Contents lists available at ScienceDirect

Tissue and Cell

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Ultrastructural observation of oocytes in six types of stony corals

Sujune Tsai^{c,d,1}, Wei-Chieh Chang^{b,1}, Suchana Chavanich^e, Voranop Viyakarn^e, Chiahsin Lin^{a,b,*}

^a National museum of Marine Biology & Aquarium, Checheng, Pingtung, Taiwan

^b Institute of Marine Biology, National Dong Hwa University, Checheng, Pingtung, Taiwan

^c Department of Biotechnology, Mingdao University, Peetow, Chang Hua, Taiwan

^d Department of Post Modern Agriculture, Mingdao University, Peetow, Chang Hua, Taiwan

e Reef Biology Research Group, Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

ARTICLE INFO

Article history: Received 6 January 2016 Received in revised form 19 May 2016 Accepted 20 May 2016 Available online 21 May 2016

Keywords: Hard coral TEM Ultrastructure Yolk formation Oocyte

ABSTRACT

In this study, the ultrastructure of the oocytes of 6 types of scleractinian corals was observed by transmission electron microscopy (TEM). Moreover, histological and ultrastructural analyses were performed to improve our understanding of the organelles involved in coral oocyte formation. In all 6 stony coral species, the microvilli were tubular and directly grew from the surface of the oocyte membrane; yolk bodies, lipid granules, and cortical alveoli accounted for most of the volume inside the oocytes, suggesting that they are associated with energy storage and buoyancy. Clear differences were observed in the size of yolk bodies and lipid granules in the oocytes. *Galaxea fascicularis* exhibited the largest lipid granule volume, but the oocytes contained only an average number of 12.45 lipid granules per unit area. Only *Montipora incrassata* oocytes may be attributed to the presence of symbiotic algae and large yolk bodies, which may help oocytes produce energy and function as a nutritional source. This study is crucial for improving the understanding of the basic biology of coral reproduction, and the ensuing datasets is critical for conservation-oriented studies seeking to cryopreserve corals during these times of dramatic global climate change.

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1. Introduction

With recent improvements in electron microscopy (EM), the association and interactions between organelles can be visualized, and variability in ultrastructure across cell types can be documented (Tsai et al., 2014). As such, EM could be used to better elucidate the roles of various cellular organelles in important biological processes, such as yolk body biosynthesis, an act thought to require activities of the nucleus, mitochondria, Golgi body, and ergastoplasm at the same or at different times (Beams and Kessel, 1963). The majority of the oocyte consists of energy storage materials, such as the yolk body, lipid granules, and corticol alveoli; such components also aid in maintaining oocyte buoyancy (Benayahu et al., 1989; Padilla-Gamino et al., 2011). Mitochondria

E-mail address: chiahsin@nmmba.gov.tw (C. Lin)

¹ These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.tice.2016.05.005 0040-8166/© 2016 Elsevier Ltd. All rights reserved. are also abundant in oocyte cytoplasm due to the need to enact high metabolic rates during oocyte development into a blastocyst (Amor et al., 2004; Najmudeen, 2008). Finally, the microvilli and oocyte membrane found beneath the vitelline layer also function as protective barriers to the external environment (Eckelbarger and Davis, 1996).

Although there are some reports on the initiation of coral egg development, they are limited to the histological level (e.g., Fadlallah and Pearse, 1982; Goffredo et al., 2012; Rinkevich and Loya, 1979; Szmant-Froelich et al., 1980). Ultrastructural studies have been recently conducted on the differentiation and histology of mature eggs of black corals as well as those of *Cirrhipathes* cfr. *anguina* (Gaino and Scoccia, 2008; Gaino and Scoccia, 2010). Transmission electron microscopy (TEM) observations have revealed at least three types of yolk materials in early- and late-stage oocytes of the gorgonian *Junceella juncea*: egg yolk bodies, lipid granules, and cortex alveoli (Tsai et al., 2014). The oocytes of *J. juncea* contain a large quantity of yolk, and there are different types of follicles, including some empty ones containing particulate materials (Tsai et al., 2014).





^{*} Corresponding author at: National Museum of Marine Biology & Aquarium, 2 Houwan Road, Checheng, Pingtung, 944, Taiwan.

