SUMATRAN RHINO REPRODUCTIVE AND HEALTH ASSESSMENT III

Sumatran Rhino Conservation Centre
Sungai Dusun, Selangor Malaysia
August 25, 2002 through September 4, 2002

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

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[2]Large Animal Surgeon, Living Fossil Productions, Ithaca, New York USA 14850

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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 Living Fossil Productions, 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 three major objectives. The primary objective of this visit was the surgical removal of a vaginal mass identified in Panjang during a previous visit. Panjang had a chronic history of hemorrhage from the vulva following breeding. Examination in March of 2002 revealed a 5 cm elliptical mass attached via a pedicle to the external cervical os. Surgical excision and follow-up care to prevent cervical adhesions was scheduled for this August 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 prostaglandin (PGF2α) 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. Use of prostaglandin therapy in the Sumatran rhino may improve managed breeding of this induced ovulator, particularly since there appears to be a propensity for the development of irregular luteal periods characterized by non-receptivity in this specie.

Finally, we make a case for pursuing 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?

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. Dr. Ellen Dierenfeld has provided valuable assistance with nutritional management and consultation for the Sumatran rhino program on a national and international basis and we thank her for this support.

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 Jerry Millhon, 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.

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.

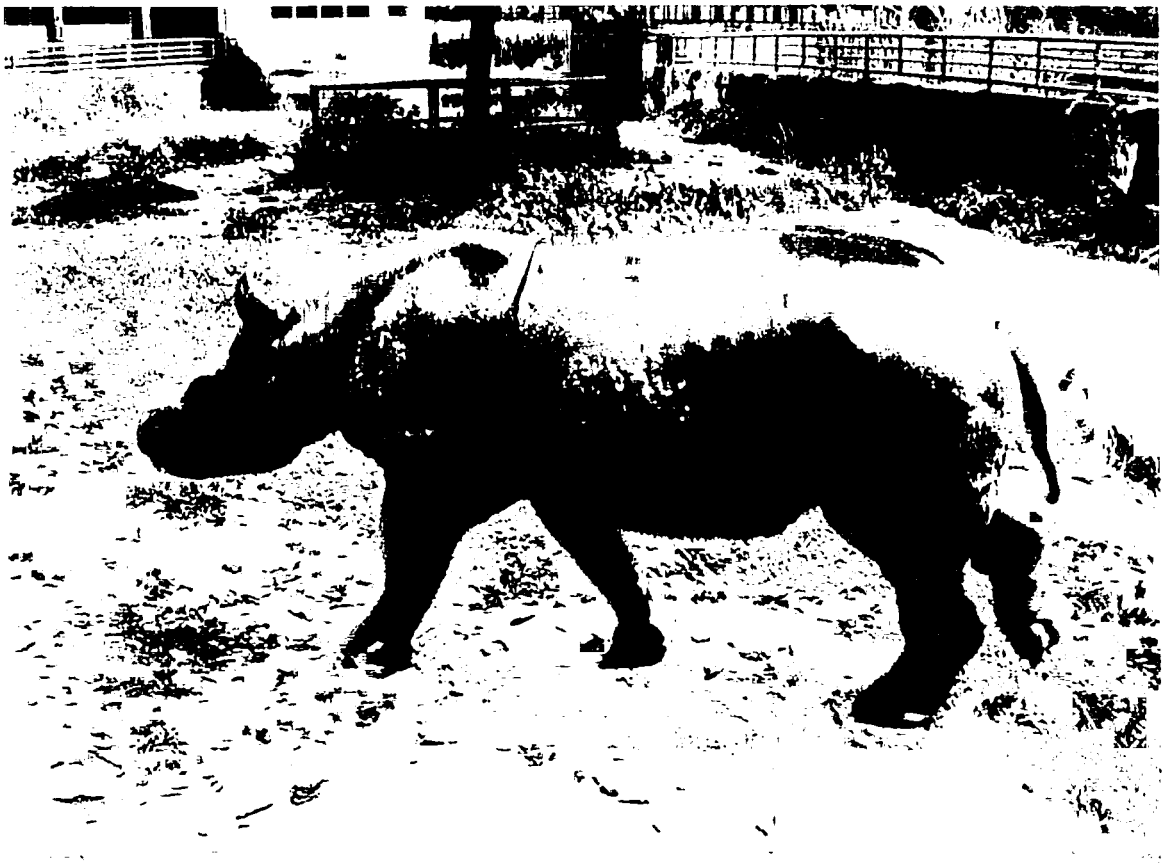
Recommendations for Rhino Management at Sungai Dusun:

1) As soon as feasible, develop the 100-acre site for housing of rhinos in individual yards with separate feeding areas (see diagrams in Appendices D and E). Look at the Way Kambas model for ideas about facility design.
2) The 100-acre site must be developed to meet both the daily management needs (ie. feeding, cleaning, health monitoring and sampling, reproductive monitoring and mixing) as well as provide adequate space to allow the rhinos to spend time in isolation (both visually and spatially) from the other animals.
3) The current housing could still be used for breeding management and housing of animals as the need arises.

Scientific Report III

Sumatran Rhino Reproductive Evaluation
Sungai Dusun, Malaysia
Date of exams: 27, 29, 31 of August and 2 of September, 2002

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. Assessment of chronic poor body condition and nutritional management of Minah.

Minah Figure 1. Thin body condition of Minah with prominent ribs and spine evident. Rhino body condition score estimate of ~3 out of 5.

Brief Summary of Findings:

Minah's reproductive tract was considered normal on ultrasound. The ovaries were small with an inactive right ovary and evidence of a small luteal structure on the left ovary. From an animal health aspect, we remain concerned with the thin body condition of Minah and the observation of prominent ribs, spine, and scapula (Figure 1). The body condition of this rhino based on previous condition scoring in the black rhinoceros (Adcock, 2001) remains at~ 3 out of 5 even one year following removal of the CIDR implant.

The urinary bladder wall appears completely normal on ultrasound (Figure 2). A physical examination was performed including cardiorespiratory auscultation, abdominal auscultation, oral exam, routine hematology [CBC (Appendix B) and serum chemistry], and ophthalmic exam. All evaluations were considered normal.

Recommendations:

The priority for Minah should be to improve her body condition as discussed during our previous visits. Efforts have been made to improve her condition via nutritional management; however, these changes have not brought about significant changes in her body condition score or body weight measurements. Therefore we will make additional nutritional modifications as outlined below:

Step One of Nutritional Changes:

1) Change the Cargill Equine pellet ration to 3 kg **three** times daily. The total amount will remain at 3 kg, but the feeding frequency will be increased from two to three times daily.
1) Add a vitamin E supplement as part of feeding program. A locally available multivitamin formulation called Stress-Pak will NOT be provided as a supplement at this time as the levels of vitamin D were considered excessive. Each adult Sumatran rhino should instead receive a daily oral supplement of 2,000 to 3,000 IU of vitamin E (α-tocopherol). This should be provided as d-α-tocopherol in either the Emcelle-E liquid formulation outlined below or as a human α-tocopherol gel capsule. At a future visit we will collect serum for comprehensive vitamin and mineral analysis. Until micronutrient evaluation, vitamin E supplementation should be sufficient.

Emcelle-E
Purina Mills Test Diet
505 North 4th
Richmond, IN 47374 USA

Dose: 2,000 IU = 4 ml per rhino daily (mix with the pelleted ration every morning)
[4ml / rhino / day = 24 ml per day for the six rhinos at Sungai Dusun, which means that 1 liter of Emcelle-E will last approximately 41 days at a cost of $82 USD per liter]

3) Add Rice bran to Minah's diet up to 1.0 kg daily. Feeding supplement should be added over a two week period starting with 500 g the first week and followed by 1.0 kg by the start of the second week.
4) Give intramuscular B-vitamin complex injection. One 10.0 ml dose of Vitamin B-complex was given to Minah on the 27th of August 2002. The 10 ml dose of B-vitamin provided: 1000 mg Vitamin B1, 50 mg Vitamin B2, 100 mg Vitamin B6, 1000 mg Niacinamide, 50 mg d-calcium Pantothenate, and 1 mg Vitamin B12.

