CHAPTER III

SEASONAL HISTOCHEMICAL VARIATIONS OF LIPIDS AND STEROID DEHYDROGENASES IN THE OVARIES OF FERAL BLUE ROCK PIGEON COLUMBA LIVIA Gm.

That gonadal lipids show well defined histophysiological changes in accordance with their functional and nonfunctional states is by now a proven fact in case of fishes, amphibians, reptiles, birds and seasonally breeding mammals. Majority of histophysiological studies concerning variations in the gonads of birds pertain to males (Chapter II).

Lofts and Murton (1973) have rightly pointed out that, except for the Rook (Corvus frugilegus); in which case some detailed observations on ovarian histophysiology have been reported by Marshall and Coombs (1957), studies on ovaries of seasonally breeding birds throughout the year are almost non existent. Recently though, Chalana and Guraya (1974, 1977, 1978) and Guraya (1976a, b and c) have focussed attention on ovarian histophysiology of many seasonally breeding birds with emphasis on steroidogenesis. Bhujle and Nadkarni (1977) have demonstrated steroid dehydrogenase activity in the developing gonads of domestic pigeon. Seasonal ovarian histomorphology has been documented in case of Indian

house crow (Ambadkar and Chauhan, 1977). That the general pattern of follicular epithelium shows changes during oogenesis has been observed in some wild species of birds (Gorbik, 1977).

After Wattenberg's (1958) classical histochemical demonstration of Δ^5-3β-hydroxysteroid dehydrogenase activity in tissue sections, the technique is being widely employed to gain precise understanding about physiological stages of gonads with reference to sex steroid hormone biosynthesis. Inevitably, the technique together with those for other steroid dehydrogenases, is used extensively to acquire an added index to the conventional criteria (histochemical/histological) in assessing the functional and non-functional states of gonads.

The present study was undertaken to investigate the seasonal ovarian histochemistry with respect to lipids and steroid dehydrogenases and to acquire histochemical data in support of our quantitative work (Chapter I). These observations possibly may help pinpoint the breeding and non-breeding times more appropriately in a year. Attempts could also be helpful in the elucidation of probable correlation between localization of enzymes and their influence on the pathway of biosynthesis of female sex hormones. Observations on cyclic variations in patterns of localization of neutral and total lipids,

Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSDH) and 17β-hydroxysteroid dehydrogenase (17β-HSDH) in the ovarian tissue are reported in the present Chapter.

MATERIAL AND METHODS

Indian feral blue rock pigeons (Columba livia G.) were shot down around the University campus with a .22 air rifle between 8.00 a.m. and 10.00 a.m. in the years 1975 &'76. They were then immediately brought to the laboratory. After decapitation, gonads were taken out, blotted free of blood and tissue fluids and weighed. Part of the ovary was fixed on a cryostat chuck maintained at -20°C and the rest was fixed in Bouin's fluid for monthly histological study. Sections were cut in cryostat-microtome after 15 minutes at 18 μm thickness. They were then taken up on coverglasses and processed for histochemical demonstration of lipids and hydroxysteroid-dehydrogenases. Δ^5-3β-HSDH was demonstrated according to the method of Wattenberg (1958) using both dehydroepiandrosterone (DHEA) and pregnenolone (P) as substrates with NAD as cofactor. 17β-HSDH was demonstrated as per the method of Kellogg and Glenner (1960) using estradiol-17β as substrate and NAD as the cofactor. Neutral and Sudanophilic lipids were demonstrated using Fettrot 7 B and Sudan Black B respectively (Pearse, 1968).

RESULTS

Histologically, avian ovary does not differ much from the basic vertebrate pattern. It possesses an outer cortex (containing developing follicles) and a highly vascular medulla (ovarian stroma with oocytes and connective tissue); follicular atresia being a common feature (Lofts and Murton, 1973) (Chapter VII).

Localization of lipids and steroid dehydrogenases:

Lipids were localized in theca interna, granulosa and yolk (yellow and white) of pre-ovulatory and post-ovulatory follicles and atretic follicles. Interstitial cells too, showed clear staining. Both, Δ^5-3β-HSDH & 17β-HSDH were found to be localized in theca interna and granulosa cells of the pre- and post-ovulatory follicles and atretic follicles. Interstitial cells reacted positively.

Seasonal changes:

Lipids (neutral and sudanophilic lipids):-

In January and February, lipids were seen in maturing ova, in the theca interna as well in granulosa layer. Dispersed atretic follicles laden with lipids were observable even with naked eye as small yellow follicles in the stroma. Developing follicles were

charged with lipids. Overall localization of sudanophilia was predominant in any part of the ovary. On the whole, presence of lipoidal atretic follicles dispersed in the stroma and absence of mature follicles with yellow yolk characterized these months. During March to May, marked changes in the ovarian lipids were observed. Ova with yellow yolk were predominant. Ovulated follicles were seen in mid March, April and early May which were full of lipids with clear thecal and granulosa layer localization (Fig. 2). Clearcut distribution of lipids was observed in theca interna and granulosa cells of pre-ovulatory follicles (Fig. 1). Nevertheless, overall lipid localization barring the yellow yolk of mature ovum, in all parts of ovary decreased considerably.

