

# Influence of suture diameter on microsurgical ureterorrhaphy in rabbits: an experimental study

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## Research article

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# Abstract

## Background

Ureteral obstruction is a common indication for surgical intervention, due to the small size of the ureter, specialized instrumentation is required such as operating microscope for intraoperative magnification and microsutures. The aim of this study is to evaluate and compare 3 sizes of nylon 6 - 0, 8 - 0, 10 - 0 for ureterorrhaphy in the ureter of rabbits, using microsurgical technique.

## Results

A total of fifteen male, adults, New Zealand rabbits were divided in three groups: all groups were submitted ureterotomy. A 2 mm longitudinal incision was made in the medial portion of the right ureter, followed by a partial perforating simple interrupted 6 - 0, 8 - 0 and 10 - 0 sutures in each group. During the experiment ultrasound evaluations were performed to assess the kidney length and width, the presence of ureteral calculi, the renal pelvic dilation, the ureter diameter and the presence of free liquid in the abdominal cavity. At the end of the experiment all kidneys and ureters were submitted to macroscopic and histopathological evaluations. In the first ultrasound assessment, all groups had an acute increase in renal length ( $p < 0.048$ ). The group with 8 - 0 suture showed a difference in ureteral diameter on the 5th day ( $p < 0.01$ ) and all groups showed a reduction in diameter on the 30th day. The group with 6 - 0 suture showed a high mortality rate during the experimental period and the histopathological evaluation exhibited areas of necrosis. According to the ultrasound evaluation caliber 10 - 0 achieved better results in, macroscopic and microscopic evaluations. The finer suture diameters had better outcomes according to the ureteral inflammation scores.

## Conclusions

The use of 10 - 0 suture resulted in lower rates of ureteral inflammation, renal changes and postoperative complications when compared to two other sizes of the same material.

## Background

In ureteral sutures, the ideal surgical and suture material are necessary for the microsurgical technique, and the diameter of the suture material is especially important due to its correlation with cases of morbidity and mortality in these procedures.

Ureteral surgeries in companion animals can be indicated for the treatment of trauma, congenital anomalies or obstructive uropathies due to intra or extramural injuries [1]. Ureterotomy is one of the most used and traditional surgical techniques, especially for removing ureteral calculi in different species [1-11].

After surgery, ureters have a delayed repair period and any intervention can cause disturbances during remodeling process [12]. This fact, associated with the small size of the structures in animals, demands the use of an intraoperative microscopy to promote visual magnification and to perform complex procedures. [13–15].

Suture materials play an important role, providing support for tissue healing during wound repair, and, in microsurgery, non-absorbable sutures have effective clinical responses [16]. The use of nylon monofilament induces minimal tissue reaction and maintains high levels of elasticity after implantation in the tissue, configuring a good choice when tissue edema and inflammation may occur [17, 18].

Despite the increasing use of microsurgery in several fields of veterinary medicine [14], descriptions of microsurgical ureterotomies [1, 3, 4, 6–11] have been associated with several postoperative complications, such as uroabdomen, edema at the suture site and post-surgical stenosis, resulting in high mortality rates [4, 19]. It is worth noting that these studies use 5.0 to 8.0 suture gauge, do not standardize the magnification of the microscope and do not specify the needle used, therefore, it is likely that complications are associated with sutures that cause extensive tissue damage and compromise the lumen, predisposing to chronic inflammation, fibrosis and stenosis [12].

The objective is to compare and evaluate the most appropriate diameter of nylon to perform ureterorrhaphy using the microsurgical technique in rabbits. The hypothesis is that the use of 10 - 0 suture provides less tissue reaction and adequate sealing of the micro sutures, reducing postoperative complications associated with ureterotomies, such as uroabdomen.

Therefore, the present manuscript contributes to elucidate the importance of suture material and size as predictors for the successfully execution of microsurgical ureterorrhaphy and the association with the correct intraoperative magnification. Researches comparing tissue reactions with suture sizes in these procedures are scarce.