Favard and Carasso (1958) explored the association between the mitochondrion and yolk body in snail oocytes, and Beams and Kessel (1962) revealed close associations between the rough endoplasmic reticulum (ER) and the formation of the yolk body in crawfish oocytes. These studies suggest that yolk materials may be transformed from the mitochondria or large follicles and interact with the ER. The source of the volk in the oocytes of many insects is extra oocytes on the oocyte surface (Andersone, 1964; Roth and Porter, 1964; Stay, 1965; Telfer, 1961). In crustaceans, extra oocytes are the origin of the yolk precursor and are related to the formation of an active yolk body in the organelles in oocytes (Beams and Kessel, 1963; Kessel, 1968). In the present study, the ultrastructure of oocytes of six types of scleractinian corals (Oxypora lacera, Echinopora gemmacea, Montipora incrassata, Montipora hispida, Galaxea fascicularis, and Merulina ampliata) was observed by TEM, and histological and ultrastructural analyses were performed improve our understanding of the organelles involved in coral oocyte formation.

2. Materials and methods

2.1. Sample collection

Eggs and sperm released from corals located at a 3–5-m depth in the Houbihu marine protected area within Kenting National Park (Southern Taiwan) were collected between April and May in both 2012 and 2013. After stony corals completed oocyte emissions, 50mL syringes were used to draw oocytes, which were confirmed to be unfertilized by observing for subsequent embryonic development. The samples were processed for ultrastructural analysis immediately after collection. The coral collection license was issued by the Kenting National Park Management Office; the license can be used for stony coral oocyte collection during the breeding season.

2.2. Species identification and classification and recording of oocytes

The samples were sorted by divers and categorized according to the phenotype and structure of the coral bone plate. Six types of stony corals were identified, as follows: *O. lacera, E. gemmacea, M. incrassata, M. hispida, G. fascicularis,* and *M. ampliata.* These 6 species are common at the sampling site, and their reproductive biology is fairly well understood (Richmond and Hunter, 1990). Furthermore, we sampled corals that vertically transmit *Symbiodinium* (endosymbiotic dinoflagellates) to their eggs (*M. incrassata*), as well as those that horizontally transmit *Symbiodinium* to their eggs (the remaining 5 species). The adults of all 6 species rely on translocated carbon photosynthetically fixed by these dinoflagellate endosymbionts for their survival, as do all reef-building corals.

Underwater photography was used to record and enumerate the gametes released from the corals for quick sample categorization before the samples were delivered back to the National Museum of Marine Biology and Aquarium. The samples were then classified according to their number. An optical microscope camera system (Micrometrics SE3, Taiwan) and a dissecting microscope camera (C31, Olympus, Japan) were used to record the oocyte color and size. Ultrastructural analyses were immediately conducted after sample recording. The organelles of microvilli, mitochondria, yolk bodies, lipid granules, and symbiotic algae in oocytes from the stony corals were compared, and the ratio of the yolk body and lipid granule to the oocyte volume was analyzed. The steps involved in transmission electron microscopy (TEM) are listed as follows:

2.3. TEM

Before observing the biological samples by TEM, we subjected them to the following preprocessing treatments: fixation, dehydration, infiltration, embedding, polymerization, sectioning, and double staining. For prefixation treatment, the oocyte samples were washed 3 times with filtered seawater $(0.02 \,\mu\text{m})$ and placed in a fixative (2.5% glutaraldehyde, 2% paraformaldehyde, 0.1 M phosphate buffer, and 5% sucrose) at 4°C for 2h. After the fixative was removed, the samples were washed with 0.1 M phosphate buffer and placed in an orbital shaking incubator for 20 min. After the 0.1 M phosphate buffer was discarded, the samples were placed in 1% osmium tetroxide (OsO₄) in the dark for 1 h as postfixation treatment. After the 1% OsO₄ was discarded, the samples were washed with 0.1 M phosphate buffer and placed in an orbital shaking incubator for 20 min. Gradient dehydration was performed at room temperature by using different concentrations of ethanol (50%, 70%, 80%, 90%, and 95%); the samples were dehydrated in each concentration of ethanol for 30 min. Subsequently, 100% ethanol and 100% acetone were mixed at a 1:1 ratio, and the samples were dehydrated 2 times at room temperature for 30 min. Finally, the samples were dehydrated in 100% acetone for 30 min. Each sample was individually permeated for 1 h in 100% acetone and Supr resin mixture at 1:1 and 1:3 ratios, followed by overnight infiltration with 100% Supr resin. The overnight permeation solution was replaced with fresh 100% Supr resin for the final stage of permeation. The samples were placed in rubber moldings and then in an oven set at 60 °C for 48 h. The embedding agent was completely polymerized into a solid shape. After ultraglass knife strips $(400 \text{ mm} \times 25.4 \text{ mm} \times 6.4 \text{ mm})$ were washed, the rough side of the glass was placed downward facing the glass cutter knife machine (Leica EMKMR2, Leica, Germany) to form a triangular glass cutter. An edge tape was used to create a groove in the glass. An ultramicrotome (Leica Ultracut R, Leica) was used to slice the samples completely embedded in resin. Through the use of a 0.22-µm filter, impurities were filtered from a uranyl acetate solution and centrifuged at 6000g for 15 min; the sections were stained with uranyl acetate in the dark for 40 min and washed with autoclaved distilled water for 5 min. This step was repeated 3 times. Moreover, through a 0.22-µm filter, impurities were filtered from a lead citrate solution and centrifuged at 6000g for 15 min. NaOH was placed on the staining plate, and several drops of distilled water were added for 5 min to remove the CO_2 on the staining plate. The plate was then stained with lead citrate for 3 min and washed with autoclaved distilled water for 5 min. This step was repeated 3 times. The stained samples were dried in the drying chamber. The samples were observed by TEM (JEM-1400, JEOL, Japan).

2.4. Statistical analyses

SPSS (version 17.0; SPSS, Inc., Chicago, IL, USA) was used for experimental data analysis. The one-sample Kolmogorov–Smirnov test (P<0.05) was used to test whether the data distribution was normal. The Levene test (P<0.05) was used to test for homogeneity of variance, and the Tukey test, a posthoc test of analysis of variance, was used to evaluate significant differences in the means of the groups.

3. Results

A total of 60 oocytes per species were used to obtain 3 replicates, each of which was repeated 3 times. For the oocytes of the 6 stony coral species, *M. hispida* exhibited the largest diameter ($442.11 \pm 6.69 \mu m$; *P* < 0.05; means \pm standard error), whereas *E. gemmacea* exhibited the smallest diameter ($274.85 \pm 6.23 \mu m$; *P* < 0.05; Table 1). The sizes of the 6 stony coral species are as fol-

Table 1	
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Comparison of oocyte diameters of 6 coral species	•
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Species/diameter (µm)				
O. lacera	407.12 ± 18.49			
E. gemmacea	274.85 ± 6.23			
M. incrassata	152.23 ± 7.51			
M. hispida	442.11 ± 6.96			
G. fascicularis	286.99 ± 40.13			
M. ampliata	307.45 ± 4.07			

n = 20; Mean \pm SE.

lows (from small to large): M. incrassata, E. gemmacea, G. fascicularis, M. ampliata, O. lacera, and M. hispida. Table 2 presents a comparison of the related organelles in the oocytes. For microvilli, E. gemmacea exhibited the smallest diameter $(1.21 \pm 0.24 \,\mu\text{m}; P < 0.05)$, whereas *M. incrassata* exhibited had the largest diameter $(3.87 \pm 0.47 \,\mu\text{m})$; P < 0.05). For the yolk body, O. lacera exhibited a smaller average diameter ($1.17 \pm 0.28 \mu m$; P > 0.05), and *M. incrassata* exhibited the largest average diameter ($1.79 \pm 0.65 \mu m$; P>0.05). Among all organelles, considerable differences were observed in the lipid granules in the oocytes of the 6 stony coral species, with M. incrassata having the smallest average diameter (6.71 \pm 0.92 μ m), and G. *fascicularis* having the largest average diameter $(13.02 \pm 3.02 \,\mu\text{m})$. The average mitochondrial size in the oocytes of the 6 stony coral species was approximately 1–1.3 µm, except for *G. fascicularis*. Because of the insufficient number of mitochondria, the average size of 1.86 µm was obtained only from 2 observed groups. Among the 6 stony coral species, symbiotic algae were found in only M. incrassate oocytes, which were larger than the other measured organelles, with an average size of $9.16 \pm 1.69 \,\mu\text{m}$.

