

2005 Annual Report on Sumatran Rhinos in the U.S.

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February 1, 2006*



Photo by: Dave Jenike, Cincinnati Zoo

Suci - Cincinnati Zoo & Botanical Garden

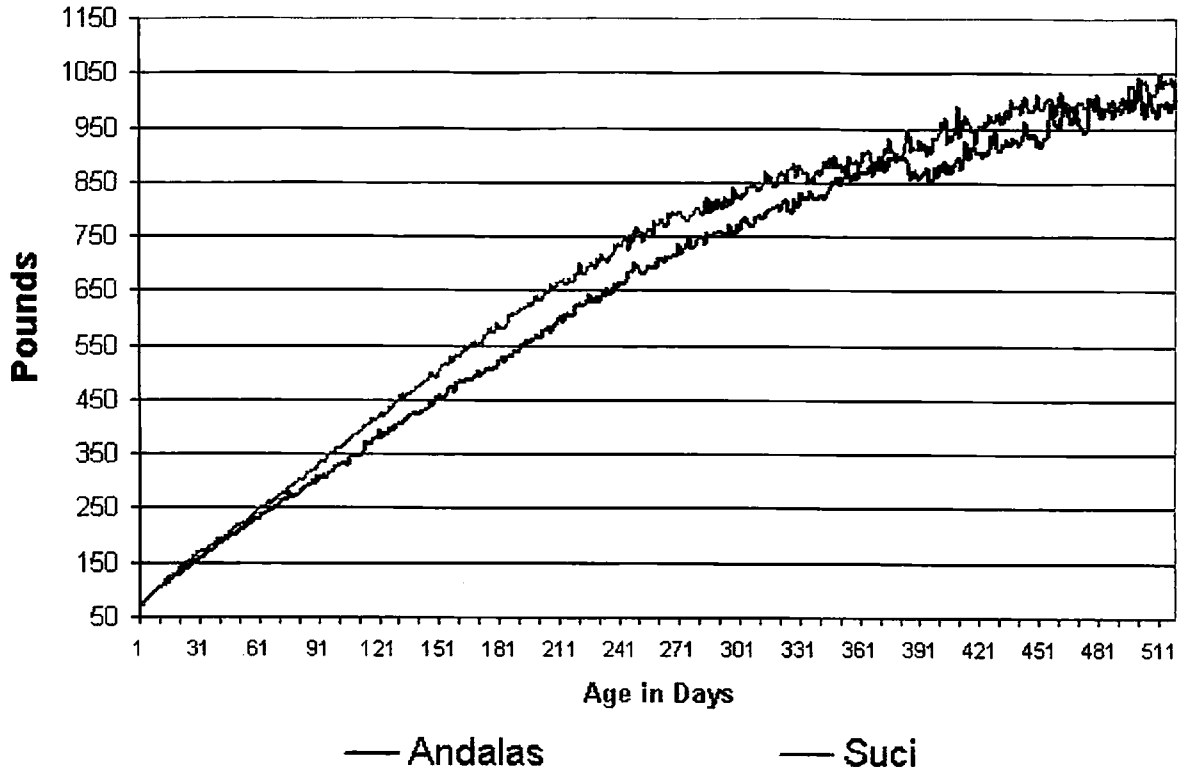


Photo by: Terri Roth, Cincinnati Zoo

Suci next to mother Emi in the mud wallow in her enclosure at the Cincinnati Zoo, July 2005

Suci, the latest U.S. addition to the Sumatran rhino program, celebrated her first birthday at the Cincinnati Zoo on July 30, 2006. Suci has been a very healthy calf gaining weight even a bit faster than her brother Andalas did when he was her age (see weight chart on pg. 3). Suci was weaned when she was about 15 months old and is now maintained separately from Emi. As expected, her weight has leveled off, but we believe she will continue gaining weight slowly until she reaches about 1500-1600 pounds. Suci has been conditioned for blood collection from a vein in the ear and samples are occasionally collected for health assessments throughout the year (see pg. 4 for results). Monthly fecal samples are being collected for hormone analysis as part of a puberty study, and ultrasound exams are slated to begin in the fall of 2006 to watch for early follicular development on her ovaries as she approaches maturity. The only medical issue we have experienced with Suci was irritation of the vulva (swollen, pink, blistered) for a period of time when she was aggressively swishing her tail throughout the day. We think this was the result of her frustration at being weaned and away from Emi. Her vulva was treated daily with an antibiotic soothing salve and has resolved.

Offspring of Sumatran Rhino Sire Ipuh and Dam Emi
Male Andalas DOB 9/13/01 and female Suci DOB 7/30/04
Weight Records
DHB 051231



Andalas and Suci weight records during their first 17 months of life. Andalas was weaned at about 390 days. Suci was weaned at about 450 days.

