https://doi.org/10.48048/tis.2024.8217

Oogenesis and Spermatogenesis of the Triploid Black Tiger Shrimp, *Penaeus monodon*

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Received: 20 March 2024, Revised: 15 April 2024, Accepted: 22 April 2024, Published: 1 October 2024

Abstract

Triploidy (3n) induction of *Penaeus monodon* was performed separately by Australian and Thai groups of researchers 14 years ago, incidentally at the same period. The Australian group employed the chemical induction method, while the Thai group the cold shock one, and both groups obtained 3n *P. monodon* in their attempts. The success has led to several studies on the physiology of the 3n *P. monodon*, including growth, survival and reproductive functions. Both groups reported sterility of the 3n shrimp. The Australian group reported defected oogenesis and spermatogenesis of the 3n shrimp examined at subadult stage. Since the evaluation of the reproductive functions should be performed at the adult stage of the animals, we, therefore, performed in-depth studies on oogenesis and spermatogenesis of adult 3n *P. monodon* induced by cold shock. The studies include gross observation of the ovary, light (LM) and transmission electron (TEM) microscopy and image analysis of sex cells. The ovary of the 3n *P. monodon* females could not develop to full-maturation and their gonadosomatic index (GSI) was significantly lower than that of the 2n shrimp. Oogenesis of the 3n females proceeded from oogonia to cortical rod oocytes, but with significantly lower percentage than that of the 2n shrimp. The GSI of the 3n males was significantly lower than that of the 2n shrimp. Their spermatogenesis, however, proceeded normally from spermatogonia to spermatozoa, but with low density of spermatozoa in the seminiferous tubule and vas deferens, and none in the terminal ampoule. The average nuclear area of the 3n spermatozoa was significantly lower than that of the 2n shrimp as well. Altogether, these results suggest that 3n *P. monodon* induced by cold shock had defect in both oogenesis and spermatogenesis, probably more in quantity than in quality.

Keywords: *Penaeus monodon*, Triploid, Oogenesis, Spermatogenesis, Synaptonemal complex

Introduction

The black tiger shrimp *P. monodon* Fabricius, an indigenous species in tropical Pacific Ocean, has been farmed in several countries in Asia-Pacific region, Africa and northern Australia. Thailand was a leading country in exporting farmed *P. monodon* from 1999 to 2010, after which Thai shrimp growers switched the species of culture from *P. monodon* to the Pacific white shrimp *Litopenaeus vannamei* [1]. However, recent problems in growing *L. vannamei*, including infections from white-spot syndrome virus [2], toxin-producing *Vibrio parahaemolyticus* [3] and *Enterocytozoon hepatopenaei* [4,5], have plagued the industry leading to the re-vitalization of farming *P. monodon* as an alternative species.

To re-introduce *P. monodon* as an alternative species, it may be a plus to employ triploid (3n), instead of ordinary diploid (2n), shrimp to improve its culture performance. Thus far, 2 groups of investigators have been seriously working in triploid induction of *P. monodon*; the Australian [6] and Thai groups [7]. The former employed chemical shock, 6-dimethylaminopurine, while the latter used cold shock, to induce the triploidy. Both methods were applied to the spawned eggs at time before the extrusion of the 2nd polar body, thus the chromosomes of the 2nd polar body were retained, rendering 3n number of chromosomes in subsequent embryonic cell divisions. The former group of investigators reported no difference in the growth rate of the 3n females, 2n males and 3n males, and, on the contrary, the growth rate of the 2n females was significantly higher than that of the 3n females [6]. The latter group, however, reported significantly faster growth of 3n shrimp of both sexes, compared to their 2n counterparts, under both experimental and field trials, and with sex being skewed toward female [7,8].

Regarding the reproductive function, Sellars *et al*. [6] reported abnormal oogenesis and spermatogenesis of the 3n *P. monodon*, rendering sterility to the shrimp. In their report, the ovaries of the 3n females were consisted of low populations of advance-staged oocytes and no mature oocytes observed, and the testes of the 3n males had low numbers of spermatogonia and advance-staged sex cells, and absence of sperm.

Since both groups of investigators employed different methods to induce the triploidy, it is therefore possible that this could lead to the different results in growth rate. Moreover, the expressions of oogenesis and spermatogenesis may be different as well. With detailed investigations employing light (LM) and transmission electron (TEM) microscopy, in combination with a software-cytometric method, we herein reported results from our analysis that were different from those of Sellars *et al*. [6].

Materials and methods

Experimental animals

The use of experimental animals for scientific purposes has been permitted by the ethical committee of the Prince of Songkla University.

Using cold shock for triploid induction of *P. monodon* was performed according to the previous method described [7]. Briefly, at 8 min post-spawning, the fertilized eggs were subjected to a sudden drop in temperature from 28 to 8 °C for 10 min, after which the eggs were suddenly placed back into 28 °C seawater. The process inhibits the extrusion of the 2nd polar body, resulting in 3 sets of chromosomes in the subsequent embryonic cell divisions. The 3n eggs were allowed to hatch into nauplii and developed into post-larvae, juveniles, subadults and adults. The whole process took about 1 year to obtain 3n *P. monodon* adults, of which the body weights (BW) were 80 - 100 g. Control 2n *P. monodon* were from the same batch of eggs undergone the same process as those being triploidy-induced, except the cold shock.

