SCIENTIFIC ARTICLE

Gasless laparoscopic renal biopsy in canine cadavers: a preclinical study

Biópsia renal laparoscópica *gasless* em cadáveres caninos: estudo pré-clínico

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Abstract

In this study we aimed to analyze the feasibility of the gasless renal biopsy technique in canine cadavers. The cadavers were randomly divided into two groups: laparoscopic GCG, in which gasless laparoscopy was performed and GCP, laparoscopy with pneumoperitoneum was performed. The procedures were randomly performed on the right and left kidneys. The total surgical time, procedural steps, and intraoperative complications were recorded. The degree of difficulty of the surgical approaches was evaluated by the surgeon, assistant, and external evaluators. Renal samples were evaluated for quality, number of glomeruli, and proportion of renal cortex. The total operative time was higher in the GCG group than in the GCP group ($p < 0.01$). Additionally, positioning of the second portal and platform positioning took longer than the other steps. The surgical groups differed from each other in the Likert scale values for almost all the parameters regarding the difficulty of the surgical approaches ($p < 0.05$), with higher scores in the GCG group than in the GCP group. Based on the video recordings, the GCP group had higher scores than the GCG group for degrees of difficulty of the approach ($p < 0.05$). Renal histological parameters were similar between the surgical groups and surgical sides. Our study findings indicate that the proposed *gasless* renal biopsy technique is feasible. The longer operative time and technical difficulties in the gasless approach did not affect the quality of the renal specimens.

Keywords: laparoscopy, gasless, kidneys, biopsy, *ex vivo*.

Resumo

Neste estudo objetivamos analisar a viabilidade da técnica de biópsia renal *gasless* em cadáveres caninos. Os cadáveres foram divididos aleatoriamente em dois grupos: GCG laparoscópico, no qual foi realizada laparoscopia *gasless* e GCP, onde foi realizada laparoscopia com pneumoperitônio. Os procedimentos foram realizados aleatoriamente nos rins direito e esquerdo. O tempo cirúrgico total, etapas do procedimento e complicações intraoperatórias foram registrados. O grau de dificuldade das abordagens cirúrgicas foi avaliado pelo cirurgião, assistente e avaliadores externos. As amostras renais foram avaliadas quanto à qualidade, número de glomérulos e proporção do córtex renal. O tempo operatório total foi maior no grupo GCG do que no grupo GCP ($p < 0,01$). Além disso, o posicionamento do segundo portal e o posicionamento da plataforma demoraram mais do que as outras etapas. Os grupos cirúrgicos diferiram entre si nos valores da escala Likert para quase todos os parâmetros relativos à dificuldade das abordagens cirúrgicas ($p < 0,05$), com escores mais elevados no grupo GCG do que no grupo GCP. Com base nas gravações de vídeo, o grupo GCP obteve pontuações mais altas que o grupo GCG para graus de dificuldade da abordagem ($p < 0,05$). Os parâmetros histológicos renais foram semelhantes entre os grupos cirúrgicos e os lados cirúrgicos. Os resultados do nosso estudo indicam que a técnica proposta de biópsia renal *gasless* é viável. O maior tempo operatório e as dificuldades técnicas na abordagem sem gás não afetaram a qualidade das amostras renais.

Palavras-chave: laparoscópico, *gasless*, rins, biopsia, *ex-vivo*.

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Introduction

Nephropathies occur frequently in the field of veterinary medicine. Although most of them are detected using non-invasive modalities, such as laboratory and imaging tests (Manashirova et al., 2011; Vaden, 2004), renal biopsy is necessary to establish a definitive diagnosis and determine the severity of the kidney injury, especially in diseases involving the renal cortex or cases of proteinuric nephropathy, renomegaly or renal tumors (Crivellenti et al., 2018; Lees et al., 2011; Rawlings et al., 2003; Vaden, 2005). A histological diagnosis favors the identification of an ideal treatment and provides prognostic information (Park et al., 2017).

Ultrasonography (US)-guided percutaneous renal biopsies are considered the standard method of sample collection in animals (Crivellenti et al., 2018; Lees et al., 2011; Vaden, 2004). However, the laparoscopic approach allows for the direct visualization of kidneys, selective sampling of a specific lesion, and visual control of the lesion, collection technique and hemostasis (Nowicki et al., 2010; Park et al., 2017; Vaden, 2005). The laparoscopic approach is the first choice for an alternative approach in patients with contraindications to or failure in performing US-guided percutaneous biopsy (Richter & Ross, 2015; Silvinato et al., 2019).

Although pneumoperitoneum is effective and safe in most healthy patients, renal side effects have been detected during and after laparoscopy (Aoun et al., 2019). Animal studies have demonstrated that high pressures can decrease venous return, compress the renal vasculature, and cause systemic hormonal changes, significantly reducing renal blood flow, urinary output, and glomerular filtration rate (Demyttenaere et al., 2007). Furthermore, it can result in increased apoptosis of the renal tubular cells (Tosun et al., 2014).

