Prediction from ultrasonographic measurements of the expected delivery date in two species of bottlenosed dolphin (*Tursiops truncatus* and *Tursiops aduncus*)

G. LACAVE, M. EGGERMONT, T. VERSLYCKE, F. BROOK, A. SALBANY, L. ROQUE, R. KINOSHITA

Ultrasonographic measurements were made at least once a month during 14 gestations in seven *Tursiops truncatus* and 12 gestations in five *Tursiops aduncus* (bottlenosed dolphins). The 121 measurements of the fetal biparietal diameter and 139 measurements of the fetal thoracic diameter in *T truncatus* and the 97 measurements of the biparietal diameter and 97 measurements of the thoracic diameter in *T aduncus* were used to establish regression lines for the increases in the diameter of the head and thorax of the fetus with time. From these relationships an easy-to-use computer program was developed to predict the date of birth of the two species of bottlenosed dolphin, and its predictions were compared with the actual dates of birth of other calves of both species. The births occurred within the range of predicted dates, and even when only a few measurements were available, the program provided accurate predictions.

DOLPHINS are used in daily demonstrations in marine mammal facilities worldwide. Special arrangements for newborn dolphin calves, such as the separation of the dams, the setting of protection nets and night observations, are important but costly and time-consuming. Unlike many species, dolphins are generally not confined to seasonal breeding and births can occur at any time of year (Schroeder 1990, Wells 1999, Lacave 2000), and an unexpected delivery can be problematic, for example, in cases of dystocia, stillbirth or trauma to the newborn calf. The arrival of a new calf in a group can lead to social aggression, including interactions between juveniles, aggression by males and calf-stealing attempts by other females (G. Lacave, unpublished observations, Brasseur and others 1998). As a result, deliveries under the control of the training and medical team are preferable, and a method for the accurate prediction of parturition would be valuable.

To predict a delivery on the basis of the onset of a gestation requires a knowledge of the day of conception. However, dolphins are known to mate regardless of the reproductive status of the female, and the mating-recognition method has not been reliable (Brook 1997). Visual observation towards the end of gestation can be helpful; signs in the pregnant female such as the swelling of the mammary glands, refusing to eat or visible contractions can indicate the onset of parturition, but are not totally reliable. Bortolotto and others (1995, 1997) and Bortolotto and Stanzani (1998) observed the frequencies of flexion and contraction in several *Tursiops truncatus* gestations and recorded a significant increase in the number of flexions towards the end of gestation. Although these observations may be useful for identifying an approaching delivery, they require an experienced observer and long periods of observation, and are not useful for the long-term prediction of delivery.

Terasawa and others (1999) investigated the use of rectal temperature as a method for predicting delivery in bottlenosed dolphins during five pregnancies in four animals. In four of the five gestations, there was a decrease in the rectal temperature of 0·7 to 1·3°C between 12 and 24 hours before parturition. A similar decrease in rectal temperature was observed in one pregnant female just before delivery at Duisburg Dolphinarium (M. Garcia Hartmann, personal communication). At Zoomarine in Portugal, comparable results were obtained during the monitoring of a twin gestation (G. Lacave, unpublished observations). On the day before aborting the calves, there was a decrease in the rectal temperature of the dam of 0·5°C in the morning and 1·5°C by the evening. However, in three other gestations monitored at this facility there has been no decrease in temperature. It appears that rectal temperature measurements could provide, at least in some animals, an accurate prediction of imminent delivery, but they would be unable to give an accurate prediction sufficiently ahead of time to allow management planning.

The method of using the time of conception to predict delivery dates in dolphins has received new attention through the work of Brook (1997, 2000, 2001), Brook and others (2001) and Kinoshita and others (2000), which has been based on the ultrasonographic determination of ovulation in *Tursiops aduncus*. As a result, it has been possible to bring a chosen male to the female at the time of ovulation and thus know the date of onset of gestation. The mean (sd) period of gestation recorded from 12 controlled matings was 371 (9) days (F. Brook, unpublished observations). However, such data are not available for *T truncatus*.

Until recently, ultrasonography had been used simply to confirm a dolphin pregnancy; however, an increasing number of marine mammal parks are now monitoring gestations by ultrasonography. The method has the advantages that it can identify abnormalities at an early stage (Brook 1997), and that regular measurements make it possible to monitor fetal growth and provide a more accurate prediction of expected delivery, so that adequate arrangements can be made in good time.

