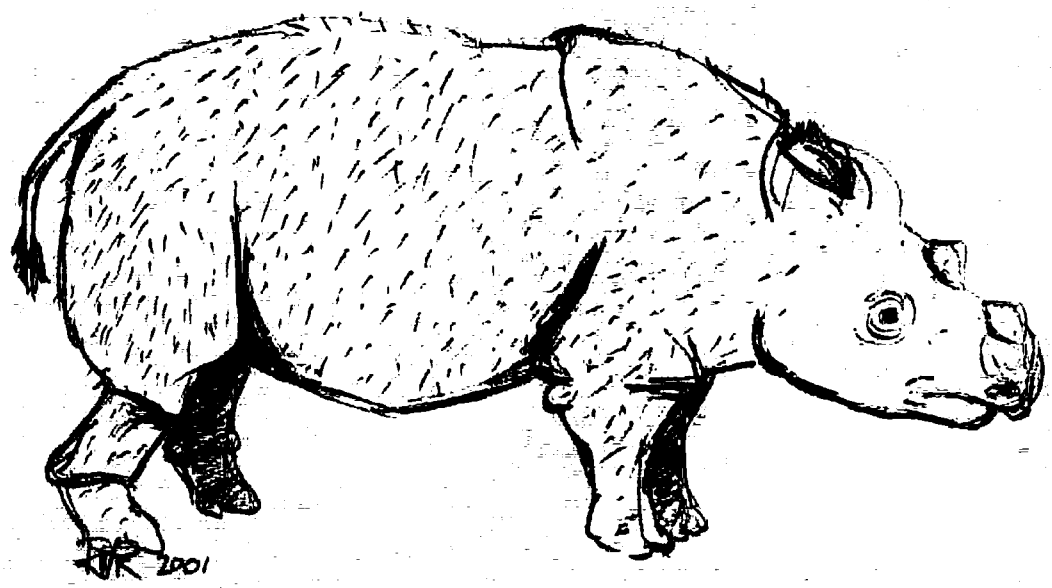
SUMATRAN RHINO REPRODUCTIVE AND HEALTH ASSESSMENT

Sumatran Rhino Conservation Centre
Sungai Dusun, Malaysia
August 29, 2001 through September 5, 2001



Link to Sumatran rhino video introduction:
../Sungai Dusun web video/Sumatran Rhino Browsing(1 min)

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Submitted by Robin W. Radcliffe, DVM, Dipl. ACZM[1] and
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At the invitation of Musa Nordin, the Director General of the Malaysian Wildlife Department, 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 the University of Guelph, respectively, visited the Sungai Dusan Sumatran Rhino Conservation Centre. For 10 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 two major objectives. First, with concerns over possible reproductive tract disease in the young female named "Minah", the first priority was to assess this female's condition and develop a treatment protocol for Minah with the goal of returning her to normal reproductive function. Second, there is a growing need to develop a detailed reproductive monitoring program 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, the transfer of techniques for ultrasonographic study in concert with serum progesterone evaluation may provide the most effective tools for reproductive assessment of Sumatran rhino. Much work had previously been done to accomplish these objectives and our involvement was only to provide guidance in further development of such a program.

We accomplished both objectives as illustrated by the comprehensive nature of this report. The story of Minah and her long-term problem is an interesting one and is outlined here. A detailed plan for use of ultrasound as a reproductive management tool in captive Sumatran rhino is highlighted in Appendix A and our suggestions for the Sungai Dusun program are summarized in Appendix D.

Sumatran Rhino Reproductive Evaluation
Sungai Dusun, Malaysia
Date of exams: 29 August , 3 September and 5 September 2001

Species	SB#	Sex	Name	Age
Dicerorhinus sumatrensis	15	F	Minah	14

Captive years without reproducing: 14
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.

Purpose of exam: Reevaluation of suspected uterine pathology identified on March 2001 ultrasound exam by Dr. Roth.

Brief Summary of Findings:

Minah's reproductive tract was considered normal on ultrasound with no evidence of the intrauterine pathology reported by Dr. Roth in March. There were no signs of intrauterine fluid collection or other findings to suggest an

endometritis. The ovaries were small and therefore consistent with an acyclic female (Figures 7 and 8).

From an animal health aspect, we were 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 ~ 3 out of 5.

The urinary bladder wall was thickened and abnormal in appearance and therefore a second exam was scheduled (3 September) to closely evaluate Minah's bladder. The mucosal surface of the urinary bladder had an increased echogenic pattern as well as a possible intraluminal mass of unknown origin (Figures 1, 2 and 3). Based on Minah's history of a failed recovery of an intravaginal CIDR, an endoscopic exam of Minah's bladder was scheduled to identify and remove any abnormal mass or foreign body (Figures 4,5 and 6).

Immediate Plan:

An endoscopic exam of Minah's bladder was conduced on 5 September 2001 at Sungai Dusun. The results of this exam are detailed below and may help explain the abnormal signs that Minah had shown in the past year including her apparent acyclicity. A urinalysis was also conducted on Minah and several other females for comparison (Table 1). The results of the urinalysis confirmed a cystitis (bladder infection) in Minah as evidenced by the numerous white blood cells, casts and bacteria identified.

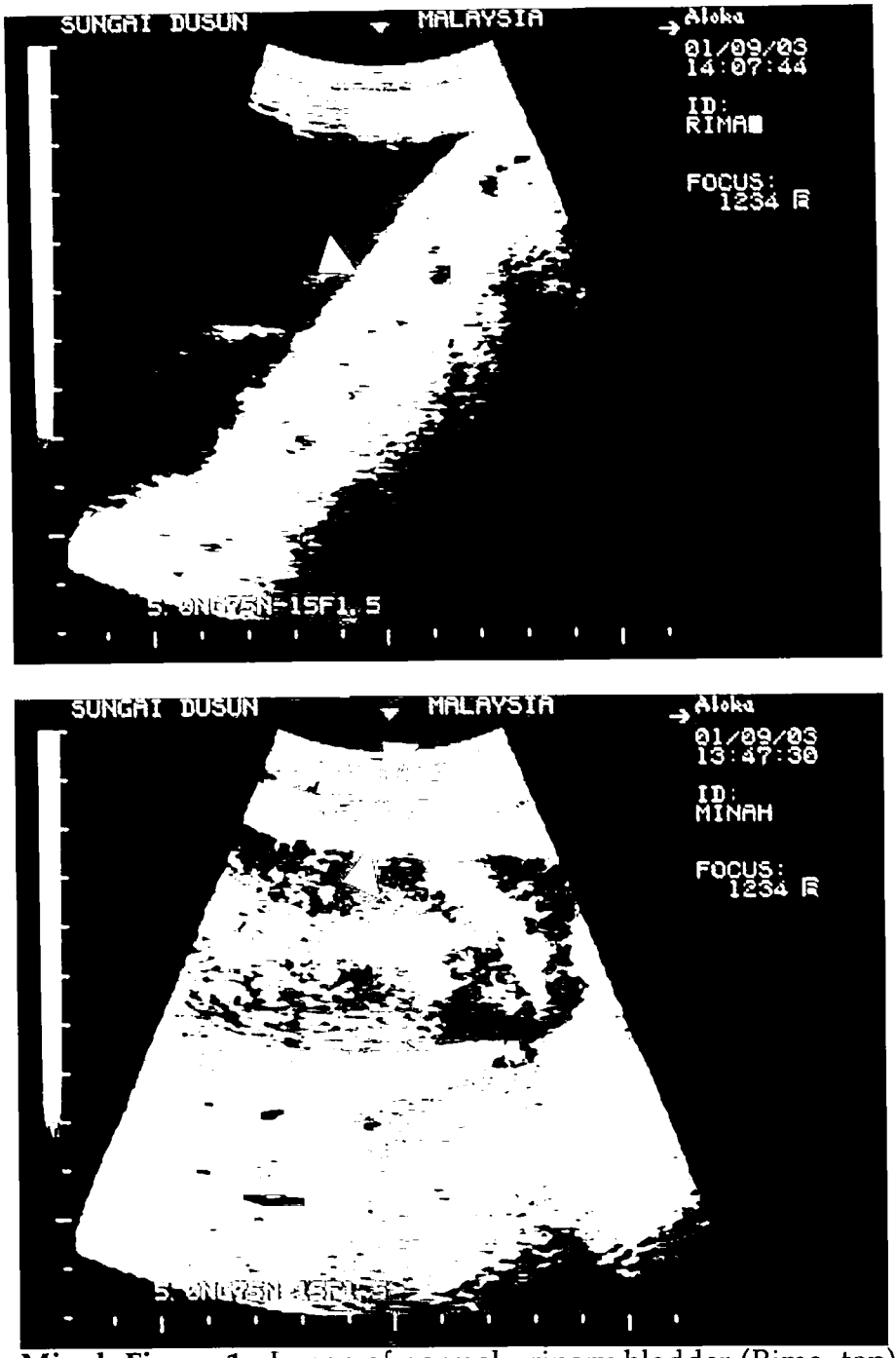
Based on the findings of normal reproductive anatomy together with the lack of any significant ovarian activity, Minah was given a dose of gonadotropin releasing hormone (GnRH):

500 micrograms (IM in the left neck) at 10 am on the 30th of August 2001

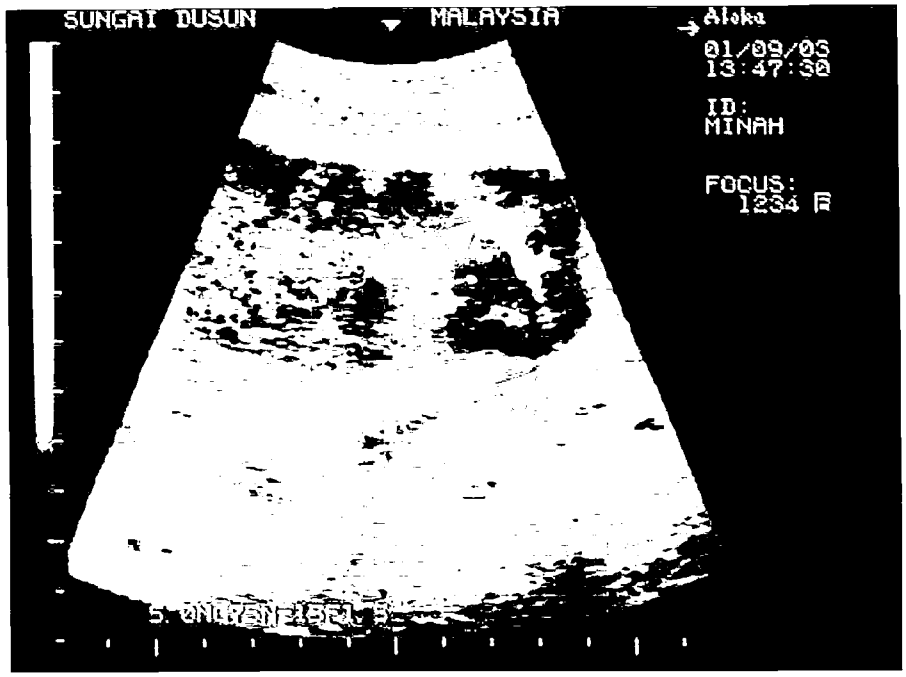
Following this therapy, it will be critical to perform follow-up reproductive monitoring of Minah via ultrasound and serum progesterone assay. See detailed recommendations for Minah outlined in Appendix B.