The adult 2n and 3n *P. monodon* were reared together in a 113-ton circular canvas pond under 30-ppt

seawater. The experimental shrimp were composed of 42 females and 12 males. For the females, 22 were 2n and 20 were 3n; for the males, 4 were 2n and 6 were 3n. The ploidy status of the animals was determined by Fluorescence Activating Cell Sorting (FACS) analysis.

Appropriate water quality was maintained, by water exchange, throughout the rearing period in order to ensure the optimum conditions for the shrimp (pH, 8.2 - 8.5, alkalinity 130 - 150 ppm, total ammonia nitrogen, < 0.5 ppm, total nitrite, < 0.5 ppm, dissolved oxygen, > 5 ppm). The shrimp were provided with commercial maturation pellets (CPF, Bangkok, Thailand) at a rate of 3 % biomass daily.

Experimental procedure

All the experimental shrimp were anaesthetized in slurry ice, –5 °C, for 10 min, individually weighed, and the ovarian maturation of each female was staged visually [9,10]. The sample numbers of the 2n and 3n females were 5 - 8 for each different stages of ovarian maturation. The reproductive tissues of the female included the ovary and oviducts, and those of the male were the testis, spermatic duct and terminal ampoule. The gonadosomatic index (GSI) was calculated as the percentage of the total weight of the reproductive tissues against the BW. Small samples (approx. 10 mg) of each part of the reproductive tissues were immediately fixed in Davidson's fixative for LM and in 2 % glutaraldehyde for TEM examinations.

LM and TEM

After fixing in Davidson's fixative for 12 h, the tissue was then dehydrated in a series of graded ethanol, embedded in melt paraffin and sectioned with microtome (3 - 5 μm). The sections were stained with hematoxylin and eosin (H & E) and viewed under LM. General histological features were observed and various types of the sex cells were examined regarding the sizes and shape of each cells type. Employing ImageJ software, the size of the sex cells (N = 100) was determined (in mm^2) and averaged.

For TEM, following 3 h in 2 % glutaraldehyde, the tissues were rinsed twice in artificial seawater, followed by 2 rinses in 0.2 M cacodylate, pH 7.8 for 30 min. The sample was then dehydrated in a series of graded ethanol, embedded in a low-viscosity epoxy resin and sectioned with diamond knives in an ultramicrotome (MT-XL RMC). Semi-thin plastic sections (0.5 - 1.0 μm) for LM are stained with 1 % toluidine blue. Thin sections (60 - 90 nm) are stained with saturated methanolic uranyl acetate, counter-stained with lead citrate and observed under TEM (Jeol, JEM-100 CXII).

Statistical analysis

All numerical data were presented as means ± standard deviation (SD). All the statistical analyses were carried out using ANOVA followed by Tukey test in multiple group comparison, and Student t-test in 2-group comparison. Differences were considered significant at $p < 0.05$.

Results and discussion

Gonadosomatic index (GSI)

The average BWs of the shrimp samples of both sexes revealed no significant difference between the 2n and 3n shrimp of the same sex, but within the same set of chromosome numbers, the BW of the females were significantly higher ($p < 0.01$) than that of the males (**Figure 1**). It is well-documented that in *P. monodon*, females are larger than males of the same age [11]. Within the same number of chromosome sets, however, no difference in the BWs was detected in both sexes, which was corresponded to that reported by Sellars *et al*. [6]. In the females, while the ovarian maturation of the 2n shrimp reached stage 4 followed by spawning, that of the 3n shrimp reached only stage 3 and no spawning was observed. Herein, ovarian maturation staging was based on external morphology of the ovary, which is divided into 4 stages

[9,10]: Stage 1, the ovarian tissue appears as a narrow medial line of extending from the cephalothorax toward the entire length of the abdomen, stage 2, thickening of the ovarian tissue line, stage 3, the tissue fills up the dorsal part of the abdomen and stage 4, the tissue extends its wings to the side of the abdomen. The GSIs of the 3n *P. monodon*, both in females and males, were significantly lower ($p < 0.05$) than those of the 2n shrimp. These findings, as well as that the ovary of 3n *P. monodon* could not reach stage 4 maturation, suggest defects in oogenesis and spermatogenesis of the 3n shrimp, which was further supported by subsequent histological examinations.

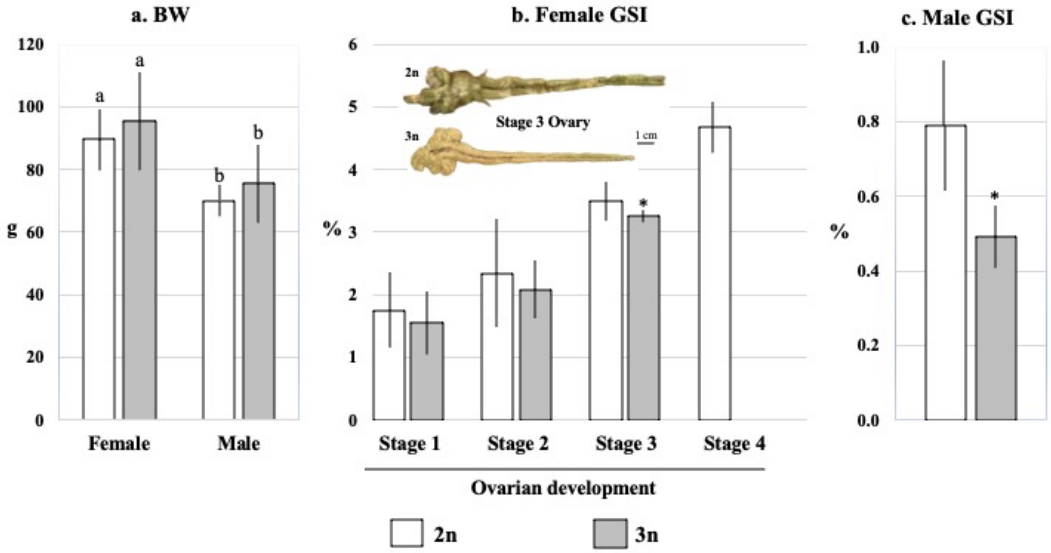

Figure 1 The average BW (a), GSI of the adult females (b) and GSI of the adult males (c), comparing between the 2n and 3n *P. monodon*. Different superscripts of the BWs indicate statistical significance ($p < 0.01$). *$p < 0.05$, compared to those of the 2n counterparts.

