A novel orchiectomy surgical procedure in donkeys (*Equus asinus africanus*) with parascrotal access

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Abstract

Donkeys are a public health concern in the Northeast region of Brazil, with thousands of stray animals. Orchiectomy is an important population control measure; however, the long postoperative period with daily treatment of open wounds in the scrotum makes it difficult to perform a large number of castrations in sheltering centers. We evaluate a novel surgical procedure for orchiectomy in donkeys using parascrotal access. Twelve donkeys were used, divided into two groups: I - submitted to orchiectomy through parascrotal surgical access (novel procedure), and II - submitted to orchiectomy through scrotal access (conventional). Postoperative evaluations consisted of a macroscopic evaluation of the surgical wound (bleeding and intensity of edema), hematological parameters, and peritoneal fluid, which occurred in both groups at the moments (M): M0 - before the surgical procedure. The others moments occurred after surgery: M12 (twelve hours); M24 (twenty-four hours); M48 (forty-eight hours); M72 (seventy-two hours); M8D (eight days); and M16D (sixteen days). The surgical techniques did not generate an important systemic inflammatory response to the point detected by the leukogram, fibrinogen dosage, and peritoneal fluid. The parascrotal technique required long surgery but promoted less bleeding, less edema, and faster healing. The techniques used did not promote sufficient systemic inflammation to alter the number of leukocytes and the fibrinogen concentration; however, evaluation of the peritoneal fluid proved to be important for evaluating inflammatory processes involving the scrotum and inguinal canal. We describe a novel surgical procedure for orchiectomy in Donkeys using a parascrotal access that promoted less risk of bleeding, shorter period of edema, and healing time, but required longer surgery time.

Key words: inflammation, donkeys, peritoneal fluid, surgical approach, castration

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Introduction

Brazil has a large population of donkeys, mules, and hinnies, with almost 1 million heads (376 thousand donkeys and 615 thousand mules and hinnies), with most of this population located in the Northeast region (58%) (IBGE 2017). Donkeys wandering on the roads are a serious risk, and northeastern states Transit Department teams have traveled several kilometers to capture these animals. Captured animals can be returned to their owners, but in abandonment cases, they are taken to properties supported by the State or NGOs and are sheltered there (Salles et al. 2013). Donkeys being cared for by charities and poor welfare conditions are also reported worldwide (Fernandez et al. 2021).

Orchiectomy emerges as the primary method to perform population control, especially in places where there are large agglomerations of these animals, and it is essential to prevent fights among males, undesirable copulation, and to promote easy handling since castrated donkeys can be placed with females (Moura Alonso et al. 2021, Trindade et al. 2021). It also contributes to the genetic improvement of animals, removing sexual activity from those who are no longer fit.

Among existing complications in castration, the tissues involved undergo surgical trauma, and the physiologically generated inflammatory response consists in a complex event, involving and promoting interactions among numerous inflammatory, hormonal, immunological, and metabolic mediators. The ultimate objective of these mechanisms is to adapt the organism to the traumatized tissues and assist it in the healing process (Di Filippo et al. 2014).

The main complications of orchiectomy are abdominal abscess (Carvalho et al. 2017), scrotal edema, infection, hemorrhage, colic, eventration or eversion, peritonitis, penile trauma, and persistent sexual behavior (Owens et al. 2018, Rosanowski et al. 2018). Another problem in equine castration is the postoperative time, which requires a prolonged period of surgical wound treatment, of around 15 to 28 days (Finger et al. 2011). Orchiectomy also induces a significant degree of pain, negatively associated with equids’ well-being (Owens et al. 2018, Rosanowski et al. 2018). Orchiectomy also induces a significant degree of pain, negatively associated with equids’ well-being (Owens et al. 2018, Rosanowski et al. 2018).

