SUMATRAN RHINO REPRODUCTIVE AND HEALTH ASSESSMENT IV

Sumatran Rhino Conservation Centre
Sungai Dusun, Selangor Malaysia
February 16, 2003 through February 28, 2003

Robin W. Radcliffe, DVM, Diplomate ACZM[1] and
Rolfe M. Radcliffe, DVM, Diplomate ACVS[2]

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At the invitation of Musa Nordin, the Director General of the Malaysian Wildlife Department; Mr. Rhashid, Acting Director General of the Malaysian Wildlife Department during the time of our visit; Mohd Khan, Chairman of the Asian Rhino Specialist Group; and Dr. Tom Foose, Program Officer of the International Rhino Foundation, Drs. Robin and Rolfe Radcliffe from the Fossil Rim Wildlife Center and Living Fossil Productions, respectively, visited the Sungai Dusan Sumatran Rhino Conservation Centre. For 14 days, Drs. Radcliffe worked closely with Dr. Aidi Mohamad, Steve Romo, Mohd Khan and the Sungai Dusun rangers to evaluate the female 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. The primary objective of this visit was the surgical removal of numerous (n~5) vaginal masses identified in Seputih during our previous visit. Surgical excision and follow-up care to prevent cervical adhesions was scheduled for this visit. A secondary and equally important objective was the continued need to modify and enhance reproductive monitoring techniques that can be used to guide daily reproductive management decisions in captive Sumatran rhinos. This will be fundamental to any future success with captive propagation of this species. Therefore, each female rhino also received a thorough reproductive exam combined with new therapies where indicated. This trip involved the use of follicle stimulating hormone (FSH) therapy in the Sumatran rhino in the hopes of restoring regular reproductive cyclic activity and predictable breeding management decisions based on hormone, ultrasound and behavioral information.

We reiterate the pressing need for facility modification at Sungai Dusun in hopes of addressing possible concerns over abnormally high rhino density. While there is limited information about wild Sumatran rhino reproductive biology, the available literature suggests that wild animals exist at relatively low population densities. Are there adverse effects to reproductive function in Sumatran rhinos from the high stocking densities associated with current captive management?

Finally, with poaching threats escalating for wild Sumatran rhinos we discuss some additional conservation techniques that may be considered for increased protection of wild rhinos – based on the extensive experiences of conservation strategies for the two species of African rhinoceros.

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 Fossil Rim Wildlife Center and Cincinnati Zoo and Botanical Garden continue to provide support to the Sumatran Rhino program in Malaysia and Indonesia through provision of technical support of the scientific mission. Special thanks to Dr. Terri Roth and Dr. Mats Troedsson for their contributions to this mission.

We would like to thank Aaron Medical for their kind donation of the Bovie model 2100 electrosurgery generator and accompanying instrumentation. This donation was critical to the success of this project. Patton Surgical and Storz endoscopy corporations both supplied instrumentation needed to perform the electrosurgery in concert with the Bovie unit. The team would like to extend their sincere thanks to each of our colleagues in Malaysia for their generous support of our work and involvement in the Sumatran Rhino conservation program. In particular, we would like to acknowledge Mohd Khan, Dr. Aidi Mohamad, the Sungai Dusun rangers and Steve Romo who manage the daily work and husbandry for the Sumatran rhinos in their care. We also thank Dr. Wahid Haron and Mr Yap Keng Chee, Universiti Putra Malaysia (UPM). We look forward to future collaborations and a spirit of cooperation for the benefit of the Sumatran rhinoceros.

The Fossil Rim Wildlife Center has provided both financial and technical resources and support in many ways to the IRF and rhino conservation efforts; we thank Pat Condy, Bruce Williams and Kelley Snodgrass for their efforts to make field conservation a reality. Dr. Steve Osofsky has provided invaluable guidance for our Sumatran rhino conservation objectives, specifically with field-orientated conservation medicine and policy. Finally, I would like to thank Annie Graham and the Tapeats fund for her continued personal and financial support for our efforts to improve conservation and health of these endangered rhinoceros species.

Scientific Report IV

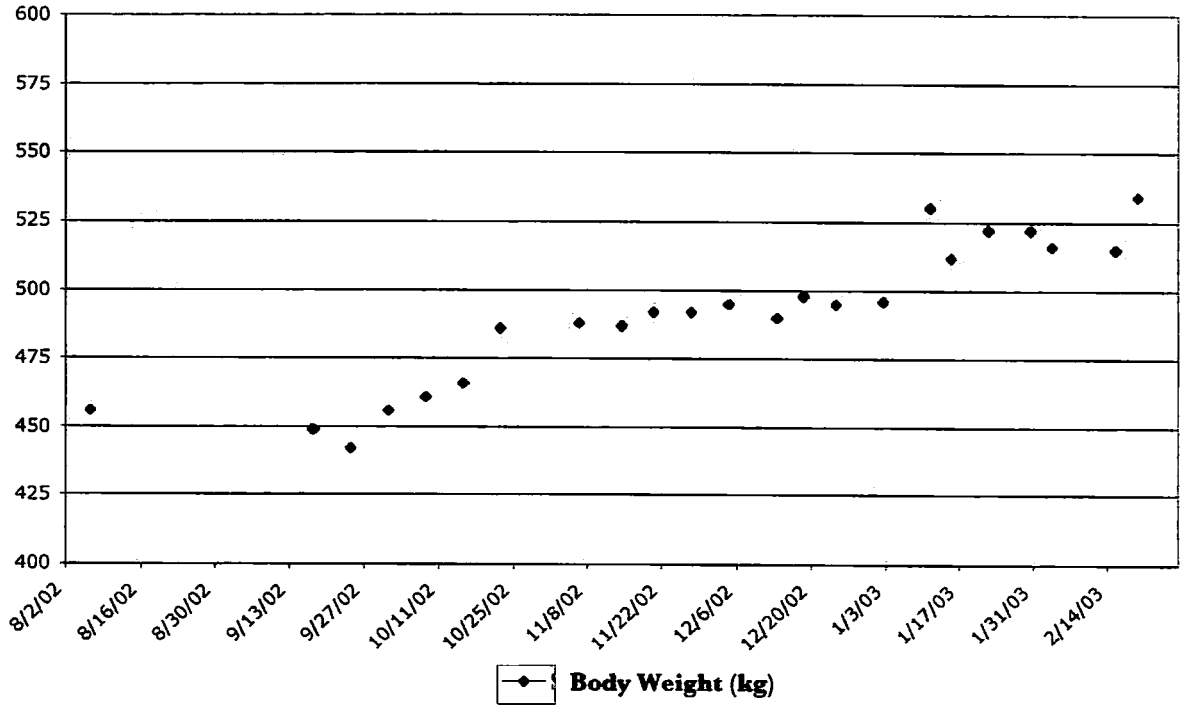
Sumatran Rhino Reproductive Evaluation
Sungai Dusun, Malaysia
Date of exams: 17 through 26 of February, 2003

Species	SB#	Sex	Name	Age
Dicerorhinus sumatrensis	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. Recent elevations in progesterone from 0.5 ng/ml to over 1.0 ng/ml since June 2002.
Hormone manipulations: History of previous use of PRID (3/2/00) and CIDR (3/30/00) with inability to recover CIDR. CIDR removed via endoscopy on 5 September 2001. GnRH therapy given prior to March 2002 team visit (see 2002 SRCC Report II for details).

Purpose of exam: Reevaluation of urinary and reproductive systems. Nutritional evaluation following 6 months of feeding Minah the Equine Senior diet (See Report III for details). Consider hormonal therapy to induce cyclic activity.