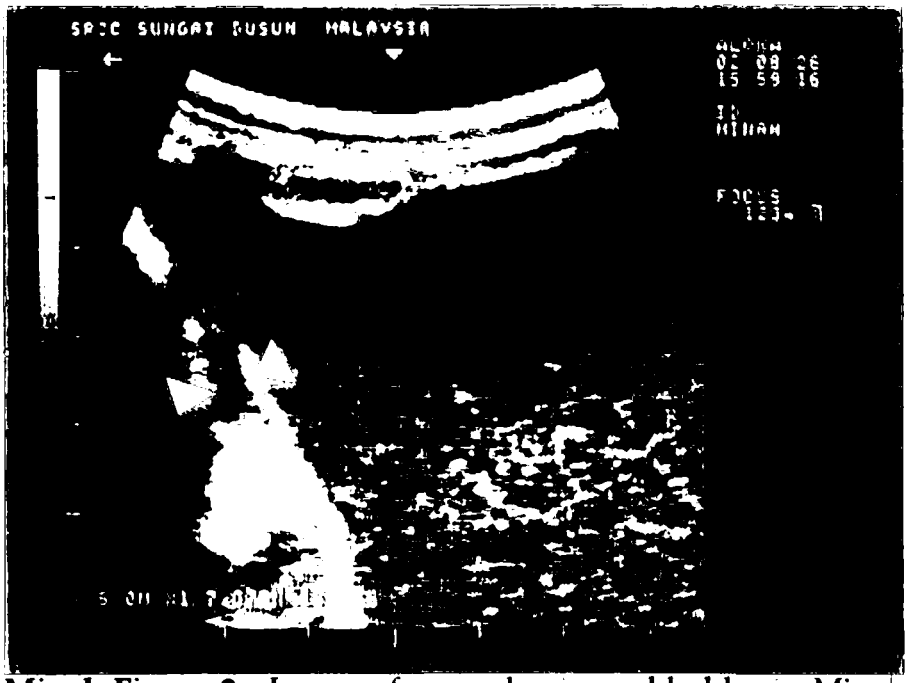
Step Two of Nutritional Changes:

1) Add Purina Mills Equine Senior diet immediately since the initial rice bran supplement was problematic (ie. Minah did not like the rice bran, and its use was accompanied by further weight loss in Minah). It is recommended that Minah receive 3 kg of the Equine Senior diet per day by gradual dietary conversion from the Cargill horse pellet to the Purina Equine Senior diet as recommended by Dr. Ellen Dierenfeld:

   Replace one quarter of the concentrate being fed with the new Purina Equine Senior diet (1:1 weight basis) for two to three days and monitor stool condition and intake. If the new diet is eaten by Minah, and no significant change in stool is observed, increase to one-half Cargill diet and one-half Equine Senior diet for a period of one week. Continue to monitor intake and stool consistency. Complete conversion from the Cargill horse pellet to the Equine Senior product should take about 2 weeks, which will allow gradual shifting in gut microbes as well. After two weeks, start feeding 100% Equine Senior as the sole source of concentrate along with usual forages (ie. Browse) and monitor for weight gain. We would expect a positive weight gain and/or visible body condition improvement after 1 month following full conversion.

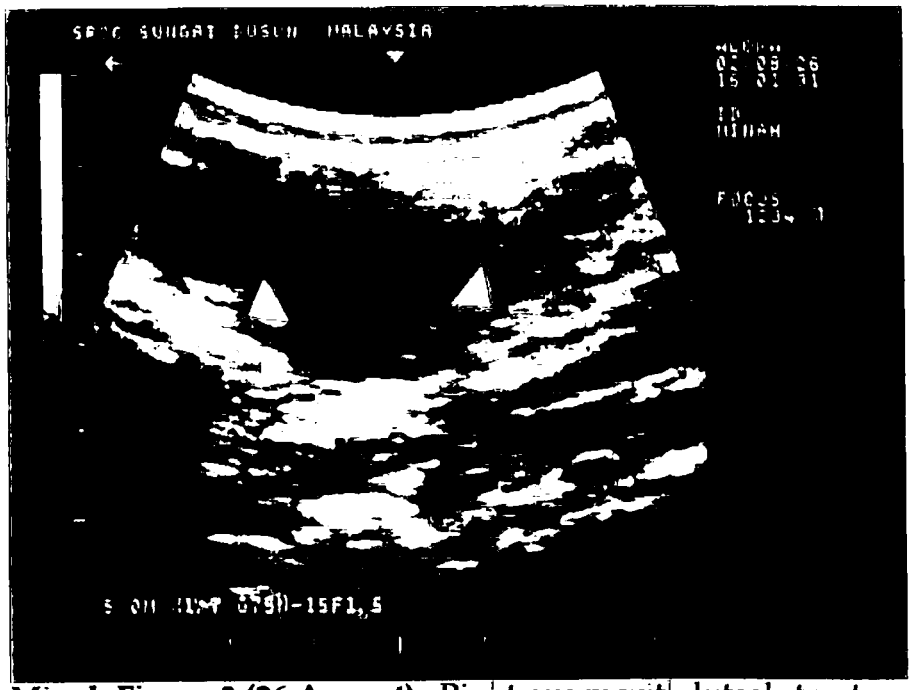
2) Continue body weight measurements once weekly.

Ultrasonographic Urinary Bladder Health Evaluation: **MINAH**



Minah Figure 2 Image of normal urinary bladder in Minah one-year post CIDR removal.

Reproductive Exam Details: MINAH



Minah Figure 3 (26 August). Right ovary with luteal structure (arrows) measuring 20 mm in diameter and characteristic of a cycling female.

Minah Figure 4 (26 August). Left ovary with small follicle measuring ~10 mm in diameter.

Reproductive Recommendations:
Based on the findings of a luteal structure on Minah's left ovary together with a persistent low level of Progesterone that does not appear to reach the low basal levels observed with estrus in other females, we decided to administer prostoglandin (PGF2α; Dinoprost tromethamine; Lutalyse) to Minah. This therapy was initiated on August 27 in Minah and was accompanied by minimal change in either behavior or ovarian activity as illustrated in Table 1. Concurrent serum progesterone analysis will be evaluated to see if there were any noticeable physiologic effects from this therapy. PGF was also given to two other females with a history of persistent progesterone levels and long delays in breeding activity (see PGF summary in Appendix C).

Table 1. Prostaglandin Therapy and Summary of Response

Date	PGF2α / HR Post-PGF	Ultrasound Data	P4 Data (ng/ml)	Behavior
27 August	10 mg PGF2α IM	R 20 mm CL2 10 mm Fo	0.37	No interest
28 August	10 mg PGF2α IM	--		No interest
29 August	48 Hrs Post-PGF	R 15 mm CL2 8 mm Fo	0.16	No interest
30 August	--	--		No interest
31 August	96 Hrs Post-PGF	R 13 mm CL 12 mm Fo		No interest
1 September	--	--		Low interest (Ara followed Minah up onto mound)
2 September	144 Hrs Post-PGF	R 15 mm Fo several 10 mm+ Fo	0.33	Low interest
3 September	7 days Post-PGF	--		No interest
5 September	9 days Post-PGF	--	0.27	No interest

Sumatran Rhino Reproductive Evaluation
Sungai Dusun, Malaysia
Date of exams: 26, 28, 30 of August and 1 of September, 2002

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

Captive years without reproducing: 15
Recent breeding activity: Last breeding 13 November 2001.
Progesterone profile: Regular 21 day cyclical pattern until late 2001. Lack of apparent cyclic pattern since last breeding with Ara on 13 November 2001.
Hormone manipulations: None

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 (Figure 1) appeared to be unchanged. There was minimal evidence of follicular activity, but ovulation was confirmed by observation of luteal tissue. Therefore, despite hormonal and behavioral evidence of prolonged or irregular luteal phases, Rima is apparently still cycling (Figure 2).

Immediate Plan:

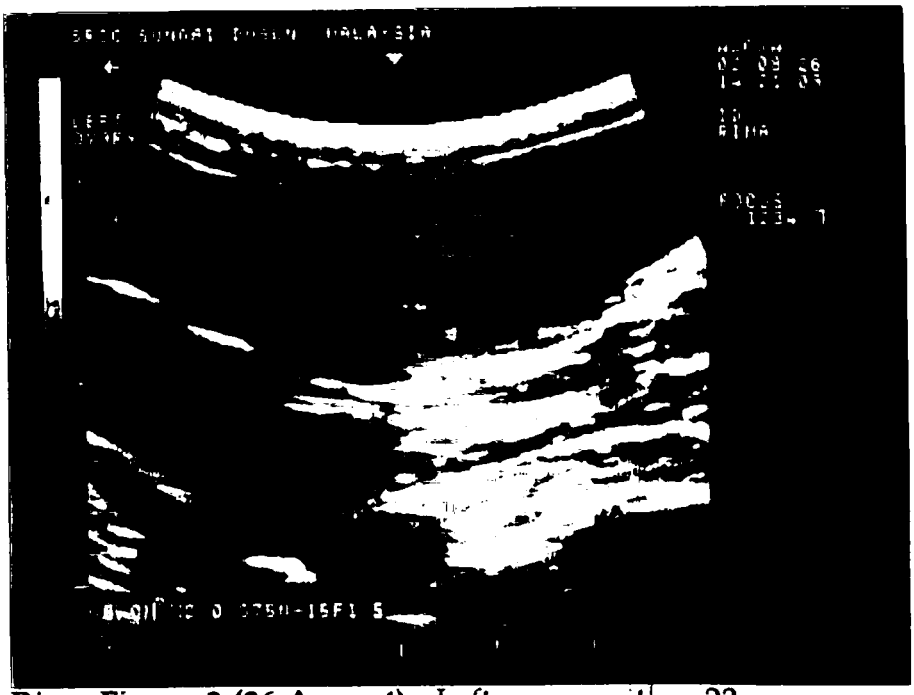
Perhaps Rima has been experiencing prolonged luteal phases following failed ovulation or missed breedings. In addition, it is also possible that Rima may be undergoing early embryonic loss. Because of the potential to miss an early pregnancy by confusion with the numerous endometrial cysts, Rima may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

Recommendations:

- Initiate prostaglanding therapy (PGF2α; 10 mg Dinoprost tromethamine; Lutalyse). Based on use in the black rhino, we empirically chose to start with two 10 mg PGF2α doses given on consecutive days. This therapy was conducted during our visit with the behavioral and ultrasonographic ovarian changes summarized in Table 2. 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) as per Dr. Roth's guidelines.

