CHAPTER 2.

Histology and histochemistry of gonads in
Channa punctatus (Bloch)
and their seasonal cycle
**Introduction**

Reproduction is a complex physiological phenomenon involving formation of gametes, fertilization and development of young ones. The reproductive cycle in fishes is dependent not only on the internal factors but also on the external factors. The physiology of reproduction in teleost fishes has drawn much attention of a number of workers and many reviews have appeared on the various aspects of this important physiological event (Hoar, 1955 and 1957; Bell, 1960; Haven, 1961; Nath, 1965; Loft and Bern, 1972; Guraya, 1976 a, b and Himel and Merrick, 1982).

Seasonal changes in the gonads during reproductive cycle have been studied by a number of workers. The ovarian cycle of teleosts has been studied by Hann (1927) in Cottus bairdii, Craig-bennett (1931) and Swaroop (1958) in Gasterosteus aculeatus, Hickling (1935) in Herlichius merluccius, Matthews (1938) in Fundulus heteroclitus, Turner (1938 a, b) in Cymatogaster aggregatus and Brachypris edinopoma, Bullough (1939) in Phoxinus laevis, Mendoza (1943) in Neotoca bilineata, James (1946) in Lepomis macrochirus and Huro salmoides, Yamamoto (1956) in Leopsetta obscura, Gokhale (1957) in Gadicus merlangs and Gadus esmarkii, Beach (1959) in Carassius auratus, Sendararaj (1959) in Heteropneustes fossilis, Bare (1960) in Scophor scophor, Sathyanesan (1961, 1962) in Barbus stigma and Avesus seenchale, Balsare (1962) and Verma et al., (1963) in Channa punctatus, Khanna and Pant (1967) in Glyptosternum pectinatus, Sinha and

These studies of seasonal changes in the ovaries and testes of fishes were done with the help of conventional histological techniques. As compared to voluminous histological studies, the histochemical studies are very less. Recently, Loft and Bern (1972) and Guraya (1976 a, b), have, however, contributed a lot to the histochemical studies of lipids in the gonads of male and female fishes.

The present work has been aimed to study the histological changes in the gonads of *Channa punctatus* (Bloch) during different phases of reproductive cycle. A qualitative assessment of lipids especially phospho-lipids by employing Sudan black "B" has also been attempted.

**Observations**

**Histological and Histochemical Study of the Ovary of Channa punctatus (Bloch).**

The ovary of *Channa punctatus* consists of an ovarian wall which is made up of the outer tunica albuginea and inner germinal epithelium. The tunica albuginea consists of connective tissue comprising of collagenous and smooth muscle fibres. The ovarian wall is richly supplied with the blood vessels. Ovarian lumen is obliterated by the presence of numerous ovi
erous lamellae which are formed by connective tissue fibres, blood capillaries and germinal epithelium (Fig. 19). The oocytes in general, are rich in phospho-lipids but the quantity and quality of lipids varies in different stages of oogenesis. Following are the different stages of oocytes present in the ovary of *Channa punctatus*.
Early chromatin nucleolus stage -

This is the earliest stage of oocyte which is almost oval in shape with a thin accumulation of clear and lightly stained cytoplasm. The oocyte has a centrally placed round nucleus and deeply stained nucleolus (Fig. 6). Histochemically, the cytoplasm is sudanophilic with a few granules supposed to be mitochondria with a light intensity of sudan black 'B'. The nucleolus also reacts faintly with this dye suggesting its lipoproteinous nature (Table 3, p. 46).

Late chromatin nucleolus stage -

The oocytes are oval in shape with deeply stained cytoplasm. The nucleus is large with a single nucleolus. The chromatin granules appear to concentrate just below the nuclear membrane (Fig. 6). The cytoplasm consists of sudanophilic phospholipid bodies and lipid granules, exhibiting more intensity of stain. The nucleoli react sharply in comparison to diffused chromatin granules (Table 3, p. 46).

Early peri-nucleolus stage -

This stage is oval in outline. A thin layer of follicular epithelium begins to develop around the oocyte and the cytoplasm takes deeper stain. The nucleus increases in size and becomes more or less eccentric. The nucleolus breaks into two to three small nucleoli placed below the nuclear membrane along with the chromatin matter (Fig. 7). The follicular epithelium is sudan +ve in nature. The cytoplasm consists of lipid bodies and lipid granules with
uniform distribution but the intensity of stain is certainly more in lipid bodies in comparison to the lipic granules. The lipo-protein granules in the chromatin complex start concentrating in the centre. The nucleoli, whose number has been increased, continue to be sudanophilic without any change in the intensity of staining (Table 3, p. 46).

**Late peri-nucleolus stage**

The shape of oocyte remains oval. The follicular epithelium covers the oocyte completely. The yolk nucleus of Dalebiani appears in the deeply stained cytoplasm as a small round body in the juxtanuclear position. The number of nucleoli now increases to 8 to 10 which still remain below the nuclear membrane. The chromatin matter, lying in the centre of nucleus, is clumped (Fig. 8). The follicular epithelium exhibits more intensity with uniformly distributed lipid granules in the cytoplasm. The yolk nucleus is sudanophilic in nature giving a dark black appearance suggesting it a mass of mitochondria, Golgi bodies and lipid bodies. The nucleoli stain weakly in comparison to the earlier stage of oocyte. The chromatin matter exhibits concentration of lipo-proteinous granules in the centre (Table 3, p. 46).