The color of the stage-3 ovary of the 2n shrimp was slightly greenish, while that of the 3n shrimp was yellowish. Since the carotenoids were accumulated in the ovary of *P. monodon* during oogenesis and turned the ovarian color from yellowish to greening while approaching ovarian maturation [12], this finding suggests a defect in this process in the ovary of the 3n shrimp. Carotenoids are obtained only by oral intake and metabolized to its main product, astaxanthin [13], it is thus possible that the 3n *P. monodon* lacks the metabolic pathway in converting carotenoids into astaxanthin. This interpretation is supported by previous reports in the shrimp *L. vannamei* and the lobster *Homarus americanus*, when the yellowish ovaries were converted to green color by oral supplement of astaxanthin [14,15].

Histology

In the female

The general histological features of the ovaries at stage 3 ovarian maturation viewed under low magnification revealed no remarkable difference between the 2n and 3n shrimp. The ovary was surrounded by the ovarian connective tissue capsule and divided into several lobules by the interlobular connective tissue. The connective tissue also contained follicle cells and haemal sinuses, in addition to fibroblasts and connective tissue fibers. Each ovarian lobule was further divided into several nodules separated by thin connective tissues, which also contained several follicle cells. Individual nodules were composed of oocytes

at different stages of development, which were oogonia, previtellogenic oocytes, vitellogenic oocytes and cortical rod oocytes. The proliferative zone of oogonia with high mitotic activities was localized at the periphery of the nodule.

The nuclei of oogonia of both the 2n and 3n shrimp were spherical in shape (8 - 20 mm in diameter) with slightly basophilic cytoplasm (**Figures 2(a)** and **2(b)**). A small number of oogonia of both groups had vacuolated cytoplasm and fragmented nuclei (**Figure 2(b)**), suggesting atrophic oogonia. The percentage of the atrophic oogonia, compared with the total number of oogonia, was not significantly different between the 2 groups (18 ± 11 of the 2n vs. 20 ± 11 of the 3n shrimp, N = 100 in each group). The previtellogenic oocytes (15 - 60 mm in diameter) of the 2n and 3n shrimp were similar in appearance, with heterochromatic nuclei and slightly more basophilic cytoplasm than that of the oogonia (**Figures 2(c)** and **2(d)**). The remarkable difference between the 2 groups was that most of the previtellogenic oocytes of the 2n shrimp were surrounded by a single layer of flattened follicle cells, while none or lesser number of the follicle cells were observed in the 3n shrimp. The vitellogenic oocytes (50 - 300 mm in diameter) of the 2n and 3n shrimp had basophilic cytoplasm that contained eosinophilic yolk granules (**Figures 2(e)** and **2(f)**). The vitellogenic oocytes of the 3n shrimp were surrounded by visually lesser number of follicle cells. Besides the yolk granules, the cortical rod oocytes (250 - 350 mm in diameter) contained oval- or cylinder-shaped cortical rods at the periphery of their eosinophilic cytoplasm (**Figures 2(g)** and **2(h)**). The cortical rod oocytes of the 3n shrimp were, as well, surrounded by visually lesser number of the follicle cells, compared to those of the 2n shrimp.

The ovaries of both 2n and 3n shrimp at stage 1 maturation were composed of only oogonia and previtellogenic oocytes, which were the majority of the sex cells. In stage 2, both groups revealed the appearance of vitellogenic oocytes, the 3n shrimp had visually higher proportion of oogonia population than the 2n shrimp. In stage 3, all the developmental sex cells appeared but the 3n shrimp had significantly lower ($p < 0.05$) percentage of the cortical rod oocytes than that of the 2n shrimp [52 (± 13) % for the 2n vs. 25 (± 14) % for 3n shrimp, N = 100 in each group].

