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STANDING LAPAROSCOPIC-GUIDED UTERINE BIOPSY IN A SOUTHERN WHITE RHINOCEROS (*CERATOTHERIUM SIMUM SIMUM*)

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Abstract: Transrectal ultrasonography of a 35-yr-old captive female southern white rhinoceros (*Ceratotherium simum simum*) with a history of chronic mucohemorrhagic vulvar discharge revealed right transmural uterine horn enlargement. Abdominal laparoscopic surgery, although extremely difficult because of inadequate instrumentation, permitted uterine visualization and biopsy. Standing anesthesia, incorporating butorphanol and azaperone together with local anesthetic infiltration, facilitated the laparoscopy. A leiomyoma was suspected on the basis of history, physical examination, ultrasonographic appearance, and histopathology. Prior rhinoceros laparoscopies have failed, primarily from limitations imposed by recumbency.

Key words: Rhinoceros, *Ceratotherium simum,* laparoscopy, laparoscopic surgery, standing restraint.

INTRODUCTION

Laparoscopy is commonly used for the diagnosis and treatment of medical and surgical conditions in humans and animals.[3,4,10,13,23] This report describes useful laparoscopic techniques and instrumentation developed for the diagnosis of uterine horn disease in a southern white rhinoceros (*Ceratotherium simum simum*) when other more conventional diagnostic approaches were unsuccessful. Continued use of laparoscopy for diagnosis and therapy in the rhinoceros will require refinement of instrumentation and techniques to overcome challenges posed by the animal's size and shape.

CASE REPORT

A 35-yr-old female nulliparous, noncycling southern white rhinoceros (studbook no. 083) at the Fossil Rim Wildlife Center with an estimated body weight of 1,500–2,300 kg[16] had an 11-mo history of chronic mucohemorrhagic vulvar discharge but no other clinical sign. An abnormal quantity of intrauterine fluid was identified on transrectal ultrasonography with a portable scanner (Aloka 500V, Aloka, Wallingford, Connecticut 06492, USA) equipped with a 5-MHz convex-array transducer (Model UST-935N-5, 47° scan angle, Aloka).[20] The rhinoceros was given prostaglandin F_2-alpha (Lutalyse, dinoprost tromethamine, Pharmacia and Upjohn Co., Kalamazoo, Michigan 49001, USA; 5 mg/ml; 50 mg i.m.) to evacuate the uterus and systemic oral antibiotic therapy with trimethoprim-sulfa (sulfamethoxazole and trimethoprim, Mutual Pharmaceutical Co., Philadelphia, Pennsylvania 19124, USA; 960 mg/tablet; 43.2 g p.o. b.i.d.). Aerobic and anaerobic bacterial cultures of the vulval discharge yielded no growth, and the discharge continued after prostaglandin and antibiotic therapy.

Transcervical endoscopy with an 11-mm-diameter, 103-cm-long endoscope (Model G1FQ30, Olympus America, Inc., Melville, New York 11747, USA) to access the uterus for culture, biopsy, and lavage was unsuccessful. Cultures of cervical fluid during the endoscopy revealed a mixed bacterial population. Subsequent transrectal ultrasonography showed a circumferential, transmural thickening of the proximal 14 cm of the right uterine horn with abnormal amounts of intrauterine fluid (Fig. 1). Uterine cross-sectional diameters as measured by ultrasound were 30 mm and 90 mm for the left and right horns, respectively.[20]

Two multiple-day protocols of i.m. prostaglandin F_2-α and estradiol (ECP, estradiol cypionate, Pharmacia and Upjohn Co., 2 mg/ml) were administered to attempt cervical relaxation for uterine diagnostic evaluation and therapy (Durrant, pers. comm., 1998). The first protocol involved giving prostaglandin F_2-α, 15 mg i.m. on the first day, then 35 mg i.m 30 min later, 15 mg i.m the next day, and 10 mg estradiol i.m. on the third day. The second

