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VOLUME: 101
NO: 4
YEAR: 1990(1990)
COPYRIGHT: CCL
PAGES: 399
ISSN: 1
OCLC #: 20335057
VERIFIED:

PATRON: Godinho,Alexandre
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Eur. Arch. Biol. (Bruxelles) **101** : 399-410 (1990)

A COMPARATIVE CYTOLOGICAL AND CYTOCHEMICAL STUDY OF THE OOGENESIS IN TEN BRAZILIAN TELEOST FISH SPECIES

BY

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(Received January 10, 1990; revised and accepted March 22, 1990)

SUMMARY — *The oocyte development of ten teleost fish species, at the Três Marias Dam, Minas Gerais, Brazil, was divided into four stages. This division was based on the vitellogenesis as well as on the changes occurring in the nucleus and in the layers surrounding the oocytes : 01- young oocyte, 02- previtellogenic oocyte; 03- oocyte with cortical vesicles and 04- oocyte with yolk globules. Cytochemical techniques for carbohydrates, proteins and lipids showed neutral glycoproteins in the yolk globules and in zona pellucida in the ten species studied. The S. brandtii zona pellucida also contained acid glycoproteins. The cortical vesicles (alveoli) content varied among the species : neutral glycoproteins, sulphated or carboxylated acid glycoproteins. The presence of lipids was also observed in the yolk globules of all species studied.*

Key words : cortical alveoli, yolk globules, fish oocyte, vitellogenesis.

INTRODUCTION

In teleosts, during the process of oocyte maturation, changes occur in the nucleus, ooplasm and layers surrounding the oocyte. One of the most important events in this process is the formation and accumulation of yolk. According to DROLLER and ROTH (1966) and ANDERSON (1968), yolk is partly synthesized in the oocyte cytoplasm by several organelles (endogenous yolk); whereas the remaining yolk material is synthesized at an extra-ovarian site

(exogenous yolk), transported by the circulation, included in the oocyte by micropinocytosis. SELMAN *et al.* (1986, 1988) pointed out that the yolk vesicles which form the cortical alveoli, are not yolk in a strict sense since their content is not used as a nutrient by the embryo, but rather are analogous to the cortical granules of other vertebrate and invertebrate species and their contents are released into the perivitelline space during the cortical reaction at fertilization.

Knowledge of the chemical nature of oocyte inclusions is of great interest. Cytochemical, electrophoretic and autoradiographical observations have shown that the proportion of proteins, lipids and carbohydrates varies among teleost species (KORFSMEIER, 1966; ANDERSON, 1968; MESTER *et al.*, 1984; SELMAN *et al.*, 1986). The zona pellucida is also important in the exogenous yolk incorporation. Complex interrelations were observed between this envelope and the oocyte plasme membrane by electron microscopy (ANDERSON, 1967; WOURMS, 1976; ABRAHAM *et al.*, 1984). Recent studies from binding of lectins with carbohydrates have demonstrated asymmetry in the organization of the zona pellucida glycoconjugates (SCHINDLER and VRIES, 1989). In view of these, the present communication includes a comparative cytological and cytochemical study of the oogenesis in ten brazilian teleost fish species.

MATERIAL AND METHODS

Ovaries in maturation of 50 adult female fishes were collected in January and February, 1986, at the Três Marias Dam (18-20 °S, 44-46 °W), on the São Francisco river, State of Minas Gerais, Brazil. Five animals of following species were used in this study :

SPECIES	FAMILY
Schizodon knerii (STEINDACHNER, 1875)	Anostomidae
Leporinus piau FOWLER, 1941	Anostomidae
Leporinus taeniatus LÜTKEN, 1874	Anostomidae
Leporinus reinhardti (LÜTKEN, 1874)	Anostomidae
Serrasalmus brandtii REINHARDT, 1874	Characidae
Acestrorhynchus britskii MENEZES, 1969	Characidae
Tetragonopterus chalceus AGASSIZ, 1829	Characidae
Triportheus guentheri (GARMAN, 1890)	Characidae
Astyanax bimaculatus lacustris (REINHARDT, 1874)	Characidae
Curimatella lepidura EIGENMANN & EIGENMANN, 1889	Curimatidae

Medium-third fragments of the
fluid, embedded in paraffin, cut into
with haematoxylin/eosin, Gomori's
and also processed for the cytoch
bohydrates and proteins. In additi
fixed in 10% formalin or in formol-
sections, cut on cryostat, were proc
ing of lipids.

The following cytochemical techn
(1960) or PEARSE (1985), were used
- periodic acid-Schiff (PAS) (after
with 1:2 glycol groups : glycogen, neu
- salivary amylase (30 min at 37 °C
following of PAS;
- alcian blue 8GX-Sigma (AB) at pl
boxylated acid glycoconjugates incl
- AB at pH 0,5 for sulphated glyco
- AB at pH 2,5 plus PAS (after M
glycoconjugates;
- hydrolysis with 0,1 N HCl (8h at
acid, followed by PAS or AB pH 2,5
et al. (1961);
- ninhydrin-Schiff for protein-boun
- sudan black B for general lipids;
- nile blue sulphate for neutral and
- sudan III for neutral lipids;
- oil red 0 for neutral lipids.