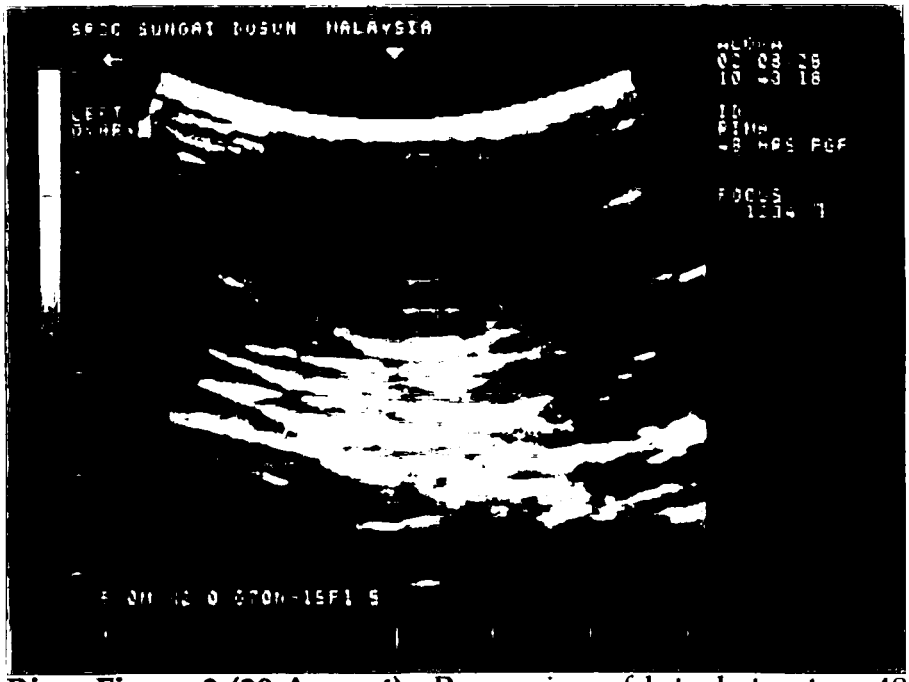
Reproductive Exam Details: **RIMA**

Rima Figure 1 (26 August). A 20 mm diameter cyst in distal location of right uterine horn.



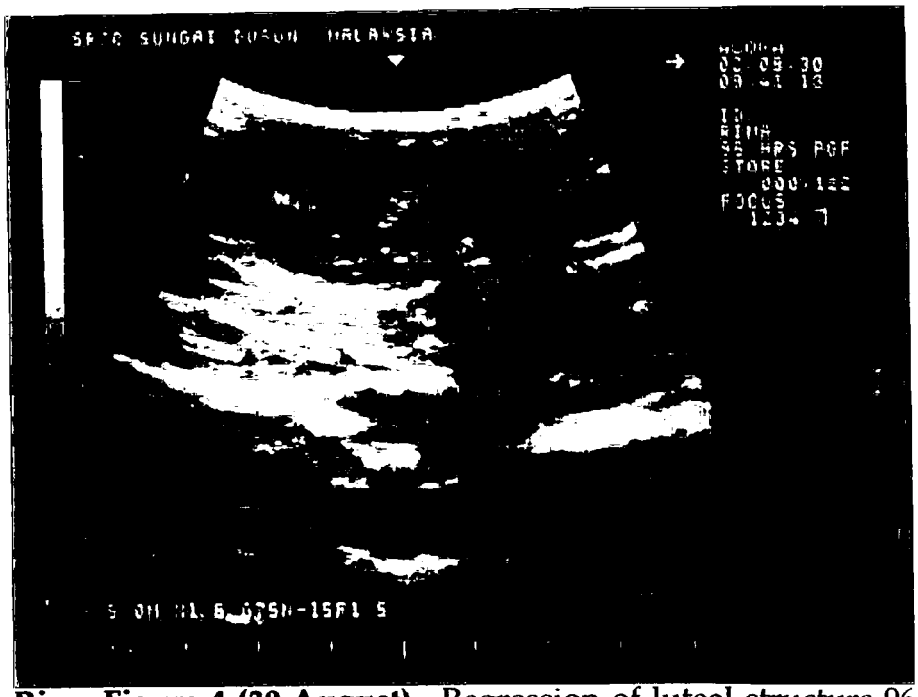
Rima Figure 2 (26 August). Left ovary with a 23 mm corpus luteum. Based on finding this structure together with hormonal evidence of prolonged luteal phases, PGF2α therapy was initiated.

48 Hours Post-PGF2α Therapy:



Rima Figure 3 (28 August). Regression of luteal structure 48 hours post-PGF2α therapy in Rima. Note obvious change in luteal tissue echogenicity.

96 Hours Post-PGF2α Therapy:



Rima Figure 4 (30 August). Regression of luteal structure 96 hours post-PGF2α therapy in Rima. Note continued change in luteal tissue echogenicity.

Rima Figure 5 (30 August) Formation of small (~10 mm) follicle on right ovary 96 hours post-PGF2α therapy correlating with first behavioral interest shown by Ara towards Rima.

Table 2. Prostaglandin Therapy and Summary of Response

Date	PGF2α / HR Post-PGF	Ultrasound Data	P4 Data (ng/ml)	Behavior
26 August	10 mg PGF2α IM	L 23 mm CL3 No Fo	0.08	--
27 August	10 mg PGF2α IM	--		No interest
28 August	48 Hrs Post-PGF	L 19 mm CL2 No Fo		No interest (Rima walked off)
29 August	--	--	0.01	No interest
30 August	96 Hrs Post-PGF	L 14 mm CL2 10 mm Fo		Strong interest (Ara followed closely)
31 August	--	--		Strong interest (Ara attempted mount)
1 September	144 Hrs Post-PGF	L 10 mm CL	0.05	Mounting by Ara, but no intromission; Rima stood with vocalizations
2 September	7 days Post-PGF	--		Strong interest by Ara, but Rima was vocal and did not stand
5 September	10 days Post-PGF	--	0.10	

Sumatran Rhino Reproductive Evaluation
Sungai Dusun, Malaysia
Date of exams: 26, 28, 30 of August and 1 September, 2002

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.

Purpose of exam: Examine for reproductive function and pathology.

Brief Summary of Findings:

Mas Merah was observed to have a significant hemorrhagic follicle on her right ovary. In other species, these structures are considered to be the result of failed

ovulatory events and are thought to be irregular but not pathologic structures. In addition, Mas Merah had a mature corpus luteum on her left ovary. These findings are consistent with Mas's observed irregular hormonal pattern over the last 6 to 9 months.

Immediate Plan:

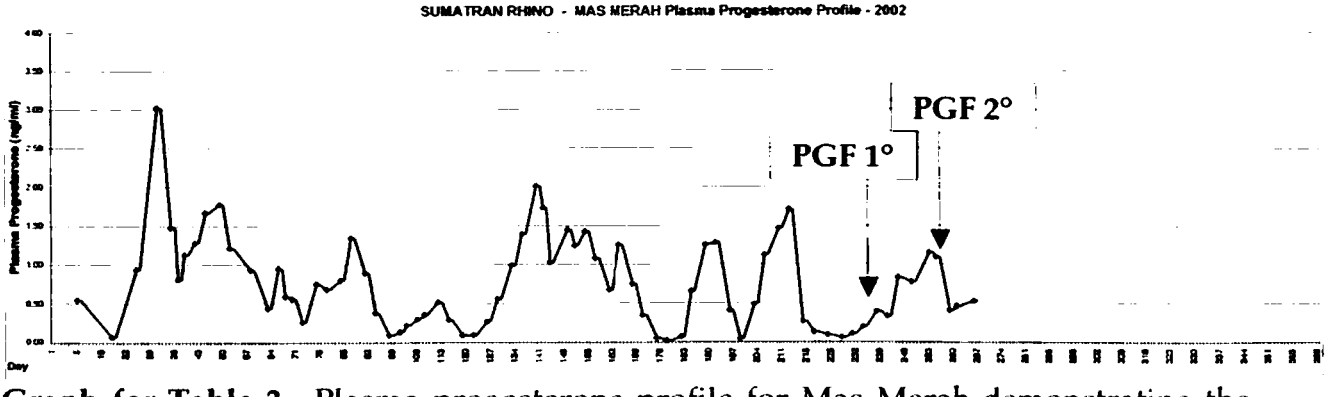
Based on the ultrasound and hormonal information outlined above, we elected to treat Mas with prostaglandin therapy in attempts to induce luteolysis and initiate a predictable sequence of receptivity and breeding. The 10 mg doses of PGF2α on consecutive days have not been associated with any signs of abdominal discomfort (ie. colic signs) or other adverse side effects that are often seen with its use in the horse.

Recommendations:

- Initiate prostaglanding therapy (PGF2α; 10 mg Dinoprost tromethamine; Lutalyse). Based on use in the black rhino, we empirically chose to start with two 10 mg PGF2α doses given on consecutive days. This therapy was conducted during our visit with the behavioral and ultrasonographic ovarian changes summarized in Table 3. This therapy may be repeated at a later date if further evidence for prolonged luteal phases are confirmed.
- Consider post-breeding oral progesterone therapy (Regumate) as per Dr. Roth's guidelines.

