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HISTOCHEMICAL STUDIES OF ENZYMES INVOVED IN HORMONAL REGULATION IN FISH (*Cyprinus carpio*)

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Abstract: **In situ changes in the enzymes i.e.Δ^5 3β- Hydroxysteroid dehydrogenase, Peroxidase, Cytochrome oxidase, Acid and Alkaline phosphatases and lipids in the Interrenal Gland and Ovary at different stages of reproductive cycle in fish, Cyprinus carpio had been studied. Peroxidase appears to be involved in the biosynthetic machinery controlling corticosteroidogenesis. Peroxidase and Cytochrome oxidase would also seem to transform adrenocortical cells and hypertrophied theca interna into highly oxidative compartments of the adrenal and ovary which attributes to the oxidation of pregnenolone to progesterone and corticosteroids towards maturation and ovulation of the oocyte from the ovary.**

Keywords: **Biosynthetic machinery, Enzymes studies, Adrenal-Ovary Interrelation, Pregnenolone to progesterone, Corticosteroids.**

I. INTRODUCTION

Reproduction is a vital activity if species are to Survive and multiply. In mammals it is controlled by the action of various endocrine glands, which through production of specific hormones, communicate with various parts of the body and regulate one or more vital metabolic processes within the animal. The regulation of the events in the estrous cycle and pregnancy is the result of a complex interaction between female sex hormones and the pituitary gonadotrophins and adrenocorticotrophic hormone. The various in vivo and in vitro studies have demonstrated that the ovary and adrenal possess the side-chain cleaving system to convert C_{27} cholesterol to pregnenolone which are mainly a C_{22}-C_{20} lyase and hydroxylases (Simmer, 1968). Other Steroidogenic enzymes viz. sulfatase, steroid Δ^5 -isomerase, 3B-OH-steroid dehydrogenase and 20 -Hydroxysteroid dehydrogenase have also been reported (Beyer et al., 1956; Burstein et al., 1963 and Weist et al., 1963) which are involved in the biosynthesis of progesterone and androgens.

The sex hormones produced by the adrenal cortex of both males and females are progesterone , testosterone and estrogens. The adrenal gland is the source of sex hormones until the testis and ovaries mature at puberty. The secretion of these hormones is controlled by ACTH and not by gonadotrophins which stimulate the testes and the ovaries. the functions of the adrenal hormones are:

1. To influence the development and maintenance of the secondary sex characteristics in both male and female.

2. To increase the deposition of protein in muscles and reduce the excretion of nitrogen especially in the male.

3. To induce heat in intact female rats and participate in the induction of ovulation at puberty (Zeilmaker, 1966).

Since ,adrenals are known to secrete large quantities of progesterone, which is an oxidation product Of pregnenolone, it appears probable that conversion of pregnenolone to progesterone may be brought about peroxidatively by the operation of peroxidase as suggested in the ovary (Agrawal and Laloraya, 1977). The role of peroxidase in the endocrine regulation of hormone action in the adrenals which is closely interlinked in reproductive functioning of different groups of animals remains largely unknown. There appear important gaps in the understanding of the hormone regulation and the enzymic mechanism which lead to the rapid formation and secretion of hormones namely progesterone and corticosteroids in the adrenal gland , is largely unknown.

In teleost fish, the anatomic association is more intimate, and the adrenal tissues are embedded in renal structures. Therefore, the adrenal – renal relationship is not only of morphological significance but it is also of physiological significance. There seems to be a functional relationship, direct or indirect between corpuscles and steroidogenic tissues.

A relationship between adrenal steroidogenesis and reproduction has been demonstrated for several species (Christian, 1963; (Christian, 1963; Ramaley, 1973). Progesterone is known to be a precursor of several steroid hormones including androgens, estrogens corticoids (Fig. 1).Samuels and uchikawa (1967) in in Vitro studies have shown that it occupies a key position in the biosynthesis of adrenal corticoids. The physiological importance of adrenal progesterone in the rodent or any other species is not yet known.

The presence of Δ^{5}-3β- Hydroxysteroid dehydrogenase, 11β-Hydroxysteroid dehydrogenase, 17 β – Hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase was demonstrated histochemically in the adrenal glands of some birds. All these enzymes occurred in the interregnal cells and no activity was observed in the chromaffin cells. The interrenal cells are probably capable of synthesizing both corticosteroids and sex steroids (Bhujle et al., 1976).

The enzyme activities like Δ^{5}-3β-hydroxysteroid dehydrogenase, 21- hydroxylase(21-OHase) and 11β-hydroxylase (11β-OHase) in the adrenals of mammals have been correlated with the synthesis of progesterone and corticosteroids in the cells of zona fasciculata and zona reticularis (Rubin et al.,1957; Chester Jones, 1957 and Rubin et al., 1963). ACTH is shown to promote Cholesterol ester hydrolysis by activating cholesterol esterase (Tyslowitz, 1943; Long 1947) thereby stimulating the synthesis of pregnenolone, the intermediate precursor of progesterone. How this pregnenolone is rapidly converted to progesterone in adrenals remains to be known.

In biological systems a group of enzymes known as the peroxidases are known to bring about rapid oxidations of organic molecules using H_2O_2. The importance of this reaction in regulating steroidogenesis has received active attention (Agrawal & Laloraya, 1977, 1980; Agrawal & Harper ,1982; Lyttle & Jellinck, 1976 and Deme et al., 1978) .

LH is shown to promote cholesterol ester hydrolysis by activating cholesterol esterase (Behrman and Amstrong , 1979) , this stimulates net progesterone secretion by increasing Conversion of cholesterol to pregnenolone . Also it stimulates the synthesis of the enzyme, Δ^{5}-3β—hydroxysteroid dehydrogenase which plays a key role in the early biosynthetic pathways of all the biologically active steroid hormones.

The objective of the present investigation therefore was:

1. To study in situ changes in various enzyme activities viz. Δ^{5}-3β-HSDH, peroxidase, acid and alkaline phosphatases and Cytochrome oxidase in Fish (Cyprinus carpio) so as to work out the biochemical mechanism controlling steroid biogenesis.

II. MATERIAL & METHODS

Fishes (Cyprinus carpio), during various reproductive cycles were collected from the Belavali tank, Department of Fisheries, Indore, during the breeding season i.e. February. They were collected at preovulatory phase as well as soon after the ovulation which occurs generally in the morning between 2-5 a.m. dissected and subjected for histochemical studies.

Histochemical Procedure: Gelatin fixed frozen sections (4µ) of the interregnal tissue were cut in the American Optical Cryocut and were then used for the localization of various enzymes.