Cincinnati Veterinary Laboratory, Inc.
 Boob test results
 October 7, 2005

	Equine Normals	Test Results		
		Ipuh	Emi	Suci
Hematology				
WBC X 10 ³	5-15	8.03	9.20	5.44
RBC X 10 ⁶	5.5-10.5	5.02	5.21	6.45
HGB gm/dl	10.0-16.0	13.6	13.9	13.9
HCT %	27-46	40.4	43.3	43.3
MCV fl	34-50	80.5	83.1	67.2
MCH pg	13-18	27.0	26.7	21.6
MCHC gm/dl	35-37	33.6	32.2	32.2
Bands	0-1			2
Neutrophils (SEGS)	22-75	53	55	55
Lymphocytes	25-70	32	35	36
Monocytes	1-7	6	5	5
Eosinophils	0-11	8	5	2
Basophils	0-2	1		

RBC Morphology

Platelet estimate		normal	normal	normal
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Chemistry

SGOT (AST)	184-566	55	80	53
SGPT (ALT)	5-30	9	13	10
T. Bilirubin	0.1-2.0	0.33	0.27	0.24
BUN	10-25	5.5	5.3	2.6
Creatinine	1.2-1.9	0.9	0.6	0.7
BUN/Creat. ratio	5.0-21.0	6.1	8.8	3.7
Cholesterol	75-150	36	60	65
Alk Phos	80-216	36	23	179
Glucose	69-122	71	71	90
Phosphorous	2.0-5.6	2.5	3.2	5.5
Calcium	10.0-13.8	14.5	13.1	13.6
Ca/PO ⁴ ratio	1.8-6.9	5.8	4.1	2.5
T. Protein	5.7-8.4	7.5	7.8	6.7
Albumin	2.3-3.8	3.5	3.6	3.6
Globulin	1.6-5.0	4.0	4.2	2.6
LDH	142-354	589	440	489
Sodium	130-146	133	133	137
Potassium	2.4-5.4	5.2	4.7	4.3
Chloride	93-109	95	98	99
CPK	40-450	513	520	416
GGT	12-45	7	8	6
CO ²	25-35	24	21	25
Anion Gap	(-)-16	19	19	17

Ipuh - Cincinnati Zoo & Botanical Garden

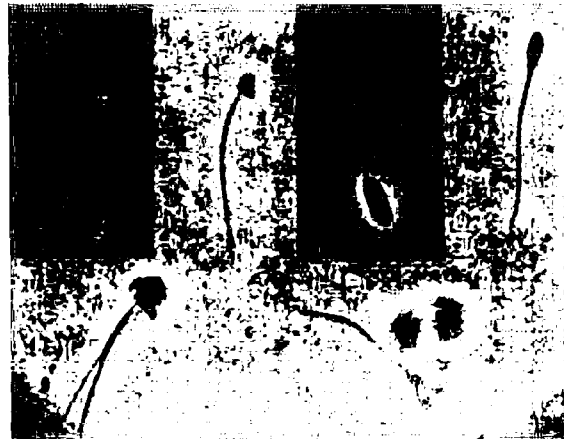


Photo by: Terri Roth, Cincinnati Zoo

Ipuh in his enclosure at the Cincinnati Zoo, February 2006

Ipuh, the only proven Sumatran rhino sire in captivity to-date, continues to do well at the Cincinnati Zoo where he has been maintained since 1991. He was estimated to be 10 years old when he was captured and is therefore over 25 years old today. Although Ipuh has remained relatively healthy since his very serious illness in 1995, he does occasionally go off feed, develop loose stool and become lethargic for a few days at a time. Such an event occurred in November 2005, and it took about 10 days before he completely recovered. Regardless of these bouts, his weight has remained relatively stable and his blood work continues to appear normal (see pg. 4 for blood results and pg. 8 for weight chart). Also of significance to the program is that Ipuh continues to breed. After the most recent mating in January 2006, semen was collected as it drained out of the female and a large quantity of semen containing motile sperm was recovered and cryopreserved at CREW. Most sperm cells are abnormal, but Ipuh has always produced poor quality sperm and, regardless, it has been good enough to produce calves (see pg. 6 for sperm sample details).

Most sperm cells (right) produced by Ipuh are abnormal in structure.



Reproductive Report
Cincinnati Zoo and Botanical Garden
Center for Conservation and Research of Endangered Wildlife
Sperm Cryopreservation
January 5, 2006

Summary Prepared by: Dr. Monica Stoops

<u>Species</u>	<u>SB#</u>	<u>Sex</u>	<u>Name</u>
<i>Dicerorhinus sumatrensis</i>	28	male	lpuh

Reproductive History: Male SB#28 'lpuh' successfully copulated with female Sumatran rhinoceros 'Emi' on 1/5/2006. The pair bred after lots of sparring and chasing. Breeding lasted for 45 minutes (12:30 p.m.- 1:15 p.m.). A post-coital semen sample was collected from 'Emi' approximately 2 hours post-copulation.