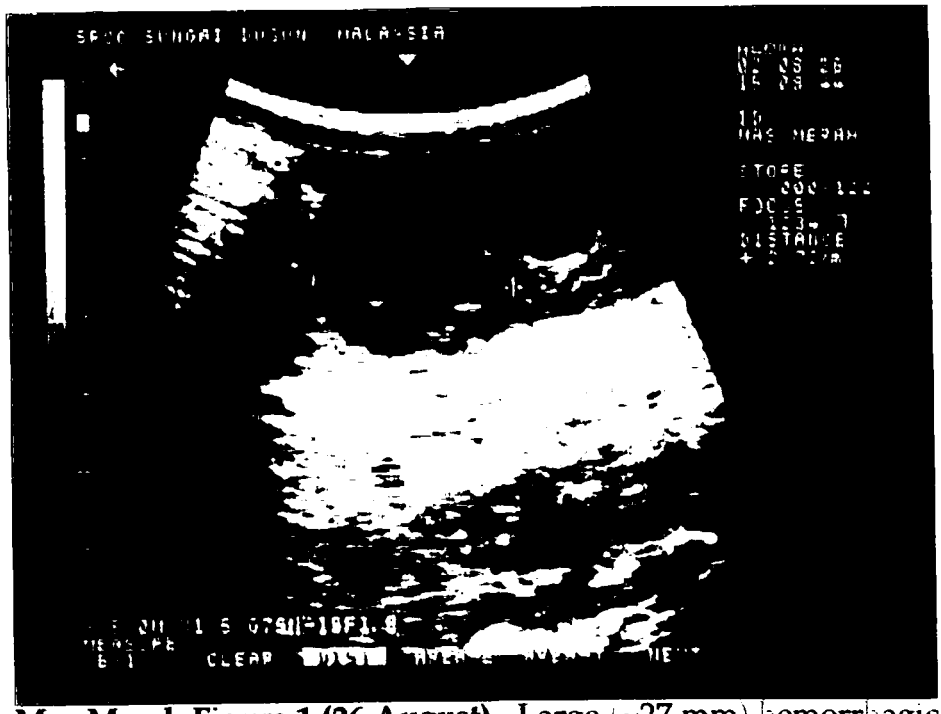
Table 3. Prostaglandin Therapy and Summary of Response

Date	PGF2α / HR Post-PGF	Ultrasound Data	P4 Data (ng/ml)	Behavior
26 August	10 mg PGF2α IM	R HAF & Fo L 24 mm CL3	0.40	--
27 August	10 mg PGF2α IM	--		No interest
28 August	48 Hrs Post-PGF	R HAF x 2 L 20 mm CL3		No interest
29 August	--	--	0.34	No interest
30 August	96 Hrs Post-PGF	R HAF x 2 L 10 mm CL2		No interest
31 August	--	--		Low interest (Ara followed closely)
1 September	144 Hrs Post-PGF	R HAF lutenized Fo?	0.83	No interest
2 September	--	--		Low interest (Ara followed closely)
5 September	10 days Post-PGF	--	0.77	



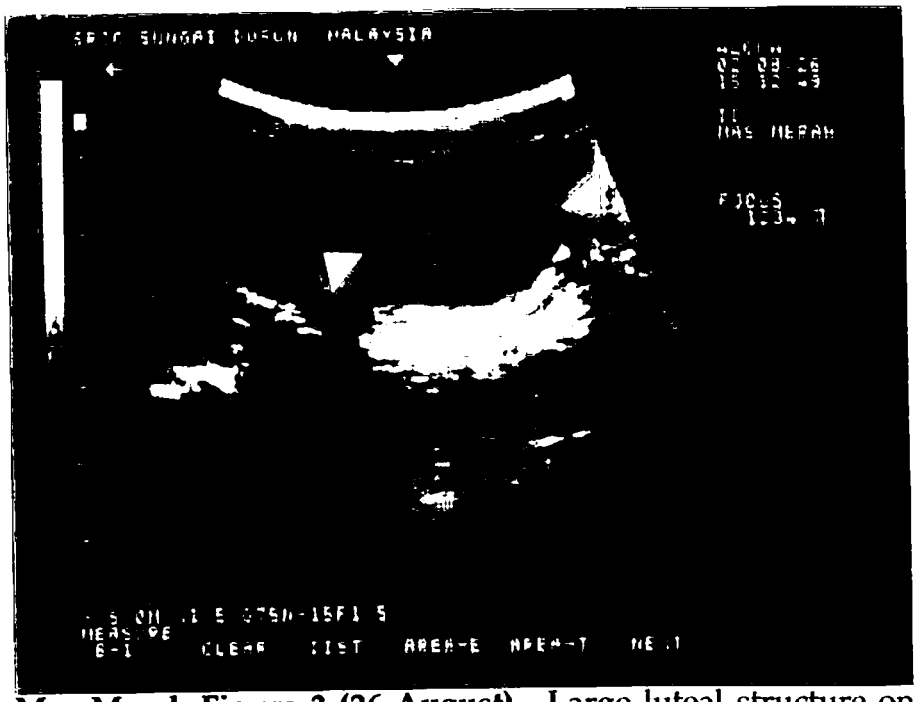
Graph for Table 3. Plasma progesterone profile for Mas Merah demonstrating the endocrine response to clinical use of prostaglandin (PGF2α) in a Sumatran rhinoceros. This preliminary data suggests that this specie may have a fairly extended period of luteal cell development and subsequent use of PGF should be based on demonstration of a mature corpus luteum. (Graph courteosy of Dr. Aidi Mohamad)

Reproductive Exam Details: **MAS MERAH**



Mas Merah Figure 1 (26 August). Large (~27 mm) hemorrhagic follicle (HAF) on Mas's right ovary. Note lutenization of HAF wall.

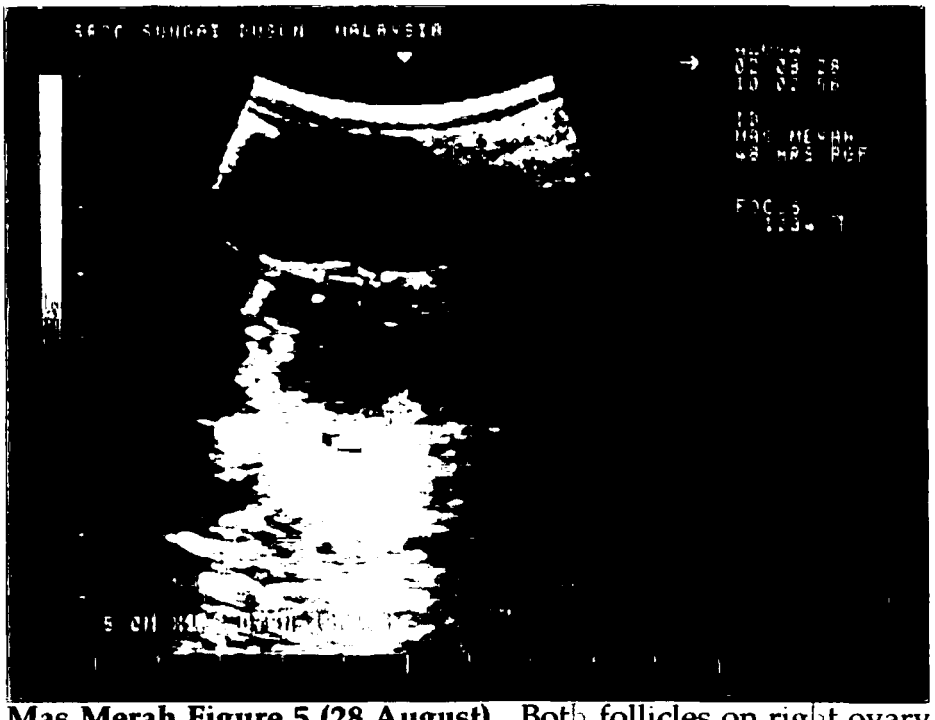
Mas Merah Figure 2 (26 August). Large follicle on right ovary Although this follicle is of preovulatory size (23 mm), Mas remains non-receptive to the male due the P4 influence from the HAF and CL3 on the contralateral ovary.



Mas Merah Figure 3 (26 August). Large luteal structure on Mas's left ovary. Based on finding these structures - the CL3 and HAF - together with hormonal evidence of prolonged luteal phases, PGF2α therapy was initiated.