The gasless laparoscopic approach avoids hemodynamic overload (Shoman et al., 2020), allowing the use of traditional instruments and the performance of complex surgeries and maneuvers (Andersson et al., 2003; Chiu et al., 1996). Specific reports of performing renal biopsies using gasless laparoscopy in animals are limited (Chiesa et al., 2009; Kassem et al., 2014). These studies have considered the relatively simple, safe, and fast procedure with adequate visualization and have obtained quality samples. The abdominal elevation platform (ES201800465 U) (BR 102019013473-9 A2) used for this study is feasible for laparoscopic diaphragmatic herniorrhaphy in a cadaveric dog model; however, it is yet to be used for renal surgery (Brun et al., 2022).

In this study, we aimed to develop a new technique for performing a gasless renal biopsy in canine cadavers and compare it to the laparoscopic technique that uses pneumoperitoneum. We hypothesized that sample collection using gasless laparoscopy would be feasible; however, it would be more difficult and time-consuming than the traditional laparoscopic technique due to the limited abdominal space. However, if this new technique is feasible, it could be clinically applied in patients who would benefit from the absence of hyperbaric pneumoperitoneum.

Material and methods

Experimental model

The study was carried out in canine cadavers from the Hospital Veterinário Universitário, who were refrigerated or frozen and destined for study and/or research. Twenty dogs of both sexes, weighing 5–16 kg, were selected. The exclusion criteria were as follows: recent abdominal surgical procedures, peritonitis or ascites, traumatic injuries to the abdominal wall or intra-abdominal organs, and abdominal alterations of another nature that could interfere with the performance of the proposed procedures.

The cadavers were randomly divided into the following two groups: GCG (n = 10) in which laparoscopic gasless renal biopsy was performed using a multidirectional traction platform (ES201800465 U) (BR 102019013473-9 A2) and GCP (n = 10) in which laparoscopic renal biopsy with pneumoperitoneum was performed. After the simple random selection of the surgical group, both kidneys were subjected to two biopsies each, totaling 80 samples.

The cadavers were thawed for 24–48 hours at room temperature before the surgical procedure, depending on the cadaver's size. The surgeries were performed by a consolidated surgical team that was on an initial learning curve for the study procedure.

After the last sample was collected, laparoscopic nephrectomy was performed in all the cadavers for another study.

Multidirectional abdominal traction platform

The specifications and characteristics of the device designed for multidirectional abdominal traction have been detailed by Brun et al. (2022). External traction and elevation of the abdominal wall are performed at certain points, from transparietal sutures fixed to hemostatic forceps, and positioned according to the surgical procedure to be performed (Figure 1).

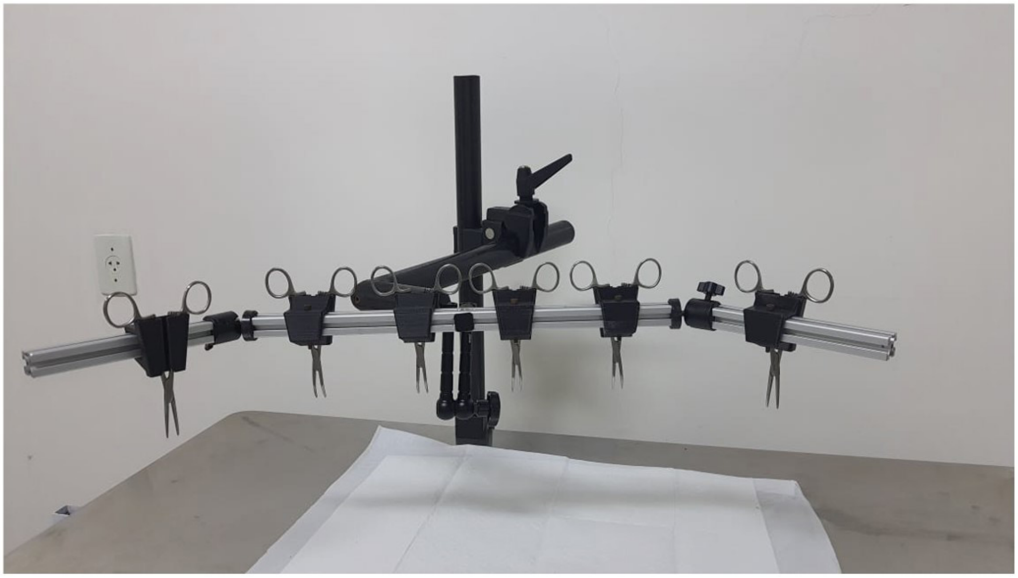

Figure 1. Multidirectional traction platform for video surgery (ES201800465 U) (BR 102019013473-9 A2), positioned on the operating table.

Surgical procedure

After the selection of the surgical group, the first kidney to be biopsied (right or left) was randomly selected.

Laparoscopic renal biopsy with pneumoperitoneum

Prior to the procedure, all the cadavers were subjected to wide shaving of the entire operative field. For right-sided renal biopsies, the cadavers were positioned in the left lateral decubitus position, keeping the flank elevated with compresses or rolled surgical drapes to facilitate renal exposure and organ isolation. For left-sided biopsies, the cadavers were positioned in the right lateral decubitus position with the flank elevated in the same way (Figure 2A).