There are few reports of ultrasonographic measurements of dolphin fetuses (Williamson and others 1990, Taverne 1991, Stone and others 1995, Lacave 2000). Williamson and others (1990) reported a limited series of measurements of the diameters of the thorax and head of the fetus in four pregnant *T truncatus*. The rate of growth was linear with time (R^2=0·888 for the thorax and 0·821 for the head). However, the study was based on only eight measurements per animal, most of them in later gestation, and the data were insufficient to confirm the accuracy of the linear model statistically. Stone and others (1999) observed a similar linear growth rate in four *T truncatus* gestations when measuring the biparietal and thoracic diameters from 46 weeks to less than a week prepartum. It is necessary to obtain more comprehensive data on the

Veterinary Record (2004) **154,** 228-233

G. Lacave, DVM, Marine Mammal Veterinary Services, Betferkerklaan 158, B-8200 Brugge, Belgium
M. Eggermont, DVM, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium
T. Verslycke, PhD, Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, J. Plateaustraat 22, B-9000 Ghent, Belgium
F. Brook, PhD, Department of Optometry and Radiography, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong
A. Salbany, DVM,
L. Roque, DVM, Zoomarine, Estrada Nacional 125, Km 65, Guia, 8200 Albufeira, Portugal
R. Kinoshita, BVMS, MRCVS, Ocean Park Corporation, Wong Chuk Hang Road, Aberdeen, Hong Kong

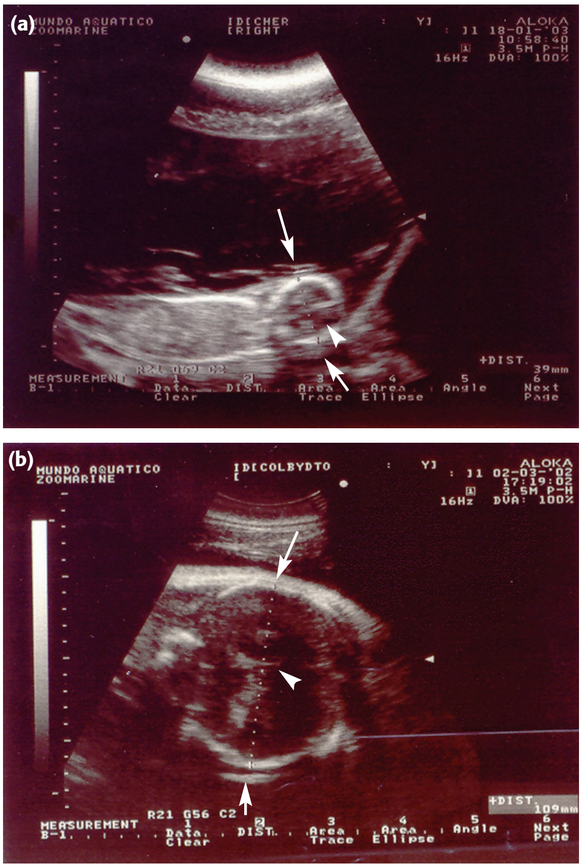

FIG 1: Ultrasonographic images of measurements of biparietal diameter (between the arrows), showing the ovoid skull and the midline echo of the falx (arrowheads) between the parietal bones, at two different times in the gestation