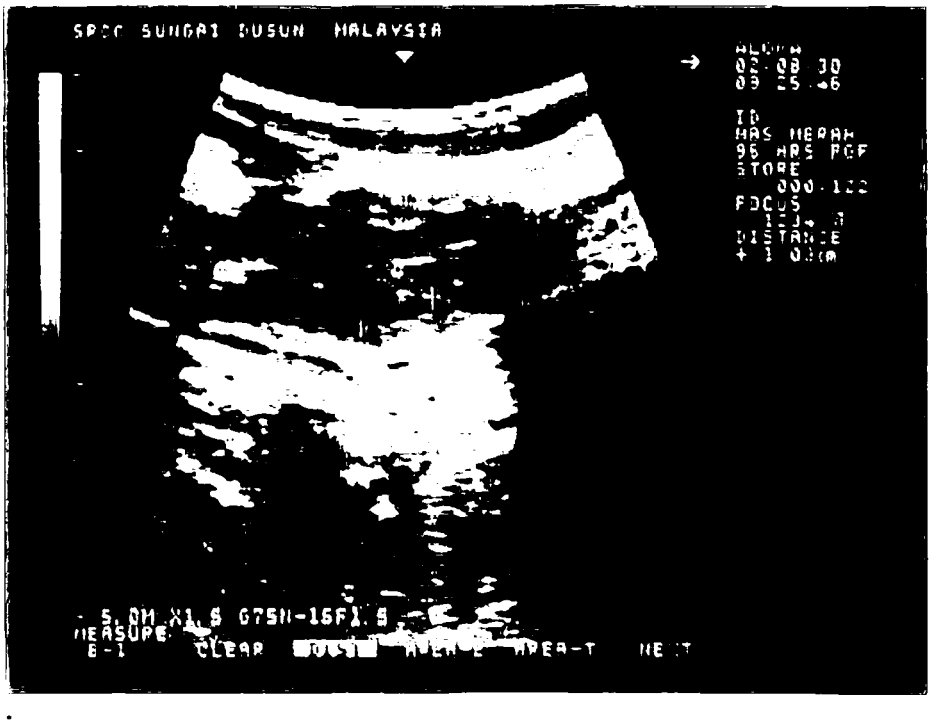
48 Hours Post-PGF2α Therapy:

Mas Merah Figure 4 (28 August). Regression of luteal structure 48 hours post-PGF2α.



Mas Merah Figure 5 (28 August). Both follicles on right ovary developed into hemorrhagic follicles by 48 hours after PGF2α.

96 Hours Post-PGF2α Therapy:



Sumatran Rhino Reproductive Evaluation
Sungai Dusun, Malaysia
Date of exam and surgery: 26 August 2002

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.

Purpose of exam: Surgical removal of cervical mass identified on previous visit. Revaluation 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 (vagina??) 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 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.

In March of 2002 another vaginal mass (suspected leimyoma) was observed via vaginoscopy to be adhered to the external cervical os. Surgery was not performed at this time as appropriate follow-up care was not available and was considered critical to successful resolution of the problem. The primary purpose of the August 2002 visit to Sungai Dusun was to remove this mass and provide this follow-up care.

Brief Summary of Findings:

A presurgical evaluation of the vagina was conducted via ultrasound and a vaginal speculum exam to facilitate surgical decision-making. Ultrasound revealed a 5 to 6 cm elliptical mass adhered to the external cervical os. Vaginosocpy further revealed the mass to be adhered via a 1cm x 4 cm pedicle that actually attached within the cervical lumen. The location of the pedicle attachment makes the post-srugical prevention of adhesions critical in order to restore natural breeding.

Surgical Procedure:

Panjang was sedated with an intramuscular combination of Butorphanol (50 mg) and Azaperone (60 mg) to facilitate vaginal surgery (see Appendix A). The vaginal mass was palpated to determine size and location. Two #3 vicryl laparoscopy loop sutures (4S Modified Roeder Knots) were placed around the pedicle of the mass and tightened. With the ligations secure the mass was transected via extended length Metzenbaum scissors through the vagina. No hemorrhage was observed following removal of the mass. The vagina was lavaged with 1% dilute betadine and isotonic saline prior to infusion with 3 grams Ticarcillin.

Post-surgical Plan:

Daily vaginal manipulations to prevent adhesion formation will be conducted for the first 10 days. This therapy will include digital manipulation of the remaining pedicle, palpation of the cervical lumen, and application of an anti-inflammatory paste (Inflamol Gel) to the surgical site. The digital examination and application of anti-inflammatory gel will be discontinued at 10 days post-surgery.

Inflamol Gel Formulation:

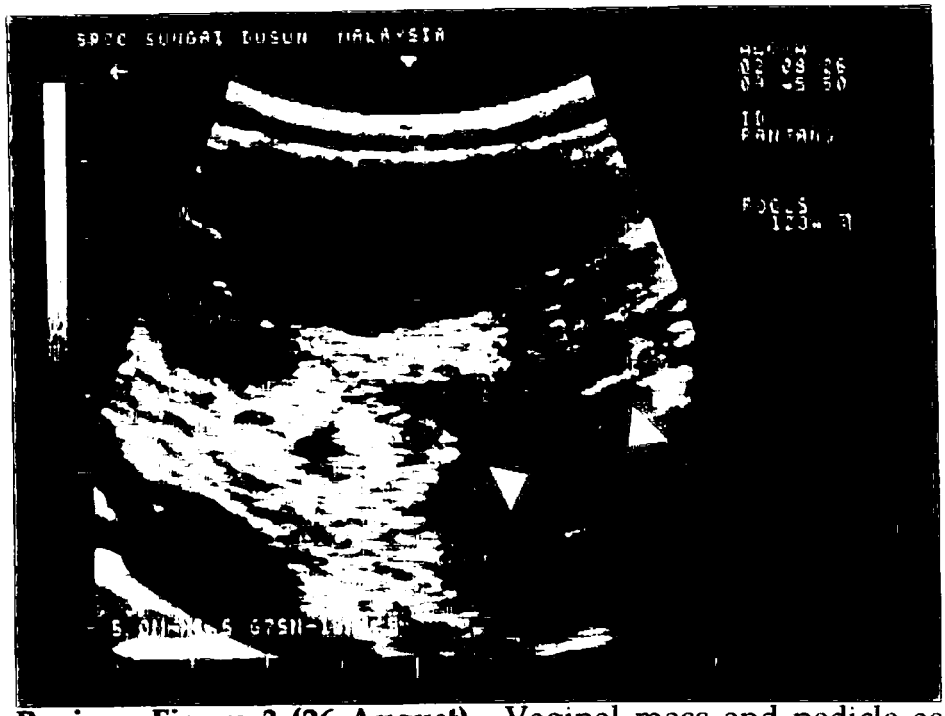
Nitrofurazone	2 mg
Neomycin SO4	5 mg
Prednisolone	2.5 mg
Undecylinic acid	20 mg
Allantoin	10 mg
Vitamin A	1000 I.U.

Panjang can be mixed for breeding at the conclusion of the above therapy at approximately 30 days post-surgery. Breeding can resume at this time as behavior and mixing response suggests.

Panjang Figure 1. Surgical removal of cervical leiomyoma in female Sumatran rhino while under Butorphanol / Azaperone sedation.

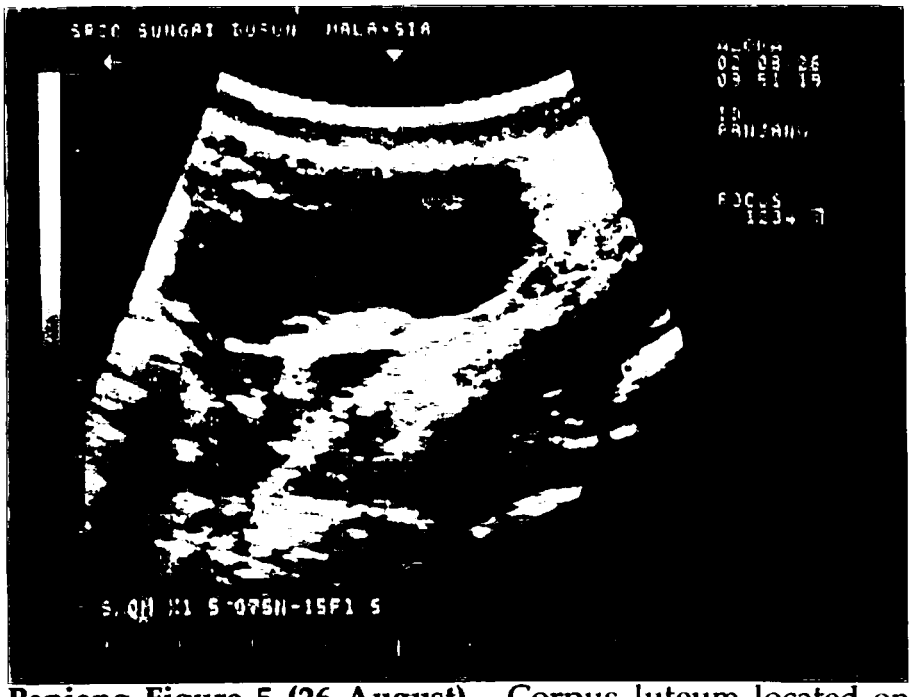
Panjang Figure 2. Detail showing surgical knot used to ligate the leiomyoma prior to surgical transection.

