CHAPTER - VIII

SEASONAL HISTOCHEMICAL ALTERATIONS IN LIPIDS AND STEROID DEHYDROGENASES IN THE GONADS OF NORMAL AND PINEALECTOMISED DOMESTIC PIGEONS, *COLUMBA LIVIA*

Majority of birds breed once or at the most twice in a year and like other seasonal breeders show periodical gonadal recrudescence prior to breeding and regression post-breeding. Such cyclic changes are marked by significant histophysiological alterations in terms of gametogenesis and steroidogenesis, processes associated with breeding. Amongst birds, histophysiological changes affecting gonads have been recorded mainly in males (Parton and Parton, 1922; Marshall, 1949; Threadgold, 1956; Johnston, 1956; Johnson, 1961; Scott and Middleton, 1968; Siverin, 1973, 1975; Gorman, 1974; Lewis, 1975a,b; Smith *et al.*, 1976; Ambadkar and Chauhan, 1976; Kotak, 1979; Mori and George, 1978). Comparatively fewer studies have been carried out on female gonads (Marshall, and Coombs, 1957; Chalana and Guraya, 1974, 1977, 1978; Guraya, 1976a,b,c; Ambadkar and Chauhan,1977; Kotak, 1979). The seasonal histological alterations are accompanied by many physiological and biochemical changes too, of which changes in lipids and steroid dehydrogenases are well recognised. Apart from the metabolic significance, lipids especially cholesterol positive lipids form the raw material for steroidogenesis . These together with the steroid dehydrogenases

(enzymes of steroid metabolism) are histochemically demonstrable and usually give a clue to the site and degree of steroidogenesis occuring within the gonads. Both the lipids and steroid dehydrogenases are usually known to show inverse relationship between them with higher content of lipids and lower activity levels of the dehydrogenases being characteristic of steroidogenically inactive gonads and the reverse set of changes being characteristic of active gonads. In this light, histochemical profile of the alterations in lipids and steroid dehydrogenases can be made use of to understand the seasonal gonadal status of seasonally breeding animals. It would also be interesting to know whether factors which affect or interfere with seasonal gonadal cyclicity would also affect the lipids and steroid dehydrogenases. Since pineal is known to influence gonadal functioning either favourably or unfavourably by acting at the hypothalamo-hypophysio-gonadal axis, it was thought pertinent to study histochemically the effect of pinealectomy on gonadal lipids and steroid dehydrogenases during the breeding and post-breeding phases in keeping with the realised pro and antigonadal roles of pineal in the domestic pigeons, Columba livia.

MATERIAL AND METHODS

Adult domestic pigeons obtained from the local animal dealer and acclimated to the laboratory conditions were used. Pinealectomy was performed during both the seasons and these

birds were sacrificed after 30, 45 and 60 days post pinealectomy along with the corresponding intact and sham operated controls. Gonads were taken out, blotted free of the tissue fluids and were then fixed on chucks of a cryostat microtome maintained at-20°C. The frozen gonads were sectioned at 10-15μ thickness. The sections were taken on a cover glass and the histochemical demonstration of Δ^5, 3β-hydroxy steroid dehydrogenase (Δ^5, 3β HSDH) was done according to the method of Wattenberg (1958) with NAD as cofactor and pregnenolone and dehydroepiandrosterone (DHEA) as the substrates. 17β hydroxy steroid dehydrogenase (17β HSDH) was demonstrated as per the method of Kellog and Glenner (1966) employing testostrone and estradiol 17β as the substrates and NAD as the cofactor. 3α-hydroxysteroid dehydrogenase (3α HSDH) was demonstrated by the method of Balough (1966) using androsterone as a substrate and NAD as a cofactor. Fettrot 7B and Sudan black B were utilised in demonstrating neutral and total lipids respectively (Pearse, 1968). Control sections for the enzymes were incubated in media devoid of the substrates.

OBSERVATIONS

Both lipids and steroid dehydrogenases could be found localised in the Leydig cells as well as the seminiferous tubules of testis and in the theca interna and granulosa of preovulatory and postovulatory follicles and atretic follicles

and even the interstitial cells of the ovary. The localisation of lipids and enzymes was noted to undergo changes on a seasonal basis in both intact and pinealectomised birds and such changes are depicted in table-1 and figures 1-28.