In the months of June, July and early August, small follicles of almost uniform size were common. June and July once again revealed increasing number of atretic follicles (Fig. 11). Total weight of the ovary (Chapter VII) was at its minimum during these months. Primary follicles and interstitial cells were heavily charged with lipids (Figs. 9 & 10).

From late August onwards, there was a remarkable change; lipids from all sites noted in earlier months were observed to decrease with concomitant accumulation

EXPLANATION TO FIGURES

Photomicrographs of T.S. of pigeon ovaries during breeding season.

Fig.1. Lipids stained with Sudan Black B. Clear staining of theca interna and granulosa layers visible in developing follicle seen on left hand top corner.

Fig.2. An ovulated follicle loaded with lipids. Sudan Black B.

Fig.3. Strong Δ^5-3β-HSDH activity using DHEA as substrate.

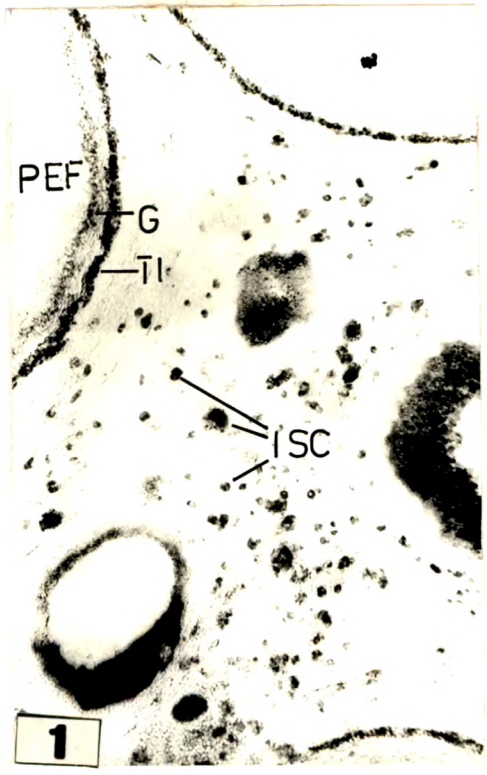
Fig.4. Same enzyme employing pregnenolone as substrate.

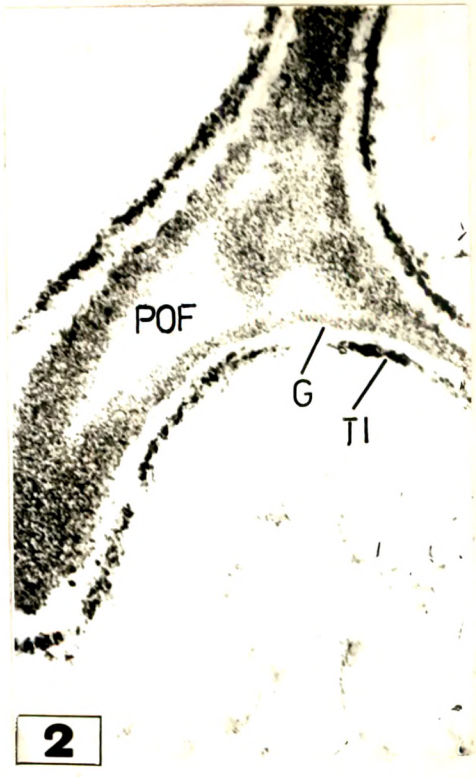
ABBREVIATIONS

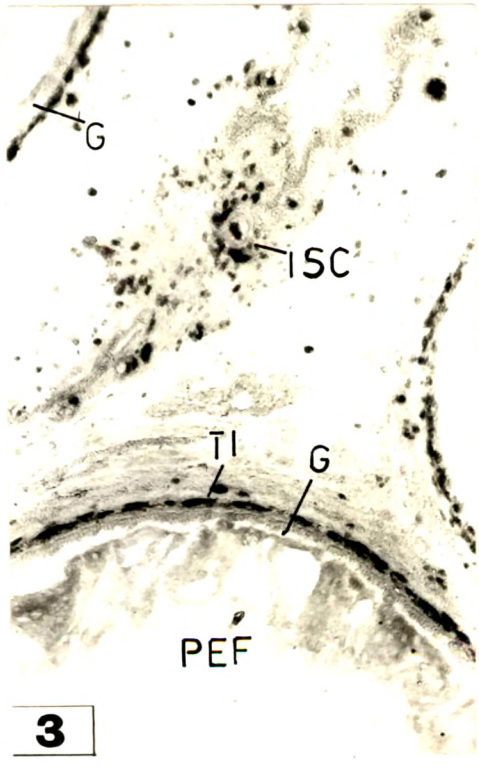
PEF - Pre-ovulatory follicle. POF - Post-ovulatory follicle