Reproductive Exam Details: PANJANG

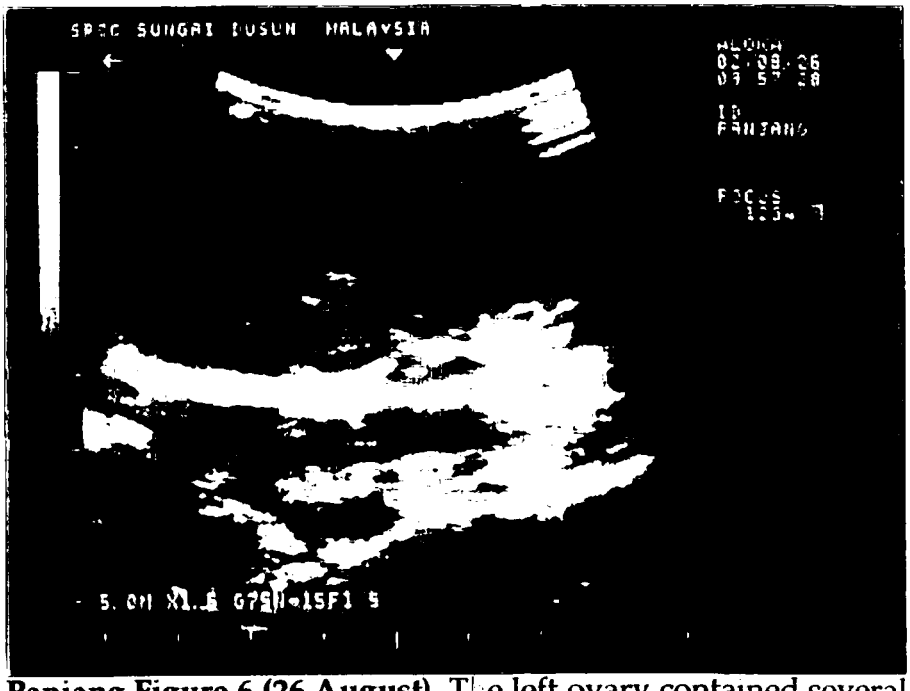


Panjang Figure 3 (26 August). Vaginal mass and pedicle as observed on ultrasound prior to surgical excision. Arrows outline the vaginal pedicle as it courses anterior into the cervical lumen.

Panjang Figure 4 (26 August). A 20 mm area of increased echogenicity (possible calcification) is present on the dorsal wall of her endometrium of left horn.



Panjang Figure 5 (26 August). Corpus luteum located on Panjang's right ovary indicating that ovulation recently occurred.



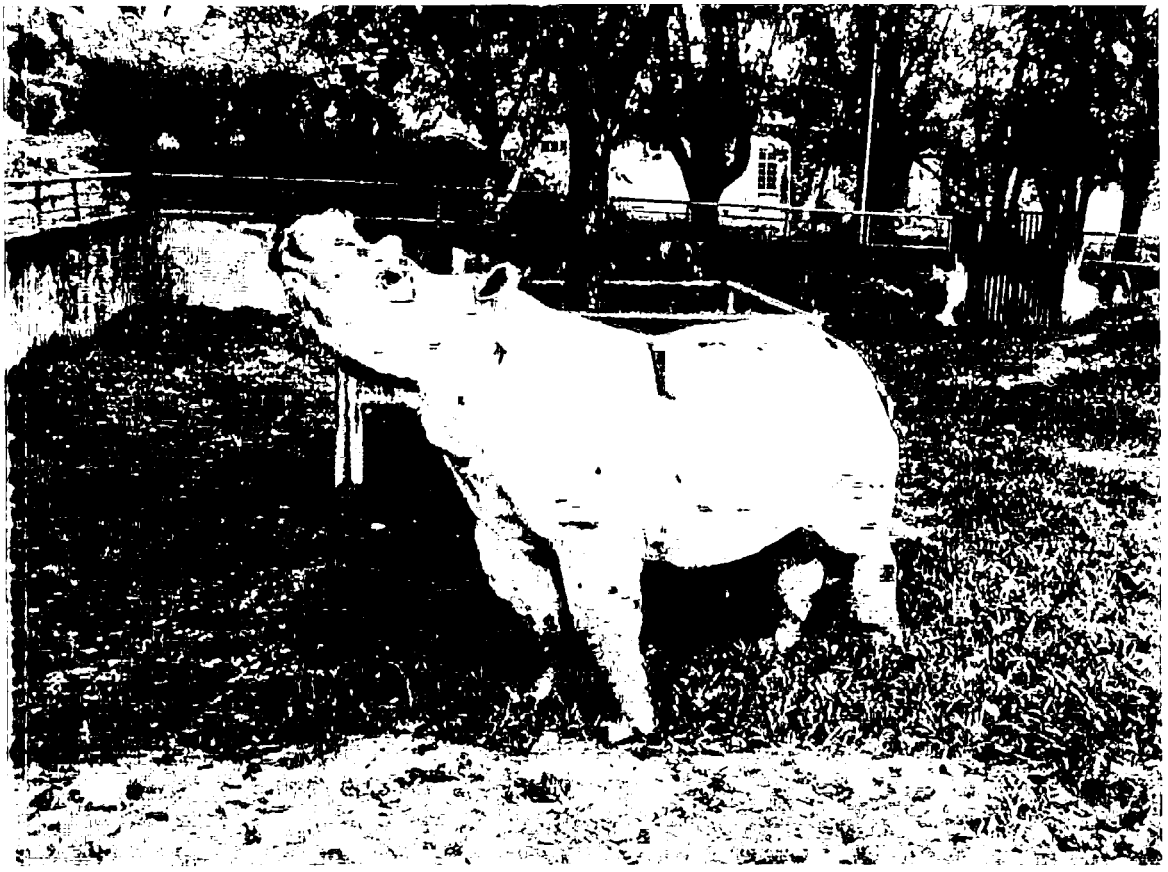
Panjang Figure 6 (26 August). The left ovary contained several follicles in various stages of growth.

Recommendations:

- Provide Panjang with rest from breeding for a minimum of 30 days to allow healing from the surgical site. On September 23, 2002 Panjang should once again be introduced with Ara for breeding.
- Post-operative treatment during the team visit included lavage of the cervical and vaginal area with diluted 1% betadine solution and a solution containing 3 g Ticarcillin. Further follow-up care consisted of daily digital manipulations of the cervix in order to prevent potential adhesion formation. These manipulations were combined with application of a topical antinflammatory paste daily for the first 10 days and then discontinued.
- If vulvar discharge is observed with Panjang during the 30 day rest period, then a vaginal / cervical lavage should be performed as demonstrated to Aidi using a 1.0 liter normal saline solution containing 3 g of Ticarcillin.
- If by 60 days after resumption of breeding attempts with Panjang 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.

Sumatran Rhino Reproductive Evaluation
Sungai Dusun, Malaysia
Date of exam: 27 August 2002

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



Captive years without reproducing: 15
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:

Seputih' did not become pregnant following her last breeding and the empirical use of oral progesterone supplement (Regumate). Numerous masses remain associated with Seputeh's uterine body and uterine horns. 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 cycling as illustrated by the observation of both follicular and luteal structures on the right ovary (Figures 2 and 3).

Vaginal Exam:

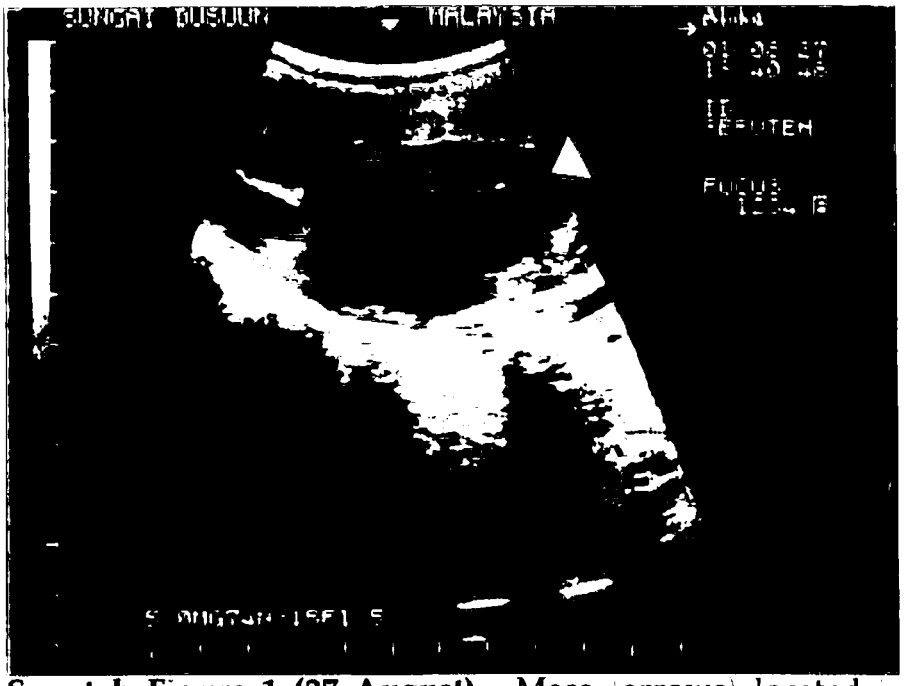
Based on the ultrasonographic observation of multiple masses within Seputih's caudal reproductive tract, we elected to perform a vaginal exam and digital palpation of the cervix. Although Seputih is a difficult female to examine in the chute due to her aggressive disposition, we were able to complete a thorough vaginal palpation without sedation. This exam revealed that Seputih has numerous masses present within the anterior vagina and cervix. An estimated 5 masses were palpable during the exam. The largest mass (also visible on ultrasound) appeared to be occluding the cervical canal at the location of the external cervical os. All five masses appear to be originating near the external cervical os. However, unlike the mass in Panjang, none of these masses appear to be pedunculated. This will complicate any future surgical removal of these masses.

A follow-up ultrasound exam was conducted specifically to help delineate the extent of the tumors and determine how many masses were located beyond the vagina and external cervical canal and therefore not easily removed with vaginal surgery. This exam revealed that one mass was present beyond the cervix in the uterine body and another small mass was present in the uterine body. However, the majority of the pathology is confined to the region of the vagina and external cervical region.

