Zygote **12** (May), pp. 125–132. © 2004 Cambridge University Press
DOI:10.1017/S0967199404002655 Printed in the United Kingdom

Relationship between the appearance of preantral follicles in the fetal ovary of Antarctic minke whales (*Balaenoptera bonaerensis*) and hormone concentrations in the fetal heart, umbilical cord and maternal blood

Yuki Muranishi[1], *Motoki Sasaki*[2], *Kengo Hayashi*[3], *Norihito Abe*[1], *Takuma Fujihira*[1], *Hajime Ishikawa*[4], *Seiji Ohsumi*[4], *Aiko Miyamoto*[3] *and Yutaka Fukui*[1]

Obihiro University of Agriculture and Veterinary Medicine, Obihiro, and Institute of Cetacean Research, Tokyo, Japan

Date submitted: 24.11.03. Date accepted: 12.01.04

Summary

The present study aimed to determine the relationship among changes in the number of preantral follicles and concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P_4), androstenedione (A) and estradiol-17β (E_2) in the fetal heart, umbilical cord and maternal blood. Primordial follicles had already appeared in a 20 cm fetus and primary follicles were observed in a 50 cm fetus. In a 70 cm fetus, the number of primordial and primary follicles increased rapidly and secondary follicles were present. The concentrations of LH and FSH did not change between 20 cm and 160 cm in fetal length. When the fetal length became > 70 cm, serum levels in the fetus, umbilical cord and mothers, and E_2 levels in umbilical cord increased synchronously ($p < 0.05$). These results showed increases in the number of preantral follicles in the Antarctic minke whale fetal ovary along with fetal growth during the early gestation period. These findings suggest that the change in preantral follicles was associated with changes in the concentration of steroids in early gestation periods. The changes in steroid concentrations in the fetal and umbilical cord blood and the increased number of preantral follicles were coincident at around 70 cm in fetal length, whereas the growth and differentiation of primordial and primary follicles appeared to be independent of FSH and LH.

Keywords: Gonadotropins, Minke whale, Preantral follicle, Steroids

Introduction

The Antarctic minke whale (*Balaenoptera bonaerensis*) is the smallest member of the family Balaenopteridae. Minke whales tend to migrate directly from low-latitude, Indian or South Pacific breeding areas during the autumn and winter months to the Antarctic feeding areas during the spring and summer months. The Antarctic minke whales are probably a short-day seasonal breeder without reproductive activity from early December to March in the Antarctic Ocean. Gestation and lactating periods are 10–11 months and 4–6 months, respectively. Fetal whales reach a body length of 2.6–3.0 m at birth. Body length at sexual maturity in both females and males is considered to be 6.5–8.5 m. Minke whales at low latitudes have a unique characteristic in that most of the adult female Antarctic minke whales have undergone oogenesis during gestation and lactation (Lockyer, 1984; Suzuki *et al*., 2001).

Thousands of small follicles are contained in mammalian ovaries (Erickson, 1966; Tanaka *et al*., 2001). These are called preantral follicles, and have

All correspondence to: Y. Fukui, Department of Food Production Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan. Tel: +81 155 495415. Fax: +81 155 495593. e-mail: fukui@obihiro.ac.jp
[1] Department of Food Production Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan.
[2] Department of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan.
[3] Department of Life Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan.
[4] The Institute of Cetacean Research, Tokyo 104-0055, Japan.

the immature shape and function of oocytes and follicles (Hyttel *et al*., 1999). Erickson (1966) reported that follicles are already present in the bovine fetus during the early stages of gestation. Rüsse (1983) reported that primordial, primary and secondary follicles appeared at day 90, day 140 and day 210 of gestation, respectively, in the bovine fetus. Follicular development is initiated and continues through the remaining fetal stage. Although there are numerous reports concerning bovine preantral follicles, almost nothing is known about them in whales. Although reports dealing with whale ovaries are numerous, there have been few histological studies (Marsh & Kasuya, 1984; Lockyer, 1987; Honma, 1994). Marsh & Kasuya (1984) provided a detailed account of ovarian changes with respect to age and reproductive activity in the short-finned pilot whale (*Globicephala macrorhynchus*). However, they did not discuss the histological characteristics of the fetal whale ovary. Information on the breeding physiology of whales is limited to date (Fukui *et al*., 1997; Asada *et al*., 2000). In particular, information on the regulation of fetal ovarian development is required to understand whale reproductive physiology. The possibility of utilizing small oocytes in primordial follicles for production of mature oocytes by *in vitro* growth culture system has been raised in mouse (Eppig, 1996; O'Brien *et al*., 2003), cow (Gutierrez *et al*., 2000; Miyano, 2003) and human (Roy & Treacy, 1993; Abir *et al*., 1997). A large number of preantral follicles in fetal ovaries could be a potential source of oocytes for *in vitro* fertilization or other reproductive technologies in whales, as well as other mammalian species.

Follicle-stimulating hormone (FSH) induces follicular development in the ovaries of adult mammals (Bao & Garverick, 1998). FSH stimulates the activity of aromatase, which converts androgens to estrogen in follicular cells (Bao *et al*., 1997). Aromatase activity has been found in fetal bovine ovaries at the early stage of pregnancy (Dominguez *et al*., 1988; Juarez-Orpeza *et al*., 1993). FSH stimulates the activity of aromatase and accelerates the production of estrogen. The proliferation of granulosa cells in the follicles is enhanced by an autocrine action of estrogen, which is stimulated by FSH. Estradiol production in fetal ovaries has also been shown in cattle (Shemesh *et al*., 1978; Shemesh, 1980). However, in the fetal whale there have been no reports on the relationship between the appearance of preantral follicles and serum concentrations of hormones in the fetal heart, umbilical cord and maternal blood.

