Gonadal Development and Sex Differentiation in the Cichlid Fish *Cichlasoma dimerus* (Teleostei, Perciformes): A Light- and Electron-Microscopic Study

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**ABSTRACT** Although the overall pattern and timing of gonadal sex differentiation have been established in a considerable number of teleosts, the ultrastructure of early stages of gonadal development is not well documented. In this study, gonads from larval and juvenile stages of laboratory-reared *Cichlasoma dimerus* were examined at the light-microscopic and ultrastructural levels. This freshwater species adapts easily to captivity and spawns with high frequency during 8 months of the year, providing an appropriate model for developmental studies. Larvae and juveniles were kept at a water temperature of 26.5 ± 1°C and a 12:12 hour photoperiod. Gonadal development was documented from 14–100 days postfertilization, covering the period of histologically discernible sex differentiation. Gonadal tissue was processed according to standard techniques for light and electron microscopy.

*C. dimerus*, a perciform teleost, is classified as a differentiated gonochorist, in which an indifferent gonad develops directly into a testis or ovary. On day 14, the gonadal primordium consists of a few germ cells surrounded by enveloping somatic cells. Ovarian differentiation precedes testicular differentiation, as usual in teleost fishes. The earliest signs of differentiation, detected from day 42 onward, include the onset of meiotic activity in newly formed oocytes, which is soon accompanied by increased oogonial mitotic proliferation and the somatic reorganization of the presumptive ovary. The ovarian cavity is completely formed by day 65. Numerous follicles containing perinucleolar oocytes are observed by day 100. In contrast, signs of morphological differentiation in the presumptive testis are not observed until day 72. By day 100, the unrestricted lobular organization of the testis is evident. The latest stage of spermatogenesis observed by this time of testicular development is spermatocyte II. J. Morphol. 264:191–210, 2005. © 2005 Wiley-Liss, Inc.

**KEY WORDS:** gonadal development; sex differentiation; histology; ultrastructure; Cichlidae; Teleostei

Although a number of articles and comprehensive reviews on early gonadogenesis and differentiation of germ cells in teleost fishes are available (Satoh and Egami, 1972; Satoh, 1974; Bruslé and Bruslé, 1978; Mezhnin, 1978; Yoshikawa and Oguri, 1978; Ryazantseva and Sakum, 1980; Timmermans and Taverne, 1983; Colombo et al., 1984; Parmentier and Timmermans, 1985; Hamaguchi, 1992; Foyle, 1993; Patiño and Takashima, 1995; Patiño et al., 1996; Strüssmann et al., 1996; Grandi and Colombo, 1997; Lin et al., 1997; Nakamura et al., 1998; Devlin and Nagahama, 2002), detailed histological descriptions of early gonadal development and of the period of sex differentiation are limited to a few gonochoristic species.

Careful histological observations of the process of morphogenesis of the gonads are of primary importance for a precise understanding of the mechanisms of gonadal sex differentiation (Nakamura et al., 1998) and could help to develop efficient methods of directing sexual development in aquaculture species, since they can provide a guide for determining the hormone-sensitive period in cases of sex manipulation by exogenous steroid treatment (see Hunter and Donaldson, 1983; Foyle, 1993; Strüssmann et al., 1996).

The South American cichlid fish *Cichlasoma dimerus*, a perciform teleost (Nelson, 1994), is common in quiet shallow waters in the Paraguay and most of the Paraná river basins (Kullander, 1983). This freshwater species adapts easily to captivity and shows notable reproductive features such as a high spawning frequency (about every 30 days during 8 months of the year, under laboratory conditions) and acceptable survival rates, providing an appropriate model for developmental studies. *Cichlasoma dimerus* lives in pairs that defend a territory. The females lay their eggs on a cleaned substrate and
Pairs were kept in 45-L aquaria, at 26.5°C by local fishermen and transferred to the laboratory. Single and bottom fishes were normally fed with pelleted commercial food. Later, fed finely ground, dried flake food. Egg and larvae were obtained from several spawnings of six males and the other half females. Spontaneous intersexes among gonochorists occur mostly in the undifferentiated species, while in the differentiated ones the occurrence of intersex is rarely or never seen (Yamamoto, 1969).

The aim of the present study was to describe the gonadal development of Cichlasoma dimerus, both at the light- and electron-microscopic levels, with special emphasis on the period of gonadal sex differentiation.

**RESULTS**

With respect to gonadal sex differentiation, Cichlasoma dimerus is classified as a differentiated gonochorist, in which ovaries and testes develop directly from undifferentiated gonadal tissue.

**Undifferentiated Gonads**

On day 14 postfertilization (day 12 posthatching), paired gonadal primordia are present in the posterior region of the abdominal cavity, immediately below the kidney, and are suspended from the dorsal peritoneal wall by short mesenteries (Fig. 1A, B). Parasagittal sections show a cord-like gonadal tissue in which germ cells form discontinuous clusters, interspersed with segments formed solely by somatic cells (Fig. 1B). Cross sections through germ cell clusters reveal that the gonadal primordium consists of large, round to oval germ cells surrounded by enveloping somatic cells (Fig. 1C). Sexually undifferentiated germ cells contain large, central euchromatic nuclei with a prominent eccentrically located nucleolus (Fig. 1A, D). The cytoplasm contains cup-shaped mitochondria with tubular cristae (Fig. 1 C, F), abundant smooth endoplasmic reticulum (Fig. 1 E), and electron-dense substance or nuage, a germ cell-specific organelle (Fig. 1D). Occasional mitotic activity occurs in these cells (Fig. 1C). Somatic cells are of various shapes. They have heterochromatic nuclei and relatively little cytoplasm that contains few organelles. Two types of somatic cells are present: coelomic epithelial cells, located at the periphery of the gonadal anlage, and supporting cells, derived from the former and located in an interior position, where they become associated with germ cells. The epithelial basement membrane is present between these two cell types (Fig. 1C). Neighboring epithelial cells are connected by desmosomes and tight junctions (Fig. 1G). Support cells tend to envelope germ cells with their cytoplasmic processes, so that only in rare cases do germ cells contact each other (Fig. 1C). Occasionally, germ cells may directly contact the epithelial basement membrane.