Although less invasive techniques are available, such as laparoscopic procedures, standing laparoscopic vasectomy, or a combination of laparoscopic and conventional techniques, the equipment required can be limiting, especially when dealing with stray donkeys treated by a public agency or NGO (Rijkenhuizen and van der Harst 2017, Vitoria et al. 2019).

In addition to the complications in orchiectomy, the postoperative period, the daily treatment of the surgical wound opened in the scrotum, with internal cleaning, and the healing time are factors that make it difficult to perform large quantities of castrations in sheltering centers. A single dressing on the suture line and the shortest healing time can be important factors in choosing the technique to be performed when aiming at castration for many animals. The above-mentioned factors led this study to evaluate a novel surgical procedure for orchiectomy in donkeys (Equus asinus africanus) using parascrotal access.

Materials and Methods

This study was approved by the Animal Ethics Committee (CEUA) of UFERSA, according to report 18/2019. Twelve male donkeys (Equus asinus africanus), weighing 133.9±11.34 kg, aged 5.5±3.67 years old, were used. The animals underwent clinical examinations, blood count, parasitological, equine infectious anemia diagnosis exam, vaccination against rabies, and were dewormed using fenbendazole (7.5 mg.kg⁻¹, Fenzol Pasta®, Agener União, Brazil) and ethanol 70% (Rialcool 70°, Rioquímica, Brazil).

Two groups of six donkeys were randomly composed. In both groups, the open orchiectomy technique was performed. Group I (GI) had the proposed approach applied – parascrotal access, and in group II (GII), conventional (scrotal) access. Both groups were submitted to the same antisepsis, anesthesia, and postoperative protocol. The animals were kept supine, and trichotomies were made in the region and adjacencies of the testicles. Antisepsis was performed with iodo-povidone degemant (Riodeine®, Rioquimica, Brazil) and ethanol 70% (Rialcool 70°, Rioquimica, Brazil).

Both jugular veins were catheterized for better control of drugs infusion. Both groups received the same anesthetic protocol. The pre-anesthetic medication was detomidine (Dormiun V®, Agener União, Brazil) 1% in an intravenous (IV) dose of 0.02 mg/kg. After 15 minutes, the induction was made associated with 10% ketamine at a dose of 2 mg/kg and 0.5% diazepam at a dose of 0.05 mg/kg, both by IV, but in separated syringes. Anesthetic maintenance was carried out with a continuous “triple drip” infusion, combining detomidine (0.02 mg/kg) with ketamine (2 mg/kg) and glyceryl guaiacol ether (GGE) 5% (100 mg/kg), at the speed of 2 mL/kg/h. The intratesticular local block was made continuously with 4 mg/kg of lidocaine (Xylestesin 2%®, Cristália, Brazil) in each testicle.

In GI, parascrotal surgical access was performed (Fig. 1). At first, a lateral region of the scrotum was identified, about 10 to 12 cm from the midline thereof. The testicle was moved to that region; an incision was made in the skin (around 5 cm) and then in the tunica vaginalis. The testicle and spermatic funicular were...
exposed. With the aid of a Kelly’s hemostatic forceps, the epididymis ligament tail was broken. The funicular was then transfixed and ligated with polyglycolic acid thread number 1. Hemostasis was also performed with the emasculator for five minutes. Vaginal tunic raffia was made simply, continuously, with polyglycolic acid thread number 1. Dermorraphy was made with the same thread in a simple continuous pattern. The same procedures were performed in the contralateral testicle.

Scrotal surgical access was made in GII according to the modified surgical technique of Hendrickson and Baird (2013). The funiculus transfixion and ligation were carried out with polyglycolic acid thread number 1, and vaginal tunic raffia was made with the same thread in a simple continuous pattern. The surgical wound was left open to heal by second intention.