In this study, the pattern of preantral follicle development in the fetal ovary of the Antarctic minke whale and changes in hormone concentrations in the fetus, umbilical cord and maternal blood were investigated. The concentrations of sex hormones (luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone ($P_{4)}$, androstenedione (A) and estradiol-17β($E_{2)}$)) relating to ovarian follicle growth were measured.

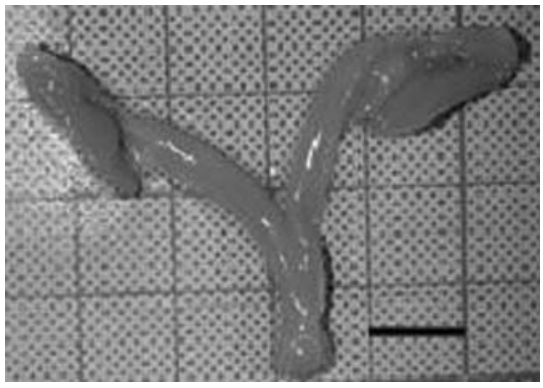

Figure 1 Uterus and ovary of an Antarctic minke whale fetus. Scale bar represents 1 cm.

Materials and methods

The present study was approved by the Animal Experimental Committee of Obihiro University of Agriculture and Veterinary Medicine, in accordance with the Guiding Principles for the Care and Use of Research Animals.

Animals

Antarctic minke whale ovaries were collected in the 2000/2001 and 2001/2002 Japanese Whale Research Program with Special Permit in the Antarctic (JARPA), organized by the Institute of Cetacean Research in Tokyo, Japan. The whales using for the present study were killed by explosive grenaded harpoons, which has been recognized by the Scientific Committee of the International Whaling Committee (IWC) as the most humane method to kill whales, and has been accepted by Schedule III (Capture) in the International Convention for the Regulations of Whaling (IWC Document 49-4, Report of the Scientific Committee, 1977). A large-caliber rifle was used when necessary as the secondary method. Special attention was given to all the sampled whales to reduce their time to death.

The fetuses (length 20–160 cm) and their mothers ($n = 48$) and umbilical cords ($n = 27$) were used after measurement of fetal length and weight. A representative reproductive tract (uterus and ovary) of a fetal minke whale is shown in Fig. 1. Fetal age was estimated from the fetal length according to the methods described by Huggett & Widdas (1951) and

https://doi.org/10.1017/S0967199404002655 Published online by Cambridge University Press

Frazer & Huggett (1974):

$$W = 0.059L^{2.676}$$

where W is fetal weight and L is fetal length. And

$$T_0 = W^{1/3}/0.243 + 74$$

where T_0 is estimated age.

Ovaries

The fetal ovaries were aseptically removed from the abdominal cavity, and separated from the surrounding connective tissue. The ovaries ($n = 12$) were immediately fixed in 10% formaldehyde (2000/2001) and Bouin's (2001/2002) fixative for 24 h and transferred to 70% alcohol. The fixed samples were processed routinely, embedded in paraffin and cut into serial sections of 7 μm in thickness and mounted on gelatin-coated slides (MUTO-GLASS, Japan). The slides were stained with haematoxylin and eosin, and examined under a microscope at ×200 (Nikon, Japan).

Classification of follicles

The diameter of preantral follicles ($n = 10$) was measured with an ocular micrometer in a section containing the nucleus of the oocyte. The follicles were classified according to previously established methods (Hulsof *et al*., 1994; Carambula *et al*., 1999; Tanaka *et al*., 2001) as follows: (1) primordial follicles: oocytes surrounded by germ epithelial cells (pre-granulosa cells); (2) primary follicles: oocytes surrounded by one layer of cuboidal or columnar granulosa cells; (3) secondary follicles: oocytes surrounded by more than one layer of cuboidal granulosa cells without differentiated theca cells.

Serum samples

The various hormone concentrations in the extracted fetal heart, umbilical cord and peripheral maternal body blood were measured by the double-antibody enzyme immunoassay (EIA) method. Blood was obtained using a 21 gauge needle connected to a plastic syringe, transferred to a 5 ml spit-tube, and centrifuged at 2000 *g* for 20 min at 4 °C. The supernatant was frozen and stored at − 30 °C until use. In the umbilical cord blood, distinction was not made between a vein and an artery.

Hormone measurements

The serum concentrations of the hormones in each sample of fetal heart blood, umbilical cord blood and peripheral maternal blood were determined in duplicate by EIA using 96-well ELISA plates (Corning Glass Works, Corning, NY). The serum levels of LH and FSH were also determined by EIA. The EIA for LH determination was based on the streptavidin–biotin technique as previously described by Mutayoba *et al*. (1990) The standard curve for LH ranged from 0.2 to 200 ng/ml and the ED_{50} of the assay was 10.5 ng/ml. The intra- and inter-assay CVs were 8.5% and 13.5%, respectively. FSH was measured by a modification of the method previously reported by Watanabe *et al*. (1997). The standard curve ranged from 0.02 to 40 ng/ml and the ED_{50} of the assay was 1.1 ng/ml. The intra- and inter-assay CVs were 13.8% and 16.7%, respectively.