Recommendations:

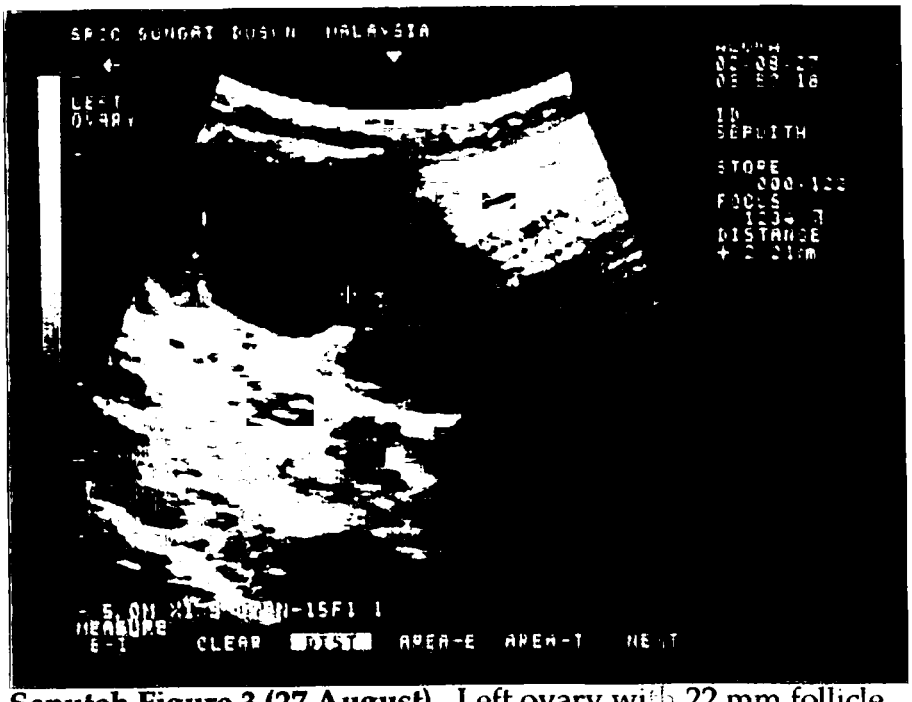
Based on the extensive pathology of this female, breeding management of other females should receive priority. We recommend that surgical excision of the vaginal masses should be conducted at a later date in order to give Seputih the best possible chance at normal reproductive function. Before this surgery is done, it is recommended that Seputih be left in a breeding situation as regular breeing activity seems to be important in order to maintain normal cyclic ovarian function in these induced ovulators. Seputih should be removed from the breeding population at Sungai Dusun ONLY if rhino density issues cannot be appropriately addressed with the modifications proposed for the 100-acre enclosure (see Appendix D). A uterine biopsy should also be attempted at a future date (perhaps at the time of vaginal surgery) in order to develop the appropriate techniques and pathology protocols that will help guide future reproductive management of captive Sumatran rhinos.

Reproductive Exam Details: **SEPUTEH**



Seputeh Figure 1 (27 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 (27 August). Mature corpus luteum on Seputih's right ovary.



Seputeh Figure 3 (27 August). Left ovary with 22 mm follicle.

Captive Program Evaluation

I think most would agree that priority for conservation programs and funding for Sumatran rhinos MUST FIRST be directed toward protection of wild rhino populations and their habitats. To date, this remains the only strategy that has produced a measurable outcome. Such policies have historically, and most likely will continue to come in the form of law enforcement and intelligence [(ie. Rhino Protection Units (RPU's)] and habitat preservation. Such policy has and hopefully will continue to provide far-reaching benefits to key species and habitat beyond the rhino as this species' range encompasses several key biodiversity hotspots that remain threatened in SE Asia.

While we believe that it would be wise to continue attempts at captive propagation of the Sumatran rhino, it should be acknowledged that many of the animals at Sungai Dusun (and in the limited *ex situ* population) have reproductive problems that make salvage of the captive program at this stage difficult. Therefore it must be recognized that if the captive program is to continue in any manageable form, there must be incorporation of new animals into the population at some future point. If "doomed" animals are considered for the captive program, it should occur only where conservation funding permits AND where such objectives complement and don't compete with field conservation efforts. In essence, captive conservation programs must support (both fiscally and scientifically) the comprehensive *in situ* conservation strategies for this species.

If "doomed" Sumatran rhinos are identified in the wild, the first priority should be the safe translocation or relocation of the rhino to a protected reserve - ideally combined with improved radiotelemetry monitoring. The authors suggest that a written policy be developed that clearly outlines what constitutes a "doomed" rhino and when such animals should be considered for relocation into captivity and when they should not.

APPENDIX A

Anesthesia report detailing butorphanol / azaperone sedation of Sumatran rhinoceros.

"PANJANG" SUMATRAN RHINO ♀
26 AUGUST 2002

Anesthesia Record - Fossil Rim Wildlife Center

Genus/species: Dicerorhinus sumatrensis Location/enclosure: Sungai Dusun barn Date: 26 August 2002
Common name: Sumatran Rhino Activity: O calm ☒ active O excited
Demeanor: O undisturbed O depressed ☒ alert O 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: 25 ☒F O C Group size: 1 (6 total) housed individually
Purpose: VAGINAL SURGERY TO REMOVE CERVICAL MASS
Time fasted: ☒ not fasted O ≤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 O 4 - chronic disease
(see COC - mild anemia)
O 5 - may not survive anesthesia Pregnant? O yes ☒ no
Body condition: O obese/fat ☒ good O fair O thin O poor/emaciated
Body weight: 528 Kg ☒ actual O estimate Vet(s): Drs. Robin & Rolfe Radcliffe Recorder: RR Dr. Aidi Mahamad
August 2002

Drug	#mg	#mL	Conc in mg/mL	Route	Time given	Time - Effect seen
BUTORPHANOL	50	5.0	10	IM ①	10:13:30	Partial injection (~10%)
AZAPERONE	60	1.5	40	②	10:15:55	100% injection (50 mg Bdy/60 mg Azap.)
						10:17:30 First effects
						10:20:45 Marked sedation, head droop
						10:30:00 Head down
						10:33:10 Sternal recumbency
NALTREXONE	250	5.0	50	IM	12:38:16	12:39:15 Moving tail, ears
						12:34:22 Head up
						12:35:05 Alert, moved to stall

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 Mass	1	10% buffered Formalin	Histopathology - UPM, Malaysia	

Summary: (FROM 2° INJECTION)
E-time to first effect: 1:35 tracheal tube size: —
E-time to recumbency: 17:15 (sternal/R or L lat?) Anesthetic rating: ☒ excellent O good
E-time to first arousal (from time of reversal): 0:59 O fair O poor
E-time to standing (from time of reversal): N/A (Panjang standing at time of reversal)
Total elapsed time (darting to ~~standing~~ full stop): 2:19:10 Recovery: ☒ normal O abnormal
HR ; MIN: SEC (see comments)
Darting from (circle): vehicle or foot BY-HAND Dart/needle type: 18 gauge 1½" needle (by-hand) Site: IM ② Neck
Distance away: BY-HAND Dart mixing time/date: Immediately before use SpO2 sensor: Nellcor NPB-40 Vet-Sat Large Sensor on ① ear (hair clipped)
Dart misses or failures: 1° — Partial injection (10%)
Comments: Excellent procedure. This dose/combination produced good relaxation and recumbency in adult Sumatran Rhino

Time	SPO2	Pulse	Resp	Comments
12:33:15				Moving tail & ears
12:34:42	92%	94		Head up
12:35:05				Alert
12:36:00				Moved out of chute to barn stall

Time (hr: min: sec)	SPO2	Temp °F °C	Pulse	Resp	Comments (drugs? observations? PE findings? etc.)
10:25					Moderate sedation
10:30	87%		74		Head down; start vaginal exam
10:31	95%		84		
10:33:10	92%		78	14	Recumbency (sternal)
10:35:30	91%		60		
10:37:05	90%		61	15	
10:39:15	94%		82		Insert speculum / endoscope exam
10:41:15	92%		96		Stand up.
10:43:20	79%		88		
10:46:00	93%		80	14	Examine the mass
10:53:12	93%		91	~~15~~	Recumbency (sternal)
10:55:15	92%		83	16	Stand up.
10:58:33	95%		87		Vaginal exam
11:00:05	96%		91		
11:11:50	92%		88		
11:14:06	91%		90	16	Start attempt to ligate the mass stalk.
11:16:43	91%		89		
11:18:15	92%		88		
11:20:35	90%		87		Put 1st ligature (2nd time)
11:23:05	91%		87	16	
11:25:45	91%		90		
11:28:00	91%		89		Cutting the 1st ligature ends.
11:32:15	90%		88		
11:35:05	91%		88	~~16~~ 17	
11:37:15	89%		87		2nd ligature. Sternal &
11:40:23	93%		89		Stand up again.
11:42:55	94%		90		
11:44:40	94%		88	?	
11:47:10	93%		87		
11:49:45	93%		84	16	
11:52:05	93%		87		
11:54:45	88%		86		
11:56:30	88%		87		Cut the 2nd ligature ends.
11:59:25	88%		89		
12:07:42	88%		84		Cutting the mass off.
12:03:30	91%		89	15	
12:06:15	89%		72		
12:09:40	89%		93		Recumbency (sternal) + stand up again. Cut the mass off.
12:10:30	91%		91		
12:14:20	90%		85		
12:16:00	90%		86		
12:19:00					Iodine flushing + saline
12:22:45	93%		80		Antibiotic flushing
12:25:40	93%		80		Inflamol Gel topical on cervix
12:29:45	86%		86%		
12:32:16	90%		86		Reversal. 250mg Naltrexone IM

