

Sumatran Rhino Reproductive
Evaluations
Sungai Dusun, Malaysia
March, 2001



Minah SB# 15



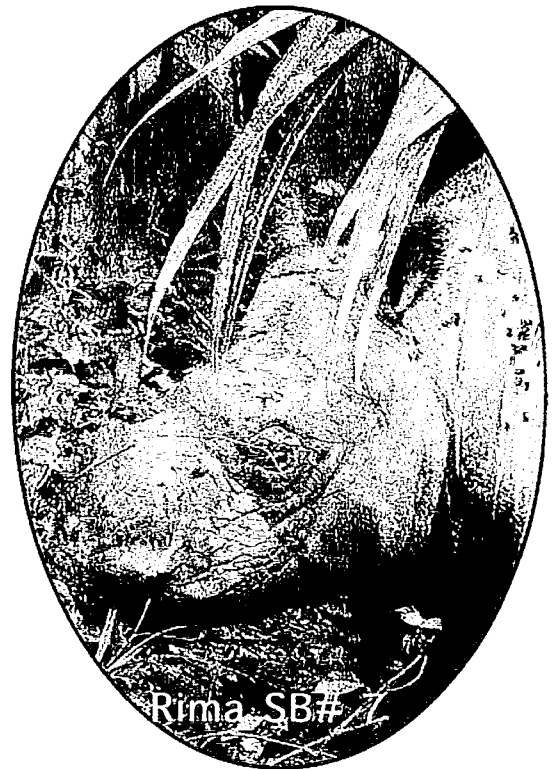
Seputih SB# 23



Mas Merah SB# 19



Panjang SB# 13



Rima SB# 7

**Sumatran Rhino Reproductive Evaluations
Sungai Dusun, Malaysia
March, 2001**

Musa Nordin
21-5-01

*Submitted by
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Center for Conservation and Research of Endangered Wildlife*



Asian Rhino Specialist Group

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, Dr. Terri Roth from the Cincinnati Zoo & Botanical Garden's Center for Conservation and Research of Endangered Wildlife (CREW) visited the Sungai Dusun Sumatran Rhino Conservation Centre in March, 2001. For 10 days, Dr. Roth worked closely with Dr. Aidi Mohamad and Steve Romo examining the female Sumatran rhinos by ultrasonography to determine the status of their reproductive tracts and to check for pregnancy. The three worked as a team with the willing assistance of the rangers and were able to examine all female rhinos at least twice and up to four times each. The examinations were conducted with care and all animals adapted very quickly to allow the procedures without demonstrating any agitation. Most examinations were limited to about 10 minutes to avoid stressing the animals. Following the exams, the ultrasound footage was reviewed and studied and the data collected. Brief sections of the footage have been edited, spliced together and labeled to produce a tape showing 5-7 minutes of ultrasound footage documenting each female. This footage can be used as a permanent record and a point of reference in future work.

Three full reports with accompanying video have been produced and provided to both Mohd Khan and Tom Foose for their distribution to whomever they feel should have an original copy of this document. They also are welcome to make additional copies as needed. The report includes background on the animals, what was observed during the examinations and recommendations for each female. Each report includes figures depicting specific ultrasound images for each female. These also are included in the live footage on the video.

Rhino Reproductive Evaluation at Sungai Dusun, Malaysia
March, 2001

<u>Species</u>	<u>SB#</u>	<u>Sex</u>	<u>Name</u>	<u>Age</u>
<i>Dicerorhinus sumatrensis</i>	23	F	Seputih	~22+

Years in captivity w/o reproducing - 13

Breeding activity lately - 10/19/00; 11/8/00; 1/26/01; 2/16/01

Progesterone profile - 21 day cyclical pattern since breeding initiated in 2000; stayed elevated after 2/16/01 breeding and she is suspected pregnant.

Hormonal Manipulations: none reported

Purpose of Exam: To check for pregnancy and pathology.

Brief Summary of Findings

Based on recent animal behavior, endocrine data and the ultrasound evaluation, the following can be concluded. First, Seputih appears to be a reproductively active female that cycles regularly when breeding (and thus ovulating) every ~21 days. Recently, progesterone levels remained elevated after breeding and Seputih may be pregnant. (The same was suspected about a year ago.) An ultrasound exam on day 26 post-breeding and again on day 28 post-breeding failed to confirm the presence of an embryo. By day 26, the embryonic vesicle should be ~25-30 mm in diameter and should contain a very small fetus. Although no embryo was detected, it is possible that one existed but was missed due to the extensive pathology noted in the uterus of this female. The ultrasound exam confirmed previous concerns that this female has developed significant pathology primarily in the caudal portion of the uterus. Several aberrant growths, masses and cysts filled the uterine body. The precise nature of these growths is difficult to diagnose. Several appear too echogenic (cloudy or gray in color) to be lymphatic cysts yet not echogenic enough (not white enough) to be classified as typical leiomyomas. Given the severity and location of the pathology, one might speculate that an embryo would have difficulty undergoing normal implantation and development. Assuming an embryo was not missed during the ultrasound examinations, it is logical to hypothesize that Seputih did become pregnant but that the embryo could not adhere to the uterus, an event that begins to occur between days 18 and 20, and was lost. Previous experience with the female Sumatran rhino in Cincinnati has revealed that early embryo loss can be followed by an extended period (up to 10-12 weeks) of elevated progesterone.

Predicted fertility – low, but may still be fertile.

Recommendations: Seputih should be managed as a pregnant animal as long as the progesterone levels remain high. If the progesterone drops, she will be put back on a regular breeding schedule in the hopes that she becomes pregnant and that the embryo survives and develops normally regardless of the uterine pathology.