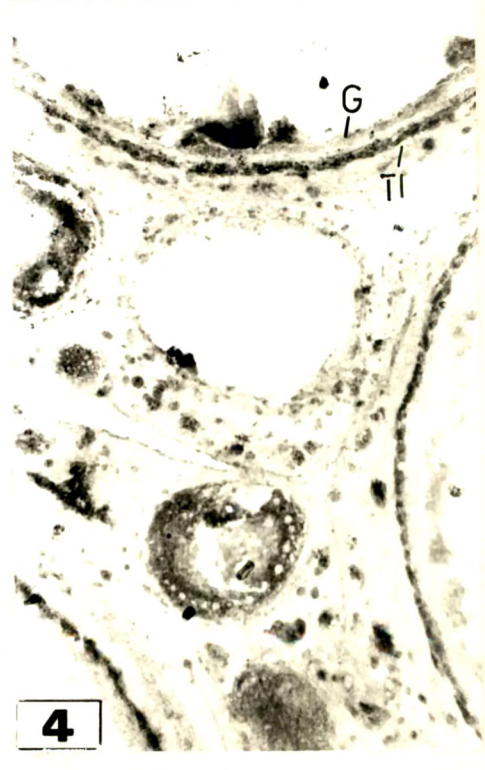
G - Granulosa cells. AF - Atretic follicle. Y - Yolk

ISC - Interstitial cells of stroma. TI - Theca interna


PEF
G
TI
ISC
1


POF
G
TI
2


G
ISC
TI
G
PEF
3


G
TI
4

EXPLANATION TO FIGURES

Photomicrographs of T.S. of pigeon ovaries during breeding season.

Fig.5. Note Δ^5-3β-HSDH activity in theca interna and granulosa of preovulatory follicle with pregnenolone as substrate.

Fig.6. Strong 17-β-HSDH (Estradiol-17β) activity. Again, theca interna and granulosa show clearcut reactivity.

Fig.7. Post-ovulatory follicle showing Δ^5-3β-HSDH (P) intensity.

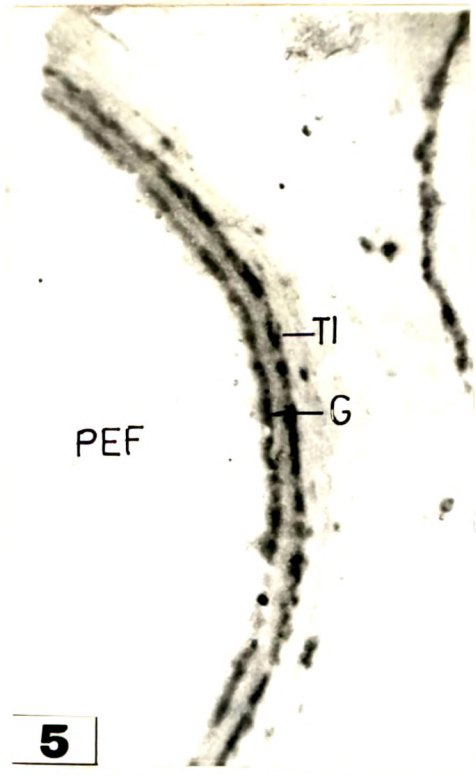
Fig.8. 17β-HSDH activity in an ovulated follicle.

ABBREVIATIONS

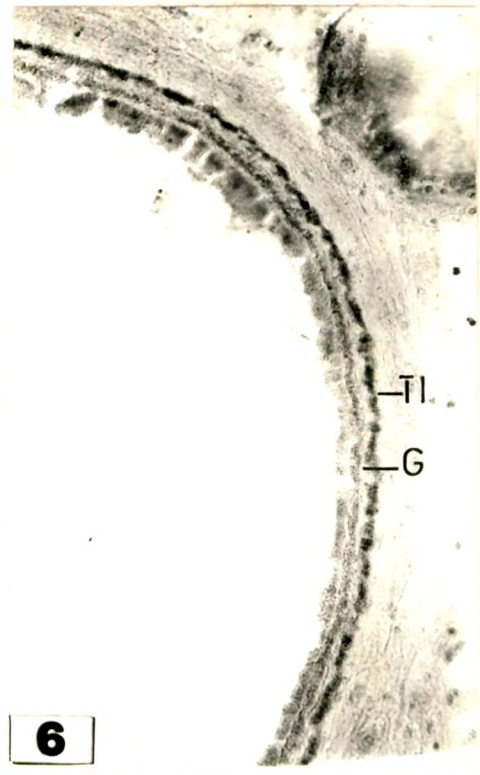
PEF - Pre-ovulatory follicle. POF - Post-ovulatory follicle

G - Granulosa cells. AF - Atretic follicle. Y - Yolk

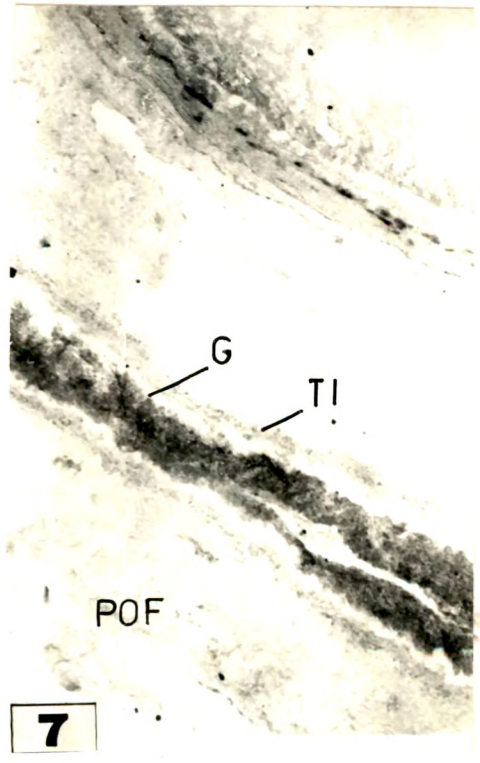
ISC - Interstitial cells of stroma. TI - Theca interna



