

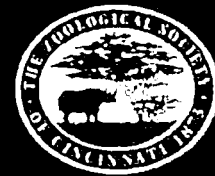
I: SUMATRAN RHINO REPRODUCTIVE
AND HEALTH ASSESSMENT
II: INTENSIVE MANAGEMENT AND PREVENTATIVE
MEDICINE FOR THE SUMATRAN RHINOCEROS



THE TAPEATS FUND



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SUMATRAN RHINO REPRODUCTIVE AND HEALTH ASSESSMENT

II

Way Kambas Sumatran Rhino Sanctuary
Way Kambas National Park, Lampung, Indonesia
Sumatran Rhino Conservation Centre, Sungai Dusun, Malaysia
March 3, 2002 through March 15, 2002

At the invitation of the Directorate General of Forest Protection and Nature Conservation (PHPK), Department of Forestry of the Republic of Indonesia; Musa Nordin, the Director General of the Department of Wildlife and National Parks, Peninsula Malaysia; Mohd Khan, Chairman of the Asian Rhino Specialist Group; Nico van Strien, field coordinator of the International Rhino Foundation; and Dr. Tom Foose, Program Officer of the International Rhino Foundation, a team of scientists visited the Sumatran Rhino Sanctuary in Way Kambas National Park in Indonesia and the Sungai Dusun Sumatran Rhino Conservation Centre in Malaysia. The team worked closely with Drs. Marcellus Adi, Dedi Candra, Muhammad Agil, Nico van Strien, Aidi Mohamad, Mohd Khan, and Steve Romo and the Way Kambas and Sungai Dusun rangers to evaluate the female/male Sumatran rhinos (*Dicerorhinus sumatrensis*) and observe and learn more about the conservation efforts for this species.

The visit was intended to accomplish three major objectives. First, with concerns over a possible persistent hymen in the Way Kambas female, "Bina", the highest priority was to assess this female's condition and develop a therapeutic plan as indicated. In addition, we reevaluated the other females at Sungai Dusun as part of our ongoing attempts to stimulate reproduction in these animals. As part of this work we prepared a GnRH treatment schedule for Minah that was evaluated during our visit and with follow-up hormonal assay. Second, we were planning to perform fertility evaluation of both male Sumatran rhinos; Torgamba in Way Kambas and Ara in Sungai Dusun. We were unsuccessful in this second goal of our trip. However, we did attempt to collect Torgamba using electroejaculation. Because of the risks of recumbent anesthesia, we recommended post-breeding collection of a semen sample from the female as has been successfully accomplished at the Cincinnati Zoo. Third, as part of the establishment of a comprehensive health protocol for the Sumatran rhinoceros, we collected samples for a fundamental data-base of health parameters upon which future health monitoring for captive Sumatran rhinos can be based. Testing included assays for all of the following: serum chemistry, mineral analysis, fatty acid analysis, Vitamin E and A assay, and serum iron analytes. These data are attached as Appendix E and are also included in a separate document entitled, *Intensive Management and Preventative Medicine Protocol for the Sumatran Rhinoceros (Dicerorhinus sumatrensis)*.

Acknowledgements

This conservation mission would not have been possible without the long-term commitment and contributions of the International Rhino Foundation. Dr. Thomas Foose and John Lucas of the IRF have been instrumental in providing support for the captive efforts and field support for this species. The Cincinnati Zoo and Botanical Garden and Fossil Rim Wildlife Center continue to provide support to the Sumatran Rhino program in Malaysia and Indonesia through provision of technical support of the scientific mission. Generous support for this particular visit was made possible by the Tapeats Foundation with special thanks to Annie Graham for her dedication to conservation and health of the rhinoceros. The team wishes to thank the Accuvet / ESC Sharplan Corporation for the generous donation of the Accuvet 50W diode laser and the accompanying support that made this mission possible.

Finally, the team would like to extend their sincere thanks to each of our colleagues in both Indonesia and Malaysia for their generous support of our work and involvement in the Sumatran Rhino conservation program. In particular, we would like to acknowledge the Bogor Agricultural University (IBP) and Taman Safari Indonesia in Indonesia as well as Dr. Vellayan, Zoo Negara, Dr. Wahid Haron and Mr Yap Keng Chee, Universiti Putra Malaysia (UPM). We also would like to thank the Singapore Zoo and Dr. Paulo Martelli for his assistance with the endoscopy procedure we performed on Minah during a prior visit. We look forward to future collaborations and a spirit of cooperation for the benefit of the Sumatran rhinoceros.

Table of Historical Body Weight Measurements
26 April, 2002

<u>Animal Name</u>	<u>Body Weight (kg)</u>
Seputih	736
Mas Merah	586
Ara	574
Rima	554
Panjang	512
Minah	476

Sumatran Rhino Reproductive Evaluation

Sumatran Rhino Sanctuary, Way Kambas National Park, Indonesia

Date of exams: 4 March and 5 March, 2002

<u>Species</u>	<u>SB#</u>	<u>Sex</u>	<u>Name</u>	<u>Age</u>
<i>Dicerorhinus sumatrensis</i>	32	F	Bina	15



History

Bina arrived at Way Kambas in January 1998 from Taman Safari, Bogor, Indonesia. Bina had never been bred in captivity and had an unknown breeding history in the wild. Torgamba, the male rhinoceros at Way Kambas, attempted breeding with partial penetration (15 cm) on multiple occasions. Vaginal examination by Drs. Dedi Candra and Muhammad Agil revealed a persistent hymen with stricture formation forming a 2 cm fistula in the vaginal vault. On January 29, 2002 a repeat vaginal exam was performed where the fistula was enlarged manually to a size of 5 cm. On February 25, 2002, full penetration was achieved with a 9 minute breeding between Torgamba and Bina. Moderate vaginal bleeding occurred and post-breeding digital examination revealed hymen tissue and blood with resolution of the vaginal hymen and stricture.

Reproductive Exam:

Date of first examination: 4 March, 2002

Sedation: None

Ultrasound examination: Normal reproductive tract with no evidence of vaginal stricture, hymen or fluid accumulation. The right ovary contained multiple small follicles. Ovulation was confirmed by documentation of two luteal structures on the left ovary, one 24 mm and the other 22 mm in diameter (Figure 1). One week earlier, Dr. Agil palpated an 18 mm follicle on the left ovary.

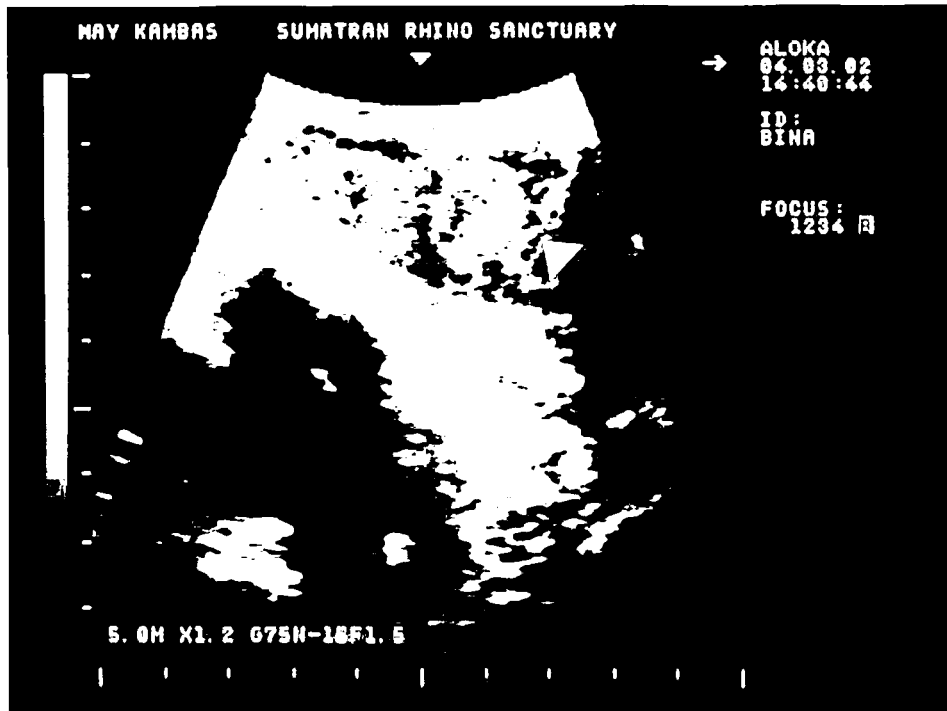


Figure 1. Ultrasound image of two luteal structures (arrows) on Bina's left ovary, indicative of a double ovulation 7 days after breeding by Torgamba.

Date of second examination: 5 March, 2002

Anesthesia/sedation: Butorphanol 40 mg and Azaperone 40 mg IM (see Appendix F for details).

External exam: Normal perineal conformation and external genitalia.

Vaginal exam:

Manual palpation: Vaginal examination revealed normal vestibule and vagina. A tight opening existed between the vestibule and vagina, but this was considered normal.

Speculum exam: A 2.5 cm diameter 40 cm long plastic speculum was used to view the vestibule and vagina. Bruises, submucosal hemorrhage and mild inflammation were discovered at the site of the previous hymen.

Endoscopic exam: An 8 mm diameter 1.0 m long Olympus videoendoscope was used to examine the caudal reproductive tract. The color of the vaginal mucosa was pale without any evidence of irritation or inflammation. Several prominent transverse folds were observed particularly in the dorsal and lateral aspect of the vagina (Figure 2). The cervix was closed and pale with mild bruises detected on the external os. No discharge was present on the external os or within the vagina.

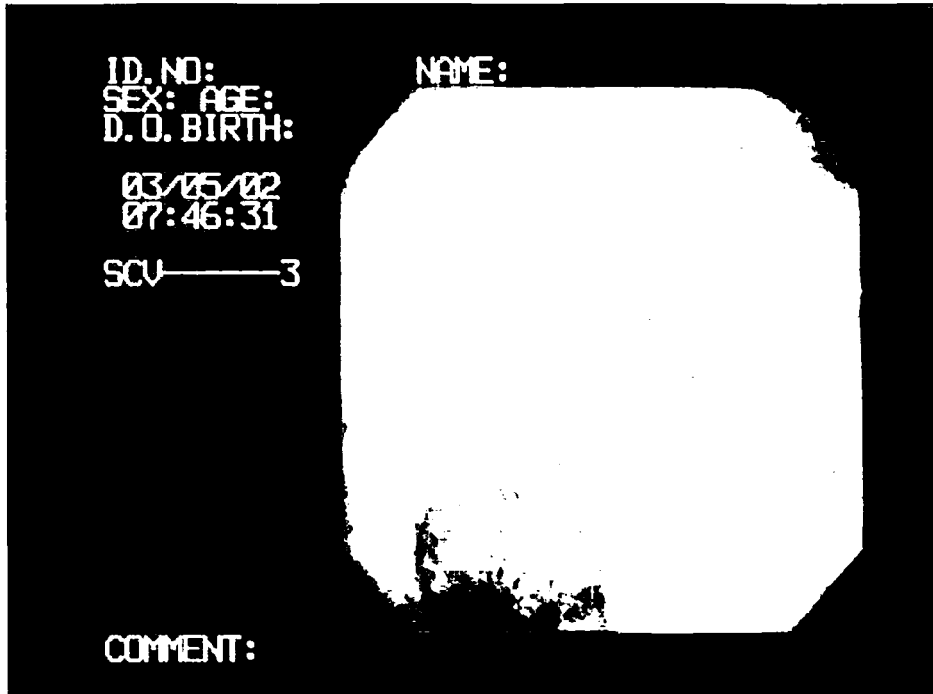


Figure 2. Endoscopic exam of Bina's anterior vagina illustrating the numerous transverse folds adjacent to the cervix. This was considered normal anatomy.

