Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Fine structure of the gonadal tissue of the horned octopod *Eledone cirrhosa* (Lamarck, 1798) (Mollusca – Octopoda) during sexual maturity

Estrutura fina do tecido gonadal do polvo com chifres *Eledone cirrhosa* (Lamarck, 1798) (Mollusca – Octopoda) durante a maturidade sexual

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Abstract

The horned octopod *Eledone cirrhosa*, a medium-sized species found in Arabian Gulf off Saudi Arabia, was collected monthly from the Arabian Gulf off Dammam city during January to December 2022. Samples were dissected and prepared for examination using transmission electron microscopy. During genital maturation, seminiferous tubules are established in the testis, with active spermatogonia dividing. Spermatocytes 1 are observed in the tubule, followed by an increase in spermatogonia and spermatocytes in August. Spermiogenesis begins, with spherical spermatids evolving into elongated spermatids. In September, active spermatogonia, meiotic divisions, and increased spermiogenesis continue. Spermatozoa appear in Needham's pouch, indicating sexual maturity. The ovary undergoes various stages of development, with oocytes at stage I in June and July, followed by stage II in October and November. In stage III, follicular cords invade the oocyte's cytoplasm, forming numerous lipid inclusions and protein granules. The cytoplasm contains cisternae of endoplasmic reticulum and a poorly developed Golgi apparatus. Stage IV occurs in November, characterized by the maximum development of follicular cords and the beginning of vitellogenesis. The ooplasm contains numerous lipid inclusions, a syncytium, and secretory cells. From December, stage V oocytes are mainly present, indicating the activity phase of maximum secretion. Yolk platelets accumulate in the oocyte ooplasm, and chorion forms at the zona pellucida. In January, the first smooth eggs are found in some octopuses' ovary, with their proportion increasing steadily. This study aimed to investigate the mitogenic action of gonadotropin and identify the periods of intense cell multiplication during the sexual cycle using cytological methods.

Keywords: *Eledone cirrhosa*, electron microscopy, seminiferous tubules, spermiogenesis, oocytes, endoplasmic reticulum, Golgi apparatus, yolk platelets.

Resumo

O polvo com chifres *Eledone cirrhosa*, uma espécie de tamanho médio encontrada no Golfo Arábico ao largo da Arábia Saudita, foi coletado mensalmente no Golfo Arábico ao largo da cidade de Dammam durante o período de janeiro a dezembro de 2022. As amostras foram dissecadas e preparadas para exame por microscopia eletrônica. Durante a maturação genital, os túbulos seminíferos se estabelecem nos testículos, com espermatogônias ativas se dividindo. Espermatócitos 1 são observados no túbulo, seguidos de aumento de espermatogônias e espermatócitos em agosto. A espermiogênese começa com espermátides esféricas evoluindo para espermátides alongadas. Em setembro, as espermatogônias ativas, as divisões meióticas e o aumento da espermiogênese continuam. Os espermatozoides aparecem na Bolsa de Needham, indicando maturidade sexual. O ovário passa por vários estágios de desenvolvimento, com oócitos no estágio I em junho e julho, seguido pelo estágio II em outubro e novembro. No estágio IIIc, os cordões foliculares invadem o citoplasma do oócito, formando numerosas inclusões lipídicas e grânulos proteicos. O citoplasma contém cisternas de retículo endoplasmático e um Aparelho de Golgi pouco desenvolvido. O estágio IV ocorre em novembro, caracterizado pelo máximo desenvolvimento dos cordões foliculares e pelo início da vitelogênese. O ooplasma contém numerosas inclusões lipídicas, um sincício e células secretoras. A partir de dezembro, os oócitos do estágio V estão principalmente presentes, indicando a fase de atividade de secreção máxima. As plaquetas da gema acumulam-se no ooplasma do ovócito e o córion se forma na zona pelúcida. Em janeiro, os primeiros óvulos lisos são encontrados no ovário de alguns polvos e sua proporção

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Received: February 04, 2024 – Accepted: April 26, 2024

aumenta continuamente. Este estudo teve como objetivo investigar a ação mitogênica da gonadotrofina bem como identificar os períodos de intensa multiplicação celular durante o ciclo sexual por meio de métodos citológicos.

Palavras-chave: *Eledone cirrhosa*, microscópio eletrônico, túbulos seminíferos, espermiogênese, oócitos, retículo endoplasmático, Aparelho de Golgi, plaquetas de gema.