RESULTS

Oogenesis starts with the proliferati
oogonia. These primordial germ cells
with a vesiculous nucleus, a central nu

The oocytes have been classifie
vitellogenesis, on the changes occurr
their surrounding layers. The followi
ed (Fig. 1) :

01- (young oocyte) : small cells, wi
vitreous cytoplasm, relatively large cen
various peripherical nucleoli.

d by the circulation, included in the
ELMAN *et al.* (1986, 1988) pointed out
orm the cortical alveoli, are not yolk
ntent is not used as a nutrient by the
gous to the cortical granules of other
pecies and their contents are released
ng the cortical reaction at fertilization.
nature of oocyte inclusions is of great
phoretic and autoradiographical obser-
roportion of proteins, lipids and car-
teleost species (KORFSMEIER, 1966;
al., 1984; SELMAN *et al.*, 1986). The
ant in the exogenous yolk incorpora-
were observed between this envelope
rane by electron microscopy (ANDER-
BRAHAM *et al.*, 1984). Recent studies
rbohydrates have demonstrated asym-
the zona pellucida glycoconjugates
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L AND METHODS

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co river, State of Minas Gerais, Brazil.
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S	FAMILY
5)	Anostomidae
	Anostomidae
	Anostomidae
	Anostomidae
4	Characidae
69	Characidae
29	Characidae
)	Characidae
HARDT, 1874)	Characidae
IGENMANN, 1889	Curimatidae

Medium-third fragments of the ovaries were fixed in Bouin's fluid, embedded in paraffin, cut into 7 μm-thick sections and stained with haematoxylin/eosin, Gomori's trichrome for cytological study and also processed for the cytochemical demonstration of carbohydrates and proteins. In addition, some fragments were also fixed in 10% formalin or in formol-calcium, and 10-12 μm frozen sections, cut on cryostat, were processed for cytochemical staining of lipids.

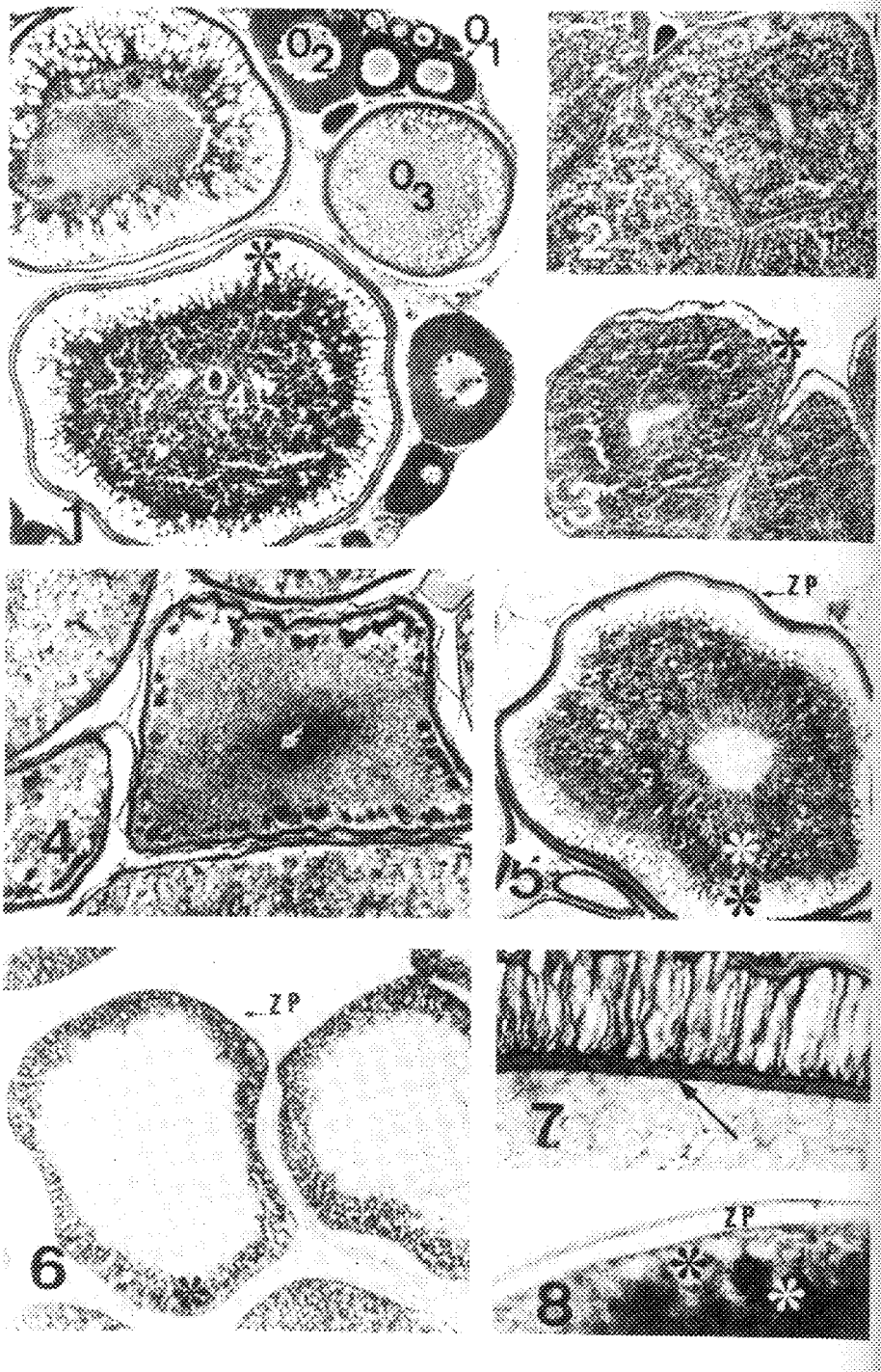
The following cytochemical techniques, as described in LISON (1960) or PEARSE (1985), were used this study :
- periodic acid-Schiff (PAS) (after McManus) for carbohydrates with 1:2 glycol groups : glycogen, neutral glycoproteins, sialomucin;
- salivary amylase (30 min at 37 °C) for extration of glycogen, following of PAS;
- alcian blue 8GX-Sigma (AB) at pH 2,5 for sulphated and carboxylated acid glycoconjugates included sialomucin;
- AB at pH 0,5 for sulphated glycoconjugates;
- AB at pH 2,5 plus PAS (after Mowry) for neutral and acid glycoconjugates;
- hydrolysis with 0,1 N HCl (8h at 60 °C) for extration of sialic acid, followed by PAS or AB pH 2,5 as described by QUINTARELLI *et al.* (1961);
- ninhydrin-Schiff for protein-bound-NH_2 groups;
- sudan black B for general lipids;
- nile blue sulphate for neutral and acidic lipids;
- sudan III for neutral lipids;
- oil red 0 for neutral lipids.

RESULTS

Oogenesis starts with the proliferation and differentiation of the oogonia. These primordial germ cells are characteristically small, with a vesiculous nucleus, a central nucleolus and scarce cytoplasm.