**Early yolk vesicle stage**

The oocyte becomes round in shape. The oocyte is marked by the appearance of minute yolk vesicles in the cytoplasm near its periphery. The yolk nucleus migrates towards the periphery of the oocyte and becomes granular. The nucleus is also round in shape lying in the centre of the oocyte. The chromatin matter is
faintly stained while the nucleoli remain as such (Fig. 9). The yolk vesicles in the cytoplasm of oocyte react faintly to the sudan black 'B'. The yolk nucleus also shows a weak (i.e. less intense) reaction in comparison to the late peri-nucleolus stage. The nucleoli as well as the chromatin matter exhibit weak staining property possibly due to less accumulation of lipo-proteinous material (Table 3, p. 46).

**Late yolk vesicle stage**

The oocyte is covered with a distinct follicular epithelium. The yolk vesicles formed near the periphery of the oocyte in early yolk vesicle stage now increase in size and fresh yolk vesicles appear in cytoplasm towards the nucleus. No change occurs in the position of yolk nucleus. The nuclear membrane begins to show folded outline. The chromatin matter and nucleoli are deeply stained (Fig. 9). The follicular epithelium exhibits a very strong intensity of phospho-lipids. The cytoplasm with increased number of yolk vesicles gives a +ve reaction for phospho-lipids with sudan black 'B' stain. The yolk nucleus as well as the chromatin matter show a weak response of dye (Table 3, p. 46).

**Early yolk stage**

The oocyte is covered with an outer theca, middle follicular layer and inner vitelline membrane. The yolk formation takes place in the form of yolk granules which appear within those yolk vesicles which are situated near the nucleus. These yolk granules are lightly basophilic in nature. Later on they increase in size in the form of yolk globules with the
Photomicrographs of sections of the ovary of _Ghanna punctatus_ showing various stages of oocytes:

**Fig. 6** - Early and late chromatin nucleolus stages

**Fig. 7** - Early and late peri-nucleolus stages

**Fig. 8** - Yolk-nucleus in the late peri-nucleolus stage

**Fig. 9** - Early and late yolk vesicle stages

**Abbreviations:**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>E.C.N.</td>
<td>Early chromatin nucleolus stage</td>
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<td>E.P.N.</td>
<td>Early peri-nucleolus stage</td>
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<td>E.Y.V.</td>
<td>Early yolk vesicle stage</td>
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<td>L.C.N.</td>
<td>Late chromatin nucleolus stage</td>
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<td>Late yolk vesicle stage</td>
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<td>Y.N.</td>
<td>Yolk-nucleus</td>
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addition of more yolk and become strongly acidophilic. The yolk nucleus starts shrinking and later becomes indistinguishable. The nuclear membrane is folded and nucleoli give a faint response to basic dyes (Fig. 10). The follicular layer gives a most +ve response to sucan black 'B' showing presence of phospho-lipids. The yolk globules are sucanophilic in nature, more over, the intensity of stain in the yolk globules present around nuclear membrane is more in comparison to the yolk globules of the peripheral region. The nucleus, however, does not show any reaction to histochemical stains for lipids.

**Late yolk stage**

The oocyte becomes irregular in shape with well distinct theca, follicular layer and vitelline membrane. Almost all the yolk vesicles contain yolk globules. The nuclear outline exhibits further foldings and nucleoli are scattered in the nucleoplasm (Fig. 10). The intensity of sucanophilic yolk globules increases exhibiting strong +ve reaction which indicates the presence of phospho-lipids. The reaction of nucleus remains the same to the stain as in the case of early yolk stage (Table 3, p. 48).

**Pre-maturation stage**

The oocyte remains irregular in shape, packed with yolk globules and disorganised yolk vesicles. The nucleus now becomes too small in size with a few and almost unrecognisable nucleoli (Fig. 11). The yolk globules, which increase in number, show intense sudanophilic activity. The phospho-lipic activity in the other parts of oocyte has not been observed.
**Nature stage**

The vitelline membrane acquires radial striations. The oocyte is filled up with acidophilic yolk globules. The yolk vesicles around the nucleus are seen with broken outlines. The nucleus shrinks further and the nuclear membrane remains no longer distinguishable. The cells of follicular epithelium placed around the oocyte also exhibit lipid activity. Whole oocyte is now filled with yolk granules strongly +ve to Sudan black 'B' stain.

**Corpora atretica**

The immature oocytes which fail to attain maturity at any stage of their development or mature oocytes which fail to spawn, undergo a process of resorption. Such oocytes are termed as corpora atretica. Bretschneider and Luyvene de Wit (1947) have described four stages in the process of atresia in *Aphrodes anguilla*. Following are the stages of atretic follicles described in the present study.

