NOTE

Pregnancy diagnosis and fetal monitoring in Yangtze finless porpoises

Xianyuan Zeng1,2, Li Chunyang3, Yuijiang Hao1,*, Ding Wang1, Fei Fan1, Chaoqun Wang1, Zhengyu Deng1, Hongbin Guo1, Zhiyuan Wang1

1Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China
2School of Life Sciences, Ningde Normal University, Ningde 352100, PR China
3Department of Student Affairs, Ningde Normal University, Ningde 352100, PR China

ABSTRACT: The Yangtze finless porpoise Neophocaena asiaeorientalis asiaeorientalis is a Critically Endangered species endemic to the Yangtze River and connecting lakes. Understanding the reproductive biology, fetal development, and physiology of this species is essential to ensure the successful development of a captive-breeding program. We studied pregnancy and fetal development in this species using endocrine monitoring and ultrasonographic imaging. Plasma progesterone concentrations increased significantly from non-pregnant (0.61 ± 0.43 ng ml⁻¹) to pregnant (37.44 ± 16.18 ng ml⁻¹) phases. Based on progesterone variation, we estimate gestation length to be approximately 12 mo. Fetal growth patterns are well described by linear regressions of thorax diameter, thorax circumference, and total body length plotted against days from parturition. Fetal growth models may be used to estimate fetal age and predict parturition date with more data in the future. Our findings provide fundamental information on pregnancy dynamics and fetal development in this Critically Endangered species to improve existing and benefit the establishment of new captive-breeding programs for Yangtze finless porpoises.

KEY WORDS: Pregnancy · Fetal development · Endocrine · Ultrasonography · Yangtze finless porpoise · Cetacean

1. INTRODUCTION

The Yangtze finless porpoise Neophocaena asiaeorientalis asiaeorientalis is endemic to the Yangtze River and connecting lakes (Jefferson & Wang 2011). The wild population of this species has experienced such severe declines over recent decades that it is now classified as Critically Endangered by the IUCN (Wang et al. 2013). To conserve this species, captive breeding efforts have been undertaken since the 1980s (Wang 2009).

In captive odontocetes, failed pregnancies and perinatal mortality are not uncommon (Robeck et al. 2001, O’Brien & Robeck 2012). Specialized management is required to increase reproductive success, such as administering nutritional and medical supplements (Wasser et al. 2017, Andrew et al. 2018), maintenance of appropriate social groupings (Waples & Gales 2002), and preparing for parturition and postpartum care (Baumgartner et al. 2018). Prenatal care requires a level of understanding of pregnancy and fetal development that is presently lacking for the Yangtze finless porpoise. We used endocrine monitoring and ultrasonographic imaging to document variation in progesterone from non-pregnant to pregnant phases, estimate gestation length based on predicted conception dates, and develop fetal growth models based on fetal measurements of Yangtze finless porpoises.

© The authors 2022. Open Access under Creative Commons by Attribution Licence. Use, distribution and reproduction are unrestricted. Authors and original publication must be credited.
Publisher: Inter-Research · www.int-res.com
2. MATERIALS AND METHODS

2.1. Animals

The 6 captive Yangtze finless porpoises in the present study were all originally wild-caught (at ca. 2 yr old), with none known to have previously given birth. Five of these individuals (F7, YY, F9, YI, JJ) were housed in a kidney-shaped pool (25 × 7.5 m; depth: 4 m) at the Yangtze Cetacean Breeding and Research Center, Wuhan, China; individual EE was housed in a rectangular net cage (width: 15 m; depth: 7 m) at Tian-E-Zhou Reserve, Shishou. These female porpoises were initially housed together with males, and once pregnancy was confirmed, the sexes were separated. All porpoises were fed diets of frozen−thawed and/or fresh fish (ca. 6−10% of porpoise body weight d−1). See Table 1 for detailed information on these porpoises.

2.2. Blood collection

Blood samples were collected monthly or bi-monthly from individuals YI, JJ, and EE during their routine health assessments. Blood collection involved temporarily restraining the porpoise (<15 min) on a wet sponge mattress, after which it was released immediately. Blood samples were centrifuged at 1500 × g for 15 min and stored in a freezer at −20°C until analysis. Porpoises were not disturbed during the late-gestation phase.

