



Embryo and fetal position during pregnancy by ultrasonographic examinations in bottlenose dolphin (*Tursiops truncatus*)

Fumio TERASAWA^{1)*}, Hiroshi AKIYAMA¹⁾, Toru SAKURAGI¹⁾, Shuto HANEDA¹⁾ and Chika SHIRAKATA¹⁾

¹⁾Enoshima Aquarium, 2-19-1 Katase-kaigan, Fujisawa, Kanagawa 251-0035, Japan

ABSTRACT. From 2012 to 2017, serial ultrasonographic evaluation of 5 healthy bottlenose dolphins, *Tursiops truncatus*, were performed over the course of 6 pregnancies in Enoshima Aquarium. A total of 98 ultrasonographic examinations were included in the study. In three out of six cases, two embryos were observed between umbilicus and genital slit, and side of umbilicus in the dam's body. All embryos were located in right below the peritoneum and observed from 308 to 325 days *pre-partum*. These days were corresponding to from 58 to 61 days after copulation respectively. The diameter of the embryo sac was approximately 4 cm. In three cases, the fetal head was located in the dam's left lateral between umbilicus and genital slit from approximately 90 days *pre-partum* to the parturition. A snout of fetus is at the top of the uterine horn, and the tail lies close to the cervix. All six calves were fluke-first births (breech presentation). It was conjectured that the contraction of the dam's uterus during parturition forced the fetus to invert, and the fetal tail fluke was expelled from the dam's body. In three cases, judging from orientation of fetal tail fluke faced towards left side of the dam, a fetal position might be RSL (Right Sacrum-Lateral) within the birth canal. In the other three cases, the left and right positions of the fetus and the dam's body are reversed during pregnancy and parturition.

KEY WORDS: embryo, fetal position, parturition, pregnancy, ultrasound

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The use of ultrasonography for monitoring bottlenose dolphin pregnancy started probably around the late 1980s [4, 19]. In Japan, ultrasonography has been used to diagnose early pregnancy of cetaceans from the 1990s. In Enoshima Aquarium, the first record of ultrasonographic examinations on a pregnant cetacean was for a finless porpoise, *Neophocaena asiaeorientalis*, at a drained pool in 1992.

Ultrasonography is a valuable non-invasive diagnostic tool on small cetaceans in general, especially pregnancy in bottlenose dolphins is best diagnosed with ultrasonography [7]. Ultrasonography has been used for early pregnancy diagnosis in dolphins, and in most cases measures ovary [3, 12], the size of follicles before ovulation [13], size of embryo [16] and fetus, fetal heart beat and movement [16, 21], as well as the diameter of the fetal head and thorax [9, 10, 21].

In cetaceans, few of the fetomaternal anatomical structures during pregnancy by ultrasonography has been reported [9]. Furthermore, there are few manuscripts which describes how a fetal position will be within the birth canal at parturition [16]. Additionally, there are few papers that comparing the breech position (fluke-first) and cephalic position (head-first) at the parturition [6], however, none described the orientation of fetal tail flukes at the breech position.

The present study aims not only to investigate where embryo and fetus located in dam's body during pregnancy by ultrasonography and photograph taken simultaneously a probe position, but also to estimate how fetal position would be within the birth canal from the orientation of fetal tail fluke which will be emerged towards dam's body at a parturition.

MATERIALS AND METHODS

Animal

Dams age ranged from 11 to estimated 30 years old at parturition. They were considered to be in good health based on routine rectal body temperature, blood data, ultrasonography and absence of clinical signs throughout pregnancy. Every dam had single neonate. Two male and 4 female neonates were born (Table 1). Case 2 was stillborn, however, all six neonates were fluke-first births (breech presentation).

*Correspondence to: Terasawa, F.: terasawa@enosui.com

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Table 1. Embryo and fetal position during pregnancy and parturition

Case	Stud book number		Date of birth	Pregnancy		Parturition
	Dam	Neonate		Embryo position where located in dam's body (CE ^a /EP ^b)	Fetal head position in dam's body within 90 days <i>pre-partum</i>	Ventral surface of fetal tail flukes face towards dam's body
1	525	1,263 F ^f	2012/6/1	Between umbilicus and GS ^c (61/325)	Left lateral abdomen	Left
2 ^e	812	1,309 F	2013/7/24	ND ^d	Left lateral abdomen	Left
3	602	1,310 F	2013/7/30	ND	Left lateral abdomen	Left
4	254	1,343 F	2014/9/29	ND	Right lateral abdomen	Right
5	600	1,358 M ^g	2015/4/12	Between umbilicus and GS (58/318)	Right lateral abdomen	Right
6	600	1,397 M	2017/5/7	Side of umbilicus (58/308)	Right lateral abdomen	Right

a) Number of days between copulation and embryo observed, b) embryo observed and parturition, c) genital slit, d) no data, e) stillborn, however the fetus was alive 2 days *pre-partum*, f) female, g) male.

