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The sheep as a model for healing studies after partial nephrectomy



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ABSTRACT

Background: The pig has been considered the best model for renal surgery. However, recent research has demonstrated that the kidney of pigs heals differently from that of humans. The objective of this study was to evaluate sheep as an alternative animal model for studying collecting system healing after laparoscopic partial nephrectomy.

Materials and methods: The caudal pole of the left kidney was removed from eight female adult domestic sheep using laparoscopic partial nephrectomy. Monopolar energy was used for hemostasis only in the parenchyma, avoiding coagulation near the collecting system, which was left opened. After 14 d, all animals were euthanized, and their left kidney was removed. Serum levels of urea and creatinine were assessed preoperatively and post-operatively (on days 2, 6, 10, and 14), and peritoneal fluid samples were collected during necropsy for urea and creatinine evaluation. An *ex vivo* retrograde pyelogram was performed, and a retrograde injection of methylene blue ink was administered to evaluate urinary leakage. Samples from the operated pole were analyzed using histologic methods.

Results: During necropsy, an urinoma surrounding the operated kidney was observed in one animal. Peritoneal fluid levels of urea and creatinine were elevated. Retrograde pyelograms exhibited contrast-medium extravasation through the operated pole in all kidneys. The opened collecting system was also confirmed by methylene blue ink injection. The operated pole was covered by collagenous tissue and adhered to adjacent organs.

Conclusions: Sheep should be considered as an adequate experimental model for research on collecting system healing after partial nephrectomy.

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1. Introduction

Laparoscopic and robotic partial nephrectomy have gained popularity as a nephron-sparing technique for treating small

renal masses [1,2]. Despite the benefits of this procedure over the open technique, some difficulties arise with the minimally invasive techniques. Adequate closure of the collecting system with sutures requires advanced skills and maybe time

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consuming. This step is especially important if the procedure was performed under renal warm ischemia [3,4]. Several methods for collecting system closure have been studied in an attempt to make this step easier [5–8].

Traditionally, the pig has been considered the best model for renal surgery owing to its kidney's anatomic resemblance to the human kidney [9–11]. However, previous studies have demonstrated that the pig kidney heals after partial nephrectomy without collecting system closure [12,13]. Instead, experiments on pigs reported that the operated pole was covered with fibrous tissue, sealing off the collecting system that had been left open. Thus, in pigs, the use of sutures, sealant agents, bolster, and/or internal drainage are not necessary for obtaining collecting system closure. This healing process is the opposite of what occurs in humans, in whom the collecting system does not heal spontaneously. Even using different methods for closing the collecting system, urinary leakage occurs in 1.9%–5.5% of patients [4,14]. Thus, the pig is not a suitable model for testing methods of collecting system closure.

Despite its disadvantages, however, no other animal has been shown to be more suitable as a model for research on collecting system repair, and therefore, pigs continue to be used for this purpose [8,15]. The aim of this study was to assess kidney healing in sheep after laparoscopic partial nephrectomy without closure of the collecting system.

2. Materials and methods

Eight adult female domestic sheep with a mean weight of 50 kg were subjected to laparoscopic partial nephrectomy. The caudal pole of the left kidney was removed, and the renal pelvis was purposefully left open to assess the animal's spontaneous healing. This project was approved by the local ethical committee in accordance with Brazilian laws for the scientific use of animals.

Under general anesthesia and using an aseptic technique, the surgical procedure used a transperitoneal laparoscopic access with four trocars, as adapted from the technique previously used in pigs [13]. The left kidney was completely exposed by dissection. Renal vessels were clamped *en bloc*, avoiding excessive dissection, and the caudal pole of the kidney was incised with cold scissors. As the collecting system was opened, the surgeon was able to feel a more dense structure during the incision. After completely removing the renal pole region, the opened collecting system was confirmed by direct visualization. To avoid coagulation near the collecting system, monopolar cautery was applied for hemostasis only in the parenchyma. The opened collecting system was once again verified, and no internal collecting system drainage catheter was inserted. The excised fragment was removed through an extension of a port-size incision. The animals received regular analgesics for 24 h after surgery. Food and water were given *ad libitum* after the animals recovered to normal ambulation, within 12 h after the procedure.

The animals were evaluated daily for 14 d after surgery, and after this period were euthanized by anesthetic overdose. Serum levels of urea and creatinine were drawn before surgery and on postoperative days 2, 6, 10, and 14, to assess renal

function and any possible peritoneal absorption due to intracavitary urinary leakage. These data were statistically compared using one-way analysis of variance considering that $P < 0.05$, using GraphPad Prism 4.0 software (GraphPad Software, San Diego, CA).