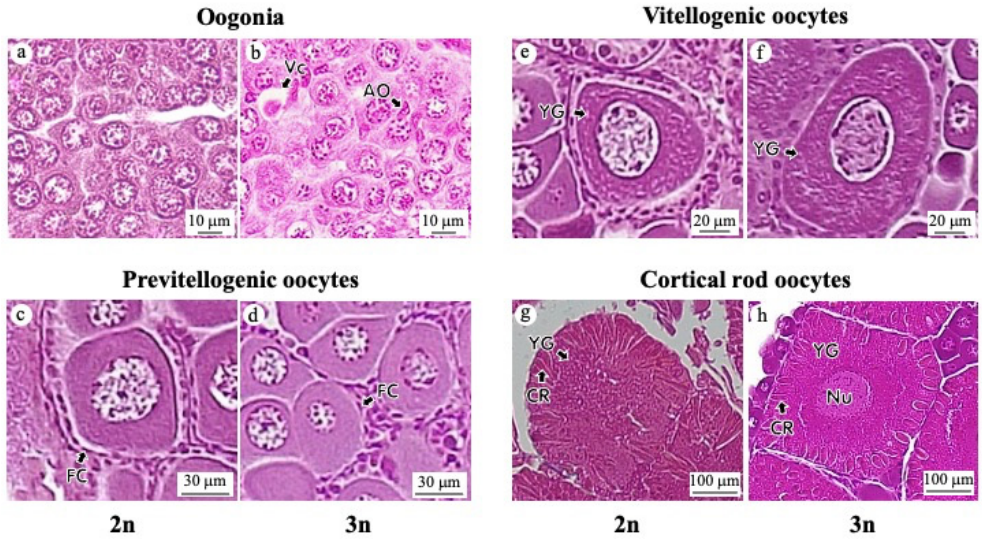

Figure 2 Histological features of oogonia (a and b), previtellogenic oocytes (c and d), vitellogenic oocytes (e and f), and cortical rod oocytes (g and h) in the ovaries of the 2n and 3n *P. monodon* females. H & E staining. Note: AO = atrophic oocyte, CR = cortical rod, FC = follicle cells, Nu = nucleus, Vc = vacuole, and YG = yolk granules.

In the male

The testis was composed of several seminiferous tubules surrounded by connective tissue capsule, or tunica albuginea (**Figure 3**). Inside the tubule, sex cells of different developmental stages and supporting cells were observed. The most peripherally located sex cells in the tubule were spermatogonia and the more advance-staged cells were localized at the central part. In the 2n shrimp, spermatogonia and spermatocytes were readily observed in the peripheral area of the seminiferous tubule, while spermatids were situated next to spermatocytes toward the center, and spermatozoa were packed in the lumen of the tubule (**Figure 3(a)**). In the 3n shrimp, spermatogonia were less discernible and the central part of the tubule was consisted mainly with spermatocytes (**Figure 3(b)**). The abundance of spermatozoa in the tubular lumen as observed in the 2n shrimp was not observed in the 3n shrimp.

All developmental stages of the male sex cells were observed in both the 2n and 3n shrimp, however, the difference between the 2 groups were in the abundance and histological features of sex cells (**Figure 4**). The spermatogonia (10 - 15 µm in diameter) of both groups were undistinguishable in size and shape and characterized by large centrally located nuclei and clear cytoplasm (**Figures 4(a)** and **4(b)**). After completion of final mitotic division of sermatogonia, the male germ cells simultaneously entered into the meiotic prophase I as primary spermatocytes. At the end of prophase I, the cells become the secondary spermatocytes, which are the transient state and cannot be identified with confidence in *P. monodon* [16]. Therefore, the 2 cell types, primary and secondary spermatocytes, were simply reported as spermatocytes in this study. The spermatocytes (8 - 12 µm in diameter) of the 2n and 3n shrimp contained heterochromatic nuclei and scant cytoplasm (**Figures 4(c)** and **4(d)**). Several fragments of nuclei were observed within the group of the spermatocytes of the 3n shrimp (**Figure 4(d)**). The spermatids (5 - 8 µm in diameter) of both the 2n and 3n shrimp were spherical (**Figures 4(e)** and **4(f)**); eosinophilic vesicles were observed in their nuclei and cytoplasm of the 3n, but not very distinctive in the 2n shrimp. At LM level, no remarkable difference in the features of spermatozoa (3 - 4 µm in diameter) between the 2n and 3n shrimp was observed (**Figures 4(g)** and **4(h)**).

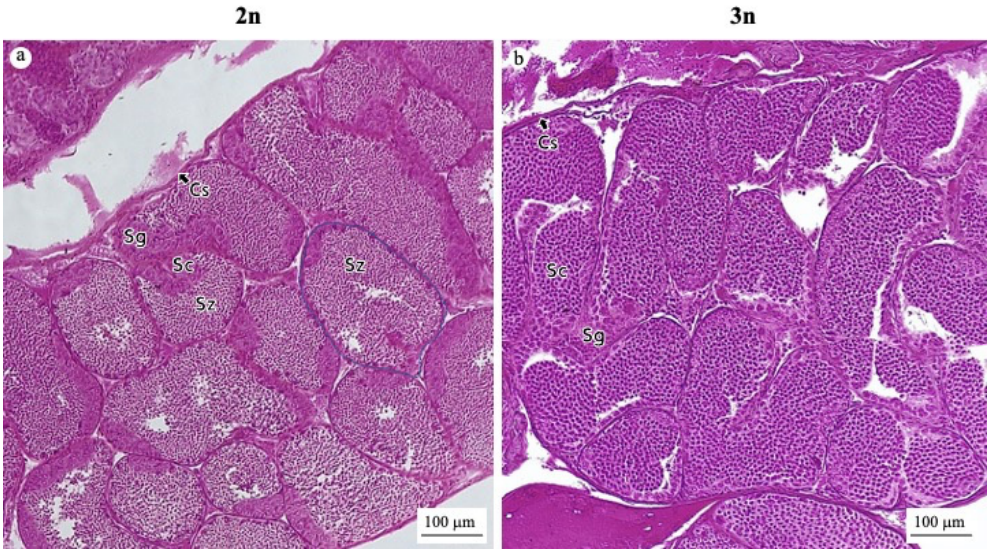

Figure 3 General histological features of the ovaries of the sexually mature *P. monodon* males, comparing between the 2n (a) and 3n (b) shrimp. Cross section of a seminiferous tubule is outlined by the blue line in (a). H & E staining. Note: Cs = capsule or tunica albuginea, Sc = spermatocyte, Sg = spermatogonia and Sz = spermatozoa.