Table 3 demonstrates the average number of yolk bodies, lipid granules, and symbiotic algae per unit area (observed under magnification of 15 K for yolk bodies and 3 K for lipid granules). The average number of yolk bodies in the oocytes of *M. ampliata* (35.12 ± 8.32) was higher than that in the oocytes of the other 5 coral species. The average number of lipid granules in the oocytes of *G. fascicularis* (12.45 ± 4.01) was lower than that in the oocytes of the other 5 coral species. The average numbers of yolk bodies and lipid granules in the oocytes of the 6 stony coral species were 20-35 and 12-22 per unit, respectively. The average number of yolk bodies are supproximately 2-fold that of lipid granules. The number of symbiotic algae in *M. incrassata* was limited to 1.62 per unit area.

Table 4 presents the individual percentage of yolk bodies, lipid granules, and symbiotic algae in the oocytes, and the total percentage of yolk bodies and lipid granules in the oocytes was calculated. The respective percentages of organelles in the oocytes of the 6 coral species are listed as follows: yolk bodies, 28%, 28%, 54%, 36%, 22%, and 37%; and lipid granules, 28%, 29%, 26%, 37%, 57%, and 28%. The total percentages of yolk bodies and lipid granules in the oocytes ranged from 55% to 80%. In addition, the percentage of symbiotic algae in *M. incrassata* oocytes was 5%. The total percentage of yolk bodies and lipid granules was 54% in the oocytes of *M. incrassata*, which is the only coral species with a higher number of yolk

Table 3

Average number of yolk bodies, lipid granules, and symbiotic algae per unit area.

Species/number			
	Yolk body	Lipid granule	Symbiotic alga
O. lacera	30.56 ± 9.65	14.02 ± 4.25	NONE
E. gemmacea	26.88 ± 6.45	22.18 ± 3.11	NONE
M. incrassata	25.68 ± 6.27	21.28 ± 4.47	1.62 ± 1.12
M. hispida	31.54 ± 7.89	18.85 ± 3.21	NONE
G. fascicularis	22.85 ± 7.23	12.45 ± 4.01	NONE
M. ampliata	35.12 ± 8.32	19.78 ± 3.55	NONE

n = 10; Mean \pm SE; NONE, not observed.

Organelles as percentages of oocytes in 6 coral species.

Species/percentage (%)					
	Yolk body	Lipid granule	Yolk body + Lipid granule	Symbiotic alga	
O. lacera	28%	28%	56%	NONE	
E. gemmacea	28%	29%	56%	NONE	
M. incrassata	54%	26%	80%	5%	
M. hispida	36%	37%	72%	NONE	
G. fascicularis	22%	57%	79%	NONE	
M. ampliata	37%	28%	65%	NONE	

Mean \pm SE; NONE, not observed.

bodies than lipid granules in the oocytes among the 6 stony coral species.

TEM observations revealed clear microvilli on the outer membrane of the oocytes of these 6 stony corals, although the length of the microvilli differed among the 6 coral species. The microvilli on the oocytes of the 6 stony coral species were long strips (Fig. 1a) and point-like (Fig. 1b). The morphology of microvilli can be attributed to the slicing angle of cylindrical microvilli. Many yolk bodies and cortical alveoli were closely attached to the oocyte membranes (Fig. 1a,b); however, this phenomenon was not observed in the G. fascicularis oocytes (Fig. 1c). Various organelles were observed in the oocytes including the Golgi body, rough endoplasmic reticulum, mitochondria, yolk bodies of various sizes and patterns, and lipid granules, which exhibited the largest size. The yolk bodies in the oocytes of the 6 stony coral species were spherical or oval, with a compact structure. In E. gemmacea, no specific particles were found inside the yolk bodies, which are gradually developed from follicles (Fig. 2a). In the remaining 5 coral species, microparticle structures were observed in the yolk bodies in the oocytes, and some microparticle structures were similar to the small follicles in the oocyte cytoplasm (Fig. 2b,c). A larger yolk body with a loose structure was also observed (Fig. 2b). The yolk body structure in the oocytes of G. fascicularis was different from that in the oocytes of the other 5 stony coral species; the yolk body of G. fascicularis exhibited a homogeneous structure and contained lipid granule-like strips (Fig. 2d). Yolk body particles were observed in some vacuolar follicles in the G. fascicularis oocytes (indicated by the arrow in Fig. 2e).