**Stage I**: The follicular epithelium of oocyte loses its syncytium-like appearance and undergoes hypertrophy. The follicle cells exhibit definite outline with small granules in them. The vitelline membrane also loses its shape. The yolk globules liquify into a continuous mass of yolk. A few vacuoles are also seen within the liquified yolk mass (Fig. 12).

The follicular epithelium exhibits less intensity of lipid granules. The yolk globules appear black in groups with spaces due to the process of vacuolization.

**Stage II**: The process of hypertrophy continues in the follicular epithelium and the follicular cells come in close contact with the
Photomicrographs of sections of the ovary of *Channa punctatus* showing various stages of oocytes:

**Fig. 10** — Early and late yolk stages

**Fig. 11** — Prematuration and mature stages

**Fig. 12** — Corpus atreticum (Stage I)

**Fig. 13** — Corpus atreticum (Stage IIInc)

**Abbreviations:**

- E.Y. — Early yolk stage
- F.L. — Follicular layer
- L.Y. — Late yolk stage
- M. — Mature stage
- O.W. — Ovarian wall
- P.M. — Prematuration stage
- V.M. — Vitelline membrane
- Y. — Yolk
vitelline membrane with an increase in the number of desintegrated granules. The vitelline membrane breaks up and gets disorganised. The process of liquification of yolk continues further (Fig. 13).

The follicular epithelium of this stage is the only region which exhibits the presence of lipid activity though with a very weak intensity.

Stage III : The follicular epithelial cells are further hypertrophied with very few granules. The vitelline membrane further breaks in fragments and yolk gets completely liquified (Fig. 14).

The follicular epithelial cells look faint black situated around the small patches of liquified yolk scattered unevenly due to high degree of vacuolation.

Stage IV : The follicle is completely filled with the invaded follicular cells and large number of vacuoles. The granules of follicle cells disappear. The vitelline membrane is not traceable. The yolk is almost exhausted (Fig. 15).

The cells of follicular epithelium are scattered exhibiting distinTEGRATED faintly stained lipid granules. As a whole, this stage looks transparent with traces of black bodies scattered unevenly.

**Post-ovulatory follicles**

The mass of follicular cells left behind after the extrusion of mature oocyte is called as post-ovulatory follicle or ruptured follicle or empty follicle. These follicle cells undergo proliferation; the whole mass then gets distorted and nuclei of
Photomicrographs of sections of the ovary of *Channa punctatus* showing:

**Fig. 14** - Corpus atreticum (Stage III)

**Fig. 15** - Corpus atreticum (Stage IV)

**Fig. 16** - Post ovulatory follicle

**Abbreviations:**

F.L. - Follicular layer

P.F. - Post ovulatory follicle

V. - Vacuole

Y. - Yolk
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<th>EPNS (3)</th>
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**TABLE 3**: Showing the qualitative assessment of phospho-lipids and lipo-proteins in the oocytes of *Channa punctatus* (Bloch).

+++ = Strong reaction
++  = Moderate reaction
+   = Weak reaction
-   = No reaction

Note: 1 to 10 are the stages of oocytes as mentioned in the text.
follicle cells lose its identity. The post-ovulatory follicles finally get absorbed in the stroma of the ovary. The lipid granules are found scattered unevenly which show a weak intensity towards sudan black 'B' stain (Fig. 16).

Concluding remarks:

Histological study of various stages of oocytes show structural changes but their chemical nature and qualitative changes also contribute to specify the significance of growth of oocyte. Histochemically the oocytes contain phospho-lipids and lipo-proteins in the cytoplasm. The phospho-lipids and lipo-proteins are present in the various stages of oocytes in the different quantity which can be examined by the tinctorial behavior of oocyte exhibited by sudan black 'B' test (McManus, 1946) and Acid-haematin test (Baker, 1946).

The early and late chromatin nucleolus stages are rich in phospho-lipids as well as lipo-proteins represented by lipid bodies and lipid granules. The lipid bodies are strong sudanophilic as compared to lipid granules. The lipid bodies are quite abundant in the cytoplasm and follicular cell layer while the lipid granules are mostly present in chromating matter. The yolk vesicles are weak sudanophilic bodies in early and late yolk vesicle stages while the yolk globules exhibit strong intensity of lipoid matter in early and late yolk stages. The quantity of lipids decreases sharply in the pre-maturation and mature stages of oocytes as a weak sudanophilic activity is exhibited.

Histological and Histochemical Study of the Testis of Channa punctatus (Bloch)

The testis is covered by a thin sheath of peritoneal membrane below which lies a comparatively thick layer of tunica albuginea composed of epithelial cells, fibrous connective tissue
and blood vessel. The testis is divided into a number of well
defined seminiferous tubules separated from each other by a layer
of interstitial tissue having interstitial cells, connective tissue
and blood vessels.

Different organelles of spermatogenetic cells exhibit
different intensity of phospho-lipid contents. Keeping in view,
the following are the different stages of spermatogenesis in the
fish, *Channa punctatus*.

**Primary spermatogonia**

These cells are located in the tubules of peripheral region
of the testis. These are smaller cells with inconspicuous cell
boundary. They have a round nucleus with a single nucleolus which
lies in the centre. The nucleoplasm consists of chromatin granules
concentrated near the nuclear membrane. (Fig. 17).