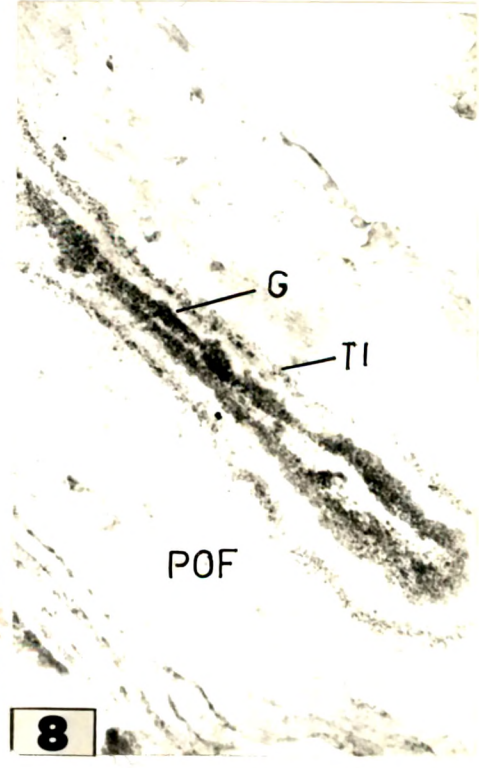
5



6



7



8

EXPLA NATION TO FIGURES

Photomicrographs of T.S. of pigeon ovaries during non-breeding season.

Fig.9. Neutral lipids stained with Fettrot 7 B.

Fig.10. Secondary follicles of regressed ovary charged with total lipids. Sudan Black B.

Fig.11. Heavily lipoidal atretic follicles stained with Sudan Black B.

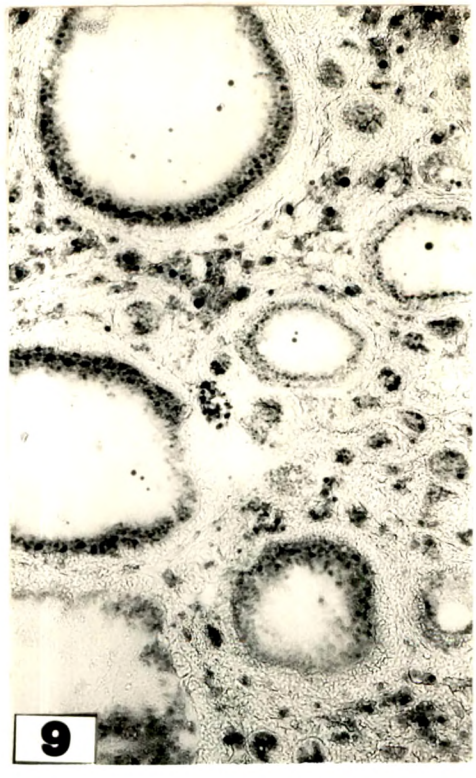
Fig.12. Feeble Δ^5-3β-HSDH activity using DHEA as substrate.

ABBREVIATIONS

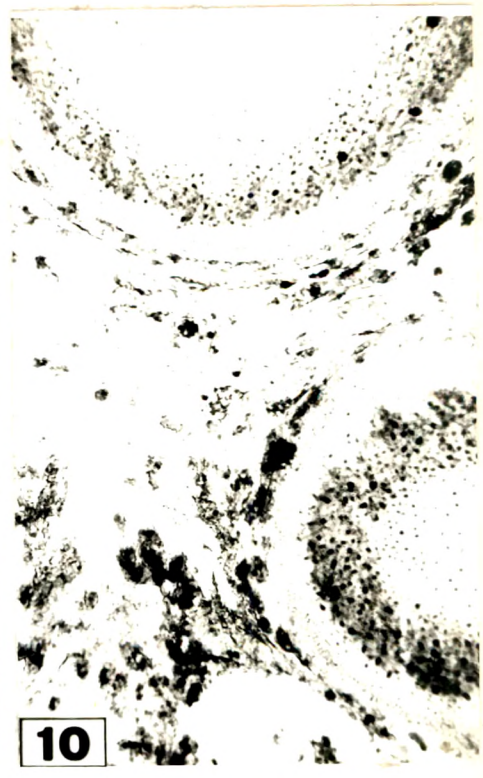
PEF - Pre-ovulatory follicle. POF - Post-ovulatory follicle