Equipment

Ultrasonographic examinations were performed with a SonoSite MicroMAXX (Sonosite, Bothell, WA, USA) and C60e/5–2 MHz transducer. A frequency of 3.5 MHz was used.

Ultrasonographic examinations

From 2011 to 2017, ultrasonographic examinations by husbandry behaviour has been performed weekly to every two weeks for six pregnancies in five bottlenose dolphins (Table 1). Subject dolphins were trained to present and maintain ventral as well as lateral abdomen at the surface of water. During ultrasonographic examinations, dolphin was held by two trainers and performed of ultrasonographic examinations by one veterinarian. In one ultrasonographic examinations of Case 1, dolphin was landed on stage and scanned (Fig. 1). Additionally, a photograph was taken simultaneously at the time of ultrasonographic examinations in order to investigate where location of fetus in the dam's body. During pregnancy, ovaries and corpus luteum were not examined in all cases.

RESULTS

A total of 98 ultrasonographic examinations were included in the study (Figs. 2, 3). As the results of the present study, it was able to determine the approximate position of embryo between 308 and 325 days *pre-partum*, and the position of the fetal head from approximate 90 days *pre-partum* to parturition in a dam's body. In the other periods, the fetal position in uterus fluctuated so much that the regularity of its position could not be determined.

Embryo position

In three out of six cases, two embryos were observed at between umbilicus and genital slit in Case 1 (Figs. 1B, 2A) and 5 (Figs. 1D, 3B), and one was observed side of umbilicus in Case 6 (Figs. 1F, 3C) where all of them were almost on the center line of dam's abdomen. All three embryos were located at right below the peritoneum (Fig. 1A, 1C, 1E) and observed from 308 to 325 days *pre-partum* (Table 1). These days were corresponding to from 58 to 61 days after copulation. The diameter of the embryo sac was approximately 4 cm. The embryos were assessed in B-mode (Fig. 1).

Fetal position

In three cases, from 96 days *pre-partum* to 4 days *pre-partum*, the fetal head was located at the dam's left lateral abdomen from umbilicus to genital slit (Fig. 2), and this position did not change. A snout of fetus was located at the top of the uterine horn, and the tail lied close to the cervix. In the other three cases, from 86 days *pre-partum* to 5 days *pre-partum*, the fetal head was located in the dam's right lateral abdomen from umbilicus to genital slit (Fig. 3). The distance between the dam's body of surface scanned by a probe and fetal head skin was within 5 cm after approximate 90 days *pre-partum*. Case 2 was a stillborn and the range of the fetal movement was narrowest during pregnancy compared to the other five cases (Figs. 2, 3).

Orientation of fetal tail flukes at parturition

In three cases (Table 1) which the fetal heads were located in the dam's left lateral abdomen within 90 days *pre-partum* (Fig. 2), and ventral surface of fetal tail flukes emerged towards left of dam's body at parturition (Fig. 4A). In the other three cases (Table 1) which the fetal heads were located in the dam's right lateral abdomen within 90 days *pre-partum* (Fig. 3), and ventral surface of fetal tail flukes emerged towards right of dam's body at parturition (Fig. 4B).

As the results of the present study, illustration of fetal position after approximate 90 days *pre-partum* is shown in Fig. 5A. Case 1, 2 and 3 corresponds to this illustration. In Case 4, 5 and 6, the left and right positions of the dam's body and fetus are reversed.

DISCUSSION

The implantation of the fertilized egg and the development of the placenta take place in the endometrium of the uterine horns

