Accepted: 20 October 2016

DOI: 10.1111/jmp.12249

ORIGINAL ARTICLE

WILEY

Urinary sex steroid hormone and placental leucine aminopeptidase concentration differences between live births and stillbirth of Bornean orangutans (*Pongo pygmaeus*)

Kodzue Kinoshita[1] | Yusuke Sano[2] | Akira Takai[3] | Mika Shimizu[4] | Toshio Kobayashi[5] | Akihiro Ouchi[6] | Noko Kuze[7,8] | Miho Inoue-Murayama[9,10] | Gen'ichi Idani[9] | Munehiro Okamoto[1] | Yasuhiko Ozaki[11]

[1]Primate Research Institute, Kyoto University, Inuyama, Japan

[2]Osaka Municipal Tennoji Zoological Gardens, Osaka, Japan

[3]Kobe Municipal Oji Zoo, Kobe, Japan

[4]Tama Zoological Park, Hino, Japan

[5]Zoorasia Yokohama Zoological Gardens, Yokohama, Japan

[6]Asahikawa City Asahiyama Zoo, Ashashikawa, Japan

[7]Japan Society for the Promotion of Science, Chiyoda, Japan

[8]National Museum of Nature and Science, Tsukuba, Japan

[9]Wildlife Research Center, Kyoto University, Kyoto, Japan

[10]National Institute for Environmental Studies, Tsukuba, Japan

[11]Department of Obstetrics and Gynecology, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan

Correspondence
Kodzue Kinoshita, Primate Research Institute, Kyoto University, Inuyama, Japan.
Email: kinoshita.kodzue.8v@kyoto-u.ac.jp

Funding information
This work was supported by a Grant-in-Aid from the Japan Society for the Promotion of Science (JSPS) Fellows (Grant Number 241484) and the Inui Tasuke Scientific Foundation.

Abstract

Background: Under the environment of pregnancy, the placenta assumes an important steroidogenic role in the maintenance of pregnancy.

Methods: Urinary placental leucine aminopeptidase (PLAP), estrone-3-glucuronide (E_1G), and pregnanediol-3-glucuronide (PdG) concentrations were compared among five pregnancies (four live births and one stillbirth) in four orangutans.

Results: The gestation period of the stillbirth (223 days) was shorter than that of the live births (239-254 days). In females who gave a live birth, average PLAP and E_1G concentrations increased until the delivery. Conversely, in the female who gave a stillbirth, PLAP concentration failed to increase, and E_1G concentration was significantly low in late pregnancy period. Regarding PdG concentrations, there was no significant difference among all pregnancies.

Conclusions: This is the first study reporting a change in urinary PLAP, E_1G, and PdG concentrations during orangutan stillbirth and live birth pregnancies. The findings will assist in developing pregnancy screening tests.

KEYWORDS

estrone-3-glucuronide, placental leucine aminopeptidase, pregnancy, pregnanediol-3-glucuronide, urine

1 | INTRODUCTION

Bornean orangutans (*Pongo pygmaeus*) are classified as an endangered species on the IUCN Red List of Threatened Species,[1] and the population of this species is decreasing in both the wild and captivity.[2] In particular, Anderson et al.[3] reported that orangutans in captivity have a higher infant mortality than those in the wild. Therefore, it is important to improve the reproductive efficiency and birth rate of Bornean orangutans.

The gestational period of Bornean orangutans is 235-270 days,[4] with an average of 245 days.[5] Orangutans are usually monotocous

© 2016 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd

animals, but in rare cases, they do produce twins; however, at least one of the twins almost always dies at birth or within 1 month or year.[6-9] The offspring are weaned relatively late (approximately 6-8 years).[10-13] In addition, reproduction appears to be particularly costly in terms of an orangutan's energy expenditure.

Orangutans in the wild give birth, on average, approximately once every 6-9 years, which is the longest interbirth interval of any mammal.[10,12,14] Therefore, orangutans typically have an older age at first reproduction, a longer interbirth interval, and a longer generation time than African apes. Derivation of the age for a wild female begins by assigning the minimum age for first reproduction (15.2 years) and then adding the estimated age of the dependent offspring.