Two portals and a 16G semiautomatic biopsy needle (Tru-Cut; ProMedical, Juiz de Fora, Brazil) were used in triangulation (Figure 2B). For right-sided renal biopsies, an 11 mm incision was made in the right lateral abdominal wall at the midpoint between the iliac tuberosity and the costal margin, just lateral to the level of the inguinal crease, for the placement of an 11 mm cannula (Karl Storz, Tuttlingen, Germany). A 10 mm, 0° rigid endoscope (Karl Storz, Tuttlingen, Germany) was passed through this portal, and the abdominal cavity was manually insufflated (Karl Storz, Tuttlingen, Germany) with medicinal CO_2 up to a pressure of 6 mmHg at 1.5 L/min. Another 11 mm trocar was inserted into the right lateral abdominal wall, dorsocranial to the first trocar.

When performing left-sided renal biopsies, the first 11 mm port was inserted into the left lateral abdominal wall, at a point similar to that described for right-sided biopsies. The second port was a 6 mm port (Karl Storz, Tuttlingen, Germany) that was inserted dorsocranial to the first portal. Thus, the portals were created according to the side of the renal biopsy in a way that sought the best triangular formation with the biopsy needle, keeping it in the caudocranial position at the time of collection.

After visualizing the entire abdominal cavity and locating the kidney, a 5 mm Kelly forceps (Storz, Tuttlingen, Germany) was used to elevate it (Figure 2C). Renal biopsy was performed using a 16G semiautomatic biopsy needle (Tru-Cut), which was inserted under optical view into the abdominal cavity slightly caudal to the caudal pole of the kidney (Figure 2D). Two samples were collected. Samples collected from the renal cortex were fixed in 10% neutral buffered formalin and evaluated.

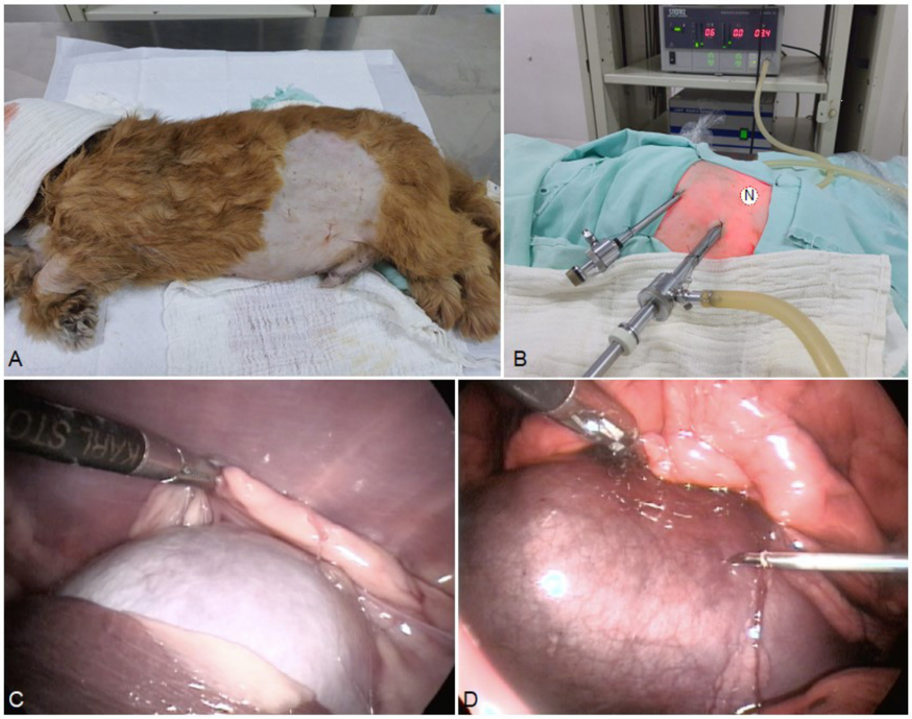

Figure 2. (A) Patient positioned in the right lateral decubitus position with the flank elevated for a left-sided renal biopsy. (B) Positioning of the two portals (11 mm and 6 mm) and the semiautomatic biopsy needle (N = needle) in a triangular pattern for performing laparoscopic renal biopsy with pneumoperitoneum. (C) Intraoperative image of a 5 mm Kelly forceps used to elevate the kidney for adequate exposure. (D) A Tru-Cut 16G semiautomatic biopsy needle was inserted under optical view into the abdominal cavity slightly caudal to the caudal pole of the kidney being biopsied.

The pneumoperitoneum was reversed, and the abdominal wall was sutured using polyglactin 910 2-0 (PGA 910 2-0; Shalon, Brazil) in a cross-mattress pattern. Subsequently, the subcutaneous tissue was sutured in the same pattern with PGA 910 3-0 (Shalon, Brazil). The skin was sutured in an isolated horizontal mattress pattern using 4-0 monofilament nylon (Nylon, Technofio, Brazil).

Gasless laparoscopic renal biopsy

The cadavers were positioned according to the side to be operated on, with the relevant flank elevated. For the gasless laparoscopic renal biopsy, two portals of the same diameter and same arrangement were placed in the lateral abdominal wall just as they were place for laparoscopic renal biopsy with pneumoperitoneum.