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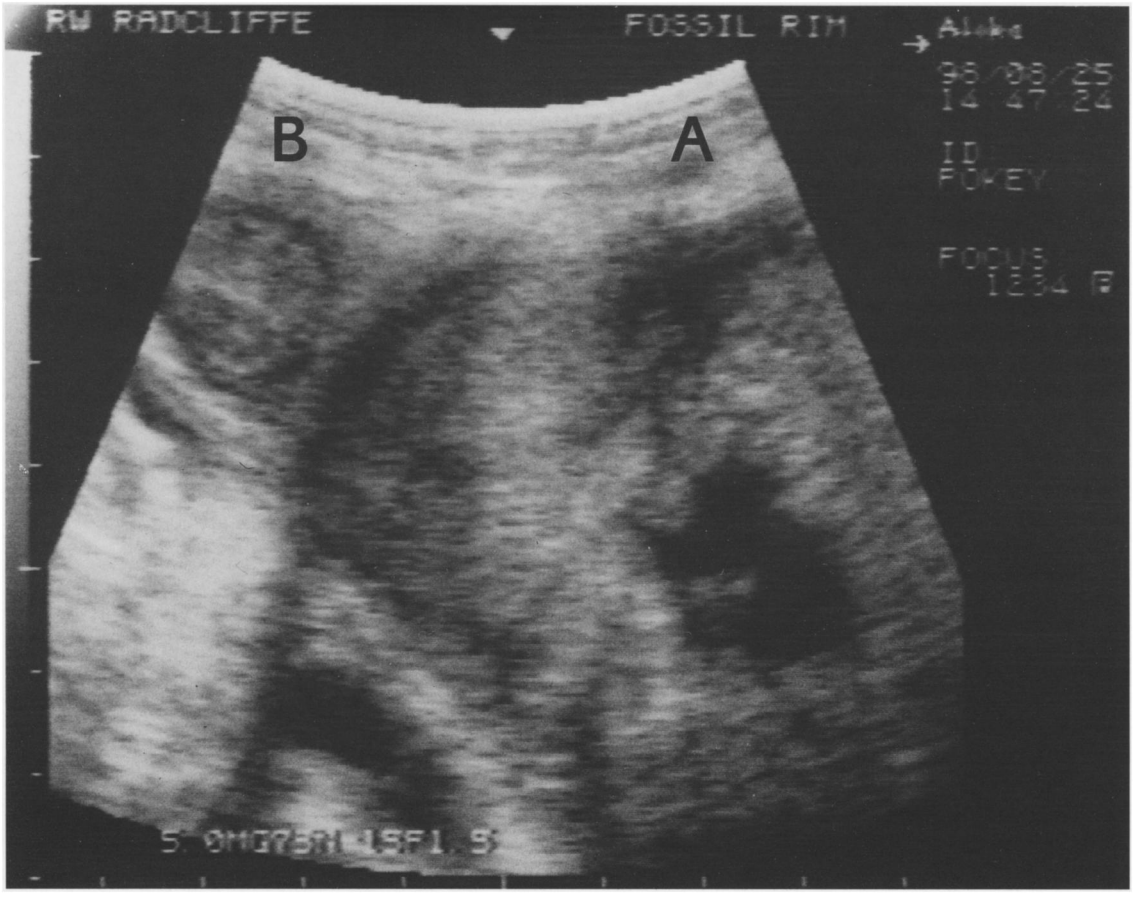

Figure 1. Transrectal ultrasound image of the right (**A**) and left (**B**) uterine horns of a southern white rhinoceros (*Ceratotherium simum simum*). Note the intrauterine fluid collection within the lumen of the right uterine horn and the enlargement of the uterine wall.

protocol was similar, although estradiol (15 mg) was given on two consecutive days after prostaglandin treatment. On the basis of endoscopy, cervical dilation was not achieved with either protocol. A third protocol involved intracervical placement of two prostaglandin E_2 pessaries (Cervidil, Forest Labs Inc., New York, New York 10022, USA; 10 mg) followed by immobilization 6 hr later to assess cervical relaxation. Local signs of relaxation and congestion were present, but access to the uterus was not possible because cervical dilation was incomplete. At this time, a decision was made to utilize laparoscopy to biopsy the uterine mass.