SEASONAL CHANGES IN NORMAL BIRDS

Both total and neutral lipids were intensely localised in the Leydig cells and seminiferous tubules during the post breeding phase. Concurrently the activities of $\Delta 5$ 3β HSDH and 17β HSDH were very much reduced. However, the activity of 3α HSDH was slightly higher than that of 3β HSDH and 17β HSDH with estradiol 17β as the substrate. The activities of all 3 enzymes were very much pronounced during the breeding phase. The activity of 3β HSDH was stronger with DHEA as the substrate. Similarly the activity of 17β HSDH with testosterone as the substrate was higher in the testis than with estradiol as the substrate. Localization of lipids was descernible in the theca interna, granulosa layers of the follicles and also in the interstitial cells and was stronger in the non-breeding season than the breeding season. Similarly the activities of the 3 enzymes too showed changes inverse to that of lipids with the result the concentrations of the enzymes were higher during breeding and reduced during non-breeding.

TABLE-1: Relative activities of various Steroid Dehydrogenases in Gonads during breeding and non-breeding periods of normal and pinealectomised domestic Pigeons Columba livia.

Enzymes	BREEDING PERIOD								NON-BREEDING PERIOD							
	Testis		Ovary						Testis		Ovary					
	LC	ST	TI	G	IT	DF	MF	AF	LC	ST	TI	G	IT	DF	MF	AF
$\Delta^5$3β HSDH (DHEA)																
Normal	++++	+++	+++	++	+++	++	+++	-	++	+	++	+	+	+	+	++
PX	+++	+	+++	+	++	++	+	++	+++	++	+++	++±	++	++	++	+
$\Delta^5$3β HSDH (Pregnenolone)																
Normal	+++	++	++++	+++	+++	+++	+++	-	++	++	+	+	±	+	±	+++
PX	++++	+++	++	++	++	+	+	+++	++	+	+++	++	++	+++	+++	+
17β HSDH (Testosterone)																
Normal	++++	+++±	+++	++	++	++	++	-	++	+	++	++	++	+	+	+++
PX	++	+	+	+	+	+	+	++	+++	++	+++	++	++	++	++	+
17β HSDH (Estradiol)																
Normal	+++	+++	+++	++	++	++	++	-	+	+	+	+	+	+	+	+++
PX	++	++	++	+	++	+	+	+++	+++	++	+++	++	++	++	++	+
3α HSDH (Androsterone)																
Normal	+++	++	+++	++	+++	++	++	-	++	+	++	+	++	+	+	+++
PX	++	+	++	++	+	+	+	+++	+++	++	+++	++	+++	++	++	+

AF - Atretic follicle; DF - Developing follicle; G - Granulosa; IT - Interestitium; LC - Leydig cells; PX - Pinealectomised ; ST - Seminiferous tubules; TI - Theca interna; MF - Mature follicle.

EXPLANATIONS TO FIGURES

Δ^5 3β HSDH activity with PREGNENOLONE as substrate

Figs.1a and b : Testis of normal birds during early and late breeding phases. 120 X

Note the reduced tubular size and decreasing enzyme activity in 1b.

Figs.2a and b: Testis of PX birds during early and late breeding phases. 120 X

Note the regressed tubules in 2a and enlarged tubules with increasing enzyme activity in 2b.

Figs.1c and 2c: Testis of normal and PX birds during the non-breeding phase. 120 X

Note the enlarged tubules with increasing enzyme activity in 2c.

EXPLANATIONS TO FIGURES

Δ^5 3β HSDH activity with DHEA as substrate

Figs.3a and b : Testis of normal birds during early and late breeding phases. 120 X

Figs.4a and b : Testis of PX birds during early and late breeding phases. 120 X

Note the decreased tubular size in 4a and enlarged tubules in 4b.

EXPLANATIONS TO FIGURES

Δ^5 3β HSDH activity with PREGNENOLONE as substrate

Figs.5a and b : Ovary of normal birds during breeding and non-breeding seasons. 120 X

Figs.6a and b : Ovary of PX birds during breeding and non-breeding seasons. 120 X

G - Granulosa; IC - Interstitial cells ; TE - Theca externa;

TI - Theca interna.