The oocytes have been classified in four stages based on vitellogenesis, on the changes occurring in their nucleus and on their surrounding layers. The following stages could be recognized (Fig. 1) :

01- (young oocyte) : small cells, with strongly basophilic and vitreous cytoplasm, relatively large central vesiculous nucleus, with various peripherical nucleoli.



02- (previtellogenic oocyte) : la
basophilic cytoplasm. The nucleus
colated to the nuclear membrane. I
can be observed as a conspicuous
the cytoplasm. The zona pellucida
membrane at the end of this stag
cytological preparations. A single la
surrounds the oocytes.

03- (oocyte with cortical vesicles) :
creased in size and are characterize
vesicles in the cytoplasm. These
routine cytological preparations.
spread centripetally to occupy mu
in 03. The nucleus shows an irregula
in which the nucleoli are located.
dent in this stage. The follicular ce
A. britskii, S. Knerii and squamou

04- (oocyte with yolk globules) : th
characterized by the presence of aci
stage, the cortical vesicles return to
the cortical alveoli in some species.
in position. The zona pellucida is thi
tions and is differentiated in layers
the zona pellucida consists of three
dle and external ones). The follicu

FIG. 1. Transversal section of *S. brandtii* ovary, s
stages (01, 02, 03 and 04). Note the cortical vesi
developed cortical alveoli in 04. Haematoxylin/e

FIG. 2. Absence of the cortical alveoli in 04 of *T.*

FIG. 3. Poorly developed cortical alveoli (black as
ylin/eosin. × 85.

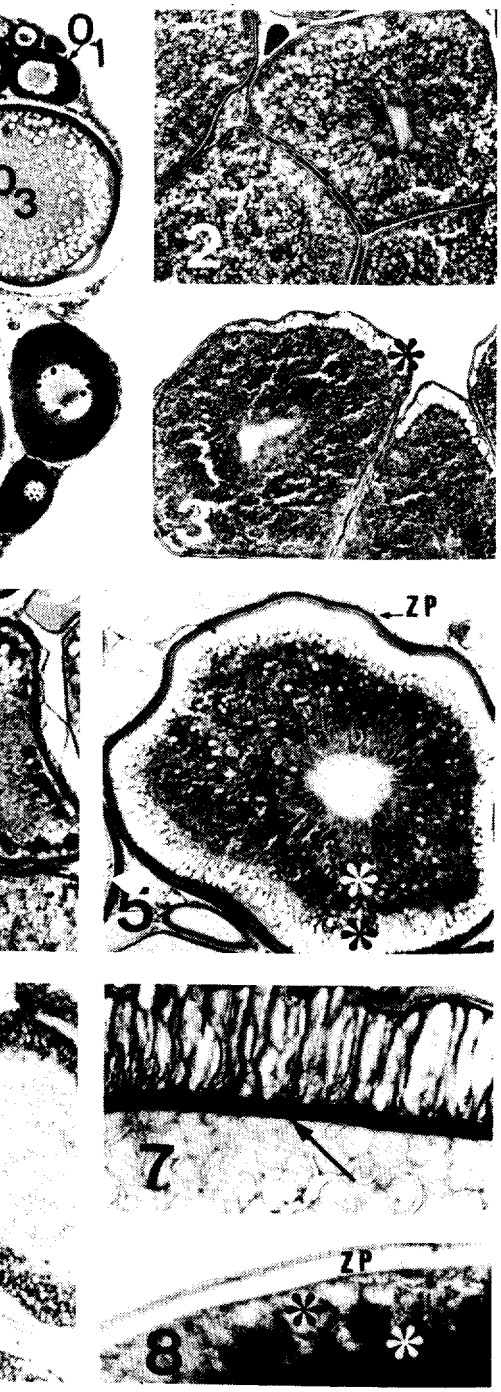
FIG. 4. PAS-positive reaction after salivary amylas
globules and in the zona pellucida of *A. britskii*

FIG. 5. 04 of *S. brandtii* showing PAS-negative react
and PAS-positive reaction in the yolk globules (w
(ZP). × 85.

FIG. 6. AB pH 2,5-positive reaction only in the corti
04. × 75.

FIG. 7. Columnar follicular cells in 04 of *S. knerii*.
in the zona pellucida (arrow). AB pH 2,5 plus P

FIG. 8. Sudan black B-positive reaction for lipids i
negative in zona pellucida (ZP) and in the cortica
04. × 319.



02- (previtellogenic oocyte) : large cells, with granular and basophilic cytoplasm. The nucleus shows peripherical nucleoli accolated to the nuclear membrane. In some oocytes, the yolk nucleus can be observed as a conspicuous structure slightly basophilic in the cytoplasm. The zona pellucida appears as a thin PAS-positive membrane at the end of this stage, but it is not very distinct in cytological preparations. A single layer of squamous follicular cells surrounds the oocytes.

03- (oocyte with cortical vesicles) : the cells have considerably increased in size and are characterized by the appearance of cortical vesicles in the cytoplasm. These vesicles are weakly stained in routine cytological preparations. They increase in number and spread centripetally to occupy much of the area of the ooplasm in 03. The nucleus shows an irregular outline forming indentations in which the nucleoli are located. The zona pellucida is very evident in this stage. The follicular cells are cuboidal in *S. brandtii, A. britskii, S. Knerii* and squamous in the other species.

04- (oocyte with yolk globules) : these are the much largest cells, characterized by the presence of acidophilic yolk globules. In this stage, the cortical vesicles return to peripherical ooplasm to form the cortical alveoli in some species. The nucleus may be eccentric in position. The zona pellucida is thicker showing transversal striations and is differentiated in layers in some species. In *S. brandtii* the zona pellucida consists of three distinct layers (internal, middle and external ones). The follicular epitelium remains simple

FIG. 1. Transversal section of *S. brandtii* ovary, showing oocytes in the four development stages (01, 02, 03 and 04). Note the cortical vesicles (black asterisk) forming a very well developed cortical alveoli in 04. Haematoxylin/eosin. × 81.