A balanced anesthetic technique, incorporating neuroleptanalgesia for sedation and visceral anesthesia combined with infiltrative local anesthesia, facilitated standing abdominal laparoscopy.[21] Standing sedation and analgesia were achieved with a combination of butorphanol tartrate (Torbugesic, Fort Dodge Animal Health, Fort Dodge, Iowa 50501, USA; 10 mg/ml, 120 mg i.m.) and azaperone (Stresnil, Janssen Pharmaceutica, Mississauga, Ontario L5N 5R9, Canada; 40 mg/ml, 160 mg i.m.). Because body weight was estimated in this animal, the dosage for butorphanol was therefore estimated to be 52–80 μg/kg and the dosage for azaperone was estimated to be 70–107 μg/kg. The animal showed signs of deep sedation, including decreased response to stimuli and head pressing, 30 min after the administration of this drug combination. Standing restraint was maintained with i.v. infusion of butorphanol at a rate that varied from 0.6 to 1.8 mg/min (100 mg butorphanol added to 1 L 0.9% NaCl delivered i.v. at 1–3 drops/sec via a 10-drop/ml infusion set) on the basis of patient response.[21] Respiratory rate and heart rate were monitored, as was percentage of oxygen saturation of hemoglobin (Spo_2) with a portable pulse-oximeter (Nellcor N-20PA, Vet-Sat Large Sensor, Nellcor Inc., Hayward, California 94545, USA) attached to the ear.[21] A large 4.0-cm-diameter rope

was placed in the inguinal region to stabilize the rhinoceros during induction of standing restraint and to prevent unexpected movement during the procedure. The rhinoceros remained standing for the duration of the procedure with occasional episodes of kneeling on its front limbs.

Prior to surgery, food and water were withheld for 72 hr and 12 hr, respectively, to reduce the likelihood of viscus organ perforation during trochar insertion. Ampicillin sodium (Amp-Equine, SmithKline Beecham, Exton, Pennsylvania 19341, USA; 60 g i.v.), amikacin sulfate (Amiglyde-V, Fort Dodge Animal Health; 250 mg/ml; 27 g i.v.), and ketoprofen (Ketofen, Fort Dodge Animal Health; 100 mg/ml; 6 g i.v.) were administered preoperatively. With standing sedation accomplished and the surgical site thoroughly cleaned and disinfected with 4% chlorhexidine gluconate (Hibiclens, Stuart Pharmaceuticals Inc., Wilmington, Delaware 19897, USA), a local anesthetic block for the first trochar placement was performed with 50 ml of 2% mepivicaine hydrochloride (Mepivicaine HCL, Steris Laboratories Inc., Phoenix, Arizona 85043, USA). The local anesthetic was injected with an 18-ga, 15.2-cm spinal needle placed through a 14-ga, 5.1-cm needle at the proposed site of trochar placement. After local anesthesia, a 2.5-cm vertical incision was made through the skin with a no. 22 blade 5.1 cm dorsal and 12.7 cm cranial to the point of the stifle (all references to stifle refer to the anteriormost aspect of the stifle skin fold; Fig. 2) for the first laparoscopic portal.

