The Pattern of Ovarian Development in the Prepubertal Antarctic Minke Whale (*Balaenoptera bonaerensis*)

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Abstract. This study describes the morphological and morphometrical changes associated with prepubertal ovarian development in the Antarctic minke whale (*Balaenoptera bonaerensis*). Ovaries were harvested from 94 immature minke whales caught in the Antarctic Ocean during the summer feeding season (December-March). Notable differences in ovarian size and morphology were found among animals. Up to 10 folds difference in ovarian weight was found among prepubertal whales of similar body size. During the prepubertal period, ovaries grew slowly and approximately doubled their weight. The morphologies of right and left ovaries were almost identical while the growth of the ovary appears to occur preferentially on the right side. The most striking morphological feature was numerous small antral follicles less than 5 mm in diameter found in ovaries of younger immature whales. The occurrence of these ovaries was highest in whales less than 6 m long and gradually decreased as body length increased. In larger whales, the occurrence of ovaries with a smaller number of follicles up to 10 mm and thick tunica albuginea increased. Thus, the ovary of the Antarctic minke whale experiences bursts of small follicular development during the early prepubertal period before becoming a more developed ovary with fewer but larger follicles, and thick tunica albuginea.

Key words: Whale, Minke whale, Ovarian development

history, much less attention has been paid to the ovary of immature whales. In the present study, we undertook a morphological observation of ovaries harvested from immature Antarctic minke whales. Here, we report an interesting morphological transformation occurring during the prepubertal period in Antarctic minke whale ovaries.

Materials and Methods

The ovaries used in this study were collected from immature Antarctic minke whales harvested in the Antarctic Ocean area IV and the eastern part of the area III (59–68°S, 41–129°E) during the 13th Japanese Whale Research Program under Special Permit in the Antarctic (JARPA) conducted from 5 December 1999 to 10 March 2000. The details of the 13th JARPA have been presented to the IWC Scientific Committee [14]. The whales were shot with explosive harpoons and ovaries were collected on the factory ship. The ovaries were marked (right or left) and individually weighed. Ovaries with no sign of ovulation (i.e. corpus luteum or corpus albicans) were regarded as immature ovaries.

Morphological observation

Both sides of each ovary were photographed by a digital camera (Cyber-shot 2.1 mega pixels DSC-F505K, Sony Corporation, Tokyo, Japan) under a standardized light condition provided by an illuminated magnifier (Model OSL-1, Otsuka Optics Co., Ltd., Tokyo, Japan) set for illumination mode. On some occasions, translucent photographs were also taken to visualize follicles by lighting through ovaries using the illuminated magnifier set for translucent mode. Visible/palpable follicles were punctured using a disposable syringe fitted with an 18G needle to collect oocytes. These oocytes were used for an in vitro fertilization study reported elsewhere [15]. On some occasions, because of tight experimental schedules, it was impossible to count precise numbers of follicles. In such cases the numbers of follicles were counted up to 200. The diameters of the largest follicles in each ovarian pair were assessed on the ovarian surface as to whether it exceeded 5 or 10 mm. On the basis of these observations ovaries were classified into two or three categories according to 1) the number and size of follicles (3 follicular types), 2) the appearance of ovarian surface tunica albuginea (3 surface types), and 3) morphology of ovarian body (2 ovarian types). The details of these criteria are mentioned in the following section.

Statistical analysis

Associations among variables in follicular, ovarian, surface types and body length were analyzed using a chi-squared test. Relationships between weight of left and right ovaries, body length and mean ovarian weight were analyzed using a regression analysis. Comparisons among group means were carried out using either a paired \( t \)-test or one-way ANOVA combined with an unpaired \( t \)-test.

Results

A total of 94 immature females were used in this study. The mean body length and weight, and ovarian weight are shown in Table 1. In 66 whales (70.2%) the right ovary was heavier than the left ovary (right dominant pair), while in 21 whales (22.3%) the left ovary was heavier (left dominant pair). The incidence of a heavier ovary on right side was significantly higher than the expected value of 50% (\( p<0.001 \): chi-squared test). When all data were combined, a highly significant difference was found between the weight of the right and left ovaries (\( p<0.001 \): paired \( t \)-test).