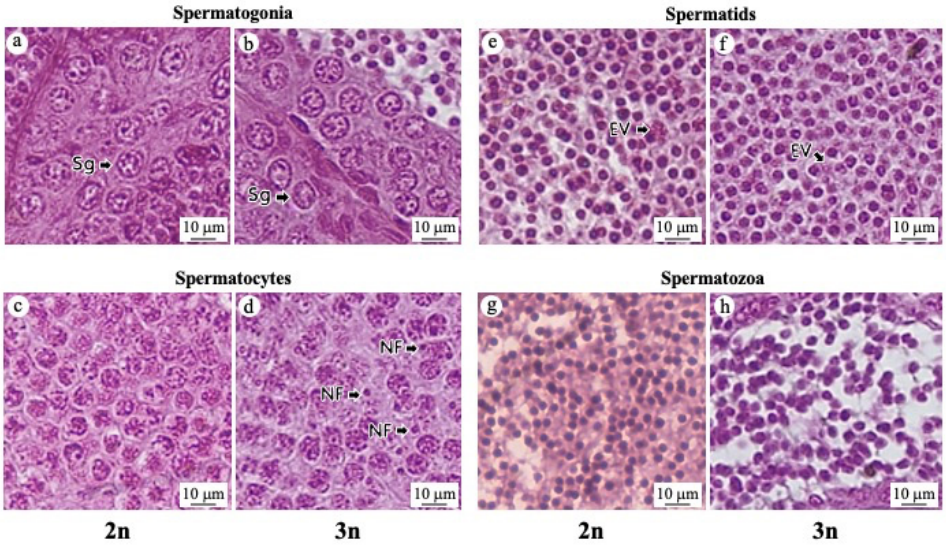

Figure 4 Histological features of spermatogonia (a and b), spermatocytes (c and d), spermatids (e and f), and spermatozoa (g and h) in the testes of the 2n and 3n *P. monodon* males. H & E staining. Note: EV = eosinophilic vesicles, NF = nuclear fragments, Sc = spermatocyte, Sg = spermatogonia and Sz = spermatozoa.

Bundles of spermatozoa embedded in the amorphous substance were observed in the vas deferens (**Figure 5(a)**) and terminal ampoules (**Figure 5(c)**) of the 2n shrimp, while low number of spermatozoa was found within the lumens of the vas deferens (**Figure 5(b)**) and none was observed in the terminal ampoule of the 3n shrimp (**Figure 5(d)**).

The sizes of the sex cells of the 2n and 3n shrimp were represented by the 2-dimensional areas of the cells, determined by ImageJ software (**Figure 6**). In the ovaries, no size difference of the same types of cells was observed between the 2n and 3n shrimp. In the testes, the spermatocytes of the 3n shrimp were significantly ($p < 0.05$) smaller than that of the 2n shrimp.

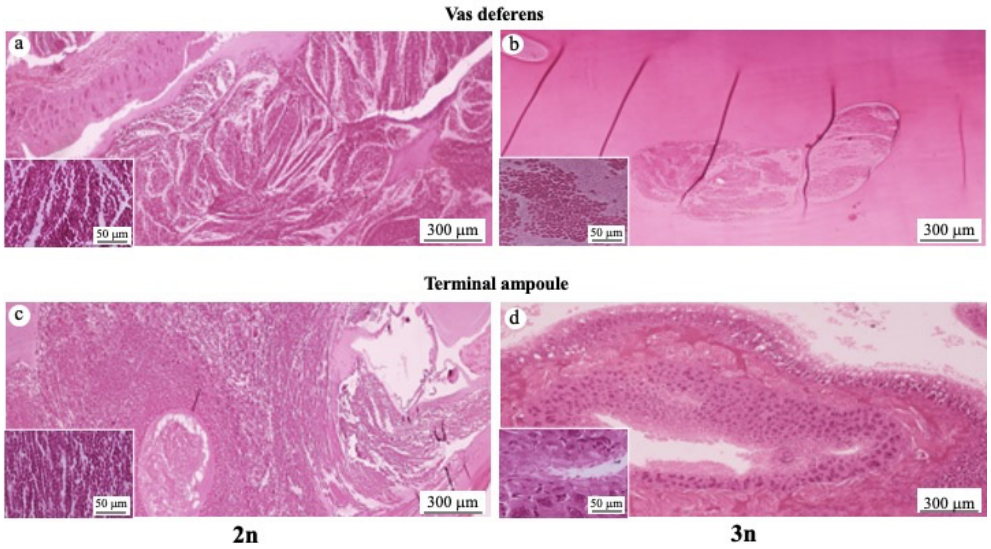

Figure 5 Histological features of the vas deferens (a and b) and terminal ampoules (c and d) of the 2n and 3n *P. monodon* males. H & E staining.

