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RESEARCH ARTICLE

Ultrasonographic Monitoring of Fetal Development in Unrestrained Bonobos (*Pan paniscus*) at the Milwaukee County Zoo

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The bonobo, *Pan paniscus*, is one of the most endangered primate species. In the context of the Bonobo Species Survival Plan®, the Milwaukee County Zoo established a successful breeding group. Although the bonobo serves as a model species for human evolution, prenatal growth is not described in detail. To develop growth graphs, the animals at the Milwaukee County Zoo were trained by positive reinforcement to allow for ultrasound exams without restraint. With this method, the well being of mother and fetus were maintained and ultrasound exams could be performed frequently. The ovulation date of the four animals in the study was determined exactly so that gestational age was known for each examination. Measurements of biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL) were used to create growth curves. Prenatal growth of *P. paniscus* was compared with the data of humans and the common chimpanzee, *P. troglodytes*. With respect to cranial structures, such as BPD and HC, humans have significant acceleration of growth

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compared with *P. paniscus* and *P. troglodytes*. In *P. paniscus*, growth of AC was similar to HC throughout pregnancy, whereas in humans AC only reaches the level of HC close to term. Growth rate of FL was similar in humans and the two *Pan* species until near day 180 post-ovulation. After that, the *Pan* species FL growth slowed compared with human FL. The newly developed fetal growth curves of *P. paniscus* will assist in monitoring prenatal development and predicting birth dates of this highly endangered species. Zoo Biol 30:241–253, 2011.

Keywords: prenatal growth; reproduction; positive reinforcement; ultrasound; pregnancy test

INTRODUCTION

The bonobo (*Pan paniscus*) and the common chimpanzee (*P. troglodytes*) are the only known living species of the genus *Pan*. Both species are closely related to humans and serve as a model for human evolution [Mitteroecker et al., 2004; D'Aout et al., 2004]. Their intelligence and social behavior have been the subject of numerous studies, and the matriarchal society of *P. paniscus* has drawn the attention of ethologists [de Waal, 1995; Palagi, 2006; Parish and De Waal, 2000]. Observations of *P. paniscus* in the wild have become increasingly difficult. Their range is restricted to a small area in central Democratic Republic of Congo, and is threatened by ongoing military actions and the hunting for bush meat [Dupain et al., 2000]. In 1995, worldwide concern over dramatically declining numbers led to the development of an action plan for *P. paniscus* by the Zoological Society of Milwaukee, Wisconsin, in conjunction with the Association of Zoos and Aquariums Bonobo Species Survival Plan®. This science-based plan serves as a guideline for preservation projects, anti-poaching measures, wildlife population assessment, and support for Congolese conservation institutions and indigenous people. However, the difficult political situation in the Congo continues to hamper the protection of *P. paniscus* in the wild.

Research conducted in zoos regarding bonobos, including their husbandry and propagation, has contributed to our understanding of bonobo biology, and behavior and conservation in the wild. To further improve bonobo breeding management, more information regarding prenatal development of this species is needed. Ultrasonography is a non-invasive means to gather information about the well-being and growth of the fetus, gestational age, placental development, and prediction of the date of parturition. Growth graphs for prenatal development have been developed for other great apes, such as the common chimpanzee (*P. troglodytes*) and the gorilla (*Gorilla gorilla gorilla*) [Bourry et al., 2006]. Prenatal growth in the bonobo has been documented in one animal in the second half of gestation [Teare et al., 1996]. Since the time of ovulation in that animal was not known, ultrasound measurements were plotted against time before parturition.

The Milwaukee County Zoo is home to one of the largest bonobo groups in captivity. The facilities and training program for bonobos at this zoo offers excellent opportunities to monitor fetal development by ultrasound. Although the ultrasound exam itself is non-invasive, the use of chemical or physical restraint to allow these examinations may adversely affect the health of the mother or fetus, and may cause physiological changes in the fetus that will affect the results of the ultrasound examination. Our method was to use positive reinforcement behavioral training to allow ultrasound examinations without chemical or physical restraint. On the basis of biometric measurements, growth graphs were developed to establish a reference for physiological prenatal development.

MATERIALS AND METHODS

The bonobos were housed at the Milwaukee County Zoo (Milwaukee, WI). The group size continued to grow over the 8 years of data collection comprised in this study, and by the end of the study in 2006, the group included 8 males and 13 females. The facilities included a 75,000 ft^3 indoor exhibit, a smaller outdoor enclosure, and ten indoor off-exhibit meshed enclosures of various dimensions. All enclosures were interconnected, allowing social fusion and fission of the group throughout the day and separation, as necessary for social interactions, breeding, and medical services. In this arrangement, daily subgroup size typically varied between three to ten animals. All individuals were occasionally separated from the group using positive reinforcement training. The duration of separations were varied to teach the animals patience and reward calm behaviors.

Between 1998 and 2006, four pregnant females who exhibited a regular history of ovarian cycles were monitored by ultrasound. Because three of the four females became pregnant twice during that period, the prenatal development of seven bonobos was documented (Table 1). The date of ovulation was determined by using a human ovulation test kit (OvuQuick® One-Step Ovulation, Nanorepro AG, Marburg, GE), based on the qualitative detection of luteinizing hormone in urine. Some of the bonobos would urinate with a verbal cue from the zookeeper. Otherwise, the bonobo was in the overhead chute and the zookeeper waited for a sample. In either case, the urine was collected from the floor with a syringe. If the female did not mense at the expected time, a human pregnancy test kit based on the qualitative detection of human chorionic gonadotropin in urine (Accustrip® hCG Pregnancy Test, JANT Pharmacal Corporation, Encino, CA) was used to determine if the bonobo was pregnant. Pregnancy was verified by transcutaneous ultrasound later in gestation.

During the ultrasound examination, the pregnant female and often her most recent offspring were directed to a chute in sight of other enclosures, so that all members of the bonobo group could watch the procedure. In the chute, the female was asked to lie in sternal recumbency to allow access to her abdomen. The females were trained for this task through positive reinforcement. The ultrasonographer

TABLE 1. Animals

Animal	Pregnancy number	Number of exams per pregnancy	Gestation length (days)
Ana Neema	1	7	229
	2	6	237
Laura	1	8	239
	2	5	228
Maringa	1	11	208
	2	6	210
Kosana	1	3	214

Fig. 1. Photograph ultrasounding a pregnant bonobo. The bonobo is trained to hold onto the mesh with her hands and to change her position upon request to allow proper probe placement. The ultrasonographer is placing the probe through the chute mesh onto the abdomen of the female. The bonobo is not restrained and volunteers for the ultrasound procedure. Note another bonobo in the background watching the procedure (arrow). The greater part of the bonobo enclosures is behind the camera and the entire bonobo group is able to watch.

stood in the hallway underneath the chute to apply the ultrasound probe through the 2″ × 2″ mesh wire (Fig. 1). Two people participated in the examination. The zookeeper ensured the procedure was conducted safely by having the bonobo hold on to the mesh with both hands, and controlled the animal's positioning with verbal praise, gentle touch, and food rewards. The ultrasonographer applied the probe to the bonobo's abdomen through the mesh and operated the ultrasound machine. If the bonobo seemed tired or uncomfortable, the keeper ended the examination session on a positive note. The female bonobos participated in the ultrasound exams voluntarily and were free to take a break or move away and end the examination at any time.

During examinations, the size and position of numerous anatomical features were documented over the course of pregnancy. These included the heart, lungs, kidneys, gastric vesicle, urinary bladder, facial features in the head, cerebellum, upper and lower extremities, and umbilical cord insertion. Fetal well-being was assessed by measurement of the fetal heart rate (HR) and the detection of fetal swallowing and body movements. The examination also included ultrasonic documentation of placental formation, and quantitative and qualitative assessment of amniotic fluid. To construct fetal growth curves, standard human fetal measurements, such as the size of the embryonic vesicle and crown–rump length (CRL), were measured during the first trimester while head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL) were measured during the second and third trimester.

Ultrasonography

Sonographs used were Philips SD 800 and Philips 2000 (Philips Ultrasound International, Irvine, CA) equipped with a 2.5 MHz sector phased array transducer.

RESULTS

Over the course of this study, we successfully documented fetal development in bonobos by ultrasonography without using chemical or physical restraint. The animals were trained by positive reinforcement to allow access to their abdomen for the ultrasound probe. During the exam, the pregnant female was not visually separated from her group and other bonobos observed the procedure with great interest. The observation of the ultrasound exam by the other bonobos familiarized these animals with the procedure and the females under examination seemed to be calmed by their companionship. Often, the respective female was accompanied in the examination chute by her recent offspring to prevent the anxiety of separation. With this method, we have demonstrated that it is possible to continuously monitor bonobo prenatal development without the risk and potential complications of sedation, anesthesia, or physical restraint. The success of the training method was underlined by the cooperation of the pregnant females. The females with second pregnancies, during the course of this study, readily participated with no retraining.

The absence of sedatives and restraints, and the calm acceptance of the female toward the procedure, enabled us to monitor the fetus without artificially changing fetal activity. The parameters used to monitor fetal activity included the measurement of fetal heart rate (HR) and the observation of intrauterine behavior. Fetal HR did not increase or decrease during the ultrasound exams but remained steady at around 150–160 bpm (beats per minute), indicating that the procedure did not stress the fetus. Fetal activity included movements of the whole body in early pregnancy and more distinct movements, such as thumb sucking, later in pregnancy. All the bonobos monitored prenatally during this study were healthy at birth.

Gestation length in the bonobos examined ranged from 208 to 239 days with an average gestation length of 224 days. Gestation length in literature ranges from 220 to 240 days. For the fetal development charts, we assumed a gestation length of 239 days, corresponding to the longest gestation period in our study.

Five biometric parameters were repeatedly measured during the course of pregnancy to monitor prenatal growth and development (Table 2). CRL was measured during early pregnancy, starting at day 46 post-ovulation (p.o.). With advancing gestation, the measurement of CRL became more difficult owing to the increasing size and movement of the fetus. CRL measurement was not attempted beyond the first trimester. BPD, HC, AC, and FL were measured from day 64 to 65 p.o., up until the prepartum period.

The latter biometric measurements were used to establish growth graphs for *P. paniscus*. Similar to other approaches, we fitted a third order polynomial to all parameters in a least squares sense. This method enabled comparison to

TABLE 2. Ultrasonographic measurements[a]

Biometric parameter	Period of gestation (days)	Range of measurements (cm/bpm)	Total number of measurements
CRL	46–80	1.4–6.8	9
BPD	64–238	1.1–7.3	33
HC	65–238	7.1–25.3	38
AC	65–238	7.4–26.1	39
FL	64–238	0.7–6.1	40
HR	46–238	146–190	40

bpm, beats per minute.
[a]Measurements in centimeters.

TABLE 3. (a) Regression coefficients for different biometric parameters and (b) Milwaukee Formulas: predicting bonobo fetal age from ultrasonographic measurements

Biometric parameter	R^2	$\sqrt{r}$	Regression coefficients *a*, *b*, *c*, *d* for biometric parameters
(a)			
BPD	0.95	1.45	2.64×10^{-7}, -2.39×10^{-4}, 8.57×10^{-2}, -3.36
HC	0.95	2.75	-2.36×10^{-6}, 6.66×10^{-4}, 8.29×10^{-2}, -1.46
AC	0.95	2.75	-2.01×10^{-6}, 6.51×10^{-4}, 6.66×10^{-2}, -0.48
FL	0.96	1.36	-1.06×10^{-6}, 3.59×10^{-4}, 1.59×10^{-3}, -0.65
Biometric parameter in centimeters	**R^2**	**$\sqrt{r}$**	**Regression coefficients *a*, *b*, *c*, *d* for fetal age determination**
(b)			
BPD	0.94	8.39	-0.26, 5.56, -4.02, 66.80
HC	0.92	8.95	1.33×10^{-3}, 1.05×10^{-1}, 3.81, 43.34
AC	0.95	8.21	5.40×10^{-3}, -1.24×10^{-1}, 7.52, 29.14
FL	0.95	8.39	0.80, -6.10, 38.29, 42.09

For the regression, a third order polynomial ($f(x) = ax^3 + bx^2 + cx + d$, with x in centimeter) has been used. R^2, coefficient of determination; r, 2-norm of residuals; BPD, biparietal distance; HC, head circumference; AC, abdominal circumference; FL, femur length.

earlier derived regression formulas for humans and chimpanzees. In an analogy to the "Hadlock Formulas" for fetal age determination in humans, we named the newly developed equations for fetal age determination in *P. paniscus* the "Milwaukee Formulas." The coefficients and statistics of the individual fits are summarized in Table 3. We fitted a third order polynomial to solve biometric measurements (Table 3, part a) and fetal age (Table 3, part b). The graphs for the different biometrical parameters are displayed in Figures 2 and 3.

A gradual increase of BPD and HC in the bonobo was observed with advancing gestation that decelerated shortly before term. On the contrary, FL displayed a constant growth rate until approximately day 180 p.o. and then decreased. The growth rate of AC showed a similar acceleration as the growth of HC, but did not decrease toward term.

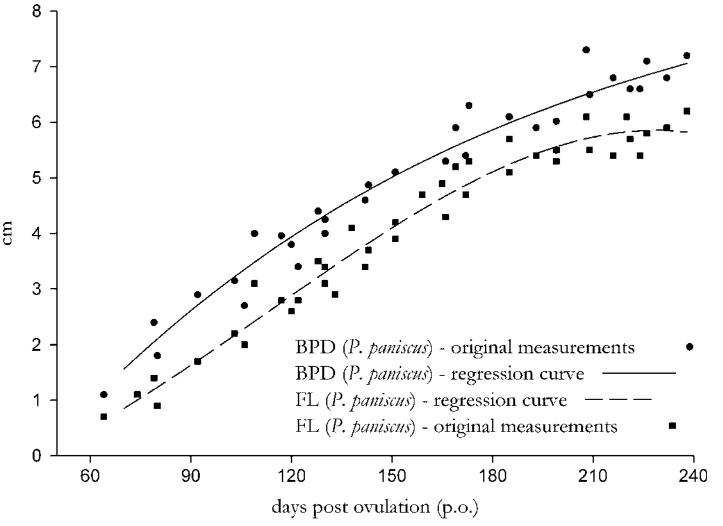

Fig. 2. Regression curves of biparietal diameter, BPD (continuous line), and femur length, FL (dashed line) of *P. paniscus* fetuses derived from our ultrasound data. For the regression, a third order polynomial has been used (Table 3, part a). Original measurements are indicated as dots (BPD, round dots; FL, square dots).

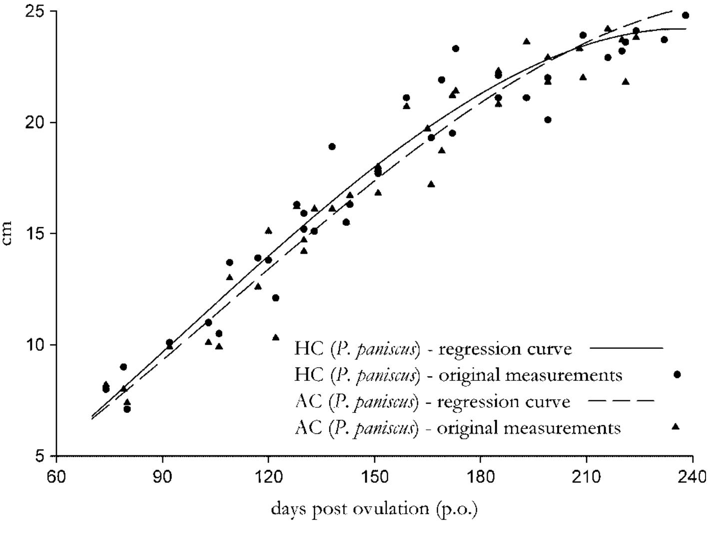

Fig. 3. Regression curves of head circumference (HC; continuous line), and abdominal circumference (AC; dashed line), of *P. paniscus* fetuses derived from our ultrasound data. For the regression, a third order polynomial has been used (Table 3, part a). Original measurements are indicated as dots (HC, round dots; AC, triangular dots).

DISCUSSION

Our study showed that ultrasonography is a safe and non-invasive tool that can be used without prior sedation, anesthesia, or physical restraint to monitor prenatal development in the bonobo, *P. paniscus*. As a result of the positively reinforced behavioral training, chemical or physical restraint of the animals was not necessary. Fetal behavior was thus not influenced by the ultrasonic examinations, and species-specific movements could be documented.

A human pregnancy test kit in combination with a complete documentation of the individual cycle histories was used to determine the date of ovulation, so that gestational age was known for each of the repeated examinations. On the basis of ultrasound measurements of BPD, HC, AC, and FL, the Milwaukee Formulas for prenatal growth in the bonobo were developed. The Milwaukee Formulas will help predict the time of parturition in zoological institutions and improve breeding success.

To demonstrate the differences in prenatal growth in *P. paniscus*, its closest relative *P. troglodytes*, and humans, we compared the Milwaukee Formulas with earlier published formulas for the two other species [Bourry et al., 2006; Hadlock et al., 1984]. The absolute dimensions are displayed in simple growth graphs (Figs. 4A, 5A, 6), showing the respective parameters for different species plotted against gestational age from the day of ovulation until birth. The change of the actual differences in size over time is illustrated by subtracting the Milwaukee Formulas from the respective Hadlock Formulas for humans and the formulas for *P. troglodytes* (Figs. 4B, 5B).

For the published formulas of *P. troglodytes*, an average gestation duration of 229 days was calculated [Bourry et al., 2006]. The average pregnancy duration of humans is 282 days post-menstruation [Kieler et al., 1995] and 274 days p.o. [Mittendorf et al., 1990]. As described above, we assumed a gestation length of 239 days p.o. for *P. paniscus*.

The comparison of the Milwaukee Formulas with earlier published data for *P. troglodytes* [Bourry et al., 2006] revealed that there are only minor differences between the two species. Regarding BPD, *P. paniscus* and *P. troglodytes* show a similar growth progression (Fig. 4A, B), with BPD of *P. troglodytes* being slightly larger (0.2–0.5 cm) than BPD of *P. paniscus* between days 70 and 229 p.o. (Fig. 4B). At the respective birthing times, the size difference would diminish to 0.33 cm, suggesting that the different assumed gestation lengths are responsible for the observed discrepancy.

P. troglodytes also exhibited slightly larger values of FL than *P. paniscus* (Fig. 6). In general, *P. paniscus* is considered to have a more slender build than its relative *P. troglodytes*. However, adult bonobos have relatively longer legs and arms compared with overall body size [Shea, 1981; Zihlman and Cramer, 1978]. In our study, FL in *P. paniscus* was 0.47–0.83 cm shorter than in *P. troglodytes* between days 70 and 229 p.o. This can be accounted for by the relatively longer gestation length of the bonobos in our study, where maximal size is only reached at day 238 p.o. Therefore, the regression formulas for *P. paniscus* and *P. troglodytes* are slightly different. No AC and HC data are available for *P. troglodytes* to compare with our data for *P. paniscus*.

Comparison of parameters correlated to skull capacity, BPD, and HC, in *P. paniscus*, and humans demonstrated that humans exhibit a significantly greater rate of growth. Human BPD is greater than BPD of *P. paniscus* at all times (Fig. 4A, B). Between days 80 and 100 p.o., the size difference is approximately 20 mm, which is quite modest. However, from day 100 p.o. onwards, human BPD exhibits greater growth rate and further extends the difference in size (Fig. 4B). At the time of birth of *P. paniscus*, this difference adds up to almost 18 cm. The same phenomenon can be observed

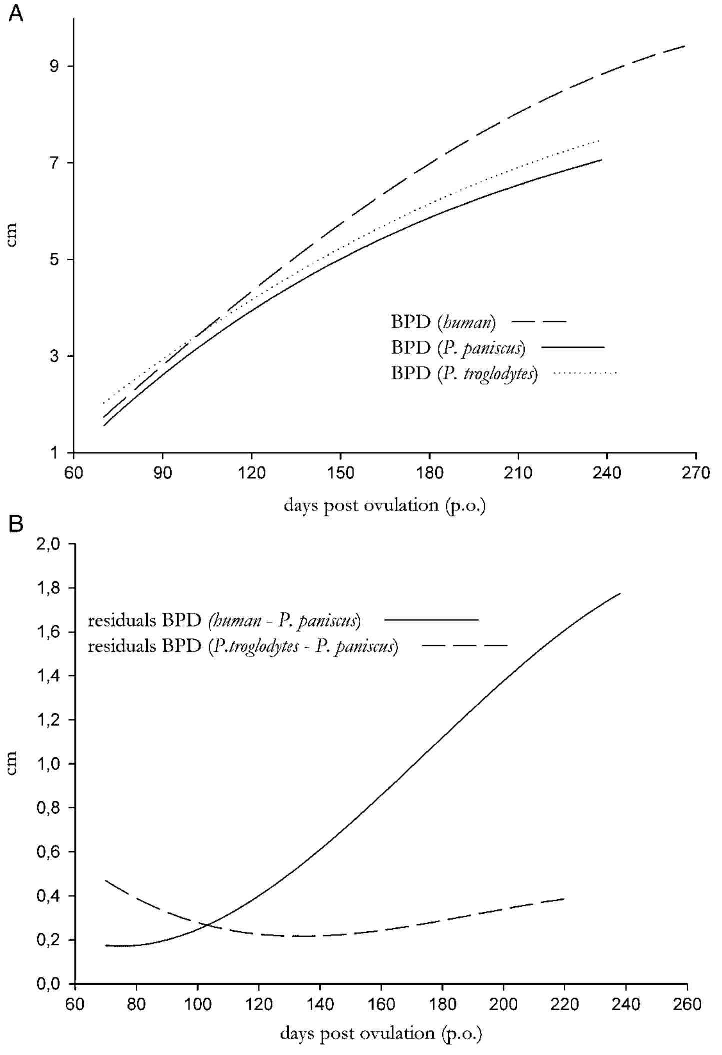

Fig. 4. (**A**) Comparison of prenatal biparietal diameter (BPD) of human fetuses, *P. paniscus* fetuses and *P. troglodytes* fetuses. The regression curve for human fetuses (dashed line) is according to Hadlock, the regression curve for *P. troglodytes* fetuses (dotted line) is according to O'Bourry et al. The regression curve for *P. paniscus* fetuses (continuous line) is derived from our own ultrasound data. Apart from a near constant offset, *P. paniscus* and *P. troglodytes* exhibit a very similar prenatal growth pattern for BPD. The values for human BPD are in the same range as in the two *Pan* species until around day 100 p.o. Human BPD growth then quickly accelerates and exhibits much larger values than in the two *Pan* species. (**B**) Graphs illustrating the actual difference in prenatal growth of BPD between human fetuses and the fetuses of the two *Pan* species. The continuous line results from the subtraction of the *P. paniscus* BPD regression from the human BPD regression (according to Hadlock). Until around day 90 p.o., the values for human BPD are only approximately 0.2 mm bigger than for BPD in *P. paniscus*. Human BPD growth then accelerates and at the time of birth of *P. paniscus* at day 239 p.o., human BPD is almost 18 cm bigger than in *P. paniscus*. The dashed line results from the subtraction of *P. paniscus* BPD regression from *P. troglodytes* BPD regression (according to O'Bourry et al.). As can be seen, *P. troglodytes'* fetuses exhibit a greater BPD over the course of pregnancy than *P. paniscus* fetuses. However, the actual difference in size remains with a range of 0.22–0.44 cm, almost the same.

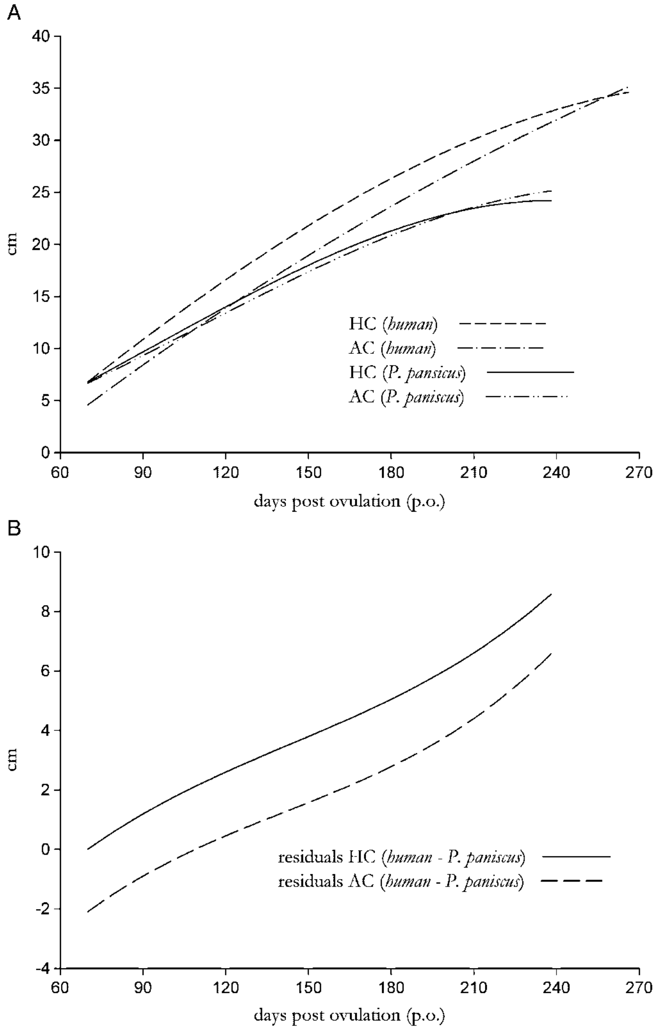

Fig. 5. (**A**) Comparison of prenatal head circumference (HC) and abdominal circumference (AC) of human fetuses and *P. paniscus* fetuses. The regression curves of human fetuses (HC, dashed line; AC, dashed and dotted line) is, according to Hadlock, the regression curves for *P. paniscus* fetuses (HC, continuous line; AC, dashed and double-dotted line) are derived from our own ultrasound data. As can be seen, human fetuses exhibit a greater HC than AC through the greatest part of pregnancy. It is only close to term that AC becomes greater than HC. In *P. paniscus* fetuses, HC and AC have the same starting point and their size does not greatly differ until around day 210 p.o., when AC becomes slightly larger than HC. (**B**) Graphs illustrating the actual difference in prenatal growth of head circumference (HC) and abdominal circumference (AC) between human fetuses and *P. paniscus* fetuses. The continuous line results from the subtraction of *P. paniscus* HC regression from human HC regression (according to Hadlock). The turning point around day 150 p.o. marks accelerated growth of human HC compared with *P. paniscus* HC. The dashed line is derived from subtracting *P. paniscus* AC regression from human AC regression (according to Hadlock). On day 60 p.o., AC in *P. paniscus* fetuses is 2.0 cm greater than in human fetuses. On day 100 p.o., AC in *P. paniscus* fetuses shows the same value as in human fetuses. From that day on, AC is greater in human fetuses than in *P. paniscus* fetuses. The turning point where human accelerated growth begins is around day 150 p.o.

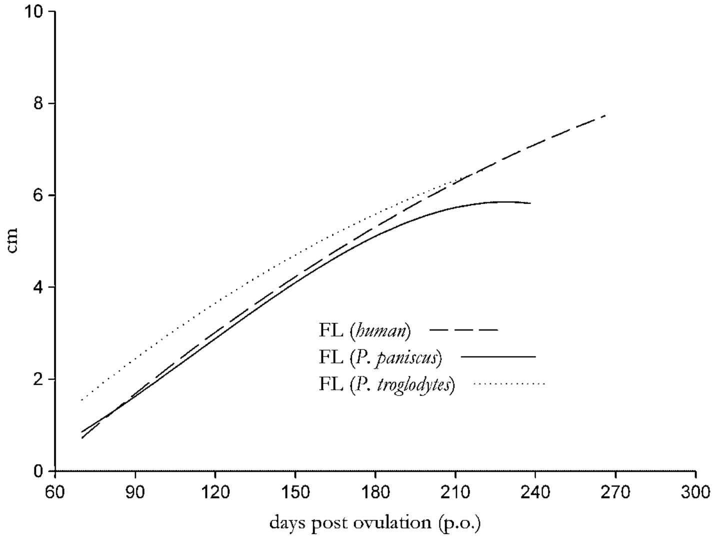

Fig. 6. Comparison of prenatal femur length (FL) growth of human, *P. paniscus*, and *P. troglodytes*. The regression curve of human fetuses (dotted line) is according to Hadlock. The regression curve of *P. troglodytes* fetuses (dashed line) is according to O'Bourry et al. The regression curve of *P. paniscus* fetuses (continuous line) is derived from our own ultrasound data. *P. troglodytes*' fetuses exhibit the greatest values for FL until birth at day 229 p.o. *P. paniscus* fetuses and human fetuses exhibit similar values for FL until around day 190 p.o., after which FL growth in *P. paniscus* decelerates. FL in human fetuses further augments until birth at day 270 p.o.

concerning HC (Fig. 5A, B). The graphs for HC have a common beginning (Fig. 5A), but then quickly separate owing to the greater growth rate of human HC.

Comparison of prenatal growth in *P. paniscus* and humans is particularly interesting because bonobos and common chimpanzees are our closest relatives. In evolutionary terms, the *Homo* and *Pan* genera have evolved from a common ancestor recently, approximately 5 million years ago [Rosenberg and Trevathan, 2002]. Approximately 95% of the human genetic blueprint is identical to the chimpanzee DNA [Britten, 2002]. Most of the difference between human and chimpanzee is not dependent on the genes themselves but on how these genes are regulated [Pollard et al., 2006]. The class of genes that has changed the fastest in humans compared with chimpanzees are the genes that control other genes. A small difference in the activity of a high-level regulatory gene could, therefore, be responsible for significant effects in the development of the brain. Recently, a novel regulatory RNA gene has been demonstrated to show the most accelerated change in the human lineage since the common ancestor of human, bonobo and common chimpanzee [Pollard et al., 2006]. This novel RNA gene is specifically expressed in the developing human and chimpanzee neocortex from gestational week 9 to 19. The strong acceleration of the human cranial growth compared with the cranial growth of the *Pan* species could be associated with the human specific expression of these regulatory genes.

Although humans exhibit an accelerated growth of the head during prenatal development, the human brain is still immature at the time of birth. A newborn child has a cranial capacity that is only 23% of the average adult value. A chimpanzee reaches a comparable value at the fetal age of approximately 159 days [Schultz, 1940]. By the age of two, the chimpanzee infant has reached 84.5% of the average cranial capacity in adults, whereas human children of the same age have only attained 73% of the adult capacity [Schultz, 1940].

The growth of AC showed a similar pattern in humans and bonobos. If AC is compared with the other circumferential parameter, HC, the greatest difference between HC and AC was observed in week 23 p.o. in humans and in week 21 p.o. for the bonobo (Fig. 5A). In humans, the maximal difference between HC and AC was 2.85 cm, whereas in the bonobo HC and AC remained almost equal, with only a small maximal difference of 0.61 cm (Fig. 5B). AC is a parameter correlated to hematopoiesis and liver growth. Augmentation of liver size is due to hematopoiesis initiated after involution of the yolk sac. From our comparison, it can be concluded that growth of AC in both humans and bonobos generally follow the same pattern. However, in humans, AC is greater and the difference between HC and AC is greater owing to overall higher values for HC.

The differences in prenatal growth of FL between humans and *P. paniscus* (Fig. 6) reflect functional differences. *P. paniscus* locomotion is primarily quadrupedal. Human locomotion is primarily bipedal and requires a longer femur [Shea, 1981].

In conclusion, ultrasonographic measurements can provide critical information for growth retardation or other developmental abnormalities. The Milwaukee growth graphs will serve as reference data to evaluate fetal development, estimate fetal age, and predict the date of parturition. The ultrasonographic monitoring of pregnancy without chemical or physical restraint can safely be used to enhance the management of bonobo breeding colonies.

NOTE ADDED IN PROOF

After online publication of this article in January 2010, the authors were contacted by Andrew Teare in regards to bonobo growth curves. This new data has been added in the following places: Abstract [lines 3–4]; Introduction [paragraph 2, lines 9–11]; References [Teare et al. and Vereecke et al.].

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