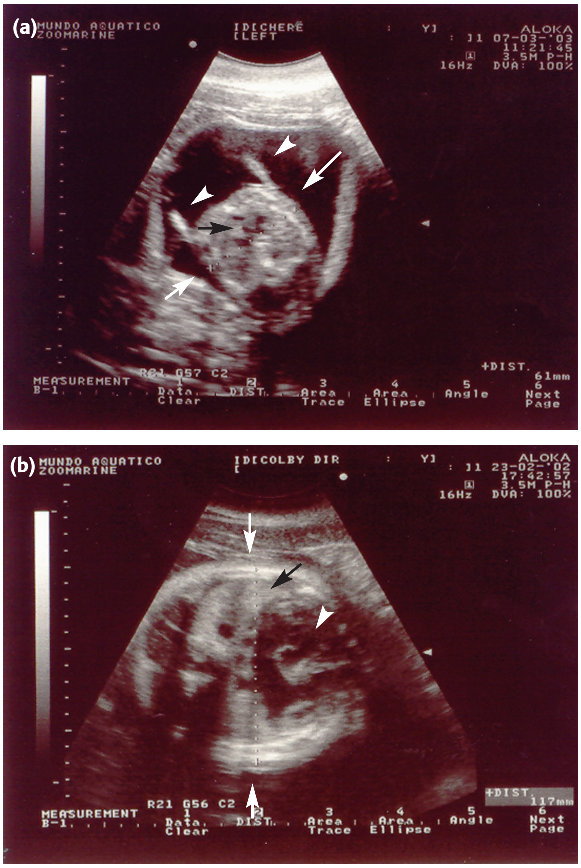

FIG 2: Ultrasonographic images of measurements of thoracic diameter (between the white arrows), showing (a) the four chambers of the heart (black arrow) and the flippers (arrowheads) during the first third of gestation, and (b) the chambers of the heart (black arrow) and parts of the lungs (arrowhead), during the the last third of gestation

growth rate of fetal dolphins to aid marine mammal workers to estimate parturition dates accurately.

For this study, 14 *T truncatus* gestations at Zoomarine (Guia, Portugal) and Boudewijnpark (Brugge, Belgium), and 12 *T aduncus* gestations at Ocean Park (Hong Kong) were monitored ultrasonographically. From these data, models of the increase in the diameters of the head and thorax of the fetus as a function of time were used to develop a computer program to predict the date of delivery and to validate the procedure. The aim of the study was to develop an easy-to-use computer program to provide better birth prediction for dolphins scanned regularly during their gestation and, if possible, to help other marine mammal workers to predict dolphin delivery dates well ahead of time, even if they could make only one or two ultrasound scans of their animals.

MATERIALS AND METHODS

Equipment

The ultrasound examinations at Zoomarine and Boudewijnpark were made with a 3·5 MHz sector transducer (Microimager 2000; Ausonics) and later with a 3·5 MHz curvilinear transducer (Aloka 900 SSD; Aloka). The examinations at Ocean Park were made with SSD 630, 900 and 1700 ultrasound units (Aloka) in conjunction with a 5 or 3·5 MHz curvilinear transducer.

Ultrasound measurements

All data were gathered through voluntary behaviour of the animals. The dolphin would position itself upside down at the side of the pool and be slightly supported by two trainers sitting on the edge with their legs in the water. At Zoomarine, dolphins will stay in this position for up to 20 minutes, regularly lifting up their heads, helped by the trainers, to breathe. The animals were scanned at least once a month and the data were recorded on video and hard-copy prints. The biparietal and thoracic diameters of the fetus were measured. The biparietal diameter can be measured as soon as it is possible to distinguish the head from the rest of the body; the head is visible as a symmetrical ovoid, showing the midline echo of the falx between the parietal bones, and it was measured at the widest section (Fig 1). For the thoracic diameter, measurements were taken at the level where the four chambers of the heart, surrounded symmetrically by the lungs, were visible (Fig 2). The pectoral flippers are often visible in the same section. On some occasions, owing to the position of the fetus, no satisfactory measurements were possible. The trainers would then ask the animal to perform a few jumps and a new scan was attempted. If the position of the fetus still prevented satisfactory measurements a new attempt was planned tentatively for the next day or booked for the following week. Several measurements were taken during each examination and the recorded images were carefully reviewed to determine the most accurate measurements, on the basis of anatomical details. These measurements were then used for the development of the growth models.

The measurements were made as soon as possible after conception. Although a fetus can be identified by the fourth week of gestation (Stone and others 1999), accurate measurements were not always possible at this stage because it was often difficult to differentiate clearly between the head and the thorax (in cross-section) owing to the technical limitations of the equipment used; earlier measurements were possible later in the study. Measurements were made at least once a month at the beginning of the gestation, weekly for the last two months and daily, whenever possible, for the last two weeks; in one case the final scan was made only a few hours before delivery.

