NOTE *Internal Medicine*

Fibrinogen Concentrations in Captive Bottlenose Dolphins during Pregnancy

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(Received 18 March 2008/Accepted 24 June 2008)

ABSTRACT. Plasma fibrinogen concentrations were measured in 136 blood samples from 360 days pre-partum to 90 days post-partum for twelve parturitions of seven bottlenose dolphins and in 50 blood samples from the dolphins they were not pregnant as a control. The median concentrations increased gradually, and the values during the fourth stage were higher than those during the other stages. The concentrations during the third stage and the puerperium were almost the same. There were significant differences between the plasma fibrinogen concentrations during the third stage and those of the controls and between those of the fourth stages and the controls ($p<0.01$). The concentrations peaked during the third stage in four cases and during the fourth stage in three cases.

KEY WORDS: bottlenose dolphin, changes in pregnancy, fibrinogen.

J. Vet. Med. Sci. 70(11): 1277–1279, 2008

An increase in the plasma fibrinogen concentration has been noted during human pregnancy [1, 3, 4, 10]. Maternal fibrinogen is required to support pregnancy by maintaining hemostatic balance and stabilizing uteroplacental attachment at the fibrinoid layer found at the fetal-maternal junction [5]. Several blood coagulation factors including fibrinogen elevate in preparation for hemorrhage [4], and changes in the fibrinogen system during pregnancy may be more complex than previously assumed [3].

This study describes longitudinal changes in the plasma fibrinogen concentrations of captive bottlenose dolphins during pregnancy and the puerperium based on clinical blood examinations at Enoshima Aquarium from 1992 to 2002 and our previous papers, dietary factors [11], seasonal changes [12] and hyperlipemia during pregnancy [13].

Seven female bottlenose dolphins, *Tursiops truncatus*, were used in this study (Table 1). Three dolphins had two parturitions, and one had three parturitions. All dolphins originated in waters around Japan. The time from entry into Enoshima Aquarium until parturition ranged between 2 and 23 years (average: 10 years). All parturitions occurred normally with the infant dolphins surviving. However, five infants from the twelve parturitions died before 90 days postpartum.

The species of fish, dietary components and amounts consumed have been previously reported [12, 13]. The size of the tank, sea water volume and air and water temperatures have also been reported [13].

One hundred and thirty six blood samples (8–10 m*l*) were obtained from the flukes of the dolphins using a plastic disposable syringe with a (21-gauge) butterfly needle and were clinically examined [6]. Additionally, 50 blood samples as a control were taken from the above seven dolphins when they were not pregnant. All blood samples were obtained between 09:00–10:00 before feeding with the dolphins fasted for the 16–17 hr since the last feeding [11]. Blood was placed in 3.8% sodium citrate tubes to prevent coagulation. Each blood sample contained 1.8 m*l* blood and 0.2 m*l* sodium citrate and was kept at 4°C for transport to a biochemical laboratory (Showa Medical Science, Tokyo, Japan). The blood was centrifuged to separate the plasma at 3,000 rpm for 10 min within 6 hr after collection. The fibrinogen concentration was measured using a Sysmex CA-6000™ (Thrombin method).

To determine the changes in fibrinogen concentrations during pregnancy and the puerperium, the blood data was categorized into the following five categories: first stage (360 to 271 days pre-partum), second stage (270 to 181 days pre-partum), third stage (180 to 91 day pre-partum) and fourth stage (90 to 1 day pre-partum) of pregnancy and the puerperium (after parturition to 90 days post-partum). The results were processed and analyzed using the Mann-Whitney U-test. All results are presented as maximum, minimum and median values, and differences were considered significance when the p-value for the two-tailed test was < 0.01.

The plasma fibrinogen concentrations of the dolphins during pregnancy ranged from 138 to 375 mg/d*l* (Fig. 1). In the control samples, the concentrations ranged from 170 to 248 mg/d*l*. The median concentrations increased gradually, and the values during the fourth stage were higher than those during the other stages and in the control samples. The median concentrations during the third stage and the puerperium were almost the same. There were significant differences ($p<0.01$) in plasma fibrinogen concentration between the third stage and the control samples and between the fourth stage and the control samples (Table 2). However, there were no significant differences in concentration between the other stages and the control samples.

The concentrations peaked during the third stage in four cases and during the fourth stage in three cases (Table 3). Additionally, peak plasma fibrinogen concentrations were observed in three cases during the second stage and in 2

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Table 1. Animals and parturitions in the present study

Parturition No	ID No	Parturition Date
1	EAM.161	1992/05/11[a)]
2	EAM.187	1993/05/24
3	EAM.138	1993/07/23
4	EAM.227	1994/12/23
5	EAM.161	1995/07/29[a)]
6	EAM.227	1998/06/13
7	EAM.237	2000/06/07
8	EAM.249	2000/07/23[a)]
9	EAM.242	2001/06/18[a)]
10	EAM.249	2001/08/01[a)]
11	EAM.242	2002/07/15
12	EAM.249	2002/10/11

In the present study, EAM.161, 227 and 242 each experienced two parturitions, and EAM. 249 experienced three parturitions. a) The infants died before 90 days post-partum.