The postoperative period consisted of antibiotic therapy, using the association between penicillin procaine, potassium, and streptomycin (Agrosil®, Vans, Brazil) in an intramuscular dose of 22,000 UI/kg every 48 hours, totaling three applications. Sodium dipyrone (D-500® Fort Dodge, Brazil), 25 mg/kg, IV, was used every 24 hours for five days for analgesic control. If the animal showed evident clinical signs of pain (looking at the flank, restlessness, ears turned back), tramadol hydrochloride (Tramadol®, Teuto, Brazil) 2 mg/kg, IV, was applied as a rescue. After surgery, all animals received anti-tetanus serum (Vencosat®, Vencofarma, Brazil) subcutaneously (5,000 IU/animal). Daily dressings and walking with all animals were performed for 30 minutes. The dressing consisted of applying physiological solution cleaning and repellent spray (Organnact® Prata em Spray, Curitiba/PR, Brazil) in both groups. The suture was removed from GI animals on the tenth postoperative day.

Animal evaluations took place at baseline, just before the surgical procedure (M0), 12 hours after surgery (M12), 24 hours after surgery (M24), 48 hours after (M48), 72 hours (M72), eight days (M8D), and 16 days after surgical procedures (M16D).

For macroscopic evaluation of the surgical wound, scores adapted from Finger et al. (2011) were followed for the bleeding degrees of the surgical wound (Table 1). The edema degrees for the foreskin and scrotum were classified as absent (0), mild (1), moderate (2), and severe (3).

The evaluations were performed by the same qualified professional. The zero moment in macroscopic
wound evaluation occurred immediately after the animal got up from anesthesia, differently from M0 in blood collection before the surgical procedure. Whole blood was collected from each animal through venipuncture for hematological response evaluation. The samples were distributed in tubes with and without anticoagulants. From blood collected with ethylenediaminetetraacetic acid (EDTA), leukocytes blood count and fibrinogen dosage were determined. Hematocrit measurement was obtained in a microhematocrit centrifuge, using 75 mm capillary tubes, where the samples were centrifuged at 10,000 rpm for 10 minutes and, later, read in a microhematocrit table (Keer 2003). For red and white blood cells counts we used the macrodilution technique with reading in a Newbauer chamber (Barros et al. 2021). Fibrinogen was measured using the precipitation method at 56°C in microhematocrit capillaries, calculating the difference between protein concentrations in plasma (Schalm 1970).

For the peritoneal fluid evaluation, animals were contained in a cattle crush and kept in a quadrupedal position. Extensive trichotomy and antisepsis were performed from the xiphoid region to the umbilical scar. The collection was carried out in the most ventral portion of the abdomen, with the aid of a 40x12 mm needle, slowly introduced into the skin at an inclination close to 90° in the midline region until the fluid was obtained (Louro et al. 2006). The samples were collected in tubes with and without EDTA.

The macroscopic evaluation of peritoneal fluid was performed after sample homogenization, observing the color and the degree of turbidity. The coloring was classified as 1: colorless, 2: yellow, 3: orange, and 4: red. The degree of turbidity was 1: clear, 2: semi-cloudy or slightly cloudy, and 3: cloudy (Lhamas et al., 2014).

Peritoneal fluid pH was measured using a dry chemical method in reagent strips (Uri-Color Check - Wama Diagnóstica®) and the density was measured using a refractometer. Total protein concentrations were obtained using the Biuret method with the aid of a diagnostic reagents set (Labtest®) and spectrophotometric readings (E-225-D, Labquest - CELM®) according to the manufacturer’s recommendations and standard laboratory practices (Ortolani et al. 2020).

Data obtained during the experimental period were analyzed for their homogeneity and for their distribution using the Kolmogorov-Smirnov test. The normal distribution data were subjected to variance analysis (F test). Tukey’s range test was performed for variable comparison between the baseline and the other moments.

Table 1. Surgical wound bleeding degrees in the groups of parascrotal and scrotal approaches to orchiectomy in donkeys (*Equus asinus africanus*) for evaluating macroscopic inflammatory response.