After positioning the first trocar using an open technique and ensuring its correct placement within the peritoneal cavity, the second portal was inserted in the right or left abdominal wall dorsocranial to the first portal. Under direct visualization, the endoscope was introduced in the first port and directed to the chosen point. The abdominal wall was elevated and moved away from the viscera. By partially withdrawing the endoscope into the trocar, a skin incision was made at this elevated point. The second trocar was inserted through this incision and directed toward the first portal to avoid possible injuries to the abdominal viscera.

For the use of the aforementioned prototype, two transparietal sustaining sutures were placed using polyester thread No. 2 (Polyester 2; Shalon, Brazil) approximately 2.5 cm dorsal to the two portals already positioned (Figure 3A). Care was taken to pass the sutures only through the skin, subcutaneous tissue, and external muscle layers, without passing them through the peritoneum. These sutures were individually fixed with conventional Kelly or Halstead hemostatic forceps, depending on the size of the cadaver. Subsequently, these were attached to the multidirectional abdominal traction platform to lift the abdominal wall at the determined points (Figure 3B) with minimum tension to visualize the target organs. Thereafter, the biopsy was performed in the same way as that described for the laparoscopic renal biopsy with pneumoperitoneum. At the end of the procedure, the transparietal suspension sutures were removed, and the surgical accesses were occluded in three planes as previously described for the laparoscopic renal biopsy with pneumoperitoneum.

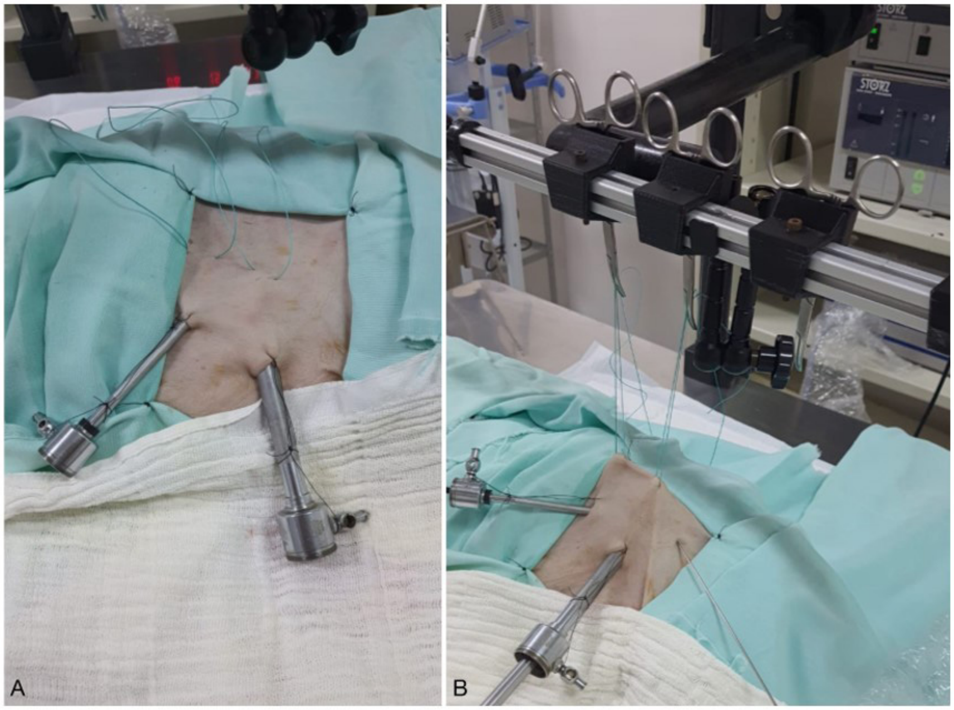

Figure 3. (A) Placement of transparietal support sutures using #2 polyester thread approximately 2.5 cm dorsal to two portals (11 mm and 6 mm) for performing a gasless laparoscopic renal biopsy using an external support platform. (B) Intraoperative image showing the sutures individually fixed to conventional Kelly or Halstead hemostatic clamps to lift the abdominal wall from two anchoring points.

Data collection

Surgical time was recorded in both groups for the following steps: T1, positioning of portal 1; T2, positioning of portal 2; T3, establishment of pneumoperitoneum or installation of the prototype; T4, collection of renal biopsies (time to collect the two samples); T5, conclusion of the closure of the surgical accesses; T6, total surgical time. The weight and sex of the animals were also recorded.

After each procedure, the performing surgeon and the assistant filled out a questionnaire to assess the degree of difficulty of the surgical approaches. For this, a Likert scale based on the model presented by Tapia-Araya et al. (2015) and Keheila et al. (2017) and adapted for laparoscopic renal biopsy, which addressed aspects of the surgical procedure, were used. Each item was rated on a score of one to five as follows: 1, no difficulty; 2, low difficulty; 3, moderate difficulty; 4, high difficulty; and 5, very high difficulty. The following items were analyzed: P1, difficulty in obtaining surgical access for portals and installation of the multidirectional traction platform; P2, difficulty in surgical maneuvers to access the kidney during biopsy and sample collection; P3, difficulty in visualizing anatomical structures; P4, difficulty in handling instruments considering the workspace; P5, physical fatigue; P6, mental fatigue.

