HISTO-MORPHOLOGICAL STUDY
HISTOMORPHOLOGICAL STUDY

During present investigation, pituitary gland and gonad of the fish, *Channa gachua* have been studied histomorphologically in corelated with endocrine mechanism and reproduction.

Hypothalamo-hypophyseal-gonadal chain is the main stream of hormone regulation, releases as in other vertebrates; control mechanism has certain distinguishing features. Fish brain (hypothalamic portion) secretes gonadotropin releasing hormones (GnRH): plays key role in the regulation of reproductive events in almost all fishes.

The histological criterion to detect the activity of any endocrine gland has own limitations especially the accuracy in attributing hormonal activity. However, increase in nuclear size, enlargement of nucleolus. Cellular hypertrophy and compact appearance of the tissue together with its highly granulation have been taken as an indication of stimulation and increase in activities.

PITUITARY GLAND

The pituitary is the master gland, control over the functional ability of the reproduction and other vital endocrine mechanism in the animals. The pituitary gland in *Channa gachua* is oval in shape, pinkish in colour, lies in a depression formed by two prootic bones dorsally and the parasphenoid bone
ventrally, the floor of infundibulum without definite stalk. Hence, it is called
platybasic type. Pituitary gland is lodged in an excavation or depression of the
parasphenoid bone, sella turcica, enclosed in a thick connective tissue capsule
(Plate No 2). The sella turcica is found in the midline, slightly high above the
parasphenoid bone.

The hypophysis is broadly divisible into adenohypophysis and
neurohypophysis.

A) ADENOHYPOPYSIS:

In teleosts it develops from the roof of the stomodaeum. The
adenohypophysis is divided into rostral pars distalis (RPD), proximal pars
distalis (PPD) and pars intermedia (PI) (Plate No. 5 A).

1) Rostal Pars Distalis (RPD):

Rostal Pars Distalis is situated in anteriodorsal region. In RPD, the main
cells are chromophobes (η) and amphophils (ε) present in this region (Plate No.
5; B).

Chromophobes (η) cells:

These acidophils are chromophobic in nature, compactly aggregated in
this region. The nucleus is rounded, usually with a distinct nucleolus. These
cells are stained with aniline blue of MTS. Some Chromophobes exhibits
follicular arrangement, which secrete prolactin (Emmart et al., 1966).
Amphophils (ε) cells:

These cells are characterized by its distinct arrangement as a palisade (fence) along border between the neurohypophysis. Cells are stained with aniline blue, grey or bluish with MTS. These cells lie in the neurohypophysis close to the connective tissue boundary of the chromophobes. There are two to three rows of cells around the neurohypophysial branches in *Channa gachua*. They are scattered among the neurohypophysial fibers along the border of RPD. These cells are generally polygonal, rounded or irregular in shape, sometimes elongated towards a nearby capillary. Identification of these cells is corticotrophs (Barker *et al.*, 1974).

2) Proximal Pars Distalis (PPD):

This region is composed by two types of randomly mixed cells viz, the basophilic (β) cells and cyanophilic (δ) cells. In addition, acidophilic (α) cells are also found in the boarder of both PPD and PI (Plate No. 5; C).

**Basophilic (β) cells:**

The cytoplasms of these cells are coarsely granular, stained with aniline blue. The number, size and intensity of granulation in the basophil (β) cells show seasonal variations which can be associated with the development of gonads, identification of these cells as gonadotrophs (Sundarraj, 1959).

These basophils are the most predominant cells type in the PPD. The cytological nature of these cells is highly diversified as they undergo seasonal
changes, correlation with the gonadal cycle. Their tinctorial affinity varies with the state of their granulation or degranulation, size of nucleus.

Two types of basophils have been recognized, as basophils-I, lying towards rostral end of PPD being polygonal in shape (with slightly elongated nucleus); basophils-II cells are elongated stained fainter than basophils–I, located towards caudal portion of the PPD.

_Cyanophilic (δ) cells:_

A more or less constant feature exhibited by the PPD in the preponderance of cyanophils. The nucleus is smaller than the basophils with less dense nuclear membrane and smaller nucleolus. Nevertheless; it is occasionally difficult to classify some basophils with certainty. The cells when become extensively degranulated stains blue. These cells are identified as thyrotrophs (Baker, 1974).

_Acidophilic (α) cells:_

These cells are stained with orange G after the application of MTS stains. In this fish, cytoplasm is densely granulated; nucleus is oval or rounded with distinct nucleolus. Although variations occurred in cell and nuclear size, the acidophilic (α) cells identified as somatotrophs (Baker, 1974).