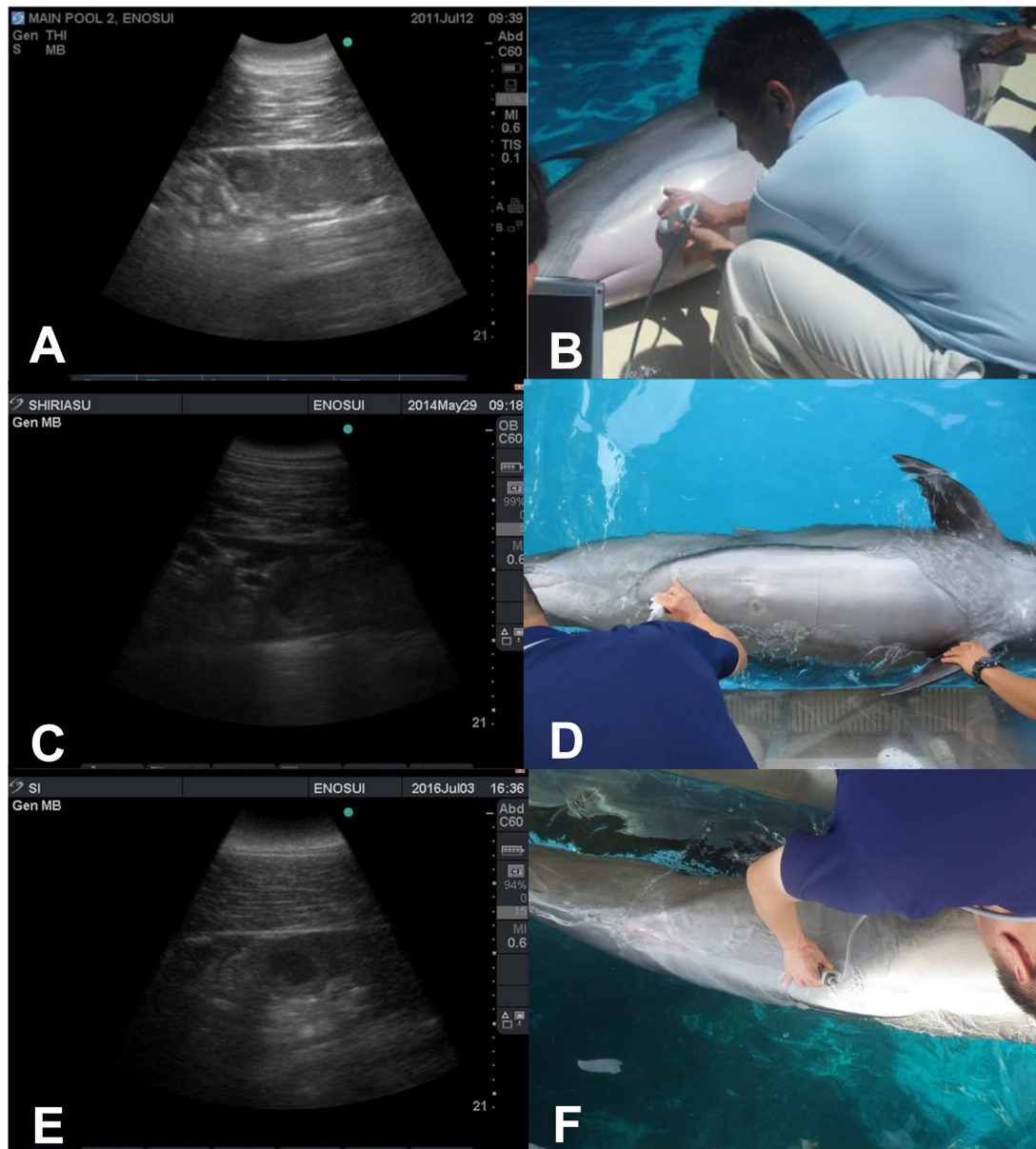


Fig. 1. Ultrasound images of an embryo and probe position for a longitudinal scan in Case 1 (A, B), 5 (C, D) and 6 (E, F). Embryo was observed between umbilicus and genital slit in Case 1 and 5, side of umbilicus in Case 6. All probe mark (Green Circle) on ultrasound images faced towards the dam's tail fluke.