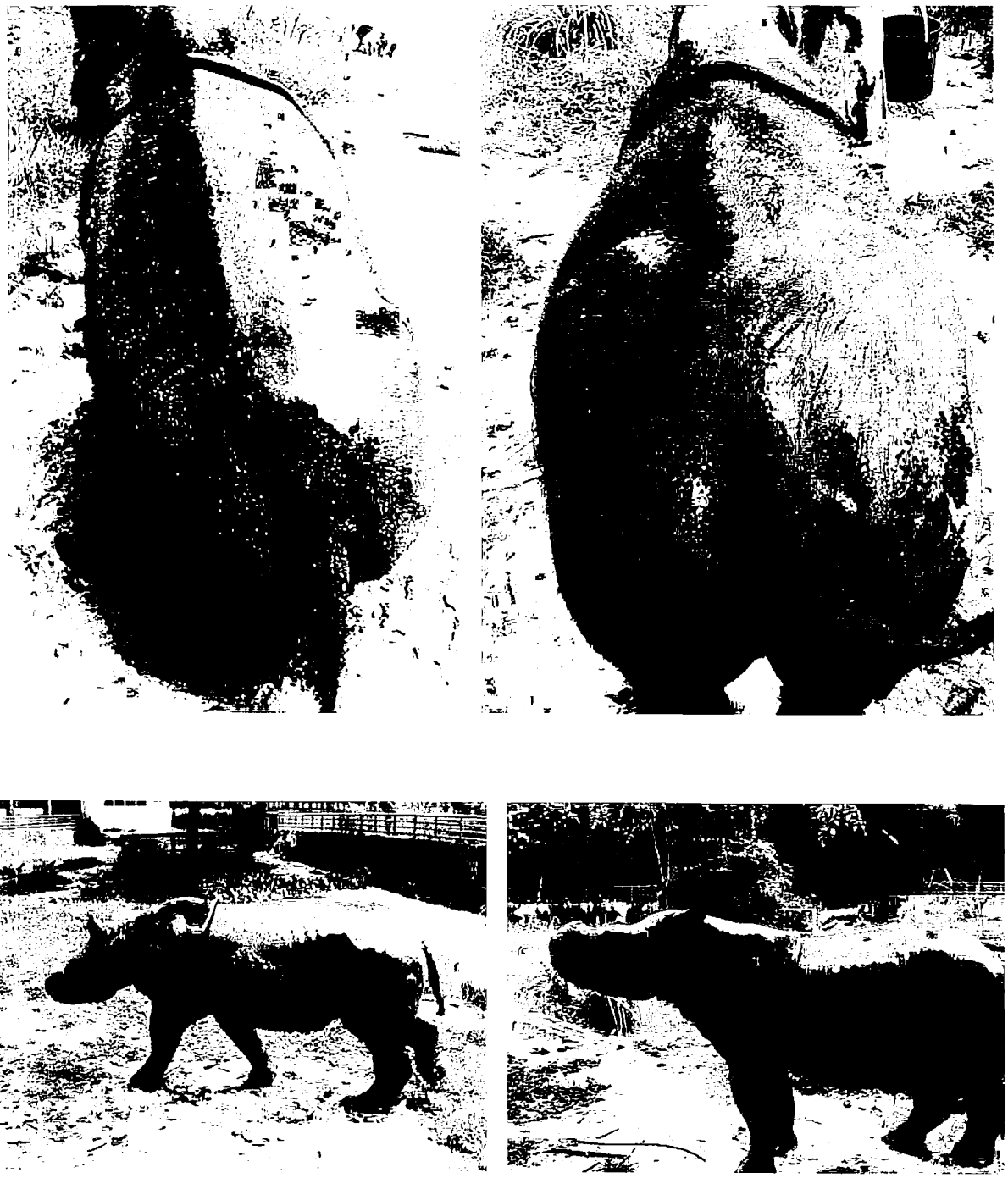
Minah Weight Chart



Minah Body Weight Summary

Date	Weight (Kg)
August 1, 2002	458
August 6	456
September 17	449
September 24	442
October 1	456
October 8	461
October 15	466
October 22	486
November 6	488
November 14	487
November 20	492
November 27	492
December 4	495
December 13	490
December 18	498
December 24	495
January 2, 2003	496
January 11	530
January 15	512
January 22	522
January 30	522
February 3	516
February 15	515
February 19	534
February 26	532

Minah: August 2002 **Minah: February 2003**



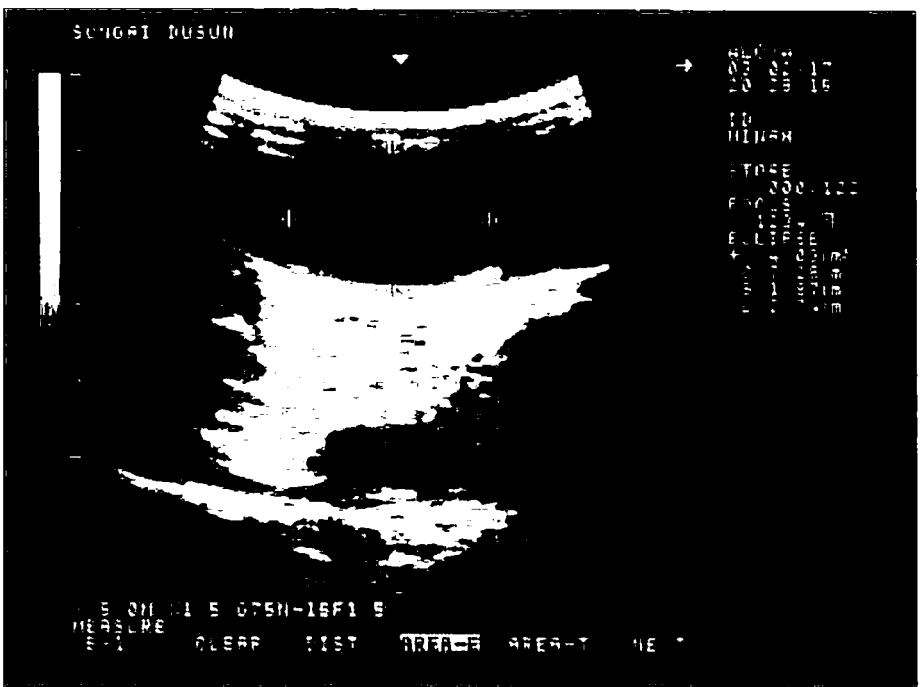
Minah Figure 1. Minah in August of 2002 with thin body condition (Left) and Minah 6 months after implementation of Equine Senior nutritional program (Right). Note marked improvement in body condition of Minah with good muscling over ribs and spine. Improved rhino body condition score estimate of ~4 out of 5.

Brief Summary of Findings:
Minah's reproductive tract was considered normal on ultrasound. From an animal health aspect, we were happy to finally see Minah with improvement in her body condition (Figure 1). The body condition of this rhino based on previous condition scoring in the black rhinoceros (Adcock, 2001) has increased from an estimated 3 of 5 to 4 out of 5 following just 6 months of the equine senior nutritional program which was implemented in August of 2002.

Concurrent with the marked improvement in Minah's body condition, we documented the first evidence of cyclic activity. A large 27 mm x 17 mm follicle was identified on her left ovary. The follicle was mature and most likely post-ovulatory based on its large size. However, we did not see any evidence of follicular lutienization or characteristics of a hemorrhagic follicle. Based on these findings, we elected to treat Minah with HCG (Chlorulon) 3000 IU IM in order to induce ovulation. We then mixed the rhinos. Ara was very interested the first day, but by 24 hours post-HCG there was no interest. No breeding took place.

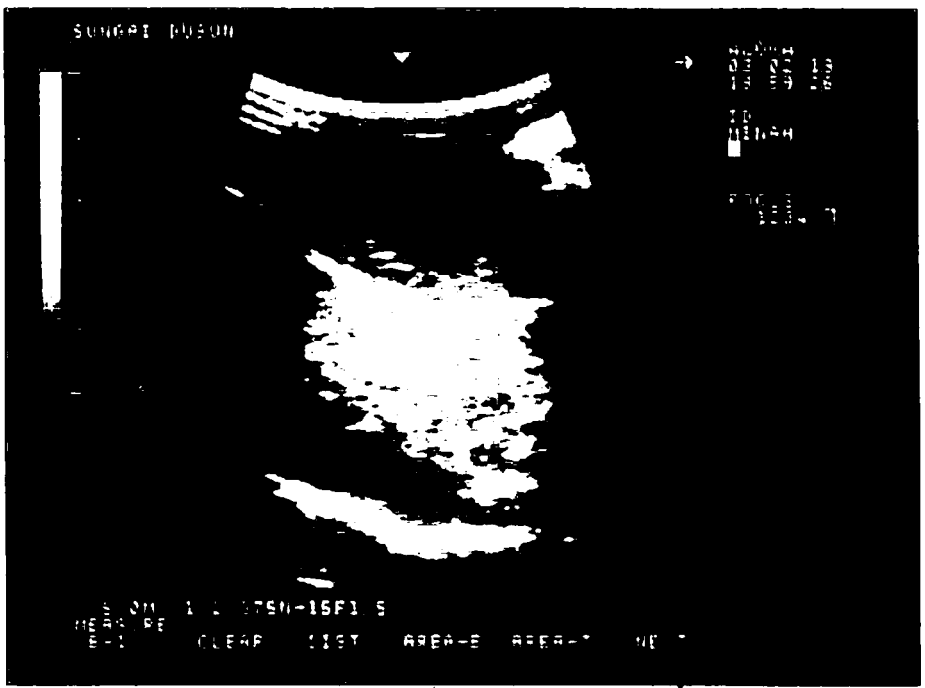
Recommendations:
Monitor Minah with ultrasound and P4 assay to document that HCG induced ovulation. Then preparations should be made to make sure that Minah is monitored closely via ultrasound and P4 assay to prepare for the next estrus period. Daily introductions should be done as P4 assay and ultrasound monitoring of the Sungai Dusun rhinos has not been consistent or accurate.