<table>
<thead>
<tr>
<th>Bleeding degree</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No bleeding</td>
</tr>
<tr>
<td>1</td>
<td>Discreet bleeding with few drops of blood</td>
</tr>
<tr>
<td>2</td>
<td>Moderate bleeding, where the drip lasted up to an hour after the procedure was completed</td>
</tr>
<tr>
<td>3</td>
<td>Constant bleeding that lasted between one and three hours</td>
</tr>
<tr>
<td>4</td>
<td>Intense bleeding for more than three hours, which required surgical reintervention</td>
</tr>
</tbody>
</table>

Adapted from Finger et al. (Finger et al. 2011b).

Table 2. Values of bleeding degree according to groups and moments (M) for assessing the macroscopic inflammatory response of donkeys submitted to orchiectomy using parascrotal (GI) or scrotal (GII) access.

<table>
<thead>
<tr>
<th>Animal</th>
<th>GI M0</th>
<th>GI M12</th>
<th>GI M24</th>
<th>GI M48</th>
<th>GI M72</th>
<th>GI M8D</th>
<th>GI M16D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Bleeding scores 0: absent, 1: discreet bleeding, 2: moderate, 3: constant, 4: intense.
Results

The average time in GI surgical procedure was 31 minutes, longer than GII time, which was 19 minutes (p<0.05). This difference can be attributed to the fact that in the parascrotal technique (GI), skin suture was performed on both sides, and this factor favored an increase in GI surgery time.

Table 2 presents the bleeding degree data, according to group and moment. In the macroscopic evaluation of surgical wounds, no animals in GI showed bleeding after the procedure, whereas animals in the GII group showed bleeding at some evaluation times (57.14%) (p<0.05).

Table 3 presents the results of edema scores in the scrotal region and foreskin. In GI and GII, animals showed edema in 66.7% and 90.5% of the evaluation times, respectively (p<0.05). Only four animals in GI showed mild edema at M8D, and none showed this alteration at the end of 16 days of study. In GII, all animals showed edema at M8D, of which half of them still exhibited this alteration in a moderated way and mild edema at M16D. Therefore, the parascrotal technique contributed to a faster resolution of this clinical sign.

Table 4 presents the hematological analysis of the animals during the study. The values of Hematocrit (HCT) and red blood count (RBC) did not differ between the groups (p>0.05). At GII, a difference was observed between moments, reducing the RBC and HCT.

Table 5 presents the peritoneal fluid analysis. The peritoneal fluid staining in GI and GII showed an increase in the color score from M12 to M72 in GI
and M8D in GII (p<0.05). There was an increase in total protein values in both groups at M24 (p<0.05). There was no difference between groups and times in turbidity and density values (p>0.05).

