The process of nucleus insertion into gonad of the Akoya pearl oysters, *Pinctada fucata* is the major procedure requiring skill and economy. In order to survive the scarce oysters, the conveniently selection of the oysters with proper gonad for nucleation was studied. Each 40 cultured Akoya pearl oysters were sampling monthly for 12 months. Average measurement of shell length and shell height were taken place around 12 months of development. Every developing oyster was studied the histological structure of gonad with various stages of reproductive cycle. In addition, the monthly increasing of the oysters’organic composition: protein, fat and glycogen was calculated. The result of proper gonadic stage for first nucleation appeared on the 11 months-old oysters, which their average shell length of 64.84 mm. and average shell height of 58.66 mm. At this stage whose gonad belonged to spawning and early resting (spent) stage: the stage that gonad is empty enough for nucleus insertion. Accordingly, the oysters at this stage had more average increasing amount of glycogen than fat and protein, because of the energy requirement for spawning activity.

**Keywords:** *Pinctada fucata*; nucleus insertion; reproductive stage; pearl oysters  
**E-mail:** kanoktorn.k@psu.ac.th
Introduction

The Akoya pearl oysters are common species along Andaman Coast and can be bred in coastal Phuket (Kanjanachatree, et al., 2004). Akoya pearl production took 2-3 years long and survival rate of the pearl oysters after nucleus insertion was so low of about 29-32% (Kanjanachatree, et al., 2006) because of several factors such as misplace nucleus insertion and pre-, post-dissection management that affected stress metabolism of the oysters. Moreover, slightly understanding about the stages of gonadic development of the Akoya pearl oysters is the most important factor. Accordingly, the gonad is appropriate location for nucleus insertion and the proper reproductive stage for nucleus insertion is spawning stage, since the insertion taken place in the gonad of mature stage caused nucleus extrusion (Meng, et al. 1994). This study aimed to determine gonadic stages of the first reproductive cycle of Akoya pearl oysters (Pinctada fucata) cultured from earlier age to 12 months old (May 2005 to April 2006). In addition, the oysters’ shell length and shell height were recorded throughout their development. These data can be use to determine the suitable body-size of the oysters that have appropriate gonadic stage for the first nucleus insertion without dissection the oysters for checking their gonad before nucleus insertion, which leaded to their mortal hazard. Finally, the farmers can easily collect the oysters with suitable gonad for nucleus insertion, so the productivity is increased and moreover, the cost of pearl culturing is lower.

Materials and methods

1. Breeding of the Akoya pearl oysters

   1.1 Preparation of the oysters’ parents for fertilization

   The oysters’ parents having shell height more than 60-65 mm. were collected. After their fecundity of the gonad was microscopic checking, separated male and female oysters. By temperature shock method (Kanjanachatree, et al., 2004).

   1.2 Spat rearing

   The fertilized eggs reared in culturing tank were going to cleavage and differentiate to D-shape larvae, then feeding with Isochrysis sp. plus Chaetoceros sp. (Kanjanachatree, et al., 2004) until they developed to Eyed larvae. After that the spats were rearing in the sea at 1 m. depth. (Kanjanachatree, et al., 2003). The 40 oysters were randomly collected monthly from the age of 1 month to 12 months, and measured their shell lengths and shell heights. In addition, the temperature, salinity, oxygen concentration and pH of the sea water were recorded monthly.
2. Histological study

Histological structures of every oyster’s gonad were examined the gametogenic stages by standard histological technique. Developmental stages of the oysters’ gonad are classified into six stages modified from the criteria used by Wada, et al. (1995) and Choi and Chang (2002).

3. Quantitative analysis of organic composition of the oysters’ mantle along 12 months of development Fat analysis by C-M mixture technique (Drouillard, et al., 2004). Protein and Glycogen analysis were resolved by Lowey’s technique (Lowey, et al., 1969).

Results

1. Growth of shell sizes and organic composition

During 12 months of the Akoya pearl oyster development, their average shell lengths and shell heights were continuously increased. Simultaneously, their accumulation of protein, fat and glycogen were fluctuated increment. (Table 1)

2. Gametogenic cycle

Akoya pearl oysters are sexual dimorphism, however, 2 oysters of the total 360 individuals were hermaphrodite which corresponded to the study in Japanese Akoya pearl oysters, *Pinctada fucata fucata* by Wada, et al. (1995). They found 2 hermaphrodite oysters from the total 232 individuals. The gonad of hermaphrodite oyster filled with sperms and oocytes in the same follicle.