1. Introduction

The genus *Eledone* of octopuses includes tiny to medium-sized benthic species that live up to 300 meters below the surface on rocky and sandy substrates (Barratt et al., 2008). *Eledone moschata*, *E. massyae, and E. cirrhosa* are most significant economically (Orsi Relini, et al., 2006; Akyol et al., 2007; Regueira et al., 2013). At a depth of 10 to 50 meters, the benthic species *Uroteuthis duvauceli* is widely distributed in Northern Red (Kilada and Riad, 2010). *E. cirrhosa* is a medium-sized species that inhabits muddy bottoms at bathyal and circalittoral levels. Its maximum size is less than 1 kilogram (Rjeibi, et al., 2013). In cephalopods, as *Bathypolypus sponsalis* and *Octopus hubbsorum* reproductive activity is typically a seasonal and cyclical occurrence that impacts the gonad, genital tract, and behavior related to reproduction (Quetglas et al., 2005; García-Flores et al., 2019). Research on octopod reproduction, particularly with regard to *Octopus* spp. revealed a notable seasonal periodicity and unpredictability in the spawning periods across various marine environments (Boyle and Rodhouse, 2005, Rodríguez-Rúa, et al., 2005). Authors interested to investigate the reproductive status of *Octopus pallidus* and its relationship to age and size (Leporati et al., 2008). Furthermore, male *Octopus vulgaris, Graneledone macrotyla* and *Enteroctopus megalocyathus* reach sexual maturity earlier than females (Gonçalves et al., 2002; Lourenco et al., 2012; Guerra et al., 2013; Ortiz, 2013).

The experimental studies carried out by Wang and Ragsdale (2018) have demonstrated the reality of the controls exerted by the optic gland in genital maturation of male and female *Octopus bimaculoides*. It seems that the existence of a mitogenic effect of gonadotropin of the optical gland of female octopuses on gonial tissues and follicular cells (Matthiessen, 2013; Alejo-Plata and Gómez-Márquez, 2015). In particular this study concluded that the optic gland undergoes remarkable molecular changes that coincide with transitions between behavioral stages. Octopuses and other soft-bodied (coleoid) cephalopods have a single reproductive cycle and are semelparous, meaning that their lives are brief (Lourenco et al., 2012). Octopuses live solitary lives and only associate with one another for mating (Krstulovic et al., 2009). In certain sections of their oviducal gland, females store sperm (Quetglas et al., 2001, Šifner and Vrgoc, 2009; Laptikhovsky, 2013; Sano et al., 2011; Olivares et al., 2017). Upon lying, eggs are fertilized after passing via the oviducal gland (Olivares et al., 2017). Using mucous secretions, the female octopus attaches her eggs to the substrate and tends to her clutch while the embryos grow (Silva et al., 2002; Son et al., 2015). She hardly ever leaves her clutch and avoids food during this brooding phase. According to Cortez et al. (1995) and Garci et al. (2015) the female perishes at hatching. The organotypic culture method appeared much too long and tedious as bioassay for hormone characterization (Hildebrand et al., 2002). In order to isolate and characterize the *octopus* gonadotropin, this study sought a more rapid and quantifiable bioassay, the very short-term cultures (a few hours) of isolated cells seem to be the better solution (Iwakoshi et al., 2002).

According to Wells et al. (1975) and Garci et al. (2015) in the ovaries of mature *Octopus vulgaris*, protein synthesis is assessed by the incorporation of [14C]leucine *in vivo* and in separated groups of eggs *in vitro*. This process happens at a high pace. *In vivo* removal of the optic glands significantly lowers amino acid incorporation *in vivo* or *in vitro* 1-3 days before testing. *In vivo* integration ceases after five days. The addition of optic gland extract accelerates the rate of incorporation *in vitro*. The hormone affects leucine's inward transport independently of its impact on protein synthesis, according to an analysis of the kinetics of leucine absorption and incorporation *in vitro*. Studies using an electron microscope on follicular cells and ova reveal that the former are where protein synthesis takes place (Faure, 2002; Barratt et al., 2007). A qualitative biological test for the optic gland hormone can be performed using alterations in the follicle cells' absorption or incorporation of the hormone into proteins (Wang and Ragsdale, 2018). Measuring uptake is very simple, but integration is a more delicate factor. As a quantitative assay, either may be appropriate for this and maybe other molluscan gonadotropins. Subsequent tests carried out on octopuses have been disappointing (Wells et al., 1975

It was necessary to choose the periods which were naturally the most favorable to experimentation by following in great detail the development of target cells during the year. The second author of this article investigated the sexual maturity indices and gametogenesis in the horned octopus *Eledone cirrhosa* (Lamarck, 1798) from the Mediterranean Sea off Alexandria, Egypt (Gaber and Elghazaly, 2021). This article concluded that males mature earlier than females,. The centripetal succession of spermatogonia, spermatocytes I, II, spermatids, and spermatozoa is visible in seminiferous tubules. Commentary is provided on the histological image of the oocytes, accessory glands, and ovarian lobules. Morphometric measures and histological overviews both identify two distinct spawning periods: a more intense spring to summer spawning and a less intense fall spawning. Males matured between June and January, and spermiogenesis continued vigorously right up until copulation. Starting in November, vitellogenesis lasts until the following laying season. It takes about eight months for females and four months. The aim of the present study was focused on the search for the mitogenic action of gonadotropin and sought by cytological method to identify the exact periods of intense multiplication of follicular and gonial cells during the sexual cycle of a population of the octopod *Eledone cirrhosa* frequenting the Anfouchy

beach. This approach makes it possible to avoid any failure linked to qualitative and quantitative variations of target cells during the sexual cycle.

