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ULTRASTRUCTURAL STUDY OF THE NEURAL GLAND-GANGLIONIC COMPLEX AND THE OVARIAN DEVELOPMENT IN THE ASCIDIAN, *STYELA PARTITA* (STIMPSON,1852)

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ABSTRACT

The cerebral ganglion is divided into three parts as cerebral cortex, medullary layer and transitional zone. It is surrounded by blood sinuses and covered with a thin fibrous sheath. The cortex is multilayered of large and small neurons. Some neurons are also scattered within the medulla. Many neurons are monopolar, and some are bi- or multipolar, some are mononucleated and others are binucleated.. The cytoplasm of the large neurons is dense with extensive rough endoplasmic reticulum, free ribosomes, mitochondria, Golgi complex and many clear or dense vesicular structures. The medulla is composed of loosely arranged nerve fibres.. The presence of neurons in the peripheral nerve fibres is consistent with a diffuse organization of the nervous system of the ascidians. The neural gland is composed of glandular lobules, closely apposing at ventral side of the cerebral ganglion. The epithelial wall of the gland showed three morphological forms. The number of neurosecretory cells in the cerebral ganglion increase rapidly while oocyte development. The germinal epithelium contains oocytes at two different stages, the dark and clear cells. The early follicular oocyte just after migration from the germinal epithelium retains most of cytological features of the larger oocyte. The reproductive cycle can be grouped into: growth (February to June), vitellogenesis (April to September), mature (July to December), spent (November to February), and recovery (December to April). The vitellogenic oocyte consists of a single fenestrated endoplasmic reticulum, cisterna and one or a few smooth vesicles containing a dense core facing the cisterna itself. Vitellogenesis occured by way of endogeous autosynthesis and exogenous heterosynthesis: The former process involves a combined activities of the Golgi complex, mitochondria, and rough endoplasmic reticulum. The latter involves endocytotic incorporation of extraovarian precursors into the basal region of the early vitellogenic oocytes prior to the formation of vitelline envelope. The follicle cells and test cells appear to play an integral role in vitellogenesis and oocyte degeneration, functioning in phagocytosis and digestion of products originating from the degenerated oocytes.

Key words: cerebral ganglion; neural glnd ; neurosecretory cells ; oogenesis ; follicle cell ; test cell ; oogonium-, Previtellogenic, vitellogenic, ripe oocytes; maturation; rough endoplasmic ; reticulum: free ribosomes; mitochondria; Golgi complex

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INTRODUCTION

Ascidians belong to the subphylum Urochordata, which diverged from the last common ancestor of all chordates (Chen et al. 2003). Styela partita is a solitary ascidian that belongs to the family Styelidae. It is generally found on rocks in subtidal water. It is a comparatively small species of ascidian. As it is usually covered with algae or barnacles and its shape and color are similar to surrounding rock, it is well camouflaged (Saad, 2008). The neural gland in ascidians was first clearly indicated by Hancock (1868). Many morphological or physiological studies have been made on this structure since then. Earlier investigators attributed various functions to the gland : It was postulated to function as a lymphatic organ (Herdman, 1883), an excretory system (Millar, 1953) or a mucus gland (Roule, 1884). In addition, the possibility that it acts as an endocrine organ has been extensively discussed (Butcher,1930; Carlisle,1951; Dodd,1955, Georges, 1974; Michael, et al., 2008 ; Saad, 2010). Since Julin (1881; Georges (1972) first postulated a homology of the neural gland of ascidians with the hypophysis of vertebrates on the basis of its position and origin. This idea is accepted by Michael, et al., 2008 and Saad, 2002 on Ciona intestinalis, Styela plicata and Styela partita. However, these studies have resulted in much disagreenient, and the function and structure of the gland in ascidians remain controversial. Neurosecretory cells have been shown to occur in the neural ganglion of ascidians, one of the main components of the neural complex in this animal (Dawson and Hisaw, 1964 ; Lane, 1972 ; Saad, 2010). Great interest concerns whether the ascidian ganglion has a gonadotropic function (Hisaw et a!., 1966; Sengel and Georges, 1966 ; Bouchard-Madrelle, 1967). However, an acceptable interpretation of function (s) of the neural ganglion and gland has not been proposed. Many recent studies on the neurosecretory phenomena in various invertebrates other than ascidians have been carried out (Tombes, 1970; Golding 1974) . Accumulating evidence from such studies indicates that the neurosecretory system in invertebrates plays an important role in reproductive activity. The present study is focused primarily on the gonadotropic function of the neural complex in the solitary ascidian, Styela partita. As an approach to this subject, the functional criteria for neurosecretory status suggested by Bern (1962) were applied and a possible role for the neural complex in reproductive activity in this species is postulated. In ascidians, the neural complex is known to be derived from the embryonic neural tube during metamorphosis (Dodd and Dodd 1966). There are a few earlier reports describing the presence of substances immunoreactive with antisera against pituitary hormones such as prolactin (Fritsch et al ., 1982; Pestarino 1983) and adrenocorticotropic hormone (Georges and Dubois 1979, 1985; Pestarino 1988) in the cerebral ganglion of ascidians. Genri, et al., 2002 demonstrated the presence of PRL-immunoreactive substance within the secretory granules in the cells of the dorsal strand as well as in the neuronal cells in the cerebral ganglion of *Halocynthia roretzi* (Terakado et al., 1997). The solitary ascidians are usually hermaphrodites that propagate by sexual reproduction, whereas colonial ascidians reproduce both sexually and asexually by budding or strobilation (Kessel, 1983; Cloney, 1990). The reproductive cycle of marine animals is controlled by both endogenous rhythms and exogenous environment (Giese and Pearse, 1974; Himmelman, 1980). The spawning periods of ascidians differ according to the reproduction strategy and geographical position (Millar,1971, Berrill, 1975), and spawning events show species specificity (Fretter, 1984). Didemnum sp. breed throughout the year, while the spawning seasons of Microcosmus sabatieri, *Halocynthia papillosa* and *Halocynthia roretzi* are limited to two- to three-month periods (Becerro and Turon, 1992; Park et al., 1991). Polycarpa *cryptocarpa kroboja, Ciona intestinalis, Styela clava* and *Styela plicata* have spawning seasons that extend over 5 to 8 months (Saad, 2008 ; Saad, et al., 2008 ; Chen and Dai, 1998; Yang and Lee, 1978; Lee, 1976, 1977). The present study investigated the ultrastructure of the neural complex, oogenesis based on gametogenesis, stage of gonadal development and monthly changes in the neural complex morphology and oocyte development in order to characterize the reproductive biology of Styela partita inhabiting the intertidal zone of the Mediterranean Sea, Alexandria.

Oogenesis in ascidians has been studied by many investigators mainly with regard to origins and roles of the accessory cells or changes in cytoplasmic organelles accompanying oocyte growth and vitellogenesis (Kessel, 1966 ;Cotell et al., 1981; Hori, and Michibata, 1981 Gianguzza,. and Dolcemascolo, 1983 ;Michael, et al., 208 ; Saad, 2008). The early phase of oogenesis in Ciona savignyi have been investigated by electron microscopy (Reverberi, 1971, Berrill, 1975 and Kessel, 1983. Sugino et al., 1987 and 1990). Oogenesis starts in the cell-aggregated region and growing oocytes migrate to the follicular region, where they form egg follicles together with three kinds of accessory cells which differentiate around them. Problems have, however, remained to be have not been distinctly identified. Moreover, there is some uncertainty regarding the classification of the cell types present in the cell-aggregated region. Sugino et al., 1987 and 1990 have reported that, in addition to early oocytes, two cell types (dark cells and clear cells) are present in the cell aggregated region. They claimed that the dark cells are progenitors of both the test cells and the inner follicle cells, but in Sugino et al., 1990, they allowed that the dark cells might be oogonia. We produced monoclonal antibodies against germ cells of Ciona intestinalis. Cortical granules are central to the block to polyspermy and do so by modifying the extracellular environment of the oocyte. The cortical granule contents modify the extracellular matrix in a significant and essential way, but the majority of the extracellular matrix is already intact and established prior to fertilization. At the other extreme are frogs and sea urchins. An extracellular matrix in these animals is present, but minimal. It does however, serve as a scaffold in the construction of a new extracellular matrix by retaining and organizing the massive content secretions of the cortical granules in these animals following fertilization. Cortical granules are membrane-bound organelles and members of the regulated family of secretory vesicles. As their name implies, the cortical granules of an oocyte are concentrated in the cortex of the cell, the outermost few microns of the cell that is distinct both morphologically and biochemically from the inner cytoplasm. (Wallace and Selman, 1990 ; Becker and Hart, 1999). Once oocyte maturation has completed, all cortical granules can be found docked beneath the plasma membrane, at which time they are referred to as "cortical alveoli" (Wallace and Selman, 1990). Danio rerio eggs show a characteristic, size-dependent cortical granule distribution (Becker and Hart, 1999): The extracellular stimulus for secretion in the case of cortical granules is sperm binding, and although the exact mechanism of signal transduction is not yet known, the calcium is derived from the endoplasmic reticulum by stimulus from inositol trisphosphate (IP3). Cortical granules are distinct, however, from most other regulated secretory

vesicles in that they are not renewed. Following fertilization cortical granules do not form again, and their contents are no longer synthesized.

MATERIALS AND METHODS

Styela partita was sampled by snorckling and diving at a depth of 2-5 meters from the intertidal zone of the Mediterranean Sea, Alexandria, Egypt. Samples were harvested monthly from June 2009 to February 2011. Pieces of the gonad and parts of mantle containing the neural complex were fixed in Bouin's solution and then embedded in paraffin. The tissues were sectioned at 5-6 µm and stained with ehrlich hematoxylin and 0.5% eosin and the specimens were examined under a light microscope. To investigate monthly changes in oocyte development, about 1,000 oocytes (sectioned through the nucleus) per month were measured by image analysis (Image scope 2.3, Image Line, Inc.). The developmental stages of the gonad were grouped according to Lee's guidelines (1976) in successive stages. In the ovary the stages were growth, vitellogenesis, mature, spent, and recovery were investigated. The stages were growth, mature, spent, and resting. For electron-microscopic observations, finely cut pieces of the ovary and neural complex were fixed first with 2.5% glutaraldehyde in 0.5 M phosphate buffer for 2 hours followed by a rinse in the buffer solution overnight, and then post-fixed in Millonig's OS04 solution for 2 hours at 4°C. After dehydration in a graded ethanol series and by two changes in n-butyl glycidyl ether, the ovarian pieces and the neural complex were embedded in Epon-epoxy resin mixture. Ultrathin sections were cut with glass knives at a thickness of about 500 to 800 A on a Porter-Blum microtome, and stained by uranyl acetate in 50% ethanol and Reynolds' lead citrate. Observation was done with a Hitachi HS-ll electron microscope.