By day 25, gonads have become suspended from the ventral edge of the forming swim bladder by short mesenteries (Fig. 2A). Germ cell mitotic figures are now more frequent in the undifferentiated gonad (Fig. 2B, C). Concomitant with mitotic divisions, the number of germ cells is augmented. As a result, the gonads increase in size and several germ cells (not one or two as in earlier stages) appear in cross sections (Fig. 2B). In parasagittal sections, germ cells acquire continuity and the gonads approach each other at the caudal tip as they converge towards the urogenital papilla (Fig. 2C). Germ cells possess large, round to oval nuclei with one, or rarely two, nucleoli (Fig. 2C, D). Granular and fibrillar components of nucleoli are evident in some germ

**MATERIALS AND METHODS**

Adult Cichlasoma dimerus used in this study were captured in Esteros del Riachuelo, Corrientes, Argentina (27°75'S, 58°15'W) by local fishermen and transferred to the laboratory. Single pairs were kept in 45-L aquaria, at 26.5 ± 1°C and a 12:12 h photoperiod. Laboratory aquaria were well aerated and provided with a layer of gravel and smooth stones for egg deposition on the bottom. Fishes were normally fed with pelleted commercial food and Tubifex worms.

Eggs and larvae were obtained from several spawnings of six pairs. On the 14th day after spawning, each lot of offspring was isolated in a 20-L aquarium, where their development was followed until the juvenile stage was reached. A part of the brood isolated in a 20-L aquarium, where their development was followed until the juvenile stage was reached. A part of the brood.
Fig. 1. Gonadal development in *Cichlasoma dimerus* on day 14 postfertilization (6 ± 0.5 mm TL). A,B: LM. C–G: TEM. A: Cross section of gonadal primordia suspended from the dorsal peritoneal wall by short mesenteries at both sides of the dorsal mesentery. Scale bar = 10 μm. B: Sagittal section of the gonadal anlage below the kidney. Note the discontinuity of germ cell clusters, which confers on the gonadal primordium the appearance of a string of beads. Scale bar = 20 μm. C: Cross section of one of the gonadal anlagen showing germ cells and somatic (epithelial and support) cells. Note that germ cells are wrapped by the cytoplasm of support cells. A basement membrane supports epithelial cells. Scale bar = 2 μm. D: Primordial germ cell. A large nucleolus is present within the nucleoplasm. Scale bar = 2 μm. E: Germ cell organelles at higher magnification. Note the presence of a vacuole within the matrix of some mitochondria. Scale bar = 0.5 μm. F: Detail of a cup-shaped mitochondrion, that appears ring-shaped since only the lip of the cup has been sectioned. Its cristae are distinctly tubular. Scale bar = 0.2 μm. G: Connections between adjacent epithelial cells. Scale bar = 0.2 μm. Arrow, smooth endoplasmic reticulum; arrowhead, basement membrane; cm, cup-shaped mitochondria; cp, cytoplasmic process of support cell; d, desmosome; E, epithelial cell; G, germ cell; K, kidney; M, gonadal mesentery; m, mitotic figure; n, nuage; nu, nucleolus; SU, support cell; tj, tight junction.
Within the cytoplasm, spherical or elongated mitochondria with lamellar cristae are present in association with nuage. Centrioles are found in pairs, called diplosomes, next to the nucleus (Fig. 2D). Epithelial cells show pinocytotic activity (Fig. 2E).

Gonads remain undifferentiated by day 38 (Fig. 3A). Many spherical or oval mitochondria are located around the nuclei of germ cells (Fig. 3B). Nuage are prominent and tend to be located near the nucleus in association with mitochondria (Fig. 3A,B). Smooth endoplasmic reticulum and annulate lamellae, forming amphitheater-like structures, are conspicuous components of the cytoplasm (Fig. 3B,C). Multilamellar bodies are also present. The flattened, peripheral epithelial cells surrounding the gonad are connected by tight junctions and desmosomes (Fig. 3D). The inner support cells contain triangular to elongated nuclei. Cytoplasmic extensions of these cells incompletely invest germ cells that have recently divided but have not completed cytokinesis (Fig. 3A,E). Desmosomes occasionally occur between neighboring support cells. Between epithelial and support cells, a thin space correspond-

Fig. 2. Gonadal development in *Cichlasoma dimerus* 25 days after fertilization (8 ± 0.7 mm TL). A–C: LM. D,E: TEM. A: Cross section of the abdominal cavity showing the indifferent gonads below the caudal tip of the swim bladder. Scale bar = 10 μm. C: Sagittal section of the gonads. Note the continuity of germ cells along the gonad. Scale bar = 20 μm. D: Undifferentiated germ cell showing two nucleoli within the nucleoplasm. Note the presence of the diplosome near the nucleus. Scale bar = 1 μm. E: Peripheral area of the gonad. Scale bar = 1 μm. arrow, smooth endoplasmic reticulum; di, diplosome; E, epithelial cell; G, germ cell; K, kidney; M, gonadal mesentery; m, mitotic figure; ml, mitochondria with lamellar crista; n, nuage; pf, pars fibrosa; pg, pars granulosa; pv, pinocytotic vesicles; sb, swim bladder; SU, support cell.

Fig. 3. Gonadal development in *Cichlasoma dimerus* on day 38 postfertilization (12.5 ± 1 mm TL). TEM. A: Cross section of the undifferentiated gonad. Separation of the germinal and interstitial compartments of the gonad is evident. The germinal compartment contains germ cells and support cells that are derived from epithelial cells. The interstitial compartment is a thin peripheral space that contains collagen fibrils and very few interstitial cells. Scale bar = 3 μm. B: Indifferent germ cell. Scale bar = 1 μm. C: Area from B, showing annulate lamellae disposed as an amphitheater. The outer pores appear in side view while the inner pores are primarily en face. Scale bar = 0.3 μm. D: Somatic (epithelial and support) cells at higher magnification. Scale bar = 0.5 μm. E: Area from A, showing the sinusuous cytoplasmic process of a support cell. Note the presence of four plasma membranes. The inner membranes define a support cell whose process is ~0.05 μm across. The outer membranes are those of adjacent germ cells resulting from a recent mitotic division. Germ cells contact each other at the point marked with opposite arrowheads. Note also the presence of a “double” basement membrane (arrowheads) corresponding to folds of the epithelial basement membrane. The outer basement membrane supports peripheral epithelial cells, while the inner basement membrane subtends internal support cells that are associated with germ cells. This basement membrane separates the germinal and interstitial compartments of the gonad. Scale bar = 0.5 μm. al, annulate lamellae; arrow, smooth endoplasmic reticulum; arrowhead, basement membrane; asterisk, collagen fibrils; cp, cytoplasmic process of support cell; E, epithelial cell; G, germ cell; I, interstitial cell; ml, mitochondria with lamellar crista; n, nuage; nu, nucleolus; pc, pore complex; SU, support cell; tj, tight junction.
Ovarian Differentiation