The cytoplasm consists of lipid bodies and lipid granules.
The lipid bodies however, are more intense in comparison to the
lipid granules. The nucleolus is also sudan black 'B' +ve but
chromatin material is −ve to sudan black 'B' (Table 4, p. 54).

**Secondary spermatogonia**

These cells are slightly large in size with a vesicular
nucleus which contains a centrally placed nucleolus. Chromatin
granules are evenly distributed around the nucleolus (Fig. 17).

The cytoplasm consists of more lipid bodies and lipid
granules. The nucleolus is also sudanophillic but the intensity
is less in comparison to the primary spermatogonia, moreover the
chromatin granules are sudan black 'B' −ve (Table 4, p. 54).
Primary spermatocytes -

These cells are round in shape with a centrally placed nucleus. The chromatin material gets clumped on one side of the nucleus due to which it appears to be crescent shaped. The nucleolus is indistinguishable (Fig. 17).

The cytoplasm is sudanophilic with feebly stained lipid granules. The nucleus also shows a weak sudan black 'B' +ve test (Table 4, p. 54).

Secondary spermatocytes -

These cells are round in shape, comparatively small with chromatin matter evenly spread in the nucleus (Fig. 18).

Large number of sudanophilic bodies appear in the cytoplasm. The nucleus is also sudan black 'B' +ve with more lipid granules.

Spermatids -

These cells are considerably small in size with deeply stained eccentric nucleus.

At this stage the cytoplasm consists of very few sudanophilic lipid bodies and sparsely distributed lipid granules. The lipid granules appear more prominent at this stage with relatively deep sudan black 'B' +ve reaction. Later on, the sudanophilic bodies get concentrated towards the posterior part of the cell (Table 4, p. 54).

Spermatozoa -

A further reduction in the size of cell with concentration of nuclear material at one end marks the formation of spermatozoon head while accumulation of cytoplasm on other end forms the middle piece.

The head is entirely sudan black 'B' -ve while middle piece is deeply sudanophilic.
Photomicrographs of sections of testes of *Channa punctatus* showing different stages of spermatogenesis during reproductive cycle:

**Fig. 17** - Primary germ cells, spermatogonia and spermatocytes during post spawning period

**Fig. 18** - Spermatids and spermatozoa along with other earlier stages of spermatogenesis during pre spawning period

**Abbreviations:**

- I.T. - Interstitial tissue
- P.G.C. - Primary germ cells
- P.SPC. - Primary spermatocytes
- S. - Spermatids
- SP. - Spermatozoa
- SP.G. - Spermatogonia
- S.S.P.C. - Secondary spermatocytes
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<td>Bouin</td>
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**TABLE 4**: Showing intensity of phospho-lipid contents in the organelles of spermatogenetic cells during spermatogenesis in *Channa punctatus* (Bloch).

+++ = Strong reaction  
++  = Moderate reaction  
+   = Weak reaction  
-   = No reaction.
Concluding remark:

The histochemical observations of the testes indicate the dominance of phospho-lipids in the early stages of spermatogenesis (Table 4, p. 57). The lipid granules are also more intense in the spermatid cells which mark the increased number of mitochondria. These granules accumulate towards the posterior part of the cell to form middle piece of spermatozoa which is deeply sudanophilic while the accumulation of nuclear material towards anterior end, forming the head of spermatozoa, gives no reaction with sudan black 'B'.

Seasonal changes in the Ovary of Channa punctatus -

On the basis of the study of morphology and histology of the ovary in different months, the reproductive cycle of Channa punctatus may be divided into following periods:

- Post spawning period ... November to February
- Pre spawning period ... March to mid April
- Spawning period - I ... Mid April to May
- Preparatory period ... June to mid July
- Spawning period - II ... Mid July to October

The ovaries show very little changes in their morphological appearance during the seasonal cycle. They remain smaller in size and smooth in appearance in the post- and pre spawning periods while elongated, swollen and granular in appearance during spawning period. The ovaries get shrunken just after the spawning period and some time look beaded in appearance.

During post spawning period, the wall of ovary is thick and the ovigerous lamellae are formed which extend from the ovarian
wall to the lumen of the ovary. The inter follicular and inter lamellar spaces are quite distinct. Oocytes of chromatin nucleolus and peri-nucleolus stages are present along with some mature stages. The corpora atretica and post-ovulatory follicles are also present (Fig. 20).

During pre spawning period the wall of ovary becomes thick and highly vascular. The inter follicular and inter lamellar spaces get reduced. Along with the earlier stages of oocytes, few oocytes of early and late yolk vesicle and early yolk stage appear. The corpora atretica are few in number while post ovulatory follicles are completely absent throughout this period (Fig. 22).

During 1st spawning period, the wall of ovary becomes thick, the ovigerous lamellae are well formed and inter follicular spaces and inter lamellar cavities are reduced. Almost all the oocytes are mature with few oocytes of early stages of oogenesis which are mostly in their peri-nucleolus stage. The corpora atretica and post-ovulatory follicles are observed during this period (Fig. 22).

