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Endoscopic Orchiectomy and Endoscope Assisted Oophorectomy in Desert Tortoises (*Gopherus agassizii*)

REPORT

Materials and Methods:

Animals: A group of fifteen adult desert tortoises (*Gopherus agassizii*), seven males and eight females, owned by the U.S. Fish and Wildlife Service, and maintained at the Desert Tortoise Conservation Center in Las Vegas was used in a prospective study approved by the Animal Care and use Committee (IACUC No. A2010 11-549-Y3-A1) of the University of Georgia (UGA), College of Veterinary Medicine, Athens, Georgia, USA. Procedures were conducted at the UGA College of Veterinary Medicine facilities.

The tortoises were transported to UGA and temporarily maintained in conditions approved by the Association for Assessment and Accreditation of Laboratory Animal Care. They were housed individually in 50 gallons plastic containers (36"x 17"x 16") with hay as substrate. Room temperature was maintained at 26.6°C (80°F). Each animal

was provided with a basking heat and broad spectrum light source[1] during the day. The animals were exposed to a 12 hour light, 12 hour dark photoperiod.

The diet during the study consisted of daily soaked commercial tortoise pellets[2] and ad libitum water. Each tortoise was physically examined and accurate weights recorded on the day of arrival. The animals were numerically identified using tape attached to the carapace, and numbers written on the carapace using an indelible permanent marker pen. Each animal was bled from a jugular vein for hematocrit, and total solids, and acclimatized to the research facilities for seven days prior to anesthesia and surgery.

Anesthesia: The tortoises were simultaneously participating in a parallel anesthesia study with specific anesthesia data to be published separately. The animals were fasted for 48 to 72 hours prior to anesthesia, although access to water was maintained. They were bathed in shallow, lukewarm water for 2 hours prior to the procedure to stimulate urination. Manual stimulation of the cloaca was also performed to promote urination before anesthetic induction. Oxytetracycline (5 mg/kg IM), and meloxicam (0.2 mg/kg SC) were administered preoperatively (12 to 24 hours prior to surgery). The animals were physically restrained and induction was achieved using a combination of ketamine (10 mg/kg) and dexmedetomidine (50 μg/kg) administered intravenously into a jugular vein. Following induction each animal was placed in a vertical (head up) position with pressure on the site of injection to reduce hematoma formation. Animal #4 (female) received propofol (10 mg/kg) intravenously, instead of ketamine and dexmedetomidine.

The tortoises were randomly assigned to receive either a combination of lidocaine (2 mg/kg)[3] and morphine (0.1 mg/kg)[4], or an equivalent volume of saline control intrathecally after induction.

After aseptic preparation of the dorsal coccygeal region, each animal was positioned in dorsal recumbency, using a vacuum bean bag positioner[5], with the head down at approximately 45°. A mid to cranial intervertebral space was identified by palpation and the tail secured in position. A 23G primed butterfly catheter was used to inject the drugs or saline into the intrathecal space. The correct placement of the hypodermic needle in the intrathecal space was confirmed by aspiration of cerebrospinal fluid (CSF) prior to injection. If blood was aspirated the needle was removed and placed again, until a clean sample of CSF was acquired.

Following intrathecal injection catheterization of the jugular vein using a 24G intravenous catheter was attempted. If successful, 3 ml/kg/hour of lactated ringers solution (LRS) was administered intravenously. If unsuccessful, one dose of 25 ml/kg of LRS (daily maintenance) was administered subcutaneously.

Each animal was intubated using an appropriately size uncuffed endotracheal tube secured using a plastic mouth gag[6] and taped to the head. The animals were maintained on 100% oxygen for the duration of the procedure. Each tortoise was ventilated using a positive-pressure ventilator[7] at 2 breaths per minute and at a peak inspiratory pressure of 4-10 mmHg. If a surgical plan of anesthesia was not achieved, isoflurane was administered in oxygen, and adjusted to patient requirements.

Pulse was monitored with a Doppler ultrasonic flow detector[8] placed over the carotid artery. A multiparametric monitor[9] was used to monitor the end-tidal partial pressure of CO_2 ($ETCO_2$), esophageal temperature, and ECG.