FIG. 2. Absence of the cortical alveoli in 04 of *T. guentheri.* Haematoxylin/eosin. × 62.

FIG. 3. Poorly developed cortical alveoli (black asterisk) in 04 of *C. lepidura.* Haematoxylin/eosin. × 85.

FIG. 4. PAS-positive reaction after salivary amylase treatment in the cortical vesicles, yolk globules and in the zona pellucida of *A. britskii* 04. × 104.

FIG. 5. 04 of *S. brandtii* showing PAS-negative reaction in the cortical vesicles (black asterisk) and PAS-positive reaction in the yolk globules (white asterisk) and in the zona pellucida (ZP). × 85.

FIG. 6. AB pH 2,5-positive reaction only in the cortical vesicles (black asterisk) of *S. brandtii* 04. × 75.

FIG. 7. Columnar follicular cells in 04 of *S. knerii.* Note strongly positive reaction to PAS in the zona pellucida (arrow). AB pH 2,5 plus PAS. × 453.

FIG. 8. Sudan black B-positive reaction for lipids in the yolk globules (white asterisk) and negative in zona pellucida (ZP) and in the cortical vesicles (black asterisk) of *S. brandtii* 04. × 319.

TABLE I. Histochemical reactions in the cortical vesicles, in the yolk globules and in the zona pellucida of ten teleost species at the Três Marias Dam, Minas Gerais, Brazil.

HISTOCHEMICAL REACTIONS	SPECIES											
	S. knerii, A. britskii, C. lepidura, T. chalceus, A. bimaculatus lacustris			*L. piau, L. taeniatus, L. reinhardti, T. guentheri*			*S. brandtii*					
	CV	YG	ZP	CV	YG	ZP	CV	YG	ZP			
									IL	ML	EL
Periodic acid-Schiff (PAS)	+	+	+ +	+	+	+ +	—	+	+	+ +	+ +
Salivary amylase + PAS	+	+	+ +	+	+	+ +	—	+	+	+ +	+ +
Alcian blue (AB) pH 2,5	+	—	—	—	—	—	+ +	—	—	+ +	+ +
Alcian blue (AB) pH 0,5	—	—	—	—	—	—	+ +	—	—	+ +	—
PAS after acid hydrolysis	+	+	+ +	+	+	+ +	—	+	+	+ +	+ +
AB pH 2,5 after acid hydrolysis	+	—	—	—	—	—	+ +	—	—	+ +	+ +
Ninhydrin-Schiff	+	+	+	+	+	+	+	+	+	+	+
Sudan black B	—	+	—	—	+	—	—	+	—	—	—
Sudan III	—	+	—	—	+	—	—	+	—	—	—
Nile blue sulphate	—	+	—	—	+	—	—	+	—	—	—
Oil red O	—	+	—	—	+	—	—	+	—	—	—

CV = Cortical vesicles; YG = yolk globules; ZP = zona pellucida; IL = internal layer; MD = middle layer; EL = external layer; + + = strongly positive reaction; + = positive reaction; — = negative reaction.

showing cuboidal cells in *S. brandti* in *S. knerri* and squamous cells in

The cortical vesicles are arranged tinuous layer around the oocyte 4 hence composing a very well develo 4). This cortical organization is not *guentheri* and *L. reinhardti* (Fig. 2) tical alveoli is poorly developed with areas of the cortical zone of the c

The cytochemical reactions obse tical vesicles and in the zona pellu in the Figs. 4 to 8. These reactions p and the zona pellucida, in all sp glycoproteins. The content of the co glycoproteins in *L. piau, L. taenia theri*; sulphated acid glycoproteins boxylated acid glycoproteins in *S. T. Chalceus* and *A. bimaculatus*

In *S. brandtii,* the cortical vesicl 0,5 positive indicating that their acid sulphated and carboxylated grou However, after using the combined plus Alcian blue pH 0,5 according t tures were shown to contain only su

The intensity of the PAS and AB alterated after acid hydrolysis, thus acid in cortical vesicles, in yolk glob of the ten species studied.

The yolk globules also contain ne reaction to Sudan black B, Oil re sulphate. The cortical vesicles and th ly to these techniques (Fig. 8).

The follicular cells of *A. britskii* even after salivary amylase treatmen may contain neutral glycoproteins sin Schiff and are negative to the AB pH

Periodic acid-Schiff (PAS)	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary amylase + PAS	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alcian blue (AB) pH 2,5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alcian blue (AB) pH 0,5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PAS after acid hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AB pH 2,5 after acid hydrolysis	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ninhydrin-Schiff	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sudan black B	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sudan III	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nile blue sulphate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oil red O	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

CV = Cortical vesicles; YG = yolk globules; ZP = zona pellucida; IL = internal layer; MD = middle layer; EL = external layer; ++ = strongly positive reaction; + = positive reaction; — = negative reaction.

showing cuboidal cells in *S. brandtii* and *A. britskii,* columnar cells in *S. knerri* and squamous cells in the other species.

The cortical vesicles are arranged peripherically forming a continuous layer around the oocyte 4 in *S. brandtii* and *A. britskii,* hence composing a very well developed cortical alveoli (Fig. 1 and 4). This cortical organization is not observed in the oocytes of *T. guentheri* and *L. reinhardti* (Fig. 2). In the other species, the cortical alveoli is poorly developed with vesicles arranged only in scarce areas of the cortical zone of the oocyte 4 (Fig. 3).

The cytochemical reactions observed in the yolk globules, cortical vesicles and in the zona pellucida are shown in Table 1 and in the Figs. 4 to 8. These reactions point out that the yolk globules and the zona pellucida, in all species studied, contain neutral glycoproteins. The content of the cortical vesicles is varied : neutral glycoproteins in *L. piau, L. taeniatus, L. reinhardti* and *T. guentheri*; sulphated acid glycoproteins in *S. brandtii*; neutral and carboxylated acid glycoproteins in *S. knerii, C. lepidura, A. britskii, T. Chalceus* and *A. bimaculatus lacustris.*

In *S. brandtii,* the cortical vesicles are AB pH 2,5 and AB pH 0,5 positive indicating that their acid polysaccharides contain either sulphated and carboxylated groups or sulphated groups only. However, after using the combined method Alcian yellow pH 2,5 plus Alcian blue pH 0,5 according to RAVETTO (1964), these structures were shown to contain only sulphated acid polysaccharides.