Development of the growth model

The models were developed in several stages. First, a fetal growth model was computed from the ultrasonographic measurements made during 11 gestations in five *T truncatus* at Boudewijnpark and Zoomarine. The fetal growth model and the predictions made by the derived birth prediction program were then compared with measurements from two further *T truncatus* gestations at Zoomarine. The data from these two gestations were then added to the primary regression lines to obtain a better prediction program, based on 13 gestations, which was then validated with another gestation at Zoomarine. After having tested the original *T truncatus* program based on 11 gestations for another subspecies, using data from seven *T aduncus* gestations from Ocean Park, a program specific for *T aduncus* was then developed. The predictions of this program were compared with two further gestations from one *T aduncus*. These data were then used to update the program, and data from a further three gestations were used to validate the new program. All of the gestations at Ocean Park had been completed by the time the program was developed; some were chosen at random for developing the program, and data from others were used at a later stage for validation.

In total, 121 measurements of the biparietal diameter and 139 measurements of the thoracic diameter were used for the development of the fetal growth model in *T truncatus*, and 97 measurements of the biparietal diameter and 97 measurements of the thoracic diameter were used for *T aduncus*. These data were plotted against time and analysed statistically (SigmaPlot; SPSS). All the data were checked for normality and homogeneity of variance by using the Kilmogorov-Smirnov test and Levene's test, respectively, with $\alpha=0{\cdot}05$. The data were analysed by linear regression, using the linear regression module of SigmaPlot, and the following regression lines were obtained.

T truncatus updated model, based on 13 gestations (11 gestations for the primary model and two gestations used for the first validation):

Biparietal diameter (mm) = 0·408 x (days before parturition) + 137·603 (R^2=0·964; P<0·0001)
Thorax diameter (mm) = 0·497 x (days before parturition) + 162·430 (R^2=0·949; P<0·0001)

T aduncus updated model, based on nine gestations (seven gestations for the primary model and two gestations used for the first validation):

Biparietal diameter (mm) = 0·411 x (days before parturition) + 135·612 (R^2=0·979; P<0·0001)
Thorax diameter (mm) = 0·474 x (days before parturition) + 155·079 (R^2=0·971; P<0·0001)

RESULTS

The results of the comparisons of the dates of birth predicted by the preliminary and updated programs, and the actual dates of birth, are shown in Tables 1 to 4.

For example, by the first prediction program based on the original 11 gestations, calf 1 had a mean estimated delivery date, based on all the head measurements available, of August 27, 2000, and from the thoracic measurements of September 4, 2000; it was born on August 31 (Table 1). The equivalent dates for another calf, calf 2, were February 25 and March 3, 2002; it was born on March 3. The regression lines for *T truncatus* and *T aduncus* are shown in Fig 3. Fig 4 shows an example of a program sheet used to record the data from which the program calculates the expected day of delivery.

TABLE 1: Comparisons of the predicted dates of birth of two *Tursiops truncatus* calves, based on a model derived from measurements made during 11 gestations, and of another calf, based on a model derived from measurements made during 13 gestations, with their actual dates of birth

Dolphin calf	Actual date of birth	Date of birth predicted from 11 gestations Head	Thorax	Date of birth predicted from 13 gestations Head	Thorax
1	31/08/00	27/08/00	04/09/00	–	–
2	03/03/02	03/03/02	25/02/02	–	–
3	06/05/02	–	–	07/05/02	11/05/02

TABLE 2: Comparisons of the predicted dates of birth of two calves to one *Tursiops aduncus*, based on a model derived from measurements made during seven gestations, and of three calves born to three other *T aduncus* based on a model derived from measurements made during nine gestations, with their actual dates of birth

Dolphin calf	Actual date of birth	Date of birth predicted from seven gestations Head	Thorax	Date of birth predicted from nine gestations Head	Thorax
4	03/04/96	02/04/96	04/04/96	–	–
5	24/04/97	25/04/97	26/04/97	–	–
6	05/01/01	–	–	09/01/01	10/01/01
7	07/06/94	–	–	03/06/94	15/06/94
8	15/09/98	–	–	25/09/98	05/10/98

DISCUSSION

Ultrasonography is not yet available in all dolphin facilities, but it has many advantages over previously described methods for predicting the date of delivery (Robeck and others 2001). It is non-invasive, poses no apparent risk to health and makes possible a more accurate estimate of birth date from early in gestation. The method has been used in human beings for many years to assess fetal development and wellbeing, and is the most accurate method by which the age of a human fetus can be estimated. In the normal human fetus, the measurements of biparietal and thoracic diameter fit a linear growth model (Hadloch and others 1982).