Ovarian differentiation precedes testicular differentiation, as is usual in teleosts. By day 42, many oogonia have entered meiotic prophase, becoming primary growth oocytes, which in addition to moderate oogonial mitotic proliferation and somatic growth results in an enlargement of the presumptive ovary. For the first time, blood capillaries occur in the dorsal region of the gonad (Fig. 4A). Oogonia contain a prominent euchromatic nucleus with a large nucleolus, mitochondria with lamellar cristae, conspicuous nuage, and abundant smooth endoplasmic reticulum (Fig. 4B). In meiotic oocytes, the nucleus becomes more electron-lucent than that of oogonia and the number of mitochondria increases, although they seem to be smaller. Nuage and smooth reticulum are less conspicuous (Fig. 4C). Synaptonemal complexes are evident within nuclei of oocytes at the pachytene stage of the first meiotic prophase (Fig. 4C,D). Support or prefollicle cells contain triangular nuclei that are less chromatic than those of the other somatic cell types (Fig. 4B,E,F). Cytoplasmic processes of prefollicle cells enclose oogonia or early oocytes, marking the onset of folliculogenesis (Fig. 4B). Epithelial cells possess an elongated nucleus (Fig. 4B,E). They are joined by tight junctions and desmosomes (Fig. 4E), and display conspicuous pinocytotic activity (Fig. 4F,G). A third type of somatic cell, the prethecal (interstitial) cell, is observed lying between epithelial and prefollicle cells. These cells, as well as the collagen fibrils that surround them, are derived from the stromal connective tissue and not from the coelomic epithelium. Therefore, they lie in a tissue compartment delimited by an inconspicuous basement membrane, i.e., the interstitial or stromal compartment of the ovary, separating them from the forming follicles (Fig. 4B,E,H). A few mitochondria with tubular cristae occur in all types of somatic cells (Fig. 4F,G,H).

Active germ cell mitosis and meiosis are observed by day 50. Likewise, somatic reorganization of the presumptive ovary begins. Gonads present a triangular or kidney-shape when observed in cross sections (Fig. 5A,B). Clusters of somatic cells are noticeable along the dorsal and ventral edges of the ovary (Fig. 5B). By day 58, somatic cells from these areas proliferate and form appendix-like structures that initially protrude from the gonad (Fig. 5C). Subsequently, they face each other and finally fuse to form the ovarian lumen (Fig. 5D). The ovarian cavity is completely formed by day 65 and numerous basophilic oocytes at the diplotene stage of meiosis are present by this time of development. The cytoplasm of these cells becomes highly basophilic and thin chromosome threads that form a random reticulum occur within the nucleoplasm (Fig. 5D).

On day 80, the ovary consists mainly of follicles that contain characteristically large, perinucleolar oocytes with a basophilic cytoplasm (Fig. 6A). These cells are arrested in the diplotene stage of the first meiotic prophase and are characterized by the presence of multiple round nucleoli in the peripheral nucleoplasm (Fig. 6A,B). Masses of an electron-dense substance, or nuage, are observed outside the nuclear envelope, associated with mitochondria (Fig. 6B,E). As folliculogenesis progresses, the typical ovarian follicle organization exists, i.e., each perinucleolar oocyte becomes surrounded by a continuous layer of follicle cells that flatten out over its surface and are separated from the stroma by a basement membrane (Fig. 6C). Interstitial connective tissue is detected in the periphery of the ovary, and in the angular interstices between three or more follicles in the inner region of the gonad (Fig. 6A). Thecal cells derived from this stromal tissue start to surround the follicles (Fig. 6C), although a complete layer of thecal cells is observed only around the more advanced follicles. Younger oocytes in the pachytene stage of meiosis are found along the luminal edge, bordering the ovarian lumen (Fig. 6A,D). Nuage forming aggregations with mitochondria, smooth endoplasmic reticulum, and Golgi complexes occur in their cytoplasm. Prefollicle cells located between pachytene oocytes are joined by desmosomal junctions (Fig. 6E).