G - Granulosa cells. AF - Atretic follicle. Y - Yolk

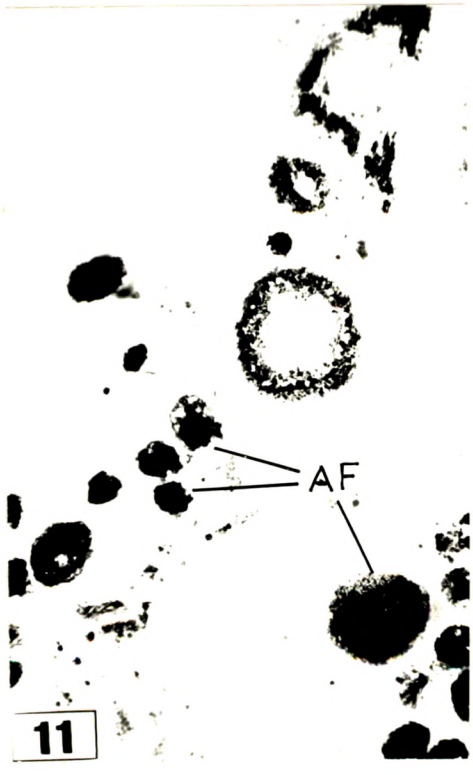
ISC - Interstitial cells of stroma. TI - Theca interna



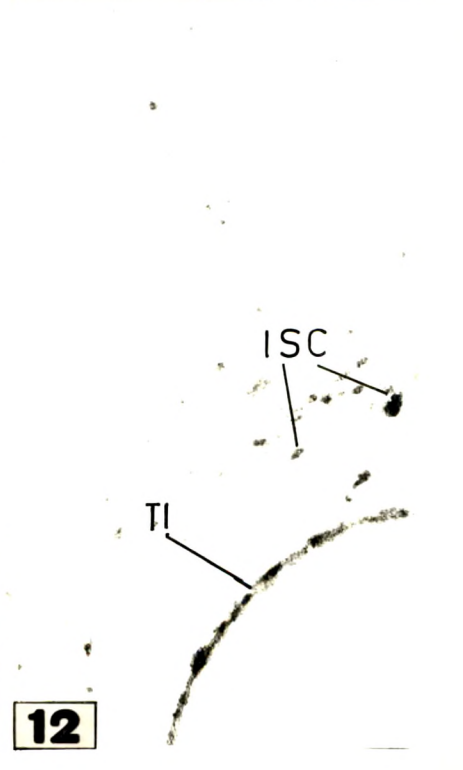
9



10



11



12

EXPLA NATION TO FIGURES

Photomicrographs of T.S. of pigeon ovaries during non-breeding season.

Fig.13. Weak intensity of the same enzyme (as compared to Figs. 4 and 5) employing pregnenolone as substrate.

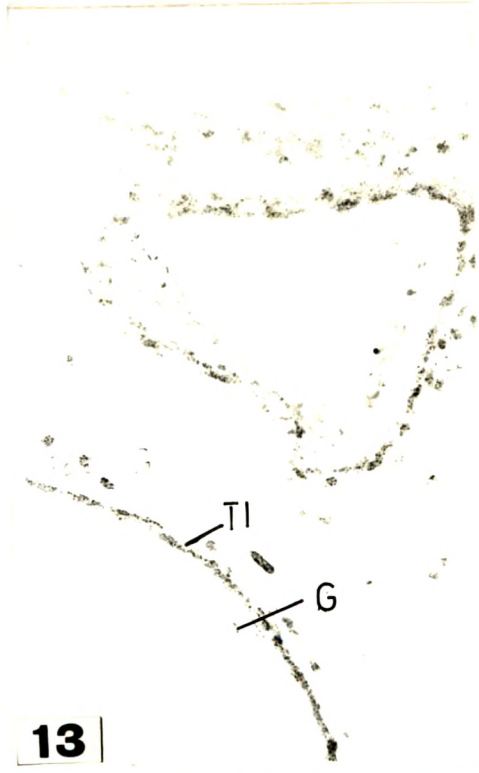
Fig.14. Poor intensity of 17β-HSDH. Compare it with Fig.6.

Fig.15. Various atretic follicles showing Δ^5-3β-HSDH (P) activity.

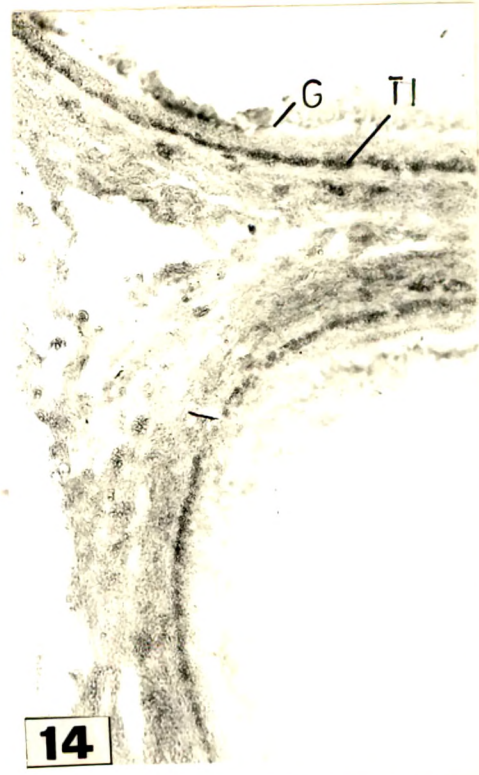
Fig.16. Note the activity of Δ^5-3β-HSDH (P) in a lipoidal atretic follicle; the intensity is stronger as compared to that of an ovulated follicle (Fig.7).