***1. Peroxidase*:** This enzyme was localized by the modified method of Van Duija (1951) using benzidinas a donor. Another method Graham & Karnovsky (1966) using diaminobenzidine as a donor was also applied. The activity was also tested with the other donor namely Guaiacol.

2. Cytochrome Oxidase: The method followed for the localization of Cutochrome oxidase was that of Burstone, (1959).

3. Δ^5***3β- Hydroxysteroid dehydrogenase:*** This enzyme was localized by the method of Wattenberg (1958).

4. Acid Phosphatase: This enzyme was localized after the method of Gomori,(1950).

5. Alkaline Phosphatase :The Calcium-Cobalt method for Alkaline phosphatase , Gomori, (1952) was followed .

6. Lipids: Lipids were stained in frozen sections (4µ) by Herxheimer's fat stain method (1903).

III. RESULTS

Interrenal Gland

The interrenal cells in teleost Study of histochemical changes in Δ^5 -3β- hydroxysteroid dehydrogenase , peroxidase, Cytochrome oxidase, Acid and Alkaline phosphatases and Lipids in interrenal and ovary at different stages of reproductive cycle in fish ,Cyprinus carpio are based on semi-quantitative analysis which indicates the nature and fluctuatuion in the metabolic reserves at specific sites . The observations reported in the present study are based on the intensity of staining reactions and are at the best semi- quantitative in nature.

Δ^5-3β-hydroxy steroid dehydrogenase

Δ^5 – 3 β- hydroxyl steroid dehydrogenase activity occurs in the interrenal organ in teleost fish, Cyprinus carpio. The enzyme activity is stronger in the cortical cells at the ovulatory phase (Plate 1B) than in the cells of the post-ovulatory female (Plate 1C) . Weak histochemically detectable activity of Δ^5-3β- hydroxysteroid dehydrogenase is seen at the follicular stage (Plate 1A).

Cytochrome oxidase

A positive Cytochrome oxidase activity is seen in the interregnal tissue. The enzymic activity varies with the sexual cycle being detectable at the follicular stage (Plate 2A). Cortical cells are rich in this enzyme than the chromaffin tissue. A high activity of this enzyme is seen in the adrenocortical tissue at the ovulatory stage (Plate 2B) which does not vary after ovulation (Plate 2C).

Peroxidase

The enzyme activity occurs in the interregnal tissue at the ovulatory stage (Plate 3B). The adrenocortical cells show a strong peroxidase activity with no detectable activity in the chromaffin tissue .These cells lack histochemically detectable Peroxidase activity at the follicular phase (Plate 3A) with diffused localization at the postovulatory stage of the cycle (Plate 3C) .

Acid and alkaline phosphatase activity

The adrenocortical tissue of Cyprinus carpio shows an alkaline phosphatase activity, while chromaffin tissue has an acid phosphatase activity at the ovulatory phase. The study of acid & alkaline phosphatase activities show clearly some groups of cells richer in enzyme than other groups adjacent to them. Those which predominate in the periphery are rich in alkaline phosphatase activity at the ovulatory phase than those of the more central chromaffin cells which are grouped in nodules

(Plate 4A).The adrenocortical tissue show a weak acid phosphatase activity(Plate 4B) at the ovulatory phase.The adrenocortical cells at the Postovulatory phase show activity of alkaline and acid phosphatase(Plate 4C&D) .

Lipids

The adrenocortical cells show abundant sudanophilic lipid granules at the follicular and ovulatory phase (Plate 5 A & B), which decrease at the postovulatory phase (Plate 5).

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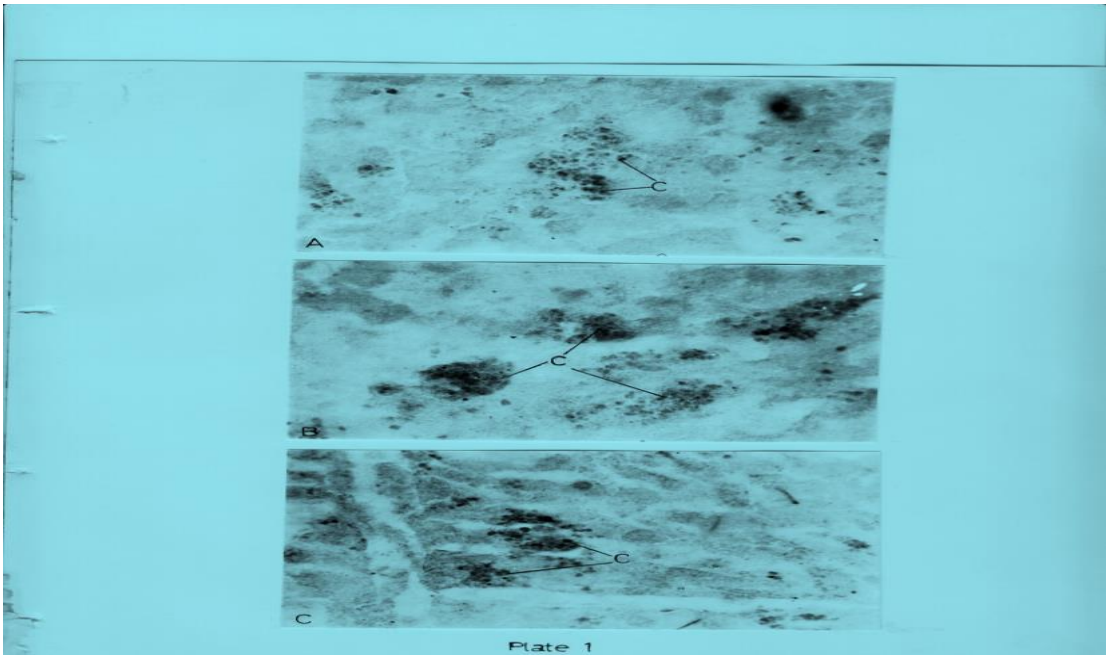

Plate 1 A, B & C

Localization of enzyme Δ^5-3β-Hydroxysteroid dehydrogenase in the Interrenal Gland of Fish during (Cyprinus carpio) during sexual cycle.

A. Section showing a weak activity of Δ^5-3β- Hydroxysteroid dehydrogenase in cortical cells (C) during the follicular Phase of the sexual cycle.

B. Section showing High activity of Δ^5-3β-Hydroxysteroid dehydrogenase in the cortical cells (C) during the ovulatory phase

C. Section showing a moderate activity of Δ^5-3β-hydroxysteroid dehydrogenase in the cortical cells (C) at the time of post-ovulatory phase.