2. Material and Methods

The benthic and neritic octopus *Eledone cirrhosa* (Lamarck, 1798) is a stationary, lonesome animal that loses its individuality during the reproductive process (Relini, et al., 2006, Rjeibi et al., 2013, García-Flores et al., 2019;). It lives in marine environments on rocky backdrops with lots of stones and fissures. During January to December 2022, each month 10 adult octopuses were collected from the Arabian Gulf off Dammam city (Figure 1), using pots, craft gear, and decoys. Aquaria are used to transport samples to the lab, where they are dissected following carbon dioxide anesthesia. Gonadal samples were taken from males and females every two weeks during the sexual cycle. The male and female gonad fragments are fixed with 2.5% phosphate-buffered glutaraldehyde in a 0.4 M sodium cacodylate buffer (pH 7.2) at 4 °C for 20–24 h. and postfixed with osmium tetroxide (1%, isotonic, pH 7.2, in the same buffer, at 4 °C for 3 h. and included in the epon (Son et al., 2015). The Semithin sections were stained with toluidine blue. The fine sections are contrasted with uranyl acetate followed by lead citrate. The observations were carried out in "Elmiskop I" (Siemens) and Electron Microscopy Center of the University of Alexandria.

3. Results

3.1. Fine structure of the testis

The testis is formed of highly developed seminiferous tubules in different sexually mature phases. These tubules are lined with a thin layer of connective-muscular tissue containing fibrocytes, muscle cells, blood vessels and nervous endings. Centripetal differentiation of germ lines is observed with a successive appearance of spermatocytes, spermatids and spermatozoa. The spermatogonia are located at the periphery of the tubules, right against the thin envelope of connective-muscular tissue. They have a kidney-shaped nucleus with one or two nucleoli and chromatin distributed at the periphery of the nuclear envelope (Figure 2A & 2B). The spermatocytes 1 have a large nucleus in which condensation was observed in progressive chromosomes (Figure 2C & 2D). spermatocytes II have a smaller nucleus. The spermatids were initially spherical and lie downwards; their nucleus has denser chromatin than that of spermatocytes (Figure 2E). Spenniogenesis was characterized by the formation in the anterior part of an acrosome separated from the nucleus and in the posterior part of a mitochondrial ring which surrounds part of the flagellum. The spermatozoa are located at the level of the lumen of the tubule and have a very elongated mitochondrial intermediate segment and a flagellum (Figure 3).

At the beginning of genital maturation, in June and July the cytological study of the testis shows the establishment of the seminiferous tubules and connective and muscular cells assemble to delimit the periphery of the tubule. Inside, islets of spermatogonia were actively dividing (Figure 2A). At the end of July, the presence of the first spermatocytes in the tubule was observed. Spermatocytes 1 can be identified by their large nucleus characteristic of phenomenon of increase (Figure 2C & 2D); the chromosomes gradually condense. In August, there is an increase in the number of spermatogonia and spermatocytes in the tubules. The dividing cells were very numerous. The spennatogonia in particular exhibit numerous mitoses. An intense division of spermatocytes by meiosis was observed and spermatogenesis accelerates.

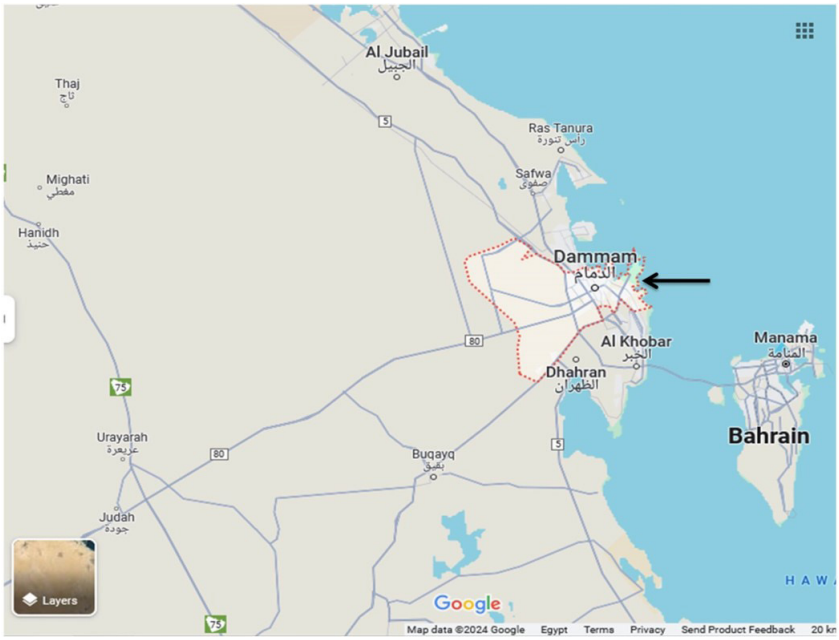

Figure 1. Google map. Arrow indicates the collection site.