By day 100 the ovary contains many developing follicles, as well as younger oocytes at early stages of meiotic prophase and only a few oogonia (Fig. 7A,B). Oogonia (Fig. 7C) and oocytes in early meiotic prophase (Fig. 7B) reside in the luminal edge of the developing ovarian lamellae, forming, along with the epithelial and prefollicle cells, the so-called "germinal epithelium." Follicles, derived from the germinal epithelium, are composed of a perinucleolar oocyte and surrounding follicle cells (Fig. 7B). Segregation of the fibrillar and granular components of nucleoli becomes evident in perinucleolar oocytes. Nuage are observed outside the nuclear envelope (Fig. 7D). A well-developed Golgi apparatus and many mitochondria occur in the cytoplasm (Fig. 7D,E). Formation of microvilli, by folding of the oolemma, has begun at the periphery of these cells. Microvilli extend from the surface of the oocyte toward the overlying follicle cells (Fig. 7C,F). Two or three squamous follicle cells (derived from epithelial cells) surround each perinucleolar oocyte. Thecal cells (derived from stromal or interstitial connective tissue cells) form a thin continuous layer around follicle cells (Fig. 7B,C). Both follicle and thecal cells have little cytoplasm, containing only a few organelles. The area between follicles, in which thecal
Fig. 4. Onset of ovarian differentiation in *Cichlasoma dimerus* 42 days after fertilization (15 ± 1.2 mm TL). **A**: LM. **B-H**: TEM. **A**: Cross section of differentiating ovaries. Note the presence of many early meiotic oocytes with thick strands of condensed chromatin inside the nucleus. Scale bar = 30 μm. **B**: Oogonium at the onset of folliculogenesis is encompassed by cytoplasmic processes of a prefollicle cell. Scale bar = 2 μm. **C**: Primary growth oocyte in early pachytene stage of the first meiotic prophase. Scale bar = 3 μm. **D**: Longitudinal section of a synaptonemal complex inside the nucleus of a pachytene oocyte. The synaptonemal complex appears as a tripartite structure formed by a thin central component and two thick lateral components that insert onto the nuclear envelope. Scale bar = 0.5 μm. **E**: Spatial relations of somatic cells, collagen fibrils, and the basement membrane. Scale bar = 2 μm. **F**: Detail of a prefollicle cell associated with pachytene oocytes. Scale bar = 1 μm. **G**: Area from **F**, showing the cytoplasm of an epithelial cell exhibiting mitochondria with tubular cristae and pinocytotic vesicles. Scale bar = 0.5 μm. **H**: Prethecal cell surrounded by collagen fibrils. Scale bar = 1 μm. arrow, smooth endoplasmic reticulum; arrowhead, basement membrane; asterisk, collagen fibrils in stromal compartment; c, blood capillary; cc, central component; cp, cytoplasmic process of prefollicle cell; E, epithelial cell; lc, lateral component; M, mesovary; mb, multilamellar body; ml, mitochondria with lamellar cristae; mt, mitochondria with tubular cristae; n, nuage; ne, nuclear envelope; nu, nucleolus; O, oogonium; PF, prefollicle cell; PO, pachytene oocyte; PT, prethecal cell; pv, pinocytotic vesicles; sc, synaptonemal complex; tj, tight junction.
cells, blood capillaries, and other interstitial elements reside, is known as the stromal extravascular space (Fig. 7B). A prominent basement membrane separates follicle from thecal or epithelial cells (Fig. 7C,D,F). A single basement membrane is frequently present at the point where a follicle is attached to the germinal epithelium, i.e., the follicle cells of the follicle and the epithelial cells of the germinal epithelium both share a common basement membrane (Fig. 7D,F). Pinocytotic activity still occurs in epithelial cells (Fig. 7F).

Testicular Differentiation

In contrast to ovarian development, signs of histological differentiation are not observed in the presumptive testis until day 72.

Undifferentiated gonads on day 65 of development are recognizable as presumptive testes. They retain the original pear-like shape of indifferent gonads and are much smaller than ovaries of the same developmental stage (Fig. 8A, cf. Fig. 5D). The overall appearance of spermatogonia is similar to that of undifferentiated germ cells at 38 days. The most characteristic features are the presence of a prominent nucleus with one or two nucleoli, a conspicuous smooth endoplasmic reticulum, and abundant mitochondria that may be associated with nuage (Fig. 8B,D). Annulate lamellae and multilamellar bodies are also present (Fig. 8E,F). Support or presumptive Sertoli cells have triangular to elongated nuclei and invest spermatogonia with cytoplasmic extensions (Fig. 8B,D). Desmosomes and tight junctions join neighboring Sertoli cells. Epithelial cells show pino-
cytotic activity and are connected by tight junctions (Fig. 8F). A thin basement membrane, as well as collagen fibrils, occur between Sertoli and epithelial cells (Fig. 8D,F). The mesorchium is composed of a double layer of epithelial cells that show many pinocytotic vesicles. Many collagen fibrils lie between epithelial cells (Fig. 8B,C).

By day 72, mitotic proliferation of spermatogonia is occurring and blood vessels become evident in the dorsal region of the testis. Some spermatogonia A undergo mitotic divisions with incomplete cytokinesis, becoming spermatogonia B (Fig. 9A). The onset of meiosis is soon noticed in the gonads of the larger specimens (Fig. 9B). Likewise, a central space that becomes recognizable as the efferent duct anlage is distinguished in some sections of the testes (Fig. 9C).

On day 80, meiotic activity is marked so that spermatocytes at early stages of meiotic prophase become numerous within the differentiating testis (Fig. 10A). Isogenic spermatocytes, together with Sertoli cells, form spermatocysts. Within each cyst,
germ cells are at the same stage of meiosis. Cytoplasmic processes of Sertoli cells form the walls of spermatocysts. Spermatocytes I at pachytene stage show synaptonemal complexes inside the nucleus (Fig. 10B). The cytoplasm contains mitochondria with lamellar cristae, annulate lamellae, and smooth endoplasmic reticulum. Swirls of smooth reticulum are detected as well (Fig. 10B,C). A Golgi apparatus is also present. Sertoli cells are joined laterally by desmosomes and tight junctions (Fig. 10D).

By day 100, the unrestricted lobular organization of the testis can be distinguished. This type of testis is subdivided into lobules, separated from each other by interstitial tissue (Fig. 11A,B). Inside the lobules, an epithelioid arrangement of Sertoli and germ cells occurs. Sertoli cells are supported by a basement membrane that limits the testicular lobules and separates the germinal and interstitial compartments of the testis (Fig. 11B). Germ cells are surrounded by cytoplasmic extensions of Sertoli cells, forming cysts (Fig. 11A,D), and never contact the basement membrane. Sertoli cells have pleomorphic nuclei and small, round mitochondria with poorly developed cristae. Desmosomes and tight junctions join these cells laterally. Tight junctions established between adjacent Sertoli cells form the so-called “Sertoli cell barrier” (Fig. 11B). Spermatagonia A are isolated cells, individually surrounded by the cytoplasm of a Sertoli cell (Fig. 11C). Spermatogenesis occurs within the cysts. Inside each cyst, germ cells at a similar stage of development are joined by cytoplasmic connections that are sometimes difficult to detect (Fig. 11D). Mitochondria with lamellar cristae, nuage, and annulate lamellae are present in the cytoplasm of spermatocytes I (Fig. 11D,E). The latest stage of spermatogenesis detected at this time of testicular development is spermatocyte II. Compared to spermatocytes I, spermatocytes II have decreased cell and nuclear sizes (Fig. 11A). Clumps of chromatin are located in the central and peripheral nucleoplasm. Cell limits of spermatocytes II are difficult to observe and the cytoplasm has few organelles (Fig. 11F). The interstitial or stromal tissue is barely differentiated. The most common cell type identified is a fibroblast-like cell. Its nucleus usually is elongated and quite irregular in shape (Fig. 11G). Myoid cells are recognized by the presence of microfilaments running parallel to the long axis of the cell and tend to be surrounded by collagen fibrils (Fig. 11H). Leydig cells cannot be identified ultrastructurally. Blood capillaries, granulocytes, and collagen fibrils are conspicuous in the periphery of the testis (Fig. 11D,I).