Endoscopic Equipment and Sterilization: The 2.7 mm (wide view) 30°, 18 cm, telescope[10], endoscopic camera[10], two monitors[10], xenon light source[10], imaging capture system[10], Vitom system®[10], and 3 mm endoscopic instrumentation[10] were used.

All equipment was cleaned using an enzymatic detergent[11], and gas sterilized using hydrogen peroxide. If multiple surgeries were performed on the same day the equipment was cold sterilized using 2% glutaraldehyde[12] for 30 minutes, and rinsed with sterile water before repeated use.

Prefemoral Coeliotomy: Each tortoise was placed on the surgery table on a heated water mattress[13] maintained at 28-30°C. Each animal was positioned in lateral recumbency using a vacuum bean bag positioner[5]. Additional elevation of the head above the horizontal was undertaken to reduce the cranial movement of intrathecal medication. The hindlimbs were extended caudally and taped to each other to allow exposure of both prefemoral fossae. The prefemoral fossa and surrounding shell were aseptically prepared and draped using standard techniques.

A 5 - 6 cm craniocaudal skin incision was made in the cranial prefemoral fossa using a #15 scalpel blade. The subcutaneous tissues were bluntly dissected and the coelomic membrane (formed by the aponeurosis of the tendinosis parts of the ventral and oblique abdominal muscles) identified. A 3 mm incision was made in the coelomic

membrane to permit insertion of the telescope[10] and confirmation of correct coeliotomy approach.

The coelomic membrane incision was carefully extended, taking care not to damage the often voluminous and closely associated bladder, nor the septum horizontale (post-pulmonary septum) or lung. A ring and elastic stay retractor[14] was positioned to provide improved exposure of the prefemoral incision. The use of a single prefemoral coeliotomy incision permitted the use of multiple instruments without the need for additional cannulae.

Endoscopic Orchiectomy: Endoscopic orchiectomy required bilateral prefemoral approaches, starting with the left prefemoral approach (right lateral recumbency).

Following prefemoral coeliotomy, endoscopic examination of the coelomic cavity, and identification of the left testis, the Vitom system® was used to secure the telescope in place. Alternatively, an assistant could be employed to hold the telescope. The optimal Vitom®-telescope positioning was achieved when the telescope was positioned perpendicular to the table, in the mid-dorsal region of the incision.

The testis was grasped at the cranial aspect using 3mm atraumatic endoscopic forceps with ratchet handle[10], inserted alongside the telescope. The left hand of the surgeon (non-dominant), was used to secure and elevate the testis to expose the mesorchium and associated blood vessels.

In all the animals a combination of 3mm short curved Metzenbaum endoscopic scissors[10], connected to a radiosurgery unit[15] (monopolar), 3mm bipolar endoscopic forceps[10], medium or large stainless steel vascular clips[16], with curved or straight hemoclip applicators[17], were used as necessary to ligate and transect the blood vessels

and the mesorchium. The surgeon's right hand (dominant) was used to manipulate these instruments and transect the mesorchium.

Stainless steel vascular clips were applied when large blood vessels were encountered, especially at the cranial, middle, and caudal aspects of the mesorchium. When used, the vascular clip was applied as close as possible to the kidney, and the mesorchium transected distal to the clip, as close as possible to the testis. Special care was taken not to leave any testicular tissue behind. The size of the clips and shape of the applicator (curved or straight) were chosen based on the size of the vessel and position of the testis in relation to the incision, respectively.

Bipolar endoscopic forceps were used when large vessels were not present and the space between the testicle and kidney was considered too small for the use of monopolar scissors[10] that create greater collateral tissue damage. Following the use of the bipolar forceps, the 3 mm Metzenbaum endoscopic scissors[10] were used to transect the cauterized mesorchium. Where there was sufficient space between kidney and testis, the Metzenbaum endoscopic scissor[10] was used as a monopolar device to both effect hemostasis and transection of the mesorchium. The radiosurgery unit was set to cut/coagulation with power settings varied from 10 to 45.