The entire surgical procedure was video recorded, and the unedited videos were evaluated by two experienced laparoscopic surgeons (blinded to the surgical group). The quality of the visualization and performance of the surgical steps were subjectively quantified using a scale described by Sherwinter (2012). The work space within the abdominal cavity were scored as follows: 0, impossible to visualize; 1, very difficult to visualize requiring excessive research and significant movement of the viscera; 2, difficult to visualize requiring extensive research and considerable movement of viscera; 3, satisfactorily visualized requiring some research and minor manipulation of viscera; 4, good visualization allowing easy localization of viscera with little manipulation; and 5, excellent visualization allowing obvious and clear visualization of viscera with minimal or no manipulation. The quality of visualization of the anatomical structures, quality of the workspace, and performance of the biopsies were scored as follows: 0, impossible to perform the procedure or visualize; 1, poor quality of execution and/ or visualization; 2, fair quality of execution and/or visualization; 3, good quality of execution and/or visualization; and 4, excellent quality of execution and/or visualization).

Intraoperative complications, such as transfixion of the kidney by the needle at the time of biopsy and injury to other abdominal organs, were recorded and classified according to their severity.

From a total of 80 kidney specimens collected, only 40 were evaluated (20 right-sided and 20 left-sided kidney samples) because some were considered insufficient for analysis due to their small size (<1 mm). This was mainly observed in samples that fragmented on transfer to histological cassettes. The samples were placed as a whole on histological cassettes, dehydrated in alcohol, clarified in xylene, and embedded in paraffin. Subsequently, 3 μm-thick histological sections were obtained and routinely stained with hematoxylin and eosin. The stained sections were evaluated using optical light microscopy.

Using a methodology adapted from Rawlings et al. (2003) and Park et al. (2017), the quality of each biopsy sample was scored as follows: 0, inadequate (non-diagnostic sample); 1, poor (fragmentation or severe crushing); 2, fair (moderate fragmentation or crushing); 3, good (fragment with insignificant crushing); 4, excellent (no damage to the sample). The quality of tissue fragmentation (sample discontinuity) and crushing (ruptured tubules or glomeruli) were also evaluated. Furthermore, the proportion of the renal cortex (with or without medullary region) and number of glomeruli per sample were evaluated.

Statistical analysis

The results of the surgical time of each step and number of glomeruli identified in the biopsy were tested for normality (PROC UNIVARIATE, SAS®; SAS Institute, Cary, NC, USA) using the Kolmogorov–Smirnov test. Differences between the surgical groups and between the surgical sides were analyzed using MIXED (SAS® version 9.4; SAS Institute, Cary, NC, USA). The means were compared using the lsmeans feature adjusted for Tukey. The statistical model of this analysis included the surgical groups ($n = 2$, GCG and GCP), surgical side ($n = 2$, left and right), and their interactions as fixed effects. Dogs ($n = 20$) and error were included as random effects. The sex and weight of the animals were included in the model as covariates.

With the exception of the synthesis variable, the surgical time of all other variables were transformed to achieve normality. The stages of evaluation of the degree of difficulty of the surgical approaches (classifying variables) were analyzed using GLIMMIX (SAS®, studio version), with the means compared using the lsmeans resource adjusted for Tukey. The evaluators (surgeon and assistant as well as external evaluators 1 and 2) of the degree of difficulty of the surgical approaches were included in the model for not having symmetrical responses (Kendall correlation). For these variables, the statistical analysis model included the surgical groups ($n = 2$, GCG and GCP), surgical side ($n = 2$, left and right), evaluator ($n = 2$, surgeon and auxiliary or external evaluators 1 and 2), and interaction between group and side as fixed effects. Dogs ($n = 20$) and error were included as random effects. Similarly, the classification of the sample quality and cortex/medulla proportion in the biopsy (classifying variables) were analyzed using GLIMMIX (SAS®, Studio version), with the means compared using the lsmeans resource adjusted for Tukey. The differences were considered significant if the p-value was <0.05.

Results

The mean body weight of the cadavers in the GCG and GCP group was 7.1 kg (± 0.86) and 9.4 kg (± 0. 99), respectively. Only one patient (1.3%) in the GCP group developed an intraoperative complication. The spleen was inadvertently perforated during the insertion of the biopsy needle. Similarly, one patient (1.3%) in the GCG group developed an intraoperative complication due to transfixion of the kidney by the needle.

The total operative time for biopsy was 3.55 minutes longer in the GCG group ($p < 0.01$; Table 1). Regarding the surgical time steps to perform the biopsy, a difference was observed for the positioning of the second portal and establishment of the platform, which was also higher in the GCG group ($p < 0.01$; Table 1). The surgical side did not influence the total surgical time and surgical time of each stage ($p > 0.05$; Table 1).

There was a significant difference in the Likert scale values of almost all the parameters regarding the degree of difficulty of the approaches ($p < 0.05$; Table 2) between the groups. With the exception of the score for mental fatigue (P6), which was similar between the groups ($p > 0.05$; Table 2), all the other items received a higher score in the GCG group than in the GCP group. The surgical side did not influence the Likert scale values of the parameters regarding the degree of difficulty of the surgical approaches ($p > 0.05$; Table 2).

Table 1. Total surgical time and surgical time of each step of the biopsy according to the evaluated surgical group and the surgical side.