At the end of 2015, 33 Bornean orangutans (15 females, 18 males) were housed in 12 zoos affiliated with the Japanese Association of Zoos and Aquariums. Thirty-three infants were born over the last three decades: seven of these infants were stillbirths. In addition, six out of every seven stillbirths were male.[15] Anderson et al.[3] also report that 13.1% of Bornean orangutan born into worldwide captivity died because of stillbirths or abortions. However, no study has investigated the endocrinological causes of these stillbirths. Under the special environment of pregnancy, the placenta assumes a steroidogenic role to maintain pregnancy. Placental leucine aminopeptidase (PLAP) is localized in the placental villi, and the activity of the enzyme is used as a screening test for placental function in humans because this protease reflects placental development.[16]

A few studies on non-human animals have shown that PLAP concentration was high during pregnancy in sheep[17] and mice.[18] In many species, estrone-3-glucuronide (E_1G) and pregnanediol-3-glucuronide (PdG) are abundant metabolites of estrogen and progesterone excreted into urine, which accurately reflect changes in circulating hormones and the maintenance of pregnancy, respectively.[19-21]

Therefore, it is important to measure the concentrations of these hormone metabolites in urine to assess the endocrinological changes during pregnancy. In this study, urinary PLAP, E_1G, and PdG concentrations were compared among four female Bornean orangutans who gave either a stillbirth or live birth.

2 | MATERIALS AND METHODS

2.1 | Humane care guidelines

Four female Bornean orangutans were used. They were fed fresh fruits and vegetables, and water was available ad libitum. The living space was divided into separate indoor sleeping rooms and an outdoor exhibition. All procedures and management of the orangutans were in accordance with the Code of Ethics of the Japanese Association of Zoos and Aquariums (http://www.jaza.jp/jaza_pdf/library_jaza/atsukai.pdf) and the management guidelines for the orangutan published by each institution. Also urine sampling was conducted in compliance with the Guidelines for Care and Use of Nonhuman Primates (Version 3) established by the Primate Research Institute of Kyoto University, Japan.

2.2 | Animals

Urine was collected from four females. Females A (International Studbook No. 2074), B (No. 3330), C (No. 2525), and D (No. 1327) were kept in the Kobe Municipal Oji Zoo, Tama Zoological Park, Asahikawa City Asahiyama Zoo, and Osaka Municipal Tennoji Zoological Gardens, respectively. Female A has given birth twice: she was 23 and 28 years old at the 1st (A-1) and 2nd (A-2) deliveries, respectively. At the first pregnancy (A-1), she was primipara. Similar to female A, female B was primipara and 12 years old at her first delivery, she was young. On the other hand, females C and D were 23 and 39 years old (estimated) and gravida three and four, respectively.

2.3 | Hormone and protease assays

Urine supernatant was used for the assays. Urine samples were stored at −20°C until use. The samples were collected during the pregnancy period: female A-1, between August 6, 2008 and April 2, 2009 (n=125); female A-2, between March 10, 2014 and November 11, 2014 (n=69); female B, between May 7, 2012 and November 14, 2012 (n=46); female C, between June 2, 2014 and February 5, 2015 (n=92); and female D, between May 10, 2009 and December 19, 2009 (n=133).

Except for female B, urine samples were collected from the final copulation day until the delivery. In female B, sampling was started 2 months after the final copulation. As for the measurement of PLAP concentration, the samples collected two to four times a month were used: female A-1, n=22; A-2, n=32; B, n=33; C, n=41; and D, n=31.

Urine concentrations of E_1G and PdG were assayed using an enzyme immunoassay using the same methods and reagents as described by Hama et al.[22,23] The polyclonal E_1G antiserum (FKA-224E; Cosmo Bio Co., Ltd., Tokyo, Japan) was raised in rabbits against estrone-3-glucuronide-BSA and subsequently cross-reacted with 100% estrone, 170% E_1G, 25% estrone-3-sulfate, 1% estradiol, 0.1% estriol, 1.2% estradiol-3-glucuronide, 0.1% estradiol-3-sulfate, 0.05% testosterone, 0.07% 4-androstenediol, 0% progesterone, 0% cortisol, and 0% 17-α-OH-progesterone. The polyclonal PdG antiserum (FKA334E; Cosmo Bio Co., Ltd.) was raised in rabbits against 5β-pregnane-3α, 20α-diol-3-glucuronide-BSA and subsequently cross-reacted with 100% 5β-pregnane-3α, 20α-diol-3-glucuronide; 16% 20α-OH-progesterone; 8.9% 5β-pregnane-3α, 20α-diol; 2.3% progesterone; 0.8% 5β-pregnane-3α, 20α-diol; 0.2% 5β-pregnane-3β, 20α-diol; 0.1% 5β-pregnane-3β-ol-20-one; 0.05% pregnenolone; 0.02% 17α-OH-progesterone; 0.01% 17α-OH-pregnenolone; 0.01% testosterone; 0.01% androstenediol; 0.01% cortisol; and 0.01% corticosterone.