Four laparoscopic portals were established, three of which proved functional for the procedure. A 15-cm long, 12-mm dilating tip trochar (Endopath, Ethicon Endo-Surgery, 4545 Creek Road, Cincinnati, Ohio 45242-2839, USA) was inserted through the skin and body wall. A 54-cm-long, 10-mm-diameter, 30-degree equine laparoscope (Karl Storz Veterinary Endoscopy America, Inc., 175 Cremona Drive, Goleta, California 93117, USA) was inserted into the cannula after trochar placement. The peritoneal space had not been penetrated, and this trochar was removed. A second attempt at trocharization was made utilizing a 20-cm-long, 11-mm-diameter equine trochar with a pyramidal-shaped tip (Storz). The laparoscope was placed through the cannula, confirming again that the abdominal cavity had not been entered. This trochar cannula was also removed. A third attempt at trochar placement involved the use of a 11-mm-diameter, 50-cm-long trochar and cannula designed specifically for megavertebrates (J. Zuba and Storz). Use of this trochar facilitated penetration of the peritoneum and entrance into the abdominal cavity. The thickness of the body wall in this location, as measured by the trochar, was 23 cm. Several forceful attempts to advance the trochar were needed before the tough peritoneal membrane was penetrated.

After identification of the peritoneum and the serosal surface of the cecum, insufflation of the abdomen was initiated with two 15-L/min CO_2 insufflators (Storz). The tubing from one insufflator was placed directly into the end of the cannula where the scope inserts, and the other insufflator was attached to the insufflation adaptor. An intraabdominal pressure of 5–10 mm Hg was achieved over 15 min, providing increased visualization of abdominal viscera. Insufflation was continued through the cannula at portal 1 while a second portal was established 5.1 cm ventral to the first and 15.2 cm cranial to the stifle (Fig. 2) after local anesthesia and a 2.5-cm vertical skin incision as previously described. Difficulty was again encountered with penetration of the peritoneum as with the first laparoscopic portal. The 20-cm-long equine cannula with the 51-cm-long trochar advanced 5 mm beyond the cannula end was used for placement of the second portal. The large cecum, however, interfered with peritoneal membrane penetration, and this portal location was abandoned.

The third portal was located 6.4 cm dorsal to the first and 8.9 cm cranial to the stifle (Fig. 2). Local anesthesia was obtained again with 50 ml of 2% mepivicaine, and a 2.5-cm vertical skin incision was made. Successful penetration into the abdominal cavity was achieved after several forceful attempts with the 20-cm-long equine cannula and 51-cm-long trochar. The intraabdominal pressure reached 10–14 mm Hg with continuous flow from the two insufflators set at 20 mm Hg. Insufflation was maintained with a single insufflator after the initial distention of the abdomen. The equine laparoscope was inserted through this portal for examination of the abdominal cavity. The cecum, rectum, mesocolon, and the broad ligament of the right uterine horn were identified.

A fourth laparoscopic portal was created to facilitate the uterine biopsy via one laparoscopic portal (portal 1) and two instrument portals (portals 3 and 4). This portal was established 8.9 cm dorsal to the third and 6.4 cm cranial to the stifle (Fig. 2) after local anesthesia and a 2.5-cm vertical skin incision as previously described. The equine laparoscope and several instruments (acute claw grasper, Babcock forceps, mare uterine biopsy punch) were rotated between the functional portals 1, 3, and 4. The acute claw grasper and Babcock forceps were of insufficient length (45 cm) to facilitate manipulations within the rhinoceros abdomen. Also, at 20