RESULTS

The ultrastructure of the neural complex and the ovary of Styela partita were studied from specimens collected monthly around one year. Electron microscopy revealed that the neural complex consists of a cerebral ganglion and a neural gland located above the anterior part of the branchial chamber (Fig. 1). The cerebral ganglion is ovoid, and has a maximum diameter of 40 µm and a maximum length of 120 µm. It consists of a cellular cortex and a fibrous medulla. Most of neurons are small about 5 µm in diameter, but several huge ones are more than 10 µm in diameter are scattered randomly on the periphery of this ganglion(Fig. 2). The neural gland consists of three parts: dorsal tubercle, ciliated duct, and a dorsal strand. The dorsal tubercle protrudes above the pharyngeal lining and bears a ciliated funnel. The funnel opens into a ciliated duct which opens into the neural gland, a blind sac in a blood sinus below the cerebral ganglion. Funnel and duct cells are joined by adhering junctions and, apically, by putative tight junctions. The neural gland wall is a loose, irregular, non-ciliated epithelium (Fig. 3). Some of these glanular cells are mononucleated and others are binucleated (Fig. 4). Secretory cells were observed in the gland. These glandular cells are polygonal and with variable sizes. It seems that these cells are budded off from the glanular wall and form the gland secretion (Fig. 5). In a previous work on *Ciona intestinalis*, *Styela plicata* and *Styela partita*, this glandular epithelium appeared sometimes of simple squamous cells, other times of simple cubical cells and other times of stratified cells (Michael, et al., 2008 and Saad, 2002). The lateral and ventral sides of the ganglion are surrounded by blood sinuses and the ganglion is covered with a thin fibrous sheath through which many nerve fibres run (Fig. 6). The ganglion is composed of a cellular cortex and a fibrous medulla. The cortex is multilayered of large and small neurons. Some neurons are also scattered within the medulla. Many neurons are monopolar, and some are bi- or multipolar (Fig. 7). The cytoplasm of the large neurons is dense with extensive rough endoplasmic reticulum, free ribosomes, mitochondria, one or more Golgi complexes, large dense bodies, and many clear or dense vesicular structures (Fig. 8) . Some neurons send their processes directly into the lumen of the sinuses. The medulla is composed of loosely arranged nerve fibres without cellular wrappings. The medullary fibres contain vesicles and granules of various sizes, and microtubules. At the anterior and posterior ends of the ganglion, the medullary fibres are assembled into thick peripheral nerve fibre bundles. The peripheral nerve fibres are enveloped and subdivided by fibrous structures (Fig. 9) Synapses are found in the medulla, in the cortex, and between the peripheral nerve fibres. The presence of neurons and axodendritic or axoaxonic synapses in the peripheral nerve fibres is consistent with a diffuse organization of the nervous system of the *Styela partita*. Neurosecretory granules are observed inside the neurons and the cortex of the ganglion (Fig. 10). These granules in the ganglion and the gland secretion vary in quantity in the different months of the year. The maximal quantity was observed in the neural complex of specimens collected during September (Fig. 11)and the lowest quantity in specimens of May (Fig. 12) . The ascidian, *Styela Partita* is a gonochoristic species, and the ovary has two cycles in the year. The ovary is embedded in the mantle. In immature animals the ovary is transparent in its external aspect, whereas in mature ones it shows a brownish colour. The oogenetic part of the germinal epithelium contains oocytes at two different stages, the dark and clear cells. The smaller oocyte contains synaptonemal complexes. The larger oocyte in the initial phase of growth has a conspicuous nucleolus, electron-dense materials and some mitochondria close to the nuclear envelope. The nucleus of the larger oocyte is round and has the smooth contour. The dark cell contains a relatively large nucleus and is sometimes connected to each other by an intercellular bridge. Therefore, the dark cell, which has been suggested to be the progenitor cell of two kinds of accessory cells, may be also the oogonium (Fig. 13). The early follicular oocyte just after migration from the germinal epithelium retains most of cytological features similar to those of the larger oocyte. This difference in the nuclear contour probably indicates that such a follicular oocyte is in the second phase of growth (Fig. 14).

The reproductive cycle can be grouped into the following successive stages in the ovary: growth (February to June), vitellogenesis (April to September), mature (July to December), spent (November to February), and recovery (December to April). The vitellogenic oocyte consists of an association between a single fenestrated endoplasmic reticulum, cisterna and one or a few smooth vesicles containing a dense core facing the cisterna itself. The latter is smooth and perforated by numerous small pores (about 30 nm in diameter) in the area of association; towards the periphery, it extends into several

branches with ribosomes bound to their membranes. In the vesicles, fibrillar material radiates from the dense core and is sometimes organized into a long, dense lamina. The membranes of both cisterna and vesicles appear to be coupled, but are in fact separated by a constant narrow space occupied by short densities (Fig. 15). Vitellogenesis occured by way of endogeous autosynthesis and exogenous heterosynthesis: vitellogensis occurred through a process of autosynthesis, which involves a combined activities of the Golgi complex, mitochondria, and rough endoplasmic reticulum (Fig. 16). The process of heterosynthesis involved endocytotic incorporation of extraovarian precursors into the basal region of the early vitellogenic oocytes prior to the formation of vitelline envelope. The follicle cells and test cells appear to play an integral role in vitellogenesis and oocyte degeneration, functioning in phagocytosis and digestion of products originating from the degenerated oocytes (Fig. 17): these functions can permit the transfer of yolk precursors needed for vitellogenesis. Follicle cells might have a lysosomal system for breakdown and might also resorb phagosomes in the cytoplasm for nutrient storage during oocyte degeneration.

Ultrastructure of germ cells and follicle cells during oogenesis can be grouped into four distinct phases based on electron microscopic observations: oogonia, previtellogenic oocytes, vitellogenic oocytes, and mature oocytes. The primary oogonia, which measured 30-50 µm in diameter, were round or oval in shape and still attached to the germinal epithelium. They possessed a large ovoid nucleus in which the chromatin was marginal (Fig. 18). The primary oogonia, which multiplicate on the germinal epithelium, were single or formed a cluster in the acini. The primary oogonia divided mitotically to produce the secondary oogonia. Several mitochondria, Golgi complex, and vacuoles appeared in the cytoplasm of the primary and secondary oogonia. As the secondary oogonia entered into the first prophase of meiosis, they developed into previtellogenic oocytes. The oocyte was somewhat elongated and peduculated, and the diameters of the oocytes were 55-150 µm. At the beginning of cytoplasmic growth of the previtellogenic oocyte, germ cells moves internally from the periphery of the ovary and, together with several epithelial cells, constitutes the primary follicle. Initially, the germ cell is surrounded only by a flattened layer of epithelium (Fig. 19). The cells of the epithelial layer, however, soon divide so as to form two layers of follicle cells, an inner and outer layer, and another follicle cell which becomes compressed in the periphery of the developing oocyte. The latter is referred to as the test cell and has no counterpart in any vertebrate ovary thus far examined. The test cell becomes separated from the follicle epithelium after formation of the vitelline membrane (chorion) (Fig. 20) . At this time, several mitochondria and vacuoles were concentrated around the nucleus and the follicle cells initially appeared close to the oocyte, and then progressively surrounded the oocyte. The follicle cells possessed a dense chromatin and marginal chromatin in the nucleus and contain characteristically parallel arrays of the rough endoplasmic reticulum and mitochondria in the cytoplasm. These follicle cells are differentiated into an outer and an inner follicular epithelia. Near the adherence zone, vacuoles were visible in the cytoplasm of the follicle cells (Fig. 21). Early vitellogenic oocytes measured 155-190 µm in diameter. Lipid droplets, the mitochondria, and the endoplasmic reticulum were usually present in the perinuclear region of the early vitellogenic oocyte. At this time, the follicle cells are connected to an early vitellogenic oocyte. Before the follicle cells detach from the oocyte, close contact was maintained with the oocyte. In particular, the mitochondria, lipid droplets, and glycogen particles appeared in the cytoplasm of the follicle cells (Saad, 2008). In particular, the vesicular connective tissue cells , which contained large quantities of glycogen particles and several lipid droplets, were found near the early vitellogenic oocyte (Fig. 22). At this time, lipid droplets appeared near the Golgi product which was formed by the Golgi complex in the cytoplasm, and also found between the mitochondria and well-developed rough endoplasmic reticulum in the cytoplasm (Fig. 23). At this time, several coated vesicles, which appeared at the basal region of the early vitellogenic oocyte, lead to the formation of membrane-bound vesicles via endocytosis. Vitellogenesis occured by way of endogeous autosynthesis and exogenous heterosynthesis: vitellogensis occurred through a process of autosynthesis, which involves a combined activities of the Golgi complex, mitochondria, and rough endoplasmic reticulum. The process of heterosynthesis involved endocytotic incorporation of extraovarian precursors into the basal region of the early vitellogenic oocytes prior to the formation of vitelline envelope. Follicle cells might have a lysosomal system to resorb phagosomes in the cytoplasm for nutrient storage during oocyte development. The uptake of nutritive material in the coated vesicle formed by receptor-mediated endocytosis appeared through the formation of coated endocytotic pits on the oolemma (Fig. 24). The developing oocyte synthesizes and stores a large mass and diversity of proteins . Most of the proteins and other macromolecules found in developing oocytes are made by the oocyte, but a large quantity of molecules - including yolk and glycogen - are heterosynthetic, contributed by the accessory cells. Thus, the pathways responsible for organizing membrane-bound proteins in an oocyte are complicated by the influx of proteins, the diversity of molecules, and the timing of regulated trafficking and secretion of these proteins.