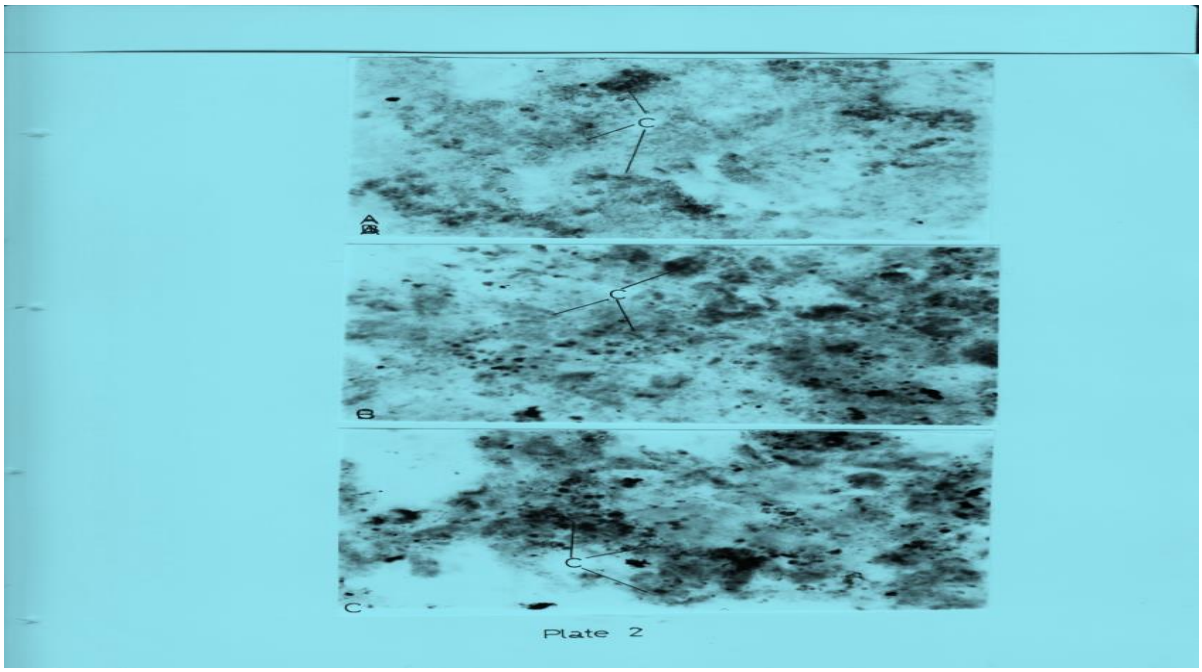

Plate 2 A, B & C

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Localization of enzymeCytochrome oxidase in the Interrenal Gland of Fish (Cyprinus carpio) during different phases of Sexual cycle.

A. Section showing low localization of Cytochrome oxidase in cortical cells (C) during follicular phase of sexual cycle.

B. A high activity of Cytochrome oxidase is observed in the cortical cells (C) during the ovulatory phase.

C. Section showing a high activity of Cytochrome oxidase in the cortical cells (C) during the post-ovulatory phase.

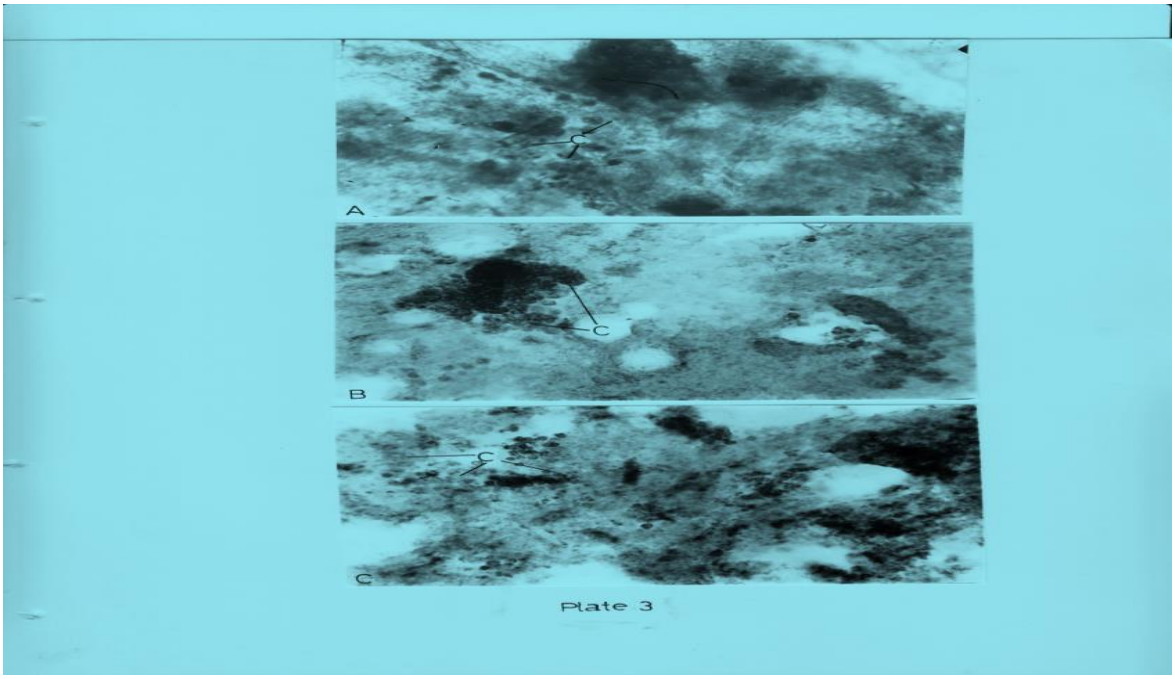

Plate 3 A, B & C

Localization of Enzyme Peroxidase in the Interrenal Gland of Fish (Cyprinus carpio) during sexual cycle.

A. A portion of interrenal showing low activity of Peroxidase in the cortical cells (C) during follicular phase.

B. Section showing high activity of Peroxidase (with guaiacol as a donor) in the cortical tissue. (C) during the ovulatory phase.