Reproductive Exam Details: **MINAH**



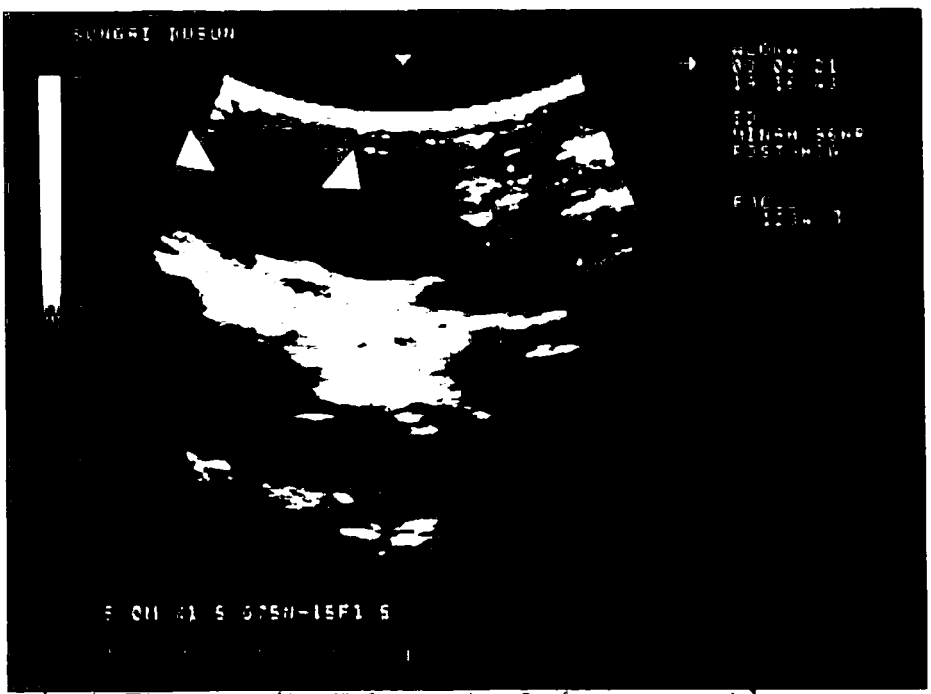
Minah Figure 3 (17 February). Left ovary with large follicle measuring 27 mm x 18 mm in diameter and characteristic of a cycling female. Marked interest by Ara, but no breeding.

Minah Figure 4 (18 February). Left ovary with large follicle 24 hours post-HCG therapy. Note thickening of follicle wall. Another 3000 IU HCG was given at this time.

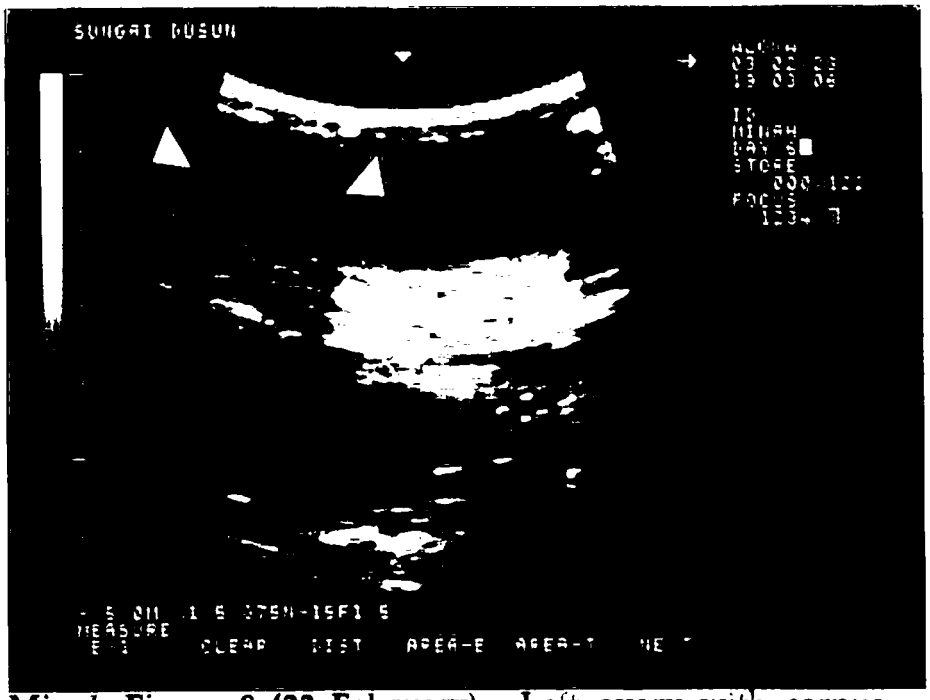


Minah Figure 5 (19 February). Left ovary with large follicle 48 hours post-HCG therapy. Note thickening of follicle wall indicative of lutienization.

Minah Figure 6 (20 February). Successful ovulation! Left ovary with ovulation site (arrows) 72 hours post-HCG therapy.



Minah Figure 7 (21 February). Left ovary with corpus luteum (arrows) and emerging 18 mm follicle 96 hours post-HCG therapy.



Minah Figure 8 (23 February). Left ovary with corpus luteum (arrows) and emerging 20 mm follicle 6 days post-HCG therapy.

Minah Figure 9 (25 February). Right ovary with marked follicular growth 8 days post-HCG therapy. The HCG induced ovulation in Minah, but its FSH activity also appears to have stimulated a follicular response.

Table 1. Human Chorionic Gonadotropin (LH) Therapy and Summary of Response

Date	HCG / HR Post-HCG	Ultrasound Data	P4 Data (ng/ml)	Behavior
17 February 10:40 am	3000 IU HCG IM	R NSF L 27 mm x 18 mm Fo	**0.33**	Good interest
18 February 8:45 am	3000 IU HCG IM	R NSF L 23 mm x 21 mm Fo Thickening of follicle wall evident.		No interest
19 February	48 Hrs Post-HCG	R NSF L 32 mm oblong Fo (27 mm x 16 mm) Follicle had thickened wall (4 to 5 mm) indicating follicular lutienization or impending ovulation		No interest
20 February	72 Hrs Post-HCG	R 12 mm , 11 mm Fo L Ovulation confirmed!! (20 mm CL3) plus 13 mm growing follicle	**0.33**	No mixing
21 February	96 Hrs Post-HCG	R 17 mm Fo L 18 mm growing follicle, CL3		No mixing
22 February	120 Hrs Post-HCG	R 19 mm Fo L 18 mm Fo + 21 mm CL3		No mixing
23 February	144 Hrs Post-HCG	R 21 mm, 20 mm Fo L 20mm, 19 mm Fo		Some interest by Ara
24 February	7 days Post-HCG	R 23 mm, 22 mm Fo L 20 mm, 19 mm Fo + 19 mm CL3	**1.24**	No interest
25 February	8 days Post-HCG	R 22 mm Fo L 24mm, 21 mm Fo + 21 mm CL3		No interest
26 February	9 days Post-HCG	R 27 mm, 23 mm Fo L 23 mm Fo + 24 mm CL3	**2.08**	No interest

HCG = 3000 IU Human Chorionic Gonadotropin; Chlorulon
NSF = No Significant Findings

Sumatran Rhino Reproductive Evaluation
Sungai Dusun, Malaysia
Date of exams: 17, 20, 22 through 26 of February, 2003

Species	SB#	Sex	Name	Age
Dicerorhinus sumatrensis	7	F	Rima	~23

Captive years without reproducing: 15
Recent breeding activity: Last breeding 20 and 21 of January 2003.
Progesterone profile: Regular 21 day cyclical pattern until late 2001, followed by a variable period of irregular cyclic activity. Renewed cycling and breeding in late 2002 and early 2003.
Hormone manipulations: PGF therapy during August visit (see SRCC Report III).

Purpose of exam: Examine for possible causes of prolonged luteal phases that would be consistent with variable periods of nonreceptivity. Reevaluate uterine pathology.

Brief Summary of Findings:

Rima was not pregnant. Previously identified endometrial cysts appeared to be unchanged. There was minimal evidence of follicular activity, but luteal tissue was observed. Therefore, despite hormonal and behavioral evidence of prolonged or irregular luteal phases, Rima is apparently still cycling.

Immediate Plan:

Perhaps Rima has been experiencing prolonged luteal phases following failed ovulation or missed breedings. In addition, it is also possible that Rima may be undergoing early embryonic loss – recent findings by Dr. Aidi Mohamad suggest that early embryo loss is a possible explanation. Because of the potential to miss an early pregnancy by confusion with the numerous endometrial cysts, Rima may be a good candidate for the planned use of Regumate post-breeding.

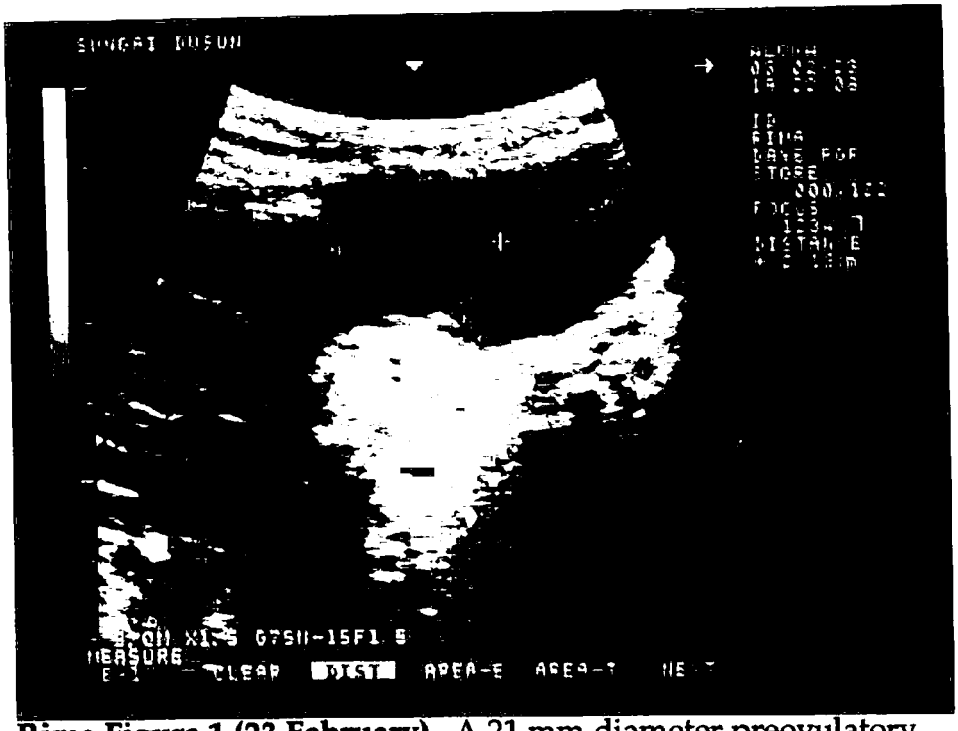
Table 2. Prostaglandin Therapy and Summary of Response