The PLAP assays were performed using commercial ELISA kits (leucyl/cystinyl aminopeptidase ELISA Kit SEH723Hu; USCN Life Science, Wuhan, China), according to the manufacturer's protocol. The protocol explained that the PLAP assay has a high sensitivity and excellent specificity for detecting leucyl/cystinyl aminopeptidase, and no significant cross-reactivity or interference between leucyl/cystinyl aminopeptidase and their analogs was observed.

Absorbance was measured using a microplate reader (Sunrise, Rainbow RC-R; Tecan Japan Co., Ltd., Kanagawa, Japan) at 450 nm. All tests for E_1G and PdG were run in duplicate, whereas all tests for PLAP were run singularly based on the protocol.

Parallel displacement curves were obtained by comparing the serial dilutions of pooled urine samples and each steroid standard preparation (R^2=.99, .96, and .90 for PLAP, E_1G, and PdG, respectively). The intra- and interassay coefficients of variation were 5.4% and 5.9% for E_1G, 4.0% and 2.1% for PdG, and 7.6% and 3.4% for PLAP, respectively.

Urine creatinine concentrations were measured using the same methods and reagents described by Hama et al.[22,23] The concentrations of steroid hormones and protease were calculated as ng or μg/mg of creatinine (Crmg).

The Steel-Dwass multiple comparison method was used to test for differences of PLAP, E_1G, and PdG concentrations among females in each gestational month. A P<.05 was considered significant. This method was conducted using a statistical analysis program, which was operated by the Genome Information Research Center of Osaka University on the Internet (http://www.gen-info.osaka-u.ac.jp/MEPHAS/s-d.html).

3 | RESULTS

Females A-1 and B each gave birth to a male infant, whereas females A-2 and C each gave birth to a female infant. On the other hand, female D gave birth to a stillborn male infant. The female gestation periods were from August 6, 2008 to April 2, 2009 (female A-1, 239 days); March 10, 2014 to November 11, 2014 (female A-2, 254 days); March 7, 2012 to November 14, 2012 (female B, 252 days); June 2, 2014 to February 5, 2015 (female C, 248 days); and May 10, 2009 to December 19, 2009 (female D, 223 days). The gestation period of female D was shorter than that of the other females who gave live births. Implantation bleeding for 7 days from 34 days after copulation was found only in female A-2.

The urinary PLAP, E_1G, and PdG concentrations for the five pregnancies are presented in Figures 1-3, respectively. In addition, Figure 4 shows the differences of median values of the urinary PLAP, E_1G, and PdG concentrations of all females in each gestational month.

The PLAP concentration ranges were 0.53-55.99, 2.15-205.92, 1.14-92.86, 18.97-297.37, and 0.32-35.20 ng/Crmg in females A-1, A-2, B, C, and D, respectively. In females A-1, A-2, B, and C, who gave live births, the PLAP concentration increased gradually until the delivery, although there were variations in the concentrations among females (Figure 1A). In female D, urine PLAP was comparatively lower than that of other females (Figure 1B). However, her median PLAP value in each gestational month was not always significantly lower than that of all other females (Figure 4A). Among the females who gave live births, there were individual differences in the PLAP concentrations.

The E_1G concentration ranges were 0.0026-4.78, 0.0014-6.26, 0.094-2.88, 0.0055-1.63, and 0.0034-0.61 μg/Crmg in females A-1, A-2, B, C, and D, respectively. In females A-1, A-2, B, and C, high E_1G concentrations were maintained until the delivery (Figure 2A). On the other hand, the E_1G concentration was lower in female D than in all other females, and the median value was significantly lower in the 2nd and 6th-8th gestational months (P<.05) (Figures 2B and 4B).