Surgical Times (min)	Group		Side		MSE	Probability[1]		
	GCG	GCP	Right	Left		G	S	G*S
Positioning of portal 1	3.18	2.70	2.91	2.97	0.26	0.29	0.94	0.44
Positioning of portal 2	2.25a	1.46b	1.87	1.84	0.15	**<0.01**	0.88	0.60
Setting up the biopsy equipment	2.01a	0.20b	1.21	1.01	0.11	**<0.01**	0.23	0.90
Collection of kidney sample	3.09	2.51	2.49	3.10	0.48	0.90	0.82	0.08
Conclusion of suturing of surgical accesses	7.07	7.16	7.06	7.17	0.31	0.85	0.79	0.52
Total time	17.65a	14.10b	15.59	16.16	0.69	**<0.01**	0.57	0.60

[1]Probability by *Tukey* test at 5%. GCG: gasless laparoscopic biopsy; GCP: laparoscopic biopsy with pneumoperitoneum; MSE: mean standard error; G: group; S: side.

Table 2. Degree of difficulty of surgical approaches during biopsy on the Likert scale, according to the surgical groups evaluated and the side of the surgery.

Parameters[1]	Group		Side		MSE	Probability[2]		
	GCG	GCP	Right	Left		G	S	G*S
P1	1.60a	1.27b	1.55	1.32	0.09	**0.03**	0.10	0.58
P2	2.00a	1.60b	1.90	1.70	0.11	**0.03**	0.22	0.99
P3	2.75a	1.64b	2.32	2.07	0.13	**<0.01**	0.14	0.55
P4	2.83a	1.86b	2.47	2.22	0.13	**<0.01**	0.19	0.99
P5	1.95a	1.62b	1.75	1.82	0.10	**0.04**	0.61	0.86
P6	1.67	1.45	1.50	1.62	0.09	0.14	0.35	0.85

[1]Likert scale parameters: P1 = difficulty in obtaining surgical access for portals and installation of the multidirectional traction platform; P2 = difficulty in performing surgical maneuvers to access the kidney during biopsy and sample collection; P3 = difficulty in visualizing the anatomical structures; P4 = difficulty in handling the instruments considering the workspace; P5 = physical fatigue; P6 = mental fatigue. [2]Probability by *Tukey* test at 5%. GCG: gasless laparoscopic biopsy; GCP: laparoscopic biopsy with pneumoperitoneum; MSE: mean standard error; G: group; S: side.

When evaluating the video recordings for the difficulty of surgical approaches, there were no significant interactions between the evaluated surgical groups and the surgical side ($p > 0.05$; Table 3). Additionally, there was no significant difference in the surgical side with respect to any parameter regarding the degree of difficulty of the surgical approaches ($p > 0.05$; Table 3).

The surgical groups differed with respect to almost all aspects of the degree of difficulty of the surgical approaches ($p < 0.05$; Table 3). With the exception of the score for the difficulty in performing the biopsies (P2), which was similar between the groups ($p > 0.05$; Table 3), all the other aspects regarding the degree of difficulty received a higher score in the GCP group than in the GCG group.

There were no significant interactions between the surgical groups and the surgical side in terms of the kidney histomorphology ($p > 0.05$; Tables 4 and 5). The sample qualities and the number of glomeruli observed were similar between the two surgical groups ($p > 0.05$; Table 4) and the two surgical sides ($p > 0.05$; Table 4). The surgical groups and the surgical sides did not alter the cortex/medulla ratio of the extracted biopsy samples ($p > 0.05$; Table 5).

Table 3. Degree of difficulty of the surgical approaches according to the surgical groups and the surgical side based on the evaluation of video recordings.

Aspects	Group		Side		MSE	Probability[1]		
	GCG	GCP	Right	Left		G	S	G*S
Workspace inside the abdominal cavity	2.39b	3.28a	2.85	2.82	0.12	**<0.01**	0.88	0.88
Visualization quality of anatomical structures	2.30b	3.04a	2.72	2.62	0.09	**<0.01**	0.42	0.42
Workspace quality	2.26b	3.06a	2.67	2.65	0.11	**<0.01**	0.87	0.62
Execution of biopsies	2.67	3.00	2.70	2.97	0.14	0.13	0.18	0.54

[1]Probability by *Tukey* test at 5%. GCG: gasless laparoscopic biopsy; GCP: laparoscopic biopsy with pneumoperitoneum; MSE: mean standard error; G: group; S: side.

Table 4. Histomorphology of the kidneys according to the surgical groups and surgical side.

Parameter	Group		Side		MSE	Probability[1]		
	GCG	GCP	Right	Left		G	S	G*S
Sample quality	1.95	2.65	2.30	2.30	0.30	0.11	0.99	0.81
Number of glomeruli	7.87	6.40	7.40	6.85	2.31	0.66	0.88	0.22

[1]Probability by *Tukey* test at 5%. GCG: gasless laparoscopic biopsy; GCP: laparoscopic biopsy with pneumoperitoneum; MSE: mean standard error; G: group; S: side.

Table 5. Proportion of renal cortex/medulla in the histomorphological examination of the kidneys according to the surgical groups and surgical side.