Future Recommendations:

Pregnancy check via ultrasound at approximately 20 days post-breeding. We suggest a comprehensive reproductive monitoring program throughout pregnancy which includes routine ultrasound examination. Please see Appendix A for a detailed discussion of suggested reproductive monitoring.

Sumatran Rhino Reproductive Evaluation

Sungai Dusun, Malaysia

Date of exams: 11 March and 13 March, 2002

<u>Species</u>	<u>SB#</u>	<u>Sex</u>	<u>Name</u>	<u>Age</u>
<i>Dicerorhinus sumatrensis</i>	15	F	Minah	15



Captive years without reproducing: 15

Recent breeding activity: None (previous interest by male in 1998)

Progesterone profile: Baseline progesterone levels since December of 2000.

Hormone manipulations: History of previous use of PRID (3/2/00) and CIDR (3/30/00) with inability to recover CIDR until September 2001 when implant was recovered from Minah's urinary bladder. GnRH treatment using pulse therapy model initiated prior to and continued during the team's visit (see protocol and findings in Appendix D).

Purpose of exam: Evaluation of ovarian function and response to GnRH therapy. Reevaluation of urinary bladder following previous removal of CIDR implant in September of 2001.

Brief Summary of Findings:

Minah's reproductive tract was considered normal on ultrasound with minimal evidence of follicular activity primarily on her right ovary (Figure 1). The GnRH protocol did appear to stimulate some follicular activity based on observed follicle growth during our visit, but these changes did not result in development of a preovulatory size follicle. Results from the serum analysis for luteinizing hormone (LH) concentrations provided strong evidence that the GnRH protocol did stimulate the desired response at the level of the pituitary (see Appendix D for detail). Minah's urinary bladder appeared normal on ultrasound other than for a small area of thickening (Figure 2) at the neck of the bladder which represents a remaining area of irritation or possibly fibrosis from the foreign body that had been lodged in her bladder for ~1.5 years.

We were again concerned with the thin body condition of Minah and the observation of prominent ribs, spine, and scapula. The body condition of this rhino based on previous condition scoring in the black rhinoceros (Adcock, 2001) was estimated to be ~ 2.5 out of 5. Minah had lost an additional 20-25 kg of body weight since the rhino's had been taken off the pelleted ration immediately following Shah's death on January 19, 2002 over concerns of a toxic etiology. The Cargill horse pellet ration was reinstated immediately during our visit as a "dry" room had been constructed at Sungai Dusun to keep pellets in a climate-controlled environment.

Reproductive Exam Details:

MINAH



Figure 1. Ultrasound image of Minah's right ovary with 13 mm follicle (arrows) at Day 7 (March 11, 2002) of GnRH therapy.

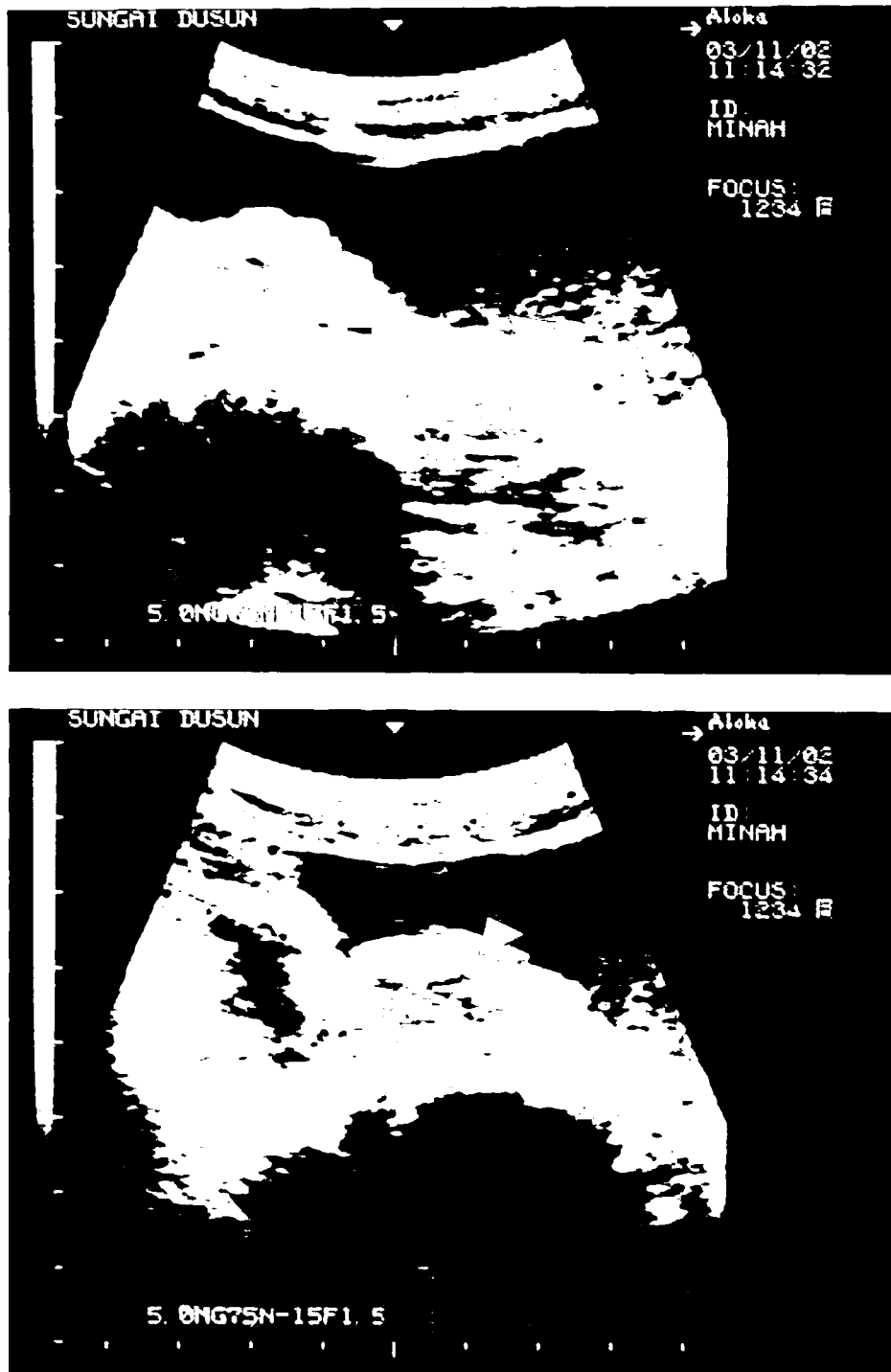


Figure 2. Ultrasound exam of Minah's urinary bladder 6 months following removal of CIDR implant. Normal bladder wall thickness is delineated by arrows (upper image). Note thickened area at neck of bladder which likely reflects a chronic reaction to prolonged foreign body irritation of the bladder mucosa (lower image, arrows).

Future Recommendations:

MANAGEMENT PROTOCOL FOR MINAH (Fall 2002)

May 7, 2002

Briefly, we would like to suggest a three-stage protocol for management of Minah in the foreseeable future as outlined below:

<u>STAGE</u>	<u>TIMELINE</u>
STAGE I. Maintain Minah in her current management situation to continue to improve her body condition.	current through May 31, 2002
<ul style="list-style-type: none">• Panjang should be moved to the 10 acres to decrease rhino density at the barn and provide rest until mass is removed in August.• Continue weekly body weight measurement of Minah.	
STAGE II. Move Minah to the 10 acre facility.	June 1 through July 31, 2002
<ul style="list-style-type: none">• Switch Panjang for Minah in 10 acres to give Minah access to large area, isolation and a change in her environment.• Continue weekly body weight measurement of Minah.• Bring Minah to the barn twice weekly to perform the following:<ul style="list-style-type: none">• Blood sampling 2 times per week as is currently being done to assess cycling while in 10 acres.• Ultrasound examination 1 time per week to detect any evidence of significant follicular growth.• "Teasing" of Minah with Ara 2 times per week during movement to barn for blood collection.	
<p>This is one area where we need to have some additional input from Steve and Aidi. What we would suggest is Minah be placed into an indoor pen in the barn while Ara is allowed to "investigate" in the hallway immediately in front of Minah's pen. Is this feasible by closing appropriate gates? Alternatively, you could attempt to "tease" the animals by placing them in adjacent yards and trying to facilitate interaction at one of the gates, although this may prove more difficult. **STEVE AND AIDI PLEASE COMMENT**</p>	
<ul style="list-style-type: none">• During this period (ie. June 1-July 31) continue attempts to breed Mas Merah, Rima and Seputih with planned implementation of the Regumate protocol as per previous discussions.	

STAGE III. Move Ara into the
10 acre facility with Minah.