Serum concentrations of steroids (E_2, A and P_4) were determined after diethylether extraction. The EIA for E_2 was carried out as described previously (Wijayagunawardane *et al*., 1998). The standard curve ranged from 2 to 2000 pg/ml, and the ED_{50} of the assay was 105 pg/ml. The intra- and inter-assay CVs were 6.5% and 7.3%, respectively. The EIA for A has been described previously (Acosta *et al*., 1998). The standard curve ranged from 2 to 1000 pg/ml, and the ED_{50} of the assay was 130 pg/ml. The intra- and inter-assay CVs were 6.8% and 8.1%, respectively. The EIA for P_4 was also performed as previously described (Miyamoto *et al*., 1992). The standard curve ranged from 20 to 20 000 pg/ml, and the ED_{50} of the assay was 850 pg/ml. The intra- and inter-assay CVs were 4.8% and 6.6%, respectively. The recovery rates of A, E_2 and P_4 were 81%, 75% and 92%, respectively.

Statistical analysis

All values are presented as the mean ± SEM. The quantitative data obtained from EIA (A, E_2, P_4, LH and FSH) were compared among the different groups using ANOVA followed by Student's *t*-test and Scheffe's test. A value of $p < 0.05$ was considered significant.

Results

Fetal length, weight and age

Figure 2 shows the fetal length and weight of the Antarctic minke whale fetuses. Figure 3 shows the fetal age estimated from fetal length by the equation given above. These whale fetuses were estimated from day 101 to day 222 of gestation. The fetal length and weight values increased with the fetal age.

Primordial and primary follicles

The number of preantral follicles in the fetal whale ovary is shown in Fig. 4. All the ovaries were examined using whale fetuses more than 23.6 cm in length. Preantral follicle diameters were divided into

https://doi.org/10.1017/S0967199404002655 Published online by Cambridge University Press

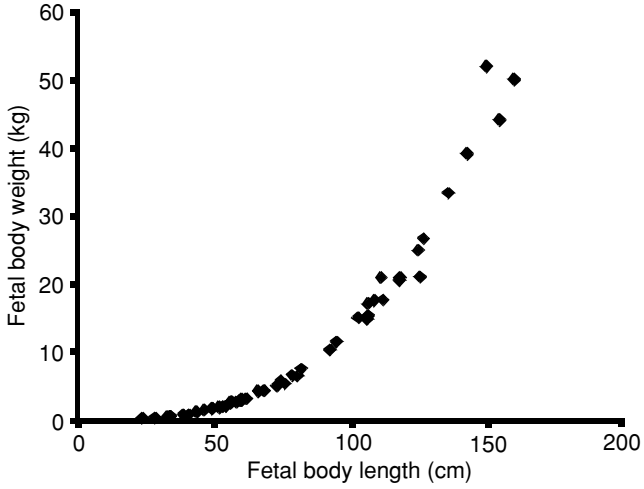

Figure 2 Fetal body length and body weight.

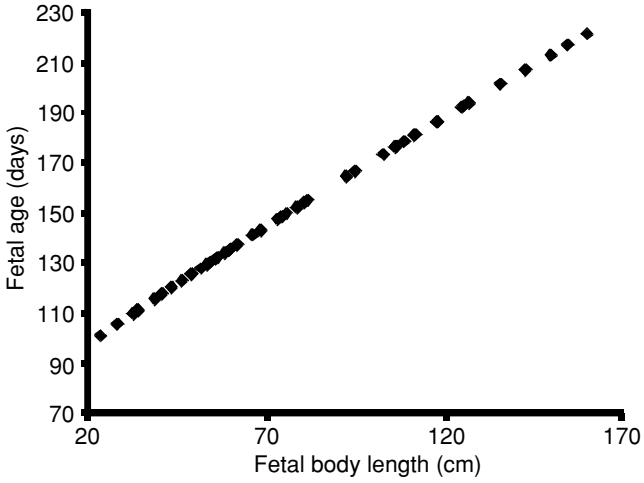

Figure 3 Estimated fetal age (gestation periods) from fetal body length.

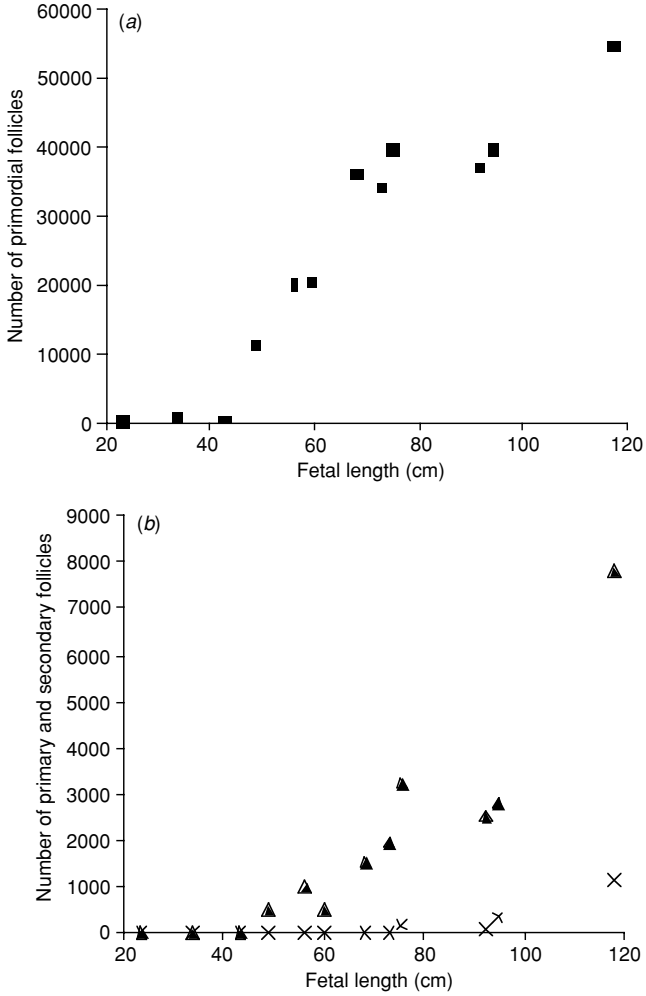

Figure 4 Number of preantral follicles in the whale fetal ovaries ($n = 12$). (*a*) Primordial (squares), (*b*) primary (triangles) and secondary (crosses) follicles.