Proportion cortex/medulla (n, %)	Group		Side		MSE	Probability[1]		
	GCG	GCP	Right	Left		G	S	G*S
Just cortex	3 (33.03)	3 (35.00)	3 (39.28)	3 (28.75)				
Cortex and medulla	1 (20.53)	1 (10.00)	1 (14.28)	2 (17.75)	0.24	0.84	0.57	0.53
Just medulla	4 (46.43)	5 (55.00)	4 (46.43)	5 (55.00)				

[1]Probability by *Tukey* test at 5%. GCG: gasless laparoscopic biopsy; GCP: laparoscopic biopsy with pneumoperitoneum; MSE: mean standard error; G: group; S: side.

Discussion

This preclinical study on canine cadavers suggests that performing a renal biopsy using a multidirectional traction platform is feasible, despite the need for a longer surgical time when compared to the conventional technique using pneumoperitoneum. The total surgical time was directly related to a greater degree of difficulty in performing some surgical steps, such as installing the abdominal elevation platform and positioning the second portal.

Fransson and Ragle (2011) determined that the mean surgical time of gasless laparoscopy was longer than that of traditional laparoscopy with pneumoperitoneum. However, they performed different surgical techniques in dogs and cats; thus, there is a need to specifically determine whether such times are comparable to those of conventional laparoscopy. In contrast, Fransson et al. (2015) did not find any differences in the surgical times when performing ovariohysterectomies in canine using gasless or pneumoperitoneum laparoscopy. This finding may be attributed to improvements in the laparoscopic techniques for elevation as the procedures were repeated. The divergent results lead us to believe that surgical times may vary according to the surgeon's training, type of lifting device used, and type of surgical procedure.

Considering that the surgical time in the GCG group was only 3.55 minutes longer than that in the GCP group, it should not considered as a contraindication for the proposed gasless technique for *in vivo* procedures. The need for obtaining histopathological diagnoses for several renal conditions has increased the demand to develop surgical techniques and skills (Maia et al., 2021).

The device used in this study has multiple structures such as a central bar, two side arms, and a support for the application of up to six lifting sutures (Brun et al., 2022). Thus, it provides ample mobility to create a sufficient intra-abdominal workspace. However, the time required for positioning and adjusting the lifting sutures is longer than that required to reach the desired pressure with CO_2 insufflation. Nonetheless, the advantages of renal biopsy via gasless laparoscopy tend to be significant, considering the negative impact of high intra-abdominal pressures of pneumoperitoneum (reduction in the renal blood flow) in patients with pre-existing renal impairment (Demyttenaere et al., 2007). Thus, we do not believe that the additional time required for platform installation is a limitation, and it does not negatively impact the total surgical time.

The lack of intra-abdominal space at first makes it difficult to position the second portal, making this step more time-consuming in gasless laparoscopy. Although studies have demonstrated the performance of laparoscopic-guided renal biopsies using only a 5 mm portal for insertion of an endoscope (Maia et al., 2021), we believe that the insertion of a second portal is useful because it allows the use of auxiliary forceps to manipulate the viscera. This ensures adequate exposure of the kidneys and allows the application of pressure at the biopsy site in cases of hemorrhage (Chiesa et al., 2009; Park et al., 2017; Rawlings et al., 2003).

Abdominal elevation laparoscopy is not approved by some researchers because they believe that it provides a reduced working space when compared to the traditional technique using pneumoperitoneum, which could prolong the operative time (Gurusamy et al., 2008; Hyodo et al., 2012; Watkins et al., 2013).

Although we found a greater degree of difficulty for all aspects related to the installation of equipment, performance of the biopsy, visualization of anatomical structures, and manipulation of instruments in the GCG group than in the GCP group, the exposure of the anatomical region of interest and the working space were adequate for collecting renal samples. Similarly, Fransson and Ragle (2011) determined that the gasless technique was associated with a slightly worse visualization of the abdominal cavity, particularly in the lateral aspects, presenting greater difficulty in exposing the organ. However, they believed that there were considerable technical advantages to the use of laparoscopy by elevation, considering that multiple biopsies of different organs were obtained.

According to Kennedy et al. (2015), a caudal abdominal lift is useful in abdominal surgeries in this location, where minimal abdominal exploration is required. Fransson et al. (2015) stated that laparoscopy performed by abdominal elevation is adequate when repeated procedures are required, such as multiple biopsies in different organs. The gasless laparoscopy device proposed in this study uses several lifting sutures and allows adjustments to various anatomical conditions and different abdominal access sites. Thus, it provides a regular distribution of forces in the ventral and lateral areas of the abdomen. This fact was verified in a study conducted by Brun et al. (2022), when the same device was found to be feasible for performing diaphragmatic herniorrhaphy in *ex vivo* canine models and providing adequate intra-abdominal space for the surgery.

In our study, the external evaluators believed that the work space and the visualization of anatomical structures were inferior in the GCP group than in the GCG group. This may be attributed to the use of a low inflation pressure of 6 mmHg; typically the abdomen is inflated to 10 and 12 mmHg (Park et al., 2017; Watkins et al., 2013). However, according to Mayhew et al. (2013), the simplest laparoscopic procedures can be performed at an intra-abdominal pressure of 4 mmHg with considerable working space, while more complex procedures requiring greater visibility can be performed at pressures of 8 mmHg. In contrast to what was observed by the external evaluators, the surgeon and the assistant the scored of the variables evaluated were lower in the GCG than in the GCP group. This difference can be attributed to the fact that these professionals performed the surgical maneuvers and understood the difficulties of each stage.