3) **Pars Intermedia (PI):**

A pars intermedia occupies the postero-ventral side (Plate No. 5; D). This region is characterized by heavy arborisation of neurohypophysial fibers. It
partly or completely surrounds the distal end of neurohypophysis and abundantly supplied with the branches of pars nervosa. It also encompasses more neurohypophysial tissue than any other region.

Two types of cells are predominated in this region. They tend to be scattered in the neurohypophysis.

**Chromophobes (Pb-H + VE cells):**

These cells are stained PbH+ve. These cells are larger, rounded or elongated with granular cytoplasm, placed with each other forming a mat. Some chromophobes cells are observed border to pars intermedia.

**Basophilic (AF+ VE cells):**

These cells are stained AF+ve. They are prominent cells for the reproduction. They appear small, rounded with large pynotic nucleus.

Anterior pituitary consists of seven major types of cell. Their secretary material or hormones are required for homeostatic regulation, development, growth and reproduction.

**B) NEUROHYPOPHYSIS:**

It consists of highly branched, loosely tangled network of fibers. These nerve fibers of neurohypophysis contain glial cells (pituicytes) and droplets of the neurosecretary material, stained with deep purple AF+ ve, large droplets of the neurosecretary material are often referred to as ‘Herring bodies’(Plate No. 5; E).
Neurohypophysis is controlled by nerves fibers from the hypothalamus. Two types of neurohypophysial fibers are distinguished as ‘stainable’ and ‘non-stainable’ depending upon the material contained in the fibers take the stain for neurosecretary fibers, transporting the neurosecretary material from the hypothalamic nuclei while unstained ones are non neurosecretary in nature. Subsequent towards the pars distalis, the neurohypophysis arbourizes the pars intermedia extensively and generally stained due to the presence of stored neurosecretary material.

OVAPRIM ADMINISTRATION (EXPERIMENTAL):

It was demonstrated that dopamine inhibitory activity was associated with the synthesis of gonadotropin. Subsequent research have been showed that the action of LH-RH analogue can be potentiated when they are combined with dopamine (A chemical that inhibits release of hormones from pituitary and thereby blocks the pituitary’s response to injected) antagonist like primozide or domparidone. During present study Ovaprim application was undertaken for induced breeding in Channa gachua.

Administration of Ovaprim brought stimulatory changes in basophils. Significant changes were observed such as increase in the cell size, cytoplasm, nuclear size and glycoproteinaceous material in these cells.

On the basis of glycoproteinaceous material concentration basophils were divided into four processes viz. hypertrophy, granulation, degranulation
and vacuolization. Results were marked by increased glycoproteinaceous material and their gonadotropin-secreting nature.

After injection, the cytoplasm was partly granulated and nuclear size was slightly enlarged in basophils (Plate 6; B) as compared with control in preparatory phase (Plate 6; A). The part of PPD was slightly increased; cells size and cytoplasmic hypertrophy was noticed. Basophils were large, densely granulated with enlarge, rounded nucleus. There was noticeable increase in the size, stained dark as compared with control fish. This indicated that there was more secretion of the gonadotropin due to administration of ovaprim.

In present study, cell size, nuclear size and cytoplasmic granulation of ova showed increase after ovaprim treatment.

During pre-spawning phase the basophils were highly granulated, cytoplasm thickly granulated and few hypertrophed basophils observed (Plate 6; D). Nuclei were large, round with dense and prominent nuclear membrane. Ovaprim injected basophil cell size became large in size, as compared with control fish (Plate 6; C), the size and percentage value of the basophils cells in the fish showed significantly increased. Basophils were accumulated with large quantity of secretary material.

In spawning phase, the basophils of PPD were highly increased in volume, tightly packed, responded strongly to aniline blue. Most of them exhibit indistinct cell boundary, showed nuclei which were comparatively larger than control (Plate 7; A). In treated fish, there was an increase in the number of the
gonadotropin or basophils cells (Plate 7; B). Their granulation was rich, intensely stained with MTS. Basophils were heavily granulated, intensively stained with MTS of aniline blue, increased number, large sized with huge granulated material.

During post-spawning phase, nucleus was still prominent and the cytoplasm of many cell were granulated. Basophils were mostly released their cytoplasm; nuclei were visible and became completely degranulated, showed that large quantity of gonadotropin was present in cell (Plate 7; D) than control one (Plate 7; C).