Cortical granule biogenesis in these cells occurs concurrently with the biogenesis of several other major types of secretory vesicles. Yet, each type of vesicle contains a unique population of proteins, is translocated to different regions, and is secreted at distinct times either before, during, or following fertilization. Each of these secretory vesicles is morphologically and biochemically distinct, yet each is made coincidentally during oogenesis. Thus, each vesicle must have select targeting signals for its contents, for its translocation to the cell surface, and for its secretion. Cortical granules thus serve as a good model for organelle biogenesis in this cell type because of their abundance, their content diversity, and their distinct morphology. Cortical granules in *Styela partita* are made continuously through oogenesis and first detected in oocytes 50-150 µm in diameter (Fig. 25) . In the late vitellogenic oocyte, yolk granules clearly appeared among the mitochondria, lipid droplets, the rough endoplasmic reticulum, and cortical granules at the basal region of the oolemma. Thereafter, yolk granules filled the cytoplasm of the oocyte, whereas the follicle cells gradually lost their intimate association with the late vitellogenic oocyte surface. After a few follicle cells detached from the oolemma of the late vitellogenic oocyte, microvilli occurred on the site of oolemma. The cytoplasm of residual follicle cells, which was attached to the oocyte, was filled with vacuoles and myelin-like organelles (Fig. 26). In the late stages of oogenesis, larger immature yolk granules were formed by mixing of small yolk granules. At this time, the tips of the microvilli, some of which bifurcated, protruded and extended just beyond the outer border of the vitelline envelope. The vitelline envelope of the

oocyte was covered with a thick jelly coat. Upon reaching maturity, mature oocytes began to separate from the follicular wall and smaller immature yolk granules were continuously intermingled with one another and became larger mature yolk granules in the cytoplasm. A mature yolk granule is composed of three components: crystalline core, electron lucent cortex; and a limiting membrane (Fig. 27). The degenerating oocytes appeared slightly irregular or polyhedric near the follicle cells, and were deformed by compression of the oocyte. During the gradual disintegration of the oocytes, the endoplasmic reticulum, the first cytoplasmic organelle, was specifically involved in the degenerative process. During the period of oocyte degeneration, in particular, a few phagosomes (lysosomes), a number of vacuoles, and a small number of lipid droplets appeared in the cytoplasm of the follicle cells, while glycogen particles decreased in the cytoplasm of the follicle cells which were attached to the degenerated oocyte (Fig. 28). The perivitelline space of degenerated oocyte increased, and numerous glycogen particles were also present in the vitellin coat. Finally, the vitelline envelope having abnormal microvillus structures and glycogen particles breakdowns, and these are released to the ooplasmic contents . Reproductive cycle with ovarian dvelopmental stages Based on electron microscopic and histological observations of the germ cells and other surrounding cells (follicle cells), the ovarian developmental stages were classified into five successive stages (Saad, 2008). The stages and the criteria used to define them are as follows: The early active stage was characterized by the expansion of the oogenic follicle, and oogonia and previtellogenic oocytes propagated along the follicular wall of the ovary. The oogonia were about 50 µm in diameter, and the previtellogenic oocytes about 100 µm in diameter. The lumina of the oogenic follicles were empty during the early active stage (Fig. 29). Individuals in the early active stage appeared from July to August when seawater temperatures were moderately high about 25 °C. Late active stage is characterized by the presence of early vitellogenic oocytes. Oogenic follicular walls were slightly thin. At a size of 155-190µm in diameter, each early vitellogenic oocyte formed an egg-stalk connected to the germinal epithelium, and the auxiliary cells, which were attached to the oocyte, appeared in the lumen of the follicle. At a diameter of 195-200 µm, each late vitellogenic oocyte had a large germinal vesicle and an eggstalk attached to the follicular wall (Fig. 30). Individuals in the late active stage were found from July to August when the seawater temperatures gradually increased. Ripe stage oocytes grew to 200 µm in diameter, became round or oval in shape, and were located in the center of the lumen. Each ripe ovum was surrounded by a gelatinous membrane and its cytoplasm was filled with a large number of yolk granules. Individuals in the ripe stage appeared from September to February when seawater temperature gradually increased over 30oC. In Partially spawned stage, the lumen of the oogenic follicle appeared mostly empty. Most ripe ova were discharged from the oogenic follicles, although a few undischarged mature oocytes as well as vitellogenic oocytes remained (Fig. 31).

The oocyte nuclear envelope consists of two distinct osmiophilic membranes, the inner and outer membranes, which are approximately 60 A wide and separated by a less dense perinuclear space usually 150 to 200 A in width. At frequent and regular intervals the inner and outer membranes join to form a pore region which, in profile view, has a diameter of approximately 600 A (Fig. 32). In the oocytes of *Styela partita*, the pore region sometimes appears to be traversed at the level of the nuclear membrane by two fine parallel membranes appearing less distinct and dense than the nuclear membrane. Some degree of electron opacity appears associated with the pore region when viewed in perpendicular sections of the nuclear envelope. It is difficult, however, to determine exactly to what extent this is due to the presence of a material within the pore and how much may be due to the section level which passes through the very edge of the pore rim. The electron-opaque material is more favorably viewed in oblique or nearly tangential sections of the nuclear envelope (Fig. 33). A homogeneous matrix of moderate density is present within the pore as well as between. The wall of the pore in some cases appears to be composed of small, spherical structures approximately 150 A in diameter. Dense granules about 150 A in diameter are also observed in the central region of many of the nuclear pores. Both the inner and outer nuclear membranes appear to engage in considerable activity during the course of oogenesis. This is reflected in a blebbing from both layers of the envelope. A large amount of blebbing takes place along the outer layer of the nuclear envelope especially during early stages of oogenesis (Fig. 34). The blebs have ribosomes associated with their surfaces while still being a part of the outer membrane and enclosing space continuous with the perinuclear space. The outer nuclear membrane sometimes has ribosomes attached to its outer surface. The oocytes in which these intranuclear vesicles were observed are smaller than those in which differentiated intranuclear annulate lamellae occurred. This observation would tend to suggest that the intranuclear vesicles precede the intranuclear annulate lamellae in time in the developing oocyte. The active vesiculation of both the inner and outer nuclear membranes in these and other oocytes indicates that the envelope must be in a highly dynamic state insofar as membrane synthesis and replacement are concerned and that undoubtedly active membrane flow is involved in this process. The endoplasmic reticulum is represented in the developing oocytes by numerous roughsurfaced vesicles scattered throughout the ooplasm. Their diameter varies depending upon the section level, but many range from 200 to 400 m/~. They were observed in oocytes of all sizes studied. In addition to the ribosomes which appear attached to the outer surface of the membranous vesicles, ribosome particles are also scattered throughout the ooplasm, where they usually occur singly rather than in rosettes or clusters. It seems that the vesicular endoplasmic reticulum appears to be derived for the most part from a blebbing of the outer nuclear membrane. Few lamellar elements of the endoplasmic reticulum appear in the developing oocytes, but, when present, they are observed in the very small oocyte before any indication of yolk deposition (Fig. 35). .

The nerve ganglion showed maximal amount of neurosecretory granules and at the same time the neural gland showed maximal width and secretions during September- February. Meanwhile the ovary occupied with huge quantity of vitellogenic and postvitellogenic oocytes. Moderate amount of neurosecretory granules and glandular secretion were found in specimens of July-August. Meanwhile the ovary occupied with huge quantity of young pre- and vitellogenic oocytes. The little amount of neurosecretory granules and glandular secretion were found in specimens of March-June. the ovary occupied with huge quantity of huge quantity of oogonia and previtellogenic oocytes. This means that the neural gland-ganglionic complex controls the ovarian maturation in the ascidian *Styela partita*.

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DISCUSSION

The neural complex can be differentiated into cerebral ganglion, dorsal strand, and neural gland (Saad, 2010, Saad and Hamed, 2009 ; Michael, et al., 2008 ; Ogawa et. al.,1985 ; Georges, 1972). The cerebral ganglion lies between the two siphons being embedded in the mantle and from which several nerves elongate to various parts of the body such as muscles, pharynx, viscera and siphons. The region lies between the two siphons in which the components of the nervous system are present is small in extension and referred to as the interoscular area of Lacaze-Duthiers (Herdman, 1882-1883). The cerebral ganglion is divided into three parts as cerebral cortex, medullary layer and transitional zone. The neural gland of *Styela partita* lies ventral to the ganglion and is composed of glandular lobules, closely apposing at ventral side of the cerebral ganglion; the neurons and nerve fibres were colored deeply, making a distinct contrast with the background. The neural gland of Ciona intestinalis undergoes morphological changes turning from an epithelial structure (reticulate stage) to a mesenchymal structure (compact stage) (Georges,1971). The ganglion is surrounded by blood sinuses and covered with a thin fibrous sheath through which many nerve fibres run. The ganglion is composed of a cellular cortex and a fibrous medulla. The cortex is multilayered of large and small neurons. Some neurons are also scattered within the medulla. Many neurons are monopolar, and some are bi- or multipolar. The cytoplasm of the large neurons is dense with extensive rough endoplasmic reticulum, free ribosomes, mitochondria, one or more Golgi complexes, large dense bodies, and many clear or dense vesicular structures. Some neurons send their processes directly into the lumen of the sinuses. The medulla is composed of loosely arranged nerve fibres without cellular wrappings. The medullary fibres contain vesicles and granules of various sizes and microtubules. At the anterior and posterior ends of the ganglion, the medullary fibres are assembled into thick peripheral nerve fibre bundles. Georges (1967, 1970, 1971, 1972, 1974a&b, 1977 and 1978) investigated the structure of the neural gland ganglionic complex of Ciona intestinalis. This author concluded that neurons located at the periphery of the nerve ganglion, showed a rhythmic alternation of voids and "puddles" which seem, according to cytochemical tests, to be secondary lysosomes. This alternating cycle phase synchronous glandular and therefore in connection with the tide, that was not observed during the oviposition period. Experiments were undertaken to ascertain the factors, internal or external setting the pace: ablation of neural gland or ovary cultures in vitro, in natural lighting or permanent, or complex neural ganglia with or without ovarian (Dawson & Hisaw,1964). In the present study it was found that the secretory granules are dispersed throughout the cytoplasm. A secretin-like immonoreactivity is demonstrated in the neural complex of *Styela plicata* and the secretinergic neurons are localized in the cortex of the cerebral ganglion Pestarino (1984).