C. Section showing diffuse localization of Peroxidase in the cortical cells (C) at post-ovulatory phase**.**

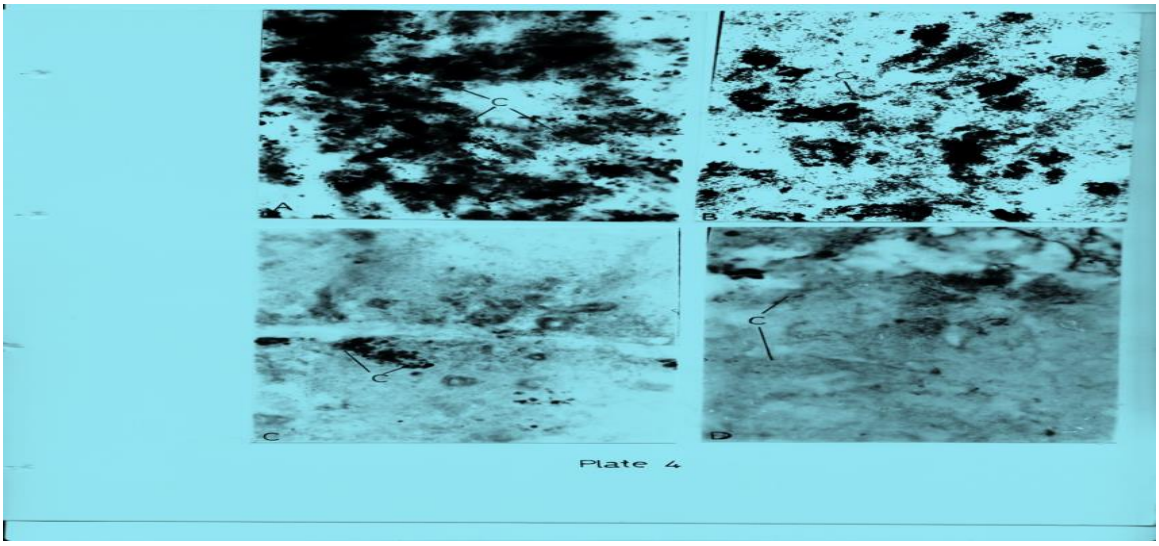

Plate 4 A, B, C & D

Showing localizationof acid & alkaline phosphatases in the interrenal gland of fish (Cyprinus carpio) during sexual cycle.

A. Section showing high activity of alkaline phosphatase in cortical Cells (C) during ovulatory phase.

B. A weak acid phosphatase activity is observed in the cortical cells (C) during the ovulatory phase.

C. A weak alkaline phosphatase activity is observedin the cortical cells (C) during the postovulatory phase.

D. Section showing low activity of acid phosphatase in the cortical cells (C) at postovulatory phase.

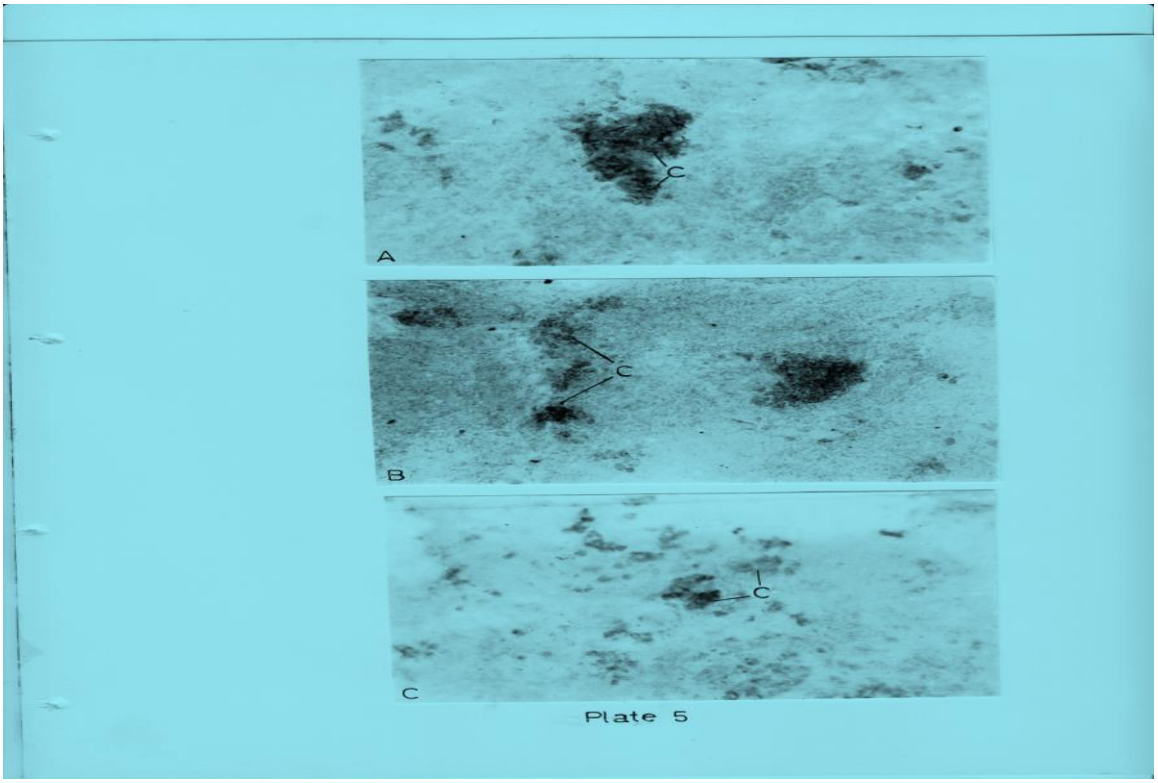

Plate 5 A, B & C

Showing localization of lipids in the interrenal gland of fish (Cyprinus carpio) during sexual cycle

A. Section showing dense deposition of sudanophilic lipid droplets in the cortical cells (C) during follicular phase.

B. Section showing abundant sudanophilic granules in the cortical cells during the ovulatory phase of sexual cycle.

C. Section showing weak localization of sudanophilic lipid granules in the cortical cells (C) during post –ovulatory phase of the sexual cycle.

Ovary

Peroxidase

Follicular Growth

In Non- mammalian species, at the termination of vitellogenesis, the ovaries are packed with large, yolk-laden primary oocytes. Each primary oocyte has an outer layer of thecal cells which abuts against an inner layer of granulosa cells which in turn is separated from the ovum by a thin vitelline membrane.The compact stromal tissues are full of growing follicles and IGT. Simultaneously, the stromal connective tissue elements also become organized to form the distinct outer thecal layer of the follicular epithelium. No peroxidase activity is seen in the membrane granulose or IGT, while a weak localization is seen in the blood Capillaries,since the blood vascular supply is increased with the accumulation of yolk (Plate 6A). A weak sparsely distributed activity of Δ^5- 3β- hydroxysteroid dehydrogenase and Cytochrome oxidase is seen in the membrane granulosa and thecal cells while high activity of Δ^5-- 3β- hydroxysteroid dehydrogenase is seen in IGT (Plate 6 B & C) .

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The thecal cells of growing follicle show high activity of alkaline phosphatase (Plate7 A) . No activity of acid phosphatase could br seen in the membrane granulosa of growing follicles and TC while a weak activity is seen in the IGT(Plate7 B) . The IGT is darkly stained for sudanophilic lipids (Plate 7C) while no localization is seen in the control section(Plate 7D). In the final stages of follicular growth, the granulosa cells form a relatively 3-4 layers called membrane granulosa . The surrounding stromal elements begin to hypertrophy and form the vascularized thecal layer.