Table 2

Sizes of organelles in the oocyte of 6 coral species.

Species/diameter (µm)					
	Microvillus	Yolk body	Lipid granule	Mitochondrion	Symbiotic alga
O. lacera	1.88 ± 0.51	1.17 ± 0.28	8.63 ± 2.02	1.22 ± 0.14	NONE
E. gemmacea	1.21 ± 0.24	1.25 ± 0.37	$\textbf{6.93} \pm \textbf{1.22}$	1.08 ± 0.24	NONE
M. incrassata	3.87 ± 0.47	1.79 ± 0.65	6.71 ± 0.91	1.08 ± 0.20	9.16 ± 1.69
M. hispida	2.25 ± 0.4	1.31 ± 0.30	8.51 ± 1.51	1.25 ± 0.24	NONE
G. fascicularis	1.76 ± 0.39	1.22 ± 0.26	13.02 ± 3.02	$1.86 \pm 0.30 [n=2]$	NONE
M. ampliata	2.40 ± 0.57	1.26 ± 0.22	7.24 ± 1.38	1.199 ± 0.23	NONE

n = 50, except in *G. fascicularis* for mitochondrion (n = 2).

Mean \pm SE; NONE, not observed.



Fig. 1. Microstructure of oocyte microvilli. (a) Long strips of microvilli in *O. lacera*. Scale bar = 1 µm. (b) Dot-like microvilli in *E. gemmacea*. Scale bar = 2 µm. (c) Yolk body and cortical alveoli were not closely associated with the oocyte membrane in *G. fascicularis*. Scale bar = 1 µm. mic, microvilli; v, vesicle; yb, yolk bodies; ca, cortical alveoli.



Fig. 2. Microstructure of yolk bodies. (a) *E. gemmacea* contained no specific particles inside yolk bodies, which may gradually develop from follicles (arrow). (b) Yolk bodies with a loose structure (arrow) and microparticle structures in *O. lacera* and (c) *M. hispida*. (d) Homogeneous structure containing strip-like lipid granules in the yolk bodies of *G. fascicularis* and vesicle containing (e) yolk body particles (arrow). Scale bar = 2 µm. yb, yolk bodies; v, vesicle.

The lipid granules were spherical and contained white strips and tissue particles (Figs. 3 and 4a), and a high number of yolk bodies surrounded the lipid granules (Fig. 3). The mitochondria in the oocytes of the 6 stony coral species were biconcave discoid and were surrounded by a high number of yolk bodies and lipid granules (Fig. 4a). The ultrastructure of the Golgi body in the oocytes of the 6 stony coral species was a sandwich-like folded structure (Fig. 4b). The endoplasmic reticulum in the oocytes of these 6 stony coral species exhibited a tight, wrinkled, and elongated structure, with numerous miniature follicles on its membrane surface (Fig. 4c,d), and yolk bodies were attached to the edge of the endoplasmic reticulum, indicating that the particle tissue inside the yolk body of oocytes may be transferred by the endoplasmic reticulum. The nucleus of E. gemmacea was located close to the oocyte plasma membrane; the nuclear membrane could be easily identified in the oocyte. The appearance of the nucleoplasm was clearly different from that of the oocyte cytoplasm (Fig. 5a), and A visibly darker region indicating the nucleolus was observed (Fig. 5b). Many nuclear pores and a single nucleolus were observed on the nuclear membrane (Fig. 5b,c). In this study, symbiotic algae were observed only in M. incrassata oocytes (Fig. 6a). The algae inside the *M. incrassata* oocytes exhibited a cell wall, chloroplast, nucleus, and vacuolar-type region (Fig. 6b); however, the function of this



Fig. 3. Lipid granules in *E. gemmacea* oocytes. Scale bar = $1 \mu m$. lg, lipid granules.