Small nuclear size was clearly noticed in control fish. They differ significantly as compared with treated fish. A less cytoplasmic material was an evident. In spawning phase after injection, cells underwent remarkable granulation which became more pronounced at the end of June. In July it was resulted in heavy granulation of cells with aniline blue with prominent nuclei and cytoplasm. Few basophils were degranulated and vacuolated. The cell size is large in treated fish as compared with the control fish. Cytoplasmic material of cells became more prominent. In Channa gachua heavy granulation in basophils indicated enhance in activities responded to rise gonadotropin level due to ovaprim administration.
A: T. S. of Pituitary Gland × 100
RPD – Rostal Pars Distalis
PPD - Proximal Pars Distalis
NH - Neurohypophysis
PI – Pars intermedia

B: Rostal Pars Distalis × 400
AC-Amphilhs cell (ε)
CC -Chromophobes cells (η)

C: Proximal Pars Distalis × 400
AC – Acidophilic cells (Somatotrophs)
BC – Basophils cells (Gonatrophs)
CC – Chromophobes cells (Thyrothrophs)

D: Pars Intermedia × 400
CC - Chromophobes cells
BC– Basophils cells

E: Neuropypophysis × 400
Hb – Herring bodies
SF- Stainable nerve fibers
NSF – Non stainable nerve fibers
PLATE NO. 6: T.S. OF PITUITARY GLAND (MTS I, II and III)

A: Preparatory Phase (Control) (×400)

HBC- Hypertrophed Basophils
GBC- Granulated Basophils

B: Preparatory Phase (Injected) (×400)

VBC- Vacuolated Basophils
GBC-Granulated Basophils

C: Pre-spawning (Control) (×400)

GBC- Granulated Basophils
GCY- Granulated Cyanophils
GAC- Granulated Acidophils

D: Pre-spawning Phase (Injected) (×400)

HBC- Hypertrophed Basophils
GBC- Granulated Basophils
HCY- Hypertrophed Cyanophils
PLATE NO. 7: T.S. OF PITUITARY GLAND (MTS I, II and III)

A: Spawning Phase (Control) (×400)
GBC-Granulated Basophils
DBC- Degranulated Basophils

B: Spawning Phase (Injected) (×400)
GBC-Granulated Basophils
GCY-Granulated Cyanophils

C: Post Spawning Phase (Control) (×400)
DBC- Degranulated Basophils
VBC- Vacuolated Basophils
GCY-Granulated cyanophils

D: Post Spawning Phase (Injected) (×400)
DBC- Degranulated Basophils
VBC- Vacuolated Basophils
GBC-Granulated Basophils
GONADS:

Gonads were selected to study possible correlations of metabolites with Leydig cells in testis and thecal, granulosa cells in ovary during reproduction. The reproduction of fishes depends upon coordinated actions of various hormones associated with brain-pituitary-gonadal axis.

GnRH reaches pituitary via portal circulation, binds to its receptor in the pituitary gonadotrophs cell membrane. This causes release of gonadotropin hormone (GtH) from gonadotroph cells. GtH then travels through circulation and finds target organ, ovary or testes, where it occupies specific receptor in the cell membrane of theca and granulosa cell of ovary or Leydig cell of testes. GtH induced, synthesis and released steroids were responsible for growth and maturation of male and female germ cells.

Various workers have been classified gonads in different developmental stages but most accepted has been advocated by ICES (International Council for Exploration of Seas) is as follows:

TESTES:

Testes are elongated, paired structure, situated on ventral side of the kidneys in the posterior region of the abdominal cavity. It remains attached to the body wall by means of mesorchia. The two sperm ducts joins at posterior and opens externally into common urinogenital opening (Plate No. 3).
The different stages of spermatogenesis are distinguished on the basis of their characteristics such as nuclear and cytoplasmic structure (Plate No.8; A). The progressive spermatogenesis takes place in to following six stages:

**Primary spermatogonia (Sperm Mother cells):**

The primary spermatogonia are the largest, spherical cells among the spermatogenic cells in the testis especially being higher concentration in spherical lobules as well as in the vicinity of lobular wall. Under light microscope, they show large nucleus with deeply stained eccentric nucleolus and nuclear membrane. The cytoplasm of these cells has less affinity towards basic dyes such as heamatoxyline and eosine. Primary spermatogonia are prominent during preparatory phase. Their number decreases gradually as per advancement in maturity. Primary spermatogonia are observed close proximity of lobule wall (Plate No. 8; B).

**Secondary spermatogonia:**

Primary spermatogonium is divided mitotically and gives rise to small, rounded secondary spermatogonia. They have less cytoplasm and nucleus with clear nucleolus (Plate No. 8; B).

**Primary spermatocytes:**

The active multiplication of secondary spermatogonia gives rise to smaller cells. The primary spermatocytes show various stages of their divisions and contain large vesicular eccentrically placed nucleus. It is distinguishing
feature of primary spermatocytes and helps in differentiation from rest of other cell types. The nucleus is visible (Plate No. 8; C).

**Secondary spermatocytes:**

The reduction division of each primary spermatocytes results in the formation of secondary spermatocytes. The chromatin materials in secondary spermatocytes are in the form of thick clumps. The nucleolus is absent and cytoplasm is less (Plate No. 8; C).