Histological examination of the 6 reproductive stages was described below.

1. Initial developing stage: Gametogenesis was first appeared in 4 month-old oysters (Figure 1A,2A). Every oyster had developing gonad with small follicles surrounded by numerous connective tissue. The gonad follicles contained small stem cells lining germinal epithelium of the follicles. The distinguishable males had the follicles lined with spermatogonia of about 3-4 µm diameters, while the females had oogonia of about 4-5 µm diameters along some parts of follicle wall.

2. Multiplicative stage: This stage was first appeared in 5 month-old male oysters (Figure 1B), while in female oysters were 6 months old (Figure 2B). The more developing gonad contained numerous follicles yet among with much connective tissue. Gonad follicles contained oogenic and spermatogenic cells which proceeded to proliferate into different stages of development. Spermatocytes in the testis were small cells with dark blue nuclei. In the ovary, small oocytes with basophilic cytoplasm were proliferating along the wall of follicle.

3. Growing stage: The expanded gonad follicles were filled with different stages of developing gametes. Gamete formation proceeded actively in the follicles among with decreasing connective tissue. This stage was first appeared in 6 month-old male oysters (Figure 1C), while in female oysters were 7 months old (Figure 2C). The enlarging dark-blue testis in which numerous developing-
Table 1. Data on protein, lipid, glycogen, shell lengths and shell heights where development of the pearl oysters, *Pinctada fucata* was taken place during 12 months

<table>
<thead>
<tr>
<th>Month</th>
<th>Protein(%)</th>
<th>Lipid (%)</th>
<th>Glycogen(g)</th>
<th>Shell lengths (mm)</th>
<th>Shell heights(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.97</td>
<td>0.22</td>
<td>0.23</td>
<td>12.45 ± 1.64</td>
<td>10.95 ± 0.92</td>
</tr>
<tr>
<td>2</td>
<td>3.21</td>
<td>0.26</td>
<td>0.30</td>
<td>18.6 ± 2.29</td>
<td>16.88 ± 2.02</td>
</tr>
<tr>
<td>3</td>
<td>3.87</td>
<td>0.33</td>
<td>0.53</td>
<td>22.86 ± 2.05</td>
<td>18.25 ± 0.70</td>
</tr>
<tr>
<td>4</td>
<td>4.11</td>
<td>0.38</td>
<td>0.89</td>
<td>26.79 ± 1.55</td>
<td>21.03 ± 2.04</td>
</tr>
<tr>
<td>5</td>
<td>6.37</td>
<td>0.59</td>
<td>1.18</td>
<td>30.79 ± 2.19</td>
<td>24.53 ± 1.05</td>
</tr>
<tr>
<td>6</td>
<td>9.52</td>
<td>0.81</td>
<td>1.34</td>
<td>33.65 ± 2.47</td>
<td>27.81 ± 2.17</td>
</tr>
<tr>
<td>7</td>
<td>9.81</td>
<td>0.90</td>
<td>1.57</td>
<td>38.48 ± 2.23</td>
<td>31.83 ± 1.82</td>
</tr>
<tr>
<td>8</td>
<td>10.22</td>
<td>1.07</td>
<td>1.62</td>
<td>44.88 ± 3.46</td>
<td>36.7 ± 2.18</td>
</tr>
<tr>
<td>9</td>
<td>10.45</td>
<td>1.15</td>
<td>1.82</td>
<td>52.76 ± 3.56</td>
<td>44.46 ± 4.48</td>
</tr>
<tr>
<td>10</td>
<td>10.77</td>
<td>1.26</td>
<td>2.14</td>
<td>57.37 ± 3.59</td>
<td>50.49 ± 1.56</td>
</tr>
<tr>
<td>11</td>
<td>11.03</td>
<td>1.33</td>
<td>2.46</td>
<td>64.84 ± 3.05</td>
<td>58.66 ± 1.91</td>
</tr>
<tr>
<td>12</td>
<td>11.11</td>
<td>1.41</td>
<td>2.73</td>
<td>71.5 ± 4.81</td>
<td>63.68 ± 3.12</td>
</tr>
</tbody>
</table>

-spermatocytes and spermatids were evenly distributed in the follicular lumen. The enlarging ovary in which various stages of developing oocytes were proceeded expanding into the follicular lumen. Primary oocytes along the follicular wall had diameters about 30 µm. Newly mature oocytes could be found at the center of the follicle. These are the large polygonal cells having expanded nuclei and cytoplasm with accumulating yolk.