Most of the cells in the neural gland of the ascidian *Halocynthia roretzi* are binucleated. The central area of the dorsal wall of the gland is a simple columnal epithelium composed of rather compact mono-nucleate cells. Peripheral to the center of the dorsal wall , the epithelium expands and becomes stratified containing binucleate cells. These cells seem to proliferate in this limited area of the dorsal epithelium and slough off into the lumen of the gland to form a loose parenchymatous tissue (Ogawa et. al.,1985 & 1987) Thorndyke and Geoges (1988) described the nerve ganglion of *Ciona intestinalis* as oval to spindle-shaped and is surrounded by a fibrous sheath of connective tissue. The neural gland. is a blind sac with folded walls that form separate lobules or acini and surrounded with a thin fibrous lamina. This lamina streches when the size of the gland increases. The glandular cells are polyhedral-shaped reticulate or stellate accumulated centrally or distributed overall and these conditions are governed with the tide The nerve ganglion itself governs the cyclical change in the gland cells morphology (Georges and Schawabe,1999).The morphological change in the gland cells is synchronized with tidal fluctuations and accompanied by modification in the nerve ganglion (Georges,1968,1970,19711974 &1977 ; Burighel and Cloney,1997) The first author concluded that most glandular cells are binucleate and the dorsal wall of the gland itself is composed of compact binucleate cells (Electron Microscope study). Ogawa et al., (1985) proved that there is no seasonal variation in the number of these binucleate cells inside the gland. In 1987 the same authors tried to discover the origin of these binucleate cells. The dorsal wall of the gland is composed of a simple columnar epithelia and this epithelium not only extends dorso-posteriorly as a dorsal cord but also connects anteriorly a neural gland duct which open in the pharyngeal cavity via a ciliated funnel. The central area of the dorsal epithelia is composed of simple columnar rather compact and non-nucleate cells. Some of these cells contain two nucleoli located at the opposite side of the nucleus. Peripheral to the center of the dorsal wall the epithelia expands and becomes irrigularly stratified. In this region binucleate cells are observed. The simple columnar epithelia is seen to be gradually transformed into a pseudostratified form in the area around the centre of the dorsal wall.The transformed cells lack uniformity and binucleate cells are encountered. Butcher (1930) studied the neural gland of *Molgula manhattensis* and experimentally ascertained that it is a pituitary. Georges (1974 & 1978) concluded that the neural gland and its funnel in ascidians have been homogenized to the vertebrate adenohypophysis, moreover it plays a regulatory role in spawning i.e., it is the set of circadian rhythm. Harris and Donovan (1966) studied the histology of the pituitary gland in prototheria-like reptiles and concluded that it is a sac- like in which delicate glandular cells and few number of pituicytes are dominate.

Electron microscope studies were made on various tunicate oocytes at different stages of growth and development. Both the inner and outer lamellae of the perforated nuclear envelope demonstrate considerable blebbing activity. The blebs of the inner lamella detach into the nucleoplasrn where they undergo a special type of fusion process resulting in the formation of numerous, usually single, differentiated annulate lamellae of various lengths. The blebbing of the outer layer of the nuclear envelope contributes to the vesicular and granular endoplasmic reticulum characteristically present in the ooplasm and perhaps to the differentiation of cytoplasmic annulate lamellae as well. In particular, various cell organelles, the Golgi complex, the rough endoplasmic reticulum, and mitochondria are thought to be involved in endogenous formation of yolk granules in the cytoplasm, while the uptake of nutritive material in the coated vesicle formed by receptor-mediated endocytosis appeared through the formation of coated endocytotic pits on the oolemma. From the ultrastructural study of this species, vitellogenesis can be classified into two processes: autosynthetic and heterosynthetic yolk formations (Eckelbarger and Davis, 1996). Vitellogenesis occurred through a process of autosynthesis, involving the

combined activity of the Golgi complex, mitochondria and rough endoplasmic reticulum. On the other hand, Pipe (1987) reported endocytotic activity in the oocytes of Mytilus edulis, and Eckelbarger and Davis (1996) presented evidence for heterosynthetic yolk formation in the oocytes of Crassostrea virginica. In the present study, extraovarian precursors are incorporated into oocytes by endocytosis at the basal region of the early vitellogenic oocytes as evidence for heterosynthetic yolk formation. Beside the process of heterosynthetic yolk formation by endocytosis, it is known that for supply of the nutrients to vitellogenic oocytes, the VCT cells and the follicle cells are involved in the process of heterosythetic yolk formation (Eckelbarger and Davis, 1996). From these observations mentioned above, it is assumed that vitellogenesis of Cyclina sinensis occurs by way of endogeous autosynthesis and exogenous heterosynthesis. The follicle cells initially appeared close to the previtellogenic oocyte, and thereafter, progressively surrounded the oocyte. At this stage, a small number of vacuoles was visible in the cytoplasm of the follicle cells near the adherence zone. The attached follicle cells also showed cytological modifications as their cytoplasmic volume increased in Crassostrea virginica (Eckelbarger and Davis,1996) and Mytilus edulis (Pipe, 1987). In Cyclina sinensis, a few follicle cells attached to previtellogenic and early vitellogenic oocytes during the early stages of oogenesis, but in the late stages of oogenesis, most follicle cells, which were attached to the late vitellogenic oocyte, were gradually detached from the mid- or late vitellogenic oocytes; only a few cells appeared near the stalk region of the oocyte. Pipe (1987) reported that endocytotic figures appeared between vitellogenic oocytes and the follicle cells, indicating a transfer nutrients in Mytilus edulis. In the present study, however, similar ultrastructural findings between the follicle cells and the vitellogenic oocytes were observed. According to observations on auxiliary cells and degenerated oocytes showing characteristics of a functional role for hydrolytic enzyme activity, a number of degenerated yolk granules showing lysosomal enzyme activity and containing a few myelin-like organelles appeared in the ooplasm of the degenerated oocytes in C. sinensis. At the same time, the number of phagosomes (or lysosomes) and lipid droplets increased in the cytoplasm of the follicle cells that were attached to the degenerated oocyte. In particular, morphologically similar phagosomes, which were easily observed in the cytoplasm of degenerated oocytes, also appeared in the follicle cells. Thus, the follicle cells appear to play an integral role in vitellogenesis and oocyte degeneration. During the period of oocyte degeneration, the follicle cells function in phagocytosis and intracellular digestion of products originating from oocyte degeneration: these cells may also have a function associated with induction of oocyte degeneration, and it is assumed that they are also active in resorption of phagosomes from the degenerated oocyte because lipid droplets and degenerating phagosomes appeared in the follicle cells. In this study, the number of lipid granules gradually increased in follicle cells during gametogenesis; this function can permit a transfer of yolk precursors necessary for vitellogenesis and allows for the accumulation of reserves in the cytoplasm as glycogen and lipids, which can be employed by vitellogenic oocytes (Gaulejac et al., 1995; Michael et al., 2008 ; Saad, 2008). Therefore, it is assumed that the follicle cells, which are attached to degenerated oocytes, may be involved in the induction of oocyte degeneration and resorption of degenerating phagosomes (lysosomes) through a lysosomal system, as seen in Meretrix lusoria (Chung, 2007). In Mytilus edulis, after spawning, gamete resorptions are common in the acini of the ovary. It is thought that Mytilus edulis resorb gametes in follicles and utilize the high nutritive reserves for developing oocytes for other metabolic activities (Pipe, 1987) as observed in other bivalves (Dorange and Le Pennec, 1989).

LEGENDS

Fig. 1

Semithin of a transverse section of the neural complex of *Steyla partita*. It consists of a cerebral ganglion and a neural gland located above the anterior part of the branchial chamber.

Fig. 2

Semithin of a transverse section of the cerebral ganglion of *Steyla partita*. The cerebral ganglion consists of a cellular cortex and a fibrous medulla. Most of neurons are small, but several huge ones.

Fig. 3

Semithin of a transverse section of the neural complex of *Steyla partita*. The neural gland wall is a loose, irregular, non-ciliated epithelium.

Fig. 4

TEM of a transverse section of the neural complex of *Steyla partita*. Some of the glandular cells are mononucleated and others are binucleated.

Fig. 5

Semithin of a transverse section of the neural complex of *Steyla partita*. The glandular cells are polygonal and with variable sizes. It seems that these cells are budded off from the glandular wall and form the gland secretion.

Fig. 6

TEM of a transverse section of the neural complex of *Steyla partita*.The lateral and ventral sides of the ganglion are surrounded by blood sinuses and the ganglion is covered with a thin fibrous sheath through which many nerve fibres run.

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Fig. 7

TEM of a transverse section of the neural complex of *Steyla partita*.The ganglion is composed of a cellular cortex and a fibrous medulla. Some neurons are also scattered within the medulla. Many neurons are monopolar, and some are bi- or multipolar.

Fig. 8

TEM of a transverse section of the neural complex of *Steyla partita*. The cytoplasm of the large neurons is dense with rough endoplasmic reticulum, free ribosomes, mitochondria and many clear or dense vesicular structures.

Fig. 9

TEM of a transverse section of the neural complex of *Steyla partita*. The medulla is composed of loosely arranged nerve fibres without cellular wrappings. The medullary fibres contain vesicles and granules of various sizes, and microtubules.

Fig. 10

TEM of a transverse section of the neural complex of *Steyla partita*. Neurosecretory granules are observed inside the neurons and the cortex of the ganglion.

Fig. 11

TEM of a transverse section of the neural complex of *Steyla partita*. Neurosecretory granules are maximal during September.

Fig. 12

TEM of a transverse section of the neural complex of *Steyla partita*. Neurosecretory granules are lowest during September.

Fig. 13

TEM of a transverse section of the ovary of *Steyla partita*. The primary oogonium contains a relatively large nucleus.

Fig. 14

TEM of a transverse section of the ovary of *Steyla partita*. The early follicular oocyte migrated from the germinal epithelium and surround the primary oocyte.

Fig. 15

TEM of a transverse section of the ovary of *Steyla partita*. The vitellogenic oocyte.