Particular care and attention were taken to position the testis as far away as possible from the colon, urinary bladder, kidneys and adrenal gland. As the mesorchial transection proceeded in a cranial to caudal direction, the grip on the testis was adjusted to permit better exposure of the remaining mesorchium. After complete removal of the right testis, the coelomic cavity was inspected to verify hemostasis. Visualization of the contralateral (right) testis, through the left pre-femoral approach was consistently

impossible, necessitating a bilateral procedure. The left prefemoral coeliotomy was closed in a routine manner. Absorbable suture, 3-0 Monocryl-Plus, on a tapered needle in a simple continuous pattern was used to close the coelomic aponeurosis and associated subcutaneous tissues in a single layer. The skin was closed using 3-0 Monocryl-Plus on a cutting needle using a simple horizontal mattress pattern.

The tortoise was then repositioned in left lateral recumbency for the procedure to be repeated via the right prefemoral fossa. Rather than moving the endoscopy tower around the surgery table, a second, slave monitor was placed on the opposite side of the table and used for the right orchiectomy.

Endoscope Assisted Oophorectomy: Unilateral or bilateral prefemoral coelioscopy was performed in order to accomplish bilateral oophorectomy. Whenever possible both ovaries were removed via a unilateral left prefemoral approach. Each animal was first placed in right lateral recumbency and a left pre-femoral coeliotomy performed, as previously described for males. If the initial entry was confirmed as correct, the incision was extended, and the ring retractor applied[14]. However, the animal was then placed in dorsal recumbency before continuing with oophorectomy.

The left ovary consisting of variably sized follicles was identified using the telescope. Atraumatic forceps were used to grasp the interfollicular tissue (not a round follicle), and gently exteriorize the gonad. Once the ovary was elevated to the level of the prefemoral incision, manual manipulation was used to exteriorize the organ. Care was taken to assure that the entire left ovary was exteriorized from the cavity.

The mesovarial vasculature was identified and ligated using medium to large stainless steel vascular clips as necessary. The left ovary was transected, distal to the

clips, using Metzenbaum scissors and removed before inspection of the coelom to verify hemostasis. The right ovary was visualized using the telescope via the same left pre-femoral incision. If the right gonad was visible and accessible it was removed via the left prefemoral incision, as described above. If the gonad could not be visualized or could not be exteriorized via the left prefemoral incision, the surgical site was closed as described above, and a right prefemoral approach and right oophorectomy was undertaken using the same techniques described above. After complete resection of both ovaries, the coelomic cavity was inspected to verify hemostasis, before routine closure of the right prefemoral fossa.

Postoperative Care: All tortoises received postoperative hydromorphone (0.5 mg/kg IM) for pain management, and 25 ml/kg of subcutaneous LRS. If present, the intravenous catheters was removed once recovered and ambulatory.

The animals were kept in recovery holding pens, without substrate, food or water, for the first 12-24 hours, until fully recovered. Each animal was given additional doses of oxytetracycline (5 mg/kg, IM) and meloxicam (0.2 mg/kg, SC) daily for three days. Incisions were inspected daily for the duration of their hospitalization at UGA (6-16 days post surgery).

Footnotes:

1. Heating lamp, Tin-Yi Metal Manufacturing, Taiwan
2. Mazuri Tortoise Pellets, Mazuri PMI Nutrition International, St. Louis, MO
3. Lidocaine 4%, Hospira Inc., Lake Forest, IL
4. Morphine 15mg/ml, West-ward, Eatontown, NJ

5. Vacuum Bin Bag Positioner, Natus Medical Inc., San Carlos, CA
6. Plastic mouth gag, PetAg, Hampshire, IL
7. Positive-pressure ventilator, Bioanalytical Systems, Inc., West Lafayette, IN
8. Doppler, Parks Medical Electronics, Aloha, OR
9. Multiparametric monitor, Digicare Biomedical Technology, Inc., Boynton Beach, FL
10. Endoscopy equipment, Karl Storz, Tuttlingen, BW, Germany
11. Enzymatic detergent, Getinge/Castle, Inc., Rochester, NY
12. Cidex, Ethicon, Irvine, CA
13. Heated water mattress, Gaymar Industries, Inc., Orchard Park, NY
14. Lone star retractor, Cooper Surgical, Trumbull, CT
15. Radiosurgery unit, Ellman International, Inc., Oceanside, NY
16. Vascular clips, Ethicon Endosurgery LLC, Guaynabo, Puerto Rico, USA
17. Hemoclips, Teleflex Medical, Research Triangle Park, NY