In the preliminary comparisons with two *T truncatus* gestations at Zoomarine, the expected birth dates fell within

TABLE 3: Comparisons of the dates of birth of three *Tursiops truncatus* calves, predicted from measurements made in the first, second and third thirds of the pregnancy on the basis of a model derived from measurements made during 12 or 13 gestations, with their actual dates of birth

Dolphin calf	Actual date of birth	Scanning date	Date of birth predicted Head	Thorax
			12 gestations	
2	03/03/02	1st third, 01/06/01	**04/03/02***	22/02/02
		2nd third, 29/08/01	14/03/02	**03/03/02**
		3rd third, 18/01/02	**02/03/02**	15/02/02
		2nd and 3rd thirds	08/03/02	23/02/02
1	31/08/00	1st third, 10/11/99	19/09/00	**28/08/00**
		2nd third, 04/03/00	06/09/00	**28/08/00**
		3rd third, 12/07/00	15/08/00	15/09/00
		2nd and 3rd thirds	26/08/00	06/09/00
			13 gestations	
3	06/05/02	1st third, 16/08/01	**08/05/02**	**04/05/02**
		2nd third, 25/10/01	**07/05/02**	**09/05/02**
		3rd third, 16/02/02	**03/05/02**	24/05/02
		1st and 2nd thirds	**08/05/02**	**06/05/02**
		2nd and 3rd thirds	**05/05/02**	16/05/02
		1st, 2nd and 3rd thirds	**06/05/02**	12/05/02

* Predicted dates which were within three days of the actual birth date are in bold

TABLE 4: Comparisons of the dates of birth of *Tursiops aduncus* calves, predicted from measurements made in the first, second and third thirds of the pregnancy on the basis of a model derived from measurements made during eight gestations, with their actual dates of birth

Dolphin calf	Actual date of birth	Scanning date	Date of birth predicted Head	Thorax
9	17/09/98	1st third 24/11/97	12/09/98	06/09/98
		2nd third 18/03/98	**20/09/98***	**16/09/98**
		3rd third 08/07/98	29/09/98	27/09/98
		2nd and 3rd thirds	**20/09/98**	21/09/98
10 (big calf)	03/06/01	1st third 12/09/00	**01/06/01**	29/05/01
		2nd third 06/12/00	29/05/01	**02/06/01**
		3rd third 27/03/01	**06/06/01**	04/05/01
		2nd and 3rd thirds	**02/06/01**	18/05/01
11	01/05/99	1st third 23/07/98	08/05/99	06/05/99
		2nd third 18/11/98	**04/05/99**	10/05/99
		3rd third 03/03/99	**02/05/99**	02/04/99
		2nd and 3rd thirds	**03/05/99**	21/04/99
12	25/05/01	1st third 06/09/00	03/06/01	**25/05/01**
		2nd third 22/11/00	**28/05/01**	02/06/01
		3rd third 07/02/01	**24/05/01**	31/05/01
		2nd and 3rd thirds	**26/05/01**	01/06/01
13 (small calf)	19/07/95	1st third 11/10/94	04/08/95	03/08/95
		2nd third 24/01/95	30/07/95	01/08/95
		3rd third 09/05/95	**17/07/95**	29/07/95
		2nd and 3rd thirds	24/07/95	30/07/95
14 (big calf)	01/05/95	1st third 11/07/94	18/04/95	15/04/95
		2nd third 16/10/94	10/04/95	21/04/95
		3rd third 26/03/95	05/04/95	18/04/95
		2nd and 3rd thirds	07/04/95	19/04/95
15 (longer than average gestation)	14/05/01	1st third 23/08/00	10/05/01	03/05/01
		2nd third 22/11/00	27/04/01	**16/05/01**
		3rd third 27/03/01	15/04/01	18/05/01
		2nd and 3rd thirds	21/04/01	**17/05/01**
5	24/04/97	1st third 17/07/96	**24/04/97**	**27/04/97**
		2nd third 16/10/96	**27/04/97**	**28/04/97**
		3rd third 17/01/97	02/05/97	**26/04/97**
		2nd and 3rd thirds	30/04/97	**27/04/97**
4	03/04/96	1st third 11/07/95	**05/04/96**	30/03/96
		2nd third 04/10/95	**03/04/96**	28/03/96
		3rd third 15/02/96	**02/04/96**	**01/04/96**
		2nd and 3rd thirds	**02/04/96**	30/03/96