Fig. 16

TEM of a transverse section of the ovary of *Steyla partita*. vitellogensis occurred through a process of autosynthesis, which involves a combined activities of the Golgi complex, mitochondria, and rough endoplasmic reticulum (Fig. 17).

Fig. 17

TEM of a transverse section of the ovary of *Steyla partita*. The process of heterosynthesis involved endocytotic incorporation of extraovarian precursors into the basal region of the early vitellogenic oocytes prior to the formation of vitelline envelope.

Fig. 18

TEM of a transverse section of the ovary of *Steyla partita*. The follicle cells and test cells appear to play an integral role in vitellogenesis and oocyte degeneration.

Fig. 19

TEM of a transverse section of the ovary of *Steyla partita*. The primary oogonia, which multiplicate on the germinal epithelium, were single or formed a cluster in the acini. The primary oogonia divided mitotically to produce the secondary oogonia.

Fig. 20

TEM of a transverse section of the ovary of *Steyla partita*. As the secondary oogonia entered into the first prophase of meiosis, they developed into previtellogenic oocytes. At the beginning of cytoplasmic growth of the previtellogenic oocyte, germ cells moves internally from the periphery of the ovary and together with several epithelial cells, constitutes the primary follicle. Initially, the germ cell is surrounded only by a flattened layer of epithelium.

Fig. 21

TEM of a transverse section of the ovary of *Steyla partita*. The follicle cells possessed a dense chromatin in the nucleus and arrays of the rough endoplasmic reticulum and mitochondria in the cytoplasm.

Fig. 22

TEM of a transverse section of the ovary of *Steyla partita*. The vesicular connective tissue cells , which contained large quantities of glycogen particles and several lipid droplets, were found near the early vitellogenic oocyte.

Fig. 23

TEM of a transverse section of the ovary of *Steyla partita*. Follicle cells might have a lysosomal system to resorb phagosomes in the cytoplasm for nutrient storage during oocyte development. The chorion and follicular epithelium are developed on the oolemma while , test cells are embedded in the ooplasm.

Fig. 24

TEM of a transverse section of the ovary of *Steyla partita*. In the late vitellogenic oocyte, yolk granules clearly appeared among the mitochondria, lipid droplets, the rough endoplasmic reticulum, Golgi complex and cortical granules at the basal region of the oolemma.

Fig. 25

TEM of a transverse section of the ovary of *Steyla partita*. yolk granules filled the cytoplasm of the oocyte, whereas the follicle cells gradually lost their intimate association with the late vitellogenic oocyte surface. After a few follicle cells detached from the oolemma of the late vitellogenic oocyte, microvilli occurred on the site of oolemma.

Fig. 26

TEM of a transverse section of the ovary of *Steyla partita*. The cytoplasm of residual follicle cells, which was attached to the oocyte, was filled with vacuoles and myelin-like organelles. In the late stages of oogenesis, larger immature yolk granules were formed by mixing of small yolk granules.

Fig. 27

TEM of a transverse section of the ovary of *Steyla partita*. Mature oocytes began to separate from the follicular wall and smaller immature yolk granules were continuously intermingled with one another and became larger mature yolk granules in the cytoplasm

Fig. 28

TEM of a transverse section of the ovary of *Steyla partita*. Late active stage is characterized by the presence of early vitellogenic oocytes, which formed an egg-stalk connected to the germinal epithelium, and the auxiliary cells, which were attached to the oocyte, appeared in the lumen of the follicle.

Fig. 29

TEM of a transverse section of the ovary of *Steyla partita*. Ripe stage oocytes became round or oval in shape, and were located in the centre of the lumen. Each ripe ovum was surrounded by a gelatinous membrane and its cytoplasm was filled with a large number of yolk granules.

Fig. 30

TEM of a transverse section of the ovary of *Steyla partita*. Most ripe ova were discharged from the oogenic follicles, although a few undischarged mature oocytes as well as vitellogenic oocytes remained.

Fig. 31

TEM of a transverse section of the ovary of *Steyla partita*. Vitellogenic oocytes remained in the ovary after reproductive season undergo gradual autolysis.

Fig. 32

TEM of a transverse section of the ovary of *Steyla partita*. Another process of oogenesis proceeds while autolysis of the remained vitellogenic oocytes occurs.

Fig. 33

TEM of a transverse section of the ovary of *Steyla partita*. A homogeneous matrix of moderate density is present within the ovulated oocyte.

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Fig. 34

TEM of a transverse section of the ovary of *Steyla partita*. Vesicular structure increase gradually in vitellogenic oocytes.

Fig. 35

TEM of a transverse section of the ovary of *Steyla partita*. Some degree of electron opacity appears associated with the pore region when viewed in perpendicular sections of the nuclear envelope. The electron-opaque material is more favorably viewed in oblique or nearly tangential sections of the nuclear envelope.

ABBREVIATIONS

BG	Binucleated Glanular Cell
BS	Blood Sinus
CC	Cellular Cortex
C	Chorion
CG	Cerebral Ganglion
CN	Cortical Granules
DC	Dark Cell
EO	Early Follicular Oocyte
EM	Electron-Dense Materials
ER	Endoplasmic Reticulum
E	Epithelium
EP	Endocytotic Pit
FE	Follicle Epithelium
FM	Fibrous Medulla
FR	Free Ribosomes
GC	Golgi Complex
G	Granules
LN	Large Multilayered Neuron
LC	Light Cell
L	Lysosome
MV	Microvilli
M	Microtubules
MI	Mitochondria
MC	Mononucleated Glanular Cell
NC	Nerve Fibres
NX	Neural Complex
NG	Neural Gland
NG	Neurosecretory Granules
NU	Nucleolus
N	Nucleus
PO	Previtellogenic Oocytes
PG	Primary Oogonium
PO	Ripe Ovum
R	Ribosome
SO	Secondary Oogonium

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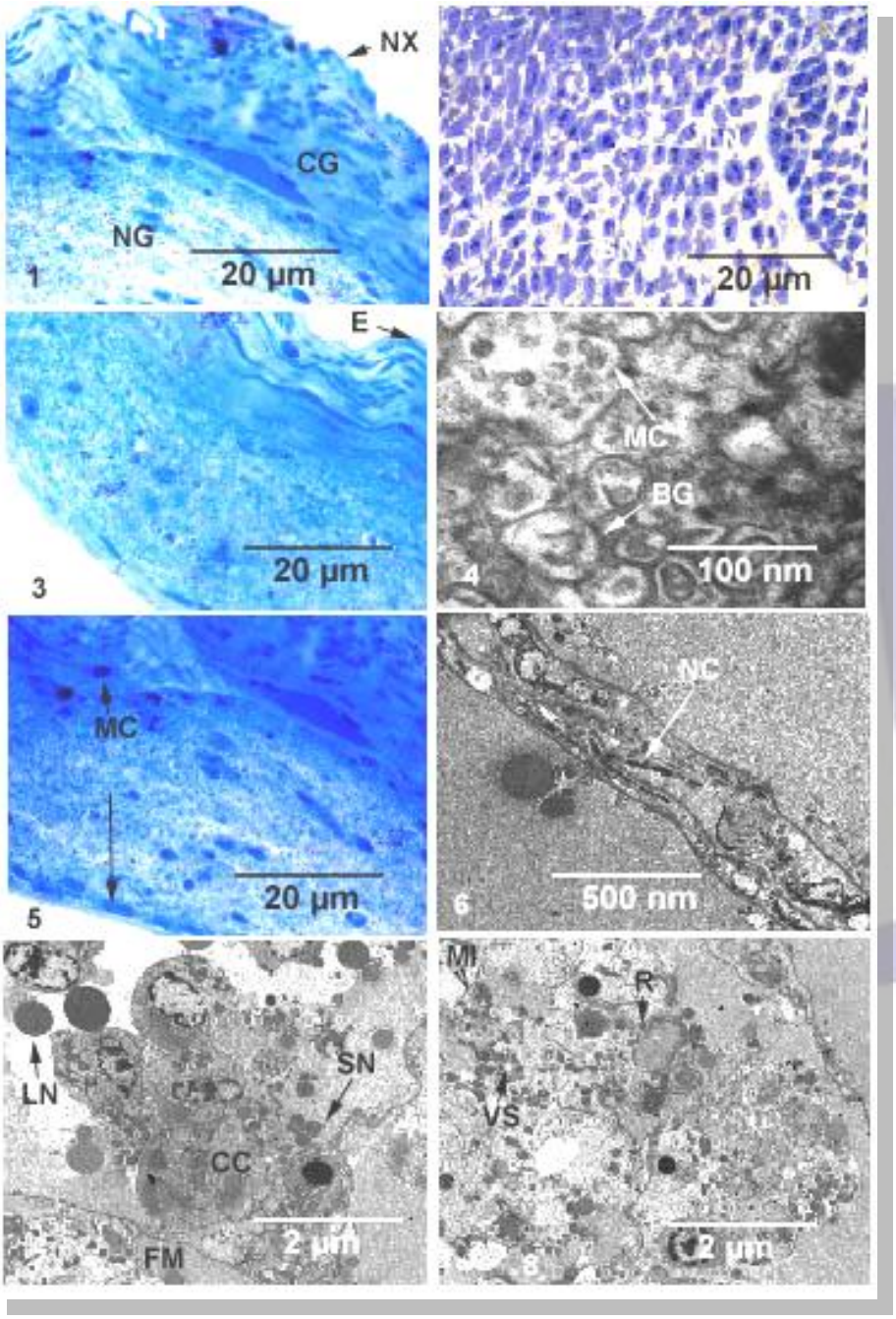
SN	Small Neuron
TC	Test Cell
VS	Vesicular Structure
VE	Vitelline Envelope
VO	Vitellogenic Oocyte
Y	Yolk