August 1, 2002 through undetermined

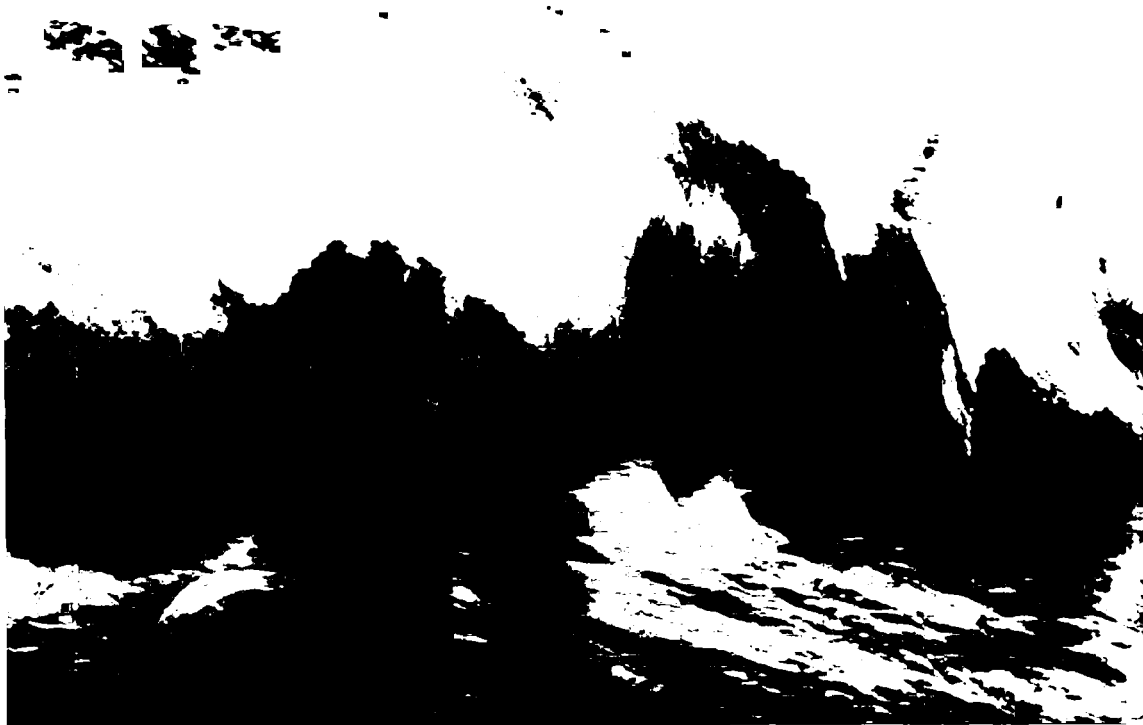
- If Minah shows evidence of cycling (ie. based on hormonal and/or ultrasound data) prior to the August 1 date, then Stage III should be implemented at that time instead of waiting for the August 1 deadline.
- This idea also needs some further discussion, as well as clarification on what may have happened historically when Minah and Ara were placed together in the 10 acres. ****STEVE AND AIDI PLEASE COMMENT****
- Continue weekly body weight measurement of Minah

Sumatran Rhino Reproductive Evaluation

Sungai Dusun, Malaysia

Date of exams: 11 March, 2002

<u>Species</u>	<u>SB#</u>	<u>Sex</u>	<u>Name</u>	<u>Age</u>
<i>Dicerorhinus sumatrensis</i>	7	F	Rima	~23



Captive years without reproducing: 15

Recent breeding activity: Regular breeding at ~21 day intervals (last breeding with Ara on 13 November 2001).

Progesterone profile: Regular 21 day cyclical pattern.

Prolonged Cycles?: One suspected extended luteal phase of ~65 days from July through September of 2001; however, serum collection for P4 assay was inconsistent during this period.

Hormone manipulations: None

Purpose of exam: Reevaluation of uterine pathology observed on previous visit.

Brief Summary of Findings:

The multiple uterine cysts observed in Rima were still prominent and had not changed significantly. A large endometrial cyst was again observed in the right distal uterine horn (Figure 1) and a well-circumscribed 2 cm diameter uterine mass was present just proximal to this cyst. Rima appeared to be in a luteal phase with minimal follicular activity observed. Therefore no attempts were made to introduce Rima during our visit.

Immediate Plan:

Based on Rima's history of irregular cycle lengths and despite the absence of confirmed early embryonic loss, it may be worth empirically placing Rima on a planned treatment with Regumate (Appendix B). If this is done, it would be very important to monitor Rima closely via regular serum progesterone assay as well as routine ultrasound examination. This monitoring will help to confirm pregnancy if there is successful conception as well as make it clear when to terminate the Regumate if Rima does not become pregnant.

Recommendations:

- Continue breeding Rima based on progesterone assay combined with ultrasound data.
 - Begin empirical Regumate therapy based on previous use in Emi at the Cincinnati Zoo. See protocol outlined in Appendix B.
- 3) Initiate post-breeding ultrasound monitoring for early pregnancy as outlined in Appendix A.

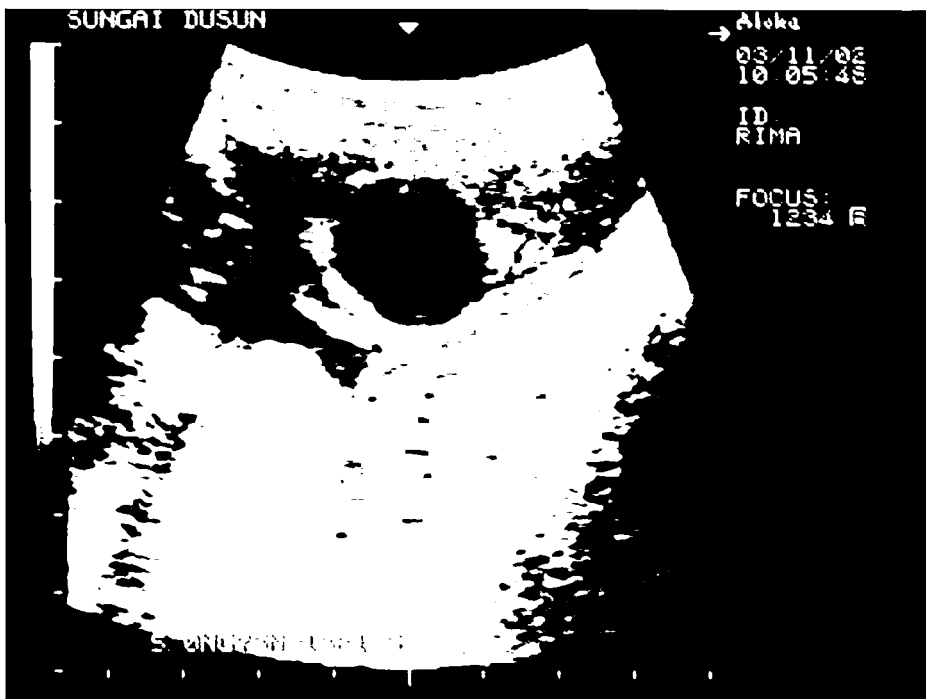
Reproductive Exam Details:**RIMA**

Figure 1. Ultrasound image of large 20 mm diameter endometrial cyst in Rima's right distal uterine horn.

Sumatran Rhino Reproductive Evaluation

Sungai Dusun, Malaysia

Date of exams: 11, 12, 13 and 14 of March, 2002

<u>Species</u>	<u>SB#</u>	<u>Sex</u>	<u>Name</u>	<u>Age</u>
<i>Dicerorhinus sumatrensis</i>	19	F	Mas Merah	~23



Captive years without reproducing: 15

Recent breeding activity: Bred on 3 occasions since September of 2001.

Progesterone profile: Irregular cyclic pattern suggestive of formation and lutenization of hemorrhagic follicles.

Prolonged Cycles?: One confirmed (based on serum P4 assay) extended cycle of ~60 days following the August 2001 breeding.

Hormone manipulations: Attempted CIDR implant plus PGF2alpha in March 2000 without success. Implanted with Synchronate in Left flank in April 2000 for 9 days with no estrus observed.

Purpose of exam: Examine for reproductive function and pathology.

Brief Summary of Findings:

Interestingly, Mas Merah had what appeared to be a 20 mm preovulatory follicle on her left ovary (Figure 1) and based on this information the decision was made to attempt an introduction of this female with Ara on the first day of our visit (March 11, 2002). Mas Merah showed some minor receptivity to Ara, but would not stand for breeding. As illustrated by the changes observed in Mas Merah's follicle during the

succeeding 3 days (Figure 2) the follicle grew larger so it was thought that Mas Merah may be nearing estrus. However, subsequent introductions resulted in declining receptivity to Ara and increased aggression. In retrospect, it appears that Mas Merah was going out of estrus. This was demonstrated nicely by observation of increased follicular echogenicity (Figure 2) which likely represented follicular lutenization. This was also later confirmed by the rising serum progesterone observed during this period.

Mas Merah had a palpable mass that was difficult to image on ultrasound, but located at both the base of the left and right uterine body (Figure 3). The masses were small (2cm x 3cm) and did not appear to be a significant problem.

Recommendations:

- Continue breeding Mas Merah based on progesterone assay combined with ultrasound data.
- Initiate post-breeding ultrasound monitoring for early pregnancy as outlined in Appendix A.
- Consider use of oral progesterone supplement (as was done with Emi in Cincinnati) if early embryo loss is observed or if progesterone levels remain elevated beyond 21 day period in absence of documented pregnancy.

Reproductive Exam Details:

MAS MERAH



Figure 1. Ultrasound image of 20 mm preovulatory follicle on Mas Merah's right ovary. Mas Merah showed some interest towards Ara, but would not allow mounting.

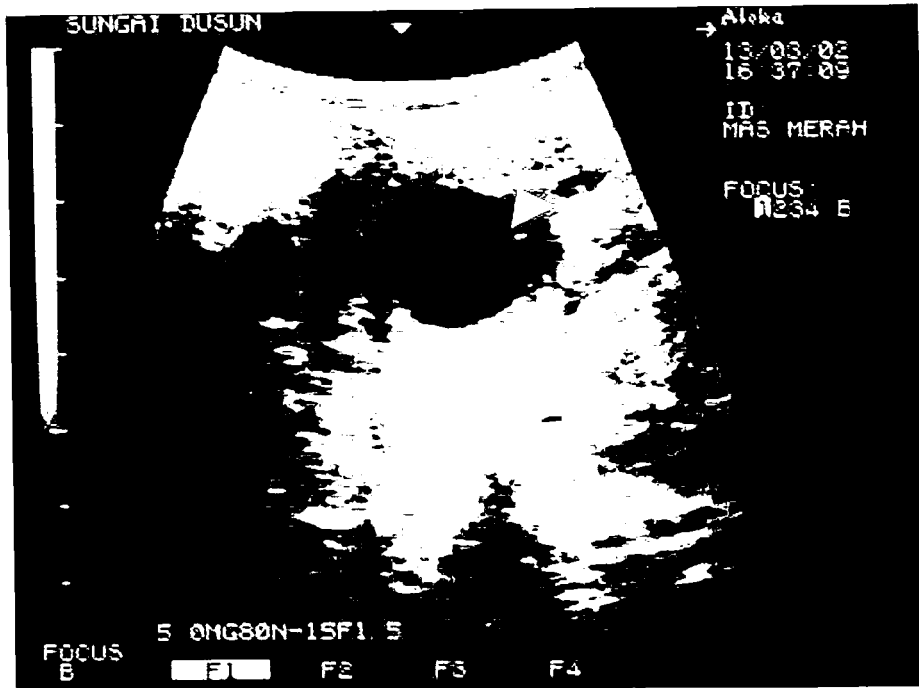


Figure 2. Ultrasound images of follicular change observed in Mas Merah during behavior that resulted in progressive resistance towards Ara. Note increased echogenicity of follicle (upper image, 13 March; arrow) and follicle shape change (lower image, 14 March) that suggests follicular lutenization and increasing progesterone influence that would explain the observed resistant behavior.