## Results

In the first ultrasound assessment, performed on the fifth post-operative day, the 3 groups showed a difference in renal length ( $p < 0.048$ ) in relation to the control, but not between them, and, although all of them presented larger measurements in width, this was not significant. It was possible to observe that the measurements increased according to the thickness of the used suture. Only the N8 showed a difference in relation to the ureteral diameter ( $p < 0.01$ ) (Fig. 1). There was renal pelvis dilation in 4/5 N6 animals; 5/5 animals from the N8 and 3/5 from the N10. None of the 15 animals presented lithiasis in the first ultrasound assessment.

During the 25-day interval between the two ultrasound assessments, 4 N6 animals died due to severe peritonitis. Thus, when comparing renal morphometric measurements, it is possible to observe a difference between all groups, including the control, with the N6 ( $p < 0.0001$ ). The measurements of renal length and width between N8 and N10 showed no difference. The ureteral diameter of these two groups

was similar at the 30th day, in which N8 decreased considerably compared to the first assessment. The only N6 ureter evaluated at the 30th day was not dilated. No pelvic dilation was found in groups N8 and N10, however, there was lithiasis formation in 2 animals operated with 8 - 0 suture (Fig. 2).

The 4 animals that died on the N6 had a large amount of exudate with blood in the abdominal cavity and diffuse peritonitis (Fig. 3). The only surviving animal developed hydronephrosis. Group N8 obtained the largest number of animals with altered kidney contour (2/5), in which, when cut, these kidneys showed changes in the corticomedullary junction pattern and the presence of caseous content. In N10, a single kidney developed hydronephrosis with the presence of a caseous content at the cut. Only the N8 presented alteration of the ureteral form macroscopically, where one of them dilated considerably and the other developed stenosis. In N6, a ureter presented blackened areas in the kidneys and at the suture site (Fig. 4).

On microscopy, a total of 11/15 of the kidneys evaluated had focal inflammations, where 3 of these belonged to N6; 5 to N8 and 4 to N10 (Fig. 5). The infiltrates were characterized mainly by heterophiles, lymphocytes and rare plasmocytes associated with tubules enlarged in size, with large and multiple cytoplasmic vacuoles, with evident and pycnotic nuclei, characterizing active chronic interstitial nephritis. Only 2 kidneys from subgroup N8 and 1 from subgroup N10 showed mild focal fibrosis, showing no difference with the control group. 2/5 of the kidneys at N6 showed areas of necrosis.

As for ureteral inflammation, the 3 groups showed a difference when compared to the control group, with the highest scores associated with thicker suture materials (Fig. 6). In N6, proliferation of giant cells encompassing the suture was observed, associated with a high proliferation of periureteral connective tissue (Fig. 7).

## Discussion

Commonly 6 - 0 to 8 - 0 gauge are used for ureteral suture in felines [1-11], whose outside diameter is equivalent to that of New Zealand rabbits, corresponding to 1 mm [20]. The thickness of the 6 - 0, 8 - 0 and 10 - 0 sutures represents, respectively, 20  $\mu$ m, 40  $\mu$ m and 70 to 99  $\mu$ m [21], and although the three diameters are considered thin for general surgical procedures, it is noted that, for millimeter structures such as the ureter, this difference is an important characteristic regarding to the non-compromise of the lumen. Using thicker sutures, coupled with traumatic needles and with the inappropriate aid of magnification, makes ureterorrhaphy a challenging procedure [11], corroborating the high rates of complications described so far [4, 6, 7, 11].

Due to the widespread use of rabbits in experimental research, some studies [22-24] describe and compare the measurements and sonographic characteristics of healthy animals. They all point to a positive correlation between kidney measurements and animal weight, which can also be observed in this study, because, despite the measures of renal length and width of the control group, consisting of the non-operated contralateral kidney and ureter, being smaller than those described for white New Zealand rabbits [24], the animals used here had a lower average weight, justifying the difference found. In

addition, another study [23], obtained similar renal measurements in 21 mixed breed animals, having an average weight equivalent to that reported in this study.

Changes in kidney size have been correlated with kidney disease and some specific pathological processes, such as hydronephrosis, are involved in increasing length and width [25]. In the first ultrasound assessment, all groups obtained differences with the control group, but not between them when comparing renal length. The width obtained more discreet increases, showing no difference with the control. Considering that acute changes result in larger kidneys, it is possible to correlate the increase in these measures with inflammation and ureteral edema resulting from the surgical procedure [11], causing luminal narrowing and urine accumulation in the kidney in different proportions. These results are reinforced by comparing the data obtained in the second ultrasound assessment, where the difference significant length and width were no longer with the control group but with the N6, due to the loss of experimental plots.