Considerable variation in ovarian weight was observed among whales with similar body lengths.

| Table 1. Body size and ovarian weight of immature minke whales (n=94) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Body size**   | **Ovarian weight** |
| Length (m)      | Weight (t)      | Right (g)       | Left (g)        | Paired (g)      |
| Mean ± SD       | 6.59 ± 0.90     | 3.43 ± 1.40     | 50.5 ± 29.0     | 48.0 ± 28.8     | 98.5 ± 57.5     |
| Max.            | 8.80            | 7.56            | 144             | 151             | 295             |
| Min.            | 5.23            | 1.70            | 11              | 7               | 18              |
| Median          | 6.38            | 3.03            | 46              | 42              | 88              |
(Fig. 1a). The ovarian weight gradually increased as the growth progressed (Fig. 1a). A highly significant correlation was observed between right and left ovarian weights in both right and left dominant ovarian pairs. When all data were combined, a near 1:1 relationship was observed (Fig. 1b).

In this study, we classified ovaries based on three morphological features. 

**Follicular type**: ovaries were classified into three categories according to the number and size of follicles. In 24 whales (25.5%), ovaries with numerous small follicles less than 5 mm in diameter were observed (type A, Fig. 2A). The majority of these follicles were antral follicles around 1–2 mm in diameter. In some animals, the numbers of follicles exceeded 1,000. In these animals, follicles were found in all areas of the ovarian surface, except the ovarian hilum. In 27 whales (28.7 %), ovaries with 50–200 follicles up to 10 mm in diameter were observed (type B, Fig. 2B). In 43 whales (45.8 %), ovaries with less than 50 follicles up to 10 mm in diameter were observed (type C, Fig. 2C). In these ovaries, follicles were often not visible and only detected by translucent lighting or ovarian palpation. The diameter of the largest follicle never exceeded more than 10 mm in any ovary. The number of oocytes recovered for each follicular type was 84.1 ± 54.3, 36.8 ± 17.6, and 16.9 ± 3.8 for types A, B, and C respectively (mean ± SD, A vs B or C: p<0.001, B vs C: p<0.05).

**Surface type**: ovaries were classified into three categories according to the appearance of ovarian surface tunica albuginea. In 10 whales (10.6%), ovaries with thin tunica albuginea were observed (thin). These ovaries were pale pink in color and small follicles were clearly visible through the tunica albuginea, giving the ovarian surface a rough appearance (Fig. 2A). In 37 whales (39.4%), ovaries with thickening tunica albuginea were observed (thickening). In some whales, thickening occurred unevenly and both thin and thick tunica albuginea were often observed in different parts of an ovary. Follicles were still visible albeit less clearly (Fig. 2B and 3a). In 47 whales (50%), ovaries with thick tunica albuginea were observed. The ovaries showed a smooth whitish appearance and follicles were often not visible due to the thick tunica cover (Fig. 2C and 3b).

**Ovarian type**: ovaries were classified into two categories according to the appearance of ovarian body. In 49 whales (52.1%), ovarian bodies gave a smooth flat appearance without a major furrow (smooth, Fig. 2A, C and 3b). In 45 whales (47.9%), ovaries had at least one major furrow and often gave a convoluted or wrinkled appearance (wrinkled, Fig. 3a).
There was a significant association between incidence of follicular and surface types. The type A ovaries were associated with either thin or thickening tunica albuginea, while the type B and C ovaries were associated with either thickening or thick tunica albuginea \((p<0.001, \text{Table } 2)\). No such association was found between incidence of ovarian and follicular/surface types.

Likewise a significant association was found between body length and incidence of follicular types \((p<0.001, \text{Table } 3)\). The type A ovaries were found in more than 60% of immature whales less than 6 m in length. The occurrence of the type A ovaries gradually decreased as the body length increased and reached zero in immature whales more than 8 m in length. Type B and C ovaries, on the other hand, were found in all size groups.

There was also a significant association between the body length and the thickness of tunica albuginea \((p<0.001, \text{Table } 3)\). Thin tunica was only found in whales less than 7 m in length. The proportion of whales with thick tunica increased as the body length increased.

There was no association between the body length and the incidence of smooth and wrinkled ovaries (Table 3).

**Discussion**

In the present study, earplug data were not available for age estimation of the immature whales. From the equation given by Lockyer [6], however, the immature whales used in the present study are estimated to have been between 6 months old, shortly after weaning, and 6 to 7 years old, just before the sexual maturation, which is currently estimated to occur at 6.5 years old [7].

There was a substantial variation in ovarian weight among whales of similar body length. A ten-fold difference in ovarian weight found among recently weaned animals (<6 m) implies difference in ovarian size already exists at the birth. The four-fold difference found among prepubertal animals...
around 8 m indicates that the size of the ovary is not a crucial factor for attainment of sexual maturation. Indeed a small ovarian pair (CL side: 213 g, non-CL side: 53 g), bearing an active CL, was observed in mature pregnant whales (8.25 m) in the present catch.