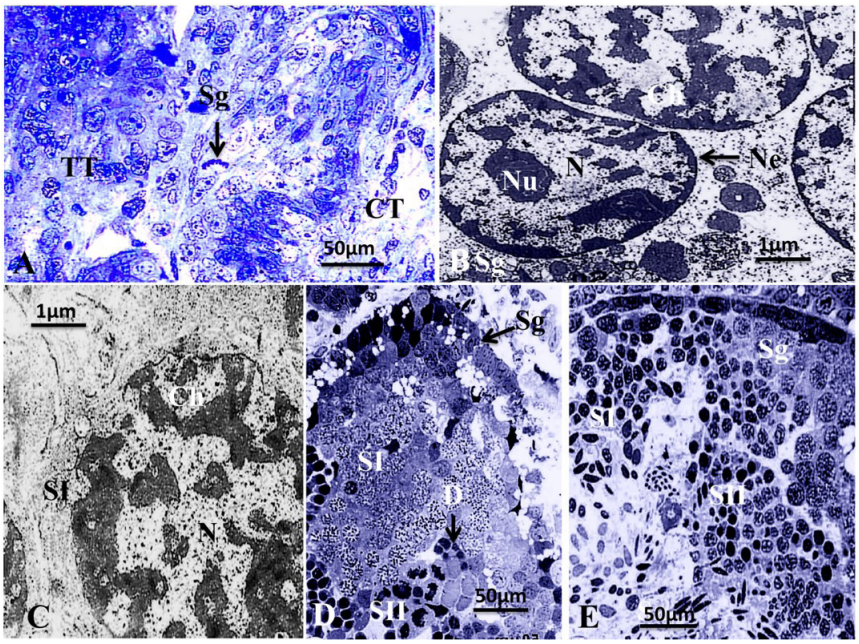

Figure 2. Cytology of the testis of the horned octopus *Eledone cirrhosa*: development of the different stages of male germ cells maturation during the sexual cycle. **A.** Semithin section stained with toluidine blue showing the structure of the testis at the beginning of maturation in July. The testicular tubule (TT), division figures (metaphase) (d). spermatogonia at the periphery of the tubule (Sg) and connective cells (CT). **B.** TEM showing the spermatogonia (Sg) with kidney-shaped nuclei (N), with one or two nucleoli (Nu) and chromatin (Ch) distributed to the periphery of the nuclear envelope (Ne). **C.** TEM showing spermatocyte 1 in maturing phase. Spermatocytes in prophase of meiosis (S1) have a nucleus significantly larger than that of spermatogonia. Chromatin (ch) begins to condense in the nucleoplasm. **D.** Semithin section stained with toluidine blue showing the testicular tubule in August in a maturing phase. Numerous divisions (D) of spermatocytes 1 (S1 and SII) are observed, the presence of spermatogonia (Sg) and spermatids. **E.** Semithin section stained with toluidine blue showing the testicular tubule of a maturing phase in September. Inside the tubule, spermatogonia (Sg) spennatocytes 1 (S1) spermatocytes II (SII) and the different stages of spermiogenesis are observed. Spermatozoa (Sa) already formed in the tubule will accumulate in the spermatophores.

This is the phenomenon most characteristic at this time of year. Rounded spermatids were progressively observed in the tubules (Figure 2D). At the end of August, spermiogenesis begins. During spermiogenesis, spermatids differentiate into spermatozoa. Spherical spermatids, with a nucleus containing very dense chromatin were firstly appeared. Then the spherical spermatids evolved to give spermatids with elongated nuclei and chromatic filaments. The chromatin then becomes more and more condensed during differentiation. The formation of the flagellum from the distal centriole was observed. In September, the continuation of active divisions of spermatogonia, meiotic divisions and increased spermiogenesis were observed. At the end of September, some octopuses have spermatozoa (Figure 2E), which coincides with the appearance of the first spermatophores in Needham's pouch and testifies to their sexual maturity. The cytological study of the testes in October shows that spermiogenesis continues while the spermatogonia and spermatocytes always present figures of division. Spermiogenesis continues until spring (May, June), when the reproduction occurs.

3.2. Fine structure of the ovary

The maturation of oocytes is accompanied very significant development of follicle cells which form cords deeply embedded in the ooplasm. This phenomenon is particularly marked among octopuses which produces very large eggs (diameter 1.1-1.5 cm). At the end of vitellogenesis, the follicular cords regress. During sexual maturation the oocyte is released into the genital coelom. It has a smooth appearance which distinguishes it from reticulated eggs. The hilum region is made up of highly vascularized connective tissue with cell connectives which are distinguished from germinal cells by their very elongated nucleus, the essential oogonia occupying the center. These are ovoid cells separated by frames of loose connective tissue. In sexually mature females, most of the ovary is occupied by oocytes at different stages: oocytes at the start of previtellogenesis (stage I), oocytes in previtellogenesis (stage II and III) and oocytes in vitellogenesis (stage IV). These stages appear gradually during sexual maturation.