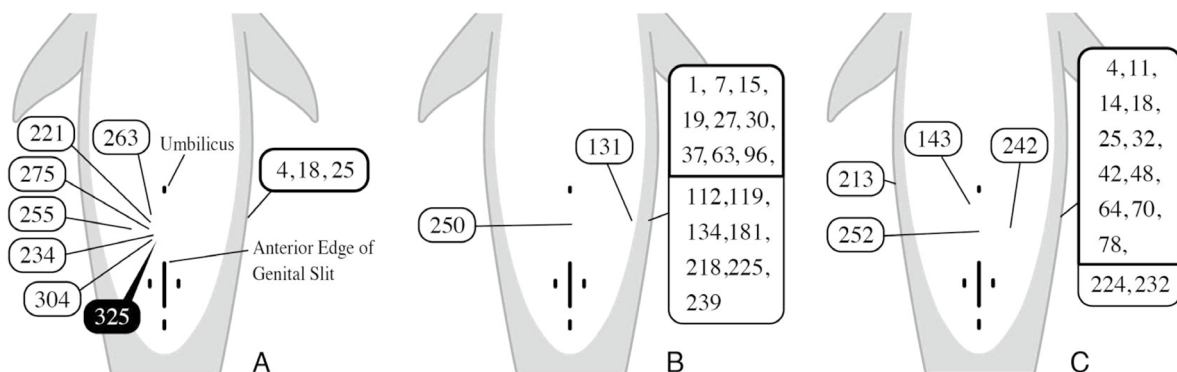


Fig. 2. Probe position at ultrasonographic examination when observed the fetal head. Numbers represents days to parturition in Case 1 (A), 2 (B) and 3 (C). Embryo was observed at 325 days *pre-partum* (61 days from copulation) in Case 1.

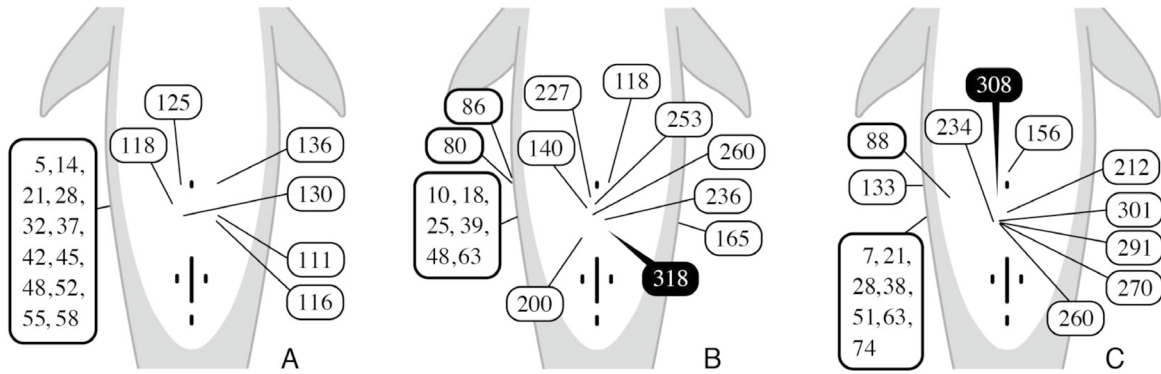


Fig. 3. Probe position at ultrasonographic examination when observed the fetal head. Numbers represents days to parturition in Case 4 (A), 5 (B) and 6 (C). Embryos were observed at 318 days *pre-partum* (58 days from copulation) in Case 5, and at 308 days *pre-partum* (58 days from copulation) in Case 6.



Fig. 4. At the parturition, ventral surface of fetal tail fluke faced towards left side of the dam's body in Case 3 (A), or right side in Case 6 (B).

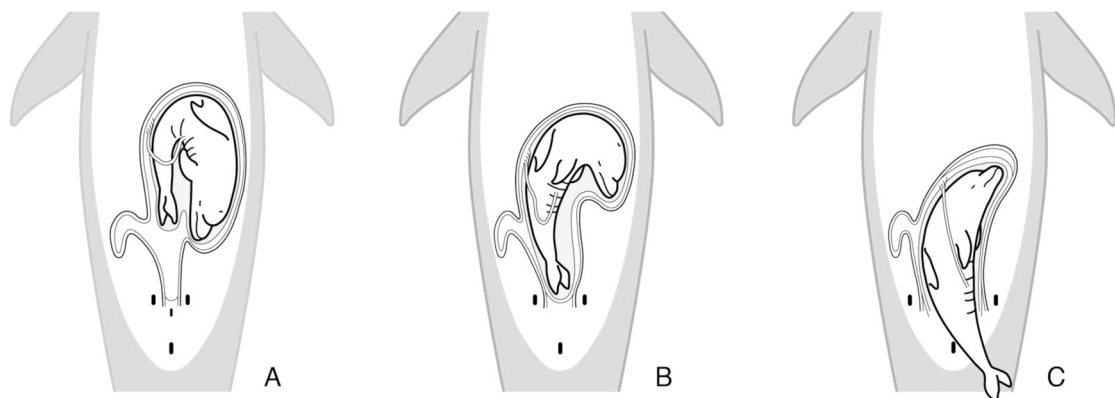


Fig. 5. Illustrations of the fetomaternal anatomical structures. A: A fetal position from ultrasonography imagine of 90 days *pre-partum* to the parturition in Case 1, 2 and 3. B: A fetal movement at the parturition. C: A fetal position might be RSL (Right Sacrum Lateral) within the birth canal. We used the terms referred to human classification [1, 8]. Dolphins do not have a sacrum, therefore, lumbar spine was used as a landmark.