* Predicted dates which were within three days of the actual birth date are in bold

the predicted interval, that is, the interval between the date predicted on the basis of head measurements and the date predicted on the basis of thoracic measurements. Because these two predictions were accurate, the data from the animals were added to the regression model on which the computer prediction program is based (now on 13 gestations), and an accurate prediction was obtained with a new *T truncatus* gestation at Zoomarine (Colby 2002) (Table 1).

The original program was also tested by using data from *T aduncus* gestations from Ocean Park, in which the exact length of the gestation was known. Although the results obtained were interesting, the original program gave less accurate results, with the birth date differing by up to two weeks from the predicted date (data not shown). This was expected, because this subspecies is generally smaller and leaner than *T truncatus*. When using the *T truncatus* delivery prediction program for *T aduncus*, the actual delivery occurred, in most cases, before the predicted interval, suggesting that prediction programs need to be species specific, or, in the case of size differences, subspecies specific. As a result of this difference, a specific delivery prediction program was developed for *T aduncus* on the basis of the regression lines resulting from measurements made at Ocean Park. As in *T truncatus*, the preliminary and updated delivery prediction programs for *T aduncus* were tested by comparisons with other births (Table 2).

The results of this study indicate that the diameter of the skull of a fetal dolphin, measured ultrasonographically, increases more slowly than the diameter of the thorax. The increase in the biparietal diameter may be measured more accurately than the growth of the thorax, because thoracic measurements are less accurate in later gestation when the relatively large dolphin fetus is folded over to be accommodated in the maternal abdomen. The results so far indicate that the prediction of birth date on the basis of head measurements is more accurate.

Another objective of the study was to determine whether, by incorporating only one or a few measurements made at different stages of a gestation into the computer program, it would be possible to predict the birth date accurately, thus allowing facilities than can scan animals only a few times during gestation to reduce the predicted interval. The delivery prediction program used for this comparison contained all the available measurements at the time, except those from the animal used for the comparison. For example, to test calf 2 (Table 3), its data were taken off the updated prediction programs (based, respectively, on 13 and nine gestations for the two subspecies) so that they would not influence the results. The comparisons with a few scanning dates were therefore done by using a prediction program based on 12 gestations for *T truncatus* and eight gestations for *T aduncus*.

The period of gestation was divided into three phases of approximately four months each. One or a few scanning dates (with both a biparietal and a thoracic measurement) were selected at random during each phase and used to predict the birth date (Tables 3, 4). For example, calf 2 (Table 3), with one measurement of the biparietal and thoracic diameters made on June 1, 2001, had a predicted birth interval between February 22 and March 4, 2002; the calf was born on March 3. For the same animal, measurements taken in the second third of the gestation, on August 29, 2001, gave a predicted birth interval between March 3 and 14, 2002. When measurements taken on two or three different dates are entered, the program automatically gives an average of the predicted birth date intervals.

The predictions were compared with the results from animals of both subspecies. The gestation of calf 3 was the last to be recorded and the one used to compare with the updated prediction program based on 13 gestations; as a result, the same program could be used for the comparisons with few data, because this calf's measurements had not been used to develop the program. The predicted birth dates that fell within three days of the actual birth date are highlighted in Tables 3 and 4. The comparisons with few measurements again gave very promising results.

In human beings, ultrasonographic measurements, for example, of the biparietal, thoracic and abdominal diameters,