The cytological study of the ovary during the sexual cycle shows that in June and July the oocytes are at stage I. At this stage, the spherical oocyte is surrounded by a single layer of follicular cells (Figure 3A). The nucleus of the oocyte is spherical and presents chromatin in nodules, filaments, or distributed homogeneously in the nucleoplasm. There are no lipid inclusions in the oocyte cytoplasm. The follicular cells, few in number, begin to stick together at the periphery

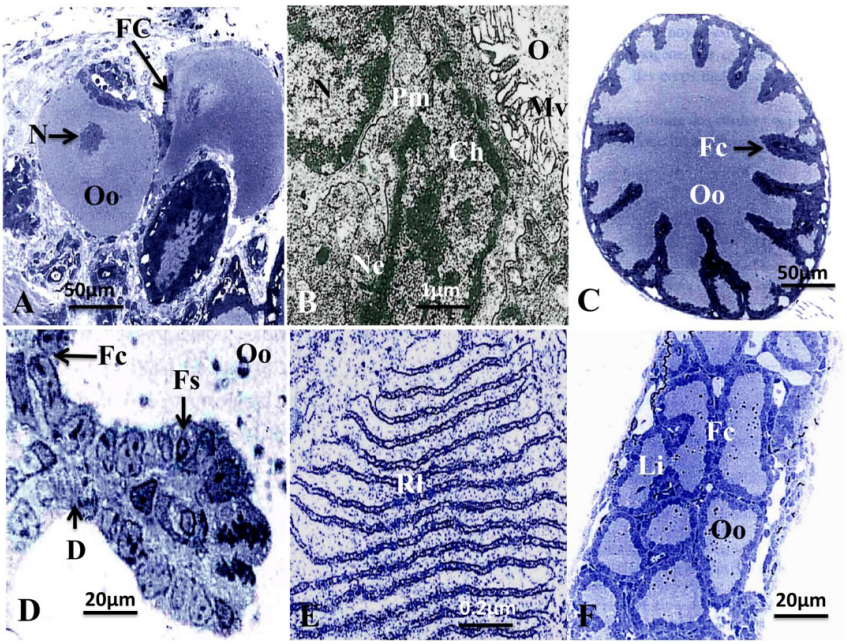

Figure 3. ovarian maturation in July. **A.** Semithin section stained with toluidine blue showing ovary maturing in July. Oocytes 1 are observed. This is the beginning of previtellogenesis. The nucleus (N) of the oocyte is spherical. The flattened follicular cells (FC) begin to stick together around the oocyte. The ooplasm (Oo) is clear. **B.** TEM showing the detail of the follicular cells surrounding oocyte at stage I in the ovary at the month of July. Follicular cells form a single layer of cuboidal cells and establish relationships with the oocyte (O). The contact area has microvilli (Mv). The nucleocytoplasmic ratio is high. Chromatin (Ch) forms clusters distributed around the periphery of the envelope nuclear (Ne). The plasma membrane (Pm) separating the cell is clearly visible. **C.** Semithin section stained with toluidine blue showing oocytes at stage I1. x 256. The follicular cords (Fc) begin to penetrate inside the ooplasm (OO) of the oocyte. **D.** Semithin section stained with toluidine blue showing the follicular cords in an oocyte at stage I1 in October. Follicular cells (Fs) actively divide (D) (metaphase) and form follicular cords (Fc). **E.** Semithin section stained with toluidine blue showing the ooplasm of the oocyte at stage I1. At this stage, ringed lamellae (Rl) in the ooplasm were observed. **F.** Semithin section stained with toluidine blue showing the oocyte at stage III in October and November. The follicular cords (Fc) occupy a large part of the ooplasm (Oo) of the oocyte. Lipid inclusions (Li) were observed.

of the oocyte and then form a continuous layer around the oocyte. They firstly have an elongated form (Figure 3A), then take a cubic form and establish relationships with the oocyte by forming cytoplasmic interdigitations in the contact zone with the oocyte (Figure 3B). Follicular cells have large nuclei with chromatin in clusters distributed against the nuclear envelop and in the nucleoplasm. In the apical region, small dictyosomes with 2 or 5 saccules are observed. At the base of cells, the mitochondria are few in number; the plasma membranes separating the cells are clearly visible (Figure 3A). In August and early September, egg development is quite slow. Most oocytes are still at stage I. Synthetic phenomenon was observed neither in the oocyte nor in the follicular cells.