The intensity of the PAS and AB pH 2,5 reactions remains inalterated after acid hydrolysis, thus excluding the presence of sialic acid in cortical vesicles, in yolk globules and in the zona pellucida of the ten species studied.

The yolk globules also contain neutral lipids due to their positive reaction to Sudan black B, Oil red 0, Sudan III and Nile blue sulphate. The cortical vesicles and the zona pellucida react negatively to these techniques (Fig. 8).

The follicular cells of *A. britskii* and *S. knerii* are PAS positive, even after salivary amylase treatment. This suggests that these cells may contain neutral glycoproteins since they react also to ninhydrin-Schiff and are negative to the AB pH 2,5 and AB pH 0,5 techniques.

Discussion

Oocyte maturation is a continuous process which seems to occur in a similar way in teleost. However, the classification in stages varies according to the criteria used. The division in four stages used in this study is similar to that proposed by Godinho *et al.* (1974).

Several morphological studies have suggested that the yolk vesicles give origin to the cortical alveoli (Wallace and Selman, 1981 in review). Selman *et al.* (1988) have recently shown by means of a combination of morphological, cytochemical, electrophoretic and immunological methods that the yolk vesicles are indistinguishable from the cortical alveoli. We have adopted the term cortical vesicles (alveoli) since these structures are not properly yolk. In this study, it was observed that the morphology of this cortical structure appears very well developed, not very distinct or absent according to the species. This observation was also related for Rastogi (1969).

A positive reaction to PAS and a negative reaction to AB pH 2,5 and to pH 0,5 as reported by Selman *et al.* (1988) in the yolk globules coincided with our results, indicating the presence of neutral glycoproteins and the absence of acid glycoconjugates. Similarly, Malone and Hisaoka (1963) and Anderson (1968) observed polysaccharides and proteins whereas Khoo (1979) reported absence of polysaccharides in these yolk structures. The presence of lipids in the yolk globules as described by Malone and Hisaoka (1963), Anderson (1968), Rastogi (1969) and Khoo (1979) were also found in this study.

Neutral glycoproteins, carboxylated acid glycoproteins and sulphated acid glycoproteins were observed in the cortical vesicles according to the species. The results found in the literature are also variable : sulphated mucopolysaccharides (Aketa, 1954); polysaccharides and proteins (Chopra, 1958a, Korfsmeier, 1966, Anderson, 1968); acid mucopolysaccharides (Rastogi, 1969); glycogen and acid polysaccharides complex (Chopra, 1958b, Khoo, 1979); polysaccharides including glycogen, proteins and RNA (Shahi *et al.*, 1979); glycoprotein containing carboxyl group (Tesoriero, 1980); acid and neutral mucopolysaccharides (Mester *et al.*, 1984); glycogen and acid mucopolysaccharides containing sialomucin in immature oocytes and sulphated acid mucopolysaccharides in the

cortical alveoli in mature ooc
polysialoglycoprotein (Inoue *et*
glycoconjugates (Selman *et al.*,
mucopolysaccharides containing sia
1988). Recent studies have also demo
composition in the sialoglycoprotei
cortical alveolus which are cleaved
fertilization (Inoue *et al.*, 1987; Kr
sity in cytochemical content of
freshwater teleost species appears
dicating specific functional role a
established that the cortical granul
and intervertebrate eggs play an imp
tion which is closely accompanied by
glycoprotein contents into the peri
establishment of the block to po
review).

The PAS-positive polysaccharid
teleosts are well known. The preser
the zona pellucida, as found in preser
vation of Pelizaro *et al.* (1981) and
a protein-acid polyssacharides cor
Khoo, 1979) and carboxylated glycop
been also found in this envelope. In
showed three layers (internal, mid
presented different responses to c
techniques (Rizzo and Bazzoli, in
was observed that in *S. brandtii* th
besides neutral glycoproteins, also ca
glycoproteins in the middle layer
glycoproteins in the external layer as
bined method Alcian yellow pH 2
(Ravetto, 1964). Several zona pe
documented in mammalian fertilizat
three glycoproteins : ZP1, ZP2 and
ZP3 serve as both primary sperm rec
inducer (Wassarman, 1988). In tele
zoon enters a region of the egg cyto
ed, the micropyle. At the base of
membrane of the unfertilized egg is c

ISCUSSION

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to that proposed by GODINHO *et al.*

dies have suggested that the yolk
tical alveoli (WALLACE and SELMAN,
(1988) have recently shown by means
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veloped, not very distinct or absent
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and a negative reaction to AB pH
by SELMAN *et al.* (1988) in the yolk
results, indicating the presence of
absence of acid glycoconjugates.
OKA (1963) and ANDERSON (1968)
d proteins whereas KHOO (1979)
arides in these yolk structures. The
obules as described by MALONE and
68), RASTOGI (1969) and KHOO (1979)

boxylated acid glycoproteins and
ere observed in the cortical vesicles
esults found in the literature are also
saccharides (AKETA, 1954); polysac-
, 1958a, KORFSMEIER, 1966, ANDER-
charides (RASTOGI, 1969); glycogen
plex (CHOPRA, 1958b, KHOO, 1979);
cogen, proteins and RNA (SHAHI *et*
aining carboxyl group (TESORIERO,
olysaccharides (MESTER *et al.*, 1984);
accharides containing sialomucin in
ed acid mucopolysaccharides in the

cortical alveoli in mature oocytes (VERMA *et al.*, 1986); polysialoglycoprotein (INOUE *et al.*, 1987); carboxylated acid glycoconjugates (SELMAN *et al.*, 1988) and non-sulphated acid mucopolysaccharides containing sialomucin (VERMA and THAKUR, 1988). Recent studies have also demonstrated different carbohydrate composition in the sialoglycoproteins of the medaka and salmonid cortical alveolus which are cleaved into repeating units following fertilization (INOUE *et al.*, 1987; KITAJIMA *et al.*, 1989). The diversity in cytochemical content of the cortical vesicles of the freshwater teleost species appears to be specie-specific, thus indicating specific functional role at fertilization. It is now well established that the cortical granules or alveoli of the vertebrate and intervertebrate eggs play an important role in the cortical reaction which is closely accompanied by the release of cortical alveolus glycoprotein contents into the perivitelline space resulting in the establishment of the block to polyspermy (GURAYA, 1982, in review).