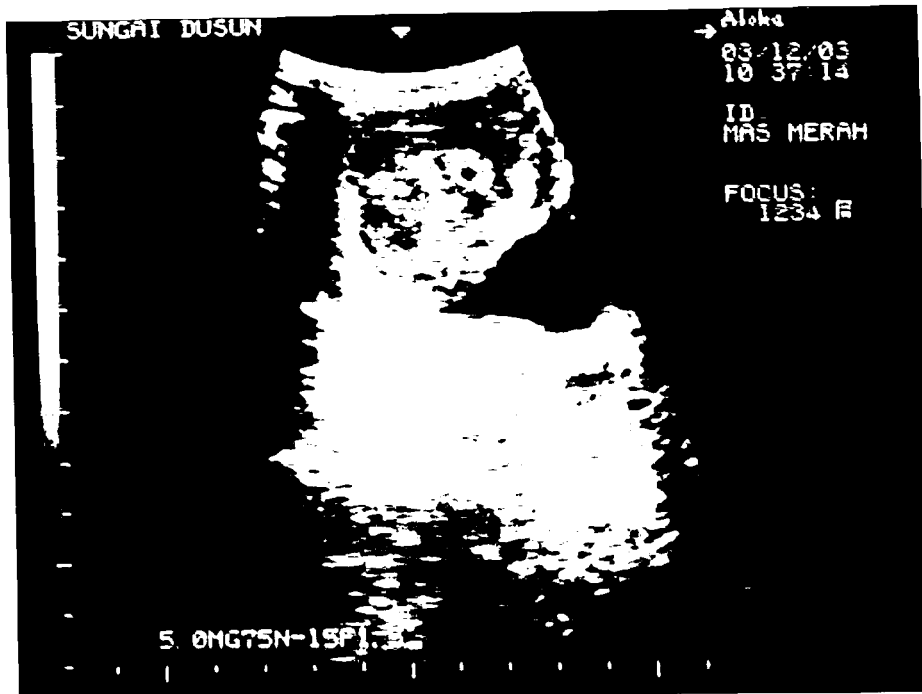


Figure 3. Ultrasound image of palpable mass located at the base of Mas Merah's right uterine horn (arrow). A similar mass was observed on the adjacent uterine horn.

Sumatran Rhino Reproductive Evaluation

Sungai Dusun, Malaysia

Date of exam: 29 August 2001

<u>Species</u>	<u>SB#</u>	<u>Sex</u>	<u>Name</u>	<u>Age</u>
<i>Dicerorhinus sumatrensis</i>	13	F	Panjang	~20



Captive years without reproducing: 15

Recent breeding activity: Last breeding 31 May 2001 Panjang and Ara.

Progesterone profile: Irregular activity suggests some ovarian activity.

Hormone manipulations: Implanted with CIDR containing progesterone and estradiol in March/April of 2000. Bloody discharge was noted in stall following a breeding in October of 2000.

Purpose of exam: Evaluation of reproductive function and pathology.

Reproductive History:

Historically, Panjang has had significant pathology present within her reproductive tract. A mass associated with her right horn was observed as previously reported in March. The mass was approximately 50 mm thick extending from the uterine body into the right horn with evidence of fluid within the lumen (Figure 1). The mass is most likely a tumor, possibly a leiomyoma, leiomyosarcoma, or adenocarcinoma. Other differentials for this mass would include chronic endometritis or pyometra, although this would be less likely given the history and clinical findings.

Brief Summary of Findings:

Under standing sedation (Butorphanol and Azaperone protocol; see Appendix F) a vaginal speculum exam was performed to evaluate the possibility of rebreeding Panjang. Based on her prior history of a large mass of suspected vaginal origin, we were concerned that Panjang may have significant pathology in her caudal reproductive tract that may impede normal copulation or conception. A speculum exam revealed a prominent mass attached directly to the cervix (Figure 2). This pedunculated structure measured approximately 9 cm in diameter. The mass was not removed during this visit as the potential for post-operative complications (cervical adhesions) were considered significant. Therefore, it is recommended that the surgery be performed when appropriate follow-up care can be provided. Also, the need for this surgery at this time is considered less critical since Panjang does not appear to be cycling.

Reproductive Exam Details:

PANJANG



Figure 1. Mineralized mass in base of right uterine horn (arrows).

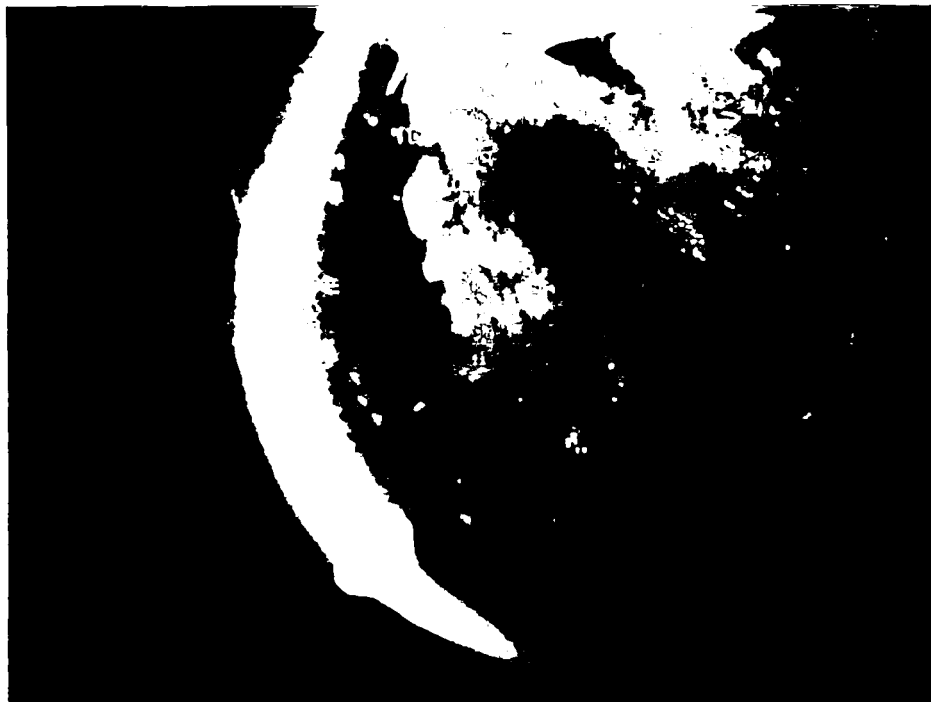


Figure 2. Ultrasound image of 9 cm diameter pedunculated vaginal mass (upper, arrow) attached to the cervix. Endoscopic view of vaginal mass via speculum examination.

Mass Histopathology Findings:

Histopathology of small biopsy samples taken from Panjang's vaginal mass was consistent with a diagnosis of a leiomyoma. There were numerous spindle shaped cells with no active mitotic figures visible. The cells were edematous and showed marked necrosis at the surface of the tissue.

Therapeutic Plan: Panjang should be mixed with Ara on a regular basis to attempt stimulation of reproductive function. However, breeding should NOT be allowed to take place since the vaginal mass is attached to the cervix and would thereby impede normal copulation. This structure was likely the source of Panjang's post-breeding hemorrhage that has been observed in the past.

Recommendations:

- 1) Mix Panjang with Ara to stimulate resumption of reproductive cycling. However, Panjang should NOT be allowed to breed as the mass would interfere with breeding and result in vaginal hemorrhage in Panjang.
- 2) Once Panjang shows evidence of cycling, plan a surgical procedure to remove the vaginal mass from Panjang.
- 3) Evaluate histopathology results from collected sample to help guide future prognosis for reproductive health.

Sumatran Rhino Reproductive Evaluation

Sungai Dusun, Malaysia

Date of exams: 30 August, 4 September and 5 September 2001

<u>Species</u>	<u>SB#</u>	<u>Sex</u>	<u>Name</u>	<u>Age</u>
<i>Dicerorhinus sumatrensis</i>	23	F	Seputih	~23



Captive years without reproducing: 14

Recent breeding activity: Multiple breedings approximately every 21 days since January of 2001. Seputih appears to consistently breed for 2 consecutive days.

Progesterone profile: History of 21 day cyclical pattern.

Prolonged Cycles?: One confirmed (based on serum P4 assay) extended luteal phase of ~50 days following a 2-day breeding in February – April 2001.

Hormone manipulations: None.

Purpose of exam: Examine for reproductive function and pathology.

Reproductive Exam Details:

SEPUTIH

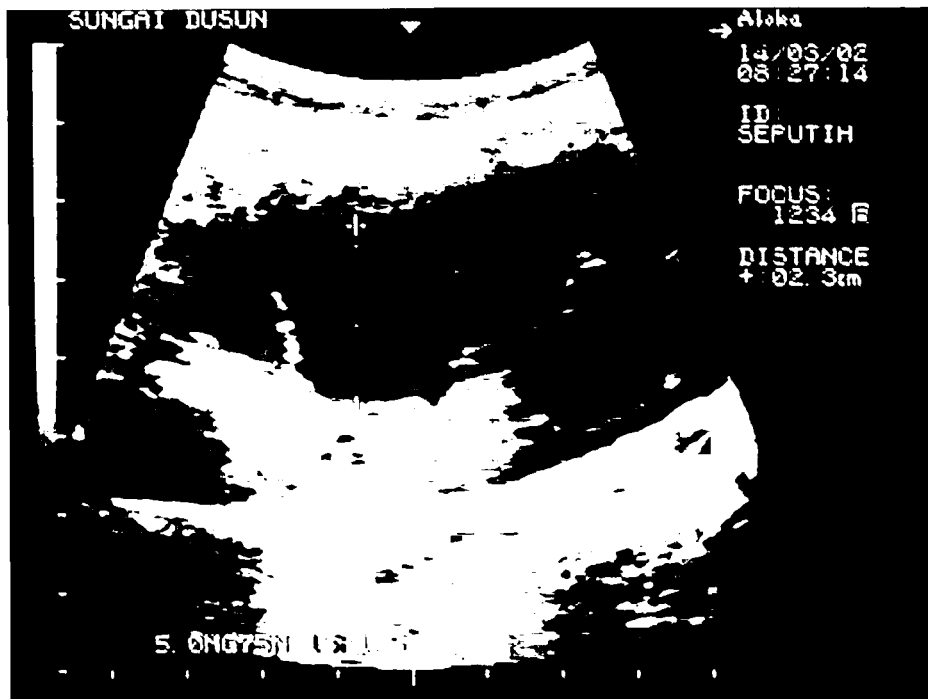
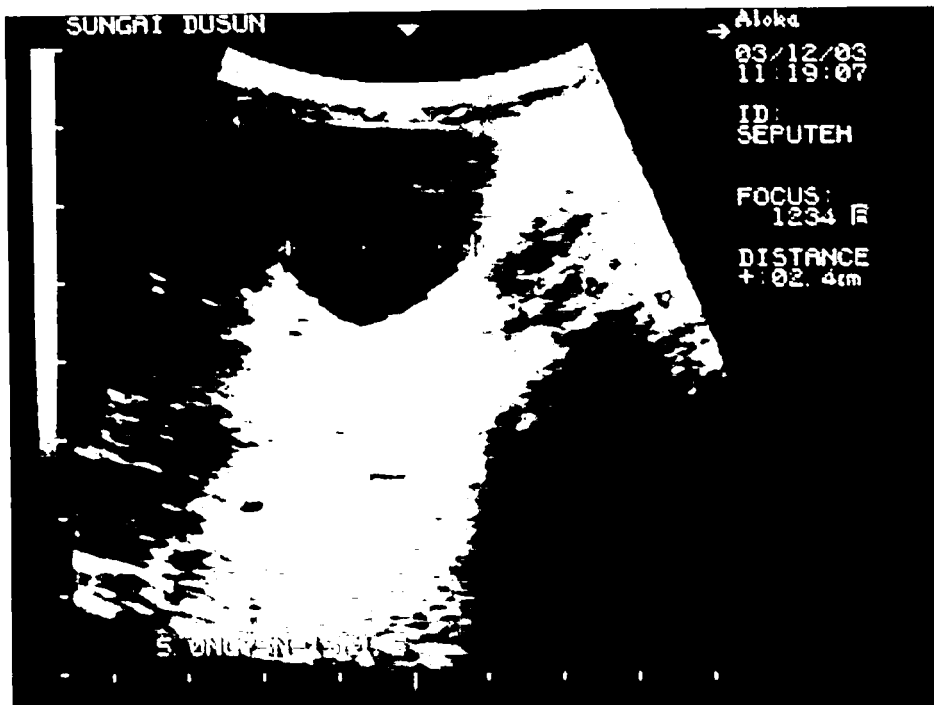


Figure 1. Follicular changes (shape change) consistent with an aging follicle beyond the period of receptivity. These changes corresponded with an alteration in observed behavior that varied from mounting (but no breeding) by Ara on 11 March, 2002 to marked aggression observed during the introduction on 13 March, 2002.