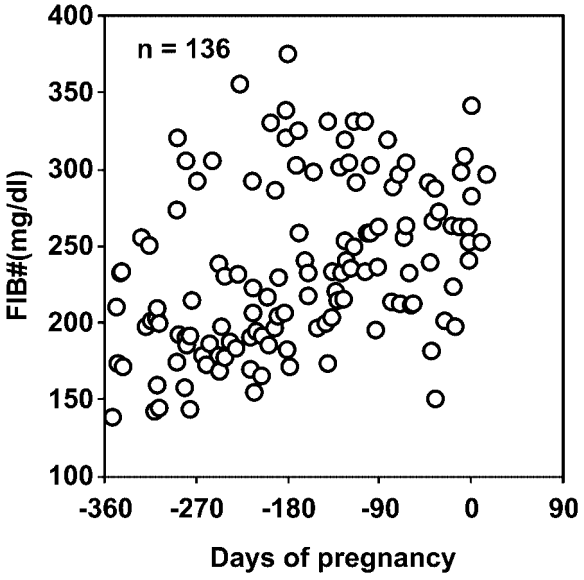

Fig. 1. The distributions of fibirinogen values from 360 days pre-partum to 90 days post-partum for the twelve parturitions of the seven bottlenose dolphins.

cases during the puerperium. However, the concentration did not peak value in any of the dolphins during the first stage. The maximum plasma fibrinogen concentration for the twelve parturitions was 375 mg/d*l*, and this was recorded during the third stage.

In the present study, the median plasma fibrinogen concentration peaked during the fourth stage, and there were significant differences between the third stage and the control samples, and between the fourth stage and the control samples. Furthermore, in four of the twelve parturitions, the plasma fibrinogen concentrations reached the maximum levels during the third stage. It is known that the fibrinogen concentration increases as human pregnancy progresses and that the concentration peaks within 48 hr after delivery [9].

Table 2. Plasma fibrinogen concentrations (mg/d*l*) of dolphins during pregnancy and the puerperium

	Non-pregnant Dolphins	Stages of Pregnancy				
		FIS[e)]	SS[f)]	TS[g)]*	FOT[h)]*	PU[i)]
MAX[a)]	248	320	355	375	319	341
MIN[b)]	170	138	154	171	150	173
MED[c)]	211	197	200.5	240	262	235
N[d)]	50	27	34	36	29	10

Results shown as a) maximum, b) minimum and c) median values. d) Number of samples. e) First stage (360 to 271 days pre-partum), f) second stage (270 to 181 days pre-partum), g) third stage (180 to 91 day pre-partum), h) fourth stage (90 to 1 day pre-partum) and i) the puerperium (after parturition to 90 days post-partum). * Significantly different from the non-pregnant dolphins used as a control ($p<0.01$, a Mann-Whitney U-test).

Table 3. Maximum values recorded in each pregnancy

Parturition No	Stages of Pregnancy				
	FIS[a)]	SS[b)]	TS[c)]	FOT[d)]	PU[e)]
1					↑[f)]
2				↑	
3			↑		
4		↑			
5				↑	
6					↑
7			↑		
8		↑			
9			↑		
10			↑		
11				↑	
12		↑			

a) First stage, b) second stage, c) third stage, d) fourth stage, and e) puerperium. f) Maximum value.

It is also known that the concentration returns to the basal level until 8 weeks post partum [4]. In one false killer whale, the plasma fibrinogen concentration was also shown to be elevated with pregnancy [14]. The pattern of the changes in the concentration for the period in which the peak occurs in bottlenose dolphins is different from that in humans; however, an increase in fibrinogen has been observed during pregnancy in small cetaceans.

The plasma fibrinogen concentration has been reported to range from 170 to 280 mg/d*l* in captive bottlenose dolphins [2]. In the present control samples, the concentrations ranged from 170 to 248 mg/d*l*, similar to those for captive bottlenose dolphins. At Enoshima Aquarium, plasma fibrinogen concentrations of over 300 mg/d*l* are judged to be abnormal. In this study, twenty-one of the one hundred and thirty six samples contained higher concentrations of plasma fibrinogen than 300 mg/d*l*, and ten of these twenty-one samples were from the third stage. A concentration of 370 mg/d*l* has been observed in a false killer whale [14] at one day pre-partum (unpublished data). In humans, plasma fibrinogen concentrations of over 400 mg/d*l* are observed within 48 hr after delivery [10]. It is necessary to examine

whether a concentration of 400 mg/d*l* is a normal maximum concentration or an abnormal concentration in the bottlenose dolphin.

It is difficult to maintain pregnancy in a human suffering from afibrinogenemia [8]. However, a minimum concentration of 200 mg/d*l* is indispensable to delivery and labor [7]. In the present study, three samples collected during the first stage had concentrations under 150 mg/d*l* and twenty-six of the twenty-nine samples collected during the fourth stage had concentrations over 200 mg/d*l*. To date, afibrinogenemia has not been observed in cetaceans at Japanese aquariums. A minimum standard value of fibrinogen and other blood coagulation factors should be established and analyzed during pregnancy in bottlenose dolphins.

ACKNOWLEDGMENTS. We thank the staff of Enoshima Aquarium for their support and dedicated care of the animals, Dr. K. Nakagaki for his advice concerning statistical analysis and Dr. S. Downes for his suggestions.

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