During necropsy, peritoneal fluid was collected for urea and creatinine analysis. The peritoneal cavity and retroperitoneal space were evaluated for evidence of urinary leakage around the operated kidney. Special attention was given to identifying any urinomas, urinary fistulae, or peritonitis and visceral adhesions.

The operated kidney was removed, after which the ureter was catheterized. To evaluate any leakage of contrast medium, an *ex vivo* retrograde pyelogram was performed by manual injection, taking care not to apply too much pressure over the syringe plunger. After the pyelogram, the kidney was injected with methylene blue ink through the ureter to distinguish the area of urinary leakage onto the kidney.

The kidneys were opened longitudinally and fixed in 10% formaldehyde for 24 h. Afterward, the operated pole was sectioned to obtain renal tissue together with adhered adjacent tissues. The section was processed for paraffin embedding, sectioned at 5 μm , and stained with hematoxylin and eosin, as well as Masson trichrome and Sirius red for histologic analyses.

3. Results

It was possible to observe the lumen of the renal pelvis in all animals after caudal polar partial nephrectomy. All animals had a normal postoperative recovery with a return to normal functioning (ambulation, food and water intake, urination, and defecation) within the first 24 h. Serum levels of urea and creatinine increased on postoperative day 2. During the remaining days, both levels gradually decreased from their early postoperative levels (Fig. 1).

Peritoneal fluid collected during necropsy contained higher urea levels than serum samples collected both preoperatively

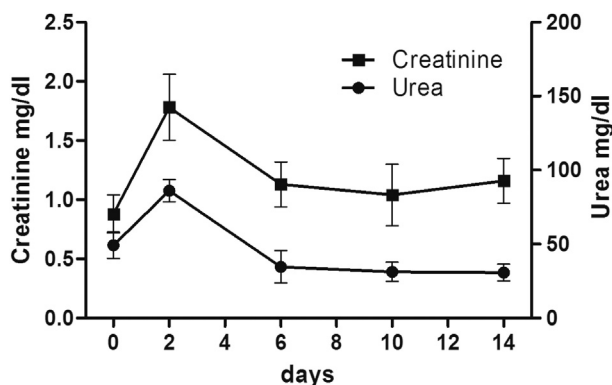


Fig. 1 – Serum creatinine and urea levels of sheep submitted to laparoscopic partial nephrectomy without collecting system closure. Both creatinine and urea levels increased on day 2 postoperatively, followed by a gradual decrease to preoperative levels. Data presented as mean \pm standard deviation.

and postoperatively. Necropsy-obtained creatinine peritoneal fluid levels were also higher than preoperative serum levels (Fig. 2).

In all animals, the operated pole had become completely covered by fibrous tissue, which hindered the identification of the collecting system. Several adhesions were observed connecting the operated pole with adjacent organs (rumen, omentum, and colon) and perirenal adipose tissue. One operated kidney presented an urinoma surrounding the caudal pole. No other macroscopic signs of urine leakage were found in the other animals. In the animal with the urinoma, the levels of urea and creatinine in the peritoneal liquid were 7.6 and 18.9 times higher, respectively, than its previous serum levels.

The retrograde pyelograms demonstrated normal sheep renal anatomy, including a collecting system with no calyceal system, but documented a small renal pelvis with multiple recesses. Contrast-medium extravasation was observed through the operated pole in all kidneys (Fig. 3). Usually, the contrast medium was observed in sinus tracts in the perirenal tissue. Injection of methylene blue ink through the ureter also confirmed that the collecting system was still open, as the contrast was easily observed extravasating into the perirenal area (Fig. 3).

Histologic sections also demonstrated adhesions to adjacent organs in which connective tissue with fibroblasts covered the operated pole. The analysis of Sirius red–stained slides under polarized light indicated that this connective tissue had areas with different amounts of collagen fibers, which were characterized as types 1 and 3 (Fig. 4).

4. Discussion

Partial nephrectomy is widely accepted as a nephron-sparing treatment for removing small renal masses and is now considered the standard of care [16]. Despite its better functional outcomes, this minimally invasive approach to partial

nephrectomy is more technically challenging than radical surgery or an open approach. Thus, several technologies have been developed to achieve adequate hemostasis and easier intracorporeal suturing [17,18].

Transferring new surgical technologies from the research setting into the clinic requires that researchers establish the appropriate experimental models. The pig kidney has been the model most often used for research and training in partial nephrectomy techniques that use open or minimally invasive approaches as follows: laparoscopic, robotic, natural orifice transluminal endoscopic surgery and laparoendoscopic single-site surgery [8,19,20]. Even so, research has demonstrated that the pig kidney heals with no urinary leakage after partial nephrectomy and without collecting system closure [12,13], but a better animal model has not been proposed. Although a sheep model was previously used for testing a radiofrequency coagulation device during partial nephrectomy without collecting system invasion [21], this is the first study of kidney healing after laparoscopic partial resection in sheep.