In contrast to above, the hypertrophied thecal cells show intense peroxidase activity. The IGT during the preovulatory phase is relatively more developed and accumulates more lipid droplets. No activity could be seen in IGT (plate 8A). The black patches observed in the IGT are again due to accumulation of yellow brown lipid droplets which is devoid of any peroxidase activity No activity is observed without H_2O_2 in the system and cyanide exerted a powerful inhibitory effect.

A high activity of Δ^5-3β- hydroxysteroid dehydrogenase and cytochrome oxidase is seen in the membrane granulosa and thecal cells of preovulatory follicles(Plate 8B & C) while sparsely distributed activity of cytochrome oxidase is seen in the IGT .

The thecal cells of mature follicles show high activity of acid and alkaline phosphatases(Plate 9A & B). A diffuse localization of sudanophilic lipids is seen in the IGT of mature follicles (Plate 9C)

Post-ovulatory Changes

It is well established that ovulation in non-mammlian vertebrates occurs as a result of endogenous pre-ovulatory LH and corticosteroids secretion. Injections of LH, progesterone and corticosterone also bring about morphological, histochemical and biochemical changes in the granulosa cells, thecal cells and the surrounding stroma.

The post-ovulatory follicles are collapsed mass of epitheloid tissue which show a weak localization of peroxidase(Plate 10A). These follicles disappear rapidly and are resorbed in the ovarian stroma exhibiting different intensities of peroxidase activity which change with the regression of luteal cells (Plate 10B). Some yolked follicles are also seen undergoing degeneration. No activity of peroxidas is seen in IGT of post-ovulatory follicles.

Corpora atretica or preovulatory CL in the fish occurs commonly during the prespawning, spawning and postspawning periods, and is characterized by the shrinkage of oocytes, hypertrophy of granulosa and thecal cells which act as phagocytes.A moderate activity of peroxidase is seen in the corpora atretica Plate 10C) . A diffuse localization of Δ^5- 3β-hydroxysteroid dehydrogenase is seen in the luteal cells and IGT (Plate 11A).The control sections without the substrate i.e. DHA and ∞- naphthol respectively show no activity. The postovulatory luteal cells and IGT show high activity of cytochrome oxidase (Plate 11B & C).Tthe control sections without the substrate i.e. DHA and α- Naphthol respectively show no activity .

A high activity of alkaline and acid phosphatases is seen in the luteal cells(Plate12 A & B) , while low content of lipids is seen in the postovulatory follicles and IGT(Plate 12C) . Only diffuse localization of lipids is seen in the regressing luteal cells.

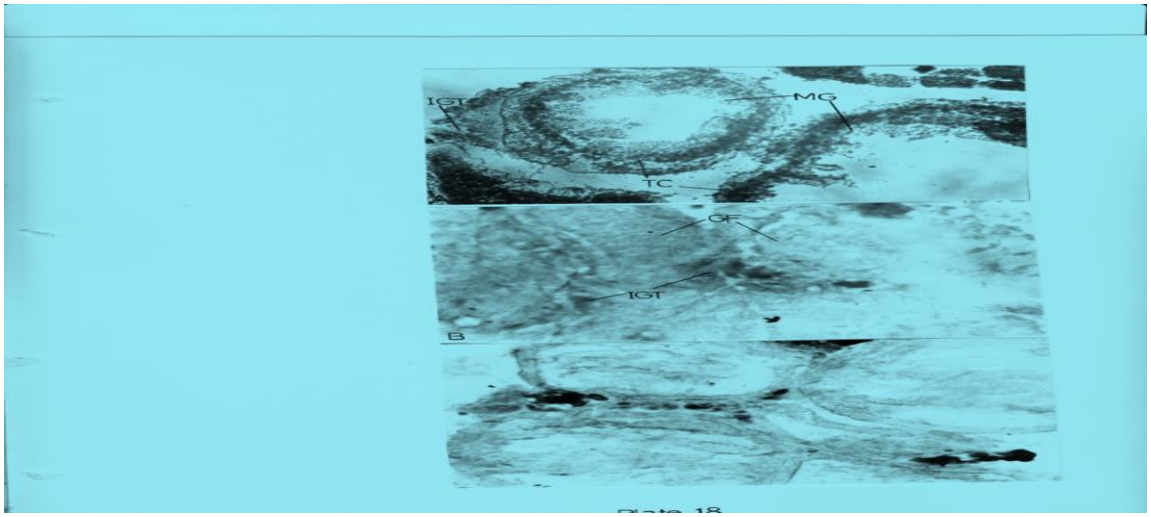

Plate 6 A, B & C

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Localization of enzymes Peroxidase, & Δ^5- 3β- Hydroxysteroid & Cytochrome oxidase in the ovary of Fish Cyprinus carpio) during follicular phase of sexual cycle.

A. Section of ovary showing no activity of peroxidase in membrana granulosa (MG) or IGT during the follicular phase.

B. Weak and sparsely distributed activity of Δ^5-3β- Hydroxysteroid dehydrogenase is seen in membrana granulosa (MG) and thecal cells (TC) with high activity in (IGT) .

C. Weak activity of Cytochrome oxidase is seen in membrana granulose (MG) and thecal cells (TC) with high activity in IGT.

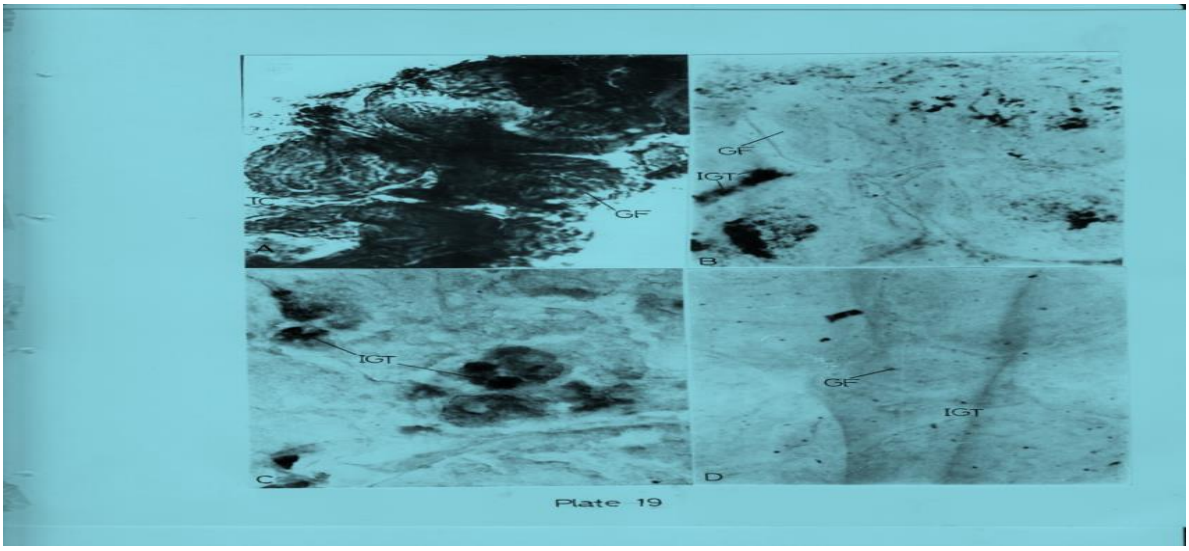

Plate 7A, B, C & D

Showing localization of alkaline and acid phosphatases and lipid in the ovary of fish (cyprinus carpio) during follicular phase of sexual cycle.