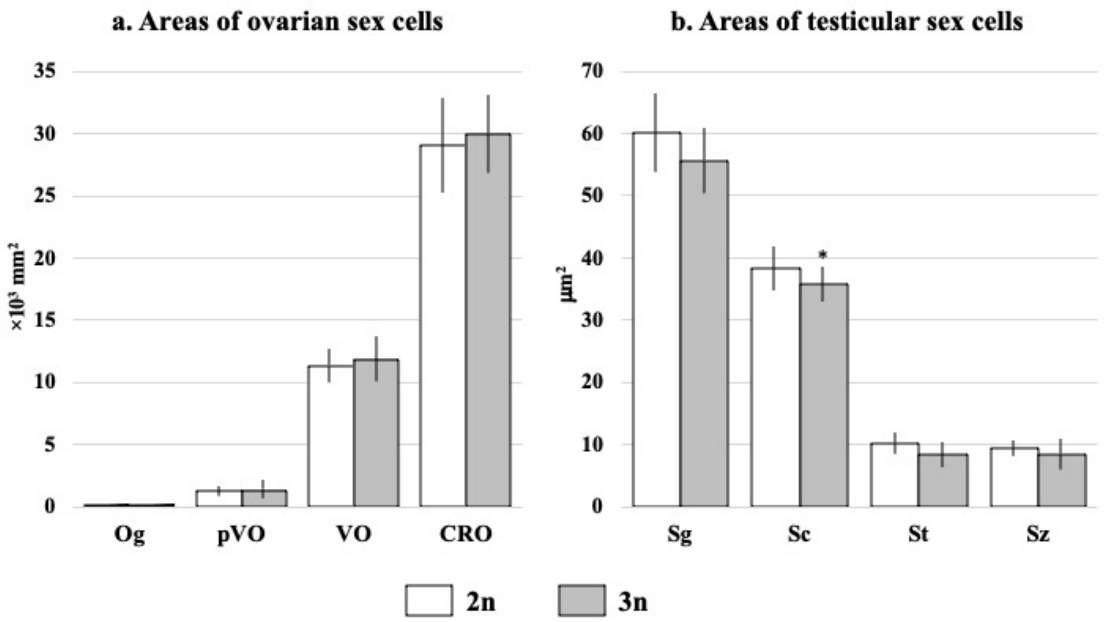

Figure 6 The cellular areas of the sex cells of the ovaries (a) and testes (b) of the 2n and 3n shrimp determined by ImageJ software. Note: CRO = cortical rod oocyte, Og = oogonia, pVO = previtellogenic oocyte, Sc = spermatocyte, Sg = spermatogonia, St = spermatid, Sz = spermatozoa and VO = vitellogenic oocyte. *$p < 0.05$, compared to the 2n counterpart.

TEM

In the female

The oogonia of the 2n (**Figure 7(a)**) and 3n (**Figure 7(b)**) shrimp had spherical nuclei. The sizes of their nuclei were varying, being due to their different stages of mitotic division. Some oogonia of both groups underwent atrophic changes, the nuclei were broken down into different compartments and finally disintegrated (**Figure 7(b)**). The previtellogenic oocytes of the 2n and 3n shrimp were similar in size and features, with peripheral heterochromatin in the nucleus, high density of ribosomes, rough endoplasmic reticula (RER), dilated RER and mitochondria in the cytoplasm, and aggregates of ribosome-like structures on the outer nuclear membrane (**Figures 7(c) - 7(f)**).

The ultrastructure of the vitellogenic oocytes of the 2n (**Figure 8(a)**) and the 3n (**Figure 8(b)**) shrimp revealed no remarkable difference. Their cytoplasm contained numerous, electron-dense, yolk granules of varying sizes (0.2 - 3.0 mm) and dilated RER of varying sizes and shapes. The cortical rod oocytes of the 2n (**Figures 8(c)** and **8(e)**) and the 3n (**Figures 8(d)** and **8(f)**) shrimp revealed no remarkable difference as well. The cytoplasm of these mature oocytes contained numerous cortical rods at the periphery with typical bottle brush structures inside.

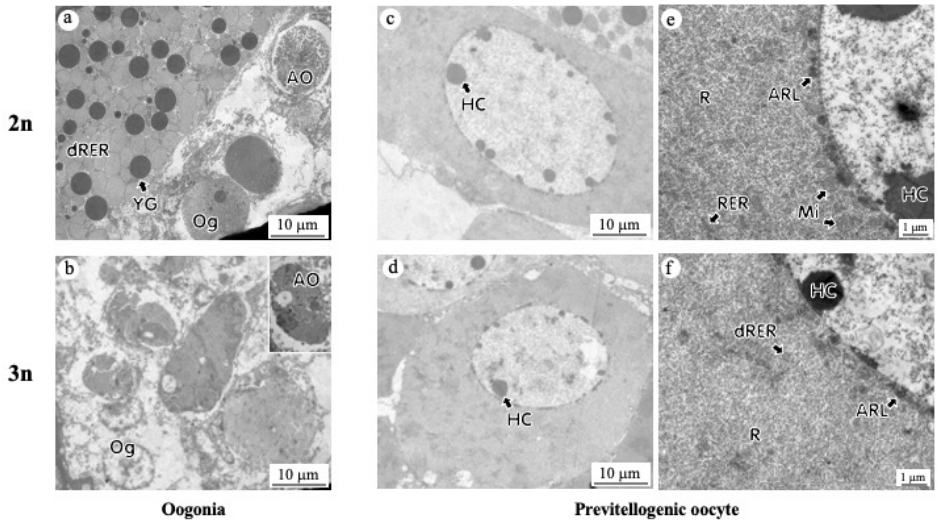

Figure 7 Ultrastructure of the oogonia (a and b) and previtellogenic oocytes (c - f) of the 2n and 3n *P. monodon* females. Note: AO = atrophic oogonia, ARL = aggregated ribosome-like structures, HC = heterochromatin, R = ribosomes, RER rough endoplasmic reticula, dRER = dilated RER, Mi = mitochondria and YG = yolk granule.

