intestine | (800X) | g. Intestine | (500X) |
| d. Kidney | (500X) | h. Kidney | (500X) |

Abbreviation :
ST : Seminiferous Tubule ; Ic : Interstitial cells ; EV : Epithelium of villi ; CV : Corium of Villi ; NT : Nephric Tissue

# PLATE **VI**

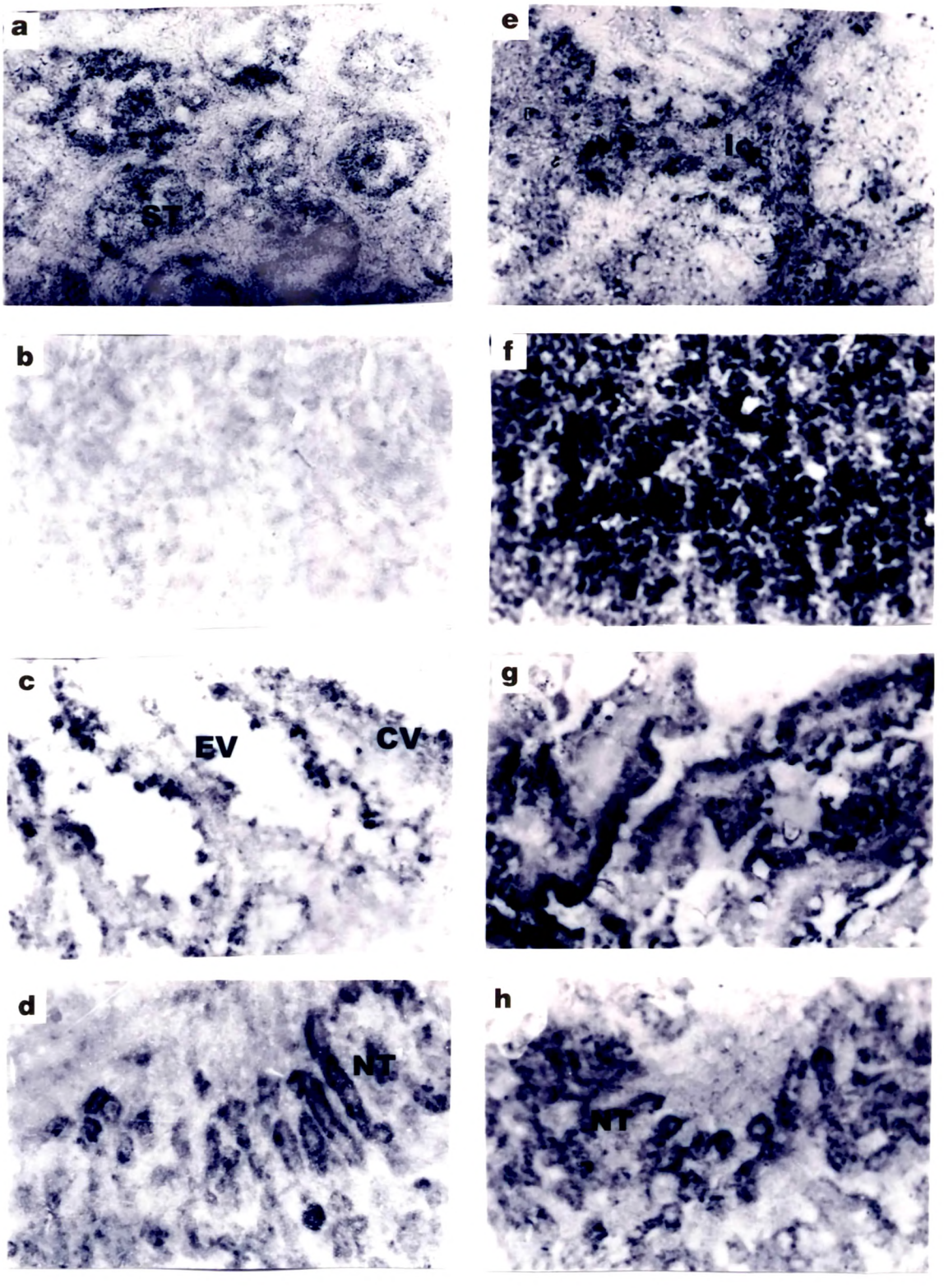


PLATE VII

3β-Hydroxysteroid dehydrogenase activities in ovarian and extra-ovarian tissues of Bank Myna, *Acridotheres ginginianus*, and Brahminy Myna, *Sturnus pagodarum*.