Detailed report of Ultrasound Examination

Cervix – not examined

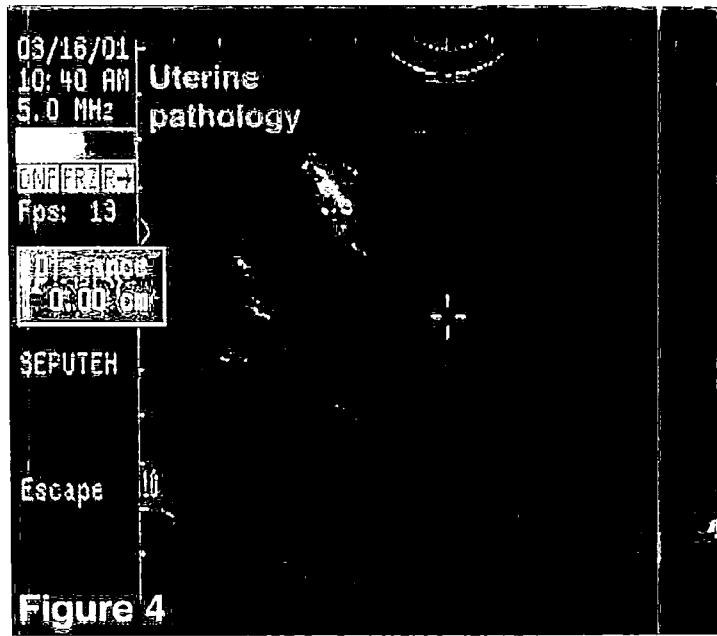
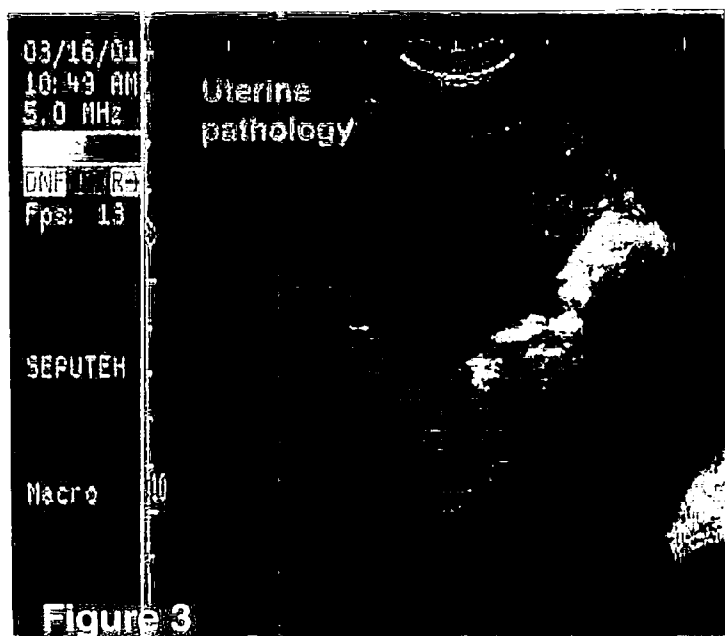
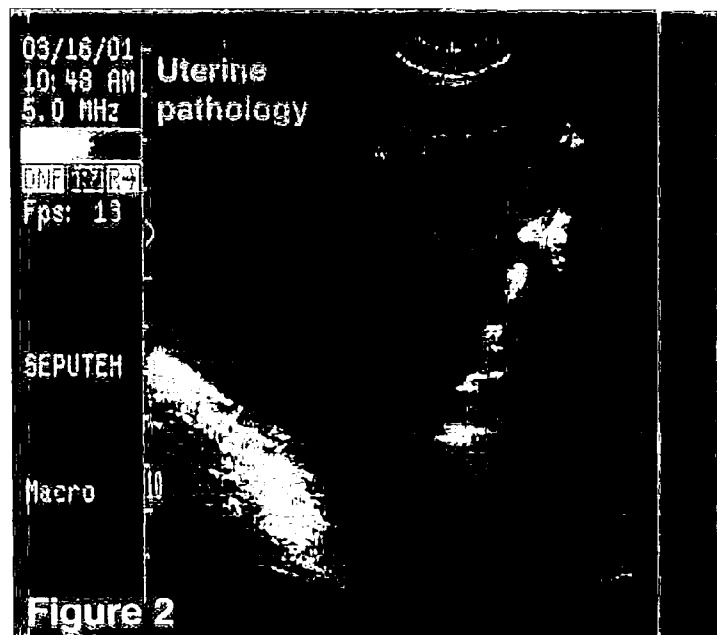
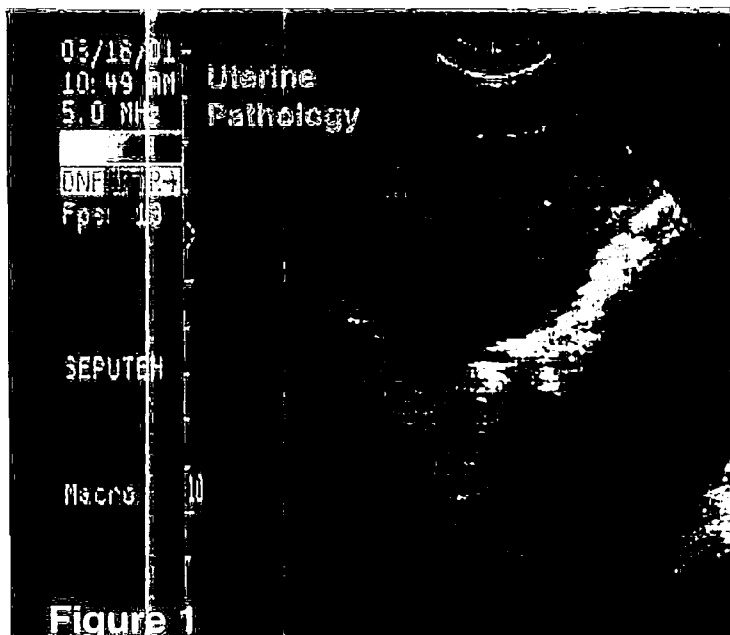
Uterus – At least 5-6 masses and cysts >20 mm in diameter were noted. The largest cystic structure measured ~55 mm and the largest mass measured 33.5 mm in diameter (see Figs. 1-5)

Uterine Horns – Both horns contained several small ~5 mm cysts but portions of horns appeared normal. The left horn contained a small amount of fluid in the central lumen (See Fig. 6) that could be considered normal in an estrual animal but should not be present in a pregnant animal. Left horn ~30 mm diameter; Right horn ~42 mm diameter.

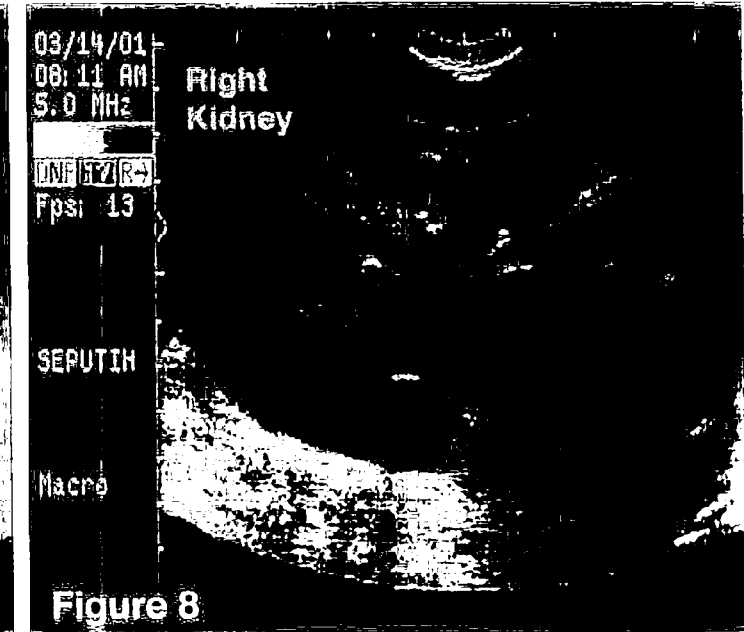
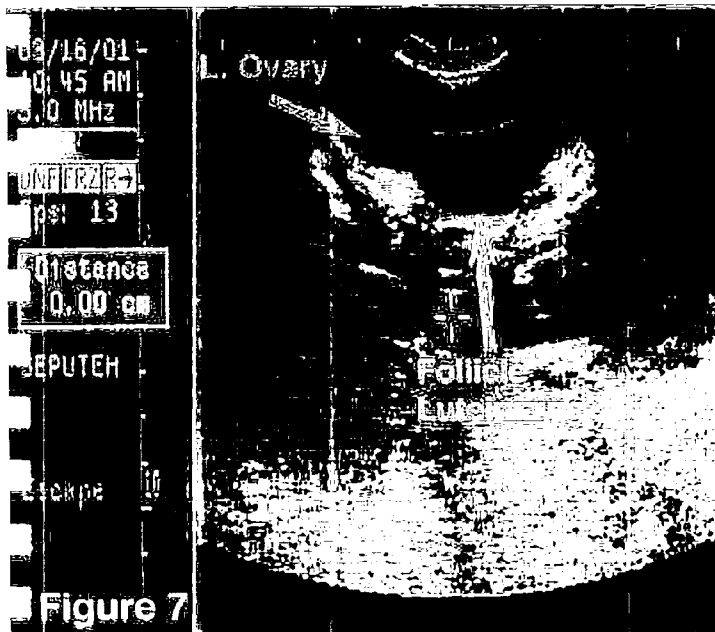
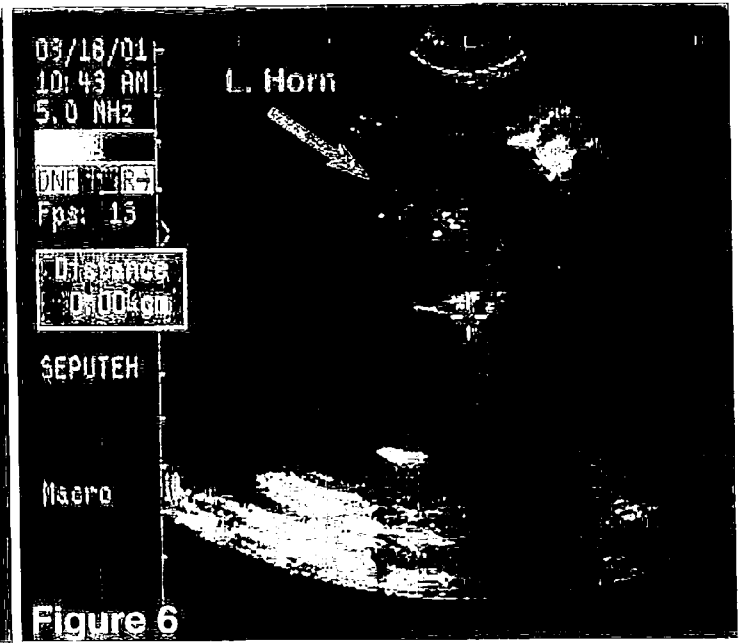
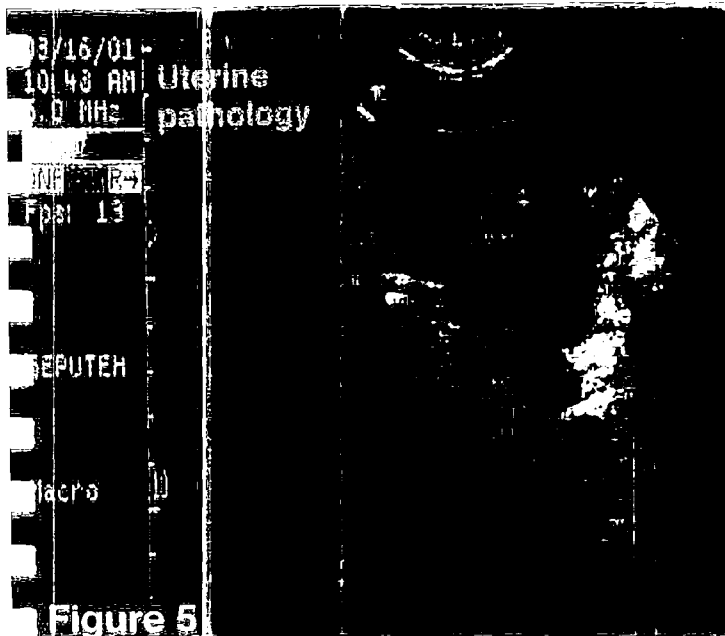
Ovaries – Left ovary appeared active with a 22.5 mm follicle present that may have been luteinizing as it appeared slightly cloudy in some scans (see Fig. 7). Such a structure can be present on ovaries of pregnant or nonpregnant animals.