Fig. 4. Microstructure of mitochondria, endoplasmic reticulum, and Golgi bodies. (a) Mitochondria of *O. lacera* oocyte are biconcave discoid. Scale bar = 0.2μ m. (b) Sandwichlike folded Golgi apparatus in *M. incrassata* oocytes. (c) *O. lacera* and (d) *E. gemmacea* oocytes contain abundant yolk bodies and miniature vacuoles on the edge of the endoplasmic reticulum, which exhibits a tight, wrinkled, and elongated structure. Scale bar = 1μ m. m, mitochondria; yb, yolk bodies; g, Golgi bodies; lg, lipid granules; er, endoplasmic reticulum.

vacuolar-type region remains unknown and therefore requires further investigation.

4. Discussion

Microvilli play a protective role during oocyte development. In the oocytes of aquatic invertebrates such as Haliotis varia (abalone), Lymnaea stagnalis (snail), and Crassostrea virginica (eastern oyster), microvilli are embedded into the oocyte membrane (Rigby 1979; Eckelbarger and Davis, 1996; Najmudeen 2008), which is different from the direct growth of microvilli from the surface of the oocyte membrane in the 6 stony coral species investigated in the present study. The microvilli on the oocyte membrane of these 6 stony coral species were tubular, similar to the microvilli of the gorgonian Junceella juncea and the soft coral Heteroxenia fuscescens in previous research (Benavahu et al., 1989; Tsai et al., 2014). However, the outer layer of the oocytes of these 6 stony coral species was different from that of oocytes of J. juncea and H. fuscescens, which have a mesogleal coat. In the present study, large amounts of yolk bodies spread to the inner membranes of the six stony coral oocytes, and this response is likely in preparation for the impending cortical reaction, whereby cortical granules are released from the egg to prevent polyspermy. This phenotype is also observed in other marine invertebrates such as sea pen Pennatila aculeate, abalone *H. varia*, and stony coral *M. capitata* (Gaino and Scoccia, 2008, 2010; Padilla-Gamino et al., 2011). In addition, when oocytes are discharged into seawater, cortical alveoli in the inner layer (Padilla-Gamino et al., 2011) produce hundreds of proteins de novo, many of which are involved in regulating osmotic pressure (e.g., aquaporin; Fabra et al., 2005). When there are differences in colloid osmotic pressure, they separate from the oocyte surface (Monroy, 1953). Follow-up studies are required to understand the association and function of the cortical alveoli aggregation process and microvilli extension.

The present study demonstrates that yolk bodies, lipid granules, and cortical alveoli accounted for most of the volume inside oocytes, suggesting that they may be associated with energy storage and buoyancy. Mature oocytes are discharged into seawater during the breeding season, and buoyancy in seawater and energy storage are crucial for fertilization. Eckelbarger and Davis (1996) described oocyte complexes in detail and reported that the homogeneous yolk bodies of mollusks contain several organelles such as the Golgi body, rough endoplasmic reticulum, smooth endoplasmic reticulum, mitochondria, autophages, and multivesicular bodies. The activity of the organelles is crucial for yolk formation. In the present study, the endoplasmic reticulum and Golgi body were observed at the periphery of yolk bodies and may facilitate yolk synthesis and promote particle development, suggesting that



Fig. 5. Microstructure of nucleus. (a) Nucleus and (c) nuclear pore (arrow) of an *E. gemmacea* oocyte. Scale bar = 10 µm. (b) A visibly darker region indicating the nucleolus in a *G. fascicularis* oocyte. Scale bar = 5 µm. n, nucleus; nl, nucleolus; np, nucleoplasm; g, Golgi bodies; ne, nuclear envelope.



Fig. 6. Microstructure of (a) symbiotic algae (scale bar = 5 µm) with (b) cell wall, chloroplast, nucleus, and vacuolar-type blocks (arrow) in *M. incrassata* oocytes. Scale bar = 1 µm. sa, symbiotic algae; lg, lipid granules; cp, chloroplast; cw, cell wall; san, symbiotic algae nucleus.

the activity of the organelles is crucial for yolk formation. Moreover, most yolk bodies in the oocytes of the 6 stony coral species contained special microparticles, which may be associated with differences in biosynthesized proteins and nutrient sources for oocytes, in turn leading to different patterns of the composite yolk body. Rigby (1979) speculated that vesicles derived from the endoplasmic reticulum and Golgi body have a vital function in protein and carbohydrate metabolism.