[14]. In the case of non-pregnant dolphin, uterine horns are located between umbilicus and genital slit, and lies close to the peritoneum [5, 15]. In the present study, embryos were at similar positions to these uterine horns, between umbilicus and genital slit, and side of umbilicus where were almost on the center line of dam's abdomen and were located in right below the peritoneum. The uterus itself contains of a short corpus dividing into the two uterine horns, which run parallel for a short part of their length,

and then bend respectively to the right and the left, curving first upwards and then downwards, to continue as the oviducts [17]. Therefore, it was not determined whether the uterine horn was on the left or the right side judging from the embryo position.

Earlier detection of uterine fluid and fetal membranes with ultrasonography is possible on bottlenose dolphin as early as day 30 after conception but usually requires 50 to 60 days for confirmation [7]. At 52 ± 3 days post-ovulation, the embryo was perfectly recognizable [16]. Also a fetus can be identified by the fourth week of gestation [20]. Timing of pregnancy diagnosis by ultrasonography is 50 to 60 days after post-breeding or AI [13]. In the present study, pregnancy could not be diagnosed by around 30 days after copulation. However, three embryos were observed from 308 to 325 days *pre-partum*. These days were corresponding to from 58 to 61 days after copulation. The present results were similar to these data [7, 13, 16].

In all cetacea the broad ligament of the uterus is attached to the lateral side of the corpus uteri and to the inner curvature of the uterine horns and tubae; it continues into the mesovarium. The tips of the horns of the nonpregnant uterus curves in the caudal direction. Both the tip and the base of the uterine horn are fixed to the abdominal wall at a point not far cranial of the pelvic bone [18]. Therefore, range of movement of the uterus will be limited by these ligamenta. In the present study, it was judged that the left horn of the uterus would be observed on the left lateral abdomen of the dam. In most of dolphin pregnancies, the fetus is located in the left horn and the right one contains only the tail and part of the allantois [5]. As the results of the present study, three out of six fetuses would be located in the left uterine horn and remaining three fetuses in the right uterine horn.

It is known that a cetacean fetus can change its position in the uterus during pregnancy, but usually moves into a tail-first position by the last months [2, 11]. From investigations of pregnant cetaceans it has, however, appeared that in some the final position is taken up very shortly before birth, and that some fetuses face the cervix during most of the periods of pregnancy. Possibly, the situation changes quite a few times during pregnancy [17]. As the results of the present study, we confirmed fetus would reposition easily in the uterus until 90 days *pre-partum*, however, fetal position would not change from 90 days *pre-partum* to parturition (Fig. 5A).

In the present study, snout of fetus is always at the top of uterine horn, while tail lies close to the cervix after 90 days *pre-partum*. Its position was similar to the illustrations of fetomaternal anatomical structure in third trimester [9] and the detailed drawing by Wislocki and Enders [22], however, the present fetal position (Fig. 5A) is similar to the detailed drawing [22], which is closer to dam's body wall than the illustrations [9].

In humans, the terms used for breech positions are the same as for cephalic positions, except the sacrum of the fetus is used as the identifying landmark, instead of the occiput [8]. In the present study, since dolphins do not have a sacrum [15], lumbar spine is used as a landmark, and we referred to a human classification [1, 8]. In Case 1, 2 and 3 of the present study, it was suspected that the contraction of the dam's uterus during parturition forced the fetus to invert (Fig. 5B), the fetus position might be RSL (Right Sacrum Lateral) within the birth canal (Fig. 5C) and fetal tail flukes might be expelled from the dam's body (Fig. 4A).

Cardiac flicker signified heart movement in an embryo too small to measure rate [9]. In the present study, dam was floating on the water during ultrasonographic examinations and therefore M-mode was not able to calculate the heart-beat of an embryo due to the baseline of ultrasound images could not be stabilized.

In conclusion, the fetal position was on either the left or the right side of dam's body after 90 days *pre-partum*, and it was in the same orientation as ventral surface of fetal tail flukes towards dam's body at parturition. From these points, in bottlenose dolphin, it was suggested that there might be regularity in the series of the fetal movements and uterine contraction during normal parturition.

POTENTIAL CONFLICT OF INTEREST. To the best of our knowledge, the named authors have no conflict of interest, financial or otherwise.

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