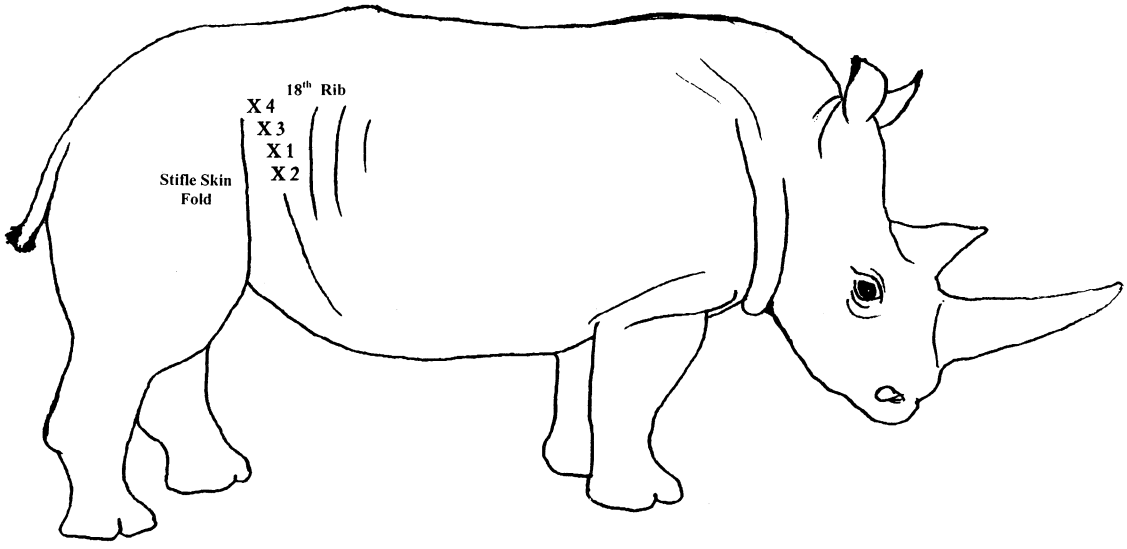

Figure 2. Schematic diagram of laparoscopic instrument portals (x = portal insertion sites) in a white rhinoceros (*Ceratotherium simum simum*). Each laparoscopic portal number (1–4) represents the sequence of trochar/cannula insertion into the rhinoceros. Note the limited space available for trochar placement in the rhinoceros.

cm in length, the equine cannulae were too short to penetrate into the peritoneal cavity; thus, with each change of instruments, reestablishment of the opening to the peritoneum was required.

The megavertebrate cannula (the only portal possible for insufflation because of its full penetration into the abdomen) bent during the latter part of the procedure, preventing access with instruments or the laparoscope but still allowing for continued insufflation. Because of the lack of instruments of appropriate length, visualization of the uterus was not possible until the uterus was elevated and directed toward the surgical site via transrectal manipulation. Through the two remaining instrument-accessible laparoscopic portals, the equine laparoscope and the mare uterine biopsy forceps (54 cm working length) were used together to identify the right horn of the uterus. Transrectal ultrasound guidance was used to help locate and visualize the abnormal uterine tissue (Fig. 1).

A biopsy of the enlarged uterus was accomplished only through a coordinated surgical team effort because of the insufficient length of the laparoscopic instrumentation. While one team member transrectally stabilized the right uterine horn against the body wall, laparoscopic visualization of the structure was achieved via portal 4, and a biopsy was obtained through portal 3. Because the large broad ligament of the uterus covered the serosal surface of the horn, the mare biopsy instrument was used to elevate the broad ligament prior to uterine biopsy. Three uterine biopsies were collected in this manner.

The four skin incisions were closed with no. 2 polydioxanone (PDS II, 5.0 metric, 70 cm on reversed cutting needle, Ethicon Inc., Somerville, New Jersey 08876-0151, USA) in a cruciate pattern placed split-thickness through the skin. No subcutaneous sutures were used in the closure of the four laparoscopic portals. At 3.5 hr after anesthetic induction, the rhinoceros was given naltrexone (Trexonil, Wildlife Pharmaceuticals, Fort Collins, Colorado 80524, USA; 50 mg/ml; 125 mg i.v., 125 mg i.m.) to antagonize the effects of the butorphanol. Recovery from anesthesia was uneventful.

DISCUSSION

Standing laparoscopy can be performed in the rhinoceros as it has been in other domestic animal species such as horses although with great difficulty, largely because of equipment limitations. With the development of innovative equipment and techniques, laparoscopy may become a valuable diagnostic and therapeutic technique for megavertebrates. Laparoscopy in very large animals presents problems compared with laparoscopy in smaller domestic and exotic animals, including anesthetic considerations, the need for specialized laparoscopic instrumentation, and variations in the surgical approach, technique, and closure.