Kidney - Right kidney was scanned and appeared normal though it did contain several <5mm hyperechoic spots (Fig. 8).

Seputih - Ultrasound Images 1



Seputih - Ultrasound Images 2



Rhino Reproductive Evaluation at Sungai Dusun, Malaysia
March, 2001

<u>Species</u>	<u>SB#</u>	<u>Sex</u>	<u>Name</u>	<u>Age</u>
<i>Dicerorhinus sumatrensis</i>	13	F	Panjang	~19+

Years in captivity w/o reproducing - 14

Breeding activity lately - 10/14/00

Progesterone profile - Irregular activity; no definitive cycles since October, 2000, but fluctuations suggest some ovarian activity.

Hormone Manipulations: Implanted with a CIDR containing 1.9 g progesterone and 10 mg estradiol benzoate for 12 days (March-April, 2000).

Other: Bloody discharge was found in the stall 18 days after the 10/14/00 breeding.

Purpose of Exam: To try to determine what caused the bloody discharge.

Brief Summary of Findings

Based on recent animal behavior, endocrine data and the ultrasound evaluation, the following can be concluded. Panjang appears to be a reproductively active female. Her ovaries contained good sized follicles during the ultrasound examinations and her reported breeding in October 2000 support this conclusion. Although her progesterone has been consistently lower over the past couple of months, levels are still above baseline and fluctuate somewhat suggesting that there is some ovarian activity ongoing. However, no clear cyclic pattern can be determined which is not unusual when a female is not breeding and ovulating regularly. The most significant and disturbing finding during the examinations was the presence of a very large uterine mass (12 cm diameter) that appeared similar to those found in the uteri of Rapunzel and Jaram. This mass appeared vascularized in some images, a sign of neoplasia. Such a large mass located in the caudal region of the uterus will almost certainly reduce the fertility of this female, perhaps making her infertile. In November of 2000, a bloody vaginal discharge was collected from this animal and it was suggested that this discharge was an aborted pregnancy. Based on the available information, and assuming it is accurate, there is strong evidence that the vaginal discharge was not an aborted fetus. First, the female bred 18 days prior to the discharge. Therefore, a pregnancy could only have been at day 18 of gestation. At this time, implantation has not yet occurred and there is no placental development. Therefore, the discharge could not have been placenta. Furthermore, the female rhino in Cincinnati has lost several early pregnancies (30-60 days after breeding) and has never produced a bloody discharge of aborted material because the pregnancy is in such early stages of development there is insufficient material to be noticed when expelled. Finally, it is unlikely that any embryo would be able to implant and undergo placentation in Panjang given the large mass present in her uterus. However, if the pathology within Panjang's uterus is undergoing change or growth, it could lead to bleeding and even tissue damage resulting in a bloody discharge. It might even be possible for breeding to aggravate the pathology and cause bleeding. This seems the most logical explanation for the bloody discharge reported in November.

Predicted fertility – poor.

Recommendations: Panjang should continue to be introduced to a male rhino daily for short periods under constant supervision until breeding occurs again. Once breeding behavior is observed, progesterone should be monitored and Panjang should be introduced to a male every

21 days until she becomes pregnant. Although her fertility outlook is poor, the possibility of a pregnancy can not be ruled out at this point. Therefore, since her ovaries are active, every effort should be made to breed her on consecutive cycles for the next 6-8 months in hopes that an embryo will be able to survive despite the significant pathology in this female's uterus. She also will be carefully observed, especially after breeding, for additional bleeding/discharge.

Detailed report of Ultrasound Examination

Cervix – not examined

Uterus – A large, relatively homogeneous uterine mass was noted in the caudal portion of the uterus possibly extending into the right horn. The mass was 11.6 cm deep (dorsal-ventral) and over 13 cm in length (caudal-cranial) and was too large for accurate measurement (Fig. 1).

Uterine Horns – Both horns measured 45-55 mm in diameter caudally and 35-40 mm cranially. Both horns contained some cysts with one horn (left?) containing a large (16 mm) cyst located centrally in the horn and surrounded by highly echogenic borders (Fig. 2).

Ovaries – Left ovary appeared active with several smaller (<10 mm) follicles and one ~20 mm follicle present (Fig. 3). There also were two highly echogenic spots noted in the ovary (Fig. 4). Such spots are not uncommon and are not problematic. The right ovary appeared active with a couple of small follicles and two 20 mm follicles noted (Fig. 5).

