飼育下の雌ベルーガ（Delphinaperus leucas）における卵巣周期検出のための基礎体温測定の有用性

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Basal Body Temperature Method for Detecting Ovarian Cycle in the Captive Beluga (Delphinapterus leucas)

Etsuko KATSUMATA¹, Chie FURUTA²,³, Hiroshi KATSUMATA¹, Gen WATANABE²,³ and Kazuyoshi TAYA²,³

¹) International Marine Biological Research Institute, Kamogawa Sea World, Chiba 296-0041, ²) Department of Basic Veterinary Science, The United Graduate School of Veterinary Sciences, Gifu University, Gifu 501-1193 and ³) Laboratory of Veterinary Physiology, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan

Abstract. The relationship between basal body temperature and circulating progesterone levels were investigated in a female beluga. Body temperature and serum concentrations of progesterone were measured daily and at 2-4 week intervals respectively, in a female beluga that was in captivity for 7 years between 1996 and 2003. The beluga first ovulated in April, 2000 (13 years old). Thereafter, serum concentrations of progesterone showed cyclic changes, indicating that the ovulatory cycle had started. Serum concentrations of progesterone ranged from 0.1 ng/ml to 15.7 ng/ml. Body temperature also showed cyclic changes during the estrous cycle. Body temperature ranged from 34.9 to 35.9 °C, and tended to reach the peak during the high progesterone phase. Mating behavior was observed during the low body temperature phase. The changes in body temperature positively correlated with the circulating progesterone levels. The length of the estrous cycle was 36.7 ± 3.9 (mean ± SEM) days based on the intervals between the days of mating behavior. This is the first report demonstrating that body temperature clearly changes during the estrous cycle in a captive female beluga. The present finding suggests that measurement of body temperature is a useful method for detecting the ovarian cycle of the beluga in captivity.

Key words: Beluga, Body temperature, Estrous cycle, Marine animals, Progesterone

The beluga, Delphinapterus leucas, is a medium-sized toothed whale that derives its name from the Russian word belukha, which means white. The beluga belongs to one of two monotypic genera of the family Monodontidae [1], which includes the narwhal, Monodon monoceros, as the other member. These two species lack dorsal fins, a common characteristic thought to be an adaptation to life in the Arctic waters.

To date, limited data on the reproduction of beluga has been available, such as the fact that the pregnancy period is around 14 months [2] and that the breeding season is generally in May, but is flexible for a few months [3, 4]. Developing controlled breeding programs for beluga depends on a thorough knowledge of reproductive physiology, including data concerning the estrous cycle, pregnancy, and parturition. Three belugas have been kept in captivity at Kamogawa Sea World (Chiba, Japan) since 1990. The present study was designed to examine the relationship between body temperature and serum concentrations of progesterone during the estrous cycle of a captive...

Materials and Methods

Animals

A female beluga (Fig. 1, Masha), wild-caught in the Sea of Okhotsk was used in the present study. She was brought to Kamogawa Sea World (Chiba, Japan) from Russia at October 1990. At that time, her body length and weight were 280 cm and 418 kg and her body color was gray, which is the characteristic of a young beluga. Based on her body length and weight, her age was estimated to be 3 years (born on 1987) [4].

Facilities, breeding environment, and cohabitating animals

The animal was housed together with 2 male belugas, one of which was caught and brought to Kamogawa Sea World from the Churchill River of the Hudson Bay, at 1988 by the authority of the Canadian government, and the other of which was captured at the same time as Masha in 1990 and brought in with her. The breeding environment of the animals consists of 2 indoor pools, one exhibiting pool (water volume; 603 m³, depth 3.5 m) and one back up pool (water volume; 230 m³, depth 3 m), with the pools connected by water gates. The pools are maintained by natural saltwater systems with regular chlorination, and the water temperature was kept at 17.0 ± 0.5 C throughout the year. Lighting was controlled in a short-day photoperiod (10 h: light, 14 h: dark) from 1990 to 2000, and the photoperiod was maintained the same as natural daylight from 2000 to 2003.

Body temperature, blood sampling, and measurement of progesterone levels

The animal had been raised to rest on its abdomen on the water surface and to present its flukes without any retention. Measurement of body temperature and blood sampling were performed from this position (Fig. 2). Body temperature was measured with a Terumo Finer CTM-303 (Terumo Japan) by inserting a PK-K041 heat-sensitive probe (heat-sensitive probe temperature range: 0 to 50 C, Terumo, Tokyo, Japan) into the rectum via the anus. Body temperature was measured daily at 0800 h before any active exercises. Blood samples were collected from the fluke blood vessel every 2 or 4 weeks from February 1996 to November 2003, and placed in a vacuum blood sampling tube (Venoject II Autosep, Terumo, Tokyo, Japan). After centrifugation at 1700 g for 5 min at room temperature, the serum obtained was kept at −20 C until assayed for progesterone. The serum progesterone level was measured by the no-extraction tube stationary phase method (COAT-COUNT®) using a DPC Progesterone Kit (Daiichi Pure Chemical Co., Ltd. Tokyo, Japan). The intra- and inter-assay
coefficients of variation were 4.2% and 6.8%, respectively. The lower limits of the assay sensitivity were 0.1 ng/ml.