Date	PGF2α / HR Post-PGF	Ultrasound Data	P4 Data (ng/ml)	Behavior
17 February 11:00 am	15 mg PGF2α IM	R NSF L CL3, MSF	**1.26**	No mixing
18 February	24 Hrs Post-PGF	NE		No mixing
19 February	48 Hrs Post-PGF	NE		No mixing
20 February	72 Hrs Post-PGF	R 13 mm Fo L NSF, CL2	**0.12**	No mixing
21 February	96 Hrs Post-PGF	NE		No mixing
22 February	120 Hrs Post-PGF	R 19 mm Fo L CL1, No Fo		Strong interest (Ara followed Rima closely and Mounted, but Rima kicked
23 February	**144 Hrs Post-PGF**	**R 21 mm Fo L CL1, 10 mm Fo**		**Mounting plus intromission; Breeding time = 19 minutes**
24 February	24 Hrs Post-breeding	R 21 mm Fo (slight Fo echogenicity) L NE	**0.05**	Strong interest by Ara, but Rima was vocal - walked out from under Ara
25 February	48 Hrs Post-breeding	R 21 mm Fo (thick wall) L 12 mm Fo		No interest
26 February	72 Hrs Post-breeding	R 28 mm luteinized Fo or HAF? (ovulation not confirmed) L NSF, 13 mm Fo	**0.06**	No mixing

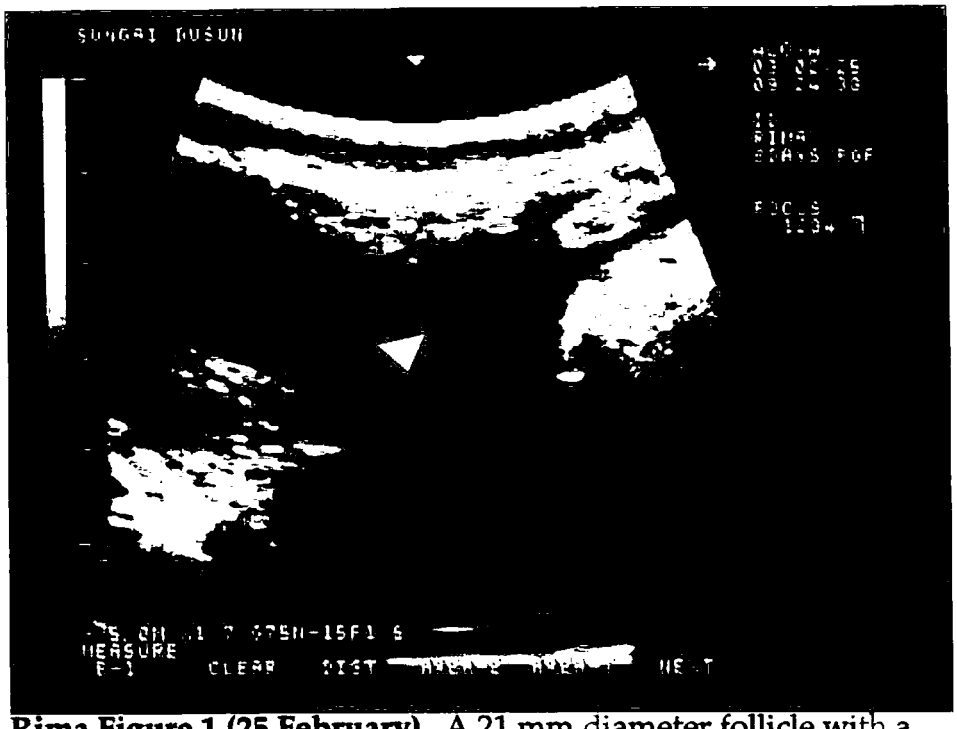
PGF = PGF2α; 15 mg Dinoprost tromethamine; Lutalyse
NE = Not Examined
NSF = No Significant Findings

MSF = Multiple Small Follicles

Reproductive Exam Details: **RIMA**



Rima Figure 1 (23 February). A 21 mm diameter preovulatory sized follicle in Rima on the day of breeding with Ara.



Rima Figure 1 (25 February). A 21 mm diameter follicle with a thickened wall (arrow) indicating that luteinization of the follicle is underway, 48 hours post-breeding.

Recommendations:

- Prostaglandin therapy was given as outlined in table 2 (PGF2α; 15 mg Dinoprost tromethamine; Lutalyse). This therapy may be repeated at a later date if further evidence for prolonged luteal phases are confirmed.

- Initiate post-breeding oral progesterone therapy (Regumate) with slight modifications to Dr. Roth's guidelines because of the high number of cysts in Rima that may preclude easy early pregnancy diagnosis:
  - Start Regumate on Day 10 post-breeding (March 5, 2003).
  - Monitor for pregnancy by ultrasound on Days 17, 21, 25 and 30. If pregnant, then continue on Regumate therapy as per Dr. Roth's guidelines. If not pregnant at 30 day exam (March 25, 2003), then discontinue Regumate and monitor for cycling. If necessary, PGF therapy may be given to Rima to induce a more rapid return to estrus, but only if she is NOT pregnant.

Sumatran Rhino Reproductive Evaluation
Sungai Dusun, Malaysia
Date of exams: 17, 19, 20, 21, and 25 of February, 2003

Species	SB#	Sex	Name	Age
Dicerorhinus sumatrensis	19	F	Mas Merah	~23

Captive years without reproducing: 15
Recent breeding activity: Bred by Ara on 3 occasions. Most recent breeding was on the 18th of January 2002.
Progesterone profile: Irregular cyclic pattern suggestive of formation and lutenization of hemorrhagic follicles.
Hormone manipulations: Attempted CIDR implant plus PGF2α in March 2000 without success. Implanted with Synchromate in Left flank in April 2000 for 9 days with no estrus observed. Given PGF therapy in August of 2002 (see SRCC Report III).

Purpose of exam: Examine for reproductive function and pathology. Consider hormonal stimulation of cycle by combined use of FSH and PGF.

Brief Summary of Findings:
Mas Merah was observed to have minimal follicular activity on either ovary. Based on these findings, we implemented a combined FSH and PGF protocol to induce follicular activity. See detailed outline of treatment and response below.

Table 3. Follicle Stimulating Hormone (FSH) Therapy and Summary of Response

Date	FSH / HR Post-FSH	Ultrasound Data	P4 Data (ng/ml)	Behavior
17 February				
10:15 am	40 mg FSH + 15 mg PGF IM	R MSF	**0.4**	No mixing
9:40 pm	40 mg FSH IM	L CL2, NSF		
18 February				
8:15 am	30 mg FSH + 15 mg PGF IM	NE		No mixing
8:40 pm	30 mg FSH IM			
19 February				
8:30 am	20 mg FSH IM	R MSF		No mixing
8:27 am	20 mg FSH IM	L CL2, NSF		
20 February				
8:54 am	10 mg FSH IM	R MSF	**0.19**	No mixing
8:08 pm	10 mg FSH IM	L 18 mm CL2		
21 February	96 Hrs Post-FSH	R MSF L CL2		No mixing
22 February	120 Hrs Post-FSH	NE		No mixing
23 February	144 Hrs Post-FSH	NE		No mixing
24 February	7 days Post-FSH	NE		No mixing
25 February	8 days Post-FSH	R MSF L CL2, MSF	**0.24**	No mixing

Note: Follutropin = porcine-derived Follicle Stimulating Hormone (FSH)
NE = Not Examined
MSF = Multiple Small Follicles

Reproductive Exam Details: **MAS MERAH**

Mas Merah Figure 1 (17 February). Corpus luteum on the left ovary. Minimal follicular activity on either ovary. Treated with FSH and PGF.

48 Hours Post-FSH Therapy:

Mas Merah Figure 2 (19 February). No significant changes 48 hours following FSH and PGF therapy.

Recommendations:

One more attempt at hormonal stimulation of Mas Merah's cycle is recommended. Consider a 15-day coarse of oral Regumate (see Dr. Roth's guidelines for Regumate dose) to mimic the luteal phase of the estrus cycle. On Day 15 of this protocol, discontinue the Regumate and give Mas one 15 mg dose of Prostaglandin F2 alpha. After the PGF treatment, Mas should be monitored with ultrasound every other day and mixed with Ara daily to see if this treatment promotes any follicular response in Mas Merah.