ABBREVIATIONS

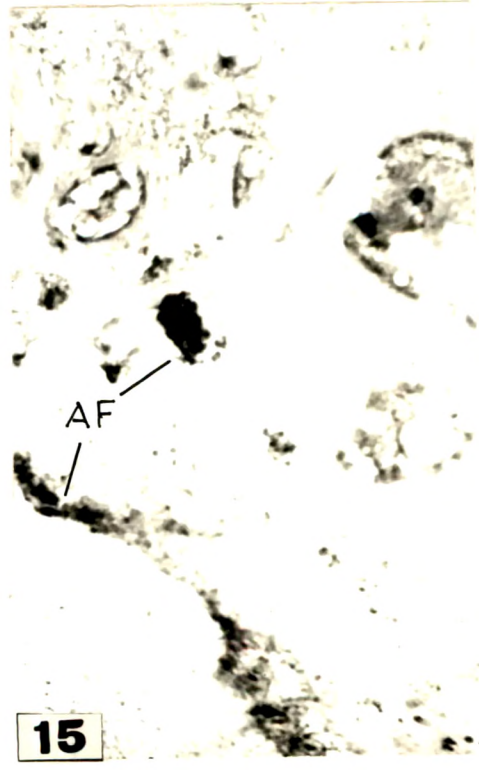
PEF - Pre-ovulatory follicle. POF - Post-ovulatory follicle
G - Granulosa cells. AF - Atretic follicle. Y - Yolk
ISC - Interstitial cells of stroma. TI - Theca interna



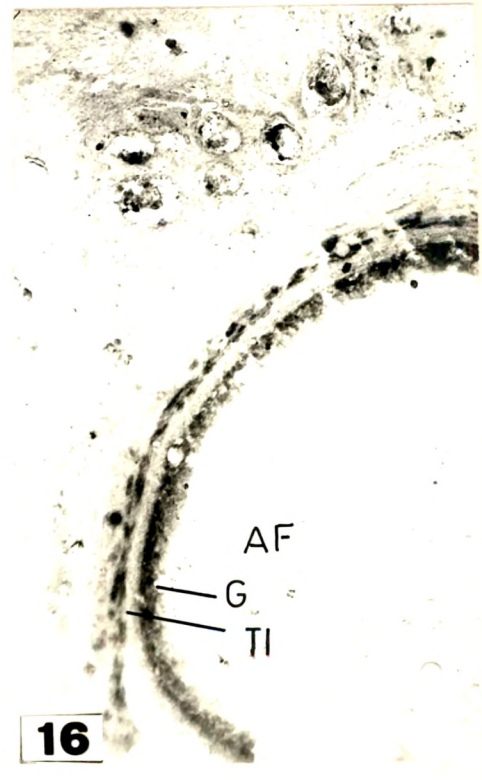
13



14



15



16

of yellow yolk in the pre-ovulatory follicles. During 1st half of the September, presence of yellow yolk-studded ova was apparent. Theca interna as well as the granulosa layer of maturing follicles showed presence of decreasing lipids, visualized though as two layers. In late September, post-ovulatory follicles were evident and they were filled with lipoidal material. These were lined with lipid positive thecal localization. October revealed further decrease in lipids of theca interna, granulosa, interstitial cells and ovulated follicles. Pattern of lipid distribution in the month of December was more or less similar to that observed during the periods March to May and late August to October.

Steroid dehydrogenases: (Δ^5-3β-HSDH and 17β-HSDH)

Intensity of both the enzymes was moderately weak in January and February; strikingly feeble in the months of June, July and early August and weak again to a certain extent in November. Poor activities were noticed in the theca interna and granulosa of follicles and in the interstitial cells (Figs. 12,13 & 14). Atretic follicles showed relatively stronger activity of Δ^5-3β-HSDH activity when pregnenolone was used as substrate (as compared to that with DHEA) and the intensity was much stronger (Figs. 15,16) than that noted in the post-ovulatory

follicles. (Fig. 7).

Contrary to this, March, April and May and late August, September and October and to a certain extent December exhibited altogether a different picture. Intensities of both the enzymes increased drastically. Intense activity of both the enzymes was most striking in theca interna and granulosa of maturing pre-ovulatory follicles (Figs. 3 to 6). When follicle was fully mature and studded with yellow yolk, localization in theca interna and granulosa was much less discernible. Δ^5-3β-HSDH activity in the post-ovulatory follicles was much stronger with pregnenolone as compared to that with DHEA as substrate. 17β-HSDH intensity in the ovulated follicles was relatively mild than Δ^5-3β-HSDH (Figs. 7 & 8). Activities of enzymes were moderately weak in November with an increase in the intensity in the month of December. Maximum intensities were seen during March-April and September-October.