The remodeling and the repairing of the operated tissue is directly related to the diameter of the suture material [26], so, with the exception of the renal width at the 30th day, it is possible to observe that, in the two ultrasound evaluations, the renal measurements developed largest changes according to the greater caliber of the suture used. In relation to the renal pelvis, when there is no dilation, this structure is not normally seen on ultrasound and subtle dilations may also go unnoticed [23, 27]. The dilation indices obtained in this study corresponded to the ureteral dilation seen in the ultrasound exams, in which the N8 had the largest ureteral diameters in the first exam and all animals in this group presented the pelvis dilated. The other two groups showed decreasing changes for pelvic dilation and ureteral diameter, according to the thickness of the suture material used (4 animals in N6 and 3 in N10). At 30 days, these two parameters decreased in all groups, particularly in N6 due to the loss of experimental plots, reaffirming that the increase in measurements occurs mainly due to acute changes [11, 27].

The ureters follow the pattern of the renal pelvis and are only seen on ultrasound when they are dilated [3, 7, 27, 28] and the group operated with 8.0 suture was the only one that developed a significant increase in diameter in the first ultrasound assessment. The animals in this group also had high scores for ureteral inflammation, presence of stenosis and dilation at the suture site, in addition to macroscopic renal changes in greater numbers. Although widely used to perform ureterorrhaphy [1–11], 8 – 0 sutures are associated with high rates of postoperative complications, making them unsuitable for this procedure in ureters of rabbits, or other species with similar ureteral diameter. Although the 10 – 0 obtained slight increases in ureteral diameter in the first and second exams, these were not significant and reduced considerably between the two evaluations. These results suggest that the use of a thinner material in the ureteral suture is more appropriate [11].

The non-dilation of the ureters of group N6 is associated with urinary leakage into the abdominal cavity and consequent uroabdomen, since the majority of animals operated with this gauge of suture died between the two evaluations and a substantial amount of exudate on necropsy exams, associated with severe peritonitis was found. The uroabdomen is the most frequently described postoperative

complication in association with ureterotomies [1, 3, 4, 7–10, 19, 29]. However, confirmatory tests comparing serum creatinine and that present in free abdominal fluid were not performed.

Although non-absorbable suture materials are not recommended for use in urinary tract surgeries due to the greater probability of acting as a starting point for the stones formation [17], a recent study [26] evaluated the behavior of 3 synthetic absorbable suture materials in urinary vesicles of rabbits in 2 phases and the formation of lithiasis was observed between the 3rd and 6th week in two of the groups evaluated. Thus, the formation of lithiasis seems to be associated with the contact of the suture with the urine, and the tissue response is directly related to the diameter used or to the presence of inflammation, infection, urinary pH and longevity of the material [17, 30]. In the present study, the group operated with 8 – 0 was the only one to present formation of lithiasis in the 4th week after surgery, a similar time to the study mentioned above, and it is likely that, because it has a thicker caliber, this suture has come into contact with the urothelium. The non-formation of stones in the N6 group may be associated with the early death of the animals. Microscopically, in the N6 group, areas of necrosis were observed, validating the blackened areas found in macroscopy. Even though this group presented the same amount of alterations in the 10 – 0 caliber renal form, this can be explained by the early death of the animals due to extravasation and peritonitis. This fact is also associated with the non-occurrence of fibrosis in this group, since this response results from prolonged inflammatory processes [7, 11].

Any ureteral surgical intervention can cause a disturbance in the healing remodeling phase, resulting in fibrosis, obstruction in various degrees and consequent hydronephrosis [12]. Therefore, despite presenting hydronephrosis and renal and ureteral inflammatory changes, according to the results presented, it is noted that these numbers are lower with the 10.0 suture and that these changes occur mainly acutely, in the immediate postoperative period, reducing with the progression of ureteral healing. The limitations of this study include not performing tests for serum creatinine and abdominal fluid to confirm urinary leakage in one of the groups, as previously discussed, and not performing a histopathological evaluation at 5 days postoperatively, to ascertain the changes caused acutely by sutures, this was not done in order to reduce the number of animals used, since it would be necessary to have a larger number of samples in each group for the histopathological evaluation in two stages.