The PAS-positive polysaccharides of the zona pellucida of teleosts are well known. The presence of neutral glycoprotein in the zona pellucida, as found in present paper, is supported by observation of PELIZARO *et al.* (1981) and LOPES *et al.* (1982). However, a protein-acid polyssacharides complex (ANDERSON, 1967 and KHOO, 1979) and carboxylated glycoprotein (TESORIERO, 1980) have been also found in this envelope. In *S. brandtii,* the zona pellucida showed three layers (internal, middle and external ones) which presented different responses to cytological and cytochemical techniques (RIZZO and BAZZOLI, in preparation). In this study it was observed that in *S. brandtii* the zona pellucida contained, besides neutral glycoproteins, also carboxylated and sulphated acid glycoproteins in the middle layer and only carboxylated acid glycoproteins in the external layer as was shown by way of the combined method Alcian yellow pH 2,5 plus Alcian blue pH 0,5 (RAVETTO, 1964). Several zona pellucida functions have been documented in mammalian fertilization. This envelope consists of three glycoproteins : ZP1, ZP2 and ZP3. The major glycoprotein ZP3 serve as both primary sperm receptor and acrosome reaction inducer (WASSARMAN, 1988). In teleosts the fertilizing spermatozoon enters a region of the egg cytoplasm that is highly specialized, the micropyle. At the base of the micropyle, the plasma membrane of the unfertilized egg is differentiated into a structure

apparently designed for sperm binding (LONGO, 1988). The micropylar apparatus is well studied in teleosts (KOBAYASHI and YAMAMOTO, 1981; NAKASHIMA and IWAMATSU, 1989). In fish, only recently has preliminary work been published that identifies the zona pellucida glycoproteins (BEGOVAC and WALLACE, 1986, 1989; HAMAZAKI *et al.*, 1987, 1989) and, the exact physiological significance of these glycoproteins still remains obscure.

The presence of neutral glycoproteins was also observed in the follicular cells of *A. britskii* and *S. knerii* coinciding with observations made by SAXENA *et al.* (1984) in *Nandus nandus*. On the other hand, CUSSAC and MAGGESE (1986) observed positive and negative reactions to AB pH 2,5 and PAS, respectively in the follicular cells of *Rhamdia sapo,* thus, detecting acid mucopolysaccharides in these cells. These authors pointed out that the follicular cells seem to degenerate to form the jelly-coat (outer thick envelope of the ovulated egg).

Therefore, it was concluded through classical cytochemical that the composition of cortical vesicles, zona pellucida and follicular cells varied in teleost fish. These variations may reflect different functions of these components in dynamics of oogenesis.

ACKNOWLEDGMENTS

The authors are thanked to the researchers : YOSHIMI SATO and ELIZABETH LOMELINO CARDOSO from the Três Marias Fishery Station (Codevasf) for the assistance during the collects of the fishes. Thanks also to the Brazilians research foundations "PRPq/UFMG-CNPq" for the finantial support.

REFERENCES

ABRAHAM, M., V. HILGE, S. LISON and H. TIBIKA — The cellular envelope of oocytes in teleosts. *Cell Tissue res.* **235** : 403-410 (1984).

AKETA, K. — The chemical nature and the origin of the cortical alveoli in the egg of the medaka, *Oryzias latipes. Embryologia,* **2** : 63-66 (1954).

ANDERSON, E. — The formation of the primary envelope during oocyte differentiation in teleosts. *J. Cell Biol.,* **35** : 193-212 (1967).

ANDERSON, E. — Cortical alveoli formation and vitellogenesis during oocyte differentiation in the pipefish, *Syngnathus fuscus,* and the killifish, *Fundulus heteroclitus. J. Morphol.,* **125** : 23-60 (1968).

BEGOVAC, P. C. and R. A. WALLACE — Vitelline envelope proteins in the pipefish. *J. Cell. Biol.,* **103** : 369a (1986).

BEGOVAC, P. C. and R. A. WALLACE — Major vi
originate within the follicle and are associat
56-73 (1989).

CHOPRA, H. C. — A morphological and histoc
Ophiocephalus punctatus, with particular re
149-158 (1958a).

CHOPRA, H. C. — Morphological and histocher
ticto (Punctius), with particular reference t
211-221 (1958b).

CUSSAC, V. E. and M. C. MAGGESE — Oogenesis
Stages of the oocyte, egg's envelopes and effe
Rev. Brasil. Biol., **46** : 139-147 (1986).

DROLLER, M. J. and T. F. ROTH — An electron m
oogenesis in *Lebistes reticulatus* guppyi. *J.*

GODINHO, H. M., S. FERRI, L. O. MEDEIROS and J.
in the ovary of *Pimelodus maculatus* Lacé
the reproductive cycle. *Rev. Brasil. Biol.,*

GURAYA, S. S. — Recent progress in the structu
cortical granules in animal egg. *Int. Rev.*

HAMAZAKI, T. S., I. IUCHI and K. YAMAGAMI —
a "spawning female-specific substance" in t
242 : 343-349 (1987).

HAMAZAKI, T. S., Y. NAGAHAMA, I. IUCHI and K
liver constitutes the inner layer of the egg env
Oryzias latipes. Dev. Biol., **133** : 101-110 (

INOUE, S., K. KITAJIMA, Y. INOUE and S. KUDO
as a major glycoprotein component in cortica
gairdneri. Dev. Biol., **123** : 442-454 (1987).

KHOO, K. H. — The histochemistry and endocrine
Can. J. Zool., **57** : 617-626 (1979).