**Spermatids:**

The spermatids are formed as a result of second meiotic division of the secondary spermatocytes. They appeared compact, dot-like structure, deeply stained with heamatoxyline. The nucleus of spermatids is more or less round in shape. The nucleolus is absent. The nucleocytoplasmic portion in spermatids is much reduced. The nucleus is observed on one side and nuclear membrane observed in contact with the plasma membrane forming the further anterior side of spermatozoa (Plate No. 8; D).

**Spermatozoa:**

The spermatids ultimately transforms into mature spermatozoa (spermiogenesis) by orientation and reorganisation of nucleus and cytoplasm together with the development of flagellum. The spermatozoa are small, rounded deeply stained structure found in cluster. In gravid male fish, the testicular lobules are full of spermatozoa, lobule wall becomes thin. The mature spermatozoa consist of three parts head, middle piece and tail.
The head of spermatozoa is spherical in shape, mainly composed of nucleus while the middle piece consists of cytoplasm. The chromatin of nucleus is highly condensed. A long cytoplasmic flagellum arises from the middle piece during the spermatogenesis which helps in movement of spermatozoa (Plate No. 8; E).

**AFTER OVAPRIM ADMINISTRATION (EXPERIMENTAL):**

A single dose of ovaprim showed changes in histomorphology of the testis. It included organization of seminiferous lobules, formation of lumen, spermatogonial proliferation, activation and elongation of leydig and sertoli cells.

In *preparatory phase*, immature testis showed testicular lobules contained spermatogonia and sertoli cells. After injection, spermatogonia took part in cyst formation. The sertoli cells were surrounded the germ cells also located in clusters filled spaces in lobules. With increased spermatogonial maturation phase, the number and size of testicular cysts were increased; groups of sertoli cells localized at the center as compared with control one as the endocrine component of the fish testis.

Interstitial cells became more active when treated with ovaprim. Increased granulation in sertoli cells observed after ovaprim treatment, thus the endocrine nature. Interlobular connective tissues were reduced considerably and primary spermatogonia were less in number as compared with control (Plate 9;
A). Secondary spermatogonia were frequently observed more than controlled preparatory phase. The large number of spermatogonia has been observed (Plate 9; B).

In pre-spawning phase, as the division of spermatogonia took place, central sertoli cells were pushed apart leaving a gap between the central parts of the lobules. This gap further widens and took the shape of tubular lumen. The testicular lobules consist of spermatogonia surrounded by sertoli cells with lumen in the centre and leydig cells were lying just outside the seminiferus tubules. Their abundance and secretary nature may the site of the production of testicular steroids (Plate 9; D).

Tubules filled with large quantity of spermatids. Secondary spermatogonia were quantitatively large. Testicular organization remains same but secondary spermatogonia were increased in number as compared with control (Plate 9; C). Sertoli cells were elongated, received invaginations of spermatogonial cytoplasm showed close physiological association between them.

At the beginning of June, sertoli cells contained clear basal nucleus, with an irregular shape and usually parallel to the basement membrane. The nucleolus was rarely seen. The cytoplasm was rather dense, residual bodies, membrane-bound dense bodies with a filamentous core and heterophagic vacuoles containing cellular debris or whole sperm cells were also frequently observed in the cytoplasm of Sertoli cell. Sertoli cells displayed an irregular
shape. A narrow, fairly regular gap closed by intimate contacts (perhaps gap junctions) separated Sertoli cells from spermatogonia, spermatocytes and spermatids. In the beginning of July, leydig's cells showed ultra structural features as active steroidogenic cells, filled with cytoplasm. The nucleus was clear and ovoid-shaped with slightly irregular outline. At the end of June, leydig's cells were further activated.

As spawning phase proceeded, active leydig's cells showed by means of steroid-producing characteristics of cytoplasm. Localization with sertoli cells in the centre widening of gap in tubular lumen (Saksena et al., 1995). Formation of a gap between sertoli cells was characteristics of more producing gonadotropin. Secondary spermatogonia developed as spermatozoa (Plate 10; B). The lipid globules were observed in sertoli cells. Leydig's cells during this stage were found in groups, assumed as characteristics of steroid synthesizing cells. Tubules filled with huge quantity of spermatozoa. Tubules mainly filled with mature spermatozoa than control one (Plate No.10; A). Ovaprim treated testicular leydig's cells showed heavily granulated, intensively registered increase in their number and size.