APPENDIX B
Sumatran Rhinoceros Hematology Summary; Sungai Dusun, August 2002

Parameter	Unit	MINAH	RIMA	ARA	MAS MERAH	SEPUTIH	PANJANG
White blood cell count	(x 10^3 ul)	12.1	9.1	12.5	10.2	9.9	8.5
Red blood cell count	(x 10^3 ul)	5.73	6.27	6.28	6.68	5.94	3.84
Hemoglobin	(g/dl)	13.7	15.3	14.4	16.7	15.4	10.3
PCV	(%)	42.8	47.7	46.8	51.7	47.1	31.3
MCV	(u^3)	74.7	76.0	74.5	77.4	79.3	81.4
MCH	(p.g.)	23.9	24.4	22.9	25.0	25.9	26.8
MCHC	(g/dl)	32.0	32.1	30.8	32.3	32.7	32.9
Neutrophils	(%)	90	80	48	80	68	32
Lymphocytes	(%)	8	12	36	12	20	60
Monocytes	(%)	2	8	8	0	0	4
Eosinophils	(%)	0	0	8	8	12	4
Basophils	(%)	0	0	0	0	0	0
Blood Protozoa		NEG	NEG	NEG	NEG	NEG	NEG

APPENDIX C
SUMMARY OF PROSTAGLANDIN EFFECTS IN THE SUMATRAN RHINOCEROS *(Dicerorhinus sumatrensis)*

Conclusions from PGF therapy following trial use in three Sumatran rhinoceros *(Dicerorhinus sumatrensis)*

The limited trial use of PGF in three Sumatran rhinoceros females was an attempt to return these females having variable periods of irregular ovarian activity to a predictable 21-day breeding cycle. Two of the females had a history of non-receptivity to the male for the previous 3 to 6 months together with apparent extension of their luteal phases as observed on routine serum progesterone monitoring.

In the absence of the progesterone data, ultrasound and behavioral data suggested that the $PGF_2\alpha$ dose (10 mg IM SID for 2 consecutive days; Dinoprost tromethamine; Lutalyse) was sufficient to induce at least partial luteolysis in all three females (see ultrasound data below).

However, serum progesterone assay in Mas Merah suggests that the 10 mg dose of $PGF_2\alpha$ was insufficient to induce complete luteolysis when given during the early period of a CL's development. Perhaps Sumatran rhinos undergo an extended period of luteal cell development following ovulation and therefore PGF only causes a short decline in P4 when given prior to CL maturation (Troedsson, pers. comm. 2002).

Unfortunately the observed regression in luteal tissue based on serial ultrasound evaluation while correlating with strong breeding behavior in one of the females, was not followed by significant follicular growth. One female had a large hemorrhagic follicle at the time of PGF administration (together with a mature corpus luteum and follicle). In the horse, hemorrhagic follicles are considered a failure of the ovulatory event and are often observed in the transitional period as the mare enters anestrus. Perhaps the induced ovulatory strategy of Sumatran rhinoceros may predispose this species to development of irregular cyclic activity if breedings are missed.

Based on these findings, we have come to the following conclusions and recommendations regarding future use of PGF in the Sumatran rhinoceros:

1) First and foremost, PGF should NOT be used in any female until proven to be NONPREGNANT following breeding. PGF is known to induce abortion in luteal-dependent species.

2) The empirical dosage of 10 mg PGF IM for 2 consecutive days in the presence of a MATURE corpus luteum appears to be sufficient to induce luteolysis and subsequent behavioral receptivity in female Sumatran rhinos. Therefore, we recommend use of the 10 mg dose administered IM for 2 consecutive doses until further work shows otherwise.

3) The dose of $PGF_2\alpha$ (10 mg IM SID for 2 consecutive days; Dinoprost tromethamine; Lutalyse) was not associated with adverse side effects such as colic, sweating or discomfort in adult Sumatran rhinos (body weight range from 460 to 580 kg).

4) Subsequent use of PGF in Sumatran rhinos should be used judiciously and perhaps may be more effective if given in the presence of both a mature corpus luteum as well as evidence of normal follicular activity (observed follicular growth of follicles beyond 15 mm in diameter).

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

RIMA




	LO: 23 mm CL	LO: 19 mm CL	LO: 14 mm CL RO:10 mm Fo	LO: 10 mm CL
Hours Post-PGF	0 Hours	48 Hours	96 Hours	144 Hours
Rhino Behavior	No interest	No interest	Strong interest; Ara followed closely	Strong interest; Ara mounted / no breeding

MAS MERAH




	LO: 24 mm CL	LO: 20 m CL	LO: 10 mm CL RO: 30 mm HAF	RO: 30 mm HAF
Hours Post-PGF	0 Hours	48 Hours	96 Hours 120 Hours	144 Hours
Rhino Behavior	No interest	No interest	No interest Strong interest?	No interest

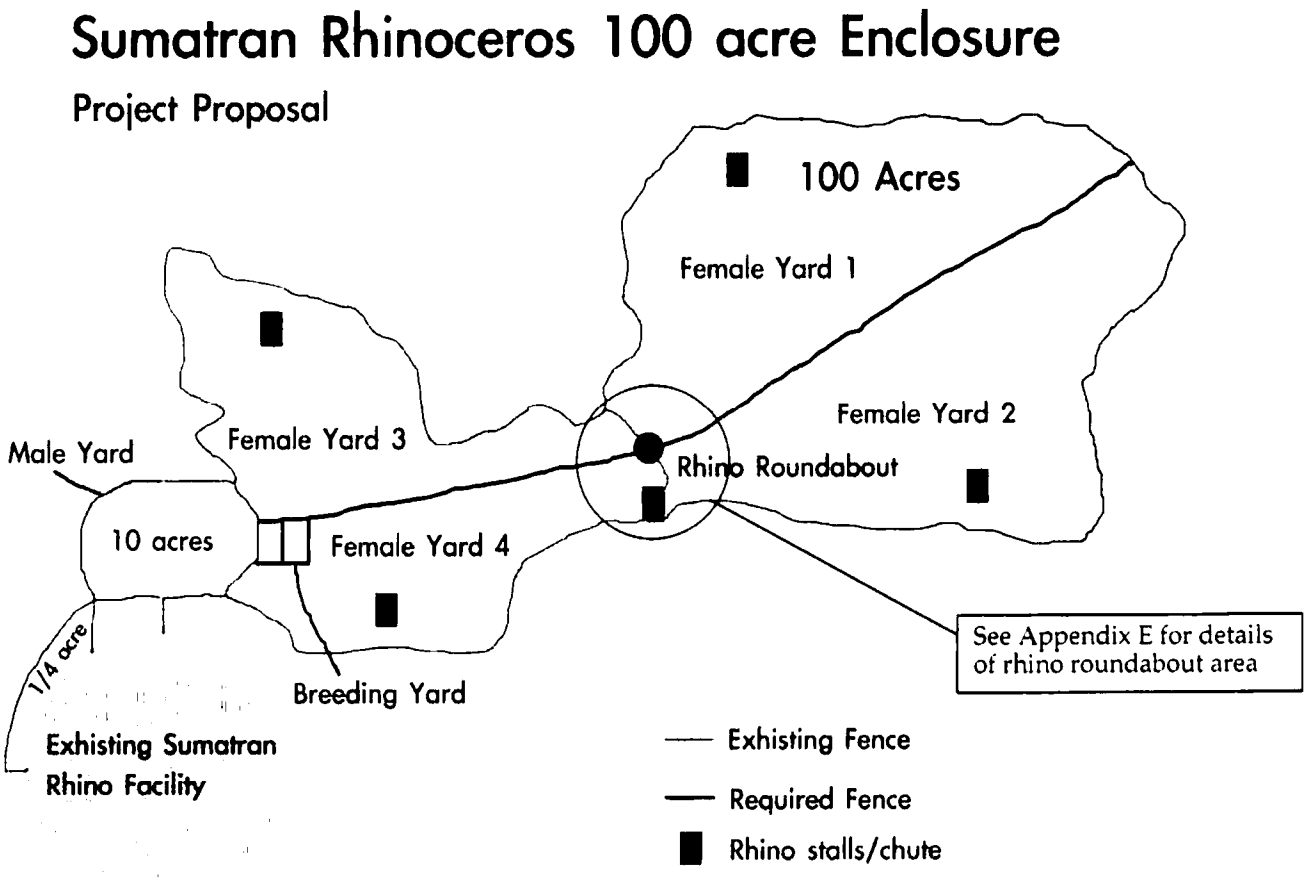
MINAH




	RO: 20 mm CL	RO: 15 mm CL	RO: 13 mm CL/12 mm Fo	RO: 15 mm Fo + small Fos
Hours Post-PGF	0 Hours	48 Hours	96 Hours	144 Hours
Rhino Behavior	No interest	No interest	No interest	Low interest

APPENDIX D

Proposed Modification to the 100-acre enclosure at Sungai Dusun to address rhino density concerns



APPENDIX E
Sumatran Rhino Conservation Center
Selangor Malaysia