A. Section showing high activity of alkaline phosphatase in in the thecal cells of growing follicles (GF).

B. Section showing no activity of acid phosphatase either in Membrane granulosa (MG) or thecal cells (TC) of growing follicles (GF) whereas it is weakly localized in IGT.

C A dense deposition of sudanophilic lipid granules is seen In IGT during the follicular phase.

D. Control section showing no localization of lipid in any region.

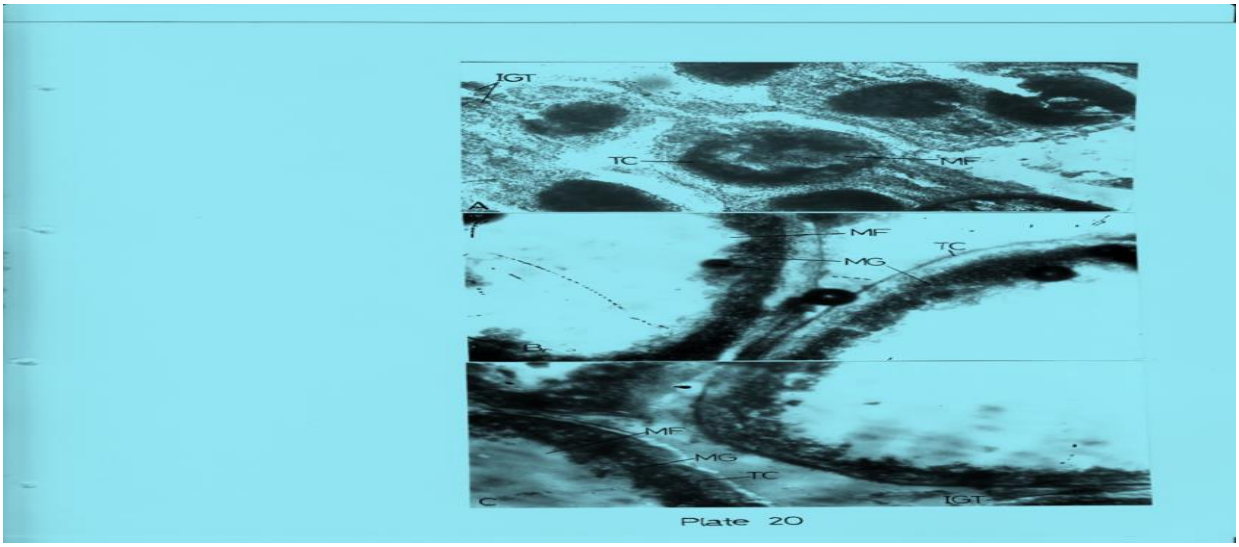

Plate 8 A, B & C

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Localization of Peroxidase, Δ^5-3β- Hydroxysteroid dehydrogenase & Cytochrome oxidase in the ovary of fish (Cyprinus carpio) during ovulatory phase of reproductive cycle.

A. A high activity of Peroxidase is seen in thecal cells (TC) of Mature follicules(MF).

B.Showing localization of Δ^5-3β- Hydroxysteroid dehydrogenase in the ovary. High activity is seen in membrana granulosa(MG) and thecal cells(TC) of mature follicles(MF).

C. Section showing localization of Cytochrome oxidase. A high activity is localized in membrana granulosa(MG) and thecal cells (TC) while sparsely distributed activity is seen in IGT.

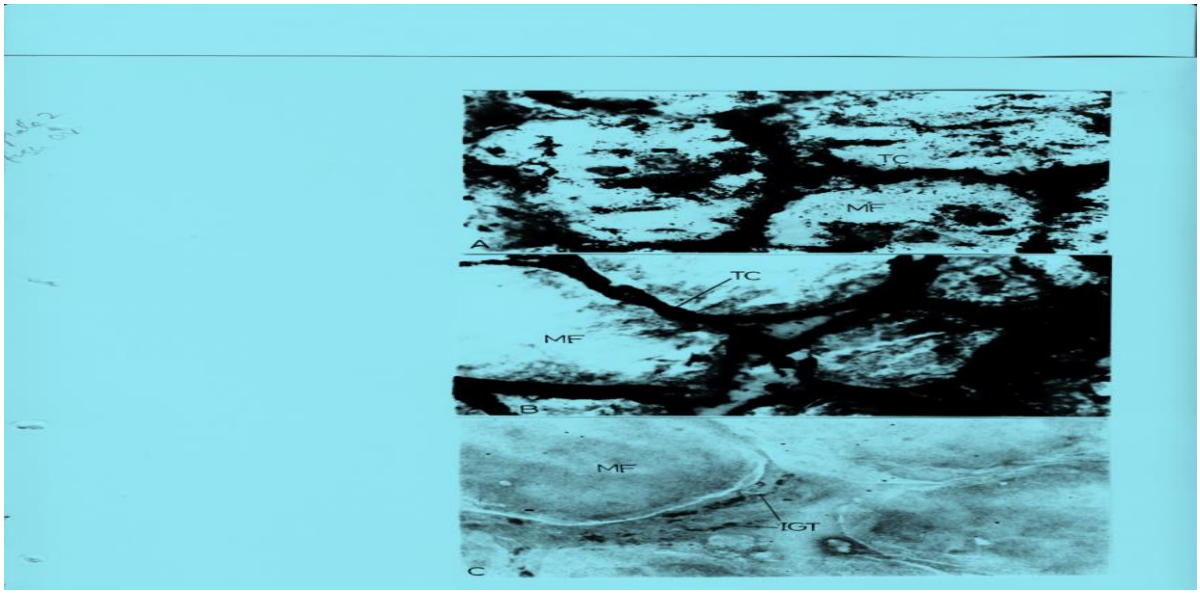

Plate 9 A, B & C

Showing localization of acid & alkaline phosphatases and lipids in the ovary of fish (Cyprinus carpio) during ovulatory phase of the sexual cycle.