Based on the lack of cyclic activity of this female - even following intensive hormonal trials - breeding management of other females should receive priority. We recommend that Mas Merah be removed temporarily from the breeding population at Sungai Dusun. This would help address the rhino density issues in concert with the modifications proposed for the 100-acre enclosure (see Appendix D). A uterine biopsy should also be attempted at a future date in order to develop the appropriate techniques and pathology protocols that will help guide future reproductive management of captive Sumatran rhinos.

The authors suggest that Mas Merah be moved to Zoo Malaca within the next 30 days to help reduce animal density at Sungai Dusun. Once the 100 acre enclosure is constructed, then consideration may be given to returning Mas Merah back to Sungai Dusun. Another alternative is to manage Mas Merah in the 10 acre enclosure to remove her from the barn area.

Sumatran Rhino Reproductive Evaluation
Sungai Dusun, Malaysia
Date of exams: 17, 19, 20, 21 of February 2003

Species	SB#	Sex	Name	Age
Dicerorhinus sumatrensis	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. Surgical excision of vaginal mass performed in August of 2002 (see SRCC Report III).

Purpose of exam: Reevaluation of reproductive function and pathology. Treatment with FSH and PGF to induce cyclic activity.

Reproductive History:
In March of 2002 another vaginal mass (suspected leimyoma) was observed via vaginoscopy to be adhered to the external cervical os. Surgery was performed in August of 2002 to remove the vaginal mass and provide follow-up care. In the 6 months since the surgery, Panjang has not started to cycle and therefore the purpose of this visit is to induce cyclic activity with FSH and PGF.

Table 3. Follicle Stimulating Hormone (FSH) Therapy and Summary of Response

Date	FSH / HR Post-FSH	Ultrasound Data	P4 Data (ng/ml)	Behavior
17 February				
9:40 am	40 mg FSH + 15 mg PGF IM	R CL2	**0.27**	No mixing
9:30 pm	40 mg FSH IM	L MSF		
18 February				
8:05 am	30 mg FSH + 15 mg PGF IM	No ultrasound		No mixing
8:25 pm	30 mg FSH IM			
19 February				
8:15 am	20 mg FSH IM	R 10 mm Fo, CL1		No mixing
8:21 am	20 mg FSH IM	L 13 mm + 10 mm Fo		
20 February				
8:24 am	10 mg FSH IM	R MSF	**0.13**	No interest
10 mg FSH IM		L 17 mm, 15 mm, & 13 mm Fo		
21 February	96 Hrs Post-FSH	R MSF	**0.12**	
		L 21 mm, 19 mm, 15 mm, MSF		

Behavior: Ara followed Panjang closely for about 10 minutes until Panjang started to "sit" like a dog. Panjang also vocalized frequently. Ara mounted for about 10 minutes, but did not breed. Ara attacked Panjang upon dismount. Ara was very aggressive and the rangers had to use plywood shields to separate the rhinos.

22 February 12o Hrs Post-FSH R MSF
L 22 mm, 19 mm, 15 mm Fo

Behavior: AM, Panjang sat immediately and Ara mounted with partial erection – no breeding. PM, Ara mounted again, but no aggression and no breeding.

23 February 144 Hrs Post-FSH L 20 mm, 17 mm, 15 mm Fo, MSF

Behavior: AM, Ara mounted with erection and searched for about 10 minutes – no breeding.
PM, Ara mounted with good searching – no breeding (Ara appeared tired after breeding Rima earlier in the day).

24 February 7 days Post-FSH R 14 mm Fo **0.38**
L 17 mm, 15 mm, 13 mm Fo, MSF

Behavior: Ara followed initially, but Panjang did not "sit" as before. Aggression.

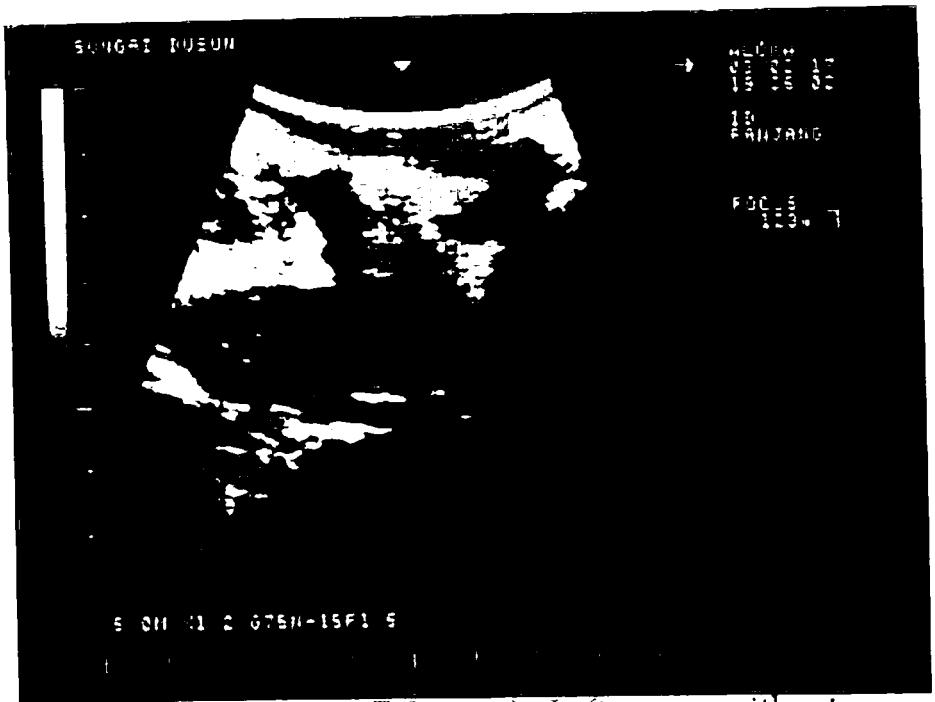
25 February 8 days Post-FSH R NE
L 20 mm, 19 mm Fo

Behavior: Ara put his head on Panjang's rump in am, but only followed her in pm.

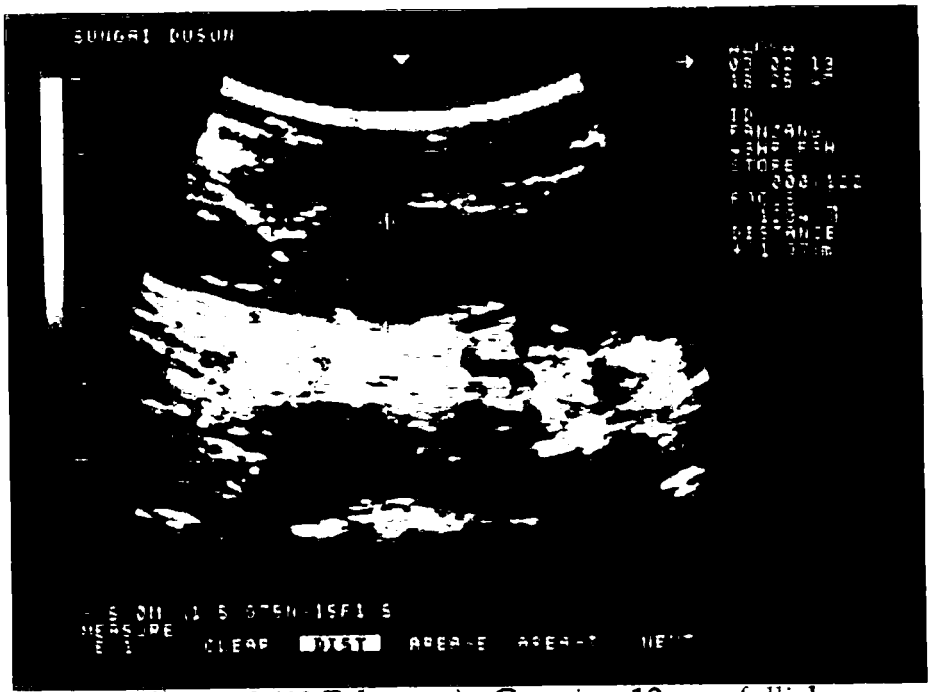
26 February 9 days Post-FSH R MSF **1.01**
Behavior: Minimal interest by Ara L 21 mm Fo + MSF

Note: Follutropin = porcine-derived Follicle Stimulating Hormone (FSH)
MSF = Multiple Small Follicles
NE = Not Examined