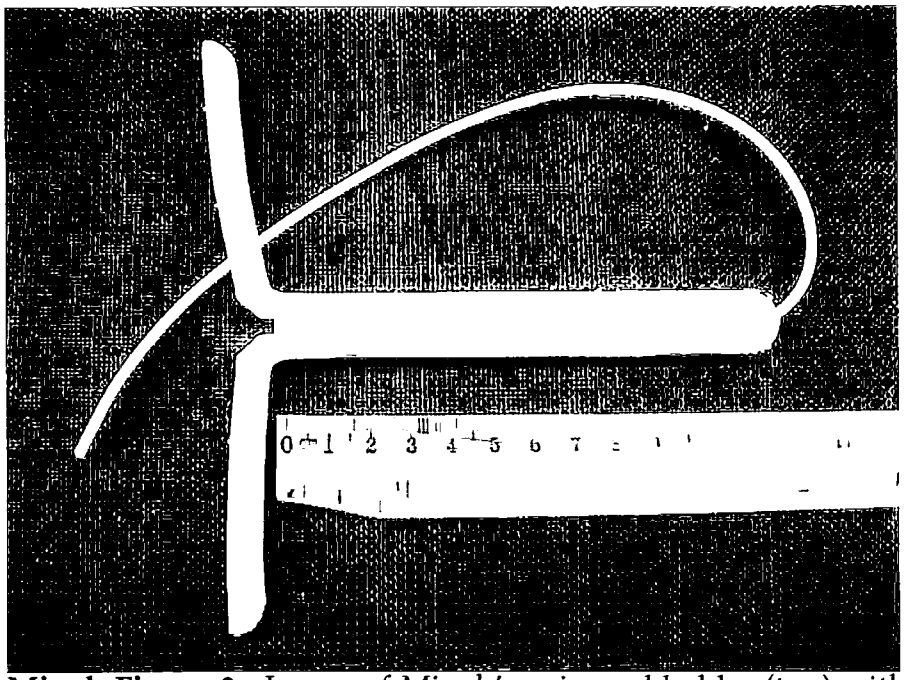
Table 1. Urinalysis of female Sumatran rhinoceros, Sungai Dusun.

Animal ID	Urine pH	Urine SG	WBC's	RBC's	Casts	Bacteria
Minah	8.5	1.006	numerous	0-2	cellular +	3+
Seputeh	7.5	1.025	rare	rare	none	+
Panjang	6.5	1.014	rare	rare	none	2+ (floor sample)
Rima	8.5	1.012	rare	rare	none	+

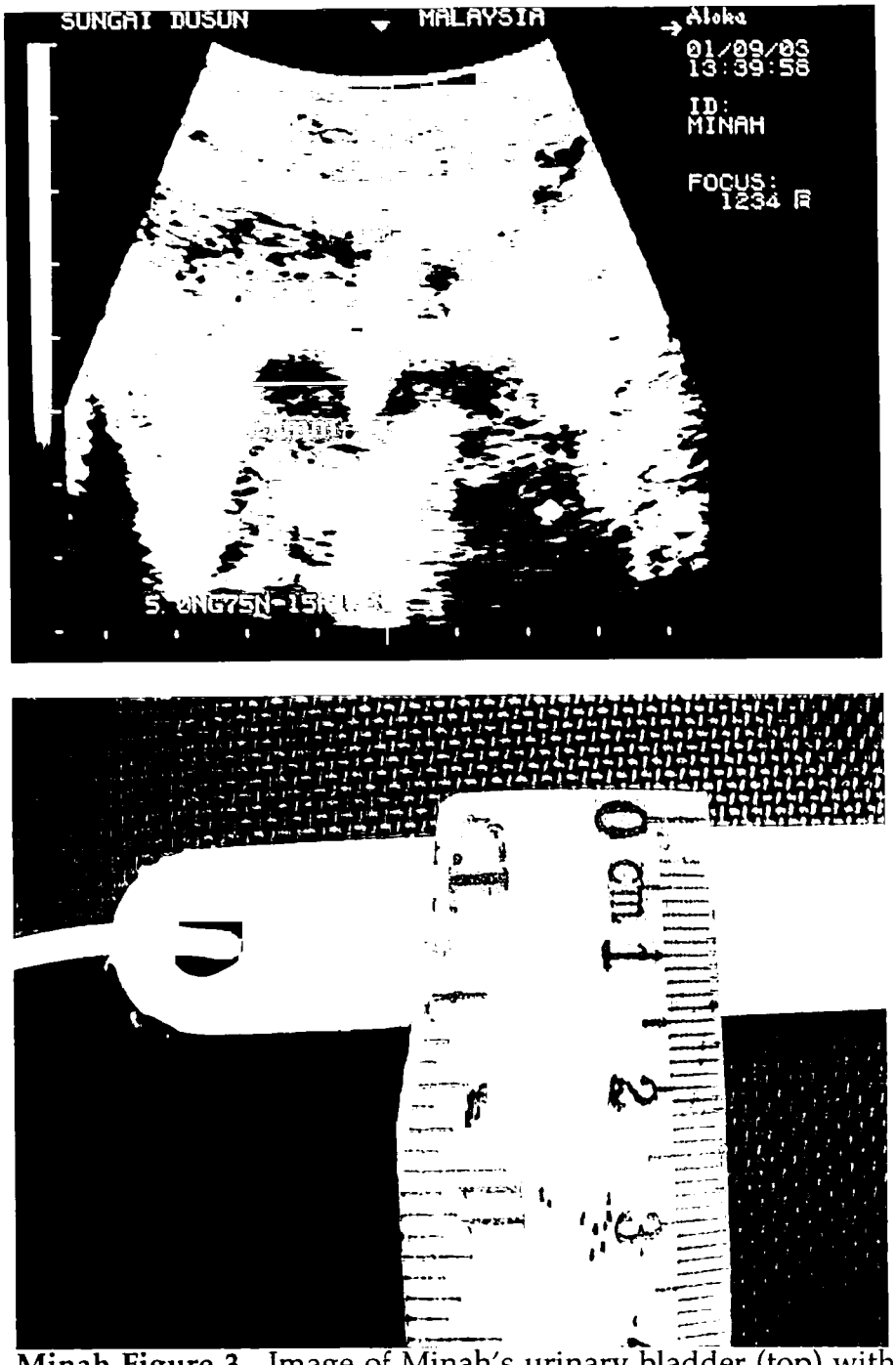


Minah Figure 1. Image of normal urinary bladder (Rima, top) and diseased urinary bladder of Minah (bottom) with echogenic mass in lumen of bladder. Note increased bladder wall thickness in Minah versus normal female as depicted by arrows.





Minah Figure 2. Image of Minah's urinary bladder (top) with echogenic mass in lumen of bladder illustrating characteristic shape and size of CIDR implant lost during placement in March of 2000 (bottom). Arrows depict borders of implant within bladder lumen.



Minah Figure 3. Image of Minah's urinary bladder (top) with echogenic mass in lumen of bladder showing characteristic cross-section and 15 mm diameter measurement of CIDR implant (bottom).

Minah Figure 4. Endoscopic removal of bladder foreign body using standing butorphanol sedation in a Sumatran rhino.

Link to procedure video: ../Sungai Dusun web video/Minah endoscopy(3 min)

Endoscopic removal of bladder foreign body:
On Wednesday September 5, 2001 we sedated Minah with Butorphanol (Radcliffe et. al., 2000; see Appendix C for anesthesia report) and performed an endoscopic exam of Minah's bladder and removed the progesterone implant that had been identified on ultrasound. The anesthesia was excellent for standing sedation in a Sumatran rhinoceros. The endoscopic procedure proved extremely difficult with multiple attempts made to facilitate removal via endoscopic visualization and retrieval of the implant with various surgical graspers. Finally, the implant was successfully removed via digital manipulation and retrieval with the surgeon's gloved hand as has been reported in the mare for removal of large uroliths (bladder stones).

The progesterone implant is designed for intravaginal use and is not to be left in the vagina for more than the recommended 7-10 days per label directions. The implant had been in Minah's bladder for approximately 1.5 years (Figure 6). The implant is impregnated with 1.9 g progesterone in an inert silicone elastomer; it is likely that the implant could continue a slow release of progesterone for several years. Therefore, all of Minah's clinical signs could be attributed to the long-term negative effects of the implant within this female's bladder.

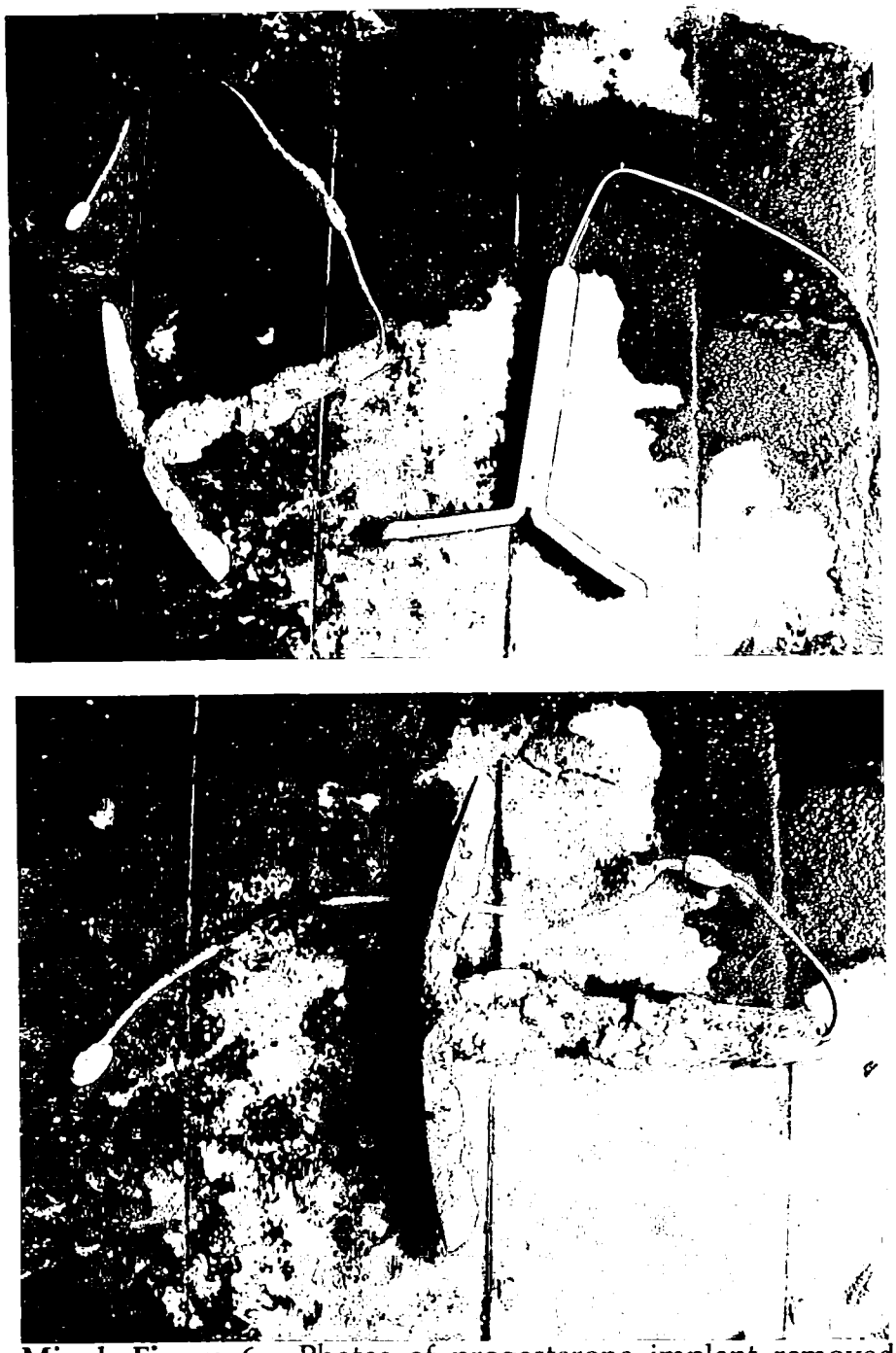
Clinical signs and disease of Minah secondary to implant:

Clinical signs and disease of Minah secondary to implant:

- Reproductive acyclicity
- Loss of body condition
- Anorexia
- Frequent urination, urine squirting, and straining to urinate
- Cystitis as illustrated by changes in bladder observed on ultrasound, endoscopy and urinalysis
- Endoscopic signs of severe bladder wall irritation and hemorrhage

All of the above clinical signs and pathology should resolve now that the implant has been removed and Minah can receive appropriate follow-up medical therapy and rest prior to any breeding attempts (see detailed recommendations for Minah in Appendix B). It is fortuitous that the implant was removed at this time, as bladder wall perforation followed by fatal peritonitis would have been the likely sequelae based on the observed changes to the bladder mucosa.

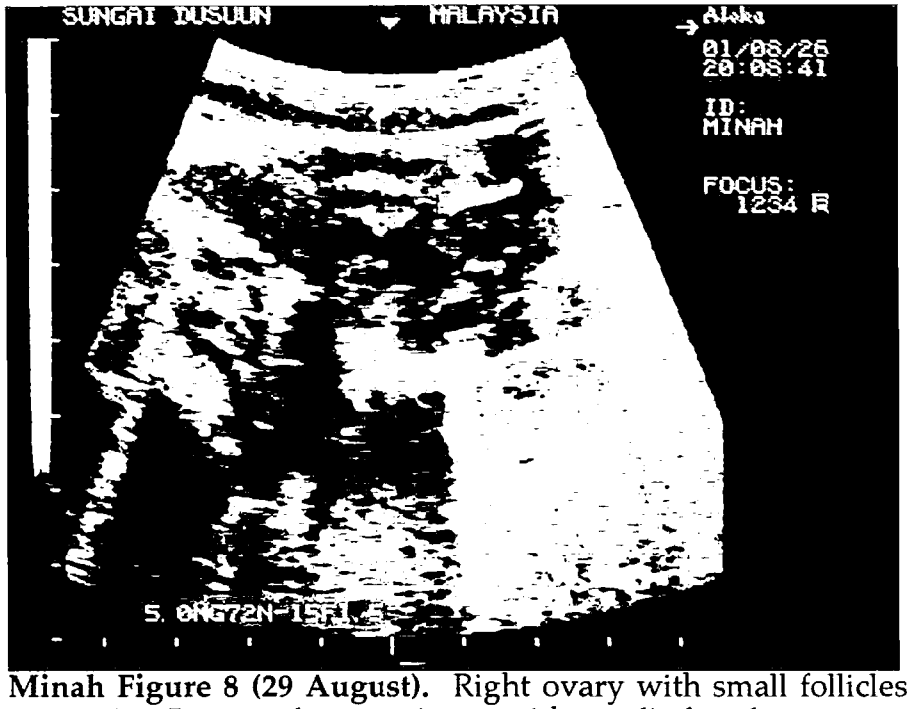
Minah Figure 5. Anesthetic monitoring of Minah during endoscopic removal of bladder foreign body under butorphanol standing sedation in chute at Sungai Dusun.



Minah Figure 6. Photos of progesterone implant removed from Minah adjacent to new implant for comparison (top) and close-up of implant illustrating accumulation of attached uroliths secondary to long-term sequestration within Minah's urinary bladder (bottom).

Reproductive Exam Details: MINAH

Minah Figure 7 (29 August). Left ovary with small follicles measuring 8 to 12 mm in diameter and characteristic of acyclic female.



Minah Figure 8 (29 August). Right ovary with small follicles measuring 5 mm or less consistent with acyclic female.

Sumatran Rhino Reproductive Evaluation
Sungai Dusun, Malaysia
Date of exams: 29 August, 30 August, 3 September and 4 September 2001

Species	SB#	Sex	Name	Age
Dicerorhinus sumatrensis	7	F	Rima	~22+

Captive years without reproducing: 14
Recent breeding activity: Regular breeding at ~21 day intervals (last breeding with Ara on 30 July 2001).
Progesterone profile: Regular 21 day cyclical pattern.
Hormone manipulations: None

Purpose of exam: Examine for possible early pregnancy and pathology. Although Rima's serum P4 is declining, it was still elevated after 29 days so we were hopeful to possibly identify an early pregnancy.

Brief Summary of Findings:

Rima was not pregnant. We did identify several endometrial cysts (Figures 1 and 2) present in Rima's uterus – one of which was located in the distal right uterine horn and measured 22 mm (Figure 2). There was evidence of follicular activity and ovulation with luteal tissue present, indicating that despite some minor pathology Rima is apparently cycling normally (Figures 3, 4 and 5).

follicular activity and ovulation with luteal tissue present, indicating that despite some minor pathology Rima is apparently cycling normally (Figures 3, 4 and 5).

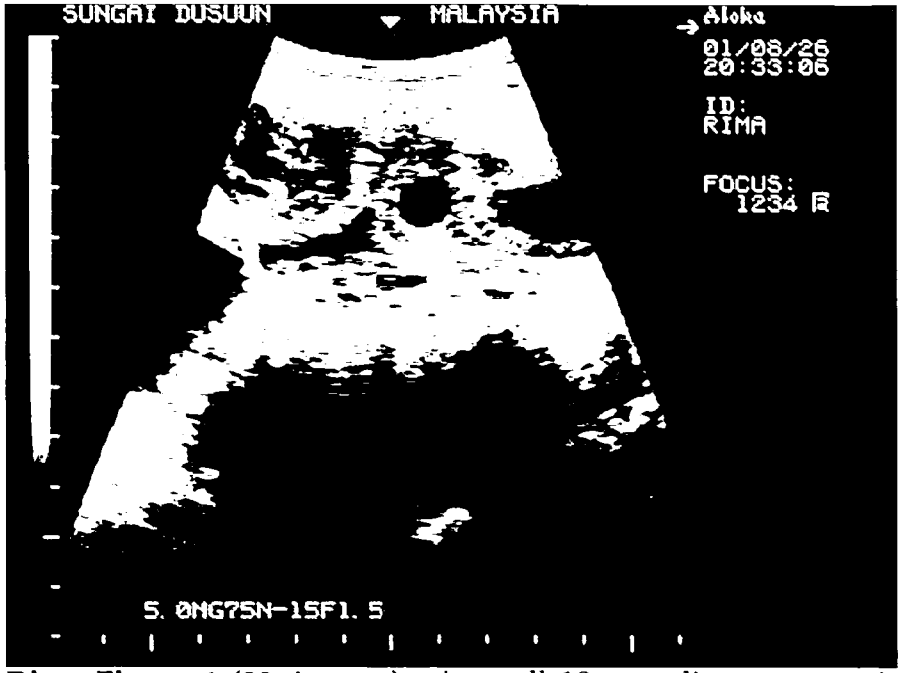
Immediate Plan:

Based on the findings of minimal uterine pathology together with a history of regular estrus behavior and progesterone levels indicating a ~21 day reproductive cycle, there is a good chance that this female could conceive and carry a pregnancy. If the progesterone levels remain elevated for longer than the 21-day cycle as it appears to have occurred on this occasion, then perhaps Rima may be sustaining early embryonic loss. The best way to assess for this possibility will be to place Rima on a regular schedule for routine ultrasound examination (see Appendix A entitled, *A Proposed Model for Reproductive Monitoring of Sumatran Rhino*).

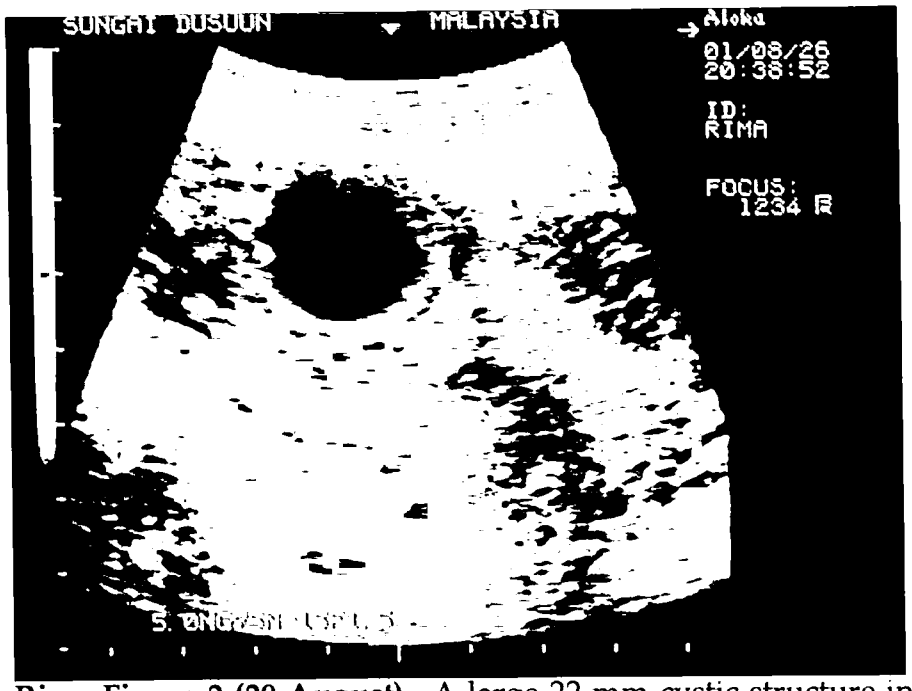
Recommendations:

- Continue breeding Rima 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.