three size classes (primordial follicles, 36.7 ± 0.4 μm; primary follicles, 58.8 ± 0.7 μm; secondary follicles, 76.8 ± 1.0 μm). Primordial follicles were observed only in the cortex layer of the ovary and were scattered (Fig. 5a). Primary follicles and secondary follicles were observed in the deep cortex layer (Fig. 5b, c). Primordial follicles appeared (primordial follicles, 30), and primary follicles were present in a 48.9 cm fetus (primordial follicles, 11 070; primary follicles, 490). Changes in the number of primordial follicles were observed in ovaries of different stage fetuses (fetal length 20–120 cm). In a 68.2 cm fetus, the number of preantral follicles increased rapidly (primordial follicles, 35 840; primary follicles, 1530). Secondary follicles were present in the 75.5 cm fetus (primordial follicles, 39 560; primary follicles, 3240; secondary follicles, 160). These preantral follicles increased with fetal age. Early antral follicles were not observed in the fetal ovaries examined in this study. Some multinuclear follicles were also observed in the fetal whale ovaries (Fig. 5d).

Hormone concentrations

Figure 6 shows the change in the serum concentration of LH, FSH, P_4, A and E_2 in the fetal heart, umbilical cord and maternal blood. Fetal length ranged from 20 cm to 160 cm and was divided into two groups (20–60 cm, 70–160 cm), because there appeared to be a change in hormone levels relating to the number of primordial follicles at about 70 cm, as shown in Fig. 4a.

The mean concentrations of LH and FSH did not change between 20 cm and 160 cm in fetal length. LH and FSH concentrations were maintained at high levels in the maternal blood ($p < 0.05$). The mean P_4 concentrations in the fetal and umbilical cord blood in the fetuses between 20 cm and 60 cm in length were 301.6 ± 57.9 pg/ml and 682.6 ± 153.7 pg/ml, respectively. The P_4 levels increased in fetuses between 70 cm

https://doi.org/10.1017/S0967199404002655 Published online by Cambridge University Press