At the end of September, stage II oocytes are observed in the ovary (stage IV and V). This stage is characterized by the formation of the first follicular cords (Figure 3C). The oocyte takes an ovoid shape, the nucleus is pushed to one pole of the oocyte and the appearance of the chromatin is very homogeneous. At this stage, the follicular cells multiply to form cords which begin to penetrate inside the ooplasm. Numerous mitoses were observed. (Figure 3D). The cytoplasm contains some lipid inclusions, protein granules, multivesicular bodies and ringed lamellae (Figure 3E).

During October and early November, the oocytes are mostly at stage II and III. At stage IIIc (stage VI), following multiplication intense, the developed follicle cords invade the cytoplasm of the oocyte where quite numerous lipid inclusions are formed (Figure 3F). Protein inclusions are degrading in the cytoplasm of the oocyte (Figure 4A). Relationships between follicular cells and the oocyte are narrower: the microvilli of the contact zone are more developed. The cytoplasm contains cisternae of endoplasmic reticulum and a poorly developed Golgi apparatus (Figure 4B). The membrane of each follicular cell remains visible during stage III.

Oocytes are observed at stage IV from the end of November. At this stage, the vitelligenesis is the highest. The follicular cords reach their maximum development. This is the beginning of vitellogenesis. In the ooplasm, the presence of numerous lipid inclusions was observed. The endoplasmic membrane is not very developed and the mitochondria are few in number. Free ribosomes were abundant and the nuclear envelop has numerous pores which translate senses of the existence of transport. The follicular cells are much taller, the plasma membrane of the cells disappears, and a syncytium is formed. Cells take on all the characteristics of secretory cells: highly developed nucleolar mass (Figure 4C), abundant granular

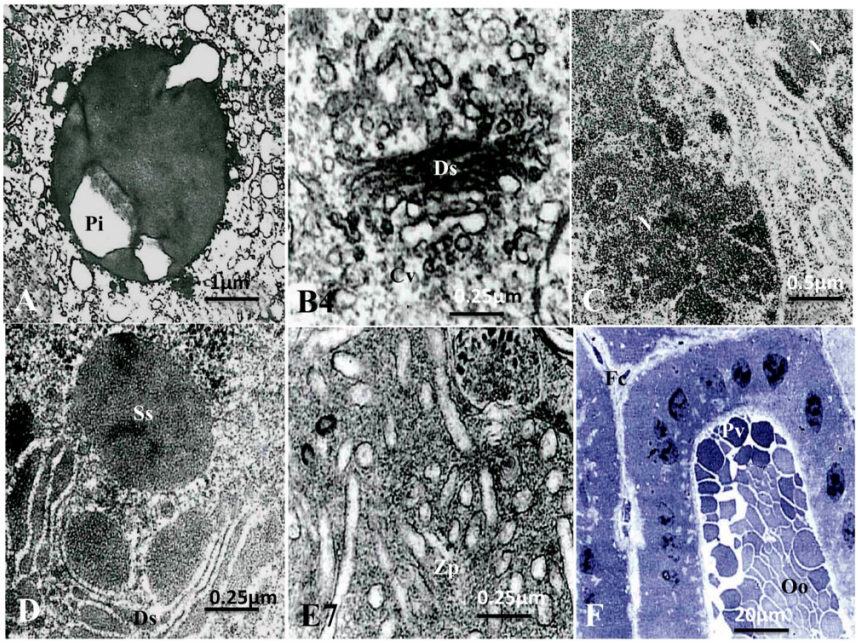

Figure 4. ovary state in October and November. **A.** TEM showing the ooplasm of the oocyte at stage III in October and November. The protein inclusions (Pi) are in the process of degradation: clear vacuoles appear in the dense mass. **B.** TEM showing dictyosomes in the cytoplasm of a follicular cell surrounding the oocyte at stage IV in November. The dictyosomes (Ds) do not produce secretory granules, but clear vesicles (Cv). This oocyte is in vitellogenesis. **C.** TEM showing the detail of the contact zone of the follicular cells surrounding the oocyte at stage IV in January. The plasma membrane separating the follicular cells has disappeared. A syncytium is then formed and the nucleolar mass is important. **D.** TEM showing dictyosomes and endoplasmic reticulum in a follicular cell surrounding the oocyte at stage IV in January. Development of the yolk: the saccules of the dictyosome (Ds) are active and form vesicles on their maturing face. The vesicles fuse to give secretion granules (Ss). **E.** TEM showing zona pellucida (Zp) in an oocyte at stage IV. **F.** Semithin section stained with toluidine blue showing the oocyte at stage V. Yolk platelets (Yp), in the ooplasm (Oo) and previtellogenic oocytes (Pv) were observed.

endoplasmic reticulum and Golgi apparatus elaborating secretory granules on its maturation side ((Figure 4D). A pellucid zone forms between the oocyte and the follicular cells (Figure 4E).