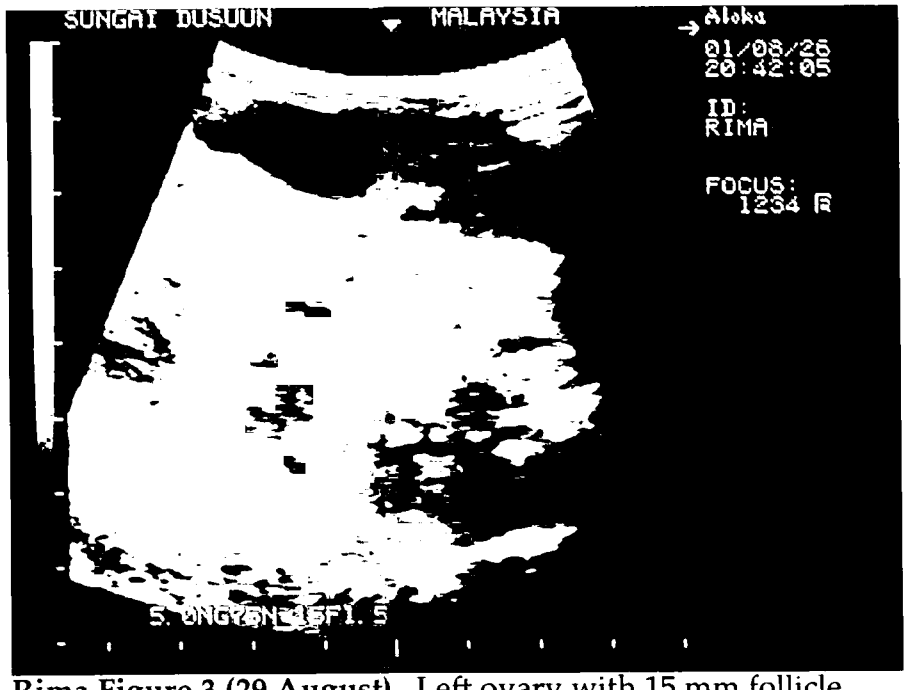
Reproductive Exam Details: RIMA



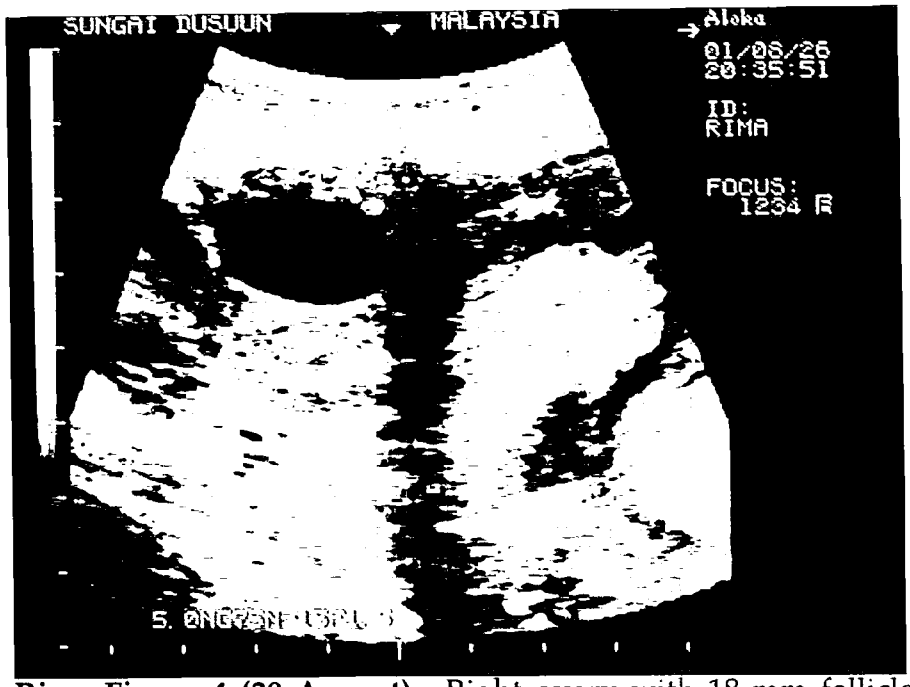
Rima Figure 1 (29 August). A small 10 mm diameter cyst in base of right uterine horn.



Rima Figure 2 (29 August). A large 22 mm cystic structure in distal right uterine horn that looks similar to an early pregnancy.

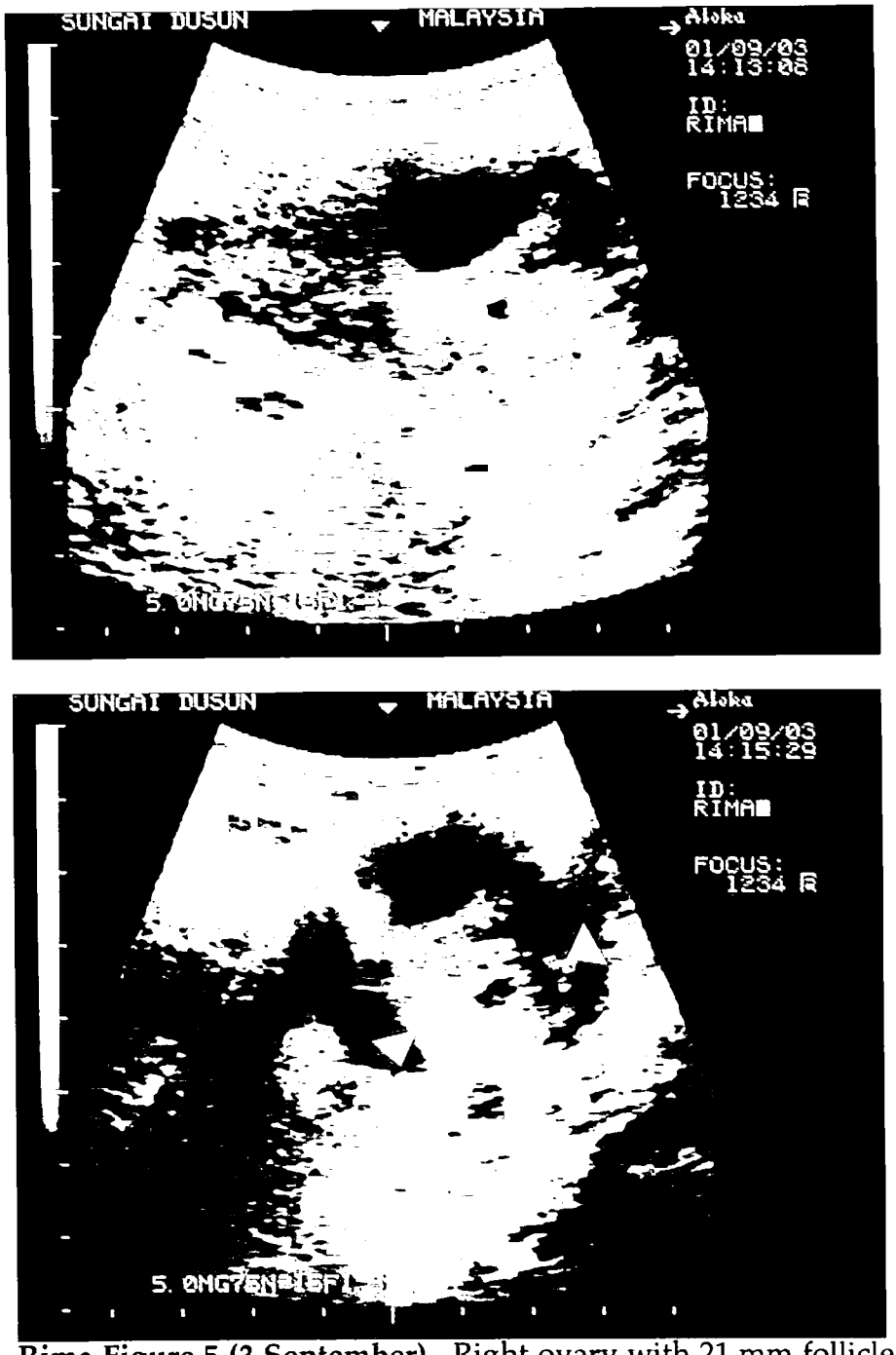


Rima Figure 3 (29 August). Left ovary with 15 mm follicle.



Rima Figure 4 (29 August). Right ovary with 18 mm follicle and 15 mm corpus luteum (CL) just below follicle. Presence of CL is consistent with luteal phase of cycle.

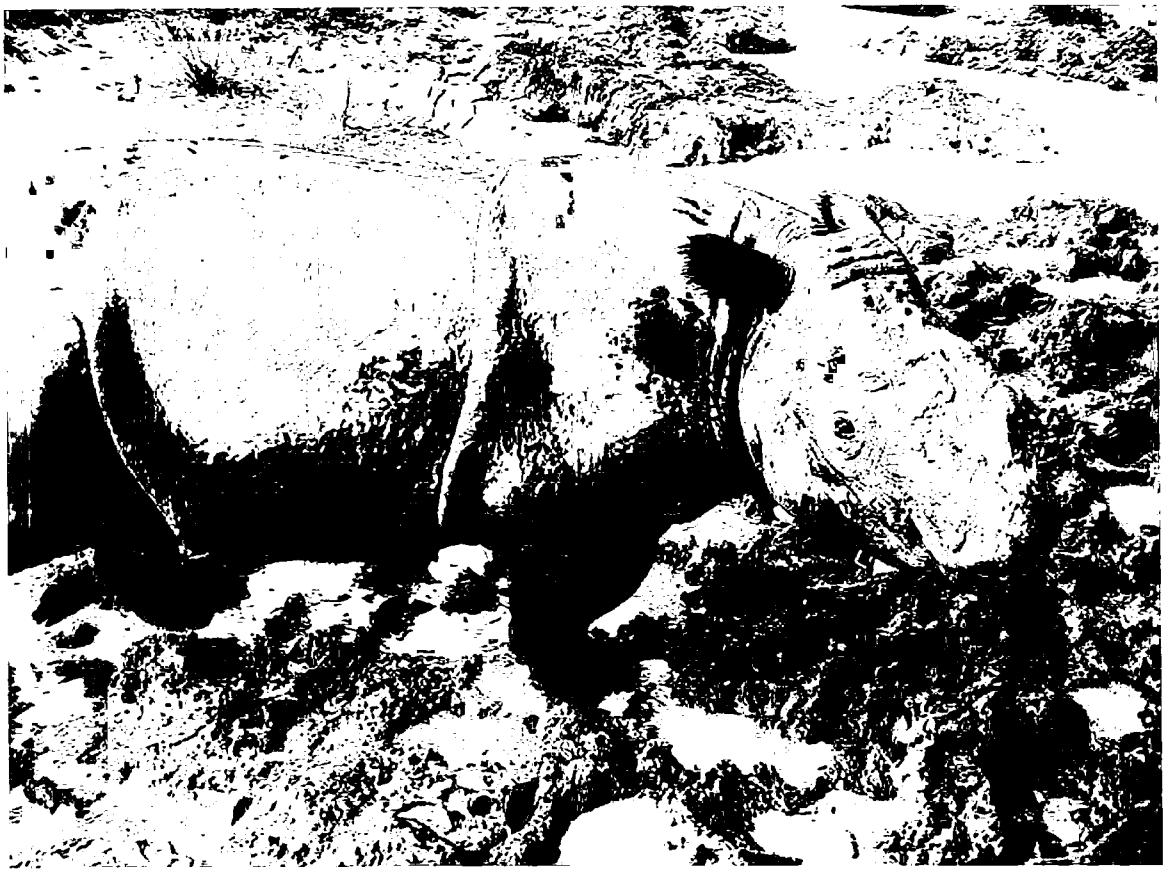
Note: 30 August exam revealed 20 mm follicle on Right Ovary indicating maturing follicle and possible approaching estrus. Ara and Rima were introduced based on the increasing size of the observed follicle without breeding. The lack of breeding activity can be explained by the persistence of the luteal structure on Rima's right ovary (Figure 5). Therefore, it is important to evaluate not only follicular size, but also any evidence of persistent luteal tissue. Serum progesterone assay results can be used to support the ultrasound findings.