The PdG concentrations ranges were 0.075-34.19, 0.069-26.07, 0.88-33.88, 0.057-17.33, and 0.085-13.69 μg/Crmg in females A-1,

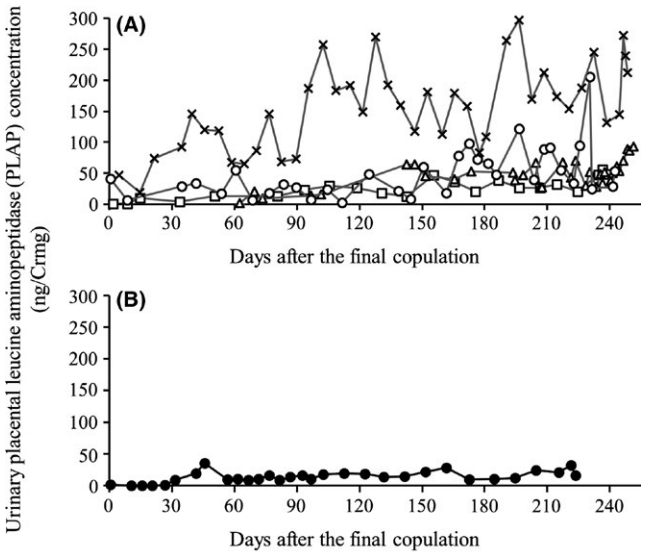

FIGURE 1 Urinary placental leucine aminopeptidase concentrations in female orangutans during five pregnancies (from the final copulation day until the delivery: day 0=the final copulation day). (A) The three females who gave a live birth in four pregnancies (female A-1: □, female A-2: ○, female B: △, and female C: ×). (B) The female who gave a stillbirth (●)

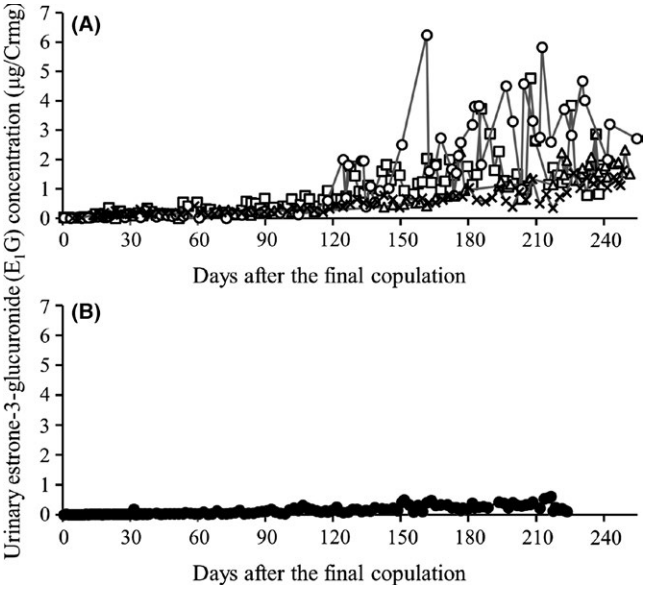

FIGURE 2 Urinary estrone-3-glucuronide concentrations in female orangutans during five pregnancies (from the final copulation day until the delivery: day 0=the final copulation day). (A) The three females who gave a live birth in four pregnancies (female A-1: □, female A-2: ○, female B: △, and female C: ×). (B) The female who gave a stillbirth (●)

A-2, B, C, and D, respectively. In females A-1, A-2, and B, high PdG concentrations were maintained until the delivery; however, in female C, who gave a live birth 248 days after the final copulation, the concentration was lower from 235 days after the final copulation, that is, 13 days before the delivery (Figure 3A). In female D, who gave a stillbirth 223 days after the final copulation, the concentration gradually decreased from 217 days after the final copulation, that is, 6 days before the delivery (Figure 3B). The median values of female D showed non-significant differences compared with other females in all gestational months; on the contrary, the median values of female D were higher than those of female C (Figures 3B and 4C).

4 | DISCUSSION

This is the first study to monitor urinary protease and sex steroid hormone concentrations during pregnancy and to compare the concentration differences between orangutan live births and stillbirths. From the results of the four live birth cases, variations were found in PLAP, E_1G, and PdG concentrations. Regardless of the variations, E_1G concentrations in the stillbirth were significantly lower from the 6th gestational month, and PLAP concentration was comparatively lower during the entire pregnancy period, although there were no significant differences from the concentrations shown in live births.