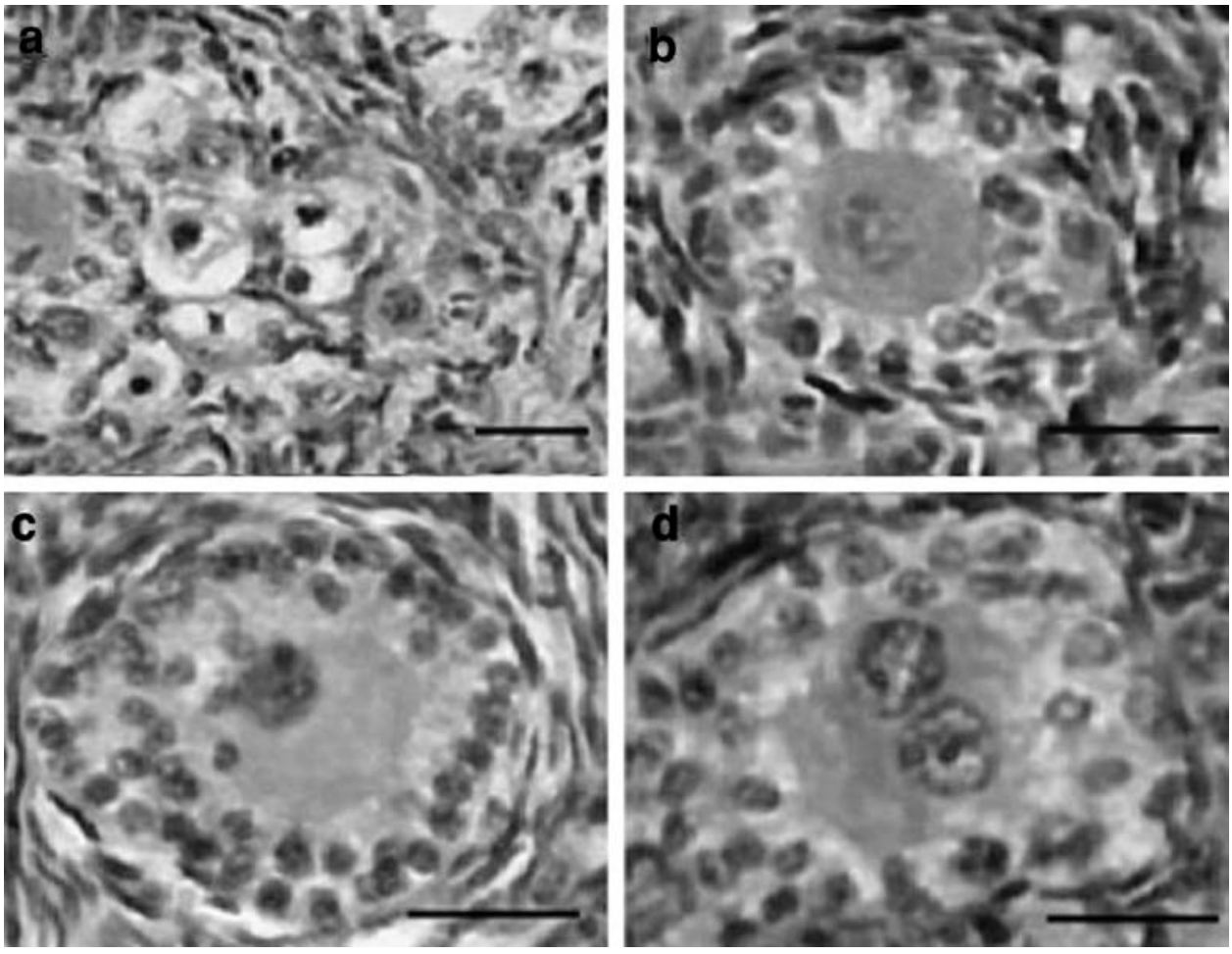

Figure 5 Representative primordial (*a*), primary (*b*), secondary (*c*) and multinuclear (*d*) follicles in the whale fetal ovaries. Scale bars represent: (*a*) 20 μm, (*b*, *c*, *d*) 40 μm.

and 160 cm in fetal length (1102.7 ± 159.47 pg/ml, 1769.9 ± 276.5 pg/ml, respectively) ($p < 0.05$). The maternal P_4 concentrations did not change during this period. In fetuses between 20 cm and 60 cm in length, the maternal P_4 level was higher than that in fetal blood ($p < 0.05$). The fetal, umbilical cord and maternal A concentrations in fetuses between 20 cm and 60 cm in length were significantly higher than those in the group of 70 cm and 160 cm fetuses ($p < 0.05$). In fetuses between 70 cm and 160 cm in length, the fetal level of A (36.2 ± 3.1 pg/ml) was higher than that in the maternal blood (23.6 ± 1.4 pg/ml) ($p < 0.05$). The mean fetal E_2 concentration (8.3 ± 1.2 pg/ml) in fetuses between 20 cm and 60 cm in length was significantly higher than that in the umbilical cord and maternal blood (3.2 ± 0.5 pg/ml and 0.5 ± 0.1 pg/ml, respectively) ($p < 0.05$). The E_2 levels in umbilical cord blood increased significantly in fetuses between 70 cm and 160 cm in length (5.8 ± 0.8 pg/ml) ($p < 0.05$). The maternal E_2 level was always lower than those in the fetal and umbilical cord blood ($p < 0.05$).

Discussion

This study is the first report on the relationship among the changes in the number of preantral follicles and concentrations of sex hormones in the Antarctic minke whale fetus. In the present study, whale fetal length and weight values increased with fetal age. Changes in the number of preantral follicles were observed in the early fetal ovary (fetal length 20–120 cm), and primordial and primary follicles significantly increased in fetuses of around 70 cm in length. The steroid levels in the fetal and umbilical cord blood changed when the fetus reached around 70 cm. Early gestation is a time of dramatic increase in the number of germ cells and preantral follicular development in the fetal ovary of domestic animals (Rüsse, 1983; Tanaka *et al*., 2001). In these fetal ovaries, Rüsse (1983) reported that the number of primordial follicles did not change between day 90 and day 150 of gestation, and primary follicles were first observed on day 140 of gestation. Since the fetal whales used in the present study were at an early gestational age, a number of primordial

https://doi.org/10.1017/S0967199404002655 Published online by Cambridge University Press

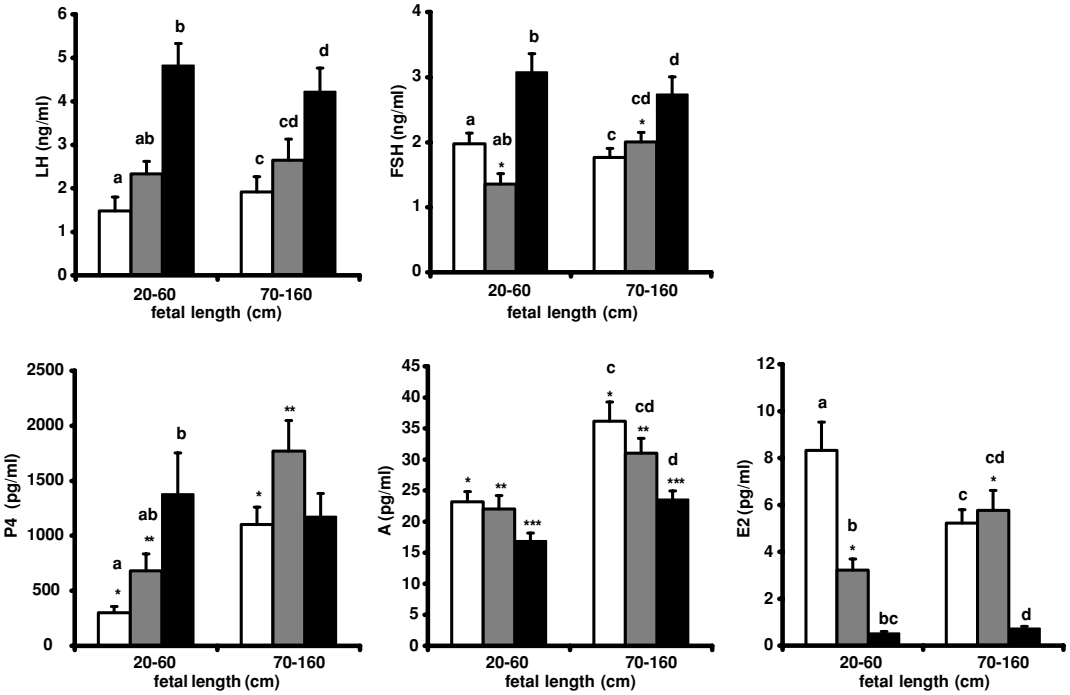

Figure 6 Changes in the serum concentrations of gonadotropins (FSH, LH) and steroid hormones (P_4, A, E_2) in the fetal heart (open columns), umbilical cord (grey columns) and maternal blood (black columns). Fetal length was divided into two groups (group I, 20–60 cm; group II, 70–160 cm). a,b and c,d: Values with the different superscripts are significantly different ($p < 0.05$). *, **, ***: Values with the same superscripts are significantly different ($p < 0.05$).

follicles were observed and the number of preantral follicles gradually increased during the observation period. As observed in this study, the occurrence of multinuclear follicles has also been reported in other mammals (Greenwald & Moor, 1989; McDougall *et al*., 1997; Lucci *et al*., 1999, 2002). Although these kinds of follicles appear to be normal in various mammalian species, in which they appear with variable frequency, their function and fate remain unknown.