Rima Figure 5 (3 September). Right ovary with 21 mm follicle and 20 mm corpus luteum just below follicle. Rima was introduced to Ara and showed resistant behavior and no breeding occurred.

Sumatran Rhino Reproductive Evaluation
Sungai Dusun, Malaysia
Date of exams: 29 August, 30 August and 31 August 2001

Species	SB#	Sex	Name	Age
Dicerorhinus sumatrensis	19	F	Mas Merah	~22+



Link to Sumatran Rhino in mud wallow at Sungai Dusun:
../Sungai Dusun web video/Wallowing Sumatran Rhino(2 min)

Captive years without reproducing: 14
Recent breeding activity: Never bred (mounted but not bred in April 2000).
Progesterone profile: Irregular cyclic pattern suggestive of formation and lutenization of hemorrhagic follicles.
Hormone manipulations: Attempted CIDR implant plus PGF2alpha in March 2000 without success. Implanted with Synchromate 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:

Brief Summary of Findings:

Interestingly, Mas Merah had what appeared to be a 26 mm preovulatory follicle on her left ovary (Figures 1 and 3) and based on this information the decision was made to attempt an introduction of this female with Ara. Mas Merah appeared to have a normal reproductive tract with only one small endometrial cyst in an area of uterine hyperplasia with no distinct masses as had been previously reported.

Immediate Plan:

Based on the findings of minimal uterine pathology together with the observation of a large preovulatory follicle on the left ovary, we put Mas Merah and Ara together for breeding at 2 pm. We were excited to see some very convincing estrus behavior by Mas Merah and breeding activity by Ara (Figure 2). The 2 animals were watched closely for 2 hours with multiple mounting attempts made as well as at least one short period of intromission. Based on the large size of the POF, it is likely that we put the 2 animals together towards the end of the period of maximum receptivity. Based on Dr. Roth's work with Emi in Cincinnati (Roth et. al., 2001), breeding and induced ovulation appears to occur at a follicular size of ~24 mm. This would suggest that perhaps we were 12 hours late in our introduction of Mas Merah for breeding.

Ovulation was documented within 48 hours of breeding as illustrated by the identification of an ovulation site and formation of a corpus luteum (Figures 4 and 5). This was a significant event that supported previous work by Dr. Roth and illustrated that such work in zoological setting can be applied directly to enhance breeding management in other captive environments. This finding also serves to highlight the importance of using ultrasound as a tool to assist rhino management efforts for the Sumatran rhino.

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.

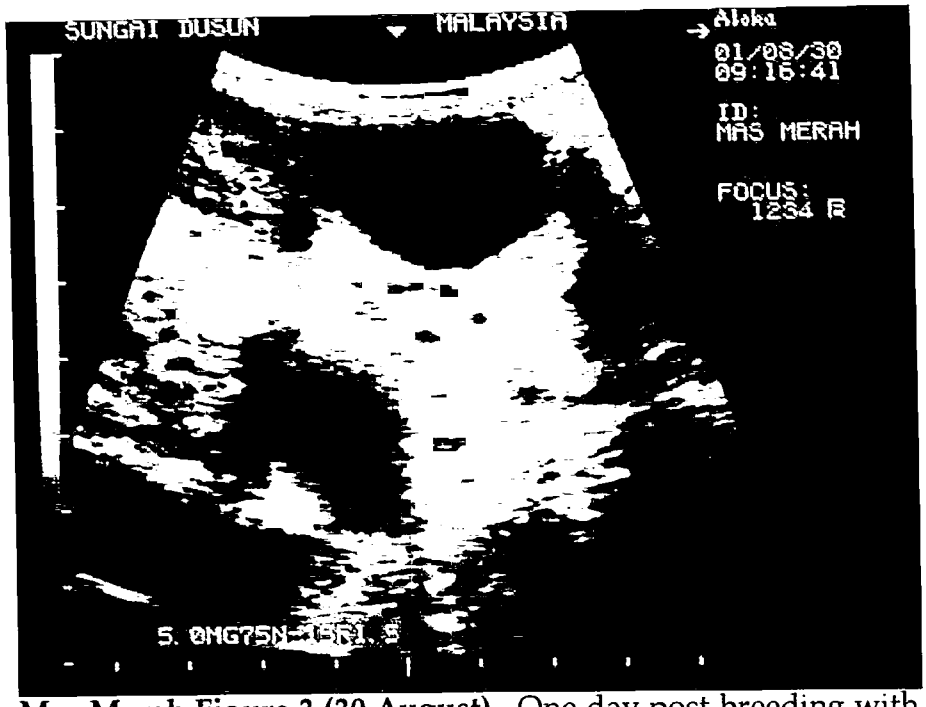
Reproductive Exam Details: MAS MERAH

Mas Merah Figure 1 (29 August). Day of breeding Mas Merah and Ara with 26 mm Preovulatory follicle on Mas Merah's left ovary.

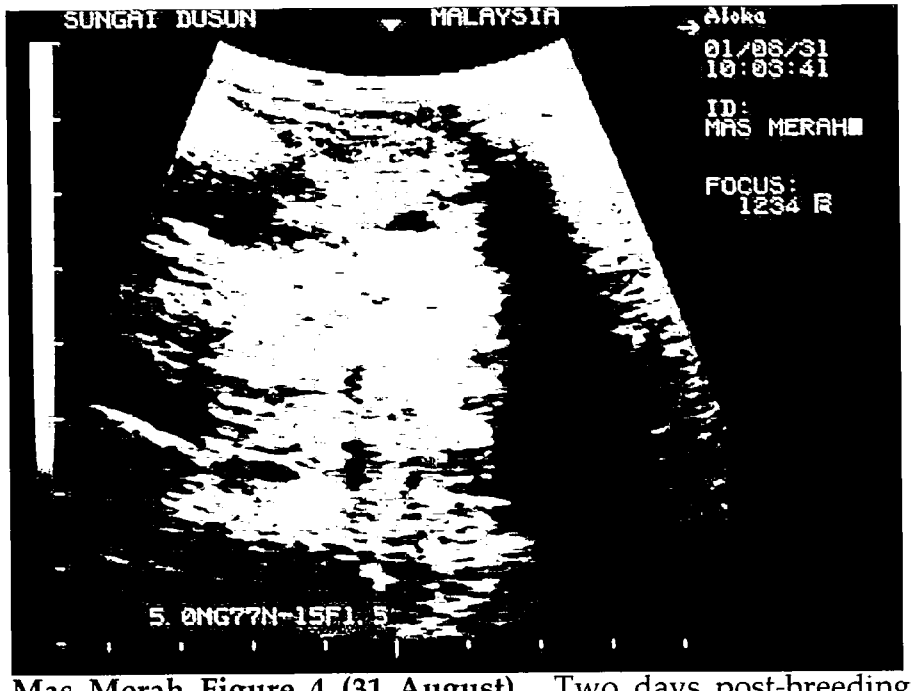
Link to Sumatran rhino video narrated by Steve Romo:

../Sungai Dusun web video/Sumatran Rhino Breeding(38 sec)

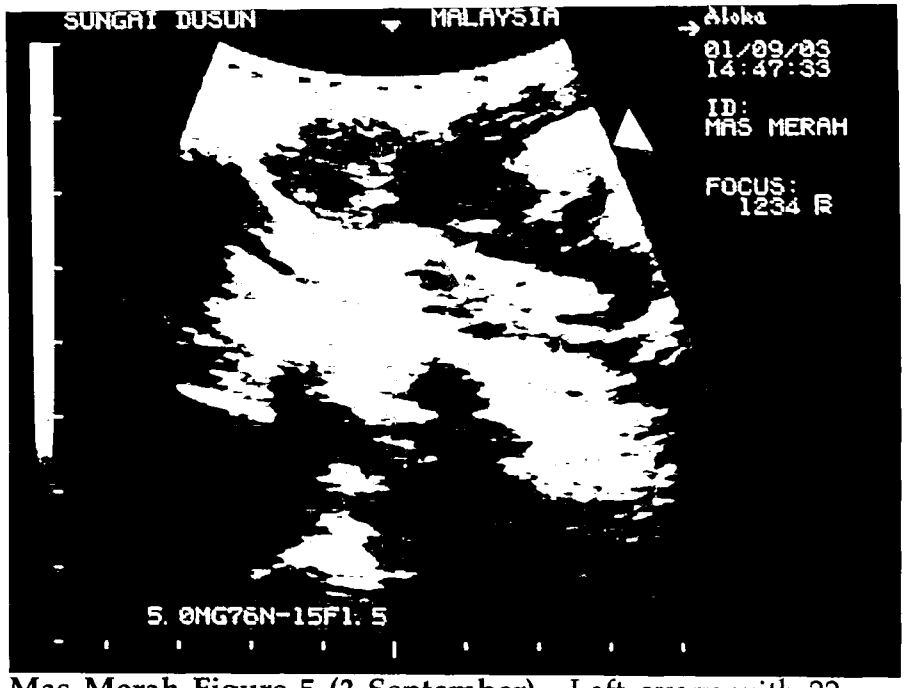
Mas Merah Figure 2 (29 August). First successful breeding of Mas Merah and Ara facilitated by timed introduction based on finding Mas Merah's 26 mm Preovulatory follicle.



Mas Merah Figure 3 (30 August). One day post-breeding with 28 mm follicle and complete lack of receptivity by Mas Merah.



Mas Merah Figure 4 (31 August). Two days post-breeding with evidence of successful ovulation by Mas Merah! Corpus luteum delineated by arrows.



Mas Merah Figure 5 (3 September). Left ovary with 22 mm corpus luteum (right at arrows) adjacent to uterine wall in cross-section at left.

