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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 postoperatively (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.

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 intracavital 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 plunge. 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

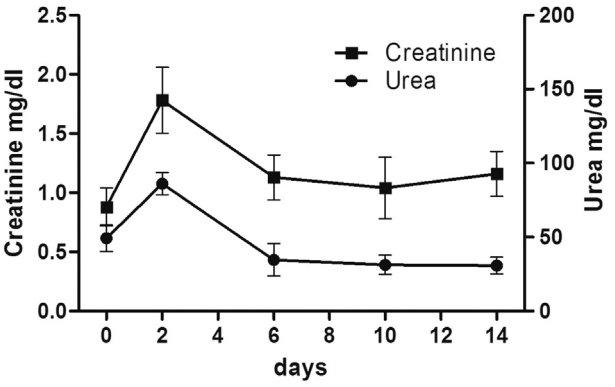

Fig. 1 – Serum creatinine and urea levels of sheep submitted to laparoscopic partial nephrectomy without collecting system closure. Both creatinine and urea levels increased on day 2 postoperatively, followed by a gradual decrease to preoperative levels. Data presented as mean ± standard deviation.