Figure 12 shows the time scale of the most important events and features during gonadal development and sex differentiation of Cichlasoma dimerus.

**DISCUSSION**

In teleost fishes, primordial germ cells arise at extragonadal locations and migrate to the genital ridges (gonadal anlagen) during specific developmental phases (Braat et al., 1999). The genital ridge forms as a longitudinal thickening of mesoderm that protrudes into the coelomic cavity ventral to the developing kidney and lateral to the dorsal mesentery (Devlin and Nagahama, 2002). Once within the genital ridge, germ cells increase in number due to mitotic proliferation (Patino and Takashima, 1995; Braat et al., 1999). In several teleost species such as the medaka (*Oryzias latipes*), intensive mitotic activity in the germ line is initiated upon arrival of the germ cells at the gonadal anlagen (Satoh and Egami, 1972; Hamaguchi, 1982). In contrast, the proliferative activity of germ cells is preceded by a period of quiescence in other species (Timmermans and Tavera, 1983; Parmentier and Timmermans, 1985; Lin et al., 1997). The initial gonadal primordium (day 14 postfertilization) of Cichlasoma dimerus increases in length without substantial proliferation of germ cells. As a result, germ cell clusters become cord-like and discontinuous, and are interspersed with gonadal segments formed solely by somatic cells (Fig. 1B). The presence of germ cells in cord-like, discontinuous clusters intercalated with germ cell-free spaces during the early gonadal development has also been reported in other teleosts (Mezhnin, 1978; Ryazantseva and Sakum, 1980; Parmentier and Timmermans, 1985; Strüssmann et al., 1996; Lin et
Fig. 8. Testicular development in *Cichlasoma dimerus* 65 days after fertilization (17.5 ± 1.5 mm TL). **A**: LM. **B–F**: TEM. **A**: Cross section of indifferent gonads (presumptive testes). Scale bar = 50 μm. **B**: Cross section of the testis at higher magnification. Scale bar = 3 μm. **C**: Detail of the mesorchium shown in **B**. Scale bar = 1 μm. **D**: Spermatogonium showing two nucleoli within the nucleoplasm and accompanying Sertoli cells. Scale bar = 2 μm. **E**: Detail of annulate lamellae located next to the nuclear envelope. Note how pore complexes of annulate lamellae appear much like those of the nuclear envelope. Scale bar = 0.5 μm. **F**: Epithelial cells. Scale bar = 1 μm. al, annulate lamellae; arrow, smooth endoplasmic reticulum; arrowhead, basement membrane; asterisk, collagen fibrils; cp, cytoplasmic process; dd, digestive duct; E, epithelial cell; M, mesorchium; mb, multilamellar body; ml, mitochondria with lamellar cristae; n, nuage; ne, nuclear envelope; nu, nucleolus; pv, pinocytotic vesicles; S, spermatogonium; SE, Sertoli cell; tj, tight junction.
al., 1997). By day 25, primordial gonads enlarge mainly by mitotic proliferation of germ cells and become suspended from the ventral edge of the swim bladder by short mesenteries (Fig. 2A). In longitudinal sections, germ cells acquire continuity, i.e., germ cell-free segments are no longer present (Fig. 2C).