EXPLANATIONS TO FIGURES

Δ^5 3β HSDH activity with DHEA as substrate

Figs.7a and b : Ovary of normal birds during breeding and non-breeding seasons. 120 X

Note the reduced enzyme activity in the theca interna and absence of activity in granulosa in 7b.

Figs.8a and b : Ovary of PX birds during breeding and non-breeding seasons. 120 X

Note the increased enzyme activity and active follicles in 8b.

AF - Atretic follicle; DAF - Developmentally arrested follicle;

DF - Developing follicle ; G - Granulosa;

Mtf - Maturing follicle ; TE - Theca externa

TI - Theca interna.

EXPLANATIONS TO FIGURES

Δ^5 3β HSDH activity with TESTOSTERONE as substrate

Figs.9a and b : Testis of normal birds during early and late breeding phases. 120 X

Note the intense enzyme activity in 9a and weak activity in 9b.

Figs.10a and b : Testis of PX birds during early and late breeding phases. 120 X

Note the shrunken tubules in 10a and enlarged tubules with increasing enzyme activity in 10b.

EXPLANATIONS TO FIGURES

17β HSDH activity with ESTRADIOL as substrate

Figs. 11a and b : Testis of normal birds during early and late breeding phases. 120 X

Note the cell degeneration in tubules and strong enzyme activity in 11b.

Figs. 12a and b : Testis of PX birds during early and late breeding phases. 120 X

Note the increased enzyme activity with tubular spermatogenic activity in 12b.

Figs. 11c and 12c : Testis of normal and PX birds during the non-breeding season.

Note the activation of tubules in 12c.

EXPLANATIONS TO FIGURES

17B HSDH activity with TESTOSTERONE as substrate

Figs. 13a and b : Ovary of normal birds during early and late breeding phases. 120 X

Note the many degenerating follicles in 13b and stronger enzyme activity in 13a.

Figs. 14a and b : Ovary of PX birds during early and late breeding phases. 120 X

Figs. 13c and 14c : Ovary of normal and PX birds during the non-breeding season. 120 X

AF - Atretic follicle; DAF - Developmentally arrested follicle ; DF - Developing follicle ; G - Granulosa ; TE - Theca externa ; TI - Theca interna.

EXPLANATIONS TO FIGURES

17β HSDH activity with ESTRADIOL as substrate

Figs. 15a and b : Ovary of normal birds during early and late breeding phases. 120 X

Note the atretic follicles in 15b.

Figs. 16a and b : Ovary of PX birds during early and late breeding phases. 120 X

Note the increased enzyme activity in 16b.

Figs. 15c and 16c : Ovary of normal and PX birds during the non-breeding season. 120 X

Note the intense enzyme activity in 16c.

AF - Atretic follicle; DF - Developing follicle; G - Granulosa ; TE - Theca externa; TI - Theca interna.

EXPLANATIONS TO FIGURES

3α HSDH activity with ANDROSTERONE as substrate

Figs. 17a and b : Testis of normal birds during early and late breeding phases. 120 X

Note the reduced tubular size in 17b.

Figs. 18a and b : Testis of PX birds during early and late breeding phases. 120 X

Note the tubules are enlarged in 18b with strong enzyme activity in the interstitial cells.

Figs. 17c and 18c : Testis of normal and PX birds during non-breeding season. 120 X

Note the increased enzyme activity in 18c.

EXPLANATIONS TO FIGURES

3αHSDH activity with ANDROSTERONE as substrate

Figs.19a and b : Ovary of normal birds during early and late breeding phases. 120 X

Figs.20a and b : Ovary of PX birds during early and late breeding phases. 120 X

Note the enhanced enzyme activity in 20 b.

AF - Atretic follicle; APF - Atretic primary follicle;

DAF - Developmentally arrested follicle ;

G - Granulosa; MF - Maturing follicle ; PF - Primary follicle ; TE - Theca externa; TI - Theca Interna.