Panjang - Ultrasound Images 1

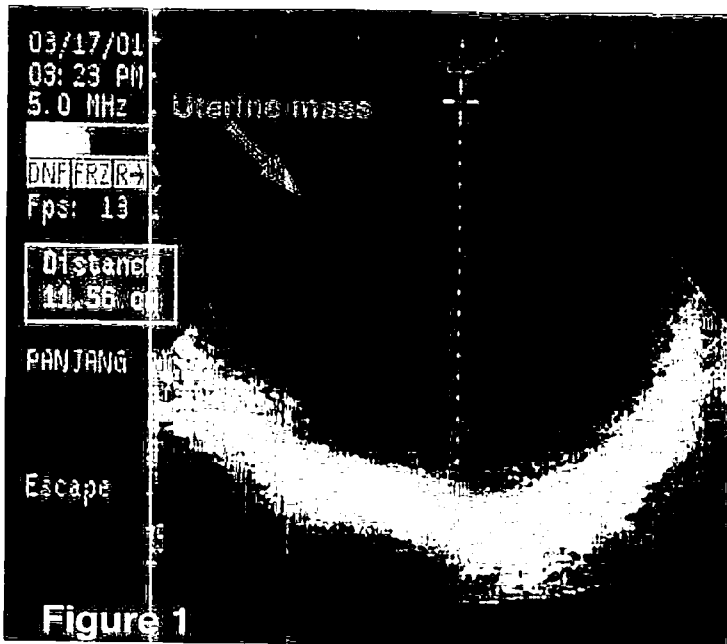


Figure 1

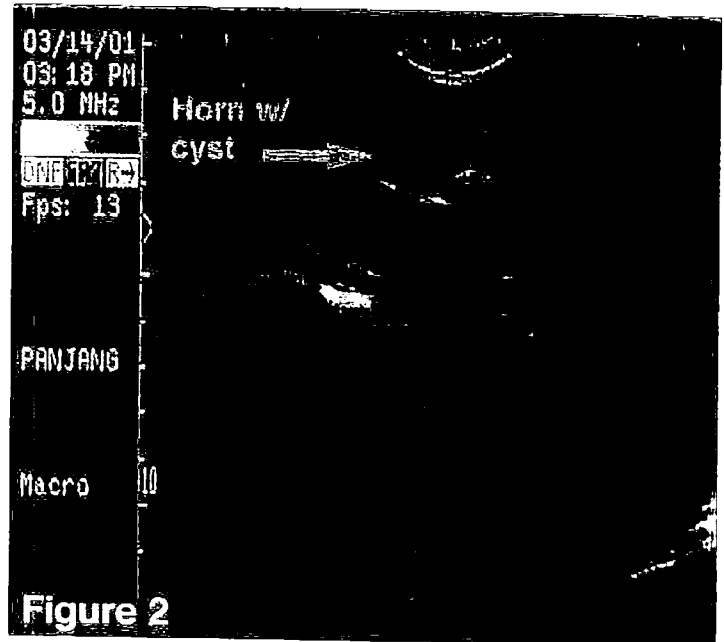


Figure 2

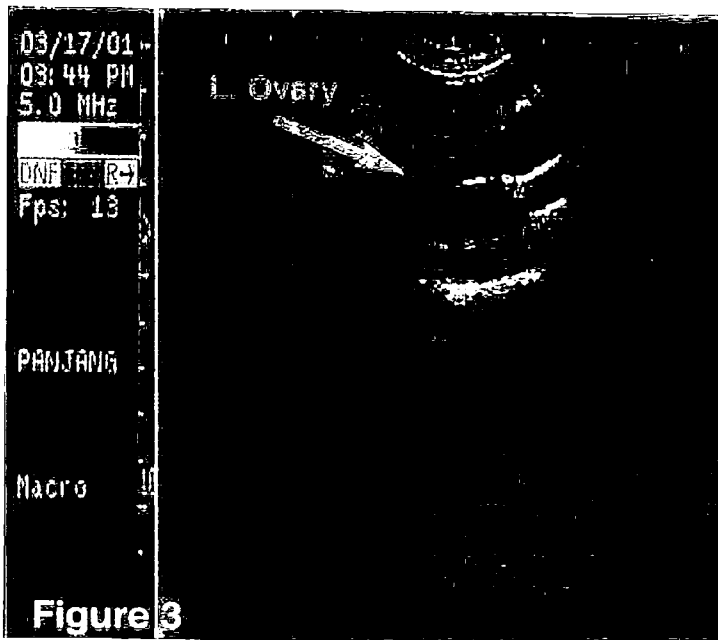


Figure 3

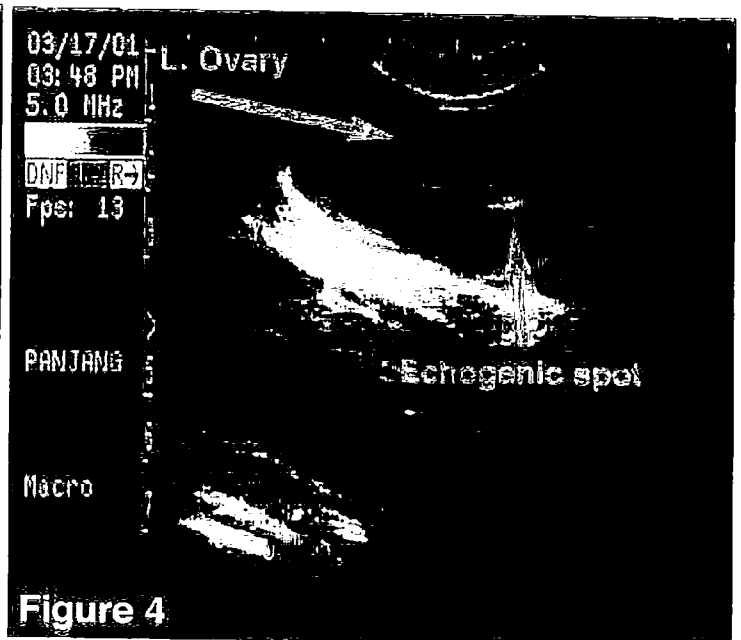
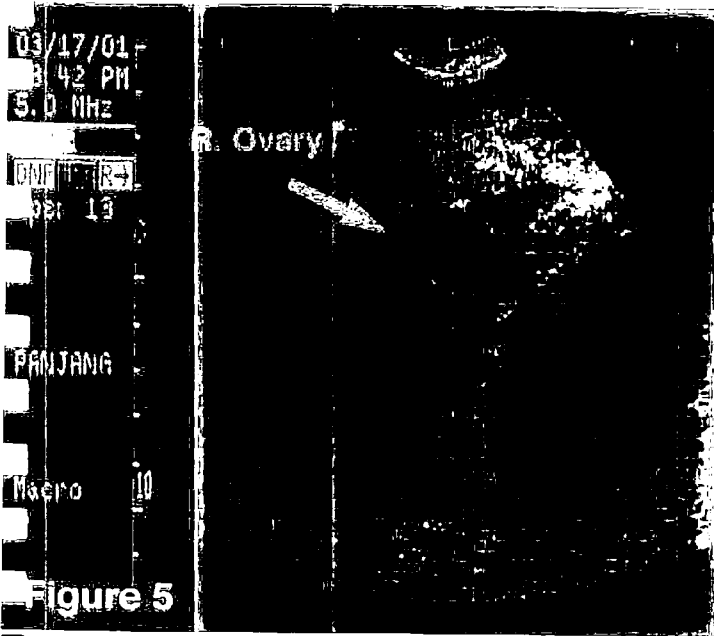


Figure 4

Panjang - Ultrasound Images 2



Rhino Reproductive Evaluation at Sungai Dusun, Malaysia
March, 2001

<u>Species</u>	<u>SB#</u>	<u>Sex</u>	<u>Name</u>	<u>Age</u>
<i>Dicerorhinus sumatrensis</i>	7	F	Rima	~22+

Years in captivity w/o reproducing - 14

Breeding activity lately - 10/5/00; 10/29/00; 1/10/01; 1/31/01; 2/22/01; 3/15/01

Progesterone profile - Fairly regular 21 day cyclical pattern.

Hormone Manipulations: none reported

Purpose of Exam: To check for pregnancy and pathology.