A. High activity of acid phosphatase is seen in the thecal Cells (TC) of mature follicles (MF).

B. A dense localization of alkaline phosphatase is seen in Thecal cells (TC) of mature follicles (MF).

C. Section showing diffused localization of lipid in IGT.

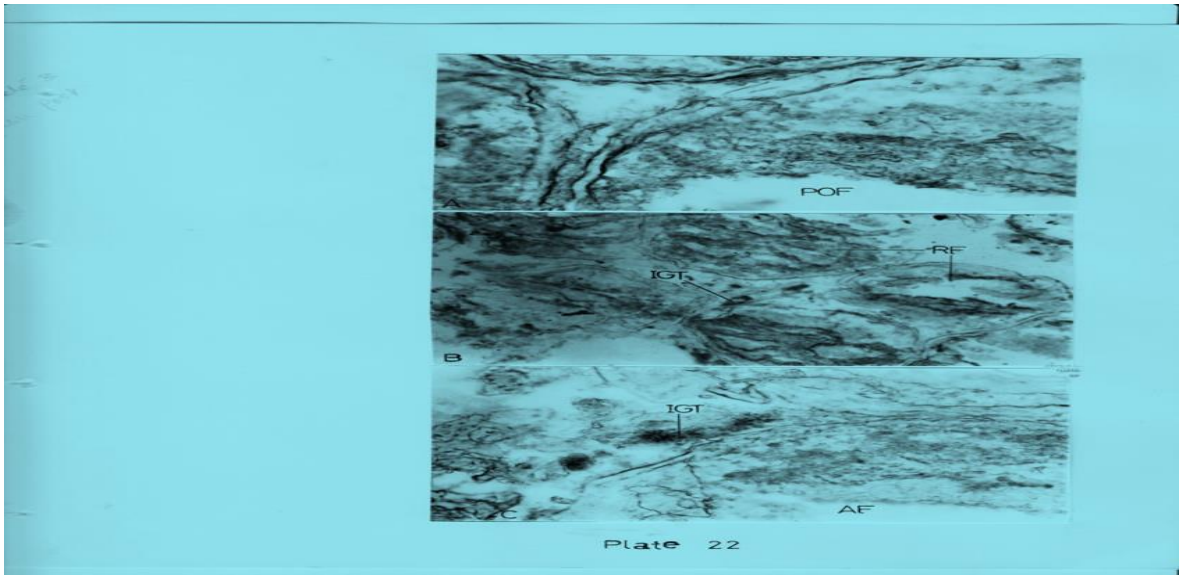

Plate 10 A,B & C

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Showing localization of Peroxidase in the ovary of Fish (Cyprinus carpio) at Post- Ovulatory of sexual cycle.

A .Section showing localization of Peroxidase in post-ovulatory follicles(POF). A weak activity is seen in epitheloid tissue of follicles.

B. A hgih activity ofPeroxidase is seen in regressive follicles (RF) of the post-ovulatory phase.

C. Moderate activity of Peroxidase is seen in corpora atretica(AF) Of post-ovulatory phase

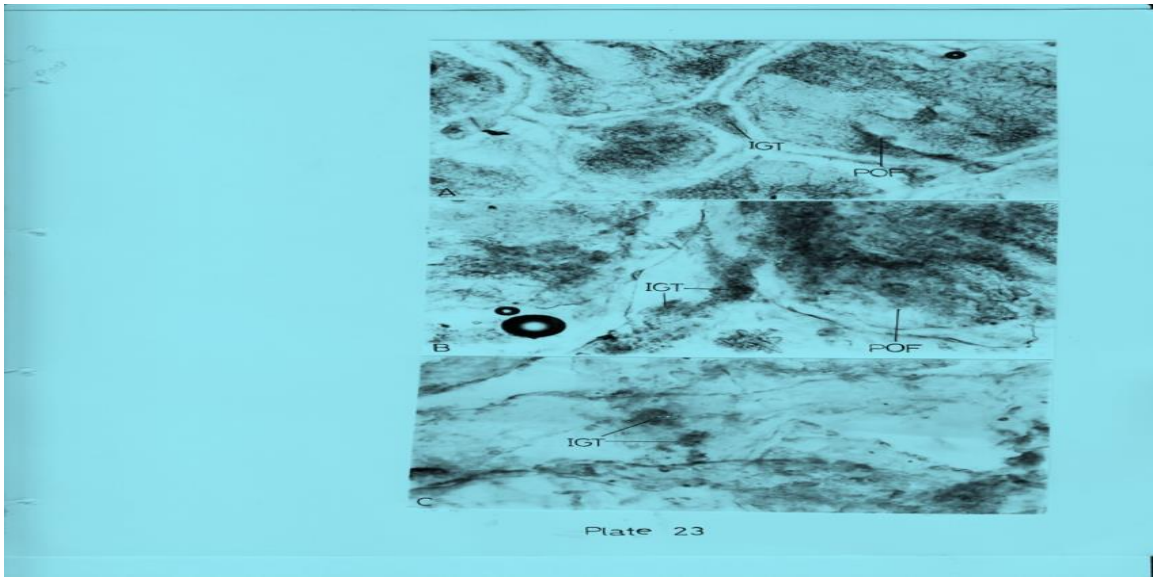

Plate 11 A, B & C

Localization of enzyme Δ^5-3β- Hydroxysteroid dehydrogenase & Cytochrome oxidase in the ovary of Fish (Cyprinus carpio) at Post- Ovulatory phase of the sexual cycle

A. A portion of ovary showing activity of $\wedge^5$ -3β- Hydroxysteroid Dehydrogenase. A diffuse activity is seen in post-ovulatory follicles (POF) and IGT.