EXPLANATIONS TO FIGURES

Figs. 21a and b : Fettrot 78 positive lipids in the testis of control birds during breeding and non-breeding phases. 120 X

Note the increased content of lipid in 21 b.

Figs. 22a and b : Neutral lipids in the testis of PX birds during breeding and non-breeding phases. 120 X

Note the increased content of lipid in 22 a.

IC - Interstitial cells ; T - Tubule

EXPLANATIONS TO FIGURES

Figs.23a and b : Neutral lipids in the ovary of control birds during breeding and non-breeding phases. 120 X

Note the increased lipid content in 23b.

Figs. 24a and b : Neutral lipids in the ovary of PX birds during breeding and non-breeding phases. 120 X

Note the reduce lipid content in 24b.

AF - Atretic follicle ; APF - Atretic primary follicle;

DF - Developing follicle ; G - Granulosa;

IC - Interstitial cells ; MF - Maturing follicle;

T - Theca

EXPLANATIONS TO FIGURES

Figs. 25a and b : Sudanophilic lipids in the testis of control birds during early breeding and late breeding phases. 120 X

Note the regressed tubules with the intense localisation of lipids in 25b.

Figs. 26a and b : Sudanophilic lipids in the testis of PX birds during early breeding and late breeding phases. 120 X

Note the regressed tubules with lipids in the tubule as well as interstitial cells in 26a and enlarged tubules with decreasing content of lipids in 26b.

Figs. 25c and 26c : Sudanophilic lipids in the testis of control and PX birds during the non-breeding season. 120 X

Note the regressed tubules and intense lipid localisation in 25c and enlarged tubules with little lipid in 26 c.

EXPLANATIONS TO FIGURES

Figs. 27a and b : Sudanophilic lipids in the ovary of control birds during early breeding and late breeding phases. 120 X.

Figs. 28a and b : Sudanophilic lipids in the ovary of PX birds during early breeding and late breeding phases. 120 X

Note the comparative loss of lipids in 28b.

Figs. 27c and 28c : Sudanophilic lipids in the ovary of control and PX birds during non-breeding phase. 120 X

AF - Atretic follicle; DAF - Developmentally arrested follicle ; DF - Developing follicle ; G - Granulosa ; MtF - Mature follicle ; T - Theca ; TE - Theca externa ; TI - Theca Interna .

CHANGES DUE TO PINEALECTOMY

Pinealectomy induced marked changes in the concentration and distribution of lipids and steroid dehydrogenases during both the seasons. Whereas the concentration of lipids in various components of testis and ovary increased in the breeding season, the levels of activity of the 3 enzymes were decreased. On the other hand pinealectomy in the non-breeding season brought about decreased lipid contents and increased enzyme activity. In general the pattern of changes post-pinealectomy during the breeding season was comparable with the picture of intact controls during the non-breeding season while the post-pinealectomy changes in non-breeding season were very much identical to those observed in intact controls during the breeding period.

DISCUSSION

Cyclic variations in the form of rise and fall of gonadal lipids contents and activities of enzymes of steroid metabolism provide an unequivocal evidence for steroidogenic status. Lofts (1968) has suggested that the sudden depletion of lipids and cholesterol prior to breeding in many species is indicative of utilization of precursor material for steroidogenesis. The present observation on domestic pigeons indicate accumulation of total and neutral lipids in the various

components of the gonads during the non-breeding phase. This phase is marked by spermatogenically inactive shrunken seminiferous tubules and reduced interstitial cell mass in the testis and many arrested follicles at various stages of development, numerous primary follicles and atretic follicles in the ovary. Hence the increased lipid contents in the gonads during this period is indicative of reduced utilization of steroid precursors and other lipids of metabolic importance resulting in their accumulation in the various components of the gonads. In the same vein, the observed reduced amounts of lipids in both the gonads during the breeding season is self suggestive. Presence of 3β HSDH which catalyses the conversion of $\Delta^{5},3\beta$ hydroxy steroid to Δ^{4}, ketosteroids (Samuels et al.,1951) is indicative of the production of progesterone and other androgenic steroids such as androsteindione. Presence of this enzyme in the Leydig cells primarily and in the tubules secondarily is strong evidence of steroidogenesis occuring in these parts during the breeding season. Such a functional significance based on the presence of this enzyme in the testis has been reported by many workers (Deane and Rubin, 1965; Botte, 1963; Arvy, 1962; Chieffi, 1964; Narbiatz and Kolodny, 1964; Tingari, 1973; Garnier et al., 1973; Gorman, 1974; Bhujle and Nadkarni, 1974; 1976; Ambadkar and Kotak, 1976; Purohit et al., 1977). Strong activity of the enzyme with both the substrates (Pregnenolone and DHEA) indicates the increased turnover of progesterone and androsteindione which could be then converted