Table 5. Means and standard deviations of peritoneal fluid in scores of color, turbidity degree, pH, density, total protein (g/dL), red blood cell count (x10⁶), and leukocyte count (x10³) of donkeys submitted to orchiectomy using paraescrotal (GI) or scrotal (GII) access.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Moments</th>
<th>M0</th>
<th>M12</th>
<th>M24</th>
<th>M48</th>
<th>M72</th>
<th>M8D</th>
<th>M16D</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>Color</td>
<td>1.0±0</td>
<td>3.6±0.5</td>
<td>3.3±0.8ab</td>
<td>3.3±1.2ab</td>
<td>3.0±1.5ac</td>
<td>1.5±1.2bcd</td>
<td>1.17±0.4cd</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.6±1.0</td>
<td>2.3±0.5</td>
<td>2.1±0.4</td>
<td>1.8±0.4</td>
<td>2.3±0.8</td>
<td>2.5±0.8</td>
<td>2.6±0.8</td>
</tr>
<tr>
<td>GII</td>
<td>Turbidity</td>
<td>1.6±1.0</td>
<td>2.1±0.4</td>
<td>2.0±0</td>
<td>2.1±0.4</td>
<td>2.4±0.5</td>
<td>2.8±0.4</td>
<td>2.1±0.9</td>
</tr>
<tr>
<td>GI</td>
<td>pH</td>
<td>7.76±0.16ab</td>
<td>7.49±0.17b</td>
<td>7.47±0.16b</td>
<td>7.77±0.15ab</td>
<td>7.80±0.29ab</td>
<td>7.96±0.35a</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.71±0.24ab</td>
<td>7.53±0.17b</td>
<td>7.74±0.25b</td>
<td>7.45±0.20b</td>
<td>7.63±0.16b</td>
<td>7.94±0.14a</td>
<td></td>
</tr>
<tr>
<td>GII</td>
<td>Density</td>
<td>0.50±0.37b</td>
<td>2.27±1.52c</td>
<td>2.62±2.03c</td>
<td>2.13±0.63b</td>
<td>1.88±0.46b</td>
<td>1.11±0.41b</td>
<td>1.03±0.39b</td>
</tr>
<tr>
<td>GI</td>
<td>Total proteins (g/dL)</td>
<td>9.50±0.37b</td>
<td>2.27±1.52c</td>
<td>2.62±2.03c</td>
<td>2.13±0.63b</td>
<td>1.88±0.46b</td>
<td>1.11±0.41b</td>
<td>1.03±0.39b</td>
</tr>
<tr>
<td>GII</td>
<td>Red blood cells (x10⁶/UL)</td>
<td>0.50±0.37b</td>
<td>2.27±1.52c</td>
<td>2.62±2.03c</td>
<td>2.13±0.63b</td>
<td>1.88±0.46b</td>
<td>1.11±0.41b</td>
<td>1.03±0.39b</td>
</tr>
<tr>
<td>GI</td>
<td>Leucocytes (x10³/µL)</td>
<td>35.0±83a</td>
<td>9283.3±8179a</td>
<td>4441±3444ab</td>
<td>1241±990b</td>
<td>1283.3±942b</td>
<td>1275±1491b</td>
<td>666.6±524b</td>
</tr>
<tr>
<td>GII</td>
<td></td>
<td>333.3±1914c</td>
<td>9508.3±6271b</td>
<td>7616±5262a</td>
<td>2958±2121c</td>
<td>3160.0±3938bc</td>
<td>1790±2133bc</td>
<td>1250±2083bc</td>
</tr>
</tbody>
</table>

Means followed by different lowercase letters on the line differ by Tukey’s test (p<0.05). Means accompanied by different uppercase letters in the column differ according to the analysis of variance (p<0.05).

Discussion

According to (Kilcoyne et al. 2013), bleeding is the most immediate complication in the surgical procedure of orchiectomy, and the large scrotal vessels may be responsible for this hemorrhage after the procedure. In this study, ligation of the spermatic funicle and vaginal tunica was made in the same way in both techniques. Thus, it is believed that the incision site, with more delicate skin and fewer caliber vessels and dermorrhaphy after orchiectomy in the paraescrotal technique (GI), is responsible for the absence of bleeding in this group.

In a study with horses, (Kilcoyne et al. 2013) it was noted that post-castration edema and inflammation are common findings, especially in the preputial and scrotal region, and occur due to inadequate drainage and tissue trauma. Moreover, according to them, the peak of these clinical signs is usually three to four days after surgery, with disappearance in 10 to 12 days. In contrast, Finger et al. (2011) stated that edema disappearance time is seven to 21 days. In the present study, the first three days proved to be of the greatest intensity for these clinical signs. However, regarding edema disappearance, our results corroborate the findings of Finger et al. (2011), as these signs were still observed after 16 days of the procedure in the scrotal technique (GII).

Surgical wound healing in GI animals was observed on the eighth day, while the GII animals were delayed between 13 and 16 days. The reason is that in GII, the surgical wounds were healing by second intention. Bacterial contamination can delay the healing process (Mueller et al. 2012). In this study, suture and surgical wound location in GI probably contributed to decreasing wound bacterial contamination and accelerating the healing process.