## Conclusions

In conclusion, the 10.0 suture resulted in lower scores of ureteral inflammation at the suture site, low postoperative complications associated with the procedure, in addition to minor acute and chronic changes in the kidney, making the diameter suitable for performing ureterorrhaphy in rabbits. Additional experimental and clinical studies need to be carried out in the long term to elucidate changes in the urinary tract and evaluate the survival rate of the animals submitted to the procedure.

## Methods

### Study design

The present study was conducted in full compliance with all applicable research ethics and animal welfare regulations, under a general stranding response authorization by the Animal Use Ethics Committee (CEUA) of the Northern Fluminense State University Darcy Ribeiro, under the protocol number: 975023, according to Brazilian federal law n ° 11794/08. The study design was an experimental study.

## Animals

The *Oryctolagus cuniculus*, 15 male, adults, New Zealand rabbits, were obtained from Northern Fluminense State University Darcy Ribeiro vivarium sector, weighing between 2.5 and 3.25 kg (average of 3.02 kg); considering anatomical similarities with cats, especially ureter diameter, they were used as an experimental model for ureterorrhaphy. The animals were kept at the experimental unit in individual cages with feeding system and water ad libitum.

## Anesthetic Protocol

The animals were contained manually and received a pre-anesthetic protocol based ketamine hydrochloride, at a dose of 30 mg/kg/ IM, associated with xylazine hydrochloride at a dose of 3 mg/kg/IM. After obtaining the desired anesthetic effect, trichotomy was performed on the left and right pinna, followed by catheterization of the marginal vein of the left external ear. This access enabled saline 0.9% infusion through syringe pump with a controlled dose of 15 ml/hr. Finally, a propofol bolus was intravenously administered at a dose of 5 mg/kg, and then tracheostomy and intubation were performed, enabling the realization of manual ventilation. Tracheal tube number 3.0 was connected to an inhalation anesthesia machine through the Baraka system, and keeping the oxygen at 100% throughout the procedure. The animals were maintained under anesthesia with intermittent bolus application of propofol.

## Ureterotomy

All surgical procedures were performed by the same surgeon and, as part of the preparation; the animals were subjected to the standard aseptic technique, caudal to the xiphoid process extending to the pubic region. The patients were positioned in dorsal recumbency position, with surgical antisepsis performed by the surgeon, with subsequent positioning of the sterile fields.

Surgical access was performed through median retro umbilical laparotomy. The right ureter was identified and isolated from the retroperitoneum. A vascular clamp was placed over the middle third of the ureter in order to stabilize and isolate the incision site, in addition to temporarily occlude urinary flow. From that moment on, the operating microscope was used (20x magnification) and a 2 mm longitudinal incision was performed in the ureter using a number 11 surgical blade attached to the number 3 scalpel handle.

The animals were randomly divided into three groups of five, each one with a different size of nylon for ureterorrhaphy 6 – 0, 8 – 0 and 10 – 0, groups N6, N8 and N10, respectively. The contralateral ureter was considered as the control for the animal itself. Three partial perforating sutures were performed (Fig. 8) in a simple discontinuous pattern with an atraumatic needle. Immediately after the vascular clamp was

removed, and the presence of possible urinary leakage was verified, the ureter was anatomically repositioned. The abdominal cavity was closed using 2 – 0 nylon suture with a Sultan suture pattern in the muscle layer; polyglactin 910 of 2 – 0 gauge with Cushing suture pattern to approach the subcutaneous tissue and nylon 3 – 0 in simple continuous pattern for skin suture.

## **Antimicrobial and analgesic protocol**

The therapies were instituted in the immediate postoperative period and lasted for 5 days, in which ceftriaxone was administered at a dose of 40 mg/kg, twice a day, IM; metamizole sodium at a dose of 25 mg/kg, once a day, subcutaneously and tramadol hydrochloride, at a dose of 5 mg/kg, twice a day, IM.