Statistical analysis
The data are presented as means ± SEM. The significance of the body temperature and concentrations of progesterone was analyzed by one-way ANOVA. The significance of differences between means was determined by Student’s t-test. Canonical correlation analysis was performed to measure the correlation between body temperatures and serum progesterone levels, and statistical significance was obtained using Wilks’ lambda test. A probability value (P) of less than 0.05 was considered significant. All statistical analyses were performed using the SAS computer package [5].

Results
A clear increase in the serum concentrations of progesterone was first observed in April of 2000 when the beluga became 13 years old. Body length and weight were 382 cm and 635 kg, respectively. This increase in serum concentrations of progesterone indicated that the first ovulation had occurred in the female beluga. After the first increase in serum concentrations of progesterone, the female beluga showed a cyclic increase in circulating progesterone 2 to 7 times each year for the next 4 years. The serum concentrations of progesterone ranged between 0.1 to 15.7 ng/ml.

Body temperature clearly changed during the estrous cycle. The cyclic changes of body temperature in 2002 are shown in Fig. 3. Body temperature ranged from 34.9 to 35.9 °C with a total of 7 ovarian cycles in 2002 (Fig. 3). The high body temperature phase corresponded to the phase of high serum concentrations of progesterone, and the low body temperature was observed during the phase of low concentrations of progesterone. The female beluga was mated with a male beluga during the low body temperature phase (35.35 ± 0.035 °C, n=15), and the body temperature rose to 35.6 °C, 11.0 ± 1.5 days (n=7, 2SD of low temperature) after mating. There was a significant difference between the body temperature observed on the day of mating behavior and the highest body temperature during the high progesterone phase (35.35 ± 0.035 °C, n=15 vs 35.86 ± 0.02 °C, n=7).
Seven ovarian cycles appeared to have occurred in the beluga in 2002, as determined by changes in body temperature and circulating progesterone. These changes in body temperature in the beluga correlated positively with those of circulating progesterone levels \( n=49, r^2=0.578, p<0.01 \) (Fig. 4). From the present results, the average length of the estrous cycle in the beluga was 36.7 ± 3.9 (n=6) days based on the intervals between the days of mating behavior.

**Discussion**

The present study demonstrated that body temperature showed a clear biphasic change associated with the ovarian cycle in a beluga. In the present study, the body temperature positively correlated with circulating progesterone. Body temperature was lower in the beluga during the low progesterone phase than in the high progesterone phase, and mating behavior was observed during the low body temperature period. Body temperature increased 11 days after mating. These results clearly showed that progesterone secreted from corpora lutea is required to increase the body temperature in this species, the same as in humans [6]. These results strongly suggest that the periods of low and high body temperature correspond to the follicular and luteal phase of the estrous cycle in this species. The length of the estrous cycle was 36.7 ± 3.9 days, although the exact length of the follicular and luteal phase was not clear in the present study. Cyclic changes in body temperature and serum levels of progesterone occurred continuously throughout the study period, indicating that the beluga is polyestrous.

The levels of circulating progesterone indicate ovulatory activity and likely pregnancy in marine animals [7–9]. Robeck et al. [10] reported that the levels of serum progesterone in female bottlenose dolphins were generally more than 3 ng/ml, and decreased to less than 1 ng/ml within 1 month, indicating ovulation. Sustained levels of serum progesterone above 3 ng/ml were indicative of pregnancy. The baseline levels of progesterone in non-cycling female bottlenose dolphins averaged 286 pg/ml. Robeck et al. [10] reported evidence that bottlenose dolphins are spontaneous ovulators [8, 11], with a 21 to 42-day estrous cycle. However, ovulatory cycles of variable length and seasonal reproductive activities may occur.

In the eastern Canadian Arctic, breeding of the beluga takes place during May [12], whereas the peak of the belugas in Alaska are between late February and early April. The breeding season apparently varies by as much as 3 months among populations [3, 12]. A recent study of captive beluga in North American zoos and aquariums reported that 70% of the beluga showed an estrous cycle between January and June, and 80% of the conceptions occurred between March and May. The mean total estrous cycle length was 48.0 ± 4.6 days (SD). In the present study, the beluga showed clear seasonal ovarian activity. In 2000, 2001, and 2003, the estrous cycle was observed in April and May. However, in 2002, 7 estrous cycles were observed between March and November.

We previously observed biphasic changes in the body temperature of killer whales [13] that resembled the basal body temperature of humans. However, the biphasic change in body temperature of the beluga in the present study was much clearer compared with the killer whales. The present study strongly suggests that a simple method of body temperature measurement may be a useful method in evaluating the timing of the ovarian cycle of beluga in captivity.

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