The current study reveals the presence of follicles of different sizes in the oocytes, and yolk material may be derived from large vesicles accumulated in the oocytes. Previous research has shown that yolk materials accumulate around the vesicles in sea anemone and gradually fill the empty space, thereby increasing its diameter and size (Larkman, 1984). Numerous microvesicles pass to the Golgi body through the endoplasmic reticulum and are secreted from the Golgi body to bind to the yolk body or directly form the new yolk body by follicular accumulation (Tsai et al., 2014). Previous studies have indicated that a continuous connection exists between the

endoplasmic reticulum and the Golgi body for yolk body synthesis in oocytes in the gorgonian coral *J. juncea* (Tsai et al., 2014).

Significant differences were observed in the lipid granule size in the oocytes of the 6 stony coral species, and the size was in the following order: G. fascicularis, O. lacera, M. hispida, M. ampliata, E. gemmacea, and M. incrassata. Moreover, the lipid granules occupied approximately 28%-57% of the inner space of the oocytes. G. fascicularis exhibited the largest lipid granule volume, but the oocytes contained only an average number of 12.45 granules per unit area. The present study also revealed the presence of white strips and spherical particles inside the lipid granules, differing from the lipid granule patterns in J. juncea oocytes in our previous research (Tsai et al., 2014). This finding is presumably related to the high number of symbiotic algae (in the tissues), which can provide nutrients to stony corals. This observation is highly different from the lipid granule structure in the oocytes of J. juncea, which do not contain symbiotic algae. Our analysis of the size and proportion of lipid granules in the oocyte reveals that the smallest

size and proportion of lipid granules in the *M. incrassata* oocytes may be attributed to the presence of symbiotic algae. As a compensation for the smallest lipid granules, *M. incrassata* exhibited the largest yolk body and was the only species containing symbiotic algae in the oocyte. The yolk bodies and symbiotic algae may help oocytes produce energy, function as a nutritional source, and provide an alternative energy source for metabolism during oocyte development. This observation may contribute to the smallest size and proportion of lipid granules for energy storage in these oocytes than those in the oocytes of the other 5 corals. Additional studies are required to investigate whether symbiotic algae affect the size of lipid granules.

Significant differences were not observed in the pattern or size of the mitochondria in the oocytes of the 6 stony coral species, and the shape of the mitochondria was similar to that in other invertebrates such as *H. varia* (abalone) (Najmudeen, 2008) and *Bolinus brandaris* (murex snails) (Amor et al., 2004). Previous research has indicated the transformation of mitochondria to yolk materials (Larkman, 1984); by contrast, we did not observe this phenomenon in the present study. Therefore, we conclude that energy production and maintenance after oocyte fertilization are functions of the mitochondria in mature oocytes released by these 6 stony coral species.

A single nucleus was observed in the oocytes of *E. gemmacea* and *G. fascicularis*, which have the same phenotype as those of *Mulinia lateralis*, *Urechis caupo*, and *Asterias forbesii* in previous studies (Vincent et al., 1969; Azevedo et al., 1984). A previous study indicated that *J. juncea* oocytes contain more than 2 nucleoli, depending on the differences between the coral species. Regardless of the presence of single or multiple nuclei, they are the main sites of RNA synthesis (Azevedo et al., 1984), and the high-electron-density particles surrounding the oocyte nuclear membrane help release ribosomes and retain them close to the nuclear membrane (Apisawetakan et al., 2001).

Future ultrastructural-based observations, combined with molecular and cellular biology-focused approaches, should more clearly elucidate the role of RNA synthesis, as well as other subcellular processes, in egg formation. Such studies are critical for improving the understanding of the basic biology of coral reproduction, and the ensuing datasets are critical for conservation-oriented works seeking to cryopreserve corals during these times of dramatic global climate change (Tsai et al., 2015; Lin et al., 2012; Kuanui et al., 2015).

Acknowledgements

This research was supported by funds from the Ministry of Science and Technology, Taiwan (MOST 103-2313-B-291 –001).

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