During the 6–7 years prepubertal period, ovaries grew slowly and approximately doubled their weight. The growth rate of paired ovaries estimated in the present study is 31.3 g per meter in body length, which is 1/5 of that reported in immature sei whales (153 g/m)[10] and 1/10 in immature humpback whales (333 g/m: calculated from the appendix 1)[12]. The morphology of right and left ovaries was almost identical in any pair of ovaries in the present study. On the other hand, the occurrence of a heavier ovary was more than three times as high in the right side as in the left side, indicating the ovarian growth takes place preferentially on the right side in immature Antarctic minke whales. Asymmetrical ovarian activity, in terms of incidence of ovulation, has been reported in many animals including cetaceans [16]. In cattle, follicular activity was shown to be higher in right side [17]. The difference between right and left ovarian weight appeared to increase as body length increased. The difference in ovarian weight, however, is not likely to reflect ovarian activity after the sexual maturation. In mature baleen whales, both right and left ovaries have been reported to be equally active [10, 13, 16], while in many species of toothed whales, the left ovary is known to be more active than the right ovary [16].

The most apparent ovarian features associated with the growth of the whales were changes in size and number of follicles, and appearance of ovarian surface. In the present study, we identified three follicular types according to the size and number of follicles (type A, B, C) and three surface types according to the thickness of tunica albuginea (thin, thickening, thick). It appears that these two features are associated with each other. The thin tunica albuginea was found to be only associated with type A ovaries, while the thick tunica was found in the type B and C ovaries.

The incidence of the type A ovary was highest in immature whales less than 6 m in length and gradually decreased as growth progressed. Type B and C ovaries were observed in all size groups. Since no features associated with type A ovary, namely numerous small follicles and thin tunica albuginea, were observed in mature whales, it is assumed that this type of ovary only occurs in the early prepubertal period. To our knowledge, this follicular feature has never been reported in any baleen whales. This is probably due to the minimum size regulations adopted during the days of commercial whaling. Small immature whales

Table 3. Relationship between body length and occurrence of each ovarian feature in immature minke whales

<table>
<thead>
<tr>
<th>Ovarian features</th>
<th>&lt;6.00 m</th>
<th>6.01–7.00 m</th>
<th>7.01–8.00 m</th>
<th>≥8.01 m</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Follicular type (FT)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>18</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>3</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>27</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>BL × OT</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Appearance of tunica albuginea (TA)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thin</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thickening</td>
<td>17</td>
<td>9</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Thick</td>
<td>3</td>
<td>25</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>BL × TA</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Ovarian type (OT)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smooth</td>
<td>11</td>
<td>20</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Wrinkled</td>
<td>18</td>
<td>15</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>BL × OT</td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

Type A: Ovaries with more than 200 follicles up to 5 mm in diameter, type B: ovaries with 50–200 follicles up to 10 mm in diameter, type C: ovaries with less than 50 follicles up to 10 mm in diameter.
have therefore rarely been a subject of investigations. Using 11 pairs of immature ovaries harvested from the Antarctic minke whales between 6.2 to 8.6 m in length, Lockyer [13] stated, “In very young females, the follicles are not macroscopically visible”. In the present study, 17 out of 24 pairs of the type A ovary were found in whales less than 6 m in length, and nearly 90% of ovaries harvested from immature whales more than 6 m were either of type B or C. Thus it is possible that the immature ovaries reported by Lockyer were the type C ovary. Laws [9] described morphology of ovaries obtained from two immature Antarctic fin whales 16.2 m and 16.8 m long as having “very many primary follicles in the superficial part of the cortex; they are already separated from the germinal epithelium by a tunica albuginea which is respectively about 150 $\mu$ and 250 $\mu$ in thickness”. Histological examinations revealed that most of these primary follicles were from 45 to 75 $\mu$m in diameter [9]. In the present study, the majority of follicles observed in the type A ovary were antral follicles around 1–2 mm in diameter and contained cumulus enclosed oocytes that could be matured and fertilized in vitro [15]. There is no description of follicles of this size in the above-mentioned immature fin whales [9].