From the end of December, the ovary contains mainly stage V oocytes (Figure 4F). This stage actually corresponds to the activity phase of maximum secretion of the follicular cells which present a very developed granular reticulum and Golgi apparatus which produces secretion (Figure 5A-D). Yolk platelets accumulate in the oocyte ooplasm, the yolk has a more homogeneous structure (Figure 5E) and the follicular cords regress and the cells are pushed back to the oocyte surface. The formation of the chorion at the level of the zona pellucida was observed (Figure 5F). In January, the presence of the first smooth eggs in the ovary of some octopuses and their proportion is steadily increasing. In spring, at the time of reproduction, the proportion of smooth eggs is maximum, but the ovary still presents numerous oocytes at different stages of development (previtellogenesis and vitellogenesis).

4. Discussion

During the sexual cycle, the greatest number of division figures is observed in spermatogonia, spermatocytes and follicular cells. The presence of spermatogonia and spermatocytes throughout genital maturation shows that spermatogenesis is continuous. It is mainly characterized by a phase of intense multiplication of spermatogonia and spermatocytes during sexual maturation (July, August, and September). The differentiation phase, which corresponds to spermiogenesis, is very active during the rest of the cycle. The proportion of spermatids and spermatozoa in the tubules increases while that of spermatocytes and spermatogonia decreases (Boyle and Daly, 2000; Quetglas et al., 2001, Šifner and Vrgoc, 2009; Alejo-Plata and Gómez-Márquez, 2015). If these observations are related to the biometric study of the gonadosomatic relationship, it can be concluded that this does not begin to increase significantly only in the month of October, therefore after the cellular multiplication phase. These changes are detectable when sexual maturity is already acquired (Silva et al., 2002; Barratt et al., 2007; Krstulovic et al., 2009; Ortiz, 2013). Therefore biometric studies do not make it possible to follow sexual maturation in the male. During oogenesis, the most striking fact is the strong multiplication of follicular cells which first surround the oocyte then invade its cytoplasm and participate to the synthesis of the yolk (Krstulovic et al., 2009; Laptikhovsky, 2013). During the sexual cycle, follicular cells can therefore be either in an intense multiplication phase or in a synthesis phase. The oocytes in stage II and III are those

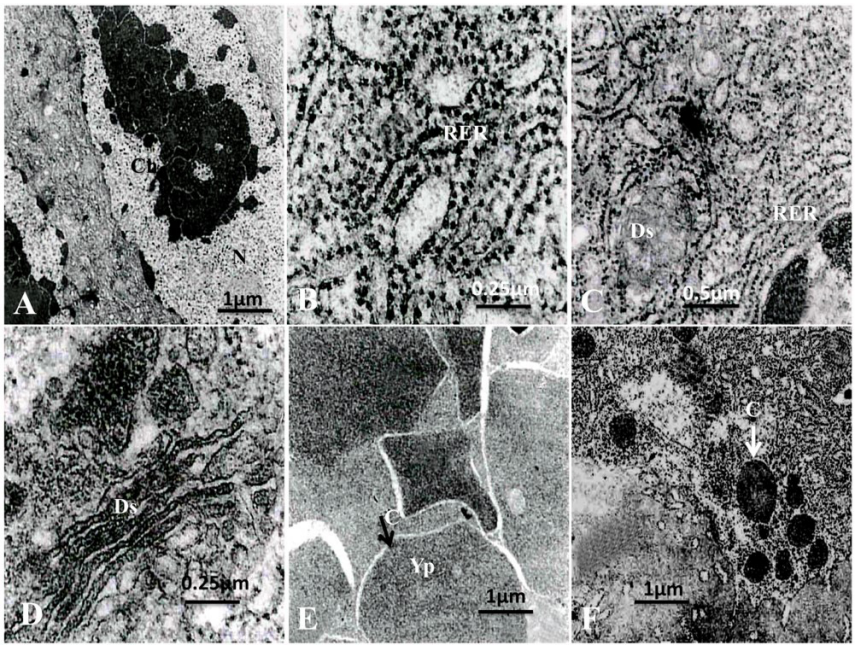

Figure 5. mature oocyte. **A.** TEM showing a syncytium surrounding a stage V oocyte. The follicular cells form a syncytium. Chromatin (Ch) is very compact in the nucleus (N). **B.** TEM showing the cytoplasm of follicular cells surrounding the oocyte at stage V. A very developed granular reticulum (RER) with much dilated cisterns and mitochondria (m) were observed. **C.** TEM showing the dictyosomes in the cytoplasm of the follicular syncytium near a very developed granular reticulum (RER) surrounding the oocyte at stage V. The actively dictyosomes (Ds) carry out the secretion. **D.** TEM showing the dictyosomes (Ds) in the ooplasm of oocyte at stage V. **E.** TEM showing the follicular cells in the oocyte at stage V in January. The Yolk platelets (Yp) accumulate in the oocyte ooplasm, the yolk has a more homogeneous structure. **F.** TEM showing the formation of the chorion (C) at the level of the zona pellucida.