Brief Summary of Findings

Rima was examined for pregnancy 20 days after breeding and no embryo was observed. Instead, a pre-ovulatory follicle (24 mm) was found on her left ovary. She was placed with a male the next day and bred. Another ultrasound exam 2 days later confirmed that she had ovulated. Based on recent animal behavior, endocrine data and the ultrasound evaluation, the following can be concluded. Rima is a normally cycling female that breeds every 21 days if not pregnant. Her reproductive tract is relatively normal. There appears to be some thickening of the endometrium in the uterine body region, perhaps some endometrial hyperplasia, but this could also simply be due to some edema associated with estrus since she bred the next day. Her uterine body is not full of cysts, masses and tumors like those of Panjang and Seputih. Several small cysts were observed in both horns and one particular cyst is larger (~10 mm) in the right horn, but it is likely these are tolerable. Assuming the thickened tissue in the uterine body is not serious, this female's reproductive tract should be able to support a pregnancy.

Predicted fertility – good.

Recommendations: This animal is being managed appropriately and the current strategy of breeding her every 21 days if her progesterone levels decrease should be continued. Every effort should be made to breed her multiple times during her 24 h receptive period since increased matings should improve chances of a pregnancy. If the progesterone does remain elevated after breeding, Rima should be managed as a pregnant female and the pregnancy should be confirmed by ultrasound between days 35-70. With regular breeding, hopefully it is just a matter of time before she becomes pregnant.

Detailed report of Ultrasound Examination

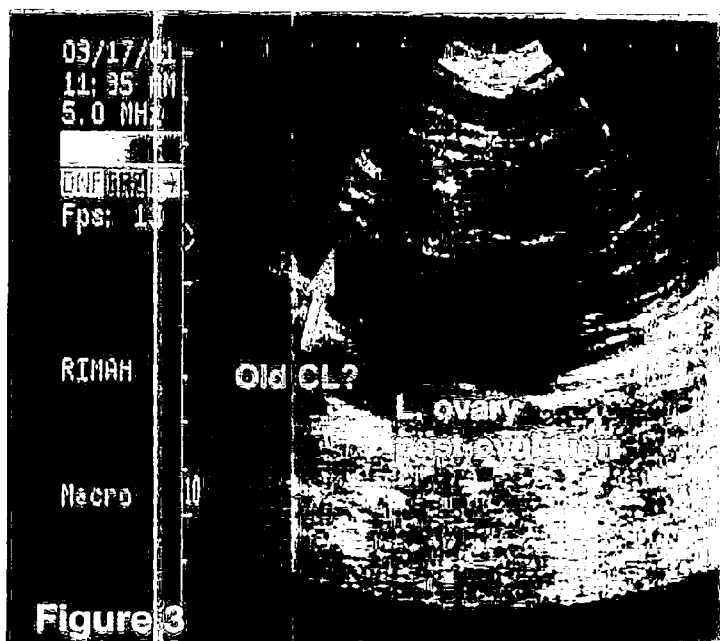
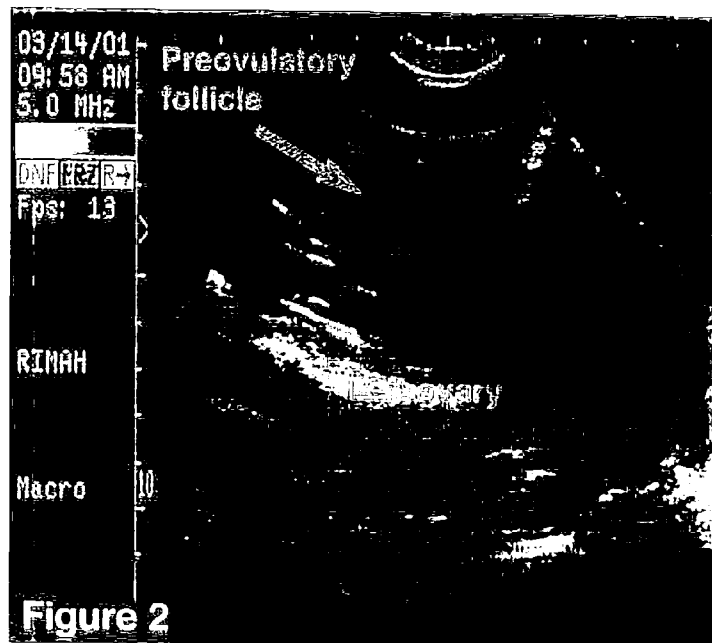
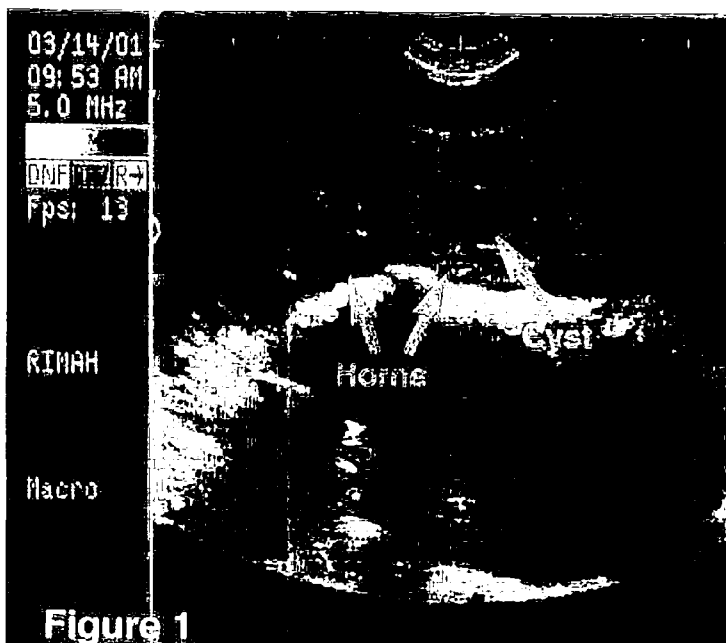
Cervix – not examined

Uterus – No serious pathology noted. Uterine tissue appeared rather homogenous and void of any significant cysts, masses and tumors. However, the tissue did seem somewhat thickened, suggestive of endometrial hyperplasia, but the extent or seriousness of this thickening could not be concluded.

Uterine Horns – Horns measured 35-42 mm in diameter and both contained several small cysts. The largest cyst (8-10 mm) was noted in the left uterine horn (Fig. 1).

Ovaries – Left ovary was imaged twice. Before breeding, it contained a pre-ovulatory follicle (24 mm; Fig. 2) and 43 h after breeding that follicle had ovulated (Fig. 3). An old CL was also present on the left ovary (Fig. 3).

Rima - Ultrasound Images 1



Rhino Reproductive Evaluation at Sungai Dusun, Malaysia
March, 2001

<u>Species</u>	<u>SB#</u>	<u>Sex</u>	<u>Name</u>	<u>Age</u>
<i>Dicerorhinus sumatrensis</i>	19	F	Mas Merah	~22+

Years in captivity w/o reproducing - 14

Breeding activity lately - 9/4/00 (mounting but intromission unsuccessful)

Progesterone profile - Definite activity but cycle is erratic

Hormone Manipulations: Attempted CIDR implant + PGF2a on 3/30/00 but unsuccessful. Implanted with Synchronate in left flank 4/8/00 and removed 4/17/00. No estrus observed.

Purpose of Exam: Check for pathology.

Brief Summary of Findings

Mas Merah's progesterone data has indicated irregular cyclicity which is common for females not breeding and ovulating regularly. The ultrasound examination of the right ovary confirmed that Mas is exhibiting ovarian activity as there were a couple of large follicles on that ovary. Breeding could be expected over time if daily introductions with a male are conducted. There was some pathology (a cystic-like structure) identified either in the cranial portion of the cervix or caudal portion of the uterine body, but it wasn't severe. Another potential mass (4 cm in diameter) was noted but difficult to confirm as it may have simply been a thickening of the uterus. A few small cysts were noted in the horns. The examination did not reveal any severe pathology and it appears this female may be fertile. Hopefully, once a consistent breeding schedule is established, she will become pregnant.