The intraoperative complications observed in the GCP and GCG groups during the sample collection were related to the insertion of the biopsy needle, leading to inadvertent spleen perforation and inadequate transfixion of the kidney. However, these complications were considered of low complexity. Similar complications were reported by Rawlings et al. (2003) and Park et al. (2017), when specimens obtained using an 18G needle occasionally contained fragments of skeletal muscle, spleen, small intestine, and other tissues. Furthermore, the main complications such as severe hemorrhage, perirenal hematoma, hematuria, secondary hydronephrosis, and formation of an arteriovenous fistula, were seen in the postoperative period (Aoun et al., 2019).

According to Lees et al. (2011) and Park et al. (2017) the renal cortex is the appropriate place for obtaining biopsy specimens because the transection of the medullary arcuate vessels can cause excessive hemorrhage and massive damage to the renal parenchyma. However, we obtained considerable amount of samples of only the renal medulla in both groups. This may have occurred due to the condition of the organ in the *ex vivo* model in association with the surgeon's initial learning curve with these specific procedures; spinal cord samples were collected mainly in the first few biopsies. According to Crivellenti et al. (2018), samples obtained by the laparoscopic approach are associated with a greater possibility of inadvertent biopsies of the renal medullary tissue. However, most studies consider that laparoscopy allows direct visualization of the kidney during biopsy, allowing selective sampling of specific portions of the organ, as well as visual control of sample collection and hemostasis (Aoun et al., 2019; Maia et al., 2021; Nowicki et al., 2010; Park et al., 2017; Rawlings et al., 2003; Vaden, 2005).

The collection of the medullary portion may be related to the operating surgeon, and as in the case of this study, the use of thawed cadavers can lead to renal alterations, culminating in a greater difficulty in collecting the tissue (Manashirova et al., 2011). Nonetheless, although the sample may be as small as possible, the tissue collected is sufficient to allow an accurate diagnosis. Thus, we recommend that the needle be inserted through the renal capsule and positioned parallel to the axis of the kidney before triggering the spring mechanism.

Veterinary medicine literature suggests that at least two good quality samples should be submitted for evaluation, and the histological analysis of 5–10 glomeruli is adequate for the evaluation and distribution of glomerular diseases (Crivellenti et al., 2018; Vaden, 2005; Zatelli et al., 2005). In our study, both the approaches allowed the collection of samples with a sufficient number of glomeruli. However, there was a tendency to obtain a more glomeruli in the GCG group than in the GCP group, proving that gasless laparoscopy is a viable option in surgical practice.

We did not observe any difference in the collection of samples between the right and left kidneys in both groups. This result was also observed in another study (Maia et al., 2021). However, the right kidney is often preferred for obtaining biopsy samples due to the resistance provided by the caudate lobe of the liver through the hepatorenal ligament, reducing organ movement during the procedure (Amorim et al., 2005; Park et al., 2017). In the study by Maia et al. (2021), the ease of collection from the right or left kidney as related to the operator and not to the procedure itself. We noticed that positioning the second portal cranial to the first, regardless of the side to be biopsied, made it easier to insert the needle into the caudal portion of the lateral abdominal wall and required less needle manipulation and instrument repositioning.

One of the limitations of our study was the use of frozen or refrigerated cadavers without any other method of conservation. It made performing the surgery and histomorphological evaluation of renal specimens more complex. Kennedy et al. (2015) used fresh cadavers one hour after euthanasia, which preserved a consistent muscle plasticity and avoided changes due to rigor mortis. Furthermore, a cadaveric study cannot capture all the possible impacts of an in vivo procedure, such as the exact duration of the procedure, intraoperative complications such as post-biopsy renal bleeding, or the assessment of pain after using a platform traction. However, the development of new techniques and training on cadavers is a very important preliminary step prior to performing procedures *in vivo*.

Conclusion

The gasless renal biopsy technique using an abdominal traction platform is a feasible option in dog cadavers. Although the total surgical time was longer and there were greater difficulties in performing some surgical maneuvers in the gasless technique than in the traditional technique using pneumoperitoneum, the quality of the renal specimens obtained were similar. We believe that with the improvement of the surgical technique and its application in vivo, patients with renal alterations may be significantly benefited due to the absence of hyperbaric pneumoperitoneum.

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Ethics statement

Those responsible for the non-human animal(s) formally consented to the study.

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Conflict of interests

The authors declare that there is no conflict of interest related to this study.

Authors' contributions

VM: Contributed to conception of the study, study design, acquisition of the imaging data, measurements, and data analysis and interpretation. Drafted the manuscript. BNA: Contributed to conception of the study, study design, acquisition of the imaging data, measurements, data analysis and interpretation. PC: Contributed to the sample collection and conception of the study. HFH: Contributed to the data analysis and interpretation. MTL: Contributed to the data analysis and interpretation. VSC: Contributed to the sample collection and conception of the study. TRC: Contributed to the sample collection and conception of the study. HCD: Contributed to the sample collection and conception of the study. MVB: Contributed to conception of the study, study design, data analysis and interpretation.

Availability of complementary results

We suggest consulting https://repositorio.ufsm.br/handle/1/24427

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