Fourteen days after laparoscopic partial nephrectomy was performed on the sheep, *ex vivo* retrograde pyelography demonstrated contrast-medium extravasation from the collecting system. Furthermore, other findings (peritoneal urea and creatinine levels and an urinoma in one animal) indicated that urinary leakage was present in the operated animals. Thus, it seems that the sheep's collecting system does not heal as it does in pigs [13] but rather behaves similarly to what is seen clinically in humans. In clinical settings, urinary leakage after partial nephrectomy is considered a common and major complication, occurring in 1.9%–5.5% of patients even after suturing the collecting system and applying sealant agents and bolsters [4,14].

According to Ames *et al.* [12], neither canine nor swine kidneys develop urinomas after partial nephrectomy without collecting system repair. These authors postulated that the absence of Gerota fascia and the diminished renal adipose capsule in pigs would allow urine to flow into the peritoneal cavity and be absorbed. Nevertheless, the most recent study in pigs refuted this theory because no significant alteration in the levels of urea and creatinine were observed in either serum or peritoneal fluid after a similar procedure [13]. Interestingly, in this study, some sheep specimens showed extravasation running around the kidney toward the cranial pole during retrograde pyelogram and methylene blue injection (Fig. 1A and C), suggesting the presence of a renal fascia similar to Gerota fascia in humans. If confirmed, this anatomic feature might help explain the similarity between kidney healing in sheep and humans. Overall, the anatomy of the sheep kidney and retroperitoneum as it pertains to renal surgery has been little described, so further studies would be of interest.

The smaller and more muscular collecting system found in pig kidneys is a possible explanation for the animal's rapid healing without urinary leakage [13]. Because this explanation has not been proven by histologic studies, it is possible to hypothesize that the collecting system histology of sheep resembles that of humans. Another factor that must be taken into account about these animal species is the age of the operated animals. Commonly, pigs used in experimental studies weigh between 25 and 50 kg, considered prepubertal

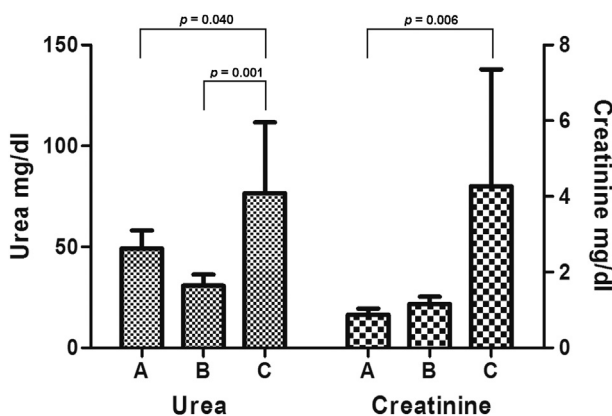


Fig. 2 – Urea and creatinine levels in serum samples collected preoperatively, (A) postmortem, (B) and in peritoneal fluid samples (C) of sheep subjected to laparoscopic partial nephrectomy without collecting system closure, indicating some urine leakage to the peritoneal cavity. Data presented as mean \pm standard deviation.