Predicted fertility – Average.

Recommendations: Mas Merah should be introduced to a male rhino daily for short periods under constant supervision until breeding occurs. Once breeding behavior is observed, progesterone should be monitored and Mas Merah should be introduced to a male every ~21 days until she becomes pregnant. Although there is some pathology in the reproductive tract, it is less severe than that of Panjang's and Seputih's. Therefore, she may be more likely to become pregnant and carry a pregnancy successfully. It is important to get her on a breeding schedule and to breed her on consecutive cycles for 6-8 months to maximize the chance of success. It also would be beneficial to mate her with the male multiple times during her receptive period.

Detailed report of Ultrasound Examination

Bladder - Unusual floccular material appeared in the bladder of this animal (Fig. 1).

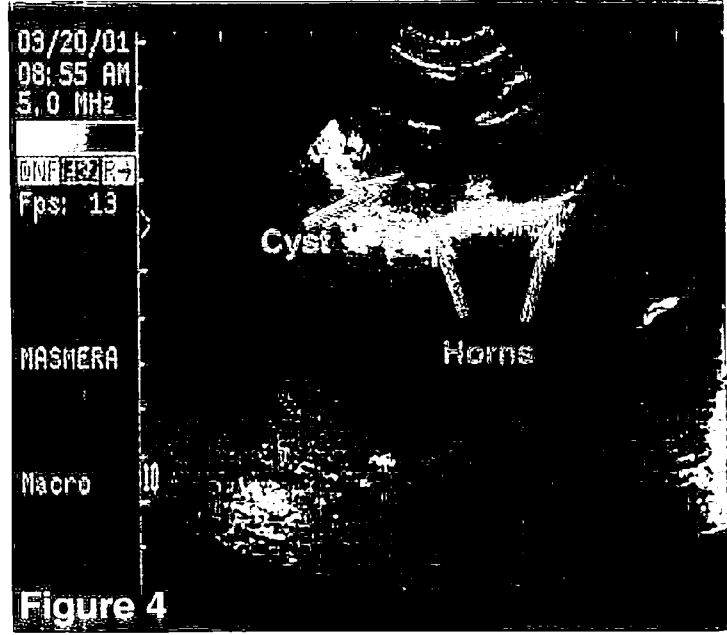
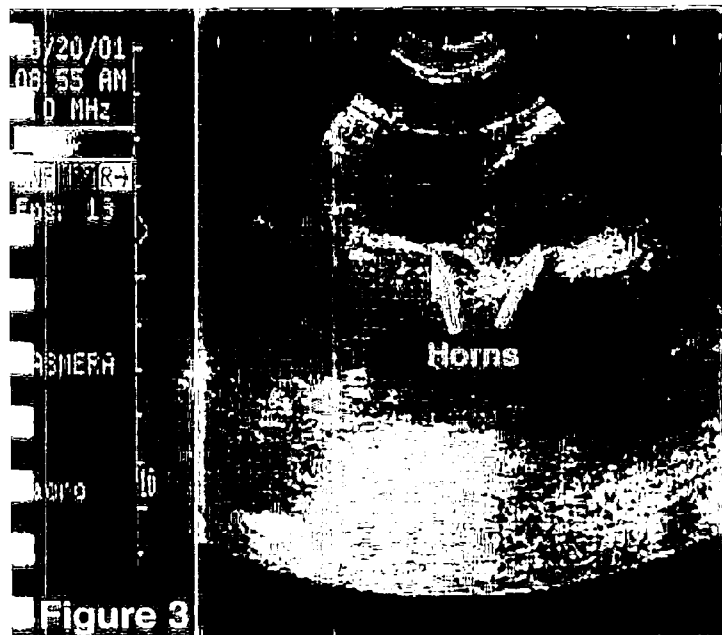
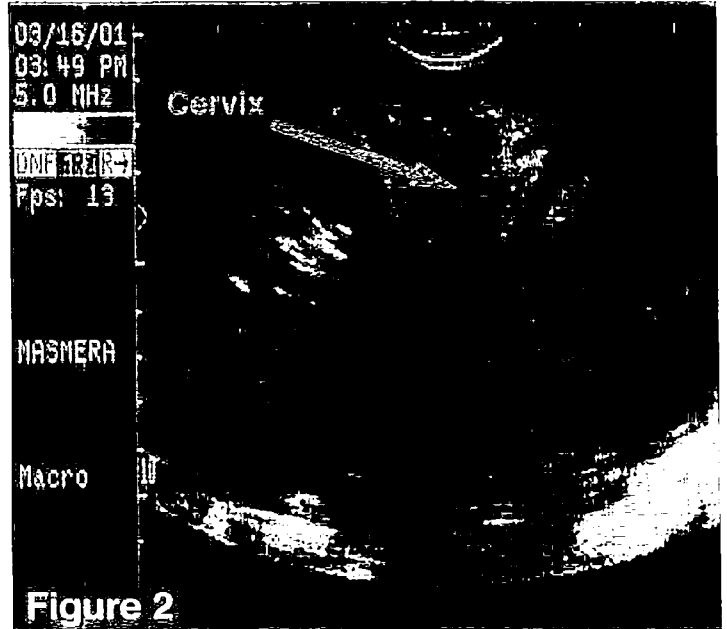
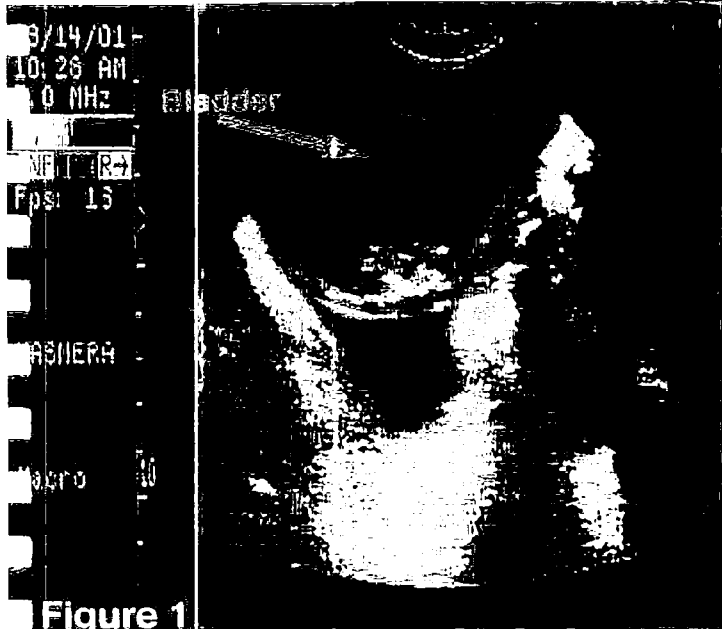
Cervix – Appeared largely normal (Fig. 2) but some pathology (cystic structure; Fig. 7) noted in the cranial portion of it. Cervix was 37.3 mm in diameter.

Uterus – Some pathology extending from the cervix possible and a potential mass (4 cm; Fig. 8) noted. However, the mass could simply be a thickened area of the uterus. Otherwise no significant pathology noted in the uterus.