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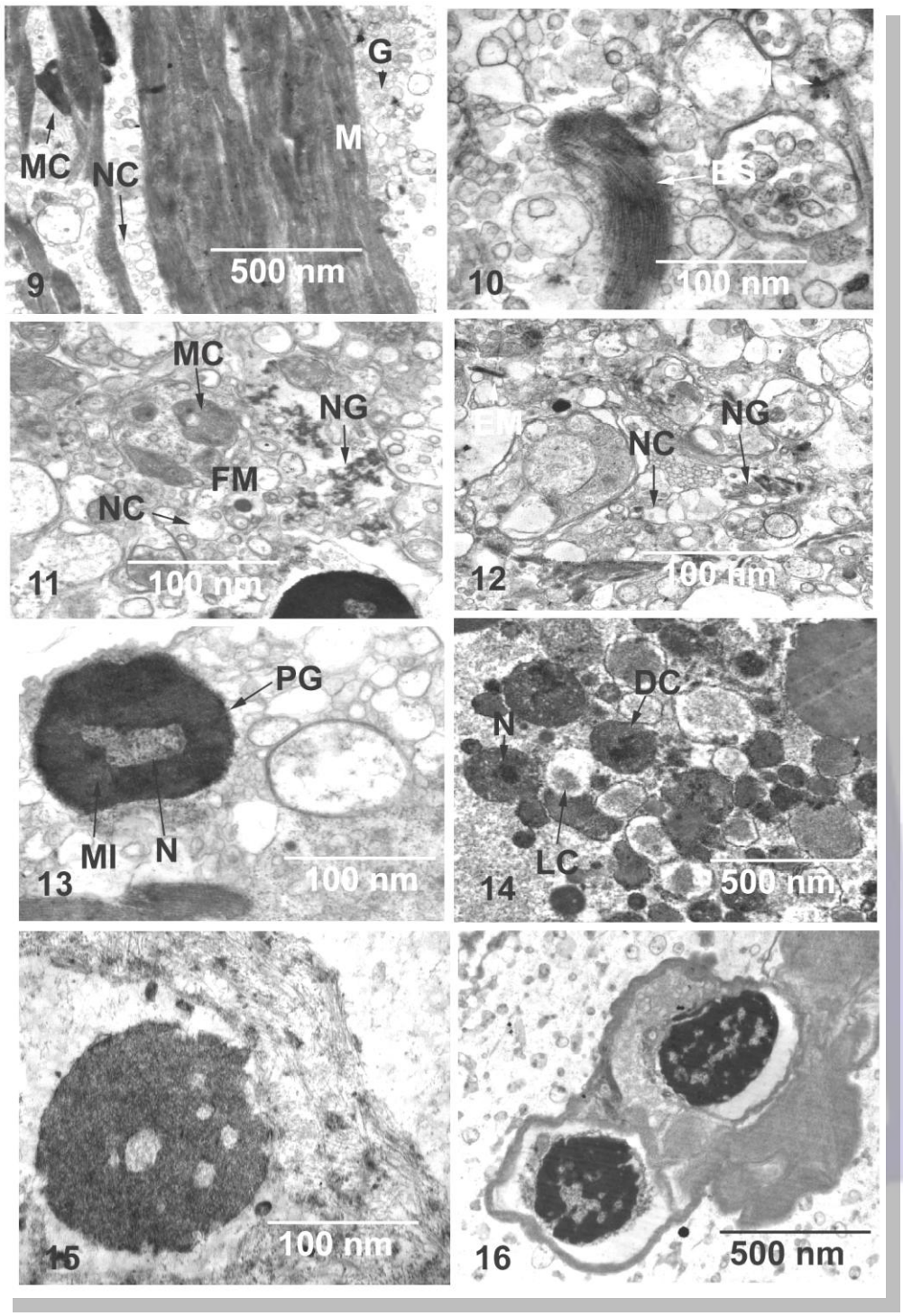

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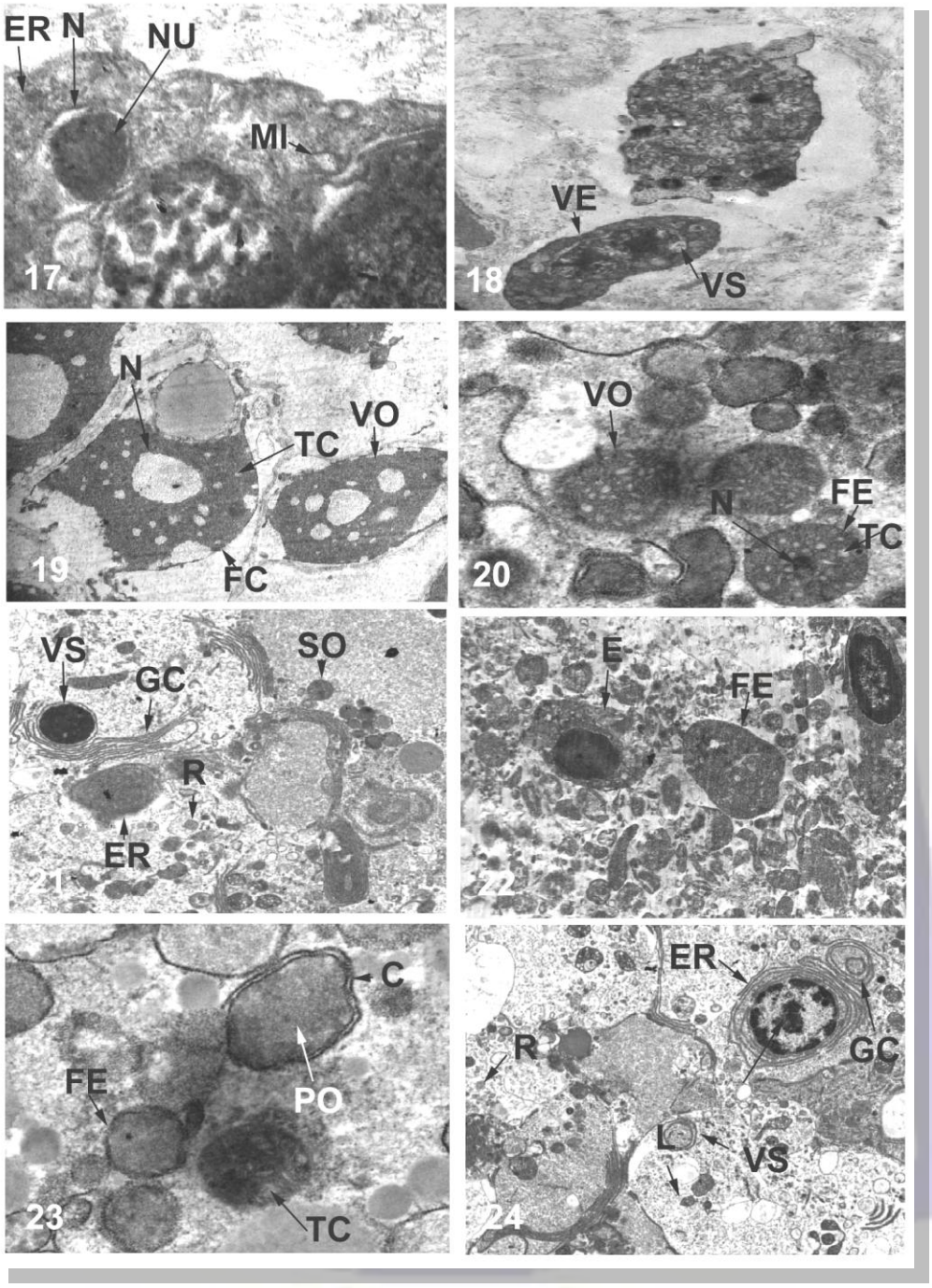

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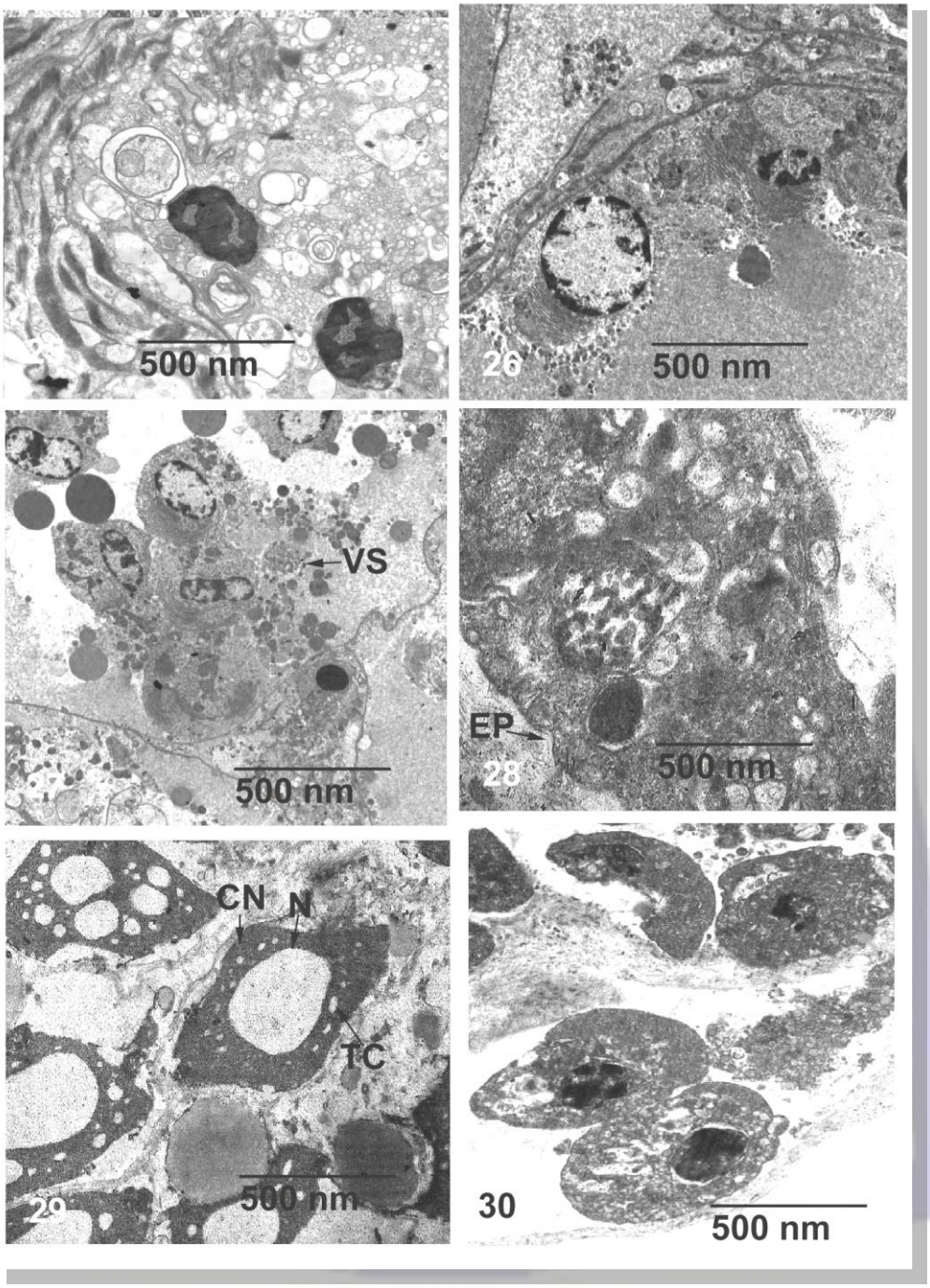