KITAJIMA, K., S. INOUE and Y. INOUE — Isolati
of sialoglycoproteins (hyosophorin) from the
tide with a large N-linked glycan chain as a tar
(1989).

KOBAYASHI, W. and T. S. YAMAMOTO — Fine stru
chum Salmon egg, with a discussion of the
Exp. Zool., **217** : 265-275 (1981).

KORFSMEIER, K. H. — Zur genese des Dottersyste
Autoradiographische Untersuchungen. *Z. Z*

LISON, L. — Histochimie et cytochimie animales.

LONGO, F. J. — Reorganization of the egg surfac
233-269 (1988).

LOPES, R. A., H. S. LEME DOS SANTOS, J. R. V. Co
— Histochemical study of oocyte zona radia
lacustris - Linnaeus, 1758 (Osteichthyes : Char

MALONE, T. E. and K. K. HISAOKA — A his
deutoplasmic components in developing ooc
J. Morphol., **112** : 61-75 (1963).

MESTER, L., D. SCRIPCARIU, A. SCARLAT and R.
of the cortical alveoli from the oocytes of s
D'Histoire Naturelle "Grigore Antipa". XX

NAKASHIMA, S. and T. IWAMATSU — Ultrastru
formation of the micropyle during oogenesis
phol., **202** : 339-349 (1989).

PEARSE, A. G. E. — Histochemistry : theoretica
Livingstone, Edinburgh, London, Melbourn

PELIZARO, M. G., H. S. LEME DOS SANTOS, R. A
of development in the oocyte of the tambiu *A.*
(Pisces : Characidae). A morphometric and h
elles) **92** : 415-431 (1981).

erm binding (LONGO, 1988). The
studied in teleosts (KOBAYASHI and
A and IWAMATSU, 1989). In fish, on-
ork been published that identifies the
(BEGOVAC and WALLACE, 1986, 1989;
989) and, the exact physiological
oteins still remains obscure.
coproteins was also observed in the
and *S. knerii* coinciding with obser-
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WLEDGMENTS

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o from the Três Marias Fishery Sta-
nce during the collects of the fishes.
search foundations "PRPq/UFMG-
ort.

ERENCES

I. TIBIKA — The cellular envelope of oocytes in
10 (1984).
ne origin of the cortical alveoli in the egg of the
ia, **2** : 63-66 (1954).
imary envelope during oocyte differentiation in
1967).
n and vitellogenesis during oocyte differentiation
nd the killifish, *Fundulus heteroclitus. J. Mor-*

telline envelope proteins in the pipefish. *J. Cell.*

BEGOVAC, P. C. and R. A. WALLACE — Major vitelline envelope proteins in pipefish oocytes originate within the follicle and are associated with the Z3 layer. *J. Exp. Zool.,* **251** : 56-73 (1989).

CHOPRA, H. C. — A morphological and histochemical study of the oocytes of the fish, *Ophiocephalus punctatus,* with particular reference to lipids. *Quart. J. Micr. Sci.,* **99** : 149-158 (1958a).

CHOPRA, H. C. — Morphological and histochemical study of oocytes of the fish *Barbus ticto* (Punctius), with particular reference to lipids. *Res. Bull. Panjab. Univ.,* **152** : 211-221 (1958b).

CUSSAC, V. E. and M. C. MAGGESE — Oogenesis in *Rhamdia sapo* (Pisces, Pimelodidae). Stages of the oocyte, egg's envelopes and effects of the human chorionic gonadotropin. *Rev. Brasil. Biol.,* **46** : 139-147 (1986).

DROLLER, M. J. and T. F. ROTH — An electron microscope study of yolk formation during oogenesis in *Lebistes reticulatus* guppyi. *J. Cell Biol.,* **28** : 209-232 (1966).

GODINHO, H. M., S. FERRI, L. O. MEDEIROS and J. M. B. BARKER — Morphological changes in the ovary of *Pimelodus maculatus* Lacépède, 1803 (Pisces, Siluroidei) related to the reproductive cycle. *Rev. Brasil. Biol.,* **34** : 581-588 (1974).

GURAYA, S. S. — Recent progress in the structure, origin, composition, and function of cortical granules in animal egg. *Int. Rev. Cytol.,* **78** : 257-358 (1982).

HAMAZAKI, T. S., I. IUCHI and K. YAMAGAMI — Isolation and partial characterization of a "spawning female-specific substance" in the teleost, *Oryzias latipes. J. Exp. Zool.,* **242** : 343-349 (1987).

HAMAZAKI, T. S., Y. NAGAHAMA, I. IUCHI and K. YAMAGAMI — A glycoprotein from the liver constitutes the inner layer of the egg envelope (zona pellucida interna) of the fish, *Oryzias latipes. Dev. Biol.,* **133** : 101-110 (1989).

INOUE, S., K. KITAJIMA, Y. INOUE and S. KUDO — Localization of polysialoglycoprotein as a major glycoprotein component in cortical alveoli of the unfertilized eggs of *Salmo gairdneri. Dev. Biol.,* **123** : 442-454 (1987).

KHOO, K. H. — The histochemistry and endocrine control of vitellogenesis in goldfish ovaries. *Can. J. Zool.,* **57** : 617-626 (1979).

KITAJIMA, K., S. INOUE and Y. INOUE — Isolation and characterization of a novel type of sialoglycoproteins (hyosophorin) from the eggs of medaka, *Oryzias latipes:* nonapeptide with a large N-linked glycan chain as a tandem repeat unit. *Dev. Biol.,* **132** : 544-553 (1989).

KOBAYASHI, W. and T. S. YAMAMOTO — Fine structure of the micropylar apparatus of the chum Salmon egg, with a discussion of the mechanism for blocking polyspermy. *J. Exp. Zool.,* **217** : 265-275 (1981).

KORFSMEIER, K. H. — Zur genese des Dottersystems in der oocyte von *Brachydanio rerio.* Autoradiographische Untersuchungen. *Z. Zellforsch.,* **71** : 283-296 (1966).

LISON, L. — Histochimie et cytochimic animales. 3a éd. Paris : GAUTHIER-VILLAIRS, 1960.