Figure 2. Ultrasound image of multiple vascular masses in Seputih's caudal reproductive tract.

Brief Summary of Findings:

Despite observed pathology, Seputih appears to be cycling normally (Figure 1). Seputih has multiple vascularized masses in her caudal reproductive tract disrupting normal uterine architecture (Figure 2). The largest mass measures 50 mm in diameter; numerous other smaller masses are associated with Seputih's uterine body and uterine horns. The significant pathology in this female associated with the reproductive tract and surrounding anatomy suggests that Seputih's chances of normal embryo development and implantation would be unlikely to occur.

Immediate Plan:

Continue attempts at breeding Seputih with the primary goal of collecting a semen sample from the male, Ara. Based on the extensive uterine pathology, Seputih is considered to be of lowest breeding priority. In fact, once a post-breeding collection from Ara is obtained, it is recommended that this female be transferred to a zoological facility.

Recommendations:

- 1) Continue breeding efforts to attempt post-breeding semen collection of Ara.
- 2) Consider biopsy of masses if pathology progresses to help guide prognosis and decisions regarding health and reproductive fitness.
- 3) Attempt post-breeding Regumate protocol prior to movement to zoo environment.
- 4) If Regumate therapy fails, move Seputih to a zoological facility to reduce rhino density at Sungai Dusun.

Sumatran Rhino Reproductive Evaluation

Sungai Dusun, Malaysia

Date of exam: 14 March, 2002

Species	SB#	Sex	Name	Age
<i>Dicerorhinus sumatrensis</i>	39	M	Ara	~22



Captive years without reproducing: 18

Recent breeding activity: Multiple breedings (n=42) with five different females during the previous 2 years with NO confirmed conceptions.

Progesterone profile: Testosterone levels were similar to a known infertile male, named Shah, at the same location.

Hormone manipulations: None.

Purpose of exam: Examine anatomy and collect reproductive measurements.

Brief Summary of Findings:

Ultrasonographic examination of both testicles did not reveal any obvious abnormalities. Measurements of each testicle were taken for future reference and comparison with other captive male Sumatran rhinoceros (see Ipuh below):

<u>Ara's Measurement</u>	<u>Right Testicle</u>	<u>Left Testicle</u>
Length (mm)	110 mm	110 mm
Diameter (mm)	41 x 42 mm	37 x 40 mm
<u>Ipuh's Measurement</u>	<u>Right Testicle</u>	<u>Left Testicle</u>
Length (mm)	107 mm	119 mm
Diameter (mm)	38 x 38 mm	37 x 37 mm



Figure 1. Cross-sectional ultrasound images with measured diameters of the left testicle (upper image) and right testicle (lower image).

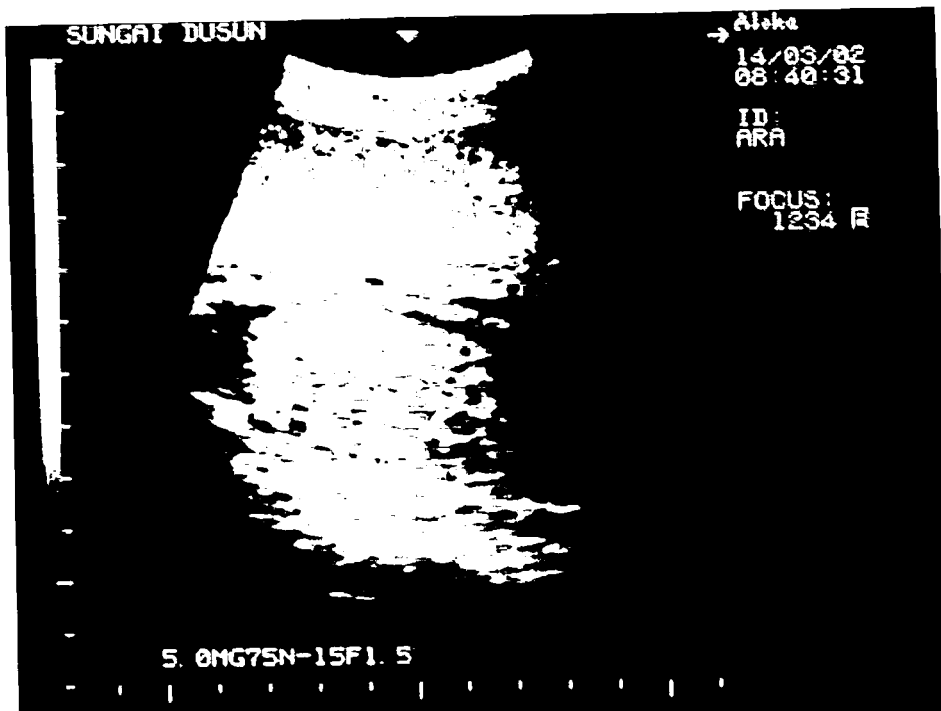


Figure 2. Longitudinal section of both testicles.

APPENDIX A

Proposed Reproductive Monitoring Strategy for Indonesian and Malaysian Captive Sumatran Rhino Breeding Programs

Goals of Ultrasonographic Reproductive Monitoring Program:

<u>Information gained</u>	<u>Management Implications</u>
Preovulatory follicle size (22 to 26 mm size at estrus)	Allows accurate prediction of estrus for accurate timed breeding
Ovulation documentation	Allows confirmation of normal female reproductive function
Early pregnancy documentation	Ultrasound can provide a precise diagnosis of early pregnancy
Early embryo loss	Allows documentation of embryo loss and possible causes and allows for informed decisions regarding potential therapies
Pathology	Allows evaluation of pathology to facilitate management and medical decision-making

I. Ultrasound as a tool for enhanced breeding management

With our current understanding of Sumatran rhino reproductive function describing *Dicerorhinus sumatrensis* as an induced ovulator (Roth et. al., 2001), the use of ultrasound to predict impending female receptivity, ovulation and timed breeding to a male will be, without question, fundamental to future captive reproductive management for this species.

Managed Sumatran rhino breeding in absence of ultrasound or progesterone assay

Without ultrasound or progesterone assay as tools for reproductive management of Sumatran rhinos, daily introductions are necessary in order to avoid missing the short period of female receptivity characteristic of these induced ovulators. However, based on discussions with Steve Romo, we feel strongly that this strategy of forced daily introductions of captive Sumatran rhino with the hopes of eventually finding the day of receptivity and breeding may be counterproductive for the following reasons:

- Increased risks associated with male injuring female
- Potential detrimental and possibly long-term behavioral modifications to subject rhinos secondary to the repeated aggression of forced introductions
- Lost time and effort that could be directed in more productive ways

- Inadvertant missed breedings secondary to staff shortages, male overuse, etc.
- Not consistent with the biology or ecology of this highly solitary and seclusive forest mammal

Prediction of preovulatory follicle for purpose of timed breeding,

Based on previous cyclic pattern (ie. 21 day cyclic period) or use of current serum progesterone assay, ovarian function should be examined via ultrasound in the late diestrus period (day 16-18) in preparation of upcoming female estrus. The preovulatory follicle will enlarge to the size of 22 to 26 mm immediately prior to the period of female receptivity. The follicle diameter will quickly become a highly accurate and quick measure of female receptivity.

Documentation of ovulation and luteal formation

In corroboration with serum progesterone assay, the documentation of ovulation and subsequent luteal formation is essential information to assure normal female reproductive function. This information also allows documentation of ovulation timing, knowledge fundamental to any future advanced reproductive techniques in Sumatran rhino.

II. Ultrasound as a tool for early pregnancy detection and loss

Early pregnancy detection

Post-breeding ultrasound will provide essential information in order to efficiently manage captive rhinos. Pregnant animals can be separated from males to reduce stress and prevent inadvertent attempts to breed pregnant animals that may contribute to pregnancy loss.

Early embryo loss

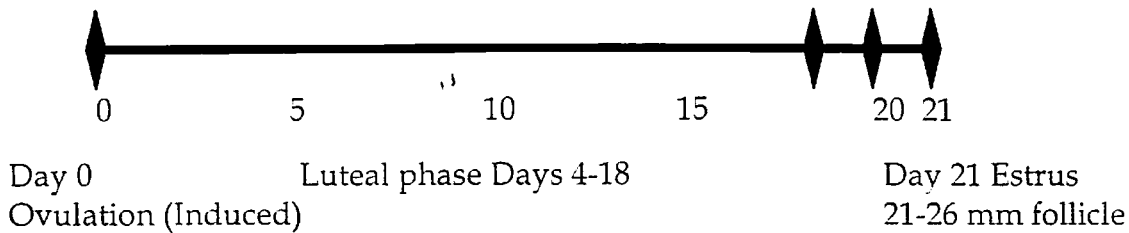
The early embryonic vesicle in the rhinoceros resembles the horse (Radcliffe et. al., 2001; Roth et. al., 2001). The early vesicle can be imaged as early as 14 days post-breeding and its documentation is critical, especially if embryo loss ensues. Without ultrasound the only indication that pregnancy occurred would be an extension of the luteal phase. As previously evidenced in the Sumatran rhino, embryo and fetal loss can be a significant cause of infertility. Perhaps the Sumatran rhino is very susceptible to captive stresses that result in reduced fertility in captive animals - a hypothesis supported by their solitary forest nature and apparent infrequent encounters with other rhinos (particularly males) except during the short period of receptivity and copulation.