2.3. Progesterone assay

Progesterone concentration was determined using radioimmunoassay (RIA) for JJ and chemiluminescence immunoassay (CIA) for EE and YI. The RIA protocol was previously developed and validated by Hao et al. (2007). CIAs were run on an IMMULITE 2000 automated analyzer (Siemens Healthcare Diagnostics) following the protocol of O’Brien & Robeck (2012). CIA was validated by parallelism between standard and sample regression curves (r = 0.97, p > 0.05) and a high recovery rate (89%) of exogenous progesterone added to the sample. The cross-reactivity of progesterone (P4) antibody was <1% with 11-deoxycorticosterone, <0.1% with cortisol, corticosterone, and cortisone, and <0.01% with estradiol-17β and prednisone for both RIA and CIA. The sensitivity of all assays was 0.1 ng ml−1, and intra- and inter-assay variation was <10% for both RIA and CIA. Plasma progesterone concentration >3.0 ng ml−1 over an extended period indicated pregnancy (Sawyer-Steffan et al. 1983).

2.4. Fetal ultrasonography

Ultrasonographic examination was performed monthly on F7, YY, and F9 using a LOGIQ Book XP ultrasound unit (General Electric) with a 3−5 MHz curvilinear array transducer, following the protocol of Robeck et al. (2015). Fetal measurements included thorax diameter (TD), thoracic circumference (TC), and total length (TL) (Fig. 1). Individuals would generally cooperate for 10−30 s for each scan for 2 or 3 scans.

2.5. Statistical analyses

An independent 2 sample t-test was used to compare plasma progesterone concentrations between pregnant and non-pregnant phases for each porpoise. Mean ± SD values were used to summarize the data set. Prior to parametric tests, assumptions of data normality and homogeneity of variance were verified by Shapiro-Wilk’s and Levene’s tests, respectively. If assumptions were not met, data were square-root transformed to meet assumptions. A value of p < 0.05 was considered to be significant. To describe the fetal growth pattern, TD, TC, and TL were regressed against days before parturition (DBP).

3. RESULTS

Four pregnancies (2 for JJ, 1 for YI, and 1 for EE) occurred during endocrine monitoring (Table 1). Proges-
terone concentrations increased significantly from non-pregnant (0.55 ± 0.39 ng ml−1 for JJ; 0.63 ± 0.46 ng ml−1 for YI) to pregnant (41.46 ± 22.75 ng ml−1 for JJ; 36.77 ± 3.97 ng ml−1 for YI) phases in JJ (t = −30.778, p < 0.001) and YI (t = −16.054, p < 0.001). For EE, progesterone concentrations also appeared to increase from non-pregnant (0.83 and 0.95 ng ml−1) to pregnant (4, 30.61, and 26.2 ng ml−1) phases. Based on the final day of baseline progesterone concentrations and the first day it exceeded 3.0 ng ml−1, we estimated the gestation lengths of JJ to be 309−393 d (median: 346 d) for the first pregnancy and 332−414 d (median: 373 d) for the second pregnancy, and for EE, 374−421 d (median: 397 d). For YI, gestation length was estimated to be at least 351 d because endocrine data were not available for the non-pregnant phase. Progesterone concentrations are detailed in Table A1 in the Appendix.

Based on ultrasonographic imaging, pregnancies in F7, YY, and F9 were confirmed on 20 August, 23 August, and 25 September 2017, respectively. Fetal measurements for F7, YY, and F9 consecutively increased throughout gestation. While fetal measurements for F9 were consistently smaller than those for F7 and YY on the same scanning day, F9 delivered earlier than either F7 or YY (Table 1), and the placenta was fragmented upon excretion. Because of this parturition abnormality, we elected not to use fetal measurements of F9 in the development of fetal growth models. Fetal measurements of F7 and YY were pooled and plotted against DBP. Each of TD, TC, and TL at DBP were well described by linear regression: TD (cm) = 12.781 + 0.040 × DBP, r² = 0.988; TC (cm) = 46.378 + 0.147 × DBP, r² = 0.983; and TL (cm) = 49.644 + 0.152 × DBP, r² = 0.97 (Fig. 2).