Sumatran Rhino Reproductive Evaluation
Sungai Dusun, Malaysia
Date of exam: 29 August 2001

Species	SB#	Sex	Name	Age
Dicerorhinus sumatrensis	13	F	Panjang	~19

Captive years without reproducing: 14
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:

Panjang appeared to be a reproductively active female earlier this year based upon animal behavior, endocrine data and ultrasound evaluation. Unfortunately, Dr. Terri Roth reported a large 12 cm diameter uterine mass visible on ultrasound within the caudal aspect of the uterus during her examination in March of 2001. This mass was thought to be associated with a bloody vaginal discharge discovered 18 days following breeding in October of 2000. Based on the large size and subsequent passage of the mass (Figure 1) the

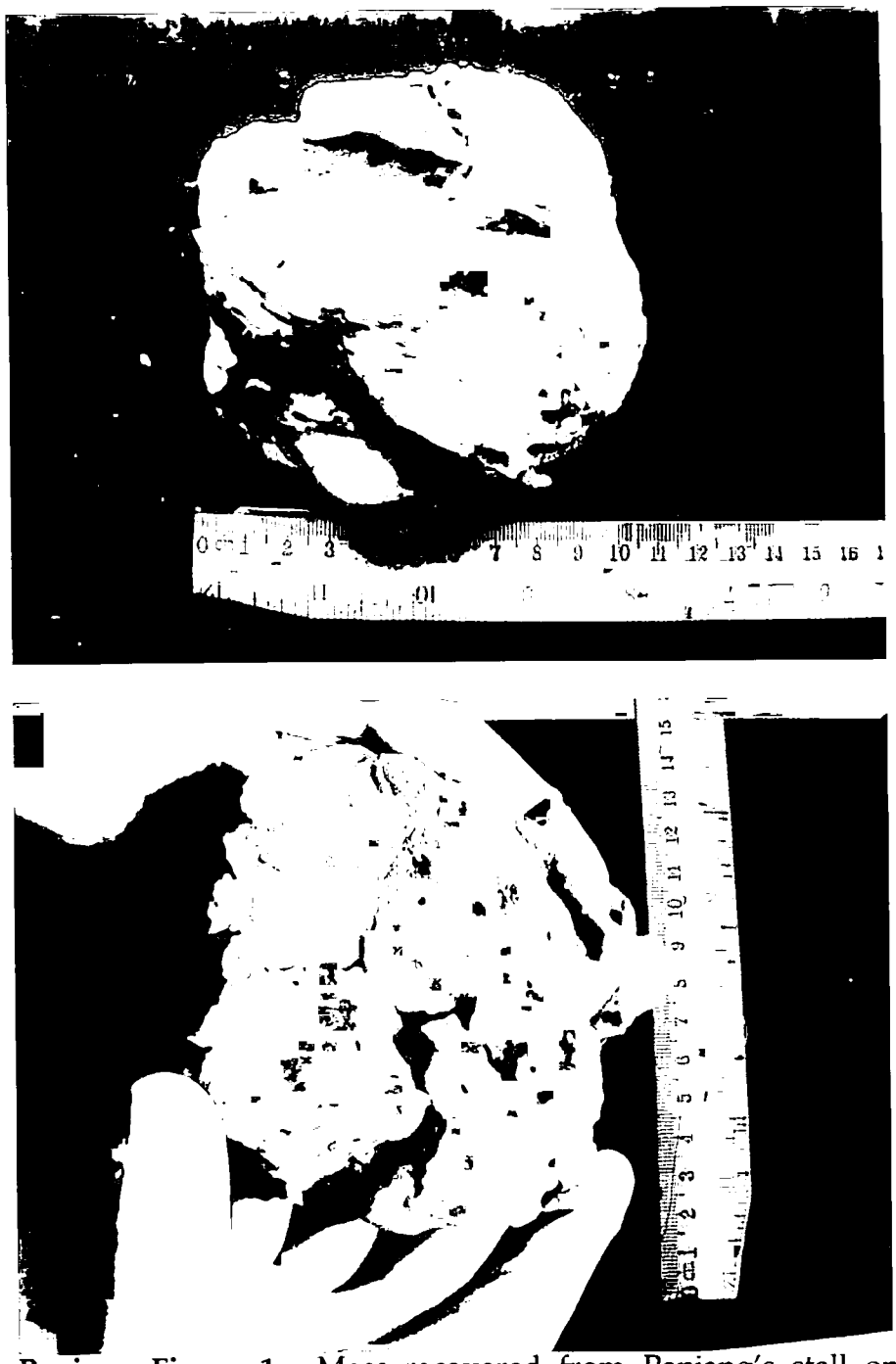
examination in March of 2001. This mass was thought to be associated with a bloody vaginal discharge discovered 18 days following breeding in October of 2000. Based on the large size and subsequent passage of the mass (Figure 1) the original location was likely within the cranial vagina and not the uterus as previously suspected. A vaginal location would also support the clinical picture of vaginal hemorrhage post-breeding.

Brief Summary of Findings:

Panjang had significant pathology present within her reproductive tract (Figures 2 and 3). A large 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 3). The mass is most likely a tumor, possibly a leimyoma, 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. The larger mass observed by Dr. Roth (12 cm) was not observed on this exam, but likely represents the large tissue mass found in Panjang's stall in mid August (Figure 1). The observation of luteal tissue on Panjang's right ovary (Figure 4) is consistent with a picture of continued cyclicity in this female despite the significant observed and historical pathology.

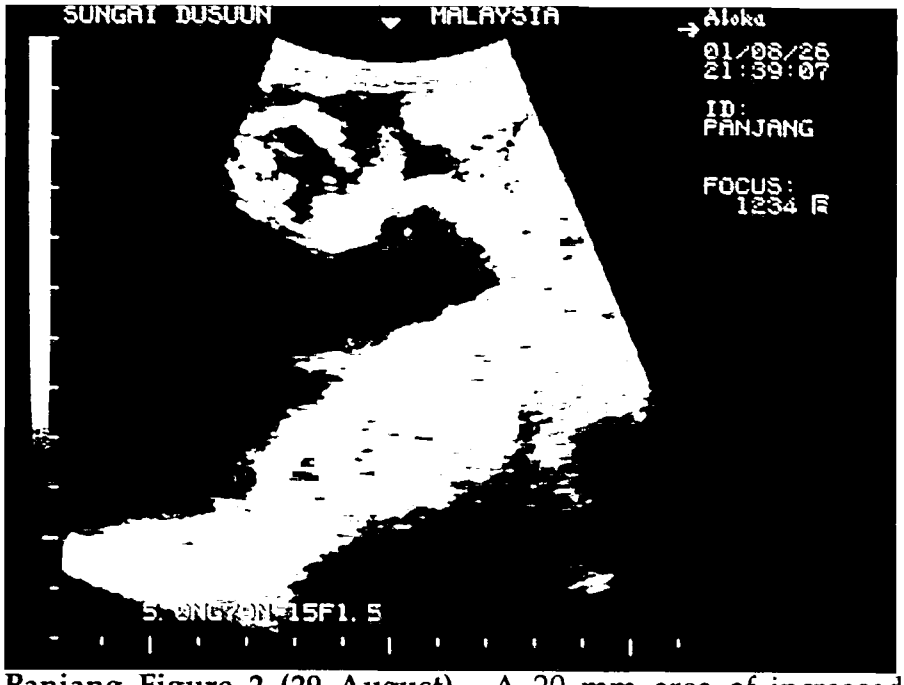
Immediate Plan:

For the immediate future, continue to monitor Panjang for any further hemorrhage or abnormal vaginal discharge. Based on the observed pathology and history of hemorrhagic discharge, Panjang may benefit from a period of rest from breeding with reevaluation of her reproductive tract via ultrasound (including a vaginal exam via speculum or endoscopy) in another 3 months. It appears that some of the pathology may be resolving. If her reproductive condition continues to progress with enlargement and or further hemorrhage, a uterine biopsy would provide necessary information to provide a prognosis for her future health and reproductive function. Histopathologic examination of the expelled mass is underway at the Veterinary Hospital of Universiti Putra Malaysia (UPM).



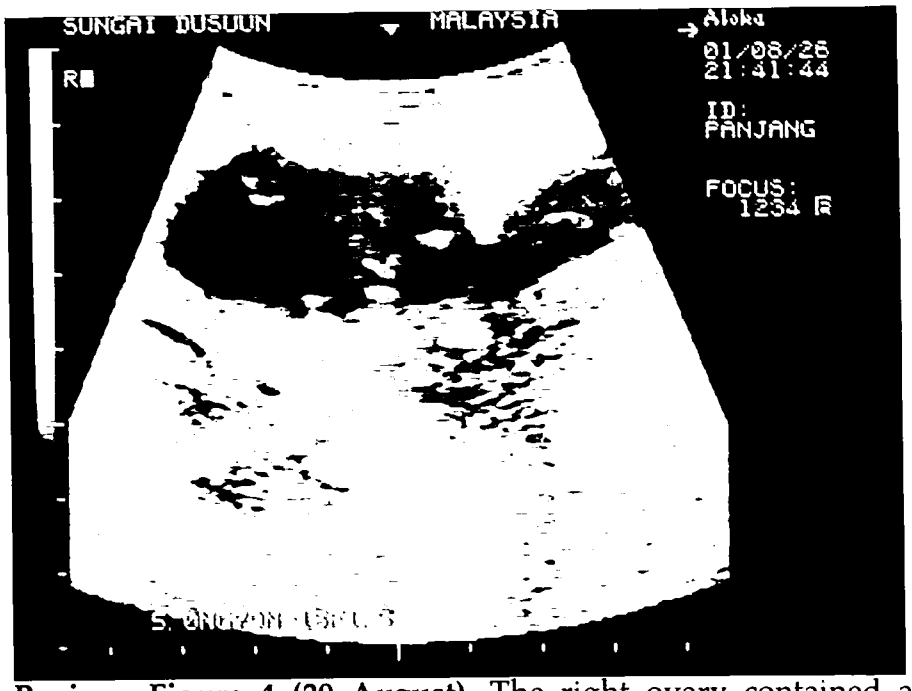
Panjang Figure 1. Mass recovered from Panjang's stall on August 20, 2001. This mass represented only ~1/3 of the mass found by the rangers. Based on large size the mass likely arose from the vagina. Mucopurulent vaginal discharge noted on 4 September 2001.

Reproductive Exam Details: PANJANG



Panjang Figure 2 (29 August). A 20 mm area of increased echogenicity (possible calcification) is present on the dorsal wall of her endometrium of left horn.

Panjang Figure 3 (29 August). The wall of the right horn is thickened (~ 20 mm) with increased echogenicity (calcification) in the endometrium associated with a 15 mm luminal fluid accumulation or necrotic/hemorrhagic core.