During preparatory period, the ovarian wall is thick and the ovigerous lamellae become obliterated. The inter follicular spaces are quite prominent. Most of the oocytes are in late yolk vesicle stage and early and late yolk stages are also found. Some corpora atretica and post-ovulatory follicles continue to be present in this period also (Fig. 23).

During IIInd spawning period, the ovigerous lamellae get disorganised as the ovary becomes packed with the mature stages of oocytes. A few early stages of oocytes are also present in between
Photomicrographs of sections of ovary of *Channa punctatus* showing:

**Fig. 19** - Early oogenetic stages with distinct ovarian wall and ovigerous lamellae during post spawning period.

**Fig. 20** - Post ovulatory follicles and early stages during early post spawning period

**Fig. 21** - Yolk vesicle stage and early stages of oogenesis during the pre spawning period

**Abbreviations**: 

- E.Y.V. - Early yolk vesicle stage
- L.P.N. - Late peri-nucleolus stage
- L.Y.V. - Late Yolk vesicle stage
- D.I. - Ovigerous lamellae
- O.W. - Ovarian wall
- P.F. - Post ovulatory follicle
Photomicrographs of sections of ovary of *Channa punctatus* showing:

**Fig. 22** - Later stages of oogenesis during early spawning period I

**Fig. 23** - Various stages of oocytes during preparatory period

**Fig. 24** - Pre maturation and mature stages during early spawning period II

**Abbreviations:**

- **C.A.** - Corpus atreticum
- **E.P.N.** - Early peri-nucleolus stage
- **L.Y.** - Late yolk stage
- **L.Y.V.** - Late yolk vesicle stage
- **M.** - Mature stage
- **P.M.** - Pre maturation stage
the mature oocytes (Fig. 24). The corpora atretica and post-ovulatory follicles are numerous in their various stages of resorption.

Concluding remarks:

The fish, *Channa punctatus* breeds twice a year. First spawning period is of shorter duration than the second spawning period which continues from July to October. The process of multiplication is fast and ovary always exhibits a new crop of oocytes. The corpora atretica are also present throughout the cycle which are more in number during post spawning and early preparatory period than in other periods of the reproductive cycle.

**Histological seasonal Changes in the testis of Channa punctatus (Bloch) during reproductive cycle**

The testes of *Channa punctatus* exhibit changes in their morphological and histological structure during different periods. On the basis of these changes the reproductive cycle is divided into the following periods.

- **Post spawning period** ... November to February
- **Pre spawning period** ... March to Mid April
- **Spawning period I** ... Mid April to May
- **Preparatory period** ... June to Mid July
- **Spawning period II** ... Mid July to October.

The testes are poorly developed during post spawning period. They are small in size and are white in colour.

The seminiferous tubules are small in diameter. The connective tissue gets thickened while the interstitial cells are
Photomicrographs of sections of testes of *Channa punctatus* showing different stages of spermatogenesis during:

Fig. 25 - Spawning period I
Fig. 26 - Preparatory period
Fig. 27 - Spawning period II

**Abbreviations:**

P.SPC. - Primary Spermatocytes
S. - Spermatids
SP. - Spermatozoa
conspicuous. The tubules which lie towards the peripheral part of
the testes are filled with early stages of spermatogenesis while
those which occupy the central region are sparsely filled with
spermatozoa (Fig. 17).

During pre spawning period the testes do not show any
appreciable change in their shape and colour from those observed
in the post spawning period.

The tubules grow larger in calibre. The connective tissue
is thick with rich supply of blood vessels. The tubules of the
peripheral region contain primary spermatogonia while spermatozoa
are present in the central region. Large number of secondary
spermatogonia, spermatocytes and spermatids are found in the tubules
present in between peripheral and central region.

During 1st spawning period the connective tissue is reduced
but with a rich vascular supply. The number of interstitial cells
is also reduced. The seminiferous tubules increase in diameter.
Even the tubules of peripheral region are filled up with spermatozoa
and other stages of spermatogenesis (Fig. 25).

During preparatory period, the interstitial tissue gets
organised again and the number of interstitial cells increases.
This period is of active phase when the rate of multiplication of
eyearly stages becomes faster beside the rebuilding of tunica
albuginea (Fig. 26).

During 2nd spawning period, the testicular wall is thin.
The connective tissue placed in between the seminiferous tubules
is also thin and highly vascularised. The tubules are much larger
in diameter. All stages of spermatogenesis are seen in the early
spawning period (i.e. August) but the tubules get fully packed with spermatozoa in the mid spawning period (i.e. September) (Fig. 27). The process of maturation is more rapid in second spawning period than the first one.

Concluding remarks -

Though the spermatogenesis in Channa punctatus is a continuous process the spermiation occurs twice a year, however, the duration, growth and spawning activity is more pronounced in IInd spawning period in comparison to ISt spawning period. All the stages of spermatogenesis are seen with certain degree of numerical variation, throughout in any of the phase of the reproductive cycle.