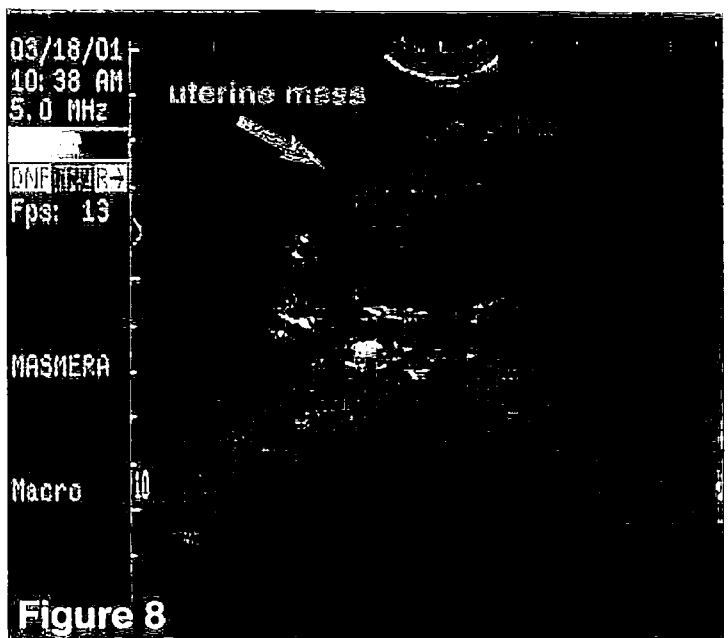
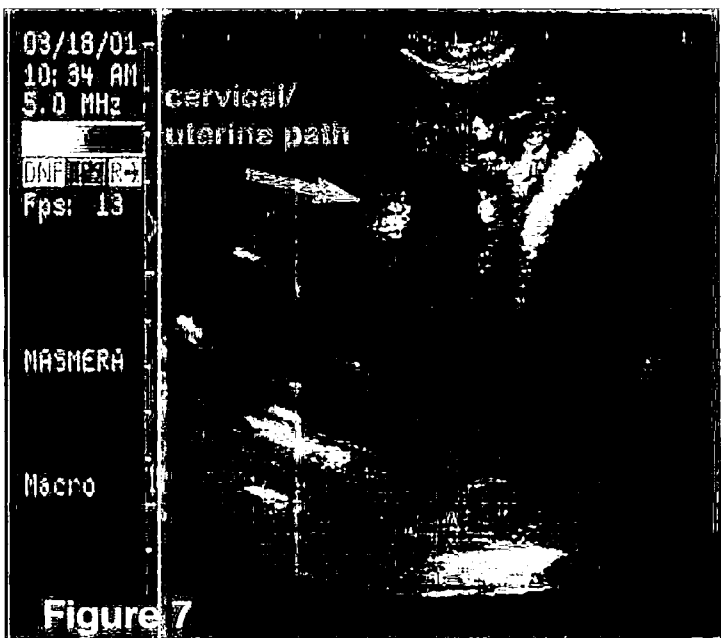
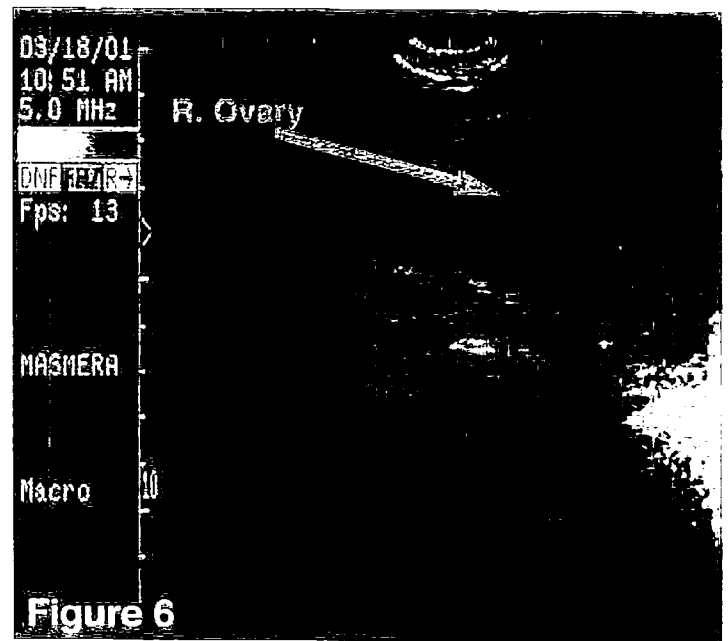
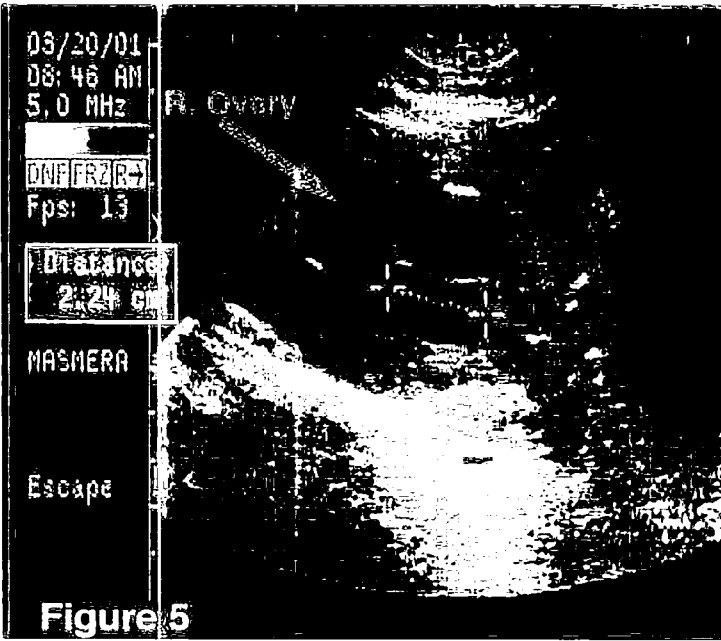
Uterine Horns – Right horn was about 30 mm in diameter and mostly normal (Fig. 3). Left horn measured about 42 mm in diameter and contained an 8 mm cyst (Fig. 4).

Ovaries – Right ovary appeared active containing two large (24 and 21 mm) follicles (Figs. 5 & 6).

Mas Merah - Ultrasound Images 1



Mas Merah - Ultrasound Images 2



Rhino Reproductive Evaluation at Sungai Dusun, Malaysia
March, 2001

<u>Species</u>	<u>SB#</u>	<u>Sex</u>	<u>Name</u>	<u>Age</u>
<i>Dicerorhinus sumatrensis</i>	15	F	Minah	14

Years in captivity w/o reproducing - 14

Breeding activity lately - Last time a male was seen trying to breed her was in 1998

Progesterone profile - Very low baseline levels of progesterone since December 2000. Prior to that, she appeared to exhibit one or two irregular cycles in Sept. and November

Hormone Manipulations: PRID implanted 3/21/00 but expelled next day. CIDR implanted 3/30/00 but could not be found 3/31/00. Intravaginal exploration and ultrasound confirmed it was expelled but the device was never recovered.

Purpose of Exam: Determine why she is not cycling.

Brief Summary of Findings

Minah's progesterone profile indicates that she has been acyclic for several months since progesterone values are consistently at or near baseline. The ultrasound evaluation provided evidence in support of this conclusion as both ovaries were small and contained mainly (<5 mm) follicles. Although, overall, the reproductive tract of this female appeared normal, there was a disturbing finding in the uterine body. There appeared to be an abnormal area with an irregular, highly echogenic border. This area probably is a pocket of fluid with air above it which would account for the highly echogenic lines. The fluid and air should be expelled from the uterus and may be the result of a low-grade uterine infection or endometritis. The observation by ultrasound in conjunction with staff reports of straining by the female without urination, suggests that this female has a uterine infection. This suggestion needs to be confirmed and, if it is, the female should be treated for the infection. This irritation might be responsible for the acyclicity but, more likely, the inactive ovaries have led to the accumulation of fluid within the uterus. If ovarian activity resumes, the situation may correct itself. If the uterine pathology clears and the ovaries become active again, this female will have a good chance of becoming pregnant and sustaining a pregnancy.

Predicted fertility – Good once cyclicity resumes.