Semen Recovery Results:

Ejaculate volume (mL)	154
Osmolality (mmol/kg)	282
pH	8.7-9.0
Sperm Motility	50%
Forward Progression	2.5-3.0
Sperm Morphology	20% normal (43% primary; 37% secondary)
Viability	58%
Acrosome Intact	23%
Total Sperm Count (x 10 ⁶)	616
Total Motile Sperm Count (x 10 ⁶)	308

Semen Processing:

Semen was diluted 1:1 in sperm TALP media (pH 7.4; 290 mmol/kg) and centrifuged (1500g) for 10 minutes to remove accessory fluid and concentrate spermatozoa in the sample. The sperm pellets (7.5mL) from all tubes were combined and diluted 1:1 in EQ extender. Extended semen was placed in a water bath to cool. The semen sample was placed in the walk-in refrigerator at 5:15 p.m.- the water bath read 21°C. Semen was slow cooled by adding ice to the water bath over a 1 hr and 30 minute interval. At 6:50 p.m., the water bath had reached 5.5°C and the first addition of glycerol was added (175uL), followed 20 minutes later with another 175uL glycerol addition. The final glycerol (400uL) fraction was added at 7:30 p.m. Pre-freeze semen evaluation showed 40% of spermatozoa were motile at a 3.0 forward progression. Straws (0.5mL) were frozen in a dry shipper (10 minutes) followed by plunging them directly into liquid nitrogen. The pre-freeze sperm concentration was 20 x 10⁶/mL.

Summary:

A high number of white blood cells were observed in the post coital sample. Due to the low concentration of semen in the sample, semen was diluted 1:1 in extender followed by the direct addition of 5% glycerol after cooling to 5°C. The sample contained a high percentage of morphologically abnormal sperm. Sperm motility declined only slightly during the processing and cryopreservation procedures. A total of 27 straws of semen were cryopreserved from this post-coital sample.