Female Bornean orangutans give birth after an average gestation period of 245 days (range: 235-270 days).[4,5] In this study, the range of gestation periods in live births was from 239 to 254 days. On the other hand, the gestation period for the stillbirth (223 days) was clearly shorter.

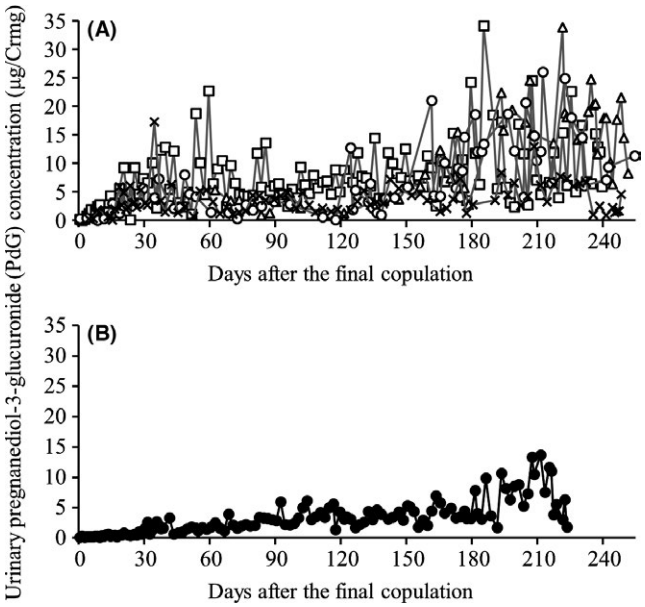

FIGURE 3 Urinary pregnanediol-3-glucuronide concentrations in female orangutans during five pregnancies (from the final copulation day until the delivery: day 0=the final copulation day). (A) The three females who gave a live birth in four pregnancies (female A-1: □, female A-2: ○, female B: △, and female C: ×). (B) The female who gave a stillbirth (●)

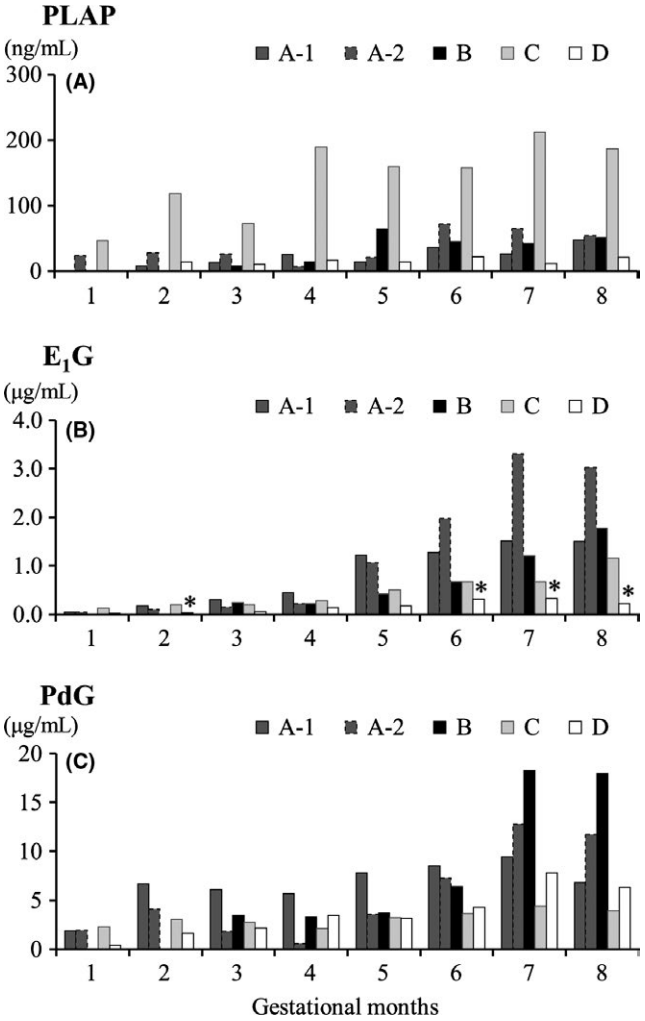

FIGURE 4 Median values of urinary (A) PLAP, (B) E_1G, and (C) PdG concentrations of all the females in each gestational month. Asterisks show the significant differences between female D and all the other females (Steel-Dwass, $P<.05$). PLAP, placental leucine aminopeptidase; E_1G: estrone-3-glucuronide; PdG, pregnanediol-3-glucuronide

The body weight and length of the stillbirth fetus were 1385 g and 26.3 cm, respectively, and edematization was observed in every part of the body (Figure 5). Given this edematization, it was thought that the fetus was already dead before birth.