In this study, an increase in diameter from primary follicles to secondary follicles was observed. Marsh & Kasuya (1984) reported that the mean diameter of primordial follicles was 58 μm in mature pilot whales. The mean diameter of primordial follicles (36.7 ± 0.4 μm) in the fetal ovary of minke whales was smaller than that in mature pilot whales. The first sign that primordial follicles and their corresponding oocytes have entered into the growth phase is the morphological change in the surrounding granulosa cells (Hafez & Hafez, 2000). The granulosa cells proliferate and change from a flattened shape in primordial follicles to a cuboidal shape in the primary follicles. Following the growth process, follicles increase in size through a series of mitotic divisions of the granulosa cells (Yamamoto *et al*., 1999), and unilaminar primary follicles are converted into multilaminar secondary follicles (Lundy *et al*., 1999; Hafez & Hafez, 2000). Since the increase in follicle diameter and the change in the morphology of granulosa cells from primordial to secondary follicle was observed, growth of preantral follicles took place during this period. *In vitro* development of oocytes from primordial follicles has been achieved in mice (Eppig, 1996; O'Brien *et al*., 2003) and cattle (Gutierrez *et al*., 2000; Miyano, 2003). In particular, O'Brien *et al*. (2003) obtained 59 living offspring from oocytes grown *in vitro* from the primordial follicles of newborn mice. These studies on *in vitro* growth of primordial follicles on their developmental competence, indicate the possibility of utilizing small oocytes in primordial follicles not only from adult or newborn animals, but also from fetal ovary of various species including whales, if the materials are available.

The concentrations of gonadotropins (FSH and LH) in the fetal, umbilical cord and maternal blood did not show clear changes during the study period. Also, the gonadotropin concentrations in the maternal blood were higher than in the fetal and umbilical cord blood. It was reported that LH was not able to cross the placental barrier in the sheep (Foster *et al*., 1972). Therefore, it is likely in the present study that the serum level of LH detected in the fetal blood was of fetal origin. FSH has been reported to promote follicular development in cultured bovine preantral follicles (Tanaka *et al*., 2001), and FSH may play a role in the early follicular development that occurs in the fetal

https://doi.org/10.1017/S0967199404002655 Published online by Cambridge University Press

bovine ovary (Fortune *et al*., 1999). However, our results and those of a previous study (Robert *et al*., 1988*a*) suggest that the development of preantral follicles is not associated with either gonadotropin.

In this study, the serum P_4 level was higher in the maternal blood than the fetal heart blood of fetuses between 20 cm and 60 cm in length. The serum levels of P_4 in the fetal and umbilical cord blood increased during the study period. The secretion of P_4 is essential to maintain gestation, and P_4 in the early gestation period is produced from the corpus luteum. Morita (2002) reported that the major source of progesterone during pregnancy in striped dolphins (*Stenella coeruleoalba*) is not only the corpus luteum but also the placental tissues at certain stages of gestation. In domestic animals, it was also reported that P_4 formed in the placenta is, in part, transported to the fetus (Robert *et al*., 1988*b*). Therefore, it is suggested that the origin of increased P_4 might have been from the placenta during the observation period in this study.

The serum levels of A increased throughout the study period in all samples. The serum A levels in the fetal heart and umbilical cord were significantly higher in fetuses of 70–160 cm than in the maternal blood. The main route for steroid production in the fetal bovine ovary appears to be the androstenedione–estrone–estradiol pathway (Dominguez *et al*., 1988). Androstenedione is mainly synthesized from P_4 through the $\Delta 4$ pathway after LH stimulation in fetal calf ovaries (Shemesh *et al*., 1978). As primordial follicles do not have differentiated theca cells, LH receptors may be absent in primordial follicles. The high serum level of A in the fetus suggested that steroid synthesis from P_4 to A increases in fetuses over 70 cm in length.

In this study, the serum E_2 level in the fetal heart was higher than that in the umbilical cord and maternal blood in fetuses between 20 cm and 60 cm in length. The serum E_2 level in the umbilical cord blood increased in fetuses between 70 cm and 160 cm in length. Frandsen & Stakemann (1961*a*, *b*) suggested the importance of estriol formation in domestic animal fetuses and steroid precursors reaching the placenta could be converted to E_2 by aromatase. Shemesh *et al*. (1978) reported that P_4 and E_2 concentrations began to increase in the fetal bovine ovary on day 30 and day 42, respectively. E_2 production in the early fetal ovaries has also been shown in cattle (Shemesh *et al*., 1978; Shemesh, 1980). Therefore, it is suggested that steroid synthesis has already started at the early gestation period in the whale fetal ovary. The increase in serum E_2 level in the umbilical cord blood may be of fetal and placenta origin.