Emi - Cincinnati Zoo & Botanical Garden

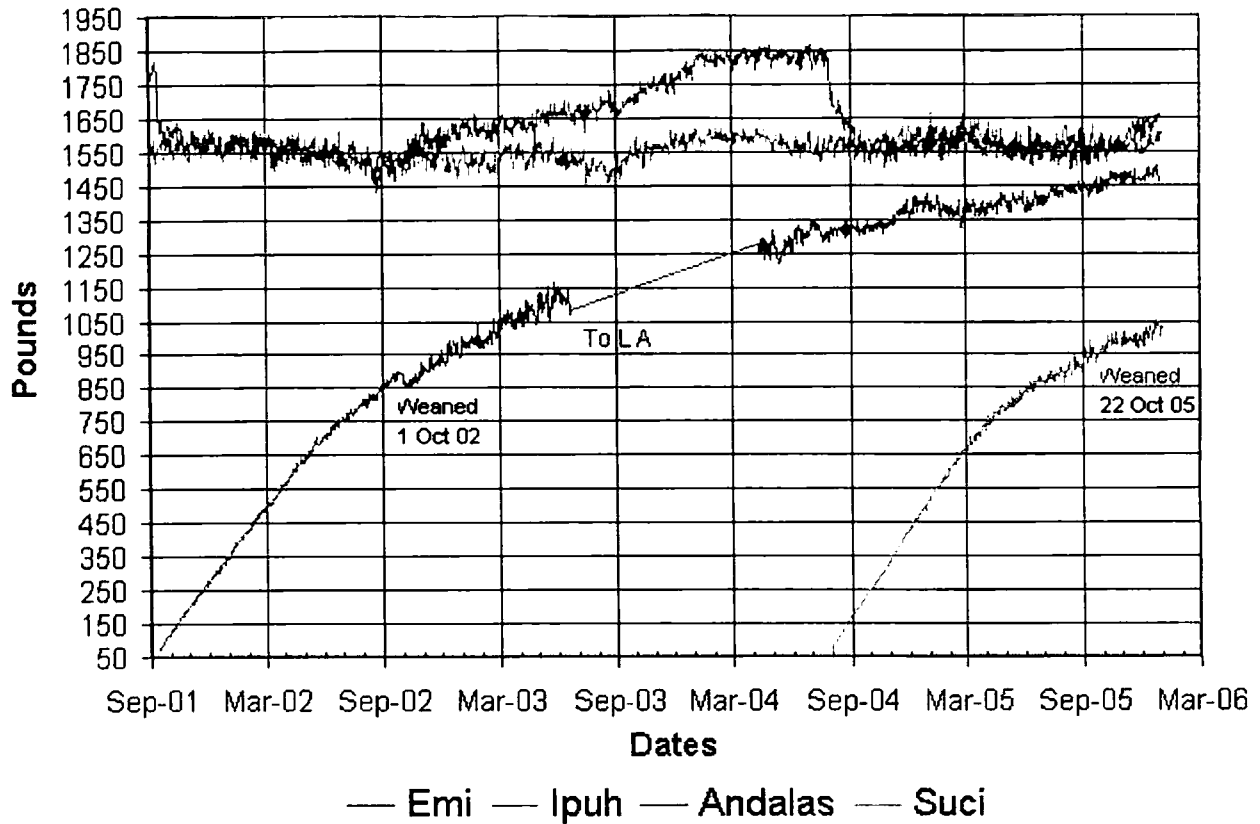


Emi, the only successfully reproducing female Sumatran rhino in captivity to-date, finished rearing her second calf at the Cincinnati Zoo in the fall of 2006. Emi has remained in good health throughout the lactation despite losing weight and body condition (see pg. 4 for bloodwork results). Regardless of how much we increase her browse during lactation, with a calf nursing every 2-3 hours, Emi does not gain weight. Just two months after Suci was weaned, Emi had already gained back about 100 lbs (see pg. 8 for weight chart). As with her first calf, Emi resumed reproductive activity within several months of giving birth. However, we believe it is important to allow her some time to gain weight and improve her body condition between calves. Therefore, there are no attempts to breed her again until her calf is weaned.

In November of 2005, ultrasound examinations were initiated to monitor ovarian activity and determine the optimal time for introducing her to the male Ipuh. The exams were regularly scheduled three times a week and then daily when follicles were progressing and estrus was expected. Because she is an induced ovulator with a highly irregular estrous cycle when not being mated, the first pairing is always difficult to time correctly. However, on January 5, 2006, Emi's ovaries contained several newly developed follicles, and the two were paired for mating. After 90 minutes of chasing and sparring, Emi and Ipuh finally settled down and mated. Numerous cycles with successful matings usually are required before Emi conceives, but she surprised us by becoming pregnant after this single mating. The embryo was first observed by ultrasound 15 days after mating, and a heartbeat was detected 25 days into the pregnancy. Because she carried her second pregnancy to term without the supplemental progesterone she was given during her first successful pregnancy, we do not plan to prescribe any hormone supplement during this pregnancy.

Sumatran Rhino Weights

Dam Emi Sire Ipuh Male Andalus DOB 9/13/01 Female Suci DOB 7/30/04
DHB 051231



Sumatran rhino weight charts over the last five years. Ipuh's weight has remained fairly constant. Emi gains weight during pregnancy but loses weight after giving birth and during lactation. Both calves gained weight rapidly their first year but growth slows after they reach ~850 lbs.

Sumatran Rhino Ocular Syndrome

The only medical issue we still sometimes encounter with Emi is the peculiar ocular syndrome that seems to occur in all captive Sumatran rhinos and appears to be associated with excessive exposure to direct sunlight. A couple years ago, the Cincinnati Zoo spent a great deal of effort and money constructing a unique shade structure over the entire outdoor yards, and these have been very effective protecting the rhinos during our peak summer months. However, the angle of the sun during the shoulder seasons cuts under the shade structure and gets into the yard (see below). Currently, additional renovations are underway to limit sun exposure even during the fall and spring seasons, and the animals are being managed to avoid any exposure by bringing them into their barn during the hours the sun is at that angle.