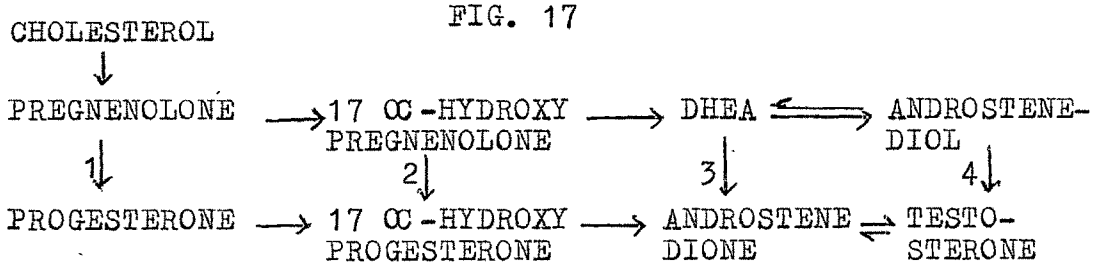
DISCUSSION

Biochemical and histophysiological studies with regard to Δ^5-3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase and other steroid dehydrogenases were conducted in the fowl substantiating

some useful facts of the steroid hormone metabolism in avian tissues in general. Both Δ^5-3β-HSDH and 17β-HSDH have been shown to be active in growing follicles of fowl ovaries (Chieffi and Botte, 1965) suggesting their role in the production of both progesterone and estrogens. Several workers have demonstrated Δ^5-3β-HSDH in theca, granulosa, pre- and post-ovulatory follicles, atretic follicles & interstitial cells of the stroma indicating that every one of the above sites could be a possible locus of endocrine function (Botte, 1963; Chieffi and Botte, 1965; Woods and Domm, 1966; Narbiatz and deRobertes, 1968; Boucek and Savard, 1970; Sayler et al., 1970 and Chalana and Guraya, 1974, 1976a, 1976b). In the fowl ovary, 3β-HSDH was found to be present in the microsomal fraction (Armstrong and Wells, 1976).

Extensive biochemical investigations have shown that the ovary has capacity to convert acetate to cholesterol and the latter to pregnenolone which could be converted into progesterone. The following reactions 1 to 4 are catalyzed by Δ^5-3β-HSDH in ovary (Baillie et al., 1966).

FIG. 17



In fowl, Chieffi (1967), Chieffi and Botte (1965, 1970) and Boucek and Savard (1970) have demonstrated 17β-HSDH activity in the granulosa cells, emphasizing its role in estrogen production. In mammals, 17β-HSDH is known to play an important role in the conversion between C-17 ketones and C-17 hydroxyl groups, occurring in both C_{17} and C_{18} steroids resulting in the formation of $C_{17\beta}$ carbonyl groups (Estrogens) (Davenport and Mallette, 1966).

Keeping in mind the above stated facts on Δ^5-3β-HSDH and 17β-HSDH, feeble intensity of these enzymes (particularly in the theca interna and granulosa) in the months of January, February, more prominantly in June, July, early August and to some extent November indicated that the ovaries of pigeon were not actively involved in sex steroid hormone synthesis. Presence of heavily lipoidal atretic follicles in some of these months could indicate that the ovary was perhaps getting ready for recrudescence but failure to do so resulted in many of the growing follicles undergoing atresia. That both the neutral as well the sudanophilic lipids were accumulated in every possible part of the ovary (Figs.9 & 10) was probably due to decreased output to sex hormones, the cholesterol positive lipids being no longer in demand. Ovarian cholesterol concentration was found to

be high in some of these months (Chapter I). All these factors appear to indicate non-breeding phases and the hormone output perhaps was at its minimum level.

In March, April and May and late August, September, October and to a certain extent in December, yellow yolk-laden ova were observed and quite a few birds shot showed presence of enclosed egg in some part of the hypertrophied oviduct. Two prominant layers (theca interna and granulosa) of lipids around the maturing follicles were strikingly obvious with decreasing localization of lipids, probably suggestive of their utilization. Cholesterol positive lipids were perhaps in great demand for hormone synthesis. This was corroborated by the fact that both Δ^5-3β-HSDH and 17β-HSDH were intensely localized in the theca interna and granulosa of maturing pre-ovulatory follicles (Figs. 3 to 6). Strong activity of Δ^5-3β-HSDH could mean that sex steroid hormone biosynthesis was progressing at an accelerated rate, probably via pathways as shown in Fig. 17. This would result in the formation of several steroid metabolites which could then be ultimately converted into estrogens. This was substantiated by the fact that activity of 17β-HSDH during these months was intense, notably so in the theca

interna and granulosa layer of maturing pre-ovulatory follicles. Unlike in the fowl in which, Chieffi (1967), Chieffi and Botte (1965, 1970) and Boucek and Savard (1970) attribute the production of estrogens on the basis of 17β-HSDH localization confined only to the granulosa cells; in the feral pigeon it is felt that estrogens may also be secreted by the theca interna, as active 17β-HSDH was observed in both these layers (Fig. 6). Botte et al. (1966) too, mentioned that isolated thecal tissue from growing follicle of hen's ovary converted cholesterol to estrone. Electron microscopic studies (Dahl, 1971) on the fine structure of fowl ovary indicated that the granulosa cells are primarily more of nurse cells for the growing oocyte than as steroid producing cells. It could be inferred from the above fact that the thecal cells after all, are more directly involved in estrogen production. In hen's ovary, estrogens are probably derived from estrone, which presumably is formed from androstenedione (Gilbert, 1971). Ainsworth et al. (1962) & Hertelendy et al.(1965) point out that one reduction product of estrone is estradiol-17β and this reduction is reversible (MacRae and Common, 1960; Hawkins and Taylor, 1967). Increased intensity of 17β-HSDH (estradiol-17β substate) in the months of March to May and late August to October and partly