Hematocrit is normally used to measure blood loss (Goodrich and Behling-Kelly 2019). The observed decrease in Hematocrit value associated with bleeding shows that the technique used in GII can cause significant blood loss. Although there was a reduction in the Hematocrit and number of red blood cells, the values remained within the reference values for the species.
Leukocyte and fibrinogen values are important for assessing animal immune response to an inflammatory and/or infectious process (Goodrich and Behling-Kelly 2019). Regarding fibrinogen values, there was no difference between groups and times, while for the number of leukocytes, higher values were observed in GI in M16D, but the values were within the normal range for the species. The inflammatory process triggered by surgical techniques used was insufficient to cause significant changes in these variables.

The evaluation of peritoneal fluid values was performed to establish a relationship of the extent to which orchiectomy performed by two different approaches interferes in this fluid since, in anatomical terms, there is continuity between the cavities. According to Barros et al. (2018), the peritoneal fluid color is related to blood in the abdominal cavity. Blood contamination is responsible for yellow, orange, and red stains, observed mainly shortly after the animals had undergone surgery and returning to normal color as they recovered (Mendes et al. 2000). Horses under normal conditions have a peritoneal fluid with a straw yellow color, varying from bright to opalescent (Mendes et al. 2000), contrasting with this study which found colorless liquid at M0.

In this study, the samples were collected and analyzed immediately before the surgery (orchiectomy) and at later times; therefore, slightly cloudy and cloudy samples were also observed in the moments after surgery. Meanwhile, samples considered within the species’ normal range should be presented transparently or clearly at M0, M8D, and M16D.

These data corroborate the work of Di Filippo et al. (2009), in which the peritoneal fluid color, considered within equine standards, ranged from straw yellow to a colorless, limpid appearance, and absence of sediment, demonstrating integrity in the vascular endothelium and peritoneum.

GII had a lower pH value at M12 and M72 compared to GI. At all times, the pH values were higher than those determined by Mendes et al. (2000), who found values between 7.25 and 7.4.

Total proteins increased at M24 in comparison to M0 in both groups. The increased level of these proteins in the peritoneal fluid may indicate inflammation since, via the inguinal canal, the scrotal cavity is close to the abdominal cavity, allowing direct communication and, therefore, secondary peritonitis following orchiectomy can occur (Di Filippo et al. 2016).

There was no difference between groups for the number of erythrocytes in the peritoneal fluid; however, in GII, there was an increase in the number of red blood cells and leukocytes at M12. Erythrocyte increment can be attributed to inflammation due to the surgical procedure. According to Lhamas et al. (2014), peritoneal fluid, when serosanguineous, indicates erythrocyte or hemoglobin presence, which can be caused by a skin vessel, laceration, or contamination. This agrees with the present study, which presented red coloration of peritoneal fluid and the presence of erythrocytes at the same time at some moments.

Although an increase in the number of leukocytes, red blood cells, and total peritoneal fluid protein was observed in both groups, these changes did not reflect an increase in turbidity. The increase in turbidity is related to blood contamination and the number of erythrocytes present in the peritoneal fluid; it is also closely linked to an increase in nucleated cells and a high amounts of protein in the collected sample (Barros et al. 2018). In the study by Louro et al. (2006), where they evaluated the peritoneal fluid in healthy donkeys, they found a variation between slightly cloudy and cloudy and stated that it differs from horses, as in these animals, slightly cloudy is considered normal.

**Conclusions**

The proposed parascutal technique required a longer surgery time but promoted lower bleeding risk, shorter edema period, and shorter healing time. The applied techniques did not generate an important systemic inflammatory response detected by leukogram and fibrinogen measurement. Peritoneal fluid was found to be important in the evaluation of acute inflammatory response involving the scrotum and/or inguinal canal caused by surgical procedure trauma.

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