At the post spawning phase, the structure of sertoli cells were similar as described above. The empty seminiferous lobule contains residual spermatozoa in center, leydig cells lying just outside the lobules. Residual spermatozoa are less in number because they all most all were spent. (Plate 10; C), as compared with control one (Plate 10; D).
PLATE 8: T.S. OF TESTIS (Heamatoxyline and Eosin)

A : T.S. of Testis × 100
ST - Seminiferous tubules
LC – Leydig cells

B : Primary Spermatogonia × 400
ST- Seminiferous tubules
PSG – Primary spermatogonia
SSG – Secondary spermatogonia

C : Primary Spermatocytes × 400
LC – Leydig cells
PSC – Primary spermatocytes
SSC – Secondary spermatocytes

D : Spermatids × 400
PSC – Primary spermatocytes
SSC – Secondary spermatocytes
SD – Spermatids

E: Spermatozoa × 400
SSC – Secondary spermatocytes
SD - Spermatids
SZ – Spermatozoa
PLATE NO. 9: T.S. OF TESTES (Heamatoxyline and Eosin)

<table>
<thead>
<tr>
<th>A: Preparatory Phase (Control) (×400)</th>
<th>B: Preparatory Phase (Injected) (×40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSG – Secondary Spermatogonia</td>
<td>SSG – Secondary Spermatogonia</td>
</tr>
<tr>
<td>PSG - Primary Spermatogonia</td>
<td>PSG - Primary Spermatogonia</td>
</tr>
<tr>
<td>LC – Leydig cell</td>
<td>LC – Leydig cell</td>
</tr>
<tr>
<td>ST – Seminiferous Tubules</td>
<td>ST – Seminiferous Tubules</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C: Pre-spawning Phase (Control) (×400)</th>
<th>D: Pre-Spawning Phase (Injected) (×400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSG - Secondary Spermatogonia</td>
<td>SSG - Secondary Spermatogonia</td>
</tr>
<tr>
<td>PSC - Primary Spermatocyte</td>
<td>PSC - Primary Spermatocyte</td>
</tr>
<tr>
<td>SC - Sertoli Cells</td>
<td>SC - Sertoli Cells</td>
</tr>
</tbody>
</table>
PLATE NO. 10 : T.S. OF TESTES (Hematoxyline and Eosin)

A: Spawning Phase (Control) (×400)

PSC- Primary Spermatocyte
SSC- Secondary Spermatocyte
SD - Spermatids
SC- Sertoli Cells

B: Spawning Phase (Injected) (×400)

PSC- Primary Spermatocyte
SSC- Secondary Spermatocyte
SD - Spermatids
SC- Sertoli Cells
L- Lumen

C: Post-Spawning Phase (Control) (×400)

RS- Residual Sperm
LC- Leydig cell

D: Post Spawning Phase (Injected) (×400)

SSG – Secondary Spermatogonia
PSC- Primary Spermatocyte
SSC- Secondary Spermatocytes
LC- Leydig Cells
RS- Residual Sperm

\[\text{Diagram Image}\]
OVARY:

The ovary is paired, elongated; sac likes structure, lying in the abdominal cavity, ventral to kidney, attached to body wall by mesovarium. The anterior ends of ovaries are free but their caudal ends united into common oviduct (Plate No. 4).

Oogonium passes through series of various developmental stages. In the present investigation, on the basis of developmental changes in oocytes are classified as fallows (Plate No. 11; A).

Chromatin nucleolus stage:

Oogonia were directly transformed in to earlier primary oocytes by growing in size; Major portion was occupied by nucleus surrounded with thin layer of basophilic cytoplasm (Plate No. 11; B).

Early Perinucleolus stage:

Oocytes during this stage are surrounded by number of flat and smaller follicle cells. During successive growth, amount of ooplasm increases, presence of large number of nucleoli in different size lying inner to nuclear membrane, which are strongly basophilic in nature (Plate No. 11; B).

Late perinucleolus stage:

The ooplasm in oocyte further increases. As maturity advances, it migrates towards the cortical ooplasm and gradually disappears some nucleoli, can be observed lying partly inside and partly outside. The nuclear membrane indicating their extrusion into ooplasm the nuclear membrane was distinct and
follicle cells were surrounded by the oocytes increase further in number and size (Plate No. 11; C).

**Early yolk vesicle stage:**

With further growth several large folds as an evagination appears in the nuclear membrane, which increases the surface area of nucleus for nucleocytoplasmic exchange of molecules during oocytes growth. Nucleoli showed their usual distribution and extruded into ooplasm of growing oocytes. The extruded nucleoli were finally losing their consistency because their material was distributed in ooplasm.

The cortex of cytoplasm termed as cortical alveoli or yolk vesicle. These cortical alveoli or yolk vesicle increases in size by fusion of smaller vesicles. Zona radiata started development. Follicular layer is better developed (Plate No. 11; D).