III. Ultrasound as a tool to assess reproductive pathology and guide therapy

Captive Sumatran rhinos appear to develop significant reproductive pathology with increasing nonparous time in captivity. The information of reproductive health is critical to dedicating appropriate resources, time and concentration of efforts toward normal animals. Appropriate therapeutic steps can be guided by monitoring reproductive function with ultrasound in concert with other medical testing.

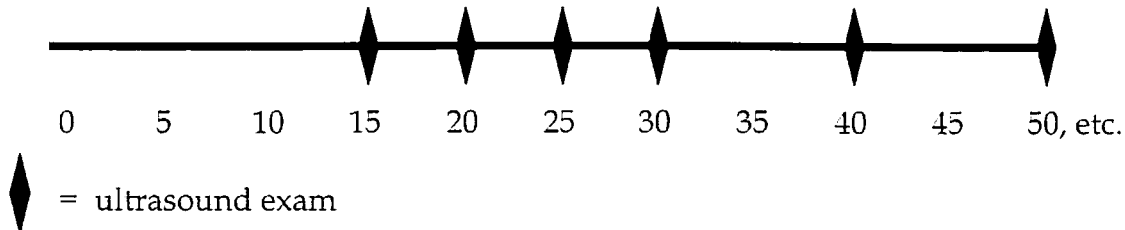
Suggested Guidelines for Ultrasonographic Monitoring of Sumatran Rhino

1) Schedule for ultrasound evaluation of Sumatran rhino estrous cycle,



Ultrasound can be used in conjunction with serum progesterone assay to precisely time male and female introductions for breeding. The serum P4 assay may be used to predict female receptivity and guide the timing of ultrasound exams for more precise determinations of timing as needed. As illustrated above, initiation of ultrasound evaluation at approximately Day 19 of a 21-day cycle could be used to critically evaluate follicular size and predict impending estrus, especially in problem females where serum P4 assay alone is considered insufficient to guide decisions (this was illustrated nicely with our predicted breeding between Mas Merah and Ara on 29 August 2001).

2) Schedule for ultrasound evaluation of rhinoceros early pregnancy,



Day 15 = earliest detection of embryonic vesicle

- Important stage as early loss can occur between days 15-20 secondary to failure of implantation with return to cycle at 21 days
- May be difficult to differentiate an early embryo from an endometrial cyst (see Rima Figure 1)

Days 20 - 30 = embryo proper first visible at this stage

- 1) Important to document this stage as this is the time period most likely to see early embryo loss
- 2) Embryo loss at this stage most likely will result in prolongation of the luteal phase with elevation of progesterone levels

Days 40+ = beginning of fetal stage

- 1) Fetal loss is much less likely at this stage unless luteal insufficiency develops as with Emi at Cincinnati
- 2) Monthly exams during fetal stage for monitoring of normal fetal health and development

APPENDIX B

Regumate Supplementation Protocol During Pregnancy in a Sumatran Rhino

This protocol was used on the female Sumatran rhino, Emi, at the Cincinnati Zoo after Emi had experienced five confirmed early pregnancy losses. The pregnancy (her sixth) was sustained to term, parturition was uncomplicated and a healthy male calf was produced. There were no apparent negative effects on the mother or calf, and at 5 months post-partum, Emi has resumed reproductive cyclicity.

The protocol used is as follows:

- 1) Confirm pregnancy by ultrasound 16 days after mating (or as early as possible which might be 20-25 days after breeding).
- 2) Start progesterone supplement immediately (on the day pregnancy is confirmed).
- 3) Continue to collect blood twice weekly for progesterone analysis. Regumate will not be detected by the progesterone hormone assay so progesterone measured in the blood sample will be that produced by the animal.
- 4) Conduct a thorough ultrasound exam on the subject twice each week (Monday and Friday). This will allow you to distinguish between the cysts in the uterus and a developing embryonic vesicle because the vesicle will continue to grow in size and will become much larger than the cysts. It will also contain a developing fetus within it, whereas, the cysts will remain mainly fluid filled.
- 5) Administer Regumate (altrenogest) orally once each day following recommendations for equine dosages (~.044 mg/kg body weight). Emi received 16 cc each day and her weight was 750-800 kg.
- 6) Using gloves, draw up 16 cc of the liquid hormone. Dispense it slowly into a food item that will absorb it (we used several slices of bread) and hand feed the food to the animal to ensure she receives the full dose.
- 7) Feed the full hormone dose every day at approximately the same time of day.
- 8) On day 450 of gestation begin to decrease the daily dosage by 1 cc each day.
- 9) By day 465, the female should no longer be receiving the supplement.

For questions or clarifications contact:

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APPENDIX C

Sungai Dusun Sumatran Rhinoceros Reproductive Summary				
Rhino ID				
Females	Pathology	Breeding(s)	Most Recent Breeding	Recommendations
Minah	None	None	None	Increasing Minah's body weight is a priority; Continue GnRH therapy for duration of protocol.
Mas Merah	Mass L/R horn base	3 cycles	18-Jan-02	Continue to breed.
Rima	Multiple cysts (intramural and intraluminal)	13 cycles	13-Nov-01	Uterine biopsy; Initiate empirical Regumate therapy at Day 14 post-breeding and continue to 45 days.
Panjang	Cervical mass; Mineralized uterine mass; cysts	2 cycles	31-May-01	Lower breeding priority; Do not breed until mass removal. Mix with Ara to stimulate cyclic activity. Consider GnRH therapy based on results of Minah's protocol.
Seputih	Multiple masses and cysts	12 cycles	8-Nov-01	Uterine biopsy; Lowest breeding priority due to marked reproductive pathology. Consider movement into zoological facility.
Male				
Ara	None	42 breedings	18-Jan-02	Semen collection via post-breeding collection from female; Semen evaluation of motility and morphology. NOTE: This evaluation should be considered of highest priority considering the large number of breedings without conception.

APPENDIX D

Minah Treatment Protocol

Prepared by Robin W. Radcliffe, Mats H. Troedsson and Terri L. Roth
January 28, 2002

Goal of therapy: To simulate the natural gonadotrophin release of Minah to induce normal estrous cycling. The following schedule was prepared based on knowledge of the mare with intended application to the Sumatran rhino (*Dicerorhinus sumatrensis*).

Product: Gonadotrophin-releasing hormone (GnRH)
Cystorelin (Rhone Merieux Inc.)
Drug concentration = 50 micrograms per ml (supplied in 10 ml vials)

Dosage and Examination Schedule:

50 micrograms of Cystorelin (1.0 ml IM) intramuscular twice daily for 14 days

NOTE: Total Cystorelin required for 14 day therapy = 1400 micrograms or 28 ml of the 50 ug per ml Cystorelin. Therefore Sungai Dusun will need 3 of the 10 ml bottles of 50 ug/ml Cystorelin.

NOTE: Please give the 50 ug dose IM alternating between the left and right sides of the neck. Any mud or dirt will need to be hosed off Minah, and the site should then be scrubbed with an antiseptic solution on gauze prior to any injection.

Treatment and Monitoring Guidelines

- 1) Please record all doses and injection times (note any partial doses if there is not full delivery of the GnRH dose).
- 2) All exams and sample collection should be done during the AM treatment period so that the blood can be processed (see below).
- 3) Collect blood as outlined in the table below. In general, blood will be collected every other day during the protocol. Please plan to collect the blood immediately prior to the morning GnRH dose. On two occasions we will collect blood prior to the first dose **AND** 30 minutes later (see chart below).
- 4) Collect a minimum of 5-10 ml of blood from Minah via the tail vein.
- 5) Please process the blood within one hour of collection. The blood should be centrifuged with all serum removed and transferred to plastic cryovials for immediate storage in the freezer (preferably -70° C, but -20° C will be adequate).
 - Please collect urine and fecal samples daily and freeze for later assay.
 - Record all ultrasound findings on the reproductive exam forms that we provided.
 - Please attempt to examine both ovaries and measure any follicles or other structures.
 - If possible, please record all ultrasound exams on HI-8 video for later review by the team.
 - If any adverse reactions are observed, please discontinue the GnRH and contact us immediately.

GnRH Treatment and Examination Schedule

<u>Day of Rx</u>	<u>Date</u>	<u>Dose/Time: AM</u>	<u>PM</u>	<u>Morning Exam/Sampling Plan</u>
0	4 March 2002	--	--	Ultrasound, freeze serum for later assay
1	5 March 2002	50 ug am	50 ug pm	Ultrasound, freeze serum x 2 NOTE: Collect blood for serum assay immediately prior to and 30 minutes following this first GnRH dose.
2	6 March 2002	50 ug am	50 ug pm	
3	7 March 2002	50 ug am	50 ug pm	Ultrasound, freeze serum
4	8 March 2002	50 ug am	50 ug pm	
5	9 March 2002	50 ug am	50 ug pm	Ultrasound, freeze serum
6	10 March 2002	50 ug am	50 ug pm	
7	11 March 2002	Tentative arrival of Sumatran Rhino Team at Sungai Dusun		
		50 ug am	50 ug pm	Ultrasound, freeze serum x 2 NOTE: On this day we will plan to collect blood for serum assay immediately prior to and 30 minutes following this GnRH dose.
8	12 March 2002	50 ug am	50 ug pm	
9	13 March 2002	50 ug am	50 ug pm	Ultrasound, freeze serum
10	14 March 2002	50 ug am	50 ug pm	
11	15 March 2002	50 ug am	50 ug pm	Ultrasound, freeze serum
12	16 March 2002	Sumatran Rhino Team departs KL		
		50 ug am	50 ug pm	
13	17 March 2002	50 ug am	50 ug pm	Ultrasound, freeze serum
14	18 March 2002	50 ug am	50 ug pm	
15	19 March 2002	No more GnRH doses		Ultrasound, freeze serum

NOTE: 50 ug is equal to 1.0 ml of Cystorelin. Refrigerate Cystorelin after each use.

NOTE: Collection of urine and fecal samples should be attempted daily during this period and frozen for later assay.