In conclusion, increases in the number of preantral follicles and follicular size in the Antarctic minke whale fetal ovary were observed with fetal growth. The changes in steroid hormone concentrations in the fetal heart and umbilical cord and the increased number of preantral follicles were coincident at around 70 cm in fetal length. This showed that steroid synthesis in the fetus and placenta gradually increases during the period. In this study, the growth and differentiation of primordial and primary follicles appeared to be independent of FSH and LH.

Acknowledgements

The authors thank the Captain and the crew on the research base ship, *NISSHIN-MARU*, for sampling the fetuses and blood, and Dr K. Okuda, Okayama University, Japan, for providing progesterone antiserum.

References

Abir, R., Franks, S., Mobberley, M.A., Moore, P.A., Margara, R.A. & Winston, M. (1997). Mechanical isolation and *in vitro* growth of preantral and small antral follicles. *Fertil. Steril.* **68**, 682–8.

Acosta, T.J., Miyamoto, A., Ozawa, T., Wijayagunawardane, M.P. & Sato, K. (1998). Local release of steroid hormones, prostaglandin E_2, and endothelin-1 from bovine mature follicles *in vitro*: effects of luteinizing hormone, endothelin-1, and cytokines. *Biol. Reprod.* **59**, 437–43.

Asada, M., Horii, M., Mogoe, T., Fukui, Y., Ishikawa, H. & Ohsumi, S. (2000). *In vitro* maturation and ultrastructural observation of cryopreserved minke whale (*Balaenoptera acutorostrata*) follicular oocytes. *Biol. Reprod.* **62**, 253–9.

Bao, B. & Garverick, H.A. (1998). Expression of steroidogenic enzyme and gonadotropin receptor genes in bovine follicles during ovarian follicular wave. *J. Anim. Sci.* **76**, 1903–21.

Bao, B., Graverick, H.A., Smith, G.W., Salfen, B.E. & Youngquist, R.S. (1997). Changes in mRNA encoding LH receptor, cytochrome P450 side chain cleavage and aromatase are associated with recruitment and selection of bovine ovarian follicles. *Biol. Reprod.* **56**, 1156–68.

Carambula, S.F., Goncalves, P.B.D., Costa, L.F.S., Figueiredo, J.R., Wheeler, M.B., Neves, J.P. & Mondadori, R.G. (1999). Effect of fetal age and method of recovery on isolation of preantral follicles from bovine ovaries. *Theriogenology* **52**, 563–71.

Dominguez, M.M., Liptrap, R.M. & Basrur, P.K. (1988). Steroidogenesis in fetal bovine gonads. *Can. J. Vet. Res.* **52**, 401–6.

Eppig, J.J. (1996). Development *in vitro* of mouse oocytes from primordial follicles. *Biol. Reprod.* **54**, 197–207.

Erickson, B.H. (1966). Development and senescence of the postnatal bovine ovary. *J. Anim. Sci.* **25**, 800–5.

Fortune, H.E., Kito, S. & Byrd, D.D. (1999). Activation of primordial follicles *in vitro*. *J. Reprod. Fertil.* **54**, 439–48.

Foster, D.L., Karsch, F.J. & Nalbandov, A.V. (1972). Regulation of luteinizing hormone (LH) in the fetal and neonatal lamb. II Study of placental transfer of LH in the sheep. *Endocrinology* **90**, 253–7.

Frandsen, V.A. & Stakemann, G. (1961*a*). The site of production of oestrogenic hormones in human pregnancy: I. *Acta Endcrinol. (Copenh.)* **38**, 383–91.

Frandsen, V.A. & Stakemann, G. (1961*b*). The site of production of oestrogenic hormones in human pregnancy: II. *Acta Endcrinol. (Copenh.)* **43**, 184–94.

Frazer, J.F.D. & Huggett, A.S.G. (1974). Species variations in the foetal growth rates of eutherian mammals. *J. Zool.* **174**, 481–509.

Fukui, Y., Mogoe, T., Ishikawa, H. & Ohsumi, S. (1997). Factors affecting *in vitro* maturation of minke whale (*Balaenoptera acutorostrata*) follicular oocytes. *Biol. Reprod.* **56**, 523–8.

Greenwald, G.S. & Moor, R.M. (1989). Isolation and preliminary characterization of pig primordial follicles. *J. Reprod. Fertil.* **87**, 561–71.

Gutierrez, C.G., Ralph, J.H., Telfer, E.E., Wilmut, I. & Webb, R. (2000). Growth and antrum formation of bovine preantral follicles in long-term culture *in vitro*. *Biol. Reprod.* **62**, 1522–8.

Hafez, E.S.E. & Hafez, B. (2000). Folliculogenesis, egg maturation, and ovulation. In *Reproduction in Farm Animals* (ed. E.S.E. Hafez & B. Hafez), pp. 68–81. Philadelphia: Lea & Febiger.

Honma, Y. (1994). Histological studies on the ovaries of Beaked Whales, *Mesoplodon stejnegeri*, stranded on the Niigata Coast, Sea of Japan. *Reports of Sado Marine Biology Station, Niigata University* **24**, 1–10 (in Japanese).

Huggett, A.S.T. & Widdas, W.F. (1951). The relationship between mammalian foetal weight and conception age. *J. Physiol. (Lond.)* **114**, 306–17.

Hulsof, S.C., Figueiredo, J.R., Beckers, J.F. & Bevers, M.M. (1994). Isolation and characterization of preantral follicles from foetal bovine ovaries. *Vet. Q.* **16**, 78–80.

Hyttel, P., Fair, T., Avery, B., Callesen, H. & Grave, T. (1999). Transcriptional activity and ultrastructure in bovine oocyte. *Reprod. Domes. Anim.* **34**, 249–54.