Fooden and Izor[24] reported that the mean birthweight was 1930 g in male single births (n=12, SD=0.29, range: 1590-2260 g), including both Bornean and Sumatran orangutans, as previously suggested by Seitz.[25] In another study on 1-month-old Sumatran orangutan infants, the average body weight for both males and females was 2100 g, and the average monthly rate of body weight increase was 470 g for males.[26]

In another case, when one Sumatran orangutan male was born by cesarean section at Higashiyama Zoo and Botanical Gardens, Japan, the fetus weighed 1440 g and the placenta weighed 245 g

FIGURE 5 Picture of the stillbirth fetus of female D immediately after the delivery. (A) Lying on his back. (B) Lying on his face

(Hashikawa H. and Kimura K., unpublished observations). Another Japanese zoo recorded a placental weight of 270 g when a female was born (M. Shimizu, unpublished observation). Compared with the above two cases, the placental weight of female D (214 g) was comparatively low. In humans, the relationship between the placenta and birthweight is significant.[27,28] Moreover, maternal age is an important factor that affects birthweight, which tends to increase with increasing maternal age in humans.[28]

Female D was approximately 39 years old at the time of delivery and older than the other females in this study. In captivity, female orangutans above 40 years of age are commonly considered "old".[14,29] Assuming that the relationship between maternal age and birthweight in orangutans is similar to that observed in humans, the birth and placental weights of female D's fetus should have been heavier. However, the placenta and fetus developed poorly during the pregnancy.

In this study, we examined the extent of differences in urinary PLAP, E_1G, and PdG concentrations between live births and a stillbirth. The protease (PLAP) is produced by the placenta. It has been said that, during late pregnancy, the mean daily PLAP activity progressively increases.[30] On the other hand, it was reported that urine PLAP gradually decreased in humans when intrauterine fetal death occurred.[30]

Urine PLAP concentration was lower in female D than in the other females during the entire pregnancy period. Although there were no significant differences in PLAP concentrations, it was suggested that the low PLAP concentration recorded in female D reflected the poor placental development. Actually, given the condition of the stillbirth fetus, that is, the low body weight, the short body length, and the low placental weight, it was considered that the placental role in developing the fetus had weakened. However, due to the high variations in PLAP concentrations among the live births, it was thought that there were no significant differences between the PLAP concentrations in the stillbirth and those in live births.

In humans, Yamahara et al.[16] showed that there was variation among individuals in the normal range of PLAP concentration, although the concentrations increased with weeks of pregnancy. Our results in orangutans also showed that the range of concentrations was wide, and the concentration gradually increased, particularly from mid-pregnancy, that is, from the 4th to 6th gestational months in the case of live birth.

In urine, E_1G and PdG are the main metabolites of estrogens and progesterone secreted from the ovaries and placenta.[31] Therefore, the hormone profiles are believed to reflect the functioning of the ovaries (ie, the corpus luteum of pregnancy) and placenta. Furthermore, it has been shown that these hormones promote increased maternal serum PLAP activities in pregnant rats.[32] In this study, the E_1G concentration in female D was particularly lower during late pregnancy, that is, from the 6th to 8th gestational months. On the other hand, PdG concentration of female D was not significantly different compared with the other females who gave live births.

Estrogens play an important role in the dynamic interchange among the mother, placenta, and fetus that leads to placental and fetal development during pregnancy.[33] In addition, it has been reported that estrogens increase uterine blood flow and supply oxygen and nutrients to the fetus.[34] On the other hand, the producing of fetus adrenal androgens (eg, androstenedione or dehydroepiandrosterone sulfate) that undergo placental aromatization can influence estrogen biosynthesis. It was shown that the estrogen increases were absent when the fetus was dead.[35] Therefore, our results for the low E_1G concentrations suggested that the placental function in female D had decreased during pregnancy and feto-placental dysfunction had occurred, and/or the fetal death causing an interruption in the provision of adrenal androgens had caused a decline in maternal estrogens for any reason.