DISCUSSION

Considerable literature exists revealing the morphology of the ovary. Brock (1879) and Calderwood (1892) confined their studies to the general structure of the ovary only. Detailed study of the histology of the ovary has been made by Hann (1927) in Cottus bairdii, Craig-Bennett (1931) and Swarup (1958) in Gasterosteus aculeatus, Hickling (1935) in Merluccius merluccius, Matthews (1938) in Fundulus heteroclitus, Turner (1938 a, b) in Cymatogaster aggregatus and Brachyrhadinus episcopi, Bullough (1939) in Phoxinus laevis, Mendoza (1943) in Nectopsa bilineata, James (1946) in Lepomis macrochirus and Huro salmoites, Ghosh and Kar (1952) and Sundararaj (1959) in Heteropneustes fossilis, Beach (1959) in Carassius auratus, Yamamoto (1956) in Lionetta obscura (1960), Bara (1960) in Scomber acomber, Sathyanesan (1961, 1962) in Barbus stoma and Mystus seenghala, Belsare (1962) in Ophioccephalus punctatus, Khanna and Pant (1967) in Glyptosternum pectinosternum.

The origin of new crop of oocyte is a subject of controversy. According to some workers, including Wheeler (1924), Yamamoto (1956), Andrew and Pinto (1957), the new crop comes up from the follicle cells of the empty follicles which are left behind after the release of the mature oocytes. Other workers, including Stuhlmann (1887), Cunningham (1898), Wallace (1903), Franz (1909), Mendoza (1943), Tromp-Blom (1959), Bara (1960), Khanna and Pant (1967), Sinha and Kastogi (1967), Raizada (1971), Saksena (1976), Bais (1977), are of the opinion that the new crop proliferates from the germinal epithelium. Hann (1927), Craig-Bennett (1931), Eggert (1931), Stenger (1959), Honma (1961), Belsare (1962) and Lehri (1968) held the view that the new crop of oocyte originates directly from the germinal epithelium and transformed into secondary oocyte following a short period of rest. Coetzee (1983) in *Chimerius nufar* also described that various stages of oocyte development project from tunica albuginea towards the centre of the ovary. In the present study in *Channa punctatus*, the earliest stage of oocyte (early chromatin nucleolus stage) is derived from the germinal epithelium of the ovary as it is seen associated with the germinal epithelial layer of the ovary. It indicates that the germinal epithelium is responsible for the production of new crop of oocytes.

The oogenesis is a continuous process which leads to the development of mature oocytes. Various components of the oogonial
cells undergo certain changes and these changes signify growth and chemical changes occurring during the process.

The origin, extrusion and role of nucleoli during oogenesis have been discussed by Eggert (1931) and Chaudhary (1951). They feel that a single nucleolus deviates by fragmentation and gives rise to a number of nucleoli. Yamamoto (1956) and Bara (1960) could not observe such a process of fragmentation. Bara (1960) has pointed that the peripheral nucleoli are fused bodies emerging from the peripheral ooplasm. Bullough (1939) in Phoxinus laevis has described a syneysis stage in which chromatin material is accumulated at one side of the nucleus during the formation of primary oocyte. Peiqiu (1960) in Pseudosciaena polyactis described the growth of oocyte as 'heterochromic', the accumulation of fat in the oocyte starts from periphery of the nucleus and the formation of yolk begins from the edge of the membrane of the oocyte. In the present study no such stages have been observed. In Channa punctatus the first stage of oogenesis has been recognised as early chromatin nucleolus stage in which nucleolus contains sudanophilic single nucleolus, embedded in the chromatin reticulum. In the peri nucleolus stage the nucleolus breaks up into two to three nucleoli arranged below the nuclear membrane. The chromatin material takes up faint stain due to lipo-proteinous nature. Finally the size of nucleoli decreases but the process of nucleolar extrusion was not clear in the present study.