**Late yolk vesicle stage:**

Cortical alveoli or yolk vesicle distributed in cytoplasm further increases in number and size. They are shifted towards periphery, size of nucleoli further get reduced. Follicular epithelium and zona pellucida deeply stained with heamatoxylin. Outer follicular epithelium lies in thecal tissue consisting stroma cells which do not show much cytoplasmic differentiation. In between oocytes and follicular epithelium, non cellular zona pellucida shows its presence which is very narrow, compact and homogenous layer (Plate No. 11; E).
Early yolk stage:

Yolk globules are in the form of very minute particles begins to appear in extra vesicular cytoplasm. Later on unite to form large spherical yolk globules loaded almost all ooplasm. These are stained with heamatoxylin, follicular becomes large, thick and layer of theca becomes differentiated (Plate No. 11; F).

Late yolk stage:

An extensive development of yolk globule increases in size and occupies the ooplasm around the nucleus. Some globules are united with each other forming layer. Rapid accumulation of yolk globules results in rapid growth of oocytes. At the same time the cortical alveoli or yolk vesicles are further pushed towards the periphery and arranged in two successive layers.

The follicular epithelial layer further increases thickness and become more distinct. The zona pellucida becomes thicker (Plate No. 11; G).

Riped egg stage:

During this stage the egg grows fully, completely packed with yolk mass. The layer of cortical alveoli or yolk vesicle is clearly observed adjacent to egg which is well developed (Plate No. 11; H).

Carpus Luteum:

The prominent feature of partially or completely spent ovary is the presence of atretic and discharged follicles. Ovum after it’s discharge leaves behind the egg membrane external to the vitelline membrane. This discharges
follicle through different transformation before resulting in formation of a more or less solid cellular structure the carpus luteum. The completely spent ovaries show the presence of atretic and discharged follicles. After the spawning, egg leaves behind the egg membrane. When the oocytes do not reach to maturity and undergoes degeneration and resorption. This atretic degeneration occurs through the degenerating ovum and finally disappears and only follicular cells are left to form carpus luteum (Plate No. 11; D).

AFTER OVAPRIM ADMINISTRATION (EXPERIMENTAL):

The ovarian follicle is the basic functional unit of the ovary and comprises of an outer layer of theca cells, separated by basement membrane from the layer of granulosa cells which in turn surrounded the oocytes-cumulus cells complex. In response to Ovaprim, the follicle was able to differentiate with regard to steroidogenesis and hormone receptor induction, as well as to produce a fertilizable ovum (Bruce et al., 1993).

Enormous growth in fish oocyte occurs during vitellogenesis. It is two step mechanisms, Ovaprim help to promote this mechanism more. In which (i) GtH binds to theca and granulose cells receptor in the ovarian follicle and thereby increase 17-β estradiol secretion, which in turn, induces synthesis and secretion of vitellogenesis. Ovaprim, most probably stimulates incorporation of vitellogenesis into oocytes from directly through circulatory blood. In the vitellogenic phase, estrodiol appears to be major regulator and syntheses in
coordinated manner, in which both theca and granulosa cell layers participate. (ii) GtH influences the secretion of androgen substrate (testosterone) from thecal cells which diffuses into the granulosa cell layer, where the aromatase (enzyme) is exclusively localized and converted testosterone to 17-β estradiol (Bhattcharya, 1999).

During preparatory phase, the follicular layer was developed with theca and granulosa cells. A vitelline membrane was clearly visible between the ooplasm and zona granulosa (follicular layer) as compared with controlled (Plate 12; A). Ovaries were found increased in weight and nearly occupied the ¾ of the body. The ovary showed ovigerius lamellae with nests of oogonia. The process of vitellogenesis started and matured, large ova appeared in the ovary after the treatment of Ovaprim. Increased size of oogonia was observed in large number (Plate 12; B).

During pre-spawning phase, the yolk appeared in the form of granules. The yolk granules fused to form large globules with heavy deposition of yolk. The size of nucleus was larger (Plate 12; D) as compared with the control one (Plate 12; C). The Ovary represented larger sized, spherical ova but in controlled ovaries of this stage consist less number of oocytes compared to ovaprim injected. In spawning, where external cues are manipulated, (e.g. temperature, rain fall water quality) in induced breeding, species spawn in captivity easily few of them may not spawn at all. For those to spawn, it may be asynchronously. Thus, breeding techniques play important role in large scale
production of gametes. This was the main difference made by ovaprim treatment observed in pre-spawning phase visible with naked eyes through the thin wall. Ovary attained maximum weight and becomes highly distended.