December pointed at an enhanced conversion of androstenedione to testosterone and/or estrone which may be converted further to estradiol and estriol. These reactions can be surmised as:

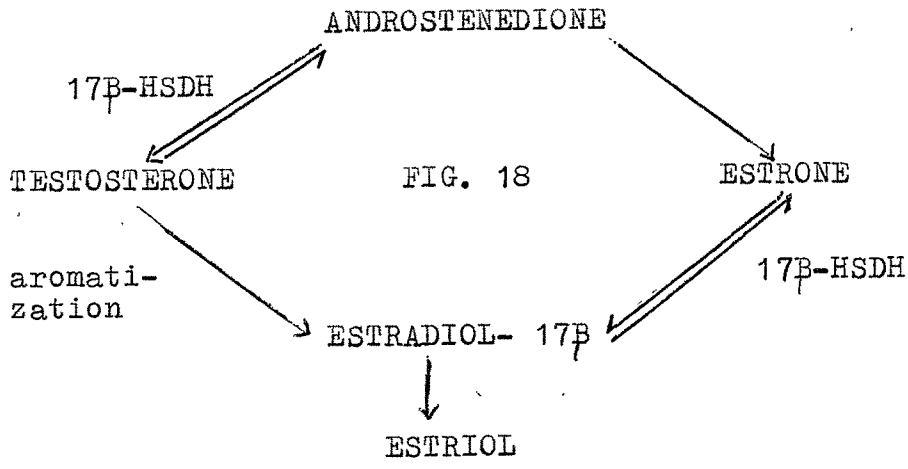


FIG. 18

As shown in the figure, the interconversion between (i) androstenedione and testosterone and (ii) estrone and estradiol-17β are reversible and are known to be catalyzed by the enzyme 17β-HSDH whereas the conversion of testosterone to estradiol-17β can be brought about by aromatization in the ovary (Layne et al., 1958; Hohn and Cheng, 1967; Ozon, 1965; Boucek and Savard, 1970). Decreased activity of the two enzymes studied in completely mature follicles full of yellow yolk suggested that such follicles were no longer active in steroid synthesis but were rather concerned with storage of yellow yolk.

Regarding the post-ovulatory follicles, Lofts and Murton (1973) have remarked that after extrusion of egg, the follicular wall collapses and gets spotted with lipoidal material; granulosa cells become inflated for about 72 hours after ovulation, but typical leutinization does not occur and hence no corpus leuteum develops. It has also been reported recently that there is no hypertrophy or proliferation of granulosa cells and no lutein cells comparable to the mammalian corpus luteum in case of post-ovulatory follicles of the domestic duck (Pal Dhananjoy, 1976). Armstrong et al. (1977) comment that 3β-HSDH levels in the post-ovulatory follicles of hen fall during first 15 hours after ovulation and then gradually decline further upto 50th hour. They have opined that the post-ovulatory follicles are the source of steroid hormones. In the present study, lipoidal ovulated follicles were encountered during the breeding months (Fig. 2) and relatively strong activity of Δ^5-3β-HSDH with pregnenolone as substrate as compared to that with DHEA in the post ovulatory follicles indicated its possible role in the secretion of progesterone. Botte (1963) reporting on the presence of 3β-ol-debydrogenase in the post ovulatory follicles of fowl ovaries has suggested that metabolism of steroids in the post-ovulatory follicles may go as far as progesterone.

One of the most notable studies on the role of the post-ovulatory follicles reports on the quantification of various steroid hormones in the same upto 52 hours after ovulation in case of hen (Dick et al., 1978). This study indicated a three fold decrease in progesterone levels in the case of post-ovulatory follicles within first 15-20 hours after ovulation whereas granulosa cells of the ante-ovulatory follicles (1 hour prior to ovulation) had 50 times more progesterone than granulosa fraction of the post-ovulatory follicle 2-3 hours after. In another study, it has been pointed out that removal of or damage to the granulosa cells of the post-ovulatory follicles leads to delayed oviposition (Gilbert et al., 1978). In the present study, relatively stronger activity of Δ^5-3β-HSDH (P) in the atretic follicles as compared to that seen in the post-ovulatory follicles (Figs. 7 and 16) suggested that atretic follicles, particulary on a long time basis, are much more likely the sites of synthesis and secretion of progesterone than the post-ovulatory follicles which are short lived.

From all the facts cited above, it seems that ovaries of feral pigeons, like testes (Chapter II) have two distinct active phases; one commencing in the month of March and extending upto May while the other starting from late August and extending upto October

with a transient fall in November which could be followed by a rather subdued functional stage in December. June-July appeared to be the chief non-functional stage. With respect to the sites of secretion of female sex hormones, it could be said that both the thecal cells and granulosa cells of the pre-ovulatory follicles seem to be actively involved in the secretion of estrogens. Progesterone appears to be liberated by the post-ovulatory follicles and the atretic follicles, perhaps more so by the latter.