to testosterone. In this connection the equally intense activity of 17β HSDH is very much related as it catalyses the interconversion between testosterone and andresteindione and dehydroepiandrosterone and androsteindiol. Similarly the active involvement of both these enzymes in the production of progesterone and estrogens in the ovary can also be envisaged. Whereas Cheiffi and Botte (1965), have demonstrated both 3β HSDH and 17β HSDH in the growing follicles of fowl ovaries, Botte (1963), Cheiffi and Botte (1965), Woods and Domm (1966), Narbiatz and De Robertis (1968), Boucek and Savard (1970), Sayler et al. (1970), Chalana and Guraya (1974, 1976a,b) and Ambadkar and Kotak (1977). have demonstrated 3β HSDH in the theca and granulosa of pre and post ovulatory follicles, atretic follicles and interstitial cells of the stroma suggesting all of them to be possible loci of steroidogenesis. Concomitantly elevated 3α HSDH activity by its involvement in the interconversions between Δ 4 ketosteroids (Androstenedione) and 3α hydroxy-steroids (Androsterone and aetiocholanolone) followed by their conjugation and ultimate excretion (Tomkins and Isselbacher, 1954) can aid in steriod metabolism during the breeding phase. Likewise 17β HSDH too by its role in interconversion between estradiol and estrone and androsteindione and testosterone can participate in steroid metabolism (Ballie et al.,1966).

Just as the seasonal changes in the control birds recorded in this chapter corroborate the findings on quantitative

analysis of lipids, the pinealectomy induced quantitative alterations in the content of lipids and steroid dehydrogenases also substantiate the quantitative findings on lipids in the gonads (Chapter-5). In keeping with the progonadal role of pineal, on its removal in the breeding season (which brought about gonadal regression; Chapter-1), histochemically demonstrable lipids increased and the activity of steroid dehydrogenases decreased; a condition identical to that obtained for the non-breeding season in the control birds. Again, pinealectomy in the non-breeding season led to decrease in lipid contents and increase in the activity levels of steroid dehydrogenases, a condition comparable to those characteristic of control birds in the breeding season. These alterations indicate that the pineal ablation does bring about profound changes in steroid metabolism of the gonads. The alteratioms seemingly reverse the normal cyclicity of gonadal functioning thus emphasising the possible role of pineal as an important modulator in the chain of events which bring about the expression of adaptive breeding activities. Apparently pineal influences the activity of steroid dehydrogenases in the gonads either directly or indirectly. In the wake of known antigonadotropic action of pineal, the increased activity of steroid dehydrogenases post-pinealectomy in the non-breeding season may be an indirect action by releasing the inhibitory action on gonadotropic hormones. The possible positive effect of gonadotropic hormones on the activity of steroid dehydrogenases can be inferred from the studies of Rubin and

Deane (1965) on the effect of gonadotropic hormones on the ovary of rats. In the same vein, the herein observed decreased steroid metabolism in the gonads of pinealectomised birds during the breeding season would indicate the loss of positive influence of pineal on secretion of gonadotropic hormones. The influence of pineal on gonadal steroid dehydrogenases can also be inferred from the reports of Sayler et al. (1970) on the influence of photoperiod on the localization of 3β HSDH in the ovaries of Japanese quail, wherein they have shown the stimulatory influence of long photoperiod on the enzyme activity associated with increased ovarian weight. It could be said that the pineal of domestic pigeons has both progonadal and antigonadal roles, and its action on gonadal physiology is either mediated through the secretion of gonadotropic hormones and/or by modification of the secretory functions of other endocrine glands.