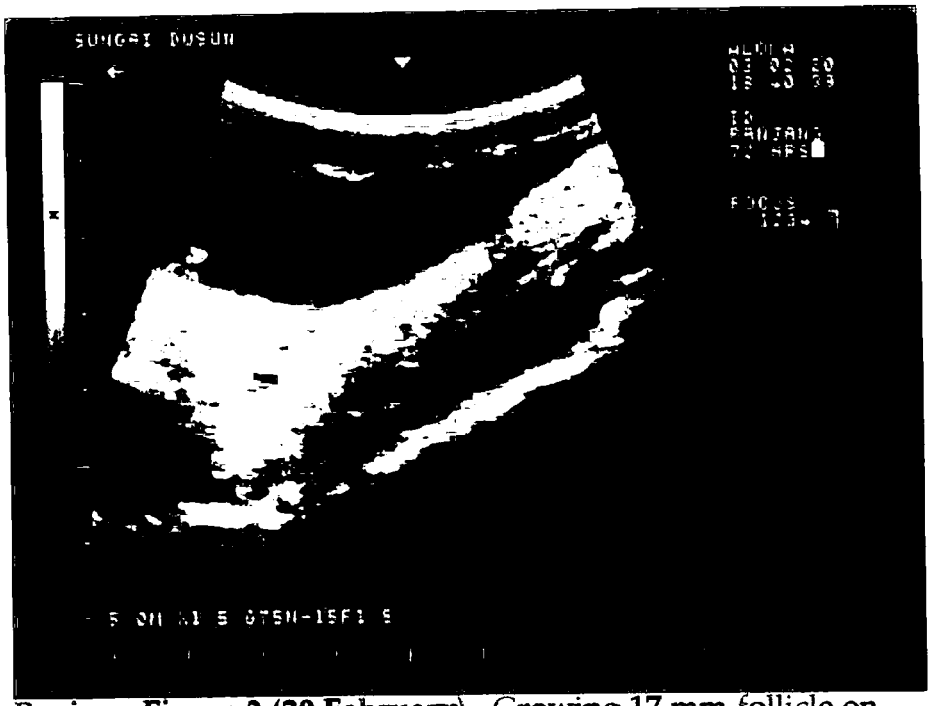
Reproductive Exam Details: **PANJANG**



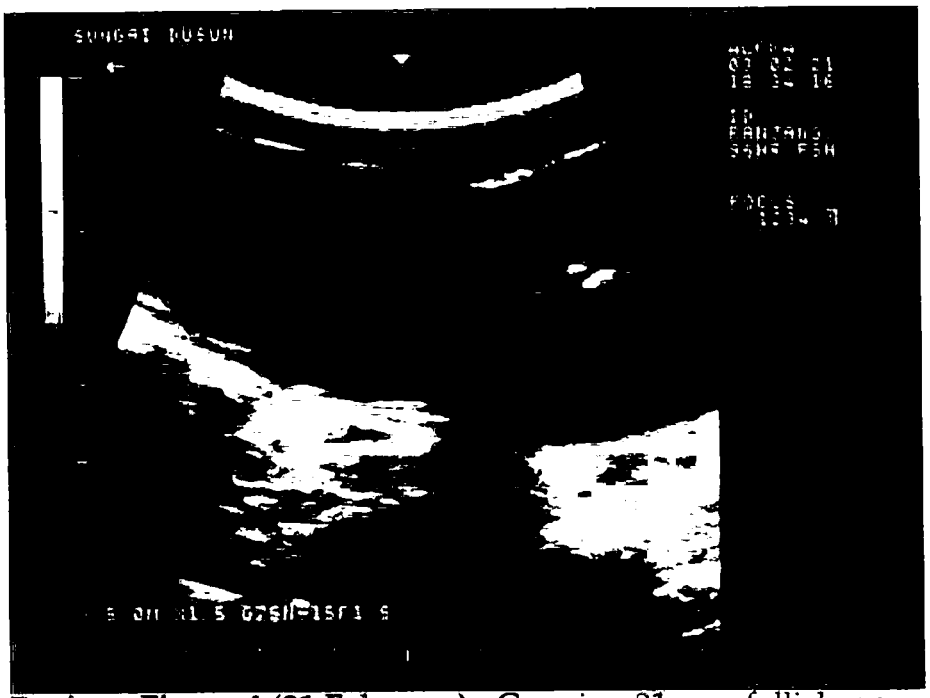
Panjang Figure 1 (17 February). Left ovary without any evidence of follicular activity.



Panjang Figure 2 (19 February). Growing 13 mm follicle on left ovary 48 hours following initiation of FSH therapy.



Panjang Figure 3 (20 February). Growing 17 mm follicle on left ovary 72 hours post-FSH therapy. There were also multiple smaller follicles ranging from 10 to 15 mm.



Panjang Figure 4 (21 February). Growing 21 mm follicle on left ovary 96 hours post-FSH therapy. There were also multiple smaller follicles ranging from 13 to 19 mm, characteristic of a superovulation response. Ara mounted Panjang today, but did not breed. Ara was aggressive and attacked Panjang on dismount.

Brief Summary of Findings:

Panjang had minimal follicular activity on either ovary and therefore she was started on a 4-day course of FSH therapy. In addition, PGF was given to remove any progesterone influence that may have been present.

Recommendations:

- Panjang should be monitored by ultrasound and P4 assay to assess if she resumes cyclic activity following this hormonal trial.
- If Panjang breeds, then careful ultrasound evaluation should be done at 15 to 19 days post-breeding to document an early pregnancy. If early embryonic loss is suspected or confirmed, then Panjang should be started on the Regumate protocol as has already been done with Rima. Refer to Dr. Roth's Regumate protocol for details.
- Consider repeating this FSH / PGF trial if Panjang does not resume normal cyclic activity.

Sumatran Rhino Reproductive Evaluation
Sungai Dusun, Malaysia
Date of surgery: 18 February 2003

Species	SB#	Sex	Name	Age
Dicerorhinus sumatrensis	23	F	Seputih	~24

Captive years without reproducing: 15
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.
Hormone manipulations: None.
Purpose of exam: Perform vaginal surgery to remove multiple tumors. These masses range in size from 3 to 7 cm in diameter and are most likely leiomyomas.

Brief Summary of Findings:

Numerous masses remain associated with Seputih's vagina and anterior uterus. The significant pathology in this female associated with the reproductive tract and surrounding anatomy suggest that Seputih's chances of normal embryo development and implantation would be unlikely to occur. Despite observed pathology, Seputih appears to be cycling and therefore surgical excision of the vaginal tumors is considered warranted to improved chances for natural breeding success.

Surgical Procedure:

Surgical removal of several vaginal masses was accomplished during a standing anesthetic procedure (see Appendix A for details of anesthesia). Five tumors ranging in size from 2 cm to 10 cm were extracted. Following surgical preparation of the vulva, a vaginal examination was performed. Three tumors were directly attached to the external os of the cervix. These were removed with electrocautery equipment (Electrosurgery generator model 2100-220 donated by Aaron Medical). Biopolar laparoscopy instruments (5 and 10 mm Coagulating and Cutting Forceps; Patton Medical, Austin, Texas) were used to separate these masses from their cervical attachments. The two remaining and largest tumors (7 and 10 cm) were located inside the lumen of the cervix. These were removed using the bipolar cautery instrument, a laparoscopic dissecting scissors (10 mm Thoracic Metz Scissors; Ethicon Endo-Surgery, Cincinnati, Ohio), and a gigly wire fetotomy technique. One 4 cm tumor was palpated within the base of the left horn of the uterus. An attempt to remove that tumor was unsuccessful due to its deep location beyond the cervix. Following surgery, the uterus and vagina were lavaged with LRS followed by an antibiotic infusion of Cefazolin (2gm IU). An anti-inflammatory cream containing Nitrofurazone and Prednisone was also applied within the vagina and cervix to help prevent the possible formation of cervical adhesions.

Recommendations:

- Provide Seputih with rest from breeding for a minimum of 45 days to allow healing from the surgical sites. On April 7, 2003 Seputih should once again be introduced with Ara for breeding based on P4 results or ultrasound findings.
- Post-operative treatment during the team visit included lavage of the cervical and vaginal area with diluted 0.9% NaCl solution and a solution containing 2 g Cefazolin. Further follow-up care consisted of digital manipulations of the cervix combined with application of a topical antinflammatory paste daily for the first 10 days and then discontinued.
- If vulvar discharge is observed with Seputih during the 45 day rest period, then a vaginal / cervical lavage should be performed as demonstrated to Aidi using a 1.0 liter normal saline solution containing 2 g of Cefazolin.
- If by 60 days after resumption of breeding attempts with Seputih there is still no activity and ultrasound and hormonal data show a persistent luteal or progesterone influence, consider PGF2α therapy. Based on the results of the PGF trial in Rima, Mas Merah and Minah, we recommend that future PGF therapy be used only in the presence of ultrasonographic evidence of both a mature corpus luteum AND a follicle measuring greater than 15 mm in diameter (see specific PGF guidelines in Appendix C).
- Evaluate histopathology results from recovered mass to help guide future prognosis for reproductive health.

Reproductive Exam and Surgery: **SEPUTIH**

Seputih Figure 1 (18 February). Surgical removal of vaginal masses via electrosurgery by Dr. Rolfe Radcliffe. A total of five masses were removed by various techniques including 2 within the cervical lumen.

Seputih Figure 2 (18 February). Dr. Robin Radcliffe (at Right) provides training on site at Sungai Dusun to Dr. Aidi Mohammad in practical anesthetic monitoring of captive Sumatran rhinoceros.