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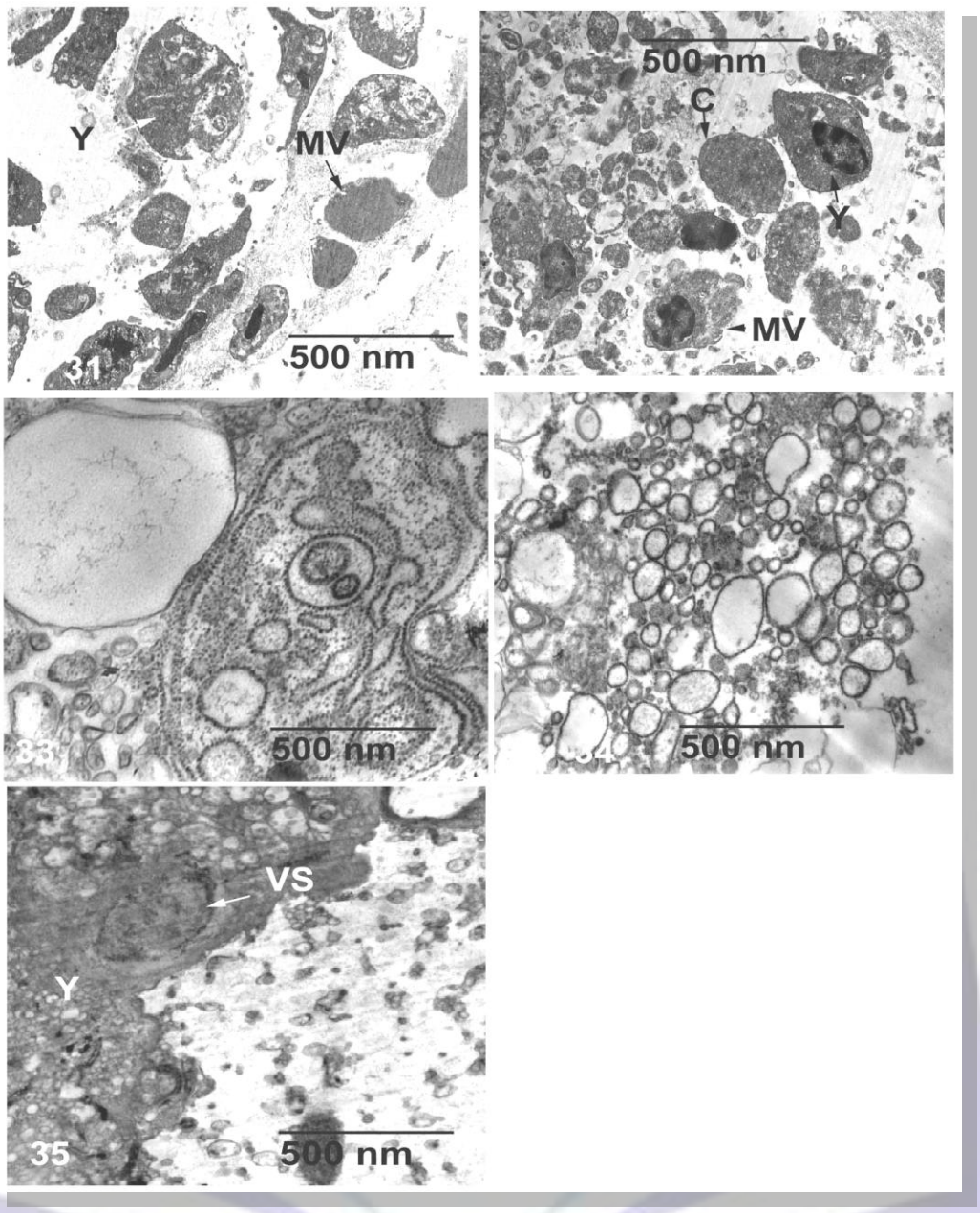

REFERENCES

{1} Becerro. M. A., Turon. X. 1992. Reproductive cycles of the ascidians *Microcosmus sabatieri* and *Halocynthia papillosa* in the Northwestern Mediterranean. Mar. Biol.,13:363–373.

{2} Becker, A. and Hart, F. 1999. Zur Fouling-Sukzession auf Hartsubstraten unterschiedlicher Oberflächenspannungen in der Kieler Bucht, Diplomarbeit: 133 pp.

{3} Bern, H. A. 1962. The properties of neurosecretory cells. Comp. Endocr., 1:117- 132.

{4} Berg, L. K., and Wessel, G. M. 1997. Cortical granules of the sea urchin translocate early in oocyte maturation. Development 124, 1845–1850.

{5} Berrill, N. J., 1975. Chordata: Tunicata. In Reproduction of Marine Invertebrates,Vol. 2 (Eds. A. C. Giese and J. S. Pearse), pp. 241-282. Academic Press, New york.

{6} Bouchard-Madrelle, C., 1967. Influence de l'ablation d'une partie ou de la totalie de Complexe neural sur le fonctionnement des gonades de *Ciona intestinalis* (Tunicier, Ascidiace). *C.R. Acad. Sci. Ser. D.,* 264 : 2055-2059.

{7} Burighel , P. and Cloney , R. A. 1997. 'The iIvertebrate Chordates'.In Microscopic Anatomy of Invertebrates .Vol. 15 Hemichordata , Chaetognatha and the Invertebrate Chordates. Harris F. W. and Ruppert E. E. eds. A John Willy and sons INC Publication New York .

{8} Butcher , E. O. 1930. The pituitary in the ascidian *Molgula manhattensis*. J. Exp. Zool., **57** : 1-11.

{9} Carlisle, D. B. 1951. On the hormonal and neural control of the release of gametes in ascidians. J. Exp. Biol., 28 (4) : 463 - 472.

{10} Chen, J.; Dai-Chien, C. 1998. The enigmatic genius. Singapore : Asian.

{11} Chu-Chin, C. ; Yi-Hong, C. ; Ling-Ling, S. and Suerl, L. 2003. Analysis of Dabsyl-Cl Derivated Amino Acids by High Performance Liquid Chromato-graphy and Tandem Mass Spectrometry. Food Sci. Technol. Res.,9 (3), 276–282.

{12} Chung, E. Y. 2007. Oogenesis and sexual maturation in *Meretrix lusoria* (Roding, 1798) (Bivalvia: Veneridae) in western Korea. J Shell Res 26: 1-10.

{13} Cloney , R. A. 1990b. Urochordata : Ascidiacea. In K. G. Adiyodi and R. G. .Adiyodi (eds.). Reproduction Biology of Urochordata.Vol. IV B. Fertilization, Development and Parental care. New York , Oxford pp 391-451.

{14} Cloney , R. A. 1990a. Larval tunic and the function of the test cells in ascidians . Acta Zool. (Stockh.) 71: 151-159.

{15} Cotelli, F. ; Andronico, F.; De Santis, R. ; Monnroy, A. and Rosati, F. 1981.Differentiation of the vitelline coat in the ascidian *Ciona intestinalis*: An Ultrastructural Study Roux's ARch. Dev. Biol., 190: 252-258.

{16} Dawson, A. and Hisaw, F. L. 1964.: The Occurrence of Neurosecretory Cells in the neural ganglia of tunicates. J. Morph., 114:411-424.

{17} Dodd, J. and Dodd, M. 1966. An experimental investigation of the supposed pituitary affinities of the ascidian neural complex. In “Some contemporary studies in marine science” Ed by H Barnes, Allen & Unwin, London, pp 233-252.

{18} Dodd, J. M. 1955. The hormones of sex and reproduction and their effects in fish and lower chordates. Pp. 166-187 in I. C. Jones and P. Eckstein, Eds., Comparative Physiology of Reproduction, Memoirs of the Society for Endocrinology. Cambridge Univ. Press, London.

{19} Dorange, G. ; Paulet, Y. and Pennec, M. 1989. Etude cytologique de la partie femelle de la gonade de Pecten maximus recolte en baie de Saint-Brieu. 2. Ovogenese et lyse ovocytaire. Haliotis 19: 299-314.

{20} Eckelbarger, K. J. and Davis, C. V. 1996. Utrastructure of the gonad and gametogenesis in the eastern oyster, *Crassostrea virginica*. 1. Ovary and oogenesis. Mar Biol 127: 79-87.

{21} Fritsch, H. ; Van Noorden, S. and, Pearse, A. 1982. Gastro-intestinal and neurohormonal peptides in the alimentary tract and cerebral complex of *Ciona ntestinalis* (Ascidiaceae). Cell Tissue Res 223: 369–402.

{22} Gaulejac, Henry, M. and Vicente, N. 1995. An ultrastructural study of gametogenesis of the marine bivalve *Pinna nobilis* (Linnaeus 1758) 1. oogenesis. J Moll Stud 61: 375-392.

{23} Genri, f. ; Harada, Y; Takagi Y et al. 2002. "Mechanisms of self-fertility in hermaphroditic chordate.". Science, 320: 548–50.

{24} Georges, D. 1971. Le rythme circadien dans la glande neurale de l'ascidie *Ciona intestinakis*. Etude anatumie microscopique. Acta Zoologica, 52:257-273

{25} Georges, D. 1978. Circadian Rhythm in the Neural Gland of *Ciona intestinalis*.Gen.Comp. Endocrin., Gaillard. P. J. and Boer, H. H., 1 : 161-163.