LONGO, F. J. — Reorganization of the egg surface at fertilization. *Int. Rev. Cytol.,* **113** : 233-269 (1988).

LOPES, R. A., H. S. LEME DOS SANTOS, J. R. V. COSTA, M. G. PELIZARO and N. CASTAGNOLI — Histochemical study of oocyte zona radiata of the lambari *Astyanax bimaculatus lacustris* - Linnaeus, 1758 (Osteichthyes : Characidae). *Zool. Anz.,* **208** : 265-268 (1982).

MALONE, T. E. and K. K. HISAOKA — A histochemical study of the formation of deutoplasmic components in developing oocytes of the zebrafish, *Brachydanio rerio. J. Morphol.,* **112** : 61-75 (1963).

MESTER, L., D. SCRIPCARIU, A. SCARLAT and R. MESTER — Ultrastructural peculiarities of the cortical alveoli from the oocytes of some fish species. Travaux du Muséum D'Histoire Naturelle "Grigore Antipa". XXVI : 233-241 (1984).

NAKASHIMA, S. and T. IWAMATSU — Ultrastructural changes in micropylar cells and formation of the micropyle during oogenesis in the medaka *Oryzia latipes. J. Morphol.,* **202** : 339-349 (1989).

PEARSE, A. G. E. — Histochemistry : theoretical and applied. Vol. 2 4th ed., Churchill Livingstone, Edinburgh, London, Melbourne and New York, 1985.

PELIZARO, M. G., H. S. LEME DOS SANTOS, R. A. LOPES and N. CASTAGNOLI — Rhythm of development in the oocyte of the tambiu *Astyanax bimaculatus* (REINHARDT, 1874) (Pisces : Characidae). A morphometric and histochemical study. *Arch. Biol.,* (Bruxelles) **92** : 415-431 (1981).

QUINTARELLI, G., S. TSUIKY, Y. HASCHIMOTO and W. PIGMAN — Studies of sialic acid containing mucins in bovine submaxilary and rat sublingual glands. *J. Histochem. Cytochem.* **9** : 176-183 (1961).

RASTOGI, R. K. — Studies on the fish oogenesis. III. Vitellogenesis in some freshwater teleosts. *Anat. Anz.*, **125** : 24-36 (1969).

RAVETTO, C. — Alcian blue-Alcian yellow : a new method for identification of different acidic groups. *J. Histochem. Cytochem.*, **12** : 441 (1964).

RIZZO, E. and N. BAZZOLI (in preparation) — The structure and development of the zona pellucida in the brazilian "white piranha" (pirambeba) *Serrasalmus brandtii*, REINHARDT, 1874.

SAXENA, R. C., Y. N. SAHAI and S. M. JAIN — Cytological and histochemical observations on the cortical zone of oocytes of a teleost fish, *Nandus nandus* (Ham) *Acta Morphol.*, **22** : 217-231 (1984).

SCHINDLER, J. F. and U. DE VRIES — Polarized distribution of binding sites for concanavalin A and wheat-germ agglutinin in the zona pellucida of goodeid oocytes (teleostei) *Histochemistry*, **91** : 413-417 (1989).

SELMAN, K., R. A. WALLACE and V. BARR — Oogenesis in *Fundulus heteroclitus*. IV. Yolk-vesicle formation. *J. Exp. Zool.*, **239** : 277-288 (1986).

SELMAN, K., R. A. WALLACE and V. BARR — Oogenesis in *Fundulus heteroclitus*. V. The relationship of yolk vesicles and cortical alveoli. *J. Exp. Zool.*, **246** : 42-56 (1988).

SHAHI, R. N. P., A. P. MISHRA and B. R. SINGH — Studies on the formation of compound yolk in the developing oocytes of an airbreathing fish *Channa punctatus* (Bloch). *Cytologia*, **44** : 397-408 (1979).

TESORIERO, J. V. — The distribution and fate of ^{3}H-glucose and ^{3}H-galactose in oocytes of *Oryzias latipes*. Formation of the cortical alveoli. *Cell Tissue Res.*, **209** : 117-129 (1980).

VERMA, G. P., K. C. SAHU and C. C. DAS — Cytochemical studies on the development of yolk in a teleost, *Notopterus notopterus* (Pallas). *Z. mikrosk. anat. Forsch.*, **100** : 111-120 (1986).

VERMA, G. P. and C. THAKUR — Origin and composition of cortical granules in oocytes of a teleost, *Mastacembelus armatus armatus* (Lacépède). *Arch. Biol.* (Bruxelles) **99** : 325-334 (1988).

WALLACE, R. A. and K. SELMAN — Cellular and dynamic aspects of oocyte growth in teleosts. *Amer. Zool.*, **21** : 325-343 (1981).

WASSARMAN, P. M. — Zona pellucida glycoproteins. *Ann. Rev. Biochem.*, **57** : 415-442 (1988).

WOURMS, J. P. — Annual fish oogenesis. I. Differentiation of the mature oocyte and formation of the primary envelope. *Dev. Biol.*, **50** : 338-354 (1976).

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Eur. Arch. Biol. (Bruxelles) **101** : 411-445 (199

LA LIGNEE GERMINAL D'EUROPE (Emys

II. — Interprétation mathé cinétiques de la migration e cellules germ

PAR

R. DUBOIS et D.

(Institut d'Embryologie cellulaire et molécu

SUMMARY — *In the present articl of Part I of on study on the germ lin rapin (I. — Origin and primary locali we confirm, on the basis of quantitat arguments, the origin in the ectobl (PGC) that can be identified at th « posterior germinal crescent » of the between the ectoblast and the endo lateral mesoblast that will give rise*

In addition, the results of measure the germ line during the phase of primordia have been analysed and inte of a kinetic theory of growth that belo relationships. The mathematical mo numerical increase in the germ line an allometric principle known as «

The parameters of the equations sh measured in hours of incubation at of reference for the observed data. « biological clock », in harmony wit that regulates the mitotic activity of t tion of the genital rudiments. It can liferation pattern of the PGC is under whose modulation is hightly linked to development.