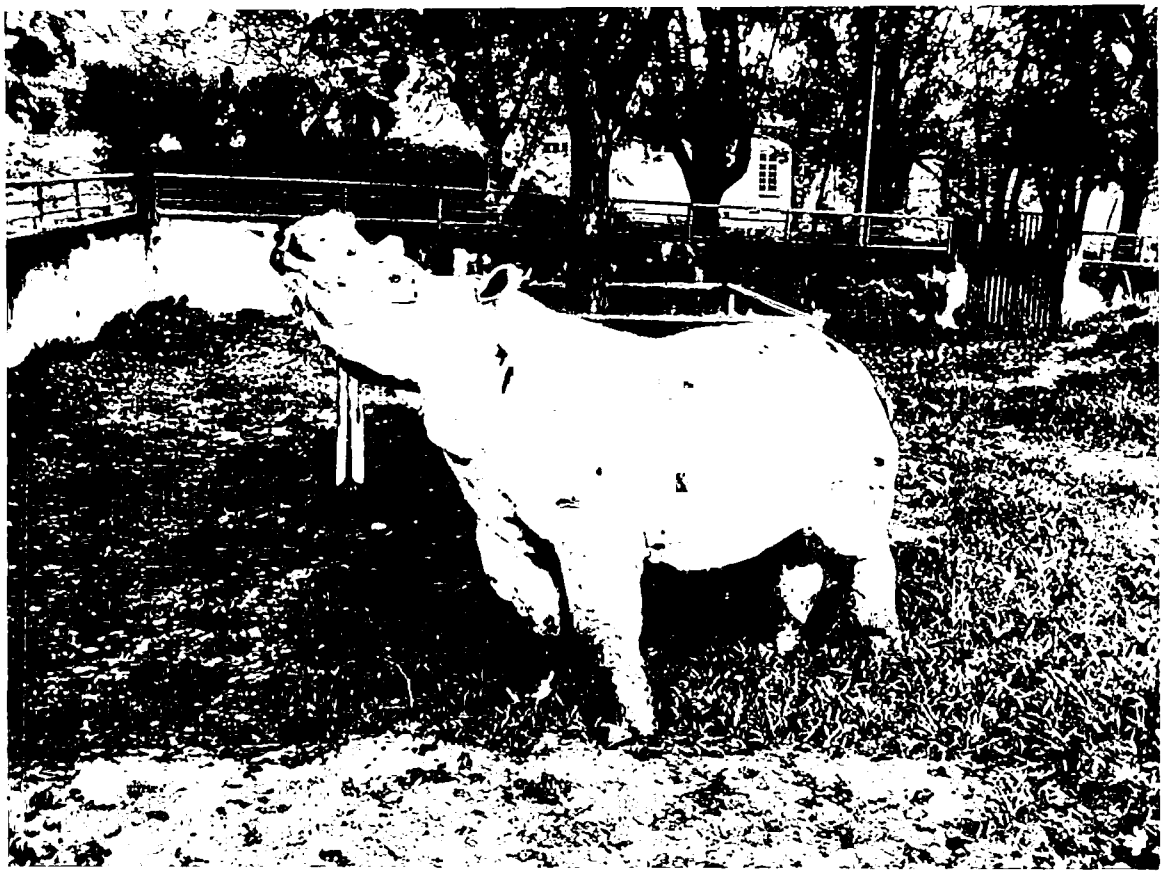
Panjang Figure 4 (29 August). The right ovary contained a regressing 12 mm corpus luteum and several small (< 10 mm) follicles.

Recommendations:

- Provide Panjang with rest from breeding.
- In 3 months, reevaluate reproductive pathology and function via serum progesterone, ultrasound, and endoscopy / speculum exam of vagina.
- General health evaluation with CBC and serum chemistry.
- Evaluate histopathology results from recovered mass 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

Species	SB#	Sex	Name	Age
Dicerorhinus sumatrensis	23	F	Seputeh	~22



Captive years without reproducing: 13
Recent breeding activity: Multiple breedings approximately every 21 days since January of 2001. Seputeh appears to consistently breed for 2 consecutive days.
Progesterone profile: History of 21 day cyclical pattern.
Hormone manipulations: None.

Purpose of exam: Examine for reproductive function and pathology.

Brief Summary of Findings:

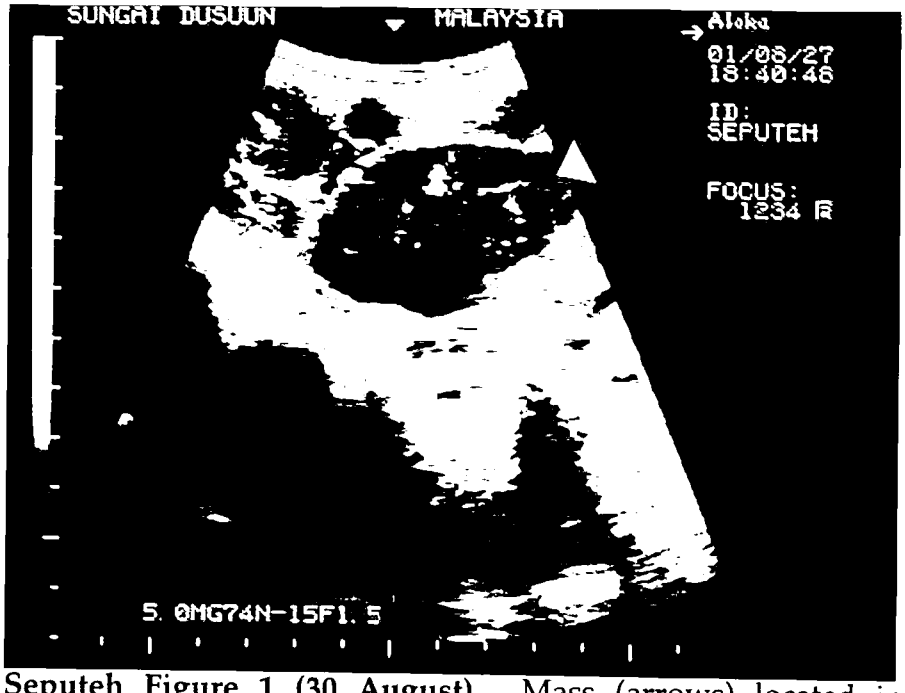
Seputeh has a vascularized mass measuring 50 mm in diameter located in the tissues surrounding her caudal reproductive tract (Figures 1 and 2). Numerous other smaller masses are associated with Seputeh's uterine body and uterine horns. As suggested by Dr. Roth, it is conceivable that Seputeh may be conceiving and undergoing early embyonic loss. The significant pathology in this female associated with the reproductive tract and surrounding anatomy suggest that Seputeh's chances of normal embryo development and implantation would be unlikely to occur. Despite observed pathology, Seputeh appears to be

this female associated with the reproductive tract and surrounding anatomy suggest that Seputeh's chances of normal embryo development and implantation would be unlikely to occur. Despite observed pathology, Seputeh appears to be cycling normally as illustrated by the observation of luteal tissue on the right ovary (Figure 3) and subsequent formation of a preovulatory follicle followed by documentation of successful ovulation within 24 hours post-breeding (Figures 4, 5 and 6).

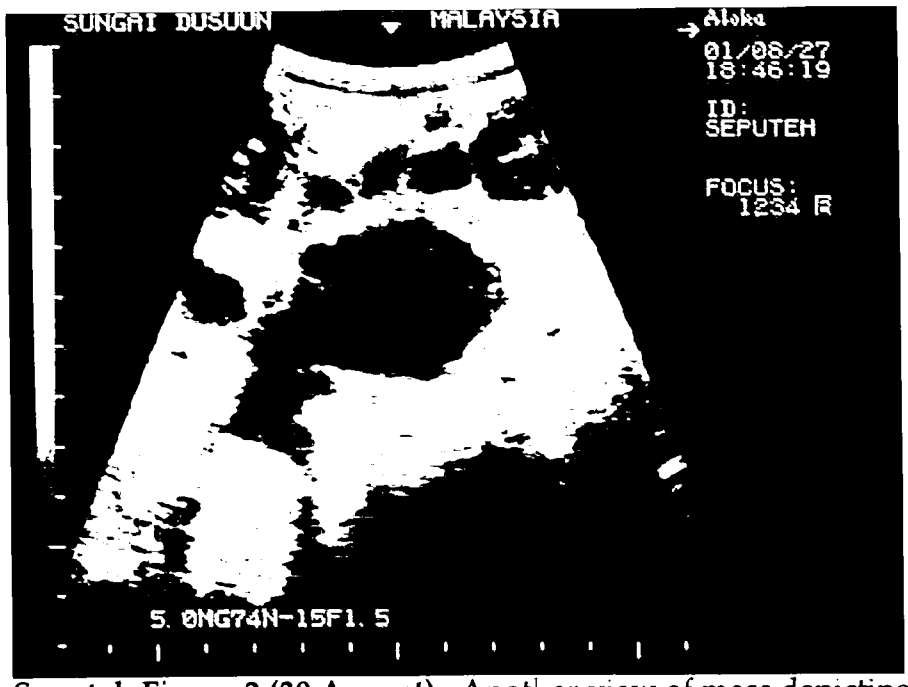
Immediate Plan:

Continue attempts at breeding Seputeh and combine with routine post-breeding ultrasound evaluations to confirm ovulation and to look for possible early embryo loss (see Appendix A entitled, *A Proposed Model for Reproductive Monitoring of Sumatran Rhino*). Based on the extensive pathology of this female, breeding management of other females should receive priority.

Reproductive Exam Details: SEPUTEH



Seputeh Figure 1 (30 August). Mass (arrows) located in periuterine region adjacent to Seputeh's uterine body. The mass may not involve the uterus directly, but appears to be associated with surrounding tissues.



Seputeh Figure 2 (30 August). Another view of mass depicting possible vascular nature of mass.

Seputeh Figure 3 (30 August). Right ovary with 20 mm corpus luteum (arrows) and multiple small follicles measuring < 10 mm in diameter.

Seputeh Figure 4 (3 September). Right ovary with preovulatory follicle measuring 22 mm in diameter. Seputeh did not breed in the morning with Ara, but did breed in the afternoon.

Seputeh Figure 5 (4 September). Right ovary with 24 mm preovulatory follicle and small regressing luteal structure. Seputeh bred immediately with Ara this morning with this size follicle.

Seputeh Figure 6 (5 September). Right ovary with confirmation of ovulation within 24 hours of Seputeh's second breeding to Ara. Corpus hemorrhagicum at ovulation site is delineated by arrows.

Recommendations:

1) Continue breeding efforts and monitor with serum progesterone.
2) Develop routine ultrasound monitoring program as detailed in Appendix A to document early pregnancy and monitor pathology. However, other females should be given priority for breeding with the available male rhinos.
3) Consider biopsy of masses if pathology progresses to help guide prognosis and decisions regarding health and reproductive fitness.

APPENDIX A

Proposed Reproductive Monitoring Program for Sungai Dusun's Sumatran Rhino Breeding Centre

Goals of Ultrasonographic Reproductive Monitoring Program:

Information gained	Management Implications
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 impeding 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. 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.

2) **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 the inadvertant breeding of pregnant animals which may contribute to pregnancy loss.