Recommendations: First, it would be best to determine conclusively if the fluid in the uterus is sterile or the result of infection. These infections typically are not associated with changes in routine blood work. Based on the ultrasound image, the uterine fluid appears purulent, but this could be confirmed if fluid could be collected from the females vagina and examined for the presence of white blood cells and/or bacteria. Perhaps with rectal massage some fluid will be expelled. Alternatively, a vaginal swab may be useful but will depend on the frequency of discharge. If an infection is confirmed, antibiotic treatments should be discussed. Regardless of whether or not it is an infection or simply a sterile collection of fluid and cellular debris, it needs to be cleared before Minah is likely to get pregnant. If she starts cycling regularly, her own hormonal changes may help clear up the situation so continued monitoring of her blood work is important. If she does start cycling, male introductions should begin as breeding activity may also help her pass the fluid out of her uterus. If she does not initiate cyclical activity, prostaglandin treatments may be worth some discussion. Either way, at some point, Minah should be re-examined to confirm that the fluid and air have been evacuated from the uterus.

Detailed report of Ultrasound Examination

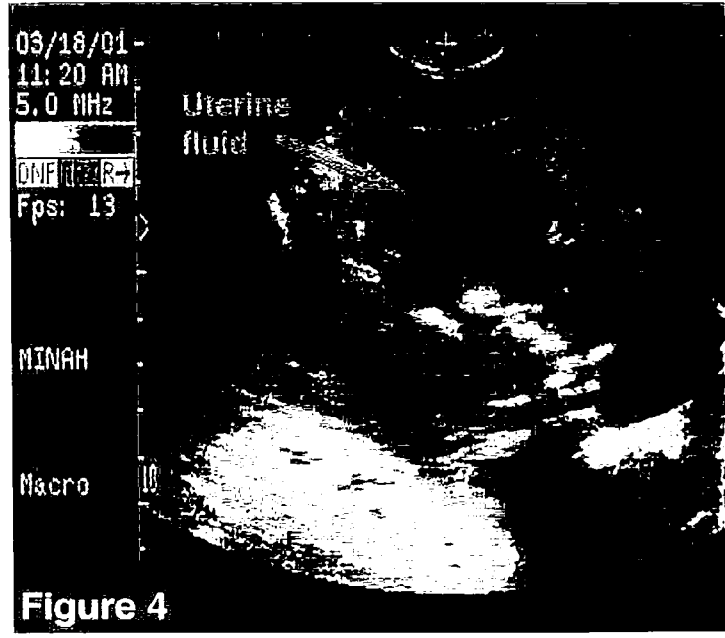
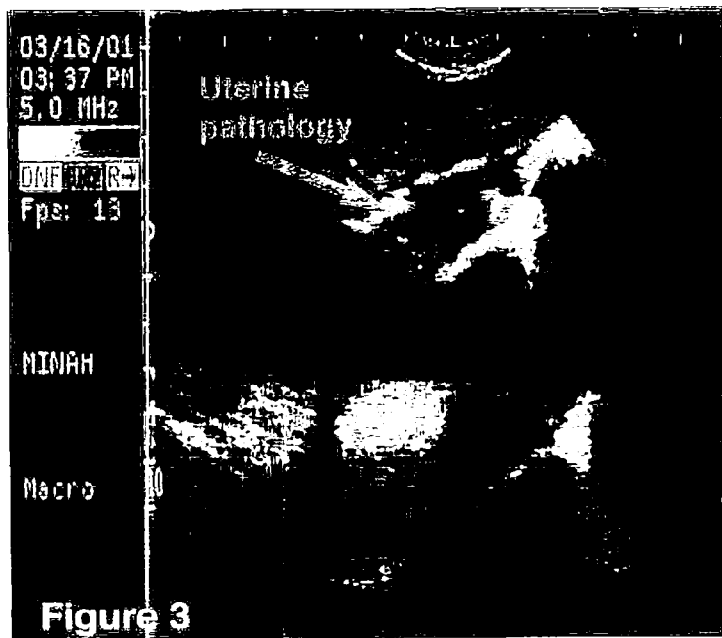
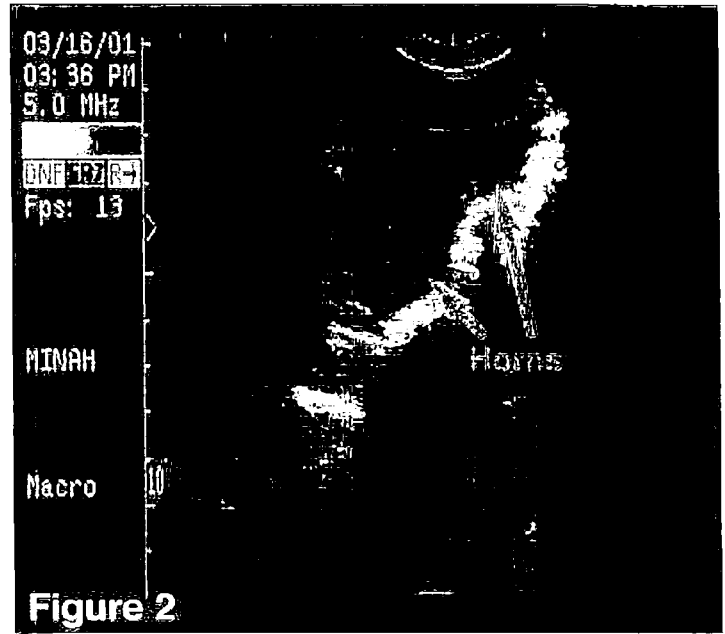
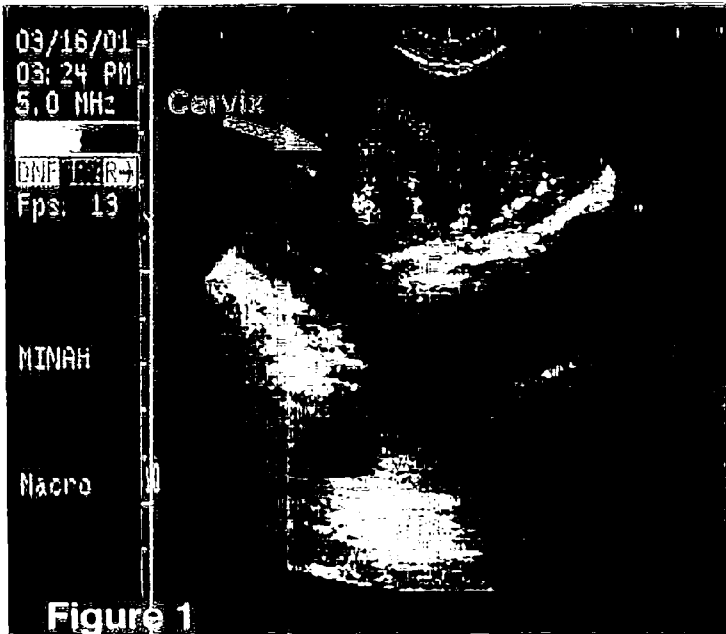
Cervix – Appeared normal and was 35 mm in diameter (Fig. 1).

Uterine Horns – Both horns contain numerous small cysts (2-3 mm) but appear largely normal. Right horn is 22.5 mm and the left horn is 26.6 mm in diameter (Fig. 2).

Uterus – Appeared largely normal except for a pocket of fluid (or possibly air) in the uterine body. This pocket was dark but slightly cloudy, irregular in shape and surrounded by highly echogenic borders (approximately 60 x 45 mm; Figs. 3 & 4). Possible endometritis resulting from a low-grade uterine infection. One small ~10 mm cyst also noted.

Ovaries – Both right and left ovaries are small and relatively inactive. However, there were small follicles on both ovaries. The largest follicle on the left ovary was ~8 mm (Figs. 5 & 6). The largest on the right ~12 mm (Fig. 7). Therefore, although no large follicles were observed, the presence of small follicles is promising and, hopefully, follicles will resume growth after uterine problem is treated and/or the female is stimulated by introductions with the male.

Minah - Ultrasound Images 1



Minah - Ultrasound Images 2