**- Non-Breeding phase –**

| **Bank Myna ♀** | | **Brahminy Myna ♀** | |
|---|---|---|---|
| a. Ovary | (500X) | e. Ovary | (500X) |
| b. Liver | (800X) | f. Liver | (800X) |
| c. Intestine | (800X) | g. Intestine | (500X) |
| d. Kidney | (500X) | h. Kidney | (500X) |

Abbreviation :
G : Granulosa ; S : Stroma ; EV : Epithelium of villi ; CV : Corium of Villi ; NT : Nephric Tissue

# PLATE **VII**

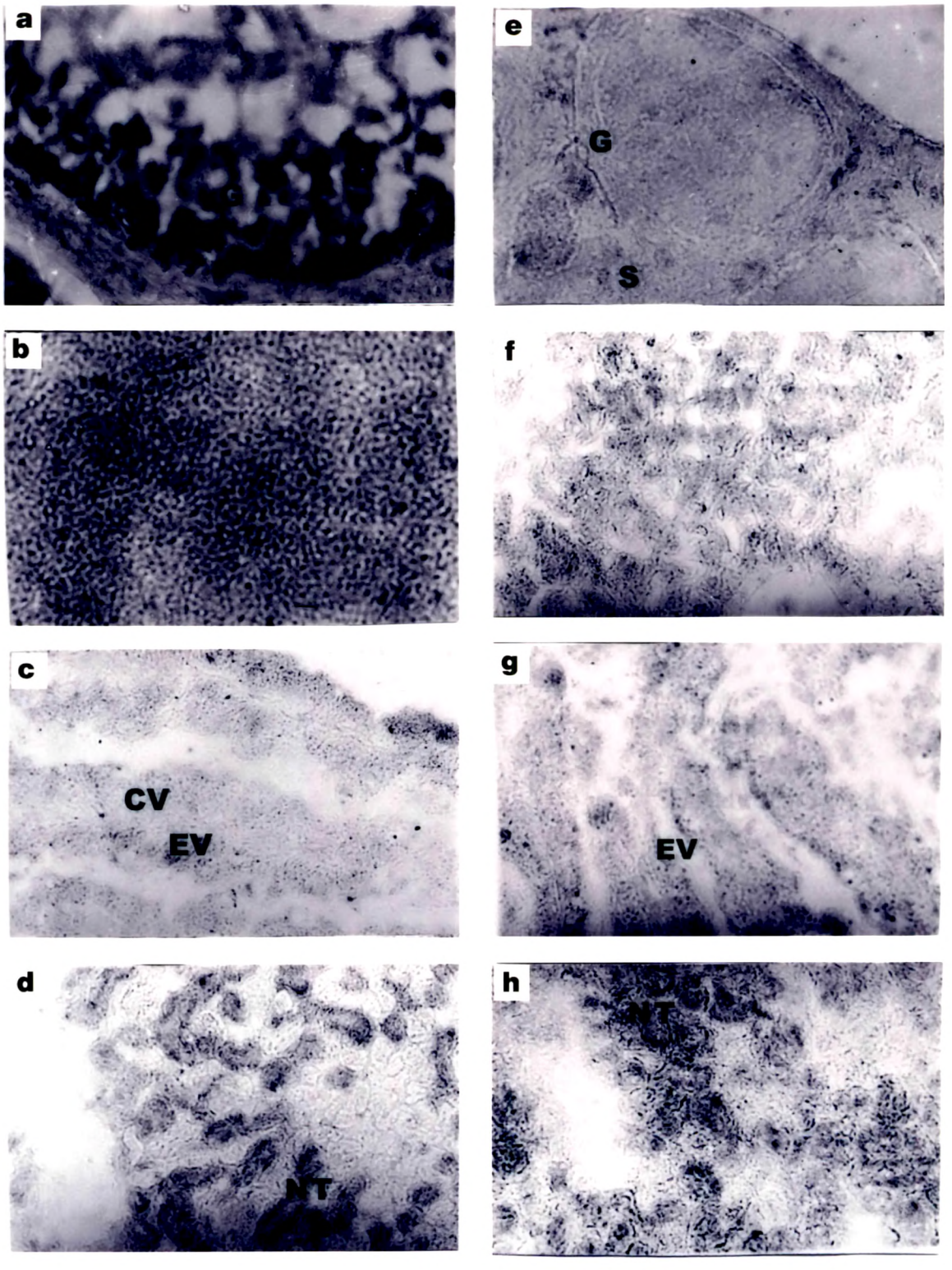


PLATE VIII

3β-Hydroxysteroid dehydrogenase activities in testicular and extra-testicular tissues of Bank Myna, *Acridotheres ginginianus*, and Brahminy Myna, *Sturnus pagodarum*.

**- Non-Breeding phase –**

| **Bank Myna ♂** | | **Brahminy Myna ♂** | |
|---|---|---|---|
| a. Testis | (500X) | e. Testis | (500X) |
| b. Liver | (800X) | f. Liver | (800X) |
| c. Intestine | (800X) | g. Intestine | (500X) |
| d. Kidney | (500X) | h. Kidney | (500X) |

Abbreviation :
ST : Seminiferous Tubule ; EV : Epithelium of villi ; IG : Intestinal Glands ; NT : Nephric Tissue ; M : Medulla

# PLATE **VIII**