During the spawning phase, the thecal (external layer), zona granulosa (follicular epithelium) and innermost zona radiata layers were became thicker which involve selective sequestration and derived synthesis of vitellogenin and yolk protein. Oocytes at this stage increased in size. In some cases, the yolk granules form larger, tightly packed granules, the whole oocytes filled with yolk granules (Plate 13; B). The ovary was fully enlarged, occupied much of the body cavity. The riped eggs were still larger in size full of yolk globules as compared with control (Plate 13; A).

In post-spawning phase, the atretic eggs were observed rarely because almost all the eggs were spent. Some large sized ova were observed (Plate 13; D). It may be explained that the dopamine antagonist block the action of dopamine, which act on thecal and granulosa cells, for the antagonistic to gonadotropin, may act as feed back by inhibiting further action of gonadotropin until the next requirement. Carpus luteum may be the seats of feed back mechanism in *Channa gachua*. Androgen ratio can be used to define a follicle as healthy and large following ovulation, hormone production was necessary to spawn. It was less in controlled (Plate 13; C).
PLATE 11: T.S. OF OVARY (Hematoxyline and Eosin)

A: T.S. of Ovary (× 100)

EYVS – Early Yolk Vesicle stage
EPNS – Early Perinucleolus Stage
LYVS-Late Yolk Vesicle Stage
EYS – Early Yolk Stage

B: EPNS – Early Perinucleolus Stage (× 400)

CNS – Chromatin nucleolus stage
EPNS – Early Perinucleolus Stage
N - Nucleus
NLI– Nucleoli

C : LPNS – Late Perinucleolus Stage (× 400) D : EYVS –Early Yolk Vesicle Stage (× 400)

A – Atresia
N – Nucleus
NLI – Nucleolus
LPNS - Late Perinucleolus Stage
CT – Connective Tissues
BV – Blood vessels

T- Theca radiate
G - Theca Granulosa
Z - Zona Pellucida
N - Nucleus
NLI – Nucleolus
YG-Yolk Globule

E : LYVS – Late Yolk Vesicle Stage (× 400) F : EYS – Early Yolk Stage (× 400)

YG – Yolk Granules
YV – Yolk Vesicles
NLI– Nucleoli
YN – Yolk Nucleus

EVC- Extra Vesicle Cytoplasm
VM - Viteline Membrane
YN – Yolk Nucleus
NLI– Nucleolus

G: LYS – Late Yolk Stage (× 400)

YG – Yolk Granule
YV – Yolk Vesicle
PLATE NO. 12: T. S. OF OVARY (Heamatoxyline and Eosin)

A: Preparatory Phase (Control) (×400)
LYVS – Late Yolk Vesicle Stage
EPNS – Early Perinucleolus Stage
EYS – Early Yolk Stage
EYVS – Early Yolk Vesicle Stage
LPNS – Late Perinucleolus Stage

B: Preparatory Phase (Injected) (×400)
CNS- Chromatin nucleolus stage
LPNS – Late Perinucleolus Stage
EPNS – Early Perinucleolus Stage
N- Nucleus
NLJ- Nucleolus
YV- Yolk Vacuoles

C: Pre-Spawning Phase (Control) (×400)
T- Theca radiate
G- Granulosa
Z- Zona Pellucida
N- Nucleus
NLJ- Nucleolus
YG- Yolk Granules

D: Pre-Spawning Phase (Injected) (×400)
T- Theca radiate
G- Granulosa
Z- Zona Pellucida
NLJ- Nucleolus
VM- Vitelline Membra
YV- Yolk Vesicle
YG- Yolk Granules
PLATE NO. 13 : T. S. OF OVARY (Hematoxyline and Eosin)

A: Spawning Phase (Control) (×400)
  BV- Blood Vessles
  N- Nucleus
  NLI- Nucleolus
  YV- Yolk Vesicle
  YG- Yolk Granules

B: Spawning Phase (Injected) (×400)
  N- Nucleus
  NLI- Nucleolus
  YV- Yolk Vesicle
  YG- Yolk Granules

C: Post-Spawning Phase (Control) (×400) (×400)
  CNS- Chromatin nucleolus stage
  LPNS- Late Perinucleolus stage
  EYS- Early Yolk stage
  A- Atresia

D: Post-Spawning Phase (Injected)
  LPNS- Late Perinucleolus stage
  EPNS – Early Perinucleolus Stage
  A- Atresia
CYCLIC CHANGES:

In pituitary gland only basophil cells of proximal pars distalis showed the significant changes in gonadal maturation during reproduction cycle.

These changes have been correlated with gonadal as well as reproductive cycle in fresh water fish, Channa gachua. These changes were observed under four different phase’s viz. hypertrophy, granulation, degranulation and vacuolization.