Until day 40, the gonad shows a relatively simple pattern of cellular associations and no signs of somatic or germ cell sex differentiation can be recognized. As in other teleosts, germ cells of Cichlasoma dimerus prior to gonadal sex differentiation are characterized by their relatively large size and a conspicuous nucleus with finely granular chromatin and one or two prominent nucleoli. At the ultrastructural level, germ cells can be distinguished by the presence of extensive smooth endoplasmic reticulum, mitochondria, nuage, and annulate lamellae (Figs. 1C, D, 2D, 3A, B). Interestingly, the shape of mitochondria in undifferentiated germ cells seems to vary during early stages of gonadal development. These organelles are initially cup-shaped and possess tubular cristae. In addition, some mitochondria appear vacuolated (Fig. 1E, F). Subsequently, mitochondria change to a round or elongated shape with typical lamellar cristae (Fig. 3B, D). This modification in the structure of mitochondria may reflect a process of maturation of these organelles during early gonadal development. The electron-dense material referred to as nuage may be the most definitive marker of early germ cells in a wide range of organisms (Eddy, 1975). It appears as discrete, electron-dense cytoplasmic inclusions that tend to be associated with mitochondria (Eddy, 1975) or with annulate lamellae (Kessel, 1983). This material, also referred to as “germinal dense bodies,” “nucleolus like bodies,” and “intermitochondrial cement,” has been observed consistently, not only in primordial germ cells but also in oogonia, oocytes, spermatogonia, spermatocytes, and even spermatids of fishes (Satoh, 1974; Bruslé and Bruslé, 1975; Hamaguchi, 1982, 1992; Azevedo, 1984; Billard, 1984; Gevers et al., 1992; Flores and Burns, 1993; Grandi and Colombo, 1997; Quagio-Grassiotto and Carvalho, 1999; Grier, 2000; Guimaraes and Quagio-Grassiotto, 2011; Lo Nostro et al., 2003; Ravaglia and Maggese, 2003). Nuage appears to be synthesized in the nucleus (Azevedo, 1984) and contains ribonucleoproteins (Toury et al., 1977) that may represent ribosomal components that are subsequently assembled in the cytoplasm (Flores and Burns, 1993). In C. dimerus, nuage is observed close to the nuclear envelope (Figs. 1D, E, 11E) and/or in association with mitochondria (Figs. 2D, E, 8D) or with annulate lamellae (Fig. 8E). Annulate lamellae, an infrequently found membranous complex, appear as stacked cisternae with pore complexes similar to those of the nuclear envelope. Stacks of lamellae commonly appear in parallel array (Fig. 8E) and sometimes may have a concentric arrangement (Fig. 3C). The function of this organelle is not clear, and how it is formed is likewise disputed. Lamellae are sometimes continuous with the rough endoplasmic reticulum, and this has led to the notion that they are derived from this organelle (Wallace and Selman, 1990). On the other hand, the structural similarities of the nuclear envelope and annulate lamellae, together with their frequent juxtaposition, has also led to the conclusion that they arise from the nuclear envelope and represent a storage form of nuclear membrane (Kessel, 1968, 1983). Annulate lamellae have been observed in rapidly dividing cells and germ cells (Satoh, 1974; Bozola and Russell, 1992). Contrary to the observations of Billard (1984), who reported the presence of annulate lamellae only in spermatogonia A of adult Poecilia reticulata, in the present study this organelle was detected both in spermatogonia (Fig. 8E) and pachytene spermatocytes (Figs. 10B, 11E) of C. dimerus. The organelle designated as annulate lamellae by Flores and Burns (1993) in spermatogonia of Xiphophorus maculatus and oogonia of X. nigrensis may actually correspond to smooth endoplasmic reticulum, since pore complexes characteristic of annulate lamellae were not clearly observed.
Fig. 10. Testicular development in *Cichlasoma dimerus* 80 days after fertilization (19 ± 2 mm TL). A: LM. B–D: TEM. A: Cross section of the testis. Scale bar = 10 μm. B: Cyst of spermatocytes at pachytene stage. Note the cytoplasmic continuity between spermatocytes belonging to the same cyst. Spermatocytes are separated by cytoplasmic extensions of Sertoli cells. Scale bar = 2 μm. C: Spermatocyte cytoplasm, showing mitochondria with lamellar cristae and swirls of smooth reticulum. Scale bar = 1 μm. D: Sertoli cells. Scale bar = 0.5 μm. al, annulate lamellae; arrow, smooth endoplasmic reticulum; arrowhead, basement membrane; asterisk, collagen fibrils; c, blood capillary; cp, cytoplasmic process; d, desmosome; E, epithelial cell; er, smooth endoplasmic reticulum swirl; I, interstitial cell; M, mesorchium; ml, mitochondria with lamellar cristae; ne, nuclear envelope; pc, pore complex; pf, pars fibrosa; pg, pars granulosa; PS, pachytene spermatocyte; SA, spermatogonium A; sc, synaptonemal complex; SE, Sertoli cell; SP, spermatocyst; tj, tight junction.
Fig. 11. Testicular development in *Cichlasoma dimerus* on day 100 postfertilization (22 ± 3 mm TL). **A**: LM. **B–I**: TEM. **A**: Sagittal section of a testis showing the unrestricted lobular organization. Scale bar = 20 μm. **B**: Interstitial tissue between adjacent lobules. A basement membrane underlies Sertoli cells from the different lobules. Tight junctions that form the Sertoli cell barrier occur at the apical ends of Sertoli cells. Scale bar = 0.5 μm. **C**: Spermatogonia A. Note that they are separated by thin cytoplasmic processes of Sertoli cells. Scale bar = 3 μm. **D**: Cyst of pachytene spermatocytes I. The nucleus appears in only one of the two cells shown. Note the presence of a granulocyte next to a blood capillary in the periphery of the gonad. Scale bar = 2 μm. **E**: Nuclear envelope and adjacent organelles from a spermatocyte I. Scale bar = 0.5 μm. **F**: Cyst of spermatocytes II. Scale bar = 3 μm. **G**: Interstitial cell (fibroblast). Scale bar = 1 μm. **H**: Myoid cell. Note the presence of cross-sectioned microfilaments (double asterisk) within the cytoplasm. Scale bar = 0.5 μm. **I**: Granulocyte. Note the voluminous dense granules within the cytoplasm. Scale bar = 1 μm. *al*, annulate lamellae; *arrowhead*, basement membrane; *asterisk*, collagen fibrils; *c*, blood capillary; *ce*, centriole; *cp*, cytoplasmic process; *d*, desmosome; *FI*, fibroblast; *GR*, granulocyte; *I*, interstitial tissue; *L*, lobule; *mL*, mitochondria with lamellar cristae; *mt*, mitochondria with tubular cristae; *n*, nuage; *ne*, nuclear envelope; *MY*, myoid cell; *PS*, pachytene spermatocyte; *SA*, spermatogonium A; *sc*, synaptonemal complex; *SE*, Sertoli cell; *SP*, spermatocyte; *SI*, spermatocyte I; *SH*, spermatocyte II; *tj*, tight junction.
One of the most common and prominent histological features marking the beginning of gonadal differentiation in teleosts is the appearance of meiotic figures in germ cells of the presumptive ovary. This feature was observed in *Cichlasoma dimerus* 42 days after fertilization (Fig. 4A). The initiation of meiotic activity has frequently been reported as the first recognizable sign of ovarian differentiation (Satooh and Egami, 1972; Nakamura and Takahashi, 1973; Yoshikawa and Oguri, 1978; Takashima et al., 1980; Colombo et al., 1984; Piferrer and Donaldson, 1989; Nakamura and Nagahama, 1993; Patiño and Takashima, 1995; Nakamura et al., 1998). The onset of ovarian meiosis is soon accompanied by an increase in germ cell mitosis (Fig. 5A). However, in ovaries of some species increased germ cell mitosis may actually precede meiosis (Lebrun et al., 1982; Hamaguchi, 1982; Strüssmann et al., 1996). Moreover, Hamaguchi (1982) reported that germ cells in female medaka *Oryzias latipes* proliferate more actively than those in males, and indicated that this difference in the rate of germ cell mitosis is the first morphological sign of sex differentiation in this species. Our findings also show a higher mitotic activity in the female lineage similar to that observed by Hamaguchi, although it becomes evident a few days after the onset of meiotic activity. In *C. dimerus*, histological sex differentiation of female germ cells coincides with the appearance of blood capillaries (Fig. 4A) and occurs 1 week before somatic cell clusters are detected in the gonad (Fig. 5B). In the channel catfish (Ictalurus punctatus), ovarian somatic differentiation (appearance of small tissue outgrowths) precedes the onset of meiosis by only 3 days (Patiño et al., 1996). Likewise, germ cell and somatic differentiations begin almost simultaneously in the tilapias *Oreochromis mossambicus* and *Tilapia zilli* (Nakamura and Takahashi, 1973; Yoshikawa and Oguri, 1978). Although relatively rare, there are some other cichlid species, e.g., *O. aureus* and *O. niloticus*, in which the somatic organization of the ovary may start well before meiosis can first be detected (Eckstein and Spira, 1965; Nakamura and Nagahama, 1985).