4. DISCUSSION

Plasma progesterone concentrations increased considerably from non-pregnant to pregnant phases in Yangtze finless porpoises, similar to increases reported for other odontocete species (O’Brien & Robeck 2012, Robeck et al. 2016). Based on changes in progesterone concentration, the medians of estimated gestation length ranged from 346−397 d for Yangtze finless porpoises that were close to 12 mo. This estimated gestation length was evidenced by seasonality of parturition, predominately from May to July (Wang 2009, Xian 2010), and by mating occurring mainly from April to August (Chen et al. 2006, Wu et al. 2010). Given the lengthy gestation, longitudinal monitoring of progesterone to continuously assess health is essential. Unfortunately, the capture and handling of animals required to sample blood can be stressful (Hao et al. 2009), leading to dysregulation of progesterone production (Michel & Bonnet 2014, Herrera et al. 2016) and even reproductive failure (Arck 2001, Uphouse 2011). An alternative to blood sampling might entail non-invasive collection of urine, which has proven to be a reliable matrix for endocrine monitoring (Robeck et al. 2009, Steinman et al. 2012). Accordingly, protocols for urine collection and urinary progesterone assay should be explored for Yangtze finless porpoises.
In addition to endocrine monitoring, real-time ultrasonography can be used to monitor fetal development during gestation. According to estimated gestation duration, the Yangtze finless porpoise fetus is likely to become visible to ultrasonography within 3–4 mo post-conception, similar to the bottlenose dolphin *Tursiops truncatus* (Williamson et al. 1990) and beluga whale *Delphinapterus leucas* (Robeck et al. 2015). Ultrasonographic measurements effectively describe Yangtze finless porpoise fetal growth using linear regression models. Such growth models for TD and TC have also been reported for bottlenose dolphins and beluga whales (Williamson et al. 1990, Lacave et al. 2004, Robeck et al. 2015).

The second-order polynomial model was the best fit for the TL regression for belugas (Robeck et al. 2015), in which growth rate increased from the first to second half of gestation. The difference between belugas and Yangtze finless porpoises probably stems from differences in sampling duration. TL data were collected throughout gestation in belugas, but we sampled these data only during the first half of gestation. Fetal growth models have been used to predict the parturition date in bottlenose dolphins and belugas, with accuracies within 7 and 18 d, respectively (Lacave et al. 2004, Robeck et al. 2015). Our Yangtze finless porpoise fetal growth models were developed based on limited data; thus, more data are required to improve estimates of fetal age and to predict parturition date with comparable accuracy.

Endocrine monitoring revealed that circulatory progesterone increased significantly from non-pregnant (0.61 ± 0.43 ng ml−1) to pregnant (37.44 ± 16.18 ng ml−1) phases, and that progesterone reliably indicates pregnancy in Yangtze finless porpoises. Ultrasonographic imaging also enables monitoring of fetal growth, with linear regression models which eventually (with more data) could be used to predict parturition date. While preliminary, our findings present fundamental information on pregnancy dynamics and fetal development that can complement existing captive breeding programs and aid in the development of future conservation models for this endangered porpoise.
Acknowledgments. The study was funded by the Ocean Park Corporation and Changjiang Conservation Foundation. Permits were provided by the Yangtze River Basin Fisheries Supervision and Administration Office, the Ministry of Agriculture and Rural Affairs of the People’s Republic of China. All procedures were performed in strict accordance with Chinese law and ethical guidelines.

LITERATURE CITED

Andrew MJ, Parr JR, Montague-Johnson C, Laler K, Holmes J, Baker B, Sullivan PB (2018) Neurodevelopmental outcome of nutritional intervention in newborn infants at risk of neurodevelopmental impairment: the dolphin neonatal double-blind randomized controlled trial. Dev Med Child Neurol 60:897–905

Arck PC (2001) Stress and pregnancy loss: role of immune mediators, hormones and neurotransmitters. Am J Reprod Immunol 46:117–123

Baumgartner K, Lacave G, Sweeney JC, Will H (2018) A suggested birth protocol for bottlenose dolphins (Tursiops truncatus)—updated 2015, Zoo Nuremberg. Aquat Mamm 44:100–109

Chen DQ, Hao YJ, Zhao QZ, Wang D (2006) Reproductive seasonality and maturity of male Neophocaena phocaenoides asiaeorientalis in captivity: a case study based on the hormone evidence. Mar Freshwat Behav Physiol 39:163–173

Hao YJ, Chen DQ, Zhao QZ, Wang D (2007) Serum concentrations of gonadotropins and steroid hormones of Neophocaena phocaenoides asiaeorientalis in middle and lower regions of the Yangtze River. Theriogenology 67:673–680