Juarez-Oropeza, M.A., Alvarez-Fernandez, G., Lopez, V., Kawa, S. & Pedernera, E. (1993). Steroid metabolism in the cortex and the medulla of the early fetal bovine ovary. *J. Exp. Zool*. **266**, 102–7.

Lockyer, C. (1984). Review of Baleen Whale (*Mystiveti*) reproduction and implications for management. *Rep. Int. Whaling Comm., Special Issue* **6**, 27–50.

Lockyer, C. (1987). Observations on the ovary of the southern minke whale. *Sci. Rep. Whales Res. Inst., Tokyo* **38**, 75–89.

Lucci, C.M., Amorim, C.A., Rodrigues, A.P., Figueiredo, J.R., Bao, S.N., Silva, J.R. & Goncalves, P.B. (1999). Study of preantral follicle population *in situ* and after mechanical isolation from caprine ovaries at different reproductive stages. *Anim. Reprod. Sci.* **16**, 223–36.

Lucci, C.M., Rumpf, R., Figueiredo, J.R. & Bao, S.N. (2002). Zebu (*Bos indicus*) ovarian preantral follicles: morphological characterization and development of an efficient isolation method. *Theriogenology* **57**, 1467–83.

Lundy, T., Smith, P., O'Connell, A., Hudson, N.L. & McNatty, K.P. (1999). Populations of granulosa cells in small follicles of the sheep ovary. *J. Reprod. Fertil.* **115**, 251–62.

Marsh, H. & Kasuya, T. (1984). Changes in the ovaries of the short-finned pilot whale, *Globicephala macrorhynchus*, with age and reproductive activity. *Rep. Int. Whaling Comm., Special Issue* **6**, 311–35.

McDougall, K., Hay, M.A., Goodrowe, K.L., Gartley, C.J. & King, W.A. (1997). Changes in the number of follicles and of oocytes in ovaries of prepubertal, peripubertal and mature bitches. *J. Reprod. Fertil., Suppl.* **51**, 25–31.

Miyamoto, A., Okuda, K., Schweigert, F. & Schams, D. (1992). Effects of basic fibroblast growth factor, transforming growth factor on the secretory function of the bovine corpus luteum *in vitro*. *J. Endocr.* **135**, 103–14.

Miyano, T. (2003). Bringing up small oocytes to eggs in pig and cows. *Theriogenology* **59**, 61–72.

Morita, Y. (2002). Sex hormone concentrations in corpus luteum and placental tissue of whales; cetaceans, pinnipeds and sirenians. *IZANA* **36**, 89–92 (in Japanese).

Mutayoba, B., Meyer, H., Schams, D. & Schallenberger, E. (1990). Development of a sensitive enzyme immunoassay for LH determination in bovine plasma using the streptavidin–biotin technique. *Acta Endocrinol. (Copenh.)* **122**, 227–32.

O'Brien, M.J., Pendola, J.K. & Eppig, J.J. (2003). A revised protocol for *in vitro* development of mouse oocytes from primordial follicles dramatically improves their developmental competence. *Biol. Reprod*. **6**8, 1682–6.

Robert, E., Gore-Langton, R.E. & Armstrong, D.T. (1988*a*). Follicular steroidogenesis and its control. In *The Physiology of Reproduction* (ed. E. Knobil & J. Neill), pp. 331–85. New York: Raven Press.

Robert, E., Gore-Langton, R.E. & Armstrong, D.T. (1988*b*). The placenta as an endocrine steroid. In *The Physiology of Reproduction* (ed. E. Knobil & J. Neill), pp. 2085–91. New York: Raven Press.

Roy, S.K. & Treacy, B.J. (1993). Isolation and long-term culture of human preantral follicles. *Fertil. Steril.* **59**, 783–90.

Rüsse, I. (1983). Oogenesis in cattle and sheep. *Bibl. Anat.* **24**, 77–92.

Shemesh, M. (1980). Estradiol-17β biosynthesis by the early bovine fetal ovary during the active and refractory phases. *Biol. Reprod.* **23**, 577–82.

Shemesh, M., Ailenberg, M., Ayalon, N. & Hansel, W. (1978). Hormone secretion by cultured bovine pre- and postimplantation gonads. *Biol. Reprod.* **19**, 761–7.

Suzuki, T., Mogoe, T., Asada, M., Miyamoto, A., Tetsuka, M., Ishikawa, H., Ohsumi, S. & Fukui, Y. (2001). Plasma and pituitary concentrations of gonadotropins (FSH and LH) in minke whales (*Balaenoptera acutorostrata*) during the feeding season. *Theriogenology* **55**, 1127–41.

Tanaka, Y., Nakada, K., Moriyoshi, M. & Sawamukai, Y. (2001). Appearance and number of follicles and change in the concentration of serum FSH in female bovine fetuses. *Reproduction* **121**, 777–82.

Watanabe, H., Miyamoto, A. & Fukui, Y. (1997). A competitive enzyme immunoassay for follicle-stimulating hormone in ovine plasma using biotin-streptavidin amplification. *Reprod. Fertil. Dev.* **9**, 597–601.

Wijayagunawardane, M., Miyamoto, A., Cerbito, W., Acosta, T.J., Takagi, M. & Sato, K. (1998). Local distributions of oviductal estradiol, progesterone, prostaglandins, oxytocin and endothelin-1 in the cyclic cow. *Theriogenology* **49**, 607–18.

Yamamoto, K., Otoi, T., Koyama, N., Horikita, N., Tachikawa, S. & Miyano, T. (1999). Development to live young from bovine small oocytes after growth, maturation and fertilization *in vitro*. *Theriogenology* **52**, 81–9.