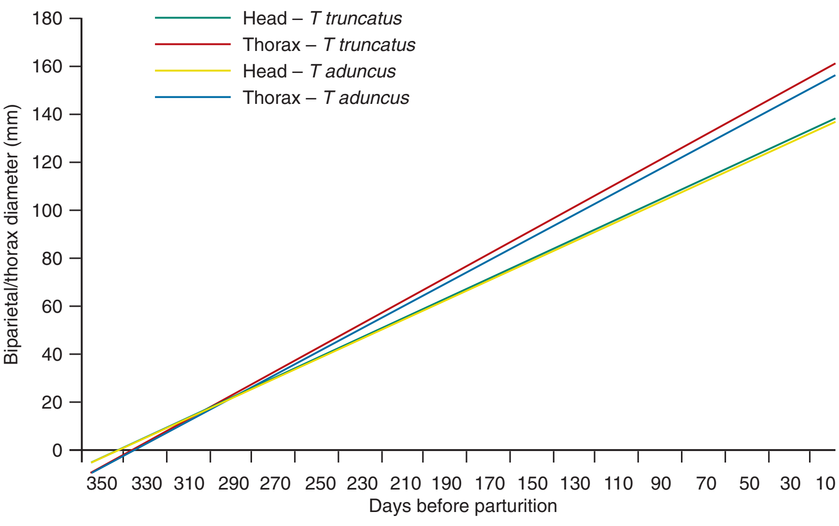

FIG 3: Comparison of the regression lines describing the relationships between the stage of pregnancy and the biparietal diameter or thorax diameter of *Tursiops truncatus* and *Tursiops aduncus* fetuses

Delivery prediction in *Tursiops truncatus*						
Name: Calf 3 2001/2002						
Other data: Zoomarine						
Actual birth date: 06/05/2002						
Fill in the first three columns						
	Diameter (mm)	Diameter (mm)	Estimation based on head size		Estimation based on thorax size	
Date	Head	Thorax	Days before birth	EDD	Days before birth	EDD
12/07/01	15	18	294	01/05/02	282	20/04/02
16/08/01	27	29	266	08/05/02	261	04/05/02
29/08/01	32	33	254	09/05/02	254	09/05/02
14/09/01	37	45	242	14/05/02	231	02/05/02
28/09/01	44	51	226	11/05/02	219	10/05/02
11/10/01	49	55	214	12/05/02	212	10/05/02
25/10/01	57	63	195	07/05/02	196	09/05/02
14/11/01	67	71	171	04/05/02	181	14/05/02
01/12/01	73	83	157	07/05/02	158	08/05/02
14/12/01	81	87	138	01/05/02	151	13/05/02
18/01/02	98	98	98	26/04/02	130	27/05/02
16/02/02	107	115	77	03/05/02	97	24/05/02
23/02/02	109	117	72	06/05/02	93	27/05/02
02/03/02	116	139	56	26/04/02	51	22/04/02
27/04/02	123	143	39	05/06/02	44	09/06/02
	Mean estimated delivery date (EDD)			07/05/02		11/05/02
	Standard deviation			9 days		13 days

FIG 4: Example of a program sheet. Three columns have to be filled in: the date, the biparietal diameter and the thoracic diameter (both in mm). The program then automatically calculates the predicted delivery date

and femur length, made in the first trimester, make it possible to predict birth date with a variability of five to seven days; measurements made in the third trimester increase the variability to up two to four weeks (Kurtz and Needleman 1988). The results obtained with these delivery prediction programs were within these intervals. At present, there are too few data to determine whether measurements of the biparietal or thoracic diameter made in early gestation are the better for predicting the birth date of *Tursiops* species. It appears that the birth date can be predicted slightly better by using measurements of biparietal diameter, but more data are needed to confirm this statistically. Ultrasonography was also helpful in determining the position and size of the fetus and for alerting staff to potential problems. In one case a very large fetus, with a thoracic diameter of 186 mm, was identified three hours before birth; however, its delivery was uncomplicated.

The ultrasonographic measurements of the biparietal and thoracic diameters of the fetus in 13 *T truncatus* and nine *T aduncus* gestations were used to develop two subspecies-specific programs for predicting the date of birth. These gave accurate predictions in one and three further gestations in these respective populations. Further comparisons with a few scanning dates were made by using a prediction program based on 12 gestations for *T truncatus* and eight gestations for *T aduncus*, and these also gave accurate results.

The programs should provide useful tools for the accurate prediction of parturition in other populations, thus providing the time needed for arrangements to be made for the fetus to be delivered under the control of the training and medical team.

ACKNOWLEDGEMENTS

The authors wish to thank Boudewijnpark in Belgium, Zoomarine in Portugal and Ocean Park in Hong Kong for providing their animals for this study, and the trainers at all three parks for their participation in this research by training the animals for voluntary scanning.

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