Hao YJ, Zhao QZ, Wu HP, Chen DQ, Gong C, Li L, Wang D (2009) Physiological responses to capture and handling of free-ranging male Yangtze finless porpoises (Neophocaena phocaenoides asiaeorientalis). Mar Freshwat Behav Physiol 42:315–327

Herrera AY, Nielsen SE, Mather M (2016) Stress-induced increases in progesterone and cortisol in naturally cycling women. Neurobiol Stress 3:96–104

Jefferson TA, Wang JY (2011) Revision of the taxonomy of finless porpoises (genus Neophocaena): the existence of two species. J Mar Anim Ecol 4:3–16

Lacave G, Eggermont M, Verslycke T, Broek F, Salbany A, Roque L, Kinoshita R (2004) Prediction from ultrasonographic measurements of the expected delivery date in two species of bottlenosed dolphin (Tursiops truncatus and Tursiops aduncus). Vet Rec 154:228–233

Michel CL, Bonnet X (2014) Effect of a brief stress on progesterone plasma levels in pregnant and non-pregnant guinea pigs. Anim Biol Leiden Neth 64:19–29

O’Brien JK, Robeck TR (2012) The relationship of maternal characteristics and circulating progesterone concentrations with reproductive outcome in the bottlenose dolphin (Tursiops truncatus) after artificial insemination, with and without ovulation induction, and natural breeding. Theriogenology 78:469–482

Robeck TR, Atkinson SKC, Brook F (2001) Reproduction. In: Dierauf LA, Gulland FMD (eds) CRC Handbook of marine mammal medicine. CRC Press, Boca Raton, FL, p 204–224

Robeck TR, Steinman K, Greenwell M, Ramirez K and others (2009) Seasonality, estrous cycle characterization, estrus synchronization, semen cryopreservation, and artificial insemination in the Pacific white-sided dolphin (Lagenorhynchus obliquidens). Reproduction 138:391–405

Robeck TR, Schmitt TL, Osborn S (2015) Development of predictive models for determining fetal age-at-length in belugas (Delphinapterus leucas) and their application toward in situ and ex situ population management. Mar Mamm Sci 31:591–611

Robeck TR, Steinman KJ, O’Brien JK (2016) Characterization and longitudinal monitoring of serum progestagens and estrogens during normal pregnancy in the killer whale (Orcinus orca). Gen Comp Endocrinol 236:83–97

Sawyer-Steffan JE, Kirby VL, Gilmartin WG (1983) Progesterone and estrogens in the pregnant and nonpregnant dolphin, Tursiops truncatus, and the effects of induced ovulation. Biol Reprod 28:897–901

Steinman KJ, O’Brien JK, Monfort SL, Robeck TR (2012) Characterization of the estrous cycle in female beluga (Delphinapterus leucas) using urinary endocrine monitoring and transabdominal ultrasound: evidence of facultative induced ovulation. Gen Comp Endocrinol 175:389–397

Uphouse L (2011) Stress and reproduction in mammals. In: Norris DO, Lopez KH (eds) Hormones and reproduction of vertebrates. Academic Press, New York, NY, p 117–138

Wang D (2009) Population status, threats and conservation of the Yangtze finless porpoise. Chin Sci Bull 54:3473–3484

Wang D, Turvey S, Zhao X, Mei Z (2013) Neophocaena asiaeorientalis ssp. asiaeorientalis. IUCN Red List of Threatened Species 2013:e.T43205774A45893487

Waples KA, Gales NJ (2002) Evaluating and minimising social stress in the care of captive bottlenose dolphins (Tursiops aduncus). Zoo Biol 21:5–26

Wasser SK, Lundin Ji, Ayres K, Seely E and others (2017) Population growth is limited by nutritional impacts on pregnancy success in endangered southern resident killer whales (Orcinus orca). PLOS ONE 12:e0179824

Williamson P, Gales NJ, Lister S (1999) Use of real-time B-mode ultrasound for pregnancy diagnosis and measurement of fetal growth rate in captive bottlenose dolphins (Tursiops truncatus). J Reprod Fertil 88:543–548

Wu HP, Hao YJ, Yu XY, Xian YJ and others (2010) Variation in sexual behaviors in a group of captive male Yangtze finless porpoises (Neophocaena phocaenoides asiaeorientalis): Motivated by physiological changes? Theriogenology 74:1467–1475

Xian Y (2010) Behavioral development of Yangtze finless porpoise calves (Neophocaena phocaenoides asiaeorientalis). PhD dissertation, Chinese Academy of Sciences, Wuhan
## Appendix. Additional data