In most teleosts the ovarian lumen is characteristically formed by the fusion of outgrowths developed from somatic cell clusters at the periphery of the gonad that enclose part of the coelom and form a lumen that is lined internally by the former coelomic epithelium (Fig. 5C,D). This state is referred to as the cystovarian condition (Hoar, 1969) or ovary of the cystovarian type (Dodd, 1977). In *Cichlasoma dimerus*, most oocytes are in the early diplotene stage of meiotic prophase by the time the ovarian cavity is completely formed (Fig. 5D).

The transition from oogonium (Fig. 4B) to oocyte (Fig. 4C) is characterized by a distinctive change in the nucleus that is associated with the onset of meiosis. Most importantly, the conspicuous nucleolus disappears as synaptonemal complexes organize at the pachytene stage. Subsequently, as early meiotic oocytes (Fig. 4A) progress through the primary growth phase of oogenesis and reach the perinuclear stage (Fig. 6A), the nucleo-cytoplasmic ratio decreases and the cytoplasm becomes highly basophilic due to the accumulation of cytoplasmic mRNA. The appearance of multiple nucleoli in the peripheral region of the oocyte nucleus, or germinal vesicle (Fig. 6B), indicates an intense transcription of ribosomal RNA (Selman and Wallace, 1989).

With respect to the origin of somatic cells within the ovary of *Cichlasoma dimerus*, it seems clear that follicle cells originate from epithelial cells which in turn are derived from the coelomic epithelium, as proposed by other authors (see Tokarz, 1978; Wallace and Selman, 1990; Hamaguchi, 1992; Grier, 2000), while thecal cells are derived from the connective tissue lying beneath the epithelial basement.
membrane, i.e., the subepithelial connective tissue that gives rise to the ovarian stroma. Nagahama (1983) and Begovac and Wallace (1988) indicated that stromal connective tissue elements become organized to form the thecal layer around salmonid and syngnathan follicles, respectively. Likewise, it has been established that somatic cells from the ovarian stroma of the common snook Centropomus undecimalis (Grier, 2000) and the swamp eel Synbranchus marmoratus (Ravaglia and Maggesi, 2002) become distributed around follicle cells and form the theca. The collagen-filled spaces between prefollicle and epithelial cells (Fig. 4B,E,H) correspond to the same interstitial compartment in which prethecal cells are observed. It is delimited by a basement membrane and has direct connections with the pericapillary spaces (Abraham et al., 1984).

In the later stages of ovarian development, this compartment is referred to as the extravascular space (Fig. 7B), as proposed by Grier (2000) and adapted from Abraham et al. (1980). Thus, prethecal cells, thecal cells, blood vessels, and other interstitial cells all reside within the stromal extravascular space. The source of the ovarian follicles is the germinal epithelium, which consists of the epithelial and derived prefollicle cells that surround either oogonia or oocytes (Fig. 7B). The germinal epithelium borders the ovarian lumen and is supported by a basement membrane that separates it from the underlying stromal compartment of the ovary (Fig. 7B,C). According to Grier (2000), thecal cells are derived from a different ovarian compartment than is the follicle and should not be considered part of it. The follicle is therefore defined by its origin from the germinal epithelium and is composed of the oocyte and surrounding follicle cells. A single basement membrane is frequently observed at the point of attachment of ovarian follicles to the germinal epithelium, i.e., epithelial cells from the germinal epithelium and follicle cells from attached follicles share a common basement membrane at the point of attachment (Fig. 7D,F). This feature was also observed in the ovary of common snook, C. undecimalis (Grier, 2000).

The appearance of microvilli due to folding of the oolemma in the periphery of perinucleolar oocytes on day 100 (Fig. 7C,F) indicates that the initiation of vitelline envelope formation is about to take place. Moreover, condensing vacuoles at the trans face of the Golgi apparatus (Fig. 7E), considered immature secretory granules (Bozzola and Russell, 1992), might contain the electron-dense material that would form the vitelline envelope when released by exocytosis and deposited around microvilli.

As is typical among teleosts, the testes of Cichlasoma dimerus remain histologically indifferent longer than the ovaries. The precocious differentiation of female germ cells (day 42, Fig. 4A) contrasts with the late appearance of the first meiotic prophase feature in spermatocytes (day 72, Fig. 9B). Thus, using meiosis of germ cells as a criterion, gonadal differentiation becomes apparent 1 month earlier in females than in males (Fig. 12). The onset of meiosis in the testis occurs concomitantly with an increase in germ cell mitosis, some of these mitotic divisions giving rise to spermatogonia B (Fig. 9A). However, it should be noticed that there is still a lack of definitive criteria for the detection of the very first discrete signs of testicular differentiation. According to Nakamura et al. (1998), the formation of efferent duct anlagen, coincident with the appearance of the first blood capillaries, are reliable criteria by which testicular differentiation can be recognized, even though germ cells remain at the gonial stage. Taking into account this sex-specific characteristic of somatic tissue, ovarian and testicular differentiation seems to start at the same time in certain tilapia species (Nakamura and Takahashi, 1973; Nakamura and Nagahama, 1985, 1989). In C. dimerus, sex differentiation of the testicular soma, i.e., appearance of blood capillaries and the efferent duct anlage, occurs concomitantly with or shortly before the onset of male germ cell meiosis (day 72, Fig. 9). Therefore, we consider that changes in the germ cells should be used as the main clues in deciding gonadal sex. Nevertheless, in those species in which changes in male germ cells occur at a considerably later period of testicular development, the behavior of somatic cells may provide valid criteria for determining testicular differentiation at an earlier phase of development.