The convoluted nature of the rhinoceros cervix[20] was primarily responsible for limiting a transcer-

vical biopsy approach, despite aggressive attempts to dilate it. A hormonally based cervical dilation protocol, previously successful in this species (Durrant, pers. comm., 1998), may have failed because of this rhinoceros's long period of acyclicity.

Laparoscopy is frequently utilized to diagnose and treat many diseases of domestic and exotic animals.[3,4,10,13,23] In zoologic medicine, it has been effective for evaluation of reproductive status, for direct visual biopsy of internal organs, for sex determination in birds, and as a surgical means of fertility control.[3,4] In horses, standing and recumbent techniques have facilitated diagnostic examination and biopsy, colic evaluation, ovariectomy, cryptorchidectomy, ruptured bladder repair, scrotal hernia repair, colopexy, and other procedures.[1,2,5–7,9,11,13–15,22–25,28,31] This procedure was based on equine standing techniques[5,7,9,13,14] with some important modifications.

The first major modification of the equine technique was in choice of anesthetic. A standing procedure was deemed essential for success because previous attempts by two of the authors (J. Zuba and G. Richardson) in recumbent white and Sumatran rhinoceros (*Dicerorhinus sumatrensis*) proved extremely difficult. Advantages of standing laparoscopy include elimination of general anesthesia and its associated risks, provision for maximal exposure and visualization of abdominal organs through small incisions, and a rapid return to function. In addition, laparoscopic surgery is relatively noninvasive and quick to perform, once the surgeon has acquired sufficient experience.[13] In horses, standing laparoscopy is generally performed with moderate sedation plus local and epidural anesthesia.[7,9,13,14] Epidural techniques were not utilized for this procedure because sedation and analgesia were adequate, and the risk of such potential complications as infection, recumbency, or unique anatomic considerations in a white rhinoceros were unknown. A neuroleptanalgesia/anesthetic combination incorporating butorphanol and azaperone has proven useful in white rhinoceros for medical procedures requiring a degree of restraint from standing sedation to full recumbency.[21]

In addition to the physical problems created for laparoscopy by recumbency in rhinoceros, recumbent large animals also have decreased functional residual lung capacity and are prone to dependent airway closure with resultant alveolar collapse, ventilation perfusion inequality, and hypoxemia.[12,18] Pulse-oximetry values remained stable throughout this procedure, with Spo_2 values ($\bar{x}$ = 89%; range = 83–92%) and respiratory rates considerably higher than have been reported with more potent narcotic agents in the white rhinoceros.[12,16,21]

A second area of modification in equine procedures for rhinoceros laparoscopy involved equipment. Most of the equipment available for standing laparoscopy in horses was utilized during this procedure.[13] A 300-watt xenon light source (Storz) provided adequate illumination. Although only one insufflator is routinely used in horses during laparoscopy,[13] the larger rhinoceros abdominal vault required a greater volume of CO_2 gas. Two insufflators were therefore used initially. The 10-mm-diameter, 54-cm-long, 30-degree rigid equine laparoscope (Storz) was sufficiently long to permit viewing of the uterus for biopsy. However, the 15-cm-long disposable trochar and cannula (Ethicon) and the 20-cm-long cannulae and trochars (Storz) commonly used to insert the telescope during flank surgery in horses[13] were too short to span the 23 cm between skin and peritoneum. In addition, the 30–45-cm-long instruments available for laparoscopic surgery in horses (acute claw grasper, Babcock forceps, serrated scissors, etc.) were also too short. The mare biopsy forceps, with a working length of 54 cm, was too short as well and could be used for biopsy of the uterine mass only after rectal manipulation of the uterus. Longer instruments with larger cusps are needed for biopsy collection in megavertebrates.

A megavertebrate laparoscope designed by Karl Storz Veterinary Endoscopy and the San Diego Wild Animal Park for use in elephants and rhinoceros was not needed. This 123-cm-long, 10-mm-diameter, 30-degree laparoscope was too long for easy manipulation, although it may be helpful during other rhinoceros procedures. The 51-cm-long sharp trochar and cannula provided with this laparoscope were essential, however, for penetrating the peritoneum and allowed for use of the 54-cm equine laparoscope and mare biopsy instrument. New instrumentation such as trocars, cannulas, forceps, and scissors designed specifically for megavertebrates are clearly needed before similar procedures are performed in rhinoceros.