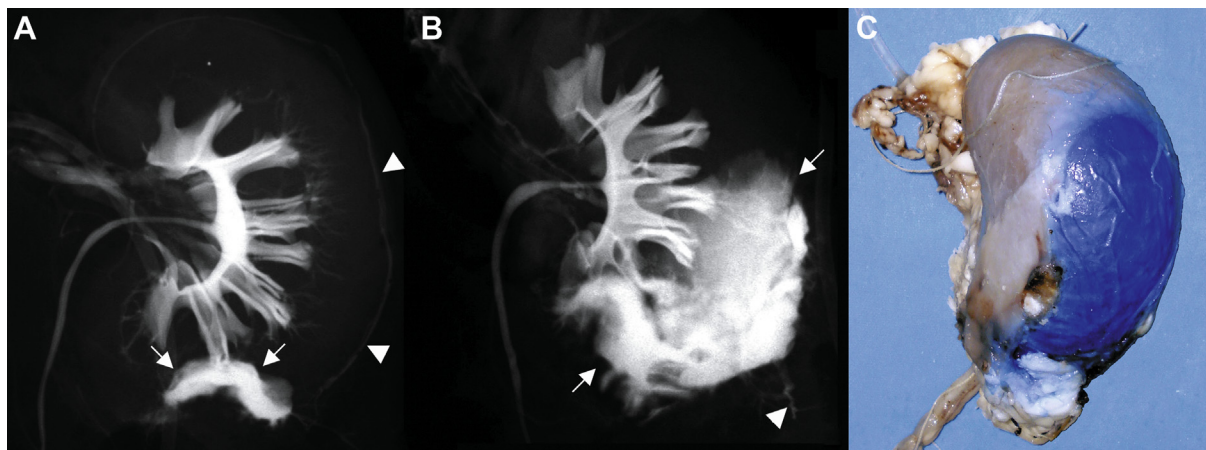


Fig. 3 – Ex vivo retrograde pyelograms (A and B) and methylene blue–injected operated sheep kidney (C) removed 14 d after laparoscopic partial nephrectomy without collecting system closure. (A and B) Ex vivo retrograde pyelograms demonstrated leakage of the opened collecting system (arrows) and sinus tracts forming (arrowheads). (C) Retrograde injection of methylene blue ink showed leakage from the collecting system into the perirenal tissue. (Color version of figure is available online.)

for most commercial breeds [22]. In contrast, the sheep used in this study were adult animals and thus better corresponded to most patients operated on for renal masses [23]. Another reason that should be taken into account for using a sheep animal model is that this animal is easier to manage.

The deposit of firm connective tissue over the operated kidney has been a common finding in other studies that featured a different method of hemostasis and collecting system repair after partial nephrectomies in pigs [19,24–26]. For example, De Souza et al. [13] reported local adhesions in pigs, but neither the deposit of connective tissue nor the adhesions appeared to cause the collecting system to close up. In our study, although connective tissue and adhesions were found in the operated pole, the contrast medium leaked during the retrograde pyelogram, indicating the presence of urinary fistula.

Other experimental studies in pigs have described the use of fibrin sealant powder or glue, intracorporeal suture with LAPRA-TY (Ethicon Endo-Surgery, Cincinnati, OH), and barbed suture as effective for collecting system closure [6,8,27,28]. Analyzing a recent study on pig collecting system closure [13]

and our present findings, we suggest that the methods used in these studies should also be evaluated in sheep.

5. Conclusions

In conclusion, this study demonstrated that the sheep, instead of the pig, should be considered an adequate experimental model for research on collecting system healing after partial nephrectomy.

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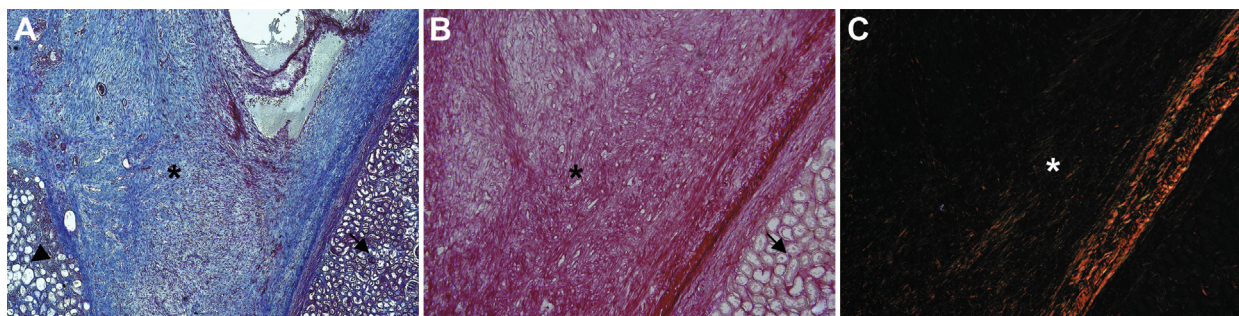


Fig. 4 – Photomicrographs of operated kidneys covered by connective tissue (*). (A) Masson trichrome stain shows an adhesion of connective tissue between the renal cortex (arrow) and adipose tissue (arrowhead). (B and C) The same histologic field observed under bright (B) and polarized light (C). Outside the renal capsule, Sirius red stain highlights loose connective tissue with types 1 and 3 collagen fibers. (Color version of figure is available online.)

Authors' contributions: D.B.D.S., M.A.P.-S., and F.J.S. contributed to the conception and design of the study. D.B.D.S. and M.A.P.-S. drafted the article. D.B.D.S., W.S.C., J.A.D.-F., A.N.J., F.O.A., M.A.P.-S., and F.J.S. did the final approval of the article. W.S.C. contributed to the interpretation of data. W.S.C., J.A.D.-F., A.N.J., F.O.A., and F.J.S. did the revising of the article. J.A.D.-F., A.N.J., and F.O.A. did the acquisition of data.

Disclosure

None of the contributing authors have any competing financial interests or conflicts of interest, including specific financial interests or relationships and affiliations relevant to the subject matter or materials discussed in this article.

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