In another great ape, the chimpanzee (*Pan troglodytes*), Shimizu et al.[36] reported a change of sex steroid hormone concentration, similar to our orangutan stillbirth case. In their study, urinary estrone conjugate concentrations from mid- to late pregnancy were significantly lower for stillbirths than for live births. However, there was no difference in PdG concentration in stillbirths when compared with pregnancies that resulted in live birth. Although it is believed that there are various types and causes of stillbirths, these findings clarified the importance of estrogen concentration during pregnancy.

In conclusion, we determined the PLAP, E_1G, and PdG concentration changes in normal live births and discussed the endocrinological reasons for female D's stillbirth. This study showed that measurement of urinary PLAP and E_1G concentrations may be a useful screening test for orangutan pregnancies.

ACKNOWLEDGMENTS

The authors are grateful to the veterinarians and keepers of Kobe Municipal Oji Zoo, Osaka Municipal Tennoji Zoological Gardens, Tama Zoological Park, Zoorasia Yokohama Zoological Gardens, and Asahikawa City Asahiyama Zoo for their generous assistance. We also thank Mr. Hisashi Hashikawa, Mr. Koichi Chaya, and Mr. Koichi Kimura (Higashiyama Zoo and Botanical Gardens) for their helpful suggestions.

REFERENCES

1. Ancrenaz M, Marshall A, Goossens B, et al. *Pongo pygmaeus*. *IUCN Red List of Threatened Species*. Cambridge: IUCN; 2008.
2. Wich SA, de Vries H, Ancrenaz M, et al. Orangutan life history variation. In: Wich SA, Utami-Atmoko SS, Setia TM, Schaik CP, eds. *Orangutans: Geographic Variation in Behavioral Ecology and Conservation*. Oxford: Oxford University Press; 2009:65–76.
3. Anderson HB, Thompson ME, Knott CD, Perlins L. Fertility and mortality patterns of captive Bornean and Sumatran orangutans: is there a species difference in life history? *J Hum Evol*. 2008;54:34–42.
4. Macdonald D. *The New Encyclopedia of Mammals*. Oxford: Oxford University Press; 2001.
5. Nowak RM. *Walker's Primates of the World*. Baltimore and London: The Johns Hopkins University Press; 1999.
6. Benoit G, Kapar MD, Suhailie K, Marc A. First sighting of Bornean orangutan twins in the wild. *Asian Primates J*. 2011;2:10–12.
7. Marshall AJ, Lacy R, Ancrenaz M, et al. Orangutan population biology, life history, and conservation. In: Wich SA, Utami-Atmoko SS, Setia TM, Schaik CP, eds. *Orangutans: Geographic Variation in Behavioral Ecology and Conservation*. Oxford: Oxford University Press; 2009:311–326.
8. Melanie RB, Judith AB. Growth and development of twin Orangutans. *Int Zoo Yearb*. 1982;22:256–261.
9. Savage A, Snowdon CT. Mental-retardation and neurological deficits in a twin orangutan. *Am J Primatol*. 1982;3:239–251.
10. Galdikas BMF, Wood JW. Birth spacing patterns in humans and apes. *Am J Phys Anthropol*. 1990;83:185–191.
11. Knott C. Female reproductive ecology of the apes. In: Ellison P, ed. *Reproductive Ecology and Human Evolution*. New York: Aldine de Gruyter; 2001:429–464.
12. Kuze N, Dellatore D, Banes GL, Pratje P, Tajima T, Russon AE. Factors affecting reproduction in rehabilitant female orangutans: young age at first birth and short inter-birth interval. *Primates*. 2012;53:181–192.
13. Robson SL, Wood B. Hominin life history: reconstruction and evolution. *J Anat*. 2008;212:394–425.
14. Wich SA, Utami-Atmoko SS, Setia TM, Rijksen HD, Schurmann C, van Schaik C. Life history of wild Sumatran orangutans (*Pongo abelii*). *J Hum Evol*. 2004;47:385–398.
15. Chaya K. *Internal Studbook of Orangutan (Pongo pygmaeus, Pongo abelii, and hybrids)*. Tokyo: Japanese Association of Zoological Gardens and Aquariums; 2012.