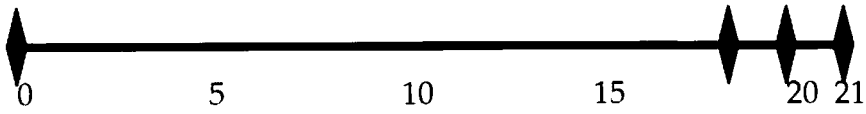
Early embryo loss
The early embryonic vesicle in the rhinoceros resembles the horse (Radcliffe et. al., 2001; Roth et. al., 2001). The early vesicle can be imaged as early as 14 days post-breeding and its documentation is critical, especially if embryo loss ensues. Without ultrasound the only indication 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 fact supported by their solitary forest nature and apparent infrequent encounters with other rhinos (particularly males) except during the short period of receptivity and copulation.

3) **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,



Day 0 Ovulation (Induced) | Luteal phase Days 4-18 | Day 21 Estrus 21-26 mm follicle

Ultrasound can 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 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,

= ultrasound exam

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 and early embryo from an endometrial cyst (see Rima Figures 1 and 2)

Days 20 – 30 = embryo proper first visible at this stage
- Important to document this stage as this is the time period most likely to see early embryo loss
- 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
- Fetal loss is much less likely at this stage unless luteal insufficiency develops as with Emi at Cincinatti
- Monthly exams during fetal stage for monitoring of normal fetal health and development

APPENDIX B

Medical Recommendations for Minah:

Next 60 days (September and October 2001)

- Start Minah on systemic antibiotics to treat the cystitis and prevent further infection of her inflamed urinary bladder. It was recommended that Minah be placed on systemic Trimethoprim-sulfamethoxazole (TMS) therapy at the following dosage administered orally in fruit twice daily:

  15 mg/kg x 400 kg (Minah estimated body weight) = 6000 mg TMS
  6000 mg TMS / 960 mg TMS per tablet = 6.25 tablets TMS

  Therefore, give 7 tablets TMS BID for 14 days.

- Repeat Urinalysis (UA) and complete blood count (CBC) weekly for the next 30 days to document clinical improvement in Minah's cystitis. Renal function should be evaluated via BUN and creatinine levels in CBC.
- If no improvement in clinical signs, CBC or UA, then perform urine culture and sensitivity to reevaluate antibiotic choice for cystitis therapy.
- Monitor Minah closely for any abnormal vaginal discharge or changes in urination habits or urine character (ie. change in urination frequency, straining, hemorrhage, purulent discharge, etc.).
- Provide Minah with a minimum of 60 days rest from any breeding activity even if signs of estrus resume.
- Monitor Minah's body weight and do not place Minah in a breeding situation until she has reached her desirable body weight (this can be determined by comparison to previous body weight measurements).
- Monitor food intake and reevaluate diet as deemed appropriate.

After October 2001

- Repeat ultrasound exam of reproductive tract and bladder after the 60 day rest period.
- Acquire ultrasound capability and develop schedule of ultrasound combined with biweekly serum progesterone evaluations to characterize Minah's ovarian activity.
- Enhance efforts to increase body condition of Minah. Specifically, the current ration needs to be evaluated and modified to meet nutritional demands of this specialized browser based on current understanding. As we await diet evaluation, hand-feeding of browse and pelleted diet should be considered a priority.
- Once cyclic activity and breeding resumes with Minah, post-breeding ultrasound monitoring should commence as outlined in Appendix A (*A Proposed Model for Reproductive Monitoring in Sumatran Rhino*).

APPENDIX C

Anesthesia report detailing butorphanol sedation of Sumatran rhinoceros.

SUMATRAN RHINO ♀ "MINAH"

Anesthesia Record - Fossil Rim Wildlife Center

Genus/species: Dicerorhinus sumatrensis Location/enclosure: Chute at SUNGAI DUSAN Rhino Center barn Date: 5 September 2001

Common name: SUMATRAN RHINO Activity: ⊘ calm O active O excited

Demeanor: O undisturbed O depressed ⊘ alert O aggressive O apprehensive

Sex: ♀ age: 14 (birthday: 23 May 1987) Basis for age if not birthday: Only captive born Sumatran rhino in 100 years

Previous ID?: MINAH ID applied today/site: —

Environmental temperature: 90° ⊘ F O C Group size: Alone in chute

Purpose: Endoscopy of urinary bladder to evaluate echogenic mass observed on ultrasound

Time fasted: O not fasted ⊘ <8 hrs O 8-24 hrs O 24 - 48 hrs O >48 hrs

Health status: O 1-normal health ⊘ 2 - mild disease O 3 - severe disease ⊘ 4 - chronic disease

O 5 - may not survive anesthesia Pregnant? O yes ⊘ no

Body condition: O obese/fat O good ⊘ fair ⊘ thin O poor/emaciated

Body weight: ~350 kg O actual ⊘ estimate Vet(s): RWR/RMR Recorder: RWR

LAST ACTUAL WT. = ; DRS. RADCLIFFE / ALIDI MOHAMMAD / DR. PAOLO / DR. WAHID

Drug	#mg	#mL	Conc in mg/mL	Route	Time given	Time - Effect seen
BUTORPHANOL	25	2.5	10	IM	10:57:30	11:00:45 Head drooping/sedate
						11:06:30 Marked sedation
						11:13:15 Eyes partially
						11:18:05 start endoscopy
BUTORPHANOL	20	2.0	10	IM	12:05:30	12:05:30 Redose due to increased movement
NALTREXONE	125	2.5	50	IM	1:16:10	1:16:45 Moving ears/head
						1:17:48 Walking back out of chute

Samples collected: None

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	
O other:				

Summary:

E-time to first effect: 3:15 Standing procedure tracheal tube size: NA

E-time to recumbency: NA (sternal/R or L lat?) Anesthetic rating: ⊘ excellent O good

E-time to first arousal (from time of reversal): 0:35 O fair O poor

E-time to walking (from time of reversal): 1:38

Total elapsed time (darting to walking): 2:20:18 HRS : MIN : SEC Recovery: ⊘ normal O abnormal (see comments)

Darting from (circle): vehicle or foot BY-HAND Dart/needle type: 1½" 18 guage needle Site: IM in Left Neck

Distance away: Hand Injection Dart mixing time/date: NA SpO2 sensor: Nellcor NPB-40 Vet-Sat Large Sensor on Left ear

Dart misses or failures: NA

Comments: Excellent dose for sedation of adult Sumatran rhinoceros.

*NOTE:
SpO_2 Large sensor placed on left ear after clipping hair with scissors

Time (hr: min: sec)	SPO2*	Temp °F °C	Pulse	Resp	Comments (drugs? observations? PE findings? etc.)
11:06	94%		69	12	9 min post IM Butorphenol (2.5 mg)
11:08	97%		64		
11:10	96%		65		Lidocaine infusion-intravaginal
11:12	97%		64		No reading
11:14					
11:18:05					Start endoscopy
11:23	92%		61		
11:25	94%		64		
11:27	93%		62		
11:29	93%		65		
11:31	93%		62		
11:33	93%		63		
11:35	94%		56		
11:37	94%		60		
11:39	95%		60		
11:41	95%		61		
11:43	94%		61		
11:45	94%		70		Trying to pull off the object via endoscopic instrument retrieval
11:47	95%		62		
11:49	93%		61		
11:51	94%		60		Note: Various surgical graspers were used unsuccessfully
11:53	91%		61		
11:55	93%		62		
11:57	93%		63		
11:59	89%		62		
12:01	88%		64		
12:03	90%		62		
12:05:30	91%		60		Butorphanol IM (20 mg)
12:07	91%		61		
12:09	89%		62		
12:11	84%		61		
12:13	93%		59		
12:15	90%		64		
12:17	92%		65		
12:19	88%		64		
12:21	93%		66		
12:23	95%		66		
12:25	91%		70		
12:27	94%		64		
12:29	92%		68		
12:31	93%		65		
12:33	93%		67		
12:35	93%		65		

Time (hr: min: sec)	SPO_2	Temp °F °C	Pulse	Resp	Comments (drugs? observations? PE findings? etc.)
12:37	93%		69		
12:39	93%		71		
12:41	93%		70		Bladder Therapy:
12:43	94%		74		
12:45	94%		69		Pull out the object - Succeed!
12:47	94%		70		Administer antibiotic.
12:49	92%		68		Flushing w saline
12:51	94%		67		~~1g Gentamycin~~
12:53	94%		68		~~Hepa~~ unit Sodium infusion
12:55	93%		66		(10 million)
12:57	96%		67		1g Gentamycin
12:59	96%		58		
1:01	93%		66		
1:03	95%		70		
1:05					
1:07					
1:10	94%		66		
1:12	93%		68		1500mg IM Flunixin at 1:14 pm
1:14					
1:16:10					125mg Naltrexone IM @ neck

PERSONEL:

Surgeon, Rolfe M. Radcliffe, DVM, Dipl. ACVS
Anesthetist, Robin W. Radcliffe, DVM, Dipl. ACZM
Aidi Mohamid, DVM
Assistant Paulo Martelli, DVM

APPENDIX D

Summary of recommended steps to maximize reproductive potential of Sungai Dusun's captive Sumatran rhinos

1) Purchase of Aloka 500V ultrasound machine and 5 MHz convex array probe (or new probe for current Aloka 210 machine) to facilitate monitoring of rhinos for impending ovulation and planned introductions. Ultrasound will prove to be a useful tool to assist in timed-breeding, pregnancy detection, and for documentation of ovulation, luteal function and pathology.
2) Establish in-house serum progesterone assay capability to facilitate timely decisions regarding stage of female estrous cycle.
3) Establish phone, Fax and e-mail service for staff at Sungai Dusun to improve work and communication efficiency.
4) Provide additional training for Dr. Aidi Mohamad in the techniques and application of ultrasonography in the rhinoceros via organization of an internship for Aidi at Fossil Rim.
5) Prioritize female breeding schedule according to the following estimate of highest to lowest fertility:

   Minah
   Mas Merah
   Rima
   Seputeh
   Panjang
6) Improve condition score of Minah by evaluation of nutritional program at Sungai Dusun under the direction of Dr. Ellen Dierenfeld.
7) Evaluate male reproductive function via vaginal sperm recovery from a post-copulatory female (Roth et. al., 2001) to evaluate semen for motility, concentration and morphology. Evaluate alternative semen collection techniques in Sumatran rhinos.
8) Design, plan and implement Sumatran Rhino Workshop for the simulation, production and dissemination of basic knowledge on the biology and conservation of Sumatran rhinos.
9) Future development of advanced reproductive techniques to assist captive breeding efforts. Techniques need to be developed for male collection, semen extension and standard evaluation protocols, female insemination, and semen cryopreservation.
10) Annual visit by Radcliffe/Citino/Roth rhino reproductive health team.
11) Make improvements to Sungai Dusun rhino conservation barn to facilitate these recommendations. The rhino barn would benefit from having a dry room (air-conditioned) for fruit and grain storage to preclude dangerous fungal growth of pelleted feed. In addition, a work area could be built into this room providing a sink and counter for laboratory use needed for medical and research procedures.