Many workers have described a cytoplasmic body in the vicinity of the nucleus in the early oogenetic stages of various group of animals and have termed it as yolk nucleus, centrosphere, archoplasm, crop vitelline and Balbiani body. Yolk nucleus was first described by Hubbord (1894) in Cymatogaster as a crescentic
mass capping the nucleus. This, later on, migrates towards the peripheral zone of the ovum. Similar structures have been reported by Cunnigham (1993), Franz (1909), Wheeler (1924), Narain (1937), Mendoza (1943), Chaudhary (1952), Yamamoto (1956 b), Sathyanesan (1959), Bara (1960), Yamamoto and Yamazaki (1961), Gopal Lutt (1964), Nayar (1964), Bhargava and Saksena (1971), Malaviya (1973) and Forberg (1982). Guraya (1979) suggested that this apparent zonation may be due to aggregation of ribo-nucleo-protein particles having been extended through the nuclear membrane when these aggregates become surrounded by cytoplasmic organelles they are variously known as yolk nuclei or Balbiani bodies. Howell (1963) found no such structure in the flounder, Limanda ferrucina. Various views have been put forward regarding function of yolk nucleus. Wallace (1904) expressed it as a seat of yolk synthesis. Chaudhry (1952) considered it as a catalytic agent in the formation of the yolk. Nayar (1964) has indicated that the yolk nuclei are well defined bodies and are the seat of activities like vitellogenesis and lipid synthesis. Gopal Lutt (1964), Bhargava and Saksena (1971) and Malaviya (1973) have suggested that involvement of yolk nucleus in the process of vitellogenesis is indirect. Guraya (1979) suggested that these bodies may function as centre for the formation, multiplication and accumulation of organelles and material needed for yolk deposition. In the present study the yolk nucleus has been observed at the time of initiation of yolk formation as it appeared in the late peri-nucleolus stage and persisted upto early yolk stage. Histochemical observation reveals that the yolk nucleus is a mass of mitochondria, Golgi bodies and lipid bodies. The appearance of yolk nucleus at the early stage and its disappearance at the onset of vitellogenesis indicate its possible role in the process of vitellogenesis.
The process of yolk formation is almost similar in all the teleosts (Merza et al., 1937; Matthews, 1938 and Lal, 1964). The formation of fish egg with reference to vitellogenesis and histochemy of yolk granules has been studied by (Yamamoto 1956 a, b, 1957 and 1958). Chopra (1958) in Ophiocephalus punctatus has described the intra-vacular and extra-vacular yolk granules and containing protein, carbohydrate and protein and lipoprotein respectively. In the present study of fish the yolk formation starts at early yolk stage as a small granule of yolk inside each yolk vesicle. The small yolk granules are at first lightly basophilic in nature and later on, they fuse to form yolk globules which are strongly acidophilic. Yamamoto (1956) and Khoo (1979) described that yolk vesicle apparently originates from the Golgi complex and contains muco-polysachharides representing first form of yolk inclusion. Khoo (1979) reported the displacement of these bodies towards the periphery of the oocyte. The present study confirms the findings of Yamamoto (1956), Chopra (1958) and Khoo (1979).

The atretic follicles or corpora atretica in fishes have been referred as corpora lutea, though they are not physiologically same with the structures met in the mammals. The only explanation for using the term corpora lutea (Hoar, 1955, 1957 and 1969) is due to their origin from granulose and theca cells. Hoar (1969) and Hoar and Naghama (1978) used two terms pre-ovulatory and post-ovulatory corpora lutea. The pre-ovulatory corpora lutea develop from the colysis of immature oocytes and mature oocytes which fail to spawn. Lixit (1956), Bell (1960, 1965), Bhargava (1966), Rajalakshmi (1966), Hastogi (1966), Lehri (1968), Kaur (1968), Raizada (1969 and 1975), Bhargava and Saksena (1972), Malaviya(1972)
Saksena (1974 and 1976), Anant Prakash (1976), Bais (1977), Goldberg (1980 and 1981), Coetzee (1982) and Howell (1983) and Saksena and Raizada (1984) have used the term corpora atretica for the oocytes undergoing resorption and post-ovulatory follicles or ruptured follicles for the follicular space left after ovulation. However, recent work of Nicholls and Maple (1972), Nagahama et al. (1976, 1978) and Hoar and Nagahama (1978) have suggested the terms pre-ovulatory and post-ovulatory follicles.

The process of formation and resorption of corpora atretica or pre-ovulatory follicles in *Channa punctatus* follows the general teleostean pattern and confirms the observations of Beach (1959), Belsare (1962), Rajalakshmi (1966), Bhargava and Saksena (1972), Raizada (1977), Goldberg (1980), Coetzee (1982) and Howell (1983). Histochemically, the follicles indicate rich amount of phospho-lipid during stage I which decreases in the next stages successively due to hypertrophy of granulosa cells, appearance of vacuoles and resorption of yolk. A phago-enzymatic activity has been suggested for the removal of yolk by Sathyanasan (1961) in *Mystus seenghala*, Hotma (1961) in *Plecoglossus altivelis*, Belsare (1962) in *Channa punctatus*, Rastogi (1966) in *Xenentodon cancila*, Lehri (1968) in *Clarias batrachus*, Raizada (1971 and 1977) in *Rasbora daniconius* and *Nandus nandus*, Saksena and Bhargava (1972) in *Glossogobius giuris*, Bais (1977) in *Mystus vittatus* and Szollosi et al., (1978) in *Salmo gairdneri*. The granules of granulosa cells have been referred as "disintegrating fragments" by Bretschneider and Duyvende de wit (1947) and Lehri (1968). Ball (1960) considered them as source of ovarian hormone. In the present study, the granules are prominent and are always present in the granulosa cells (follicle epithelial cells. The present histochemical study of corpora
atretica and post-ovulatory follicles for phospho-lipids does not indicate any functional significance regarding the process of resorption except the fact that there is a gradual decrease in the phospho-lipid contents of the resorptive oocyte. Nicholls and Maple (1972), Saidapur and Nadkarni (1976) Hoar and Nagahama (1976) and Schreibman et. al. (1982) have suggested that the corpora lutea or post-ovulatory follicles are capable of synthesis of steroid hormones. With the staining method used, the steroid synthesizing role of corpora atretica and post-ovulatory follicles could not be established in the fish under study.