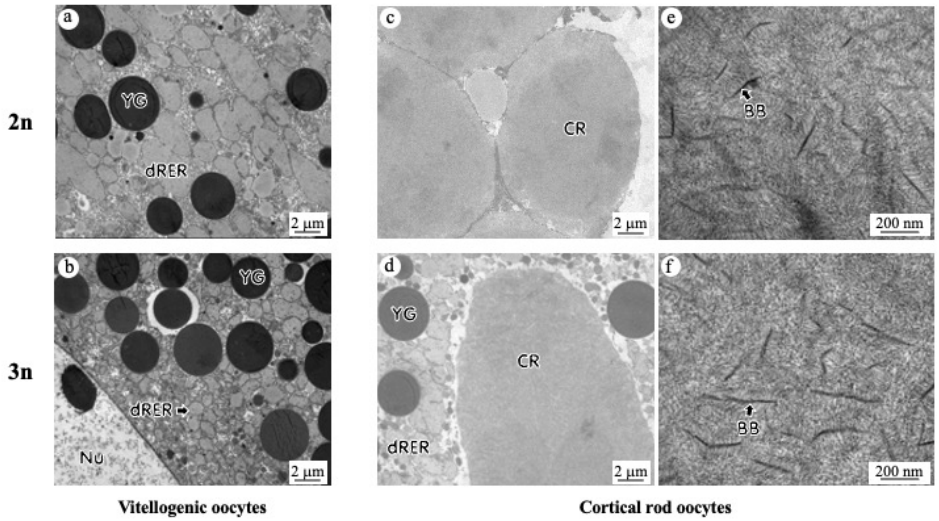

Figure 8 Ultrastructure of the cytoplasm of the vitellogenic (a and b) and the cortical rod oocytes (c - f) of the 2n and 3n *P. monodon* females. Note: BB = bottlebrush structures, CR = cortical rods, dRER = dilated rough endoplasmic reticula and YG = yolk granules.

The comparable abundance of yolk granules in the mature oocytes of the 2n and 3n *P. monodon* in this study suggests the normal accumulation of vitellin in the oocytes. In penaeid shrimp, vitellin is derived from vitellogenin, which is produced by the hepatopancreas and follicle cells [17,18], vitellin is chemically linked to lipid molecules to become lipoprotein and form yolk granules [19,20].

Likewise, normal formation of the cortical rods was also observed in this study. The role of the cortical rods is to form the hatching envelop of the spawned eggs [21,22]. The contents of the cortical rods are from those in the dilated RER [23], which are composed of 25 - 30 % of carbohydrate and 70 - 75 % protein on dry matter basis [24].

Overall, our morphological evidence revealed that oogenesis of the 3n shrimp was not different from that in the 2n shrimp, suggesting that the 3n oocytes could develop normally until they need to go through the meiotic division process, which occurs after spawning [21]. This finding is different from that reported previously in 3n *P. monodon* [6] and 3n *F. chinensis* [25-27], in which the development was arrested at the previtellogenic oocytic stage. This discrepancy may be due to the different methods of the triploid induction. Since 3n cells should have normal mitotic division as revealed in many triploid species, such as in triploid oyster [28], therefore, the mitotic division of oogonia of the 3n shrimp should be normal, which has been shown in this study.

In the male

The ultrastructure of spermatogonia (**Figures 9(a)** and **9(b)**), spermatocytes (**Figures 9(c)** and **9(d)**), spermatids (**Figures 9(e)** and **9(f)**), and spermatozoa (**Figures 9(g)** and **9(h)**) of the 2n and 3n *P. monodon* males revealed no remarkable difference. The supporting or Sertoli cells (**Figure 9(b)**) characterized by electron-dense nuclei and numerous vesicles in the cytoplasm were in close proximity to the spermatogonia. The nuclei of the spermatocytes were characterized by several groups of condensed chromatins (**Figures 9(c)** and **9(d)**) and cytoplasmic dense bodies during the process of meiosis I. These dense bodies were also observed in the previous report and suggested to be the centrioles [16]. The nuclei of the spermatids were characterized by heterochromatin at the periphery of the nuclei (**Figures 9(e)** and **9(f)**). Likewise, the nuclei of the spermatozoa (**Figures 9(g)** and **9(h)**) in the seminiferous tubules of both groups were similar and contained all components of the spermatozoa, i.e., the nucleus, the spike, the acrosome chamber, the acrosome body, the dense body between the spike and acrosome body and the sub-acrosome chamber. The size and shape of the spermatozoa from the seminiferous tubules of the 2n shrimp were more uniform than those of the 3n shrimp (**Figure 10**). The average area of the nuclei of the spermatozoa of the 3n shrimp as determined by ImageJ software was more variable and significantly ($p < 0.05$) smaller than that of the 2n shrimp. Besides, the shape of the spermatozoa of the 3n shrimp was less symmetrical than that of the 2n shrimp.