where the follicular cells are in the intense multiplication phase. During genital maturation, oocytes at stages IIIb and IIIc occupy a large part of the ovary between the end of September and the end of November. The ovary therefore contains at this period numerous follicular cells with potential division. (Gonçalves et al., 2002) used a stereological method applicable only to female *Octopus vulgaris* and making it possible to quantify the proportion of follicular cells relative to the ooplasm during follicle development. The method consists to calculate the ratio of the volume of the folliculogenesis to the total volume of the vitelligenesis, which is called the maturity index. This study showed that folliculogenesis increases regularly during previtellogenesis, during intense multiplications of follicular cells. The folliculogenesis stabilizes at the start of vitellogenesis and then decrease. The continuation of development (vitellogenesis) is marked by a great synthesis activity of follicular cells that form a syncytium and establish close relationships with the oocyte whose cytoplasm also undergoes numerous modifications. The formation of this syncytium is reported for the first time in cuttlefish, while it had been observed in other Cephalopods (Frösch and Marthy 1975; Nsoy, 2005; Akyol et al., 2007; Barratt et al., 2008; Cuccu et al., 2013). The process of yolk synthesis is still poorly known in cephalopods, but it nevertheless seems that follicular cells are responsible for this synthesis process which would therefore be heterosynthetic as observed in other cephalopods (Kilada and Riad, 2010). The gonado-somatic ratio, which remained very low throughout previtellogenesis, suddenly goes increase from January (Olivares, et al., 2001), reflecting the effect of vitelline synthesis. It therefore appears that during previtellogenesis and vitellogenesis, the egg is blocked in prophase of the first division of meiosis. Intense multiplications only concern follicular cells. These results confirm those in *Octopus mimus* (Cardoso and Estrella , 2004; Boucaud-Camou et al.,1988), *Loligo forbesi* (Orsi Relini et al., 2006; Gabr and Riad, 2008), *Octopus vulgaris* (Smale and Buchan, 1981; Son et al., 2015) and *Sepia offïcil1alis* (Lin et al., 2019), authors emphasized the particular development of the follicular cords and the participation of follicular cells in the formation of the yolk, whereas in the freshwater bivalve *Caelatura* (*Horusia*) *parreyssi* (Ilham et al., 2017), *Octopus ocellatus* (Son, et al., 2015), *Adelieledone polymorpha* (Barratt et al., 2008), in the chiton *Sypharochiton septentriones* (Selwood, 1970), the participation of follicular cells in vitellogenesis remains uncertain. This cytological study provides precise data on genital maturation of the horned octopus *Eledone cirrhosa* from the Arabian Gulf off Dammam city. The results on the structure of germinal and follicular cells all show that the intense multiplications of female germ cells take place in August and early September, while those of male germ cells are carried out in October and November in the ovary. At the end of November, the follicular cells enter an active synthesis phase. The use of a biochemical technique, the determination of aspartate transcarbamyiase activity (ATCase), gave us results similar to those obtained by this

method. Indeed, measuring ATCase activity in the ovary and testis during the sexual cycle of *Sepia officÎlwlis* from the Mauritania shows that the peaks of enzymatic activity correspond to the intense divisions of spermatogonia and spermatocytes during maturation and reproduction in the male, and with those of the follicular cells during previtellogenesis in females (Lin et al., 2019). Variations of ATCase activity during the sexual cycle show that the peaks of enzymatic activity correspond perfectly to the periods of division of follicular cells and male germ cells (spermatogonia and spermatocytes) observed by cytological monitoring of the testis and the ovary. The ATCase activity therefore appears to be a good indicator of gametogenic activity, and more especially of the cell multiplications which are there associated. Thus, it appeared that the experimental study of the characterization of the mitogenic factor of the optic gland had to be carried out in males in August and September and in females in October and November in order to obtain optimal, easily detectable responses, to the mitogenic action of gonadotropin. Studies on the stimulatory effect of syntheses of protein should be carried out from November and during the winter in females. However, at this stage, the formation of a follicular syncytium prohibits work on dissociated cells

5. Conclusion

This study looked at the fine structure of *Eledone cirrhosa* gonadal development in the Arabian Gulf off Dammam city. It concentrated on the stages of genital maturation, namely spermiogenesis, sexual maturity, and seminiferous tubules. Oocytes are produced at stages I - V of the ovary. Through the use of cytological techniques, the study seeks to understand the mitogenic effect of gonadotropin and pinpoint times during the sexual cycle when cell multiplication is most vigorous. This investigation will assist the researcher in identifying important reproductive biology topics that numerous other researchers were unable to investigate. As a result, a novel theory on the enhancement of cephalopod productivity in marine ecosystems might be developed.

Acknowledgements

The authors of this manuscript acknowledge the support provided for this article by the departments of zoology at Imam Abdulrahman Bin Faisal University's Deanship of Scientific Research and Alexandria University's Faculty of Science.

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