Proposed assays

Follicle Stimulating Hormone (FSH)

Luteinizing Hormone (LH)

Progesterone metabolites (P4 and others)

Estrone sulfate and/or other estrogens

Rx Day	Date	GnRH Dose		Ultrasound Findings and Behavior	Serum Banked		Comments
1	5 March	AM	0.9 ml	U/S: Rhino jumping up & down, restless. MSF observed on right ovary.	0	√	
		PM	1.0ml		3	√	
					0		
2	6 March	AM	1.0ml				
		PM	1.0ml				
3	7 March	AM	1.0ml	U/S: Not cooperative, difficult to enter chute	Serum taken		
		PM	1.0ml				
4	8 March	AM	1.0ml				
		PM	1.0ml				
5	9 March	AM	1.0ml	U/S: Rhino vocalize a lot in the chute. Stop the U/S until the team arrived.	Serum taken		
		PM	1.0ml				
6	10 March	AM	1.0ml				
		PM	1.0ml				
7	11 March	AM	1.0ml	U/S exam: Right ovary: 13 mm Fo Left ovary: NSF	0	√	
		PM	1.0ml		3	√	
					0		
8	12 March	AM	1.0ml				
		PM	1.0ml				
9	13 March	AM	1.0ml	U/S exam: Right ovary: 13 & 15mm Fo Left ovary: Not examine (NE)	Serum taken		
		PM	1.0ml				
10	14 March	AM	1.0ml		Serum taken		
		PM	1.0ml				
11	15 March	AM	1.0ml	U/S exam: Right ovary: 15 mm Fo Left ovary: NE			
		PM	1.0ml				
12	16 March	AM	1.0ml		Serum taken		
		PM	1.0ml				
13	17 March	AM	1.0ml				
		PM	1.0ml				
14	18 March	AM	1.0ml	U/S exam: Right ovary: 15 mm Fo Left ovary: MSF	Serum taken		
		PM	1.0ml				

Endocrine data during GnRH therapy in a female Sumatran Rhinoceros (*Dicerorhinus sumatrensis*)

Minah Luteinizing Hormone Assay

The following data suggest that the GnRH hormone protocol developed for use in the Sumatran rhinoceros, Minah, appears to have induced some pituitary release of Luteinizing Hormone (LH). Although results are preliminary as this data represents only one animal, it does provide useful information on possible avenues for future hormonal induction of reproductive cycling in captive Sumatran rhinoceros.

LH Levels in Sumatran Rhino Minah using Emi as a "control"

(Dr. A.F. Parlow; National Hormone & Peptide Program, Harbor-UCLA Medical Center, Torrance, CA 90509)

Date	Sample ID	oLH RIA	95%L	eLH RIA	95%L
17 Sep 1997	Emi (positive control)	3.4 ng/ml	2.0-5.5	4.8 ng/ml	3.9-5.9
19 Sep 1997	Emi (negative control)	<0.1 ng/ml		<0.1 ng/ml	
5 Mar 2002	Minah 8:20 am (Before GnRH)	<0.1 ng/ml		<0.1 ng/ml	
5 Mar 2002	Minah 9:20 am (60 min after GnRH)	1.1 ng/ml	0.6-1.9	1.9 ng/ml	1.5-2.3
7 Mar 2002	Minah	<0.1 ng/ml		<0.2 ng/ml	
9 Mar 2002	Minah	<0.1 ng/ml		<0.1 ng/ml	
11 Mar 2002	Minah (Before GnRH)	<0.1 ng/ml		<0.1 ng/ml	
11 Mar 2002	Minah (30 min after GnRH)	2.2 ng/ml	1.3-3.7	2.4 ng/ml	1.9-2.9
13 Mar 2002	Minah	<0.1 ng/ml		<0.1 ng/ml	
14 Mar 2002	Minah	<0.1 ng/ml		<0.1 ng/ml	

APPENDIX E

Sumatran Rhinoceros Health Assessment

Clinical Pathology Summary (all units as marked; February 1999 samples in []; Texas Veterinary Medical Diagnostic Laboratory, College Station, TX 77841)

	ARA	PANJANG	SEPUTIH	MINAH	RIMA	MAS MERAH
Total Protein (g/dl)	7.2	7.6	7.4	7.3	7.3	8.9
Albumin (g/dl)	3.1	2.9	3.2	3.3	3.1	2.4
Calcium (mg/dl)	[12.6] 12.5	[15.2] 15.6	[13.4] 15.6	[11.8] 11.9	[12.4] 12.4	[13.4] 13.7
Phosphorus (mg/dl)	[2.5] 4.63	[3.7] 5.62	[2.8] 5.82	[6.1] 6.18	[2.6] 4.13	[4.2] 7.51
Glucose (mg/dl)	53	78	94	77	69	65
BUN (mg/dl)	7.5	12.9	3.2	4.9	13.4	7.1
Creatinine (mg/dl)	0.7	1.7	0.6	0.6	0.8	1.1
T. bilirubin (mg/dl)	0.1	0.1	0.1	0.2	0.1	0.1
D. bilirubin (mg/dl)	0.1	0.1	0.0	0.1	0.1	0.1
ALP (U/l)	115	58	111	119	72	124
CK (U/l)	570	684	809	746	400	387
AST (U/l)	55	83	81	93	53	71
Globulins (g/dl)	4.1	4.7	4.2	4.0	4.2	6.5
A/G Ratio	0.76	0.62	0.76	0.82	0.74	0.37
GGT (U/l)	8	9	10	17	9	9

Mineral Analysis (all units in mg/dl; February 1999 samples in []; Woodson-Tenent Laboratories, Memphis, TN 38103)

Chromium	<0.048	<0.048	<0.0545	<0.0706	<0.048	0.0677
Copper	0.29	0.204	0.256	0.222	0.185	0.185 [0.157]
Iron	2.28	0.338	0.523	0.305	0.171	0.268
Magnesium	3.07	5.59	3.81	2.55	3.41	4.66 [3.81]
Potassium	20.0	19.0	22.2	18.6	18.7	18.2 [17.2]
Sodium	305	304	371	275	300	298
Zinc	0.175	0.113	0.128	0.145	0.124	0.116 [0.072]

Serum Iron Analytes (Dr. Donald Paglia; UCLA Hematology Research Laboratory, Los Angeles, CA 90073)

Rhino ID	Iron (ug/dl)	TIBC	Transferrin sat. (%)	Ferritin (ng/ml)	Hapto- globin (mg/dl)	Cerulo- plasmin (mg/dl)
Bina						
(2/15/99)	143	179	79.9	886	80	166
(3/5/02)	164	244	67.2	851	119	95
Torgamba						
(3/5/02)	120	255	47.1	600	103	171
Ara						
(3/14/02)	70	194	36.1	257	61	79
Mas Merah						
(2/15/99)						
(3/14/02)	79	242	32.6	837	156	92
Minah						
(3/14/02)	153	181	84.5	2880	59	123
Panjang						
(3/11/02)	101	246	41.1	1003	69	103
Rima						
(3/14/02)	64	217	29.5	515	117	82
Seputih						
(3/14/02)	154	237	65.0	1177	106	114

Vitamin A & E Report

(Dr. Ellen Dierenfeld ; Wildlife Conservation Society, Nutrition Laboratory, Bronx, NY 10460)

	Vitamin E (ug/ml)		Retinol (Vitamin A; ng/ml)
	α -tocopherol	g-tocopherol	
Ara (3/14/02)	0.791	n.d.	n.d.
Mas Merah (3/14/02)	0.613	n.d.	n.d.
Minah (3/13/02)	0.400	n.d.	n.d.
Panjang (3/11/02)	0.750	n.d.	55
Rima (3/14/02)	0.769	n.d.	n.d.
Seputih (3/11/02)	0.455	n.d.	n.d.

n.d. = none detected

Comments: Vitamin E values are high-normal for the species. The lack of retinol may indicate a health concern, although rhinos normally display low circulating levels of this nutrient (<100 ng/ml).

Comparative Sumatran Rhino Serum Evaluation

(Samples collected in February 1999)

	<u>Vitamin E (ug/ml)</u>	<u>Retinol (ug/ml)</u>	<u>Selenium (ng/ml)</u>
Bina (Way Kambas, Indonesia)	0.71	66	66
Dusun (Way Kambas, Indonesia)	0.31	87	53
Mas Merah (Sungai Dusun, Malaysia)	0.33	41	54
Emi (Cincinnati, United States)	1.32	48	50
Rapunzel (Cincinnati, United States)	0.53	29	68

Serum Leptospirosis Titers (Dr. David Alt; Zoonotic Diseases Research Unit,
National Animal Disease Center, Ames, IA 50010)

Leptospirosis Serovar	Animal Name					
	ARA	PANJANG	SEPUTIH	MINAH	RIMA	MAS MERAH
L. autumnalis	1:50	N	N	N	1:50	N
L. bratislava	1:25	N	N	N	N	N
L. canicola	N	N	N	N	N	N
L. grippo	N	N	N	N	N	N
L. hardjo	N	N	N	N	N	N
L. ictero	N	N	N	N	N	N
L. pomona	N	N	N	N	N	N
L. tarassovi	N	N	N	N	N	N

NOTE: N = Negative for serologic reaction to the listed Leptospirosis serovar.

APPENDIX F

Anesthesia reports detailing butorphanol/azaperone anesthesia of Sumatran rhinoceros.

Bina; Page 1

"Bina" Sumatran Rhinoceros
5/march/2002 ♀ 15 years

Anesthesia Record - Fossil Rim Wildlife Center

Way Kambas

Genus/species: Dicerorhinus sumatrensis Location/enclosure: Holding Pen Date: 5 March, 2002
 Common name: Sumatran rhinoceros Activity: calm active excited
 Demeanor: undisturbed depressed alert aggressive apprehensive
 Sex: F age: 15yr (birthday: —) (Basis for age if not birthday: estimated at capture)
 Previous ID?: "Bina" ID applied today/site: —
 Environmental temperature: 26° OF SC Group size: 1
 Purpose: Vaginal examination for possible persistent hymen
 Time fasted: not fasted < 8 hrs 8-24 hrs 24 - 48 hrs > 48 hrs
 Health status: 1-normal health 2 - mild disease 3 - severe disease 4 - chronic disease
 5 - may not survive anesthesia Pregnant? yes no Possibly pregnant
 Body condition: obese/fat good fair thin poor/emaciated
 Body weight: 630 kg actual estimate Ven(s): Dr. Robin Radcliffe Recorder: Dr. Catherine Radcliffe
Dr. Mats Troedsson

Drug	mg	mL	Conc in mg/mL	Route	Time given	Time - Effect seen
<u>Butorphanol</u>	<u>40</u>	<u>4.0</u>	<u>10</u>	<u>IM</u>	<u>7:53:00</u>	<u>7:58:00 sternal recumbency</u>
<u>Azaperone</u>	<u>40</u>	<u>1.0</u>	<u>40</u>			<u>but alert</u>
						<u>8:03:00 calm + sedate</u>
<u>Flunixin</u>	<u>600</u>	<u>12.0</u>	<u>50</u>	<u>IM</u>	<u>8:11:11</u>	<u>N/A</u>
<u>Naltrexone</u>	<u>200</u>	<u>4.0</u>	<u>50</u>	<u>IM</u>	<u>9:13:00</u>	<u>9:15:15 standing</u>
						<u>9:15:35 alert</u>

90% delivery

Samples collected:

sample	Amount	Media/additive	Purpose	#Cryovials banked
<input checked="" type="checkbox"/> Blood: <input checked="" type="checkbox"/> red tops	<u>6mL</u>	<u>(none)</u>	<u>serum iron</u>	<u>via Don Paglia</u>
<input type="checkbox"/> green tops	<u>6mL</u>	<u>(Na Heparin)</u>		
<input checked="" type="checkbox"/> purple tops	<u>6mL</u>	<u>(EDTA)</u>		
<input type="checkbox"/> feces		<u>none</u>	<u>parasitology</u>	
<input type="checkbox"/> ticks		<u>Ethyl alcohol</u>	<u>parasitology</u>	
<input type="checkbox"/> other:				

Summary:
 E-time to first effect: 5:00 minutes tracheal tube size: N/A
 E-time to recumbency: 5 minutes (sternal/R or L lar?) Anesthetic rating: excellent good
 E-time to first arousal (from time of reversal): 2min 15sec fair poor
 E-time to standing (from time of reversal): 2min 35sec
 Total elapsed time (darning to standing): 1hr 22min 35sec Recovery: normal abnormal

Darning from (circle): hand injection 18 gauge 1 1/2" needle (see comments)
 Dart/needle type: 6mL syringe Site: (L) semitendinosus
 Distance away: N/A Dart mixing time/date: 5 March 2002
 Dart misses or failures: N/A (90% delivery of sedation) SpO2 sensor: Nellcor Puritan Bennett

Comments: Very stimulated by needle placement. This dose caused complete recumbency, but could be stimulated to stand. Excellent dose.