B. High localization of Cytochrome oxidase is seen in post-ovulatory Follicles (POF) and IGT.

C. Section showing high activity of Cytochrome oxidase in IGT during Post-ovulatory phase.

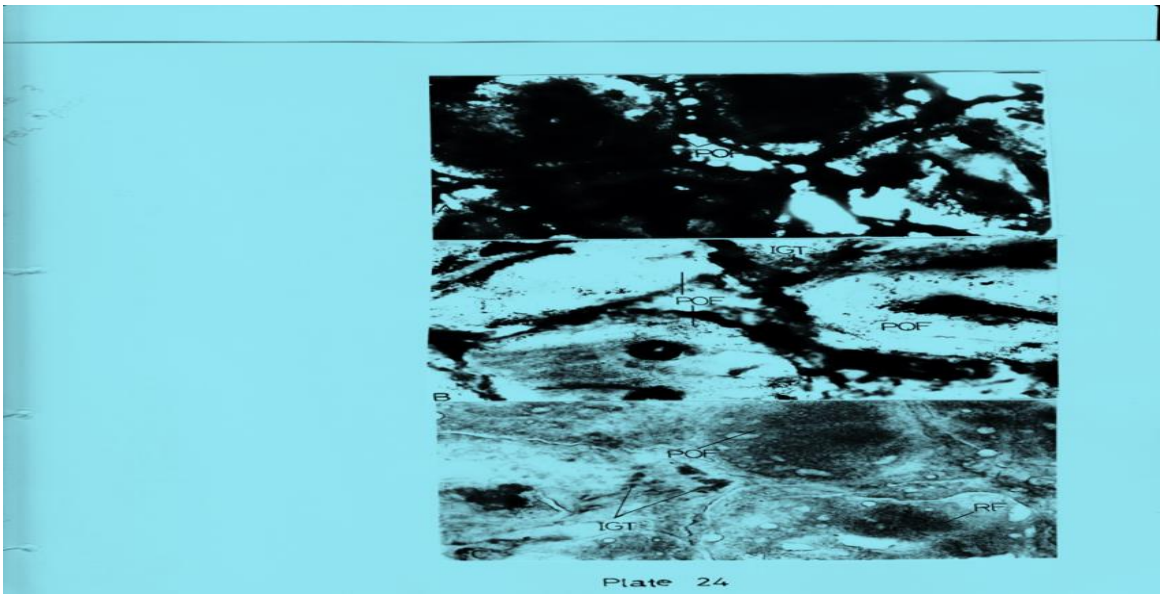

Plate 12 A, B & C

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Showing localization of acid & alkaline phosphatases and lipid In the ovary of fish (Cyprinus carpio) during postovulatory phase of the sexual cycle

A . Intense localization of alkaline phosphatase is seen in post-ovulatory follicles (POF).

B. A ghigh activity of acid phosphatase is seen in postovulatory follicles (POF).

C. Section showing localization of lipid in postovulatory follicles (POF). A diffused localization is observed in regressive Follicles (RF) and IGT.

IV. DISCUSSION

peroxidase is present in the inner layer of adrenocorticalCells but not in chromaffin cells of ovulatory animals of different groups of vertebrates which are associated with the functioning of ACTH hormone and progesterone,corticosteroid secretion.The Δ^{5}-3β – Hydroxysteroid dehydrogenase and Cytochrome oxidase are present in the adrenocortical cells during the entire period of sexual cycle.Thus the characteristic function of adrenocortical cells regulating large secretion of progesterone during increased sexual activity appears to be related to the presence of peroxidase in these ompartments.The adrenal cortex of many non-mammalian species are recognized as the chief site of conversion of ^{14}C acetate to progesterone (Vinson and Whitehouse, 1973a). Also the biochemical studies have shown that adrenal cortex is the chief site for the synthesis of steroid hormones namely Progesterone, cortisol and corticosterone (Hayano et al., 1956; Resko, 1969; Holzbauer, 1969) .

Histochemical studies in fish have shown the presence of Δ^{5}- 3β-Hydroxysteroid dehydrogenase,11β- Hydroxysteroid dehydrogenase,17β- Hydroxysteroid dehydrogenase and G-6- PDH in the interrenal cells (Hooli et al., 1974; Hooli et al., 1976; Bhujle et al.,1980). Δ^{5} - 3β – Hydroxysteroid dehydrogenase being present during the entire sexual cycle viz., follicular,prespawning and spawning period,the specific function of the adrenocortical cells appears to be characterized by the presence of peroxidase ,which is observed in the cortical cells alone during the ovulatory phase and pregnancy, the well known site for the secretion of Progesterone and corticosteroids . Presence of high peroxidase activityin the hypertrophied theca interna of ovulatory follicles and also the CL after ovulation in frog suggest that basic factor involved in leutinization of granulosa cells and also progesterone synthesis are the same for mammalian and non-mammalian vertebrates and that peroxidase appears to be one of the common factors involved in both. Progesterone and corticosteroids of adrenal origin has been attributed a function in ovulation and spawning in non-mammalian vertebrates namely fish and amphibian (Sundararaj and Goswami, 1966b; Goswami and Sundararaj, 1971) . The presence of high peroxidase activity in the adrenocortical cells of the non-mammalian vertebrates during the ovulatory phase may be correlated with the synthesis of progesterone which act synergistically with corticosteroids to cause ovulation in these species as suggested in the fowl (Soliman et al., 1974).

Furthermore, since peroxidase mediated reactions are many fold faster than dehydrogenase reactions, the association of high peroxidase activity in these regions, and lack of activity in adrenocortical cells at follicular phase, in growing follicle of the ovary and IGT of the ovary would suggest that the high rate of progesterone formation may be associated with the functioning of this enzyme at specific sites. Peroxidase thus appears to be involved in the biosynthetic machinery controlling corticosteroidogenesis.

The histochemical changes in acid and alkaline phosphatases and lipids in the adrenocortical cells and ovary at various reproductive phases have been shown by a number of workers (Galli Mainini, 1951; Botte, 1964) .High acid phosphatase activity is shown to be present at ovulatory Furthermore phase in the adrenocortical cells and ovary of fish, amphibians and reptiles, while alkaline phosphatase attains zenith during the secretory phase (spawning phase) . Sudanophilic granules have been shown to increase markedly in the adrenocortical cells,TI and IGT of the ovary At the follicular phase and disappears during the spawning period. Under the hormonal stimuli (Guraya ,1974) the marked decrease in the lipids in ovary with increase in acid and alkaline phosphatase activity of spawning period confirm these reports.

The presence of active cytochrome oxidase in the adrenocortical cells and TI,CL,IGT of the ovary is suggestive of high metabolic activity in these tissues. The operation of active Cytochrome oxidase suggest that the necessary respiratory energy in the form of ATP molecules for the biosynthesis of lipids would be available at the site.The hypertrophied TI and CL of non-mammalian vertebrates are characterized by high vascularization and increased bloodFlow is also visible in

adrenocortical cells of all vertebrates. The oxidative sites thus provided with adequate Oxygen supply with the activated blood flow thus converting thesesites into intense oxidative sites and thus the intense Cytochrome oxidase activity in these sites becomes meaningful. The Peroxidase and Cytochrome oxidase would also seem to transform adrenocortical cells and hypertrophied TI into a highly oxidative compartments of the adrenal and ovary which attributes to the oxidation of pregnenolone to progesterone and corticosteroids towards maturation and ovulation of the oocyte from the ovary.

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Abbreviations: AF- Atretic Follicle, CL- Corpus luteum, GF- Growing follicle, IGT- Interstitial gland tissue, Lc – Luteal cells, MF – Mature follicle.

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