Study of ovarian endocrine tissue by classical methods of cytology and enzymatic histochemistry have been made by Hoar (1969) Billard et. al. (1972), Guraya (1976 a), Nagahama et. al. (1976), Saidapur and Nadkarni (1976), Szollosi et. al. (1978), Hoar and Nagahama (1978) and Upadhyaya and Haider (1985). Their study reveals that there are special cells present in thecal layer and these cells are responsible for the production of steroids. An ultrastructural study of pre-ovulatory follicles and post-ovulatory follicles also suggests that thecal cells are the possible site of steroidogenesis (Nagahama et. al., 1976). In the present study, such thecal cells could not be identified, however, the changes in the granulosa cells i.e., hypertrophy, vacuolization, presence of lipid droplets in the pre-ovulatory and post-ovulatory follicles suggests their probable role in steroidogenesis.

Fish spermatogenesis in fish has been studied extensively using conventional methods of histology and cytology by Turner (1919) in Perca; Geiser (1921) 1924 in Gambusia affinis; van

There have been varied opinions in connection with the presence and possible role of interstitial cells in the testes of teleosts. Marshall and Loft (1956) have denied the presence of true interstitium in *Esox lucius*, *Salvelinus willughbii* and *Labeo* sp. but they have reported certain cells of tubular boundary as Leydig cells which are homologues to the vertebrate endocrine tissue of males. Robertson (1958) in *Salmo salar* and Jai (1965) in *Tor tor* have mentioned the presence of lobular cells and interstitial cells respectively. Henderson (1962) in *Salvelinus fontinalis* and Ruby and Donald (1970) in *Eucalia inconstans*, however, have denied the presence of such cells as male endocrine tissue.
Craig-Bennett (1931), observed Leydig cells in the testes of *Gasterosteus aculeatus* and found a correlation between the interstitial cells and the testicular cycle of the fish. Essenbeck and Champy (1923), van Gorst (1925) could not establish any such correlation. Matthews (1938) also could not observe any secretory activity in the interstitial cells. Ghosh and Kar (1952) in *Heteronemurus fossilis* described interstitial cells as homologous to the Leydig cells of higher vertebrates but no functional relationship was established with the annual testicular cycle. Gokhale (1957) in *Gaurus* sp. found vacuolization in the interstitial cells after spawning and suggested that their contents play a nutritive role for the developing germ cells. Sathyanesan (1959) has also noticed vacuolization in these cells but has not discussed their role. Bai (1965) in *Tor tor* has shown both the process of vacuolization and disintegration during active phase in these cells and termed them as lobule boundary cells. Rastogi (1968) in *Amphionogaster cuchia* has also suggested the formation of new cells from the preexisting interstitial cells. Such a cellular activity of interstitial cells was not observed in *Channa punctatus* under present study.

Recent histochemical studies of Nicholls and Maple (1972) in cichlid fish, *Cichlasoma nigrofasciatum*, Grier (1976, 1984) in *Oryzias latipes* and *Heronichthys satnai*, van den Hurk et al., (1974) in *Holleniornis latipinnia*, Hoar and Nagahama (1978) in *Oncorhynchus* and *Carassius auratus* have confirmed that the interstitial cells or Leydig cells are the site for the production of male hormone. Hence, these cells are considered as cellular
source of sex steroids in male teleosts. Ultrastructural studies by Hoar and Nagahama (1976) have suggested that the sertoli cells, though containing lipid droplets, are involved in the transport of metabolites. Schreibman et al. (1962) in platy fish, *Xiphophorus maculatus* localized the 4,5-3 β hydroxy steroid dehydrogenase (G 6 P D) in the Leydig cells. The number of these cells increases which display more activity towards the periphery. In the present study male hormone synthesizing cells could not be located with staining methods employed here.

In *Channa punctatus*, the spermatozoa are seen throughout the reproductive cycle though the development of crop is in a sequence. The histochemical observation reveals that the lipid bodies are more intense in the early stage (i.e., spermatogonia) and become reduced at the later stage (i.e., spermatids). The reduced number of lipid bodies in the later stages may be due to their utilization during the process of spermatogenesis. In spermatids, the lipid bodies are concentrated towards the posterior region of the cell and mark the formation of middle piece of spermatozoa. The possible role of these lipid bodies may be in transphosphorylation process during spermatogenesis as suggested by Guraya (1976).

A quiescent period in the process of spermatogenesis has been observed by Turner (1919), Foley (1926), Craig-Bennett (1931), Loft and Marshall (1957), Rai (1965), Ahsan (1966) and Raizada (1975). In *Channa punctatus*, an inactive period was not observed and the spermatogenesis continued throughout the year.

Study of seasonal changes in male and female gonads of fish has been useful in determining different periods of the
reproductive cycle and rhythm of maturation and spawning. Marza (1938) has classified the rhythm of the maturation of oocytes into the total synchronism, the group synchronism and asynchronism. Prabhu (1956) has studied the spawning periodicities of a large number of fishes and found difference in the spawning periods of various species. He identified four type of spawning viz., spawning once in a year with smaller duration, spawning once in a year with longer duration, spawning twice a year and spawning throughout the year. In the present study, the fish Channa punctatus is found to breed twice a year hence falls under third category of the Prabhu's classification. Such spawning periodicities in fishes provide a better understanding of functional significance of reproductive cycle.