Rhino density at Sungai Dusun:
A summary of concerns and recommendations for consideration

The following summarizes recent concerns of high rhino density as a possible factor in the observed acyclicity and irregular reproductive activity that has been observed at Sungai Dusun. A brief outline of possible reasons for concern are outlined below:

- What little is currently known about reproductive patterns and biology of this species in the wild suggests that density of *in situ* Sumatran rhino populations is very low, usually not higher than 1 rhino per 40 square kilometers and often only 1 rhino per 80-100 km^2 (Flynn, 1983). Strien (1985) estimated a density of 13 to 14 rhino per 100 km^2.
- It is well recognized that the interaction of male and female Sumatran rhinos in captive environments is often characterized by marked aggression, particularly when females are not in estrus. This aggression and any possible related stresses would seem likely to be increased in the artificial confines of a captive facility such as Sungai Dusun.
- Density-dependent suppression of reproductive cycling has been documented to occur in other species [Noakes, Parkinson and England (eds.). 2001. Arthur's Veterinary Reproduction and Obstetrics]. The following is an excerpt from this text: *The social stresses to which cattle are subject have been well known to herd managers for a very long time. Situations in which dominance hierarchies cannot be stabilized are believed to be particularly stressful. Situations in which this occurs include excessively large groups of cows, groups that are continually mixed and the introduction of new animals to the group.*
- One female rhino in a USA zoo setting (Emi @ Cincinnati) is known to have experienced at least 5 early pregnancy losses, before finally maintaining a pregnancy under exogenous hormonal supplementation. It should be noted that another female (Rima) that was captured while in early pregnancy was able to maintain a pregnancy despite exposure to intensive post-capture management changes (Romo, pers. comm. 2002).
- Finally, given the fact that the current management scheme at Sungai Dusun has failed to produce a documented conception or pregnancy, we feel that a change in rhino holding should be made as soon as possible to reduce the concerns of these animals being housed in close confines.

Recommendations for Rhino Management at Sungai Dusun:

PHASE 1

Develop the first half of the 100-acre site by placing a fence (~155 m length) at the midway location where the facility is at it narrowest point. This would split the 100-acre site into separate 50-acre pieces that could be developed in two phases. In addition, the perimeter of this fence will need to be modified to prevent any falling trees from damaging the outer fence. This may involve clearing some trees around the fence or perhaps structurally changing the fence to prevent damage from windfalls and other natural events (see Figures 1 and 2).

PHASE 2

As funds become available the second half of the 100-acre site could be fenced to increase rhino management options or further expand the SRCC at Sungai Dusun (see Appendix E).

Figure 1. The perimeter fencing of the 100-acre enclosure illustrating the foreseeable problems with maintaining a safe and effective barrier.

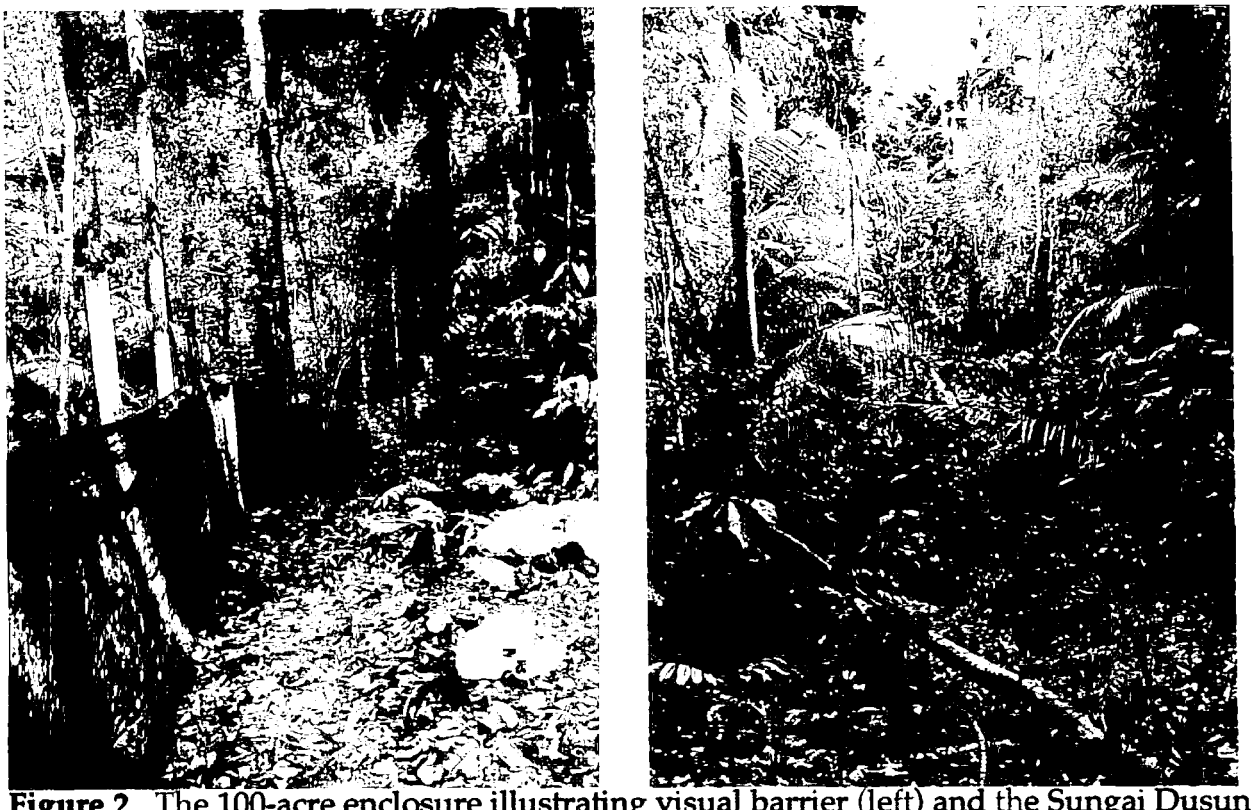


Figure 2. The 100-acre enclosure illustrating visual barrier (left) and the Sungai Dusun reserve proper (right).

A Sumatran Rhino IPZ?

The IPZ Concept

IPZ is an acronym for Intensive Protection Zone. The IPZ is a well-recognized conservation strategy for the protection and management of highly threatened black rhinoceros in African range states. The fundamental goal is to heavily patrol key rhino habitat (usually fenced). The fence acts as an initial deterrent to would-be poachers and provides a physical barrier to rhinos that may otherwise wander out of the protected area. It also provides a definable boundary that can be more easily patrolled and managed for a specific conservation purpose. It does not replace the important role of the Rhino Protection Unit or routine anti-poaching patrols in key rhino habitat.

Summary and Justification

One thing has become all too clear about conservation of the Sumatran rhinoceros: to date, these efforts have fallen short of expectations. Clearly a combined approach of utilizing all cost-effective conservation tools and techniques are needed. Based on the authors experience with African rhino species, we feel that the protection of wild Sumatran rhinos would benefit from the incorporation of accepted conservation strategies currently used to intensively protect and manage black and white rhinos in southern Africa. In particular, the authors suggest application of the Intensive Protection Zone (aka IPZ) concept for protection of key Sumatran rhino animals and habitat. While there are certainly challenges to these methods – both financial and implementation challenges in a rainforest habitat – the reality of today's intensive poaching pressures may mandate such an approach.

Fencing the Sungai Dusun Reserve?

The question remains whether the high cost of fencing an IPZ for protection of wild Sumatran rhinos is warranted in the face of dwindling conservation dollars and the obvious other needs that are crucial to this species long-term survival (ie. higher penalties for rhino poachers and enhanced legal prosecution are examples of two critical conservation needs).

Cost Estimate for creating a Sungai Dusun IPZ

Outer perimeter of cyclone fencing = 35 Ringitt (RM) per meter (or $10 USD per meter)
Sungai Dusun Reserve:
Area = 10,000 acres = 4,000 hectares = 40,000,000 square meters = 40 square kilometers

Area = ΠR^2 R^2 = Area / Π R = square root of Area / Π
Radius (R) = 3,570 meters

Circumference = $2\Pi R$
Circumference (C) = 22,430 meters

Sungai Dusun IPZ Reserve Cost: USD Cost = $224,300 USD
RM Cost = $785,050 RM

APPENDIX A

Anesthesia report detailing butorphanol / azaperone standing anesthesia for reproductive surgery on an adult female Sumatran rhinoceros.