The present observation indicates that the ovary of the immature minke whale experiences a burst of follicular development in the early prepubertal period. These small follicles, however, never exceed more than 5 mm in diameter and presumably undergo follicular atresia. Whether this follicular development occurs only once or several times during the prepubertal period is not clear. It is possible that this is a continuous process, rather than a temporal or periodic event, in which continuous follicular development up to 5 mm and atresia takes place. After this period of numerous follicular turn over, the immature ovary gradually moves into the next step of ovarian development in which the tunica albuginea thickens and follicular development proceeds up to 10 mm (type B and C). The type B and C ovaries are likely to be in the same stage of ovarian development but in different phases of follicular development. Although wave-like follicular development and regression, i.e. follicular wave, hasn’t been demonstrated in cetaceans, it is known to occur in prepubertal cattle [18, 19].

In the type B and C ovaries, the diameter of the largest follicles never exceeded more than 10 mm in diameter. In the present study, only approximate follicular diameter, observed from the ovarian surface, was measured. Since these follicles were mostly embedded in the ovarian body, the given values are likely to underestimate the true diameter [20]. Using serially sectioned formalin fixed ovaries, Lockyer [13] reported the mean diameter of the largest follicles in the immature Antarctic minke whales caught during the feeding season to be 6.41 mm. Thus it can be concluded that the follicular development beyond 10 mm is suppressed in immature minke whale during the feeding season. The diameters of the largest follicles in immature fin and sei whales during this period were also reported to be around 10 mm [8, 9, 10]. The follicular activity appears to be minimal during summer months in the Antarctic Ocean and increases as whales migrate to the lower latitude breeding area during the winter months [8–10].

It is almost certain that these follicular developments are regulated by gonadotropins and other endocrine, paracrine, autocrine factors as reported in other mammalian species. In cattle, a continuous infusion of a GnRH agonist was shown to suppress peripheral concentration of FSH to less than 0.6 ng/ml and eradicate LH pulses, resulting in a suppression of follicular development beyond 4 mm [21]. Likewise, more than 1.0 ng/ml FSH is necessary for follicles to grow beyond 2.5 mm in sheep [22]. The peripheral concentrations of FSH and LH in female immature minke whales caught during the feeding season (December to March) were reported to be 0.63 $\pm$ 0.09 and 0.70 $\pm$ 0.06 ng/ml respectively [23]. Although little is known about the gonadotropin requirement for follicular development in the baleen whale, these results indicate that a lack of gonadotropin support is, at least, one of the reasons limiting follicular development beyond 10 mm in immature Antarctic minke whales during the feeding season.

The occurrence of numerous small follicles in younger immature animals may be attributable to endocrine factors such as growth hormone (GH) and growth factors such as insulin like growth factors (IGF). The growth of the immature whales in their first year is phenomenal, being in excess of 3 m in body length and nearly 3 t in body weight [6]. In cattle, growth hormone has been shown to increase the number of small antral follicles without increasing the number of large antral...
follicles [24]. Gonadotropins may also play a supporting role in this process. It is known, in many mammalian species, that a transient increase in concentrations of circulating gonadotropins occurs in the early prepubertal period [19, 25]. A transient increase in follicular number was also reported in these animals [19].

Taken together, we propose that the ovarian features described in the present study reflect the endocrine environment of immature whales. During the early prepubertal period, small follicular development is enhanced by factors associated with rapid body growth such as GH and IGF. Gonadotropins may interact with these factors to accelerate follicular recruitment but their levels are not sufficient to support further follicular development beyond 5 mm. Once the quick growth phase is over, the rate of follicular recruitment decreases and follicular development comes under the control of gradually increasing gonadotropins. During the Antarctic feeding season, however, gonadotropin levels are suppressed and follicular development never exceeds beyond 10 mm.

As with the weight of the ovaries, the shape of the ovary varies between animals. In the present study ovaries were classified into two types, one with a flat smooth ovarian cortex and the other with a wrinkled ovarian cortex. Since the occurrence of both ovarian types was constant in any body size group, this morphological difference was not likely to be caused by a difference in age. The factor that determines the shape of the ovarian body is not clear. There might be a functional difference between the two types, e.g. number of follicles, for the wrinkled ovary is likely to have larger areas of ovarian surface and cortex than the smooth ovary. No attempt, however, was made to clarify this point in the present study.

In conclusion, the ovary of the Antarctic minke whale experiences a burst of small follicular development during the early prepubertal period before becoming a more developed ovary with fewer but larger follicles, and a thick tunica albuginea. The ovarian weight approximately doubles during the prepubertal period. The growth of ovaries appears to occur preferentially on the right side.

Acknowledgement

The authors thank Captain Tohyama and the crew of the research mother ship, Nisshin-maru, for providing the ovarian samples.

References