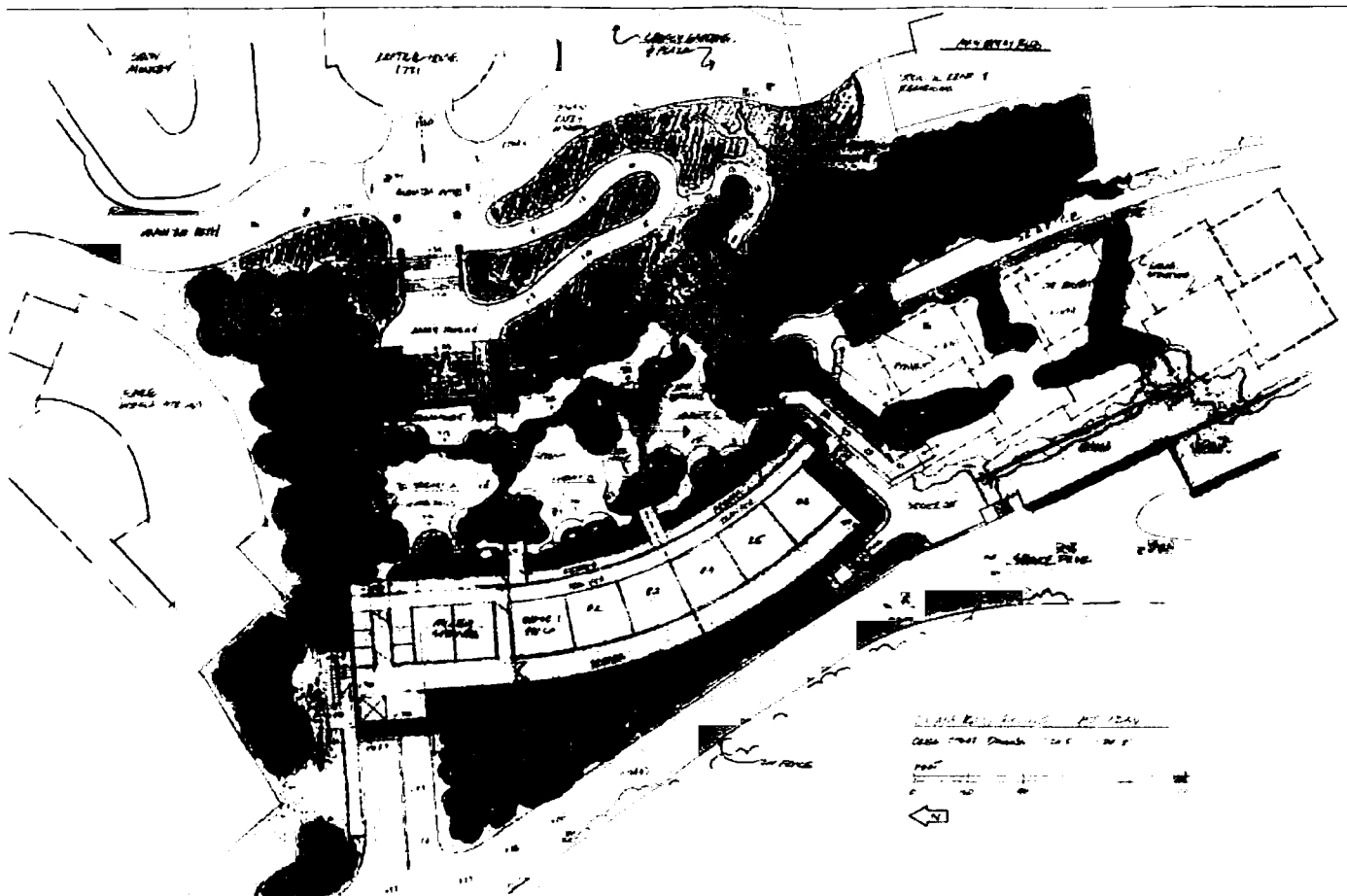


Symptoms of the ocular syndrome include tearing, redness and cloudiness of the cornea. It can take several days or several weeks to resolve.



The large shade structure covers most of the rhino enclosure at the Cincinnati Zoo.

Cincinnati Zoo & Botanical Garden Wild Sumatra Exhibit Draft



On the Horizon

In addition to looking forward to a third calf in the near future, we are also looking forward to a new, enlarged Sumatran rhino breeding and exhibition center at the Cincinnati Zoo (see above). As part of our next capital campaign, we have designed an addition to our current Sumatran rhino holding area that will include a large, climate-controlled tropical greenhouse with rhino yards. The advantages to the animals will be substantial including year-round access to mud wallows and pools, constant tropical environment, protection from direct sunlight and more spacious off-exhibit holding and handling facilities. The rhinos will also continue to have access to their outdoor yards in good weather. The advantages to our visitors will likewise be significant and include year-round live viewing of Sumatran rhinos in an environment that more closely mimics their native land and more opportunities for educational programs about the rhinos and Southeast Asia's amazing biodiversity. Although it may be several years before the construction for this new exhibit begins, it is one of just five major capital projects in our current campaign.

Andalas - Los Angeles Zoo & Botanical Garden

*Report submitted by Jeff Holland, Mammal Curator, Los Angeles
Zoo and Botanical Garden, September 2005*



Andalas in his enclosure at the LA Zoo, August 2005.

Housing

Andalas is housed in a ½ acre enclosure that is terraced. The yard consists of several ficus trees and a large mulberry and Chinese elm tree outside the yard that provides shade for most of the yard. The upper terraced part of the yard has been fitted with a shade cloth structure to provide an additional area with shade and Andalas often uses this area for resting during the day. The lower part of the yard has another shade structure (camouflage netting) over his wallow.