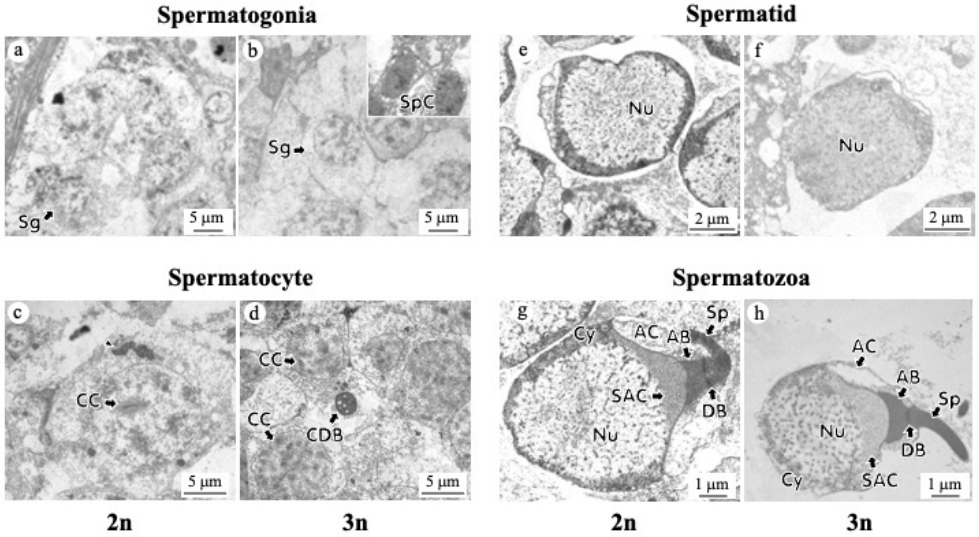

Figure 9 Ultrastructure of spermatogonia (a and b), spermatocytes (c and d), spermatids (e and f) and spermatozoa (g and h) of the 2n and 3n *P. monodon* males. Note: AB = acrosome body, AC = acrosome chamber, CDB = cytoplasmic dense body, CC = condensed chromatin, Cy = cytoplasm, DB = dense body, Nu = nucleus, SAB = sub-acrosome chamber, Sg = spermatogonia, Sp = spike and SpC = supporting cells.

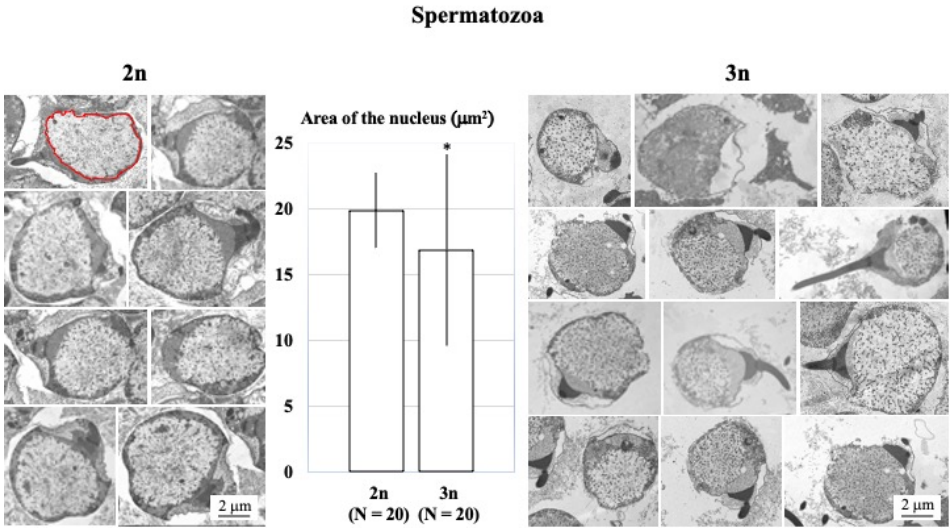

Figure 10 Ultrastructure of spermatozoa samples from the seminiferous tubules of the 2n and 3n *P. monodon* males, under the same magnification. The histogram shows the average areas of their nuclei (as outlined by the red line in 1 sample of the spermatozoa). *$p < 0.05$.

Probably, the most remarkable features of spermatogenesis of the 3n *P. monodon* males in this study was the low number and abnormal shape, and significantly smaller size, of the spermatozoa. The results of this study added new information to what reported earlier in 3n *P. monodon* [6] and 3n *F. chinensis* [25,27], which revealed only the significant reduction in the abundance of spermatozoa. The failure of spermatogenesis in the 3n shrimp is likely due to the problem of meiosis since the whole process of spermatogenesis occurs in the testis.

The causes of abnormality in the spermatogenesis in 3n animals include the abnormal transcriptomes/proteomes and synapsis of homologous chromosomes [29,30]. The decreased fertility in triploid animals was frequently due to abnormal chromosome pairing, or synaptonemal complex, among the 3 complete sets of chromosomes during meiosis with the formation of imbalanced and non-viable gametes [31]. The differences in morphology of synaptonemal complex in 3n *F. chinensis* included unsynapsed univalents, bivalents, totally paired trivalents with non-homologous synapsis, partner switches and triple synapsis at early pachytene stage, and triple synapsis at late pachytene stage [32].

Conclusions

Abnormalities in the spermatogenesis, but not oogenesis, of the triploid *P. monodon* were described. The oocytic development in the shrimp ovary are composed mainly of mitotic process and vitellogenesis; these 2 activities were found not being affected by the triploidy condition. On the contrary, spermatogenesis event in the testis, which include both mitotic and meiotic processes, was found to be abnormal in the 3n shrimp. It may be concluded that triploidy condition of *P. monodon* induced by cold shock affects meiotic, but not mitotic activity of the sex cells.

Acknowledgements

The study was supported by the Scholarship for Best Academic Performance for Graduate Study (to Muthita Saearlee) from the Prince of Songkla University and research budget of the fiscal year 2017 from the Prince of Songkla University, Surat thani Campus, Thailand.

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