Time (hr: min: sec)	SPO ₂	Temp OF °C	Pulse	Resp	Comments (drugs? observations? PE findings? etc.)
7:45:00				60	excited prior to sedation
					following needle placement
7:53:00		"			90% sedative delivery
7:55:00				32	standing quietly in chute
7:59:00				60	stimulated by handler
8:00:00					Standing-bags placed
8:01:00					Sternal recumbency
8:03:00					Standing-bags removed
8:05:40				32	
8:07:50				24	ear prepared for pulse ox.
8:08:10	82%		60	24	Pulse oximetry placed
8:11:11	95%		100		flunixin given
8:13:04	90%		69	20	quiet in sternal
8:16:44	96%		59	20	
8:19:59	92%		56		
8:21:10					Vulvar wash begins
8:23:40	93%		54	20	
8:25:59					Speculum examination attempted (needed to make her stand)
8:29:12	91	36.8	57		
8:30:15	74		87		vaginal exam begins
8:30:30					bags placed
8:32:00	82		88		speculum examination
8:33:00	89		89	20	comfortable in sternal
8:34:30	96		68	24	
8:35:15				16	
8:35:50	95		66		endoscopy begins
8:37:20	94		62	12	endoscope adjusted
8:39:15	94		66	12	
8:42:47	92		58	16	quiet - eyes closed
8:43:45	94		59		endoscopy continued
8:44:56	94		60	16	endoscopy ends
8:47:00	89		59		exam ends - hymen determined to be non-pathologic
8:48:15				16	
8:49:45	93		53		Ⓡ ear venipuncture
8:54:00	95		47	16	Ⓛ ear venipuncture
8:58:40					additional venipuncture
9:00:45	91		44	16	procedure ends
9:05:09	90		44	16	cleanup finishing
9:13:00					Reversal given IM in neck
9:15:15					standing

"Torgamba" Sumatran Rhino
5 March 2002 ♂ 20 years

Anesthesia Record - Fossil Rim Wildlife Center

Genus/species: Dicerorhinus sumatrensis Location/enclosure: Way Kambas Holding Pen Date: 5 March 2002
 Common name: Sumatran Rhinoceros Activity: calm active excited
 Demeanor: undisturbed depressed alert aggressive apprehensive
 Sex: M age: 20 (birthday: —) (Basis for age if not birthday: estimated at capture)
 Previous ID?: Torgamba ID applied today/site: N/A
 Environmental temperature: 30 °F °C Group size: 1
 Purpose: Standing Sedation for electroejaculation (Dr. Terri Roth)
 Time fasted: not fasted < 8 hrs 8-24 hrs 24-48 hrs > 48 hrs
 Health status: 1 - normal health 2 - mild disease 3 - severe disease 4 - chronic disease
 5 - may not survive anesthesia Pregnant? yes no
 Body condition: obese/fat good fair thin poor/emaciated
 Body weight: 680 kg actual estimate Vet(s): Dr. Robin Redcliffe
Dr. Catherine Radcliffe
Dr. Mike Radcliffe

Drug	#mg	#mL	Conc in mg/mL	Route	Time given	Time - Effect seen
<u>Butorphanol</u>	<u>25</u>	<u>2.5</u>	<u>10</u>	<u>IM</u>	<u>1:45:51</u>	<u>1:47:55 stationary</u>
<u>Azaperone</u>	<u>40</u>	<u>1.0</u>	<u>40</u>			<u>1:51:06 sedate</u>
<u>Naltrexone</u>	<u>125</u>	<u>2.5</u>	<u>50</u>	<u>1/2 IV, 1/2 IM</u>	<u>2:55:37</u>	<u>2:57:30 alert</u> <u>2:58:00 walking around paddock</u>

Samples collected:

sample	Amount	Media/additive	Purpose	#Cryovials banked
<input checked="" type="radio"/> Blood: <input checked="" type="radio"/> red tops	<u>1-10cc</u>	<u>none</u>	<u>serum iron</u>	<u>via Don Paglia</u>
<input type="radio"/> green tops		<u>Na Heparin</u>		
<input type="radio"/> purple tops		<u>EDTA</u>		
<input type="radio"/> feces		<u>none</u>	<u>parasitology</u>	
<input type="radio"/> ticks		<u>Ethyl alcohol</u>	<u>parasitology</u>	
<input type="radio"/> other:				

Summary:

E-time to first effect: 2 min 4 sec tracheal tube size: N/A
 E-time to recumbency: N/A (sternal/R or L lat?) Anesthetic rating: excellent good
 E-time to first arousal (from time of reversal): 2 min 23 sec fair poor
 E-time to standing (from time of reversal): N/A
 Total elapsed time (darting to standing): 1 hr 12 min 9 sec Recovery: normal abnormal

Darting from (circle): hard injection 18 gauge 1 1/2" (see comments)
 Dart/needle type: 6 mL syringe Site: semitendinosus
 Distance away: N/A Dart mixing time/date: 5 March 02 SpO2 sensor: Nellcor
 Dart misses or failures: N/A

Comments: insufficient sedation for electroejaculation

Time (hr: min: sec)	SPO ₂	Temp OF °C	Pulse	Resp	Comments (drugs? observations? PE findings? etc.)
1:37:34		36.8			prior to sedation
1:46:25					released from chute
1:47:06				32	standing sedate
1:49:00				32	
1:49:42					penis examined
1:50:34				28	penis massage begins
1:52:09					walking around slowly
1:55:20				24	extending legs - swinging tail
1:57:30					blindfold applied
1:59:06				20	rope applied / mild salivation
2:04:11					rectum evacuated
2:07:29	89		83	32	very strong response to electrostimulation
2:09:10	92		82		
2:09:48	93		63	20	
2:10:51	91		56	20	
2:12:21	93		64	16	
2:13:29	93		70		moaning
2:14:42	88		73		
2:15:54	89		68	28	
2:17:13					slightly pale, minus mem.
2:18:20	89		94		
2:19:08	90		66	28	
2:21:43	92		75	28	
2:23:04	89		59	32	
2:24:02	88		56	28	
2:25:20	88		54	24	
2:27:00	90		66	20	
2:28:29	90		51	?	moved to chute
2:31:48				20	quiet in chute
2:37:40		37.0			
2:38:09	95		60	16	2nd electrostimulation attempt
2:39:34	91		50	16	
2:41:32	92		46	20	
2:43:39					① ear venipuncture
2:49:30	87		50		
2:50:00					3rd electrostimulation attempt
2:51:40					procedure aborted

NOTE

DRUGS DISPENSED TO SUNGAI DUSUN: AND WAY KAMBAS:

BUTORPHANOL	100 mg	100 mg
AZAPERONE	400 mg	400 mg
NALTRAXONE	1000 mg	1000 mg

"PANJANG" SUMATRAN RHINO
 13 March 2002 ♀

Anesthesia Record - Fossil Rim Wildlife Center

Genus/species: Dicerorhinus sumatrensis Location/enclosure: Sungai Dusun Date: 13 March 2002
 Common name: Sumatran Rhino Activity: calm active excited
 Demeanor: undisturbed depressed alert aggressive apprehensive
 Sex: F age: 19 yrs (birthday: 1983) (Basis for age if not birthday: estimated at capture)
 Previous ID#: PANJANG ID applied today/site: N/A
 Environmental temperature: 23 OF OC Group size: 1 (of 6 total) housed indiv.
 Purpose: Vaginal Endoscopy Reproductive Exam
 Time fasted: not fasted < 8 hrs 8-24 hrs 24 - 48 hrs > 48 hrs
 Health status: 1 - normal health 2 - mild disease 3 - severe disease 4 - chronic disease
 5 - may not survive anesthesia Pregnant? yes no
 Body condition: obese/fat good fair thin poor/emaciated
 Body weight: 512 Kg actual estimate Ver(s): Dr. Matis Treeshop Recorder: Dr. Catherine Radcliffe
WT. = 512 Kg on 4/26/02 Rita Radcliffe

Drug	#mg	#mL	Conc in mg/mL	Route	Time given	Time - Effect seen
<u>BUTORPHANOL</u>	<u>40</u>	<u>4.0</u>	<u>10</u>	<u>IM</u>	<u>7:34:05</u>	<u>starting to head press 7:37:50</u>
<u>AZAPERONE</u>	<u>40</u>	<u>1.0</u>	<u>40</u>			<u>more constant head press 7:38:30</u>
						<u>7:50:30 Steady head press</u>
<u>NALTREXONE</u>	<u>200</u>	<u>4.0</u>	<u>50</u>		<u>8:29:05</u>	<u>8:30:03 Moving with stimulus</u>
						<u>8:30:35 End of head press</u>
						<u>8:32:36 Walked out of chute</u>

Samples collected:

sample	Amount	Media/additive	Purpose	#Cryovials banked
<input type="radio"/> Blood: <input type="radio"/> red tops		none		
<input type="radio"/> green tops		Na Heparin		
<input type="radio"/> purple tops		EDTA		
<input type="radio"/> feces		none	parasitology	
<input type="radio"/> ticks		Ethyl alcohol	parasitology	
<input checked="" type="radio"/> other: <u>Biopsy-vaginal mass</u>	<u>- 1g</u>	<u>10% formalin</u>	<u>histopathology via Don Paglia</u>	

Summary:

E-time to first effect: 3:45 standing procedure tracheal tube size: N/A
 E-time to recumbency: N/A (sternal/R or L lat?) Anesthetic rating: excellent good
 E-time to first arousal (from time of reversal): 0:58 fair poor
 E-time to standing (from time of reversal): N/A
 Total elapsed time (darting to standing): N/A Recovery: normal abnormal - BUTORPHANOL 60mg
 (see comments) AZAPERONE 60mg
 Darting from (circle): Hand Injection vehicle or foot Dart/needle type: 1 1/2" 18 gauge neck Site: D Neck
 Distance away: By Hand Dart mixing time/date: Immed. before use before SpO2 sensor: Nellcor NPB-40
 Dart misses or failures: None
 Comments: Excellent dose for standing sedation. Increase dose if surgery is done at a later date.