{26} Georges, D. 1967. Influence de l'eclairement sur la ponte de *Ciona intestinalis* L. (Tonicier- Ascidiace). Extrait des Cahiers de Biologie Marine. Tome IX : 106-113.

{27} Georges, D. 1970. Le rythme circadien dans la glande neurale de l'ascidie *Ciona intestinakis*. Etude anatumie microscopique. Acta Zoologica, 52:257-273.

{28} Georges, D. 1971. Fonctionnement Rythmique du Complexe Neural et Control Neuro- Endocrinien de la ponte chez *Ciona intestinalis* L.(Tunicier). Science 3: 27-39.

{29} Georges, D. 1974. Active rythmique du complexe neurall de *Ciona intestinalis* (Tunicier). Relation avec la ponteLaboratoire de Zoologie. B.P. 53,38041 Grenoble Cedex pp. 83-99.

{30} Georges, D. 1977. Analyse fonctionelle du Complexe Neural chez *Ciona intestinakis* (Tunicier, Ascidiace). Gen. Comp. Endocrin. 32:454-473.

{31} Georges, D. 1978. Circadian Rhythm in the Neural Gland of *Ciona intestinalis*. Gen.Comp. Endocrin., Gaillard,P. J. and Boer,H. H.,eds 161-163.

{32} Georges, D. and Dubois, M. P. 1984. Methionine-enkephalin-like immunoreactivity in the nervous ganglion and the ovary of a protochordate, *Ciona intestinalis*. Cell Tissue Res. 236:165-170.

{33} Georges, D. and Schwabe, C. 1999. Porcine relaxin, a 500 million-year-old hormone? The tunicate *Ciona intestinalis* has pocine relaxin. The Fased Journal 13: 1269-1275.

{34} Gianguzza, M. and Dolcemascolo, G. 1983. Ultrastructural comparative analysis on the adhesive papillae of the swimming larvae of three ascidian species. Dipartimento di Biopatologia e Metodologie Biomediche Sez. di Biologia e Genetica, Università di Palermo via Divisi 83, 90133 Palermo, Ital.y

{35} Giese A. C Pease J. S.. 1874. Reproduction of Marine Invertebrates. II : Entoprocts and Lesser Coelomates. Academic Press New York San Francisco London.

{36} Golding, W. 1974. Der Sonderbotschafter. Sprache: Deutsch. Verlag: Düsseldorf. http://www.worldcat.org/title/william- golding-the-dark-fields-of-discovery/oclc/3091016&referer=brief_results. Datenbank: WorldCat

{37} Hancock, A., 1868. On the anatomy and physiology of the Tunicata. J. Linn. Soc. Lond. Zoo!., 9 : 309-346.

{38} Herdman, W. A. 1882-1883. On the homology of the neural gland in the tunicata with the hypophysis cerebri. Edinburgh., **12** : 145-151 juvenile giant scallops (Placopecten magellanicus) in suspended culture. Aquaculture 121, 327-342.

{40} Hisaw, F. L., Borricelli, C. R. and Hisaw, F. L. 1966. A study of the relation of the neural gland-ganglion complex to gonadal development in an ascidian, *Chelyosoma productum* Simpson. Gen. Comp. Endocrinol., 7 : 1-9.of *Ciona robusta,* with special reference to the localization of vanadium and iron. Protoplasma, 108: 9-19.

{42} Julin, C., 1881. Recherches sur l'organization des Ascidies simples. Sur l'hypophyse et quel ques organes qui s'y rattachent, dans les genres *Core!la, Phallusia*, et *Ascidia.* Arch. Biol., 2 : 59â€”126.

{43} Kessel , R. G. 1966. Electron microscope studies on the origin and maturation of yolk in oocytes of the tunicate *Ciona intestinalis*. Zellforsch Mikrosk. Anat., 71: 525-544.

{44} Kessel, R.G., 1983. Intranuclear membranes (vesicles, lamellae, annulatelamellae) in oocytes of the ascidian, *Styela partita*. J. Submicrosc. Cytol.15: 773– 785.

{45} Lane, N. J., 1972. Neurosecretory cells in the cerebral ganglion of adult tunicates : Fine structure and distribution of phosphatases. J. U!trastruct. Res., 40 : 480-497.

{46} Lee, T.Y., 1976. The breeding and development of *Styela mammiculata* Carlisle. J. Marine Biol. Ass., of the United Kingdom, 41 : 187-190.

{47} Lee, T.Y., 1977. Gametogenesis and reproductive cycle of Urochordata. 2. Ascidian, *Styela clava* Herdman. Publications. Institute of Marine Sciences, National Fisheries University, Busan, Korea, 10, 514.

{48} Michael, M. I.; Hofmann, D.K. ; Khalil, S.H.; El-Bawab, F.M. and Saad, G.A. 2008. Comparative study of the nervous and reproductive systems of *Styela plicata*, *Styela partita* and *Ciona intestinalis* (Urochordata, Ascidiacea) collected from the Mediterranean Sea near Alexandria, Egypt. 5th Int. Con. Biol., (Zool.).5 : 330-374.

{49} Millar, R.H. 1971. The Biology of Ascidians. Adv. mar. Biol., 9 : 1-100.

{50} Satou, Y., Yamada, L., Mochizuki, Y. 2002. A cDNA resource from the basal chordate *Ciona intestinalis*. Genesis, 33(4):153-4.

{51} Ogawa, M.; .Orikasa, C. and Terakado, K. 1985. Binucleate cells in the Neural Gland of the Ascidian *Halocynthia*

roretzi (Drasche). Zool. Science 2: 213-217.

{52} Ogawa, M.,Terakado, K. and Okada, J. 1987.: Origin of Binucleate cells in the neural gland of the Ascidian *Halocynthia roretzi* (Drasche). Zool. Science, 4 ::731-734.

{53} Park, T. M.; Connell, S.D. , Hollyway, M.G. and Hewitt, C.L. 1991. Estudio de la comunidad de peces de dos localidades de la Laguna de La Restinga. Bol. Inst. Oceanog., Venez. 20(1-2):91-112.

{54} Pestarino, M. 1984. Immunocytochemical demonstration of prolactin-like activity in the neural gland of the ascidian *Styela plicata.* Gen. Comp. Endocrinol., 54:444–449.

{55} Pestarino, M. 1983. Somatostatin-like Immunoreactive neurons in a protochordate. Experientia, 39:1156-1158.

{56} Pestarino, M. 1988. Occurrence of melanotropin-and corticotropinlike material inthe cerebral ganglion of a protochordate. Neurosci. Lett., 87: 210–214.

{57} Pipe, R. K. 1987. Oogenesis in the marine mussel *Mytilus edulis*: an ultrastructural study. Mar. Biol., 95: 405-414.

{58} Reverberi , G. 1971.: Ascidians . In Experimental Embryology of marine and fresh water invertebrates. Ed. G. Reverberi- North Holland. Oubl. Co. Amesterdam pp 507-550.

{59} Roule, L., 1884. Recherches sur les Ascidies simples des cot©s de Provence (Phallusiades). Ann. Mus. Marseille, 2 : 263-270.

{60} Saad, G. A. 2002. Comparative studies of the nervous and reproductive systems of some species of urochordates with emphasis of the role of the nervous system on reproduction and larval metamorphosis. Ph.D. Thesis , Faculty of Science, Alexandria University.

{61} Saad, G. A. 2008. Histological and histochemical studies of the process of deutoplasmogenesis (vitellogenesis) in the oocyte of *Styela plicata*Lesuaer,1823), *Styela partita* (Stimpson, 1852) and *Ciona intestinalis* (Linnaeus, 1767). Urochordata- Ascidiacea. Int. J. Aquatic Res., 34(2): 387-411.

{62} Saad, G.A. , Radwan, Kh. H. ;Mahmoud, G. A.; Radwan, E.H. 2008. Molecular characterization of *Ciona intestinalis* proteins using Polyacrylamide Gel Electrophoresis. J. Egy. Germ. Soc. Zool., 57 (C): 297-320.

{63} Saad, G.A. and Hamed, S.S. 2009. Flourescence Axiomicroscopy and SEM studies of metamorphosis of *Ciona intestinalis* (Linnaeus,1767) and the role of NO/cGMP Signaling with the molecular chaperone heat shock protein 90 (HSP90) activity in *Phallusia mammilata* (Cuvier, 1815). Egy. .J. Zool. Soc., 53:461-490.

{64} Saad, G. A. 2010. Light and electron microscopical studies on the development of the neural complex of *Ascidia mentula* (Urochordata – Ascidiacea). Int. J.Aquatic Res., 36(1), 133-146.induced in epithelial cells is an extracellular matrix component for stem cell aggregation in tunicates. Development 113: 995-1005.

{66} Sugino, Y. M. , Tominaga, A. and Takashima, Y. 1987. Differentiation of the accessory cells and ascidian *Ciona savign* during early oogenesis. J. Exp.Zool., 242:205-214.ascidian *Ciona savignyi* Invertebrate Reproduction and Development. Invertebrate Reproduction and Development: 17 (2): 165 – 170.

{68} Terakado, K., Ogawa, M., Inoue, K., Yamamoto, K., and Kikuyama, S. 1997. Prolactin-like immunoreactivity in the granules of neural complex cells in the ascidian *Halocynthia roretzi*. Cell Tissue Res. 289: 63–71.

{69} Tombes, A. S. 1970. An Introduction to Invertebrate Endocrinology. Academic Press. New York. 203 pp.

{70} Wallace, R. A. and Selman, K. 1990. Ultrastructural aspects of oogenesis and oocyte growth in fish and amphibians. J. Electron Microsc., 16: 175-201.

{71} Wessel, G. M., Berg, L., Adelson, D. L., Cannon, G., McClay, D.R., 1998. Amolecular analysis of hyalin—A substrate for cell adhesion in the hyalinelayer of the sea urchin embryo. Dev. Biol., 193, 115–126.

{72} Yang, C. N. And Lee, B. 1978. in: Internationales Biographisches Archiv 38/1987 vom 7., im Munzinger-Archiv (Artikelanfang frei abrufbar). https://portal.dnb.de/opac.htm?method=simpleSearch&query=133780120