The third modification of equine standing laparoscopic procedures for use in rhinoceros involved methods of approach, closure, and technique. Surgical approach was modified only slightly.[5,7–9,13,14] The thick, unyielding rhinoceros integument mandated use of 2.5-cm skin incisions, larger than 11-mm portals, to provide added mobility during instrument manipulation. The trochars were placed in the paralumbar fossa caudal to the 18th rib (Fig. 2). Because this fossa is small compared with that of horses (because of craniocaudal compression), limited space was available for placement. The transverse spinous processes dorsally, the 18th rib cra-

nially, and the stifle skin fold caudally bounded the fossa. The second attempt at inserting the trochar ended because of concerns that the cecum might be perforated inadvertently at this location. Trochar placement by a more dorsal approach was therefore deemed to be safer. Trochar placement in the rhinoceros was less safe than in horses, where insufflation with CO_2 is usually performed before trochar/cannula placement to reduce the risk of perforation of abdominal organs.[13] In this rhinoceros, the peritoneal cavity was perforated with a trochar/cannula before insufflation because of limitations in insufflation catheter equipment. This risky maneuver should be avoided in the future, especially considering the force needed for perforation. If a sufficiently long stylet becomes available, the trochar could be placed after insufflation, as it is in horses. Also, guarded trochars sufficiently long for megavertebrate abdominal perforation would be useful for trochar placement.

The placement of the trochar/cannula system required more force in the rhinoceros compared with horses, probably because of anatomic differences. The body wall is twice as thick in the rhinoceros as in the average adult horse. In addition, the peritoneum was extremely difficult to penetrate even with the sharp, pyramidal-shaped laparoscopic trochars. Previous attempts by two of the authors (J. Zuba and G. Richardson) to enter the abdominal cavity of recumbent rhinoceros and elephants failed partly because of this thick, fibroelastic peritoneum. Once the abdominal cavity was entered, however, rhinoceros and horse laparoscopies were similar[8,13] except that the larger rhinoceros body size required longer instrumentation. And, unlike in horses, the rhinoceros laparoscopic portals were closed with split-thickness epidermal skin sutures because of the thick integument.

The exact nature of the uterine mass was not identified. The biopsied tissue was consistent histologically with a leiomyoma, a benign nodular tumor of the myometrium.[29] However, such small samples from a leiomyoma cannot be differentiated from normal serosa and muscular tunic of the uterus. Neoplasia, including leiomyoma, has been reported in captive Rhinocerotidae.[17,19,27] A leiomyoma apparently caused the acute death of one rhinoceros after rupture of the uterine artery secondary to tumor invasion (J. Zuba, unpubl. data). In mares, leiomyomas are usually small (2.5–5.0 cm in diameter), solitary or multiple, and often pedunculated.[29,30] Large leiomyomas, as in this rhinoceros, are rare in horses. Uterine tumors in mares are generally excised through a celiotomy or vaginally, and successful breeding has occurred after removal of solitary leiomyomas.[29] Surgical removal of this rhinoceros's tumor was not attempted, but similar excisions might be possible in the future if the necessary specialized instrumentation is developed.

The rhinoceros recovered quickly, returning to normal levels of feeding and activity within 24 hr, and the laparoscopic portals appeared completely healed within 7 days. Although complications, such as infection, hemorrhage, perforation of viscera, damage to abdominal organs, and injury to abdominal wall vessels,[7,26] can occur during and after equine laparoscopy, none occurred in this case. Laparoscopy can be a useful tool in rhinoceros and other large species for a variety of diagnostic and surgical procedures provided the limitations of anesthesia, surgical equipment and instrumentation, and surgeon training are addressed.

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