16. Yamahara N, Nomura S, Suzuki T, et al. Placental leucine aminopeptidase/oxytocinase in maternal serum and placenta during normal pregnancy. *Life Sci*. 2000;66:1401–1410.
17. Atakisi O, Ozcan A, Atakisi E, Ogun M, Kaya N. Determination of serum leucine aminopeptidase activity in zinc given Tuj sheep during pregnancy. *Kafkas Univ Vet Fak Derg*. 2007;13:17–20.
18. Matsumoto H, Mori T. Changes in cystine aminopeptidase (Oxytocinase) activity in mouse serum, placenta, uterus and liver during pregnancy or after steroid hormone treatments. *Zoolog Sci*. 1998;15:111–115.
19. Fabres C, Zegershochschild F, Altieri E, Llados C. Validation of the dual analyte assay of the estrone-pregnanediol ratio in monitoring ovarian-function. *Hum Reprod*. 1993;8:208–210.
20. Munro CJ, Stabenfeldt GH, Cragun JR, Addiego LA, Overstreet JW, Lasley BL. Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major urinary metabolites as measured by enzyme-immunoassay and radioimmunoassay. *Clin Chem*. 1991;37:838–844.
21. Shimizu K, Udono T, Tanaka C, et al. Comparative study of urinary reproductive hormones in great apes. *Primates*. 2003;44:183–190.
22. Hama N, Kanemitsu H, Sakamoto K, et al. Development and evaluation of a rapid enzyme-immunoassay system for measurement of the urinary concentration of estrone-3-glucuronide in a female giant panda (*Ailuropoda melanoleuca*). *J Reprod Dev*. 2008;54:281–285.
23. Hama N, Kanemitsu H, Tanikawa M, et al. Development of an enzyme immunoassay for urinary pregnanediol-3-glucuronide in a female giant panda (*Ailuropoda melanoleuca*). *J Vet Med Sci*. 2009;71:879–884.
24. Fooden J, Izor RJ. Growth-curves, dental emergence norms, and supplementary morphological observations in known-age captive orangutans. *Am J Primatol*. 1983;5:285–301.
25. Seitz A. Notes on the body weights of new–born and young orangutans *Pongo pygmaeus*. *Int Zoo Yearb*. 1969;9:81–84.
26. Sabapathy D, Nurhafizie H, Rosimah R. *The orangutans of Bukit Merah*. Jalan Bukit Merah: Bukit Merah Orang Utan Island Foundation; 2012.
27. Cross JC. Placental function in development and disease. *Reprod Fertil Dev*. 2006;18:71–76.
28. Sanin LH, Lopez SR, Olivares ET, Terrazas MC, Silva MAR, Carrillo ML. Relation between birth weight and placenta weight. *Biol Neonate*. 2001;80:113–117.
29. Shumaker RW, Wich SA, Perkins L. Reproductive life history traits of female orangutans (*Pongo* spp.). *Interdiscip Top Gerontol*, 2008: 36:147–161.
30. Mizutani S, Hayakawa H, Akiyama H, et al. Simultaneous determinations of plasma oxytocin and serum placental leucine aminopeptidase (P-Lap) during late pregnancy. *Clin Biochem*. 1982;15:141–145.
31. Wilcox AJ, Baird DD, Weinberg CR, et al. The Use of biochemical assays in epidemiologic studies of reproduction. *Environ Health Perspect*. 1987;75:29–35.
32. Mizutani S, Mizutani E. New insights into the role of sex steroid hormones in pregnancy: possible therapeutic approach by sex steroid hormones for the treatment of both preeclampsia and preterm labor. *Exp Clin Endocrinol Diabetes*. 2015;123:159–164.
33. Albrecht ED, Aberdeen GW, Pepe GJ. The role of estrogen in the maintenance of primate pregnancy. *Am J Obstet Gynecol*. 2000;182:432–438.
34. Resnik R, Killam AP, Battagli FC. Stimulation of uterine blood-flow by various estrogens. *Endocrinology*. 1974;94:1192–1196.
35. Walsh SW, Stanczyk FZ, Novy MJ. Daily hormonal changes in the maternal, fetal, and amniotic fluid compartments before parturition in a primate species. *J Clin Endocrinol Metab*. 1984;58:629–639.
36. Shimizu K, Douke C, Fujita S, et al. Urinary steroids, FSH and CG measurements for monitoring the ovarian cycle and pregnancy in the chimpanzee. *J Med Primatol*. 2003;32:15–22.