"SEPUTIH"
SUMATRAN RHINO ♀
18 February 2003

Anesthesia Record - Fossil Rim Wildlife Center

Genus/species: Dicerorhinus sumatrensis Location/enclosure: Sungai Dusun barn Date: 18 February 2003
Common name: Sumatran Rhino Activity: O calm ✓ active O excited
Demeanor: O undisturbed O depressed ✓ alert O aggressive ✓ apprehensive
Sex: F age: ~24 (birthday: —) (Basis for age if not birthday: Wild-caught)
Previous ID?: SEPUTIH ID applied today/site: —
Environmental temperature: ~85°F ✓F O C Group size: 1 (6 total) housed individually
Purpose: Remove vaginal tumors
Time fasted: ✓ not fasted O <3 hrs O 3-24 hrs O 24-48 hrs O >48 hrs
Health status: ✓ 1-normal health O 2 - mild disease O 3 - severe disease O 4 - chronic disease
O 5 - may not survive anesthesia Pregnant? O yes ✓ no
Body condition: ✓ obese/fat ✓ good O fair O thin O poor/emaciated
Body weight: 762 kg ✓ actual O estimate Vet(s): Dr. Robin Radcliffe Recorder: RR
27 Jan 03 Dr. Rolfe Radcliffe Dr. Aidi Mohamad

Drug	#mg	#mL	Conc in mg/mL	Route	Time given	Time - Effect seen
BUTORPHANOL	80	8.0	10	IM	9:43:40	9:47:30 Mild sedation/head droop
AZAPERONE	80	2.0	40			9:48:43 Sedated/Quiet Still Standing
CEFAZOLIN	2g	1L NaCl	—	IV		
FLUNIXIN MEGLUMINE	500	10.0	50	IM		
NALTREXONE	250	5.0	50	IM	12:33:00	12:34:08 Moving ears 12:35:27 Moving head 12:35:48 Fully alert 12:36:27 Moved out of chute to pen

Samples collected:

sample	Amount	Media/additive	Purpose	#Cryovials banked
O Blood: O red tops		none		
O green tops		Na Heparin		
O purple tops		EDTA		
O feces		none	parisitology	
O ticks		Ethyl alcohol	parisitology	
✓ other: Excisional Masses	5	10% Buffered Formalin	Histopathology - UPM, Malaysia	

Summary:
E-time to first effect: 3:50 Standing sedation
E-time to recumbency: 5:03 (sternal/R or L lat?) tracheal tube size: —
E-time to first arousal (from time of reversal): 1:08 Anesthetic rating: ✓ excellent O good O fair O poor
E-time to ~~standing~~ fully alert (from time of reversal): 2:48
Total elapsed time (darting to ~~standing~~ fully alert): 2:52:08 HR:MIN:SEC Recovery: ✓ normal O abnormal (see comments)
Darting from (circle): vehicle or foot By Hand Dart/needle type: needle (By-Hand) 18 guage 1½" Site: (R) Hip
Distance away: By Hand Dart mixing time/date: Immed. before use SpO2 sensor: Nellcor NPB-40 Vet-Sat Large
Dart misses or failures: None
Comments: Excellent dose for large adult Sumatran Rhino.

Time (hr: min: sec)	SPO2	Temp °F °C	Pulse	Resp	Comments (drugs? observations? PE findings? etc.)
9:50				32	Mucous membranes pink
9:53	95%		54		start SpO_2 on (L) ear
9:55	87%		74		start cleaning
9:57	93%		85		
9:59	93%		65		finished cleaning.
10:04	93%		66		
10:06	91%		59		insert electro
10:09	93%		61		removed 1st tumor
10:12	91%		58		insert electro
10:16	92%		61		
10:20	93%		59		pull out the electro
10:24	92%		57		insert the eelectro
10:29	92%		55		
10:33	91%		58		removed 2nd tumor
10:36	92%		55		insert electro
10:41	92%		56		
10:43	92%		55		Urinate
10:46	92%		57		
10:49	93%		53		Removed 3rd tumor
10:54	93%		54		insert electro
10:56	95%		52		
11:01	92%		58		
11:06	93%		51		removed 4th tumor
11:09	91%		55		insert electro
11:14	90%		52		
11:19	92%		59		
11:24	88%		56		cut use wire
11:25	90%		57		removed 5th tumor (big)
11:36	92%		61		
11:41	86%		63		
11:44	87%		56		insert wire loop
11:49	91%		57		vulva started to move
11:51	94%		61		start cutting
11:56	93%		71		
12:01	93%		65		
12:03	93%		61		start flushing 5000 ml
12:07	91%		58		
12:12	90%		59		
12:19	91%		67		finished flushing 5000 ml
12:20	94%		55		flushing antibiotic
12:25	93%		52		Fortrin ampicillin 500 mg
12:30	94%		56		
12:33	92%		56		NALTREXONE 250 mg IM
12:35	93		50		2 minutes post-reversal

APPENDIX B

Sumatran Rhino Management Plan Summary

February 26, 2003

Rhino Name	Short-term Plan	Long-term Plan
Minah	Mix with Ara daily Ultrasound daily P4 assay 2x weekly	Breed with Ara and confirm Pregnancy!!
Rima	Ultrasound 2x weekly	If pregnant, leave on Regumate as per Dr. Roth's Protocol Start Regumate on Day 10 (March 5) Keep on Regumate through Day 30 (March 25) Repeat PGF only if NOT PREGNANT
Panjang	Ultrasound 3x weekly	If no breeding after 30 days, then repeat the FSH/PGF trial Mix with Ara based on ultrasound findings Mix with Ara based on ultrsound findings
Mas Merah	Start on 15 day Regumate trial – give 15 mg PGF on the day following Regumate withdrawal (Give PGF on Day 16) Ultrasound 3x weekly and mix with Ara based on ultrasound findings	If no change in Mas after the Regumate trial, then move Mas to 10 acre enclosure or to Zoo Negara Consider PMSG therapy
Seputih	Give Seputih 6 weeks of rest (April 1, 2003) However, start ultrasound 1x per week on March 20 and mix with Ara based on ultrasound and P4 findings	Breed with Ara and monitor closely for early pregnancy

APPENDIX C SUMATRAN RHINO SERUM CHEMISTRY and IRON ASSAY

Parameter February17, 2003	**Unit**	**MINAH**	**RIMA**	**ARA**	**MAS MERAH**	**SEPUTIH**	**PANJANG**
Total serum protein	(g/dl)	7.0	7.2	6.3	8.1	7.1	7.4
Albumen	(g/dl)	3.4	3.1	2.9	2.4	3.4	2.9
Calcium	(mg/dl)	12.5	13.1	11.3	13.3	12.8	14.9
Phosphorus	(mg/dl)	4.0	4.8	4.2	5.6	4.1	5.5
Glucose	(mg/dl)	72	85	63	89	74	90
BUN	(mg/dl)	6.2	12.5	7.9	8.4	4.4	12.3
Creatinine	(mg/dl)	0.6	1.0	0.7	1.2	0.6	1.7
Total Bilirubin	(mg/dl)	0.1	0.1	0.1	0.1	0.1	0.1
Direct Bilirubin	(mg/dl)	0.0	0.0	0.0	0.0	0.0	0.0
ALP	(U/l)	111	80	41	239	117	229
CK	(U/l)	480	497	293	517	385	298
AST (SGOT)	(U/l)	75	61	37	99	90	76
Globulins	(g/dl)	3.6	4.1	3.4	5.7	3.7	4.5
Albumen:Globulin Ratio		0.94	0.76	0.85	0.42	0.92	0.64
GGT	(U/l)	18	14	6	10	16	12
Sodium	(meq/l)	127	126	129	124	127	123
Potassium	(meq/l)	10.9	10.3	4.7	10.5	12.9	8.4
Na/K Ratio		11.7	12.2	27.4	11.8	9.8	14.6
Chloride	(meq/l)	94	93	96	87	96	87

Sumatran Rhinoceros Serum Iron Summary; Sungai Dusun, February 2003

Rhino ID	Serum Fe (ug/dl)	TIBC (ug/dl)	Ferritin (ng/ml)	Transferrin Saturation (%)	Haptoglobin (mg/dl)	Ceruloplasmin (mg/dl)
MINAH	190	214	4,251	89	54	151
RIMA	79	182	361	43	142	183
ARA	105	176	257	60	124	114
MAS MERAH	174	194	3,293	90	235	74
SEPUTIH	196	247	2,527	79	103	152
PANJANG	185	269	4,042	69	125	160

APPENDIX D
SUMMARY OF FSH and PGF EFFECTS IN THE SUMATRAN RHINOCEROS *(Dicerorhinus sumatrensis)*

Conclusions from combined FSH and PGF therapy trial in three Sumatran rhinoceros *(Dicerorhinus sumatrensis)*

The combined use of PGF (Dinoprost; Lutalyase) and FSH (Follicle Stimulating Hormone; Follutropin) was attempted in two female Sumatran rhinos to induce cyclic activity. One animal (Panjang) responded nicely to the therapy and within 5 days had developed preovulatory sized follicles and was receptive to the male (see Panjang's detailed summary above).
Dose and Schedule for PGF/FSH Protocol:
Reference: Dr. Mel Fahning; University of Minnesota College of Veterinary Medicine; (612) 625-3795

DAY OF PROTOCOL	TIME	PGF	FSH
Day 1	AM	15 mg (3.0 ml Lutalyse) IM	40 mg (2.0 ml Follutropin) IM
	PM		40 mg IM
Day 2	AM	15 mg IM	30 mg (1.5 ml Follutropin) IM
	PM		30 mg IM
Day 3	AM		20 mg (1.0 ml Follutropin) IM
	PM		20 mg IM
Day 4	AM		10 mg (0.5 ml Follutropin) IM
	PM		10 mg IM

NOTE: PGF = Prostaglandin F2 alpha (Dinoprost tromethamine; Lutalyse; 5 mg/ml)
FSH = Follicle Stimulating Hormone (porcine-derived FSH; Follutropin; 20 mg/ml)

NOTE: New PGF dose schedule = 15 mg Lutalyse IM on one occasion (rather than the previous dose of 10 mg IM on 2 consecutive days)

Further trials and evaluations are needed before routine use of this or other hormonal therapies should be considered in the Sumatran rhino.

APPENDIX E Proposed modification to the 100-acre area at Sungai Dusun