1. **Preparatory Phase** (late February to April):

*Pituitary:*

- Proximal pars distalis showed weak affinity for stain i.e. MTS I, MTS II and MTS III.
  - The glycoproteiniceous granule shows gradual restoration (Plate No. 14; A).

*Testis:*

- The presences of primary and secondary spermatogonia were observed.
  - The lobules were small in size with thick interlobular septa (Plate No. 14; B).

*Ovary:*

- Oogonia were conspicuous, nuclei and cellular ramments of chromatin nucleolus, follicles beginning.
• Oogonia were gradually transformed into oocytes.

• Ooplasm found full of yolk content (Plate No. 14; C).

2. Pre-spawning phase (May to June):

Pituitary:

• Basophil cells of proximal pars distalis increased in volume.

• Increased volume get tightly packed, and responded strongly with MTS

• Cytoplasm was thick and granulated.

• Occasionally, certain degranulated and vacuolated cells were also observed (Plate 15; A).

Testis:

• Progressive stages of spermatogenesis and thin interlobular septa.

• At this phase, mostly the primary and secondary spermatocytes were observed (Plate 15; B).

Ovary:

• Showed progressive vitellogenesis resulting in the formation of fully matured ova (Plate 15; C).
3. **Spawning phase** (late June to July):

**Pituitary:**
- Basophils of proximal pars distalis showed marked degree of hypertrophy and preponderate over other cell type. They were still strongly MTS II + ve
  - All most all cells are full of granulation.
  - A few disintegrating cells were observed (Plate 16; A).

**Testis:**
- Mostly the spermatids and spermatozoa were observed.
- Indicated the beginning of the spawning phase (Plate 16; B).

**Ovary:**
- Fully yolk loaded oocytes were observed.
- Thecal and granulosa layers were observed very prominent.
- Indicated the beginning of the spawning phase (Plate 16; C).

4) **Post-spawning phase** (August to September):

**Pituitary:**
- The basophils became degranulated and stained feebly.
- The cells were mostly denuded of their cytoplasm (Plate 17; A).

**Testis:**
- Spent condition with most of the lobules having empty lumina.
• A few lobules, however, contain relic masses of residual spermatozoa. (Plate 17; B).

**Ovary:**

• Ovary showed spent condition with atretic oocytes (Plate 17; C).
PLATE NO. 14: SEASONAL CHANGES (Preparatory Phase)

A: T.S OF PITUITARY GLAND (× 400) (MTS)

HBC- Hypertrophed basophils
GBC-Granulated basophils

B: T.S. OF TESTES (× 400) (Heamatoxyline and Eosin)

ST- Seminiferous Tubules
PSG- Primary Spermatogonia
SSG- Secondary Spermatogonia
LC- Leydig Cell

C: T.S. OF OVARY (× 400) (Heamatoxyline and Eosin)

EPNS- Early Perinucleolus stage
EYVS- Early Yolk Vesicle stage
LYVS- Late Yolk vesicle stage
EPS- Early Perinucleolus stage
PLATE NO. 15: SEASONAL CHANGES (Pre-spawning Phase)

A: T.S OF PITUITARY GLAND (× 400) (MTS)

GBC-Granulated basophils
DBC- Degranulated Basophils
VBC- Vacoulted basophils

B: T.S. OF TESTES (× 400) (Heamatoxyline and Eosin)

SSG- Secondary Spermatogonia
SSC- Secondary spermatocyte
PSC-Primary spermatocyte

C: T.S. OF OVARY (× 400) (Heamatoxyline and Eosin)

N- Nucleus
NL- Nucleolus
YV- Yolk Vesicle
PLATE NO. 16: SEASONAL CHANGES (Spawning Phase)

A: T.S OF PITUITARY GLAND (× 400) (MTS)
- GBC-Granulated basophils
- DBC-Degranulated basophils
- DCY-Degranulated Cyanophils

B: T.S. OF TESTES (× 400) (Heamatoxyline and Eosin)
- SD-Spermatids
- SZ-Spermatozoa

C: T.S. OF OVARY (× 400) (Heamatoxyline and Eosin)
- T-Theca radiate
- G-Granulosa
- Z-Zona Pellucida
- N-Nucleus
- NLI-Nucleolus
- YV-Yolk Vesicle
- YG-Yolk Granules
PLATE NO. 17: SEASONAL CHANGES (Post spawning Phase)

A: T.S OF PITUITARY GLAND (× 400) (MTS)

DBC- Degranulated basophis
VBC- Vacuolised basophis

B: T.S. OF TESTES (× 400) (Heamatoxyline and Eosin)

RS- Residual Spermatozoa
CT- Connective tissues

C: T.S. OF OVARY (× 400) (Heamatoxyline and Eosin)

EPNS- Early Perinucleolus stage
EYS- Early Yolk stage
A- Atresia