## **Ultrasound evaluation**

Ultrasound evaluations occurred in two parts, on the 5th and 30th postoperative days, respectively. The examinations were evaluated the length and width of the kidney, the presence of nephrolith, dilation of the pelvis, the diameter of the ureter and the presence of free liquid in the abdominal cavity. The animals were not sedated for the examination, then, positioned in dorsal recumbency on a padded trough and an acoustic gel was applied to improve the contact between the gel and the transducer. The evaluations were performed using Mindray® model Z6 equipment, with linear and convex multifrequency transducers, ranging between 3 and 10 megahertz (MHz's)

## **Euthanasia**

The animals were euthanized 30 days after the surgical procedure. Rabbits were pre-medicated with ketamine hydrochloride (30 mg/kg) and Midazolam (3.0 mg/kg), IM. After 15 minutes, the animals had the marginal vein of the left auricular pavilion cannulated with a 24 Gauge (24G) catheter and an overdose of propofol was administered. After achieving the right anesthetic plan, 5 mL of IV potassium chloride was administered.

## **Macroscopic evaluation**

During necropsy, the entire abdominal cavity was examined in order to compile macroscopic changes related to the surgical procedure. The kidneys and ureters were analyzed for the form and presence of hydronephrosis or dilation, respectively. The abdominal cavity was assessed for the presence of exudate.

## **Microscopy evaluation**

Tissue fragments were obtained from the kidneys and the ureteral portion containing the suture and proceed to fixation in 10% neutral formalin buffered for at least 48 hours. Then, the samples were cleaved and subjected to histological processing in the TP 1020 Leica automatic processor, obtaining histological sections of 5 micrometers thick. The sections were subjected to hematoxylin and eosin staining (H / E) and analyzed by an experienced pathologist through light microscopy.

## **Statistical analysis**

Using the software Graphpad Prism version 5.0, the analysis of the quantitative variables was done through one-way Analysis of Variance (ANOVA), with a subsequent Newmann-Keuls, Tukey and T Student average test, depending on each case, if  $p < 0.05$  (99.95% reliability). For qualitative variables, the Mann-Whitney, Friedman and Wilcoxon tests were used.

Histological results were analyzed using a median of scores (descriptive statistics), by grading the severity of the lesions found, according to the independent observer's assessment. Scores were assigned according to the estimated percentage of lesions appearing in the observed field.

(Tables 1e 2).

Table 1  
Scores attributed according to the estimated percentage of the appearance of inflammatory cells in the observed ureters.

Percentage of Lesions	Classification	Score
Until 25%	Light	1
From 25 to 50%	Moderate	2
From 50 to 75%	Intense	3
Above de 75%	Severe	4

Table 2  
Scores attributed according to the estimated severity of fibrosis and degeneration in the evaluated kidneys.

Classification	Score
Absent	0
Discrete focal	1
Discrete multifocal	2
Moderate multifocal	3
Accentuated diffuse	4

## Abbreviations

IM: Intramusculary

G: Gauge

IV: Intravenously

MHz: Megaheartz

## DECLARATIONS

# Declarations

### **Ethics approval and consent to participate**

The present study was submitted to Animal Use Ethics Committee (CEUA) of the Northern Fluminense State University Darcy Ribeiro and approved under the protocol number: 975023, according to the federal law n ° 11794/08. The experiment was conducted in full compliance with all applicable research ethics and animal welfare regulations developed by The National Animal Experimentation Control Board (CONCEA).

### **Consent for publication**

Not applicable

### **Availability of data and materials**

The datasets supporting the conclusions of this article are included within the article. The raw data are available from the corresponding author on reasonable request.

### **Competing Interests**

Not applicable

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### **Authors Contributions**

TMBP was responsible for the experiment execution, handled the animals, performed the literature review, data collection, data analysis and wrote the paper; JPS was the surgeon responsible for executing all the surgical procedures as well as a co-supervisor for the execution of this idea; LMM was responsible for the anesthesia of the animals in all surgical procedures; RPD made a major contribution to the logistics and execution of the experiment; MRF performed all ultrasound assessments during the experiment; MBRGP was responsible for the macroscopic and histopathological analysis of the obtained renal and ureteral samples; ICM was essential helping to write the article; FA was responsible for the statistical analysis of

all the obtained data; ALAO idealized, proposed and helped to perform the project, also reviewed the article.

## Acknowledgments

Not applicable