Similar to observations in other teleosts (Bruslé and Bruslé, 1978; Billard, 1984), changes from spermatogonia (Fig. 8B,D) to spermatocytes (Fig. 10B) during testicular development in Cichlasoma dimerus include an increasing nucleo-cytoplasmic ratio, the disappearance of the nucleolus as synaptoneuclear complexes organize during the pachytene stage of meiosis, and the reduction in the number of mitochondria and the amount of endoplasmic reticulum. According to Patiño and Takashima (1995), zygotene spermatocytes can be recognized with light microscopy by the "bouquet" distribution of the chromosomes, despite the absence of the large nucleolus typical of zygotene oocytes. Our findings show that zygotene spermatocytes exhibit both the bouquet arrangement of chromosomes and a conspicuous nucleolus within the nucleoplasm (Fig. 9B). Sertoli cells are derived from epithelial cells (Hamaguchi, 1992; Grier, 1993) and their cytoplasmic processes form the spermatocyst wall. To maintain the integrity of the cyst, it is incumbent upon Sertoli cells to establish firm junctional contacts. This is accomplished by the development of inter-Sertoli desmosomes and tight junctions. The presence of tight junctions between Sertoli cells has been shown in several teleosts to result in the formation of a blood–testis barrier (Nagahama, 1983; Pudney, 1993). According to Grier (1993), the term "blood–testis barrier" should be replaced by "Sertoli cell
barrier” since the barrier does not include the participation of blood vessel endothelial cells or lobule boundary (myoid) cells. In most teleost species analyzed thus far, tight junctions are usually accompanied by the presence of desmosomes (Pudney, 1993), as observed during \textit{Cichlasoma dimerus} testicular development (Figs. 10D, 11B).

Unlike the restricted lobular testis typical of atherinomorphs, in which spermatogonia are confined to the distal termini of the lobules which contain an orderly progression of developing spermatoocytes (Grier, 1993), the testis of \textit{Cichlasoma dimerus} is of the unrestricted lobular type, i.e., spermatogonia and spermatocytes at various stages of development are distributed throughout the length of the lobules (Meijide, unpubl. obs.), which has been observed in most teleosts (Grier, 1993). By day 100 postfertilization, testicular lobules are quite well developed, spermatocyte II being the latest stage of spermatogenesis found within them (Fig. 11A,F). Based on a strict definition of an epithelium, as indicated in histology textbooks, one criterion is that epithelia border a body surface, lumen, or tube. In 100-day-old juvenile \textit{C. dimerus}, solid cords of germ cells and Sertoli cells are observed within the lobules (Fig. 11A). When most, but not all, of the criteria that define an epithelium apply to a tissue, then the term “epithelioid” applies (Ross et al., 1995). Therefore, germ and Sertoli cells in juvenile \textit{C. dimerus} are organized as an “epithelioid” tissue until maturation, when a lobular lumen develops. Solid cords of spermatogonia and Sertoli cells that are also epithelioid tissue have been reported in adult cobia (\textit{Rachycentron canadum}) during the regression and regressed classes of their reproductive cycle (Brown-Peterson et al., 2002). In contrast to the germinal compartment in the testis of juvenile \textit{C. dimerus}, the interstitial tissue appears scarcely differentiated, most interstitial cells having the appearance of fibroblasts. In this study, steroid-producing cells (Leydig cells) were not detected ultrastructurally in the testicular sections analyzed. However, further inspection of the testes might be necessary since Leydig cells were detected before the onset of meiosis in other cichlid species (Nakamura and Nagahama, 1989; Nakamura et al., 1998) and in the medaka \textit{Oryzias latipes} (Satoh, 1974). Interstitial steroidogenic cells are usually characterized by having a round nucleus, numerous mitochondria with tubular cristae, extensive smooth endoplasmic reticulum, and many free ribosomes (Satoh, 1974; Nagahama, 1983; Nakamura and Nagahama, 1989; Patiño and Takashima, 1995; Pudney, 1996; Lo Nostro et al., 2004). Interestingly, Nicholls and Graham (1972) found evidence for the origin of Leydig cells from fibroblast-like connective tissue elements in the testis of \textit{Cichlasoma nigrofasciatum}.

The role of the basement membrane in establishing the separation between the germinai compartment and the interstitium or stroma of the gonad has been adequately addressed in male (Grier, 1993), female (Grier, 2000), and hermaphroditic (Lo Nostro et al., 2003, 2004) adult fishes. The significance of the basement membrane forming this separation is that it is apparently a constant throughout vertebrate evolution. In this study, it is shown that separation of tissue compartments by the basement membrane is readily apparent in the undifferentiated gonad, i.e., before sexual differentiation of the gonads takes place. The germinai compartment contains germ cells and support cells, the latter being derived from epithelial cells. Within the interstitial compartment, collagen fibrils and a few interstitial cells are initially observed (Fig. 3A,E). During ovarian differentiation, germ cells (oogonia) transform into oocytes when entering meiosis, while the encompassing support cells become prefollicle cells. Within the ovarian stroma, interstitial cells, which become pretheccal cells, are numerous. Collagen fibrils and the first blood capillaries are observed as well (Fig. 4). In the testis, support cells become Sertoli cells, which encompass spermatogonia and spermatocytes (Figs. 10A,B, 11C,D), while interstitial cells give rise to fibroblasts (Fig. 11G), myoid cells (Fig. 11H), and presumably Leydig cells. Collagen fibrils, blood capillaries and granulocytes are also detected within the testicular interstitium (Fig. 11D,I).

Since the onset of meiotic activity is coincident with the appearance of the first blood vessels in the gonad (around 42 days in females and 72 days in males), one might hypothesize that germ cell sex differentiation in \textit{Cichlasoma dimerus} is dependent on the influence of certain endocrine factors (namely, gonadotropins) that reach the gonad via the circulatory system. In this context, the possibility that the hypothalamo-pituitary-gonadal axis is involved in triggering gonadal sex differentiation is currently under debate (see Fitzpatrick et al., 1993; Kah et al., 1993; Feist and Schreck, 1996; Baroiller and Guigné, 2001; Miranda et al., 2001; Devlin and Nagahama, 2002; Pandolfi et al., 2002).

During this study it was observed that gonadal development of \textit{Cichlasoma dimerus} was both age- and size-dependent. The chronology of gonadal sex differentiation is variable from one species to the next, and also within a given species where the growth rate is an important factor (Bruslé and Bruslé, 1982). Individuals that grow faster sexually differentiate earlier. Therefore, different degrees of gonadal development may be recognized among individuals from a same clutch, even when reared under the same conditions. In \textit{C. dimerus}, asynchrony of gonadal development is noticed from 50 days postfertilization onward. Then the development and differentiation of the gonad is more related to body size than to age, as evidenced in other teleost species (see Colombo et al., 1984; Grandi and Colombo, 1997).
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LITERATURE CITED