4. Mature stage: Ripe gonad follicles, surrounding with a small thin layer of connective tissue, were densely packed with maturing gametes. This stage was first appeared in 8 month-old male oysters (Figure 1D), while in female oysters were 9 months old (Figure 2D). The largest testis was fully filled with dark blue spermatocytes, especially spermatozoa in center of the follicle. The ovary was packed with a large number of mature oocytes which appeared freely in the follicular lumen. The oocytes are polygonal shape and some are spherical with diameter about 40-45 µm. However, some oocytes are stalk shape, having cytoplasmic process attached to the follicular wall.

5. Spawning stage: This stage was first appeared in 9th month of development (Figure 3) and continuously spawned until 12th month. Male (Figure 1E): most spermatozoa were released, some follicles were partially empty, in which immature spermatocytes or spermatids were restricted to the lining of the follicular wall. Female (Figure 2E): gonad follicles were distended, most of ripe oocytes
developed free from the follicular wall and move into the center, and then were released, while a few ripe oocytes remained and follicles become looser.

6. Resting stage or spent stage: This stage was first appeared in 11\textsuperscript{th} month of development and continuously spent until 12\textsuperscript{th} month. Follicles were almost empty or completely spawned (Figure 3). The testis (Figure 1F) or the ovary (Figure 2F) become contracted, in which the follicular lumen had residual spermatozoa or oocytes and showed signs of regression. Some shrunk follicles had stem cells along follicular wall, so this stage was preparing the reconstruction of new follicular tissue of the next reproductive cycle. The intervened connective tissue had been increased.

Growth of shell sizes and organic composition

Discussion and Conclusion

1. The 4 month-old Akoya pearl oysters were 85% gonadic indefiniteness, while 15% of them had definite sex contributed to the initial stage of gametogenesis. The 5 month-old oysters had 22.5% of indefinite gonad, while being 67.5% of initial stage and the rest 10.0% being multiplicative stage.

2. The procedure of culturing pearl oysters for spherical pearl production, the most suitable site for nucleus insertion is their gonad. Because the appropriate gametogenic stage for easily nucleation is the period of late spawning stage to spent stage, in which the gonad follicles are empty, so the 11 month-old Akoya pearl oysters were the best situation for the first nucleus insertion. Simultaneously, during development of the oysters, their shell sizes were continuous increased. As a result, the proper pearl oysters with average shell length of 64.84 mm. and average shell height of 58.66 mm. are being the first harvested for nucleation. Easefully, the farmers can harvest the pearl oysters which have shell length more than 64 mm. or shell height more than 58 mm. for the first nucleation. By this knowledge, it decreases the oyster's injury from conventionally random dissecting check.

3. Along 8 months of the pearl oyster development, they mostly accumulated protein much more than fat and glycogen. However, the highest accumulation of fat was on the 8\textsuperscript{th} month of development, in contrast to lowest glycogen accumulation. This reflected body growth and gonad development. On the other hand, the 9-12 month-old oysters continuously increased glycogen accumulation, which was more than protein and fat. This implied that the spawning process required enormous energy (Saucedo, et al., 2001).
Figure 1. Histological structures of testicular follicles of *Pinctada fucata* showing spermatogenesis during 12 months of the pearl oyster’s development. (H&E), (A-B 400×, C-F 100×)

A = initial developing stage, tf = testicular follicle, gl = digestive gland

B = multiplicative stage

C = growing stage, numerous spermatocytes with dark blue nuclei and spermatids distributed in the lumen of testicular follicles.

D = mature stage, the follicles filled with different stages of spermatocytes (dark blue color)

E = spawning stage  

F = resting stage or spent stage
Figure 2. Histological structures of the ovary of *Pinctada fucata* showing ovarian follicles with oogenesis during 12 months of the pearl oyster’s development. (H&E), (A, B, E 100×, C, F 400×, D 1000×)

A = initial developing stage, of = ovarian follicle
B = multiplicative stage
C = growing stage, mo = mature oocyte
D = mature stage, large mature oocytes with distinct nuclei and nucleoli
E = spawning stage
F = resting stage, ao = atretic oocyte with shrinkage cytoplasm
Figure 3. Monthly changes in frequencies of gametogenic stages (%) of the total individuals of pearl oysters, *Pinctada fucata*. Most oysters at the age of 11-12 months (March to April) were spawning and spent stages.
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References