Table A1. Yangtze finless porpoise progesterone concentrations. Progesterone concentrations were determined by radioimmunoassay for porpoise JJ and chemiluminescence immunoassay for porpoises YI and EE. Dates given as yyyy/mm/dd.

|Sampling date| Porpoise JJ| Progesterone concentration (ng ml⁻¹)| Sampling date| Porpoise YI| Progesterone concentration (ng ml⁻¹)| Sampling date| Porpoise EE| Progesterone concentration (ng ml⁻¹) |
|-------------|------------|-------------------------------------|-------------|------------|-------------------------------------|-------------|------------|-------------------------------------|
|2002/3/7     | 0.75       |                                     | 2003/4/8    | 0.70       |                                     | 2015/2/6    | 0.83       |                                     |
|2002/4/1     | 0.55       |                                     | 2003/4/28   | 1.30       |                                     | 2015/3/28   | 0.95       |                                     |
|2002/5/15    | 0.42       |                                     | 2003/5/28   | 1.80       |                                     | 2015/5/14   | 4.00       |                                     |
|2002/6/24    | 0.58       |                                     | 2003/6/26   | 0.30       |                                     | 2015/6/28   | 30.61      |                                     |
|2002/7/31    | 0.18       |                                     | 2003/7/23   | 0.80       |                                     | 2015/9/18   | 26.20      |                                     |
|2002/8/21    | 0.21       |                                     | 2003/8/21   | 0.80       |                                     |             |            |                                     |
|2002/9/13    | 0.68       |                                     | 2003/8/23   | 0.40       |                                     |             |            |                                     |
|2002/9/30    | 0.33       |                                     | 2003/9/18   | 0.80       |                                     |             |            |                                     |
|2002/10/8    | 0.17       |                                     | 2003/10/9   | 1.10       |                                     |             |            |                                     |
|2002/10/18   | 0.12       |                                     | 2003/10/27  | 0.42       |                                     |             |            |                                     |
|2002/11/25   | 0.82       |                                     | 2003/11/24  | 0.44       |                                     |             |            |                                     |
|2002/12/23   | 0.83       |                                     | 2003/12/8   | 0.15       |                                     |             |            |                                     |
|2002/12/27   | 0.52       |                                     | 2003/12/18  | 0.17       |                                     |             |            |                                     |
|2003/3/5     | 0.08       |                                     | 2004/1/15   | 0.15       |                                     |             |            |                                     |
|2003/4/8     | 0.60       |                                     | 2004/2/17   | 0.33       |                                     |             |            |                                     |
|2003/4/28    | 1.10       |                                     | 2004/3/15   | 0.66       |                                     |             |            |                                     |
|2003/5/28    | 1.60       |                                     | 2004/4/15   | 1.00       |                                     |             |            |                                     |
|2003/6/26    | 0.30       |                                     | 2004/6/16   | 0.62       |                                     |             |            |                                     |
|2003/7/23    | 0.70       |                                     | 2004/8/26   | 0.33       |                                     |             |            |                                     |
|2003/8/21    | 1.40       |                                     | 2004/11/9   | 0.32       |                                     |             |            |                                     |
|2003/9/18    | 0.50       |                                     | 2005/1/18   | 0.27       |                                     |             |            |                                     |
|2004/6/17    | 0.77       |                                     | 2005/3/14   | 0.20       |                                     |             |            |                                     |
|2004/8/30    | 0.65       |                                     | 2005/10/22  | 1.60       |                                     |             |            |                                     |
|2004/11/9    | 66.40      |                                     | 2005/12/1   | 0.46       |                                     |             |            |                                     |
|2005/3/14    | 22.90      |                                     | 2006/3/6    | 0.17       |                                     |             |            |                                     |
|2005/12/1    | 0.25       |                                     | 2006/4/14   | 0.10       |                                     |             |            |                                     |
|2006/3/6     | 0.09       |                                     | 2006/7/5    | 1.40       |                                     |             |            |                                     |
|2006/4/14    | 0.29       |                                     | 2006/9/15   | 0.60       |                                     |             |            |                                     |
|2006/7/5     | 21.70      |                                     | 2011/7/7    | 37.04      |                                     |             |            |                                     |
|2006/9/15    | 30.50      |                                     | 2011/10/18  | 40.60      |                                     |             |            |                                     |

\*Pregnant phase