The holding area consists of four holding stalls and two outdoor yards. The outdoor yard nearest the enclosure has been covered with a shade structure to provide the needed shade in this area. The adjacent outdoor holding yard is not covered with shade and thus Andalas is not given access to this area. Both the stalls and outdoor holding yards have a concrete floor and the stalls are lined with rubber mats. Two of the stalls are fitted for cold weather with a heating element in both stalls and all outside openings are covered to prevent drafts. The fourth stall has a crate attached to the side. The crate is outfitted with a scale and is used to weigh Andalas every morning.

Diet

Andalas is currently consuming about 70-90 pounds of fresh browse each day along with 5 pounds of alfalfa/grass mix hay. Intake of hay has varied, but has improved with the addition of alfalfa hay mixed in with the grass hay. His diet is as follows:

AM

1 ½ # Beet pulp soaked in warm water
3 # Mazuri ADF-16 mixed in with beet pulp
20 # fresh browse

PM

60-80# fresh browse
5# alfalfa/sudan grass hay (50-50)

During the training sessions he is given 6 bananas, 6 apples, 4 yams, 4 carrots and 2 papayas.

Browse is collected 5 days a week by the rhino keeper and is stored in a walk in refrigerator. The types of browse currently being fed are listed below:

Staple browse items:

Ficus elastica
Ficus rubiginosa
Ficus macrophylla
Ficus benjamina
Ficus nitida
Ficus retusa
Mulberry
Chinese elm
Kaffir plum

When available browse items:

Ficus watikinsina
Ficus thonningii
Ficus coronata
Ficus cocculifolia
Ficus auriculata
Ficus benghalensis
Ficus lutea
Ficus pumila
Ficus mysorensis
Ficus religiosa

Training

Andalas is given training sessions seven days a week. He enjoys the sessions and seems to look forward to them. All sessions are scheduled at 2:30 pm and several keepers are involved in the process. During the sessions observers are allowed to watch so that Andalas is used to the presence of more than just the trainers. He has grown accustomed to this and is now more likely to react to something if there are not more than two people around. At this time Andalas has been trained to go from point A to point B, to open his mouth for exam, to move from one side of the stall to another and to hold steady. With this we are able to get a daily weight on Andalas, conduct eye and foot exams and attempt blood collection two times a week. Andalas has been very patient and cooperative with the blood collection despite the lack of getting any good blood flow for collection.



Andalas training for blood collection in the new chute at the LA Zoo, December 2005.

Medical Management

Blood collections are conducted while Andalas is standing in a holding position. The veterinarian attempts the blood collection from the foot inserting a needle between the toes. This method has proven effective in black rhinos and in a female Sumatran rhino in Malaysia. Recently, the training program and consistent work with Andalas paid off and blood samples are now being collected regularly while Andalas stands cooperatively in his new chute (see above).

Over the last winter we had some issues with his feet drying out and cracking. This was resolved by being able to allow daily care with the cleaning of the cracks with Nolvasan solution and then having hooflex applied to the cracks. In addition we made certain that the yard was always kept moist. The foot exams are given primarily while Andalas is lying down in his stall. We are able to get him down by rubbing his inner hind leg. Once down we continue with the rubbing of his leg along with feeding of his favorite fruits while another keeper cleans the feet and applies any necessary hooflex.

The first eye problem with Andalas occurred in March 2002 after a ten day period of cloudy skies followed by a sudden sunny bright day. His eye became cloudy with fluid draining. Aggressive treatment by the veterinary staff resolved the eye problem in about a two-month period. A second eye problem occurred in 2003 when either dirt or debris entered his eye. This was cleared up in a short period with the treatment of NaCl drops. As a preventive measure Andalas is treated with NaCl drops twice a day. Additional shade structures have been placed in the yard to cover more of the yard from direct sunlight exposure. In the months from November to April, when we often have cloudy skies in the morning to early afternoon followed by bright sunny afternoons Andalas is given access to his holding yard which is completely shaded. He will always choose to stay inside and thus avoids the period of time when the skies clear and become sunny. From May to November, when we have consistent sunny days Andalas is locked out on exhibit.

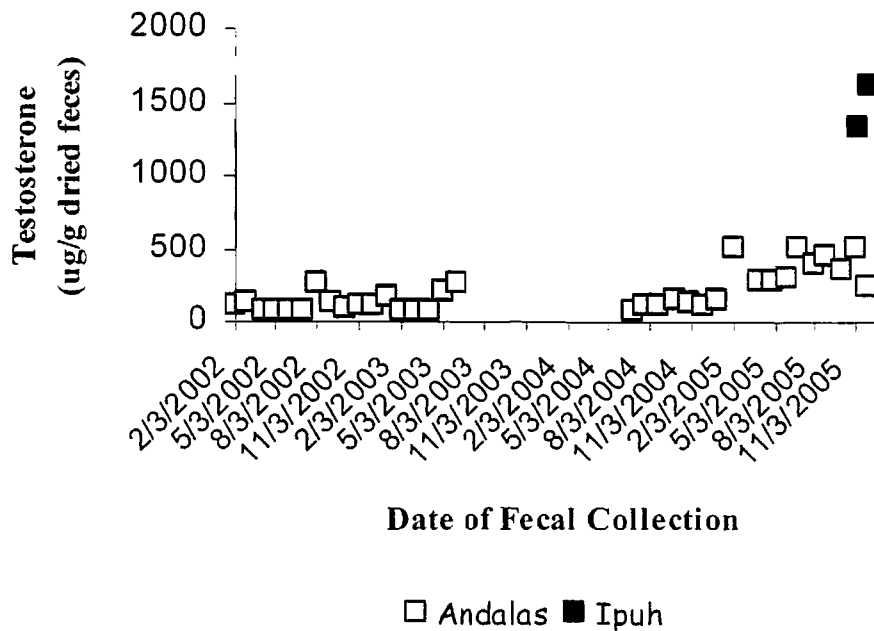
Due to the recent West Nile Virus problem in California Andalas is given a yearly vaccination for West Nile.

Updates on Andalas since the September Report

Monthly fecal samples have been collected from Andalas at both Cincinnati and LA Zoos since he was about 6 months old to monitor testosterone concentrations and determine when he reaches puberty. Recently, the fecal testosterone assay was established and validated by Dr. Monica Stoops at Cincinnati's CREW and the LA Zoo staff sent the latest samples for analysis. For comparison, samples from the adult male Ipuh were also analyzed. Based on the assay results, it appears Andalas is beginning to attain sexual maturity. In 2005, his fecal testosterone levels clearly increased from previous years. Although Andalas's testosterone concentration is still well below that of the adult, breeding male's, it is clear that at four years of age Andalas is progressing towards maturity (see graph below).

Additionally, since blood now can be collected, blood tests can be run regularly to more closely monitor Andalas's health and shifts in hormone concentrations.

**Male Sumatran Rhinoceros
Testosterone**



Rapunzel - Bronx Zoo



Photo by: Terri Roth

Rapunzel in her enclosure at the Bronx Zoo in April 2005

Sadly, the old post-reproductive female Sumatran rhino, Rapunzel, passed away at the end of 2005 at New York's Bronx Zoo. Rapunzel had been maintained at the Bronx Zoo during about half of her 15 years in the U.S. During the other half, Rapunzel was cared for at the Cincinnati Zoo where her reproductive status was assessed and monitored in an attempt to breed her with the male, Ipuh. Rapunzel's age at death is estimated at over 30 years. A brief chronology of Rapunzel's life in the U.S. and the effort to breed her is provided in this report (see pg. 16) as is her necropsy report from the Bronx Zoo (see appendix). Although Rapunzel appeared to have had calves in the wild based on the development of her mammary gland, and may have been reproductively active when first captured, she was clearly an older animal. When monitored extensively in Cincinnati by ultrasound and hormone analysis, Rapunzel never exhibited any signs of reproductive activity on her ovaries or hormonal fluctuations indicative of reproductive cyclicity. An attempt to stimulate reproductive cyclicity through exogenous hormone treatment failed, and a significant (8 cm) uterine mass was detected by ultrasound. Together, these results led us to the conclusion that Rapunzel was post-reproductive, and she was returned to the Bronx Zoo in 2001 to serve as an ambassador for her species for her remaining years. The Bronx Zoo staff took excellent care of this aging rhino until she had to be euthanized on December 22, 2005. Upon her death, her ovaries were sent to the Cincinnati Zoo in an effort to try to retrieve oocytes. Unfortunately, no eggs were found in her ovarian tissue. However, skin biopsies sent to the San Diego Zoo did produce viable cell cultures and these cell lines have now been cryopreserved, so we have not lost the genetic information from this important animal. Summaries of the gamete rescue and biopsy cell culture efforts follow on pgs. 17 and 18, respectively.

Brief Chronology of Rapunzel's Life and Attempts to Breed Her in Captivity

- Aug. 26, 1989 Captured on Sumatra
- Nov. 29, 1989 Arrived at the LA Zoo
- May 16, 1990 Arrived at the Bronx Zoo
- Feb. 6, 1993 Transferred to Cincinnati Zoo on breeding loan.
- 1994-1995 Several introductions with male rhino Ipuh. Some aggression leading to deep lacerations, some mountings, but no successful intromissions.
- Jan. 1995 Ultrasound examinations conducted by Dr. Nan Shaffer detected large uterine mass.
- Dec. 1996 Conditioning for serial ultrasound exams initiated.
- Feb.-May 1997 Intensive ultrasound monitoring. Exams three times each week by Dr. Terri Roth. Uterine mass still present, ovaries small/inactive.
- May 22, 1997 GnRH challenge to stimulate ovarian activity. Good pituitary response, but no follicular development on ovaries.
- May 1997-Feb. 1998 Blood collected twice weekly and analyzed for progesterone at CREW. Progesterone always at baseline. Ovaries remained inactive during thrice weekly ultrasound exams.
- Feb.-Mar. 1999 Blood collected twice weekly and analyzed for progesterone at CREW. Progesterone remained at baseline.
- March 13, 2001 Returned to Bronx Zoo
- Mar. 2001-Dec. 2005 Served as ambassador for her species at the Bronx Zoo.
- Dec. 20, 2005 Rapunzel extremely weak, with a very fast heartrate and extremely labored breathing. Unresponsive to antibiotics, anti-inflammatory agents, and bronchodilators. Condition worsened over next 48 hrs.
- Dec. 22, 2005 Rapunzel euthanized. Ovaries sent to Cincinnati Zoo's CREW for gamete rescue. Skin biopsies sent to San Diego's CRES to develop cell line. Body sent to the American Museum of Natural History.

REPRODUCTIVE REPORT
Center for Conservation and Research of Endangered Wildlife
Gamete Rescue Attempt – December 23, 2005
Summary: by Dr. Monica Stoops

<u>Species</u>	<u>Sex</u>	<u>Studbook #</u>	<u>Name</u>
<i>Dicerorhinus sumatrensis</i>	Female	27	Rapunzel

I. Shipping

Ovaries were shipped from the Bronx Zoo to the Cincinnati Zoo via UPS and arrived late morning on December 23, 2005. Ovaries were placed in separately labeled zip-lock bags. The zip-lock bags were shipped in a Styrofoam container. The ovaries arrived in excellent condition and were slightly cool due to the low ambient temperatures probably encountered during shipment. Attempts to rescue oocytes began immediately at the CREW laboratory.

II. Ovarian Assessment

Both ovaries were removed from their zip-lock bags with gloved hands and a visual assessment was conducted to observe the presence/absence of surface structures. The ovaries were devoid of any external follicular or luteal structures.

III. Oocyte Retrieval

Attempts were made to rescue any smaller internal follicles that might be present. Internal sections of the ovaries were exposed by slicing the ovary with a scalpel blade. A visual assessment confirmed the absence of internal follicles. The ovarian tissue segments were then sliced into small segments within a Petri dish containing washing media (TCM-199; pH 7.35). The tissue segments were minced in TCM-199 media to rescue any oocytes that might be present. The ovarian tissue of both ovaries was very difficult to cut with the scalpel blade. Microscopic evaluation of the media containing the minced ovarian tissue revealed no oocytes.

IV. Summary

The gamete rescue attempt for female Sumatran rhinoceros 'Rapunzel' was not successful in obtaining any oocytes. The female had been monitored by ultrasound many years prior to her death, and at that time, she showed no evidence of ovarian activity. The ovaries of the female at the time of death confirm earlier ultrasound findings, as no evidence of follicular or luteal structures was present. In fact, the ovarian tissue was quite tough in texture upon palpation and when cutting with a scalpel blade. The minced ovarian segments were frozen (-20°C) following the attempted gamete rescue and have been subsequently stored at -80°C.

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CASE REPORT: Sumatran rhinoceros, *Dicerorhinus sumatrensis*
REFERENCE: Lab#14999-7021
DATE: 7 February 2006

History

Biopsy samples were received 23 December 2005 from a female *D. sumatrensis*, "Rapunzel", (ISIS: 901217, Stdbk#27, Lab#14999). Five samples taken from unknown biopsy sites arrived in red top vacuatiners without media and were cleaned and transferred to biopsy vials immediately. On Dec. 24th a portion of each biopsy was diced and digested with collagenase. One T25 flask was set up from each biopsy and incubated at 37° C with 6% CO₂. On Dec. 26th fungus was noted in three of the flasks. The contaminated flasks were treated with a fungizone rinse and three additional flasks were set up from remaining biopsy pieces. Over the next two weeks, four of the eight flasks were discarded due to mold, fungus and/or bacterial contamination. Four separate fibroblast cell lines were accessioned into the CRES Frozen Zoo® cell repository, freeze #7021. The viability and primary flasks were expanded to sixteen T75 flasks that were banked (OAR#3077) along with several remaining pieces of the biopsy for future DNA extraction.

Chromosomal analysis and Diagnosis:

Non-banded karyotype analyses indicate a **normal 2n=82,XX** constitution based on number and morphology. G-band analysis is in progress.

Marlys L. Houck, CLSp(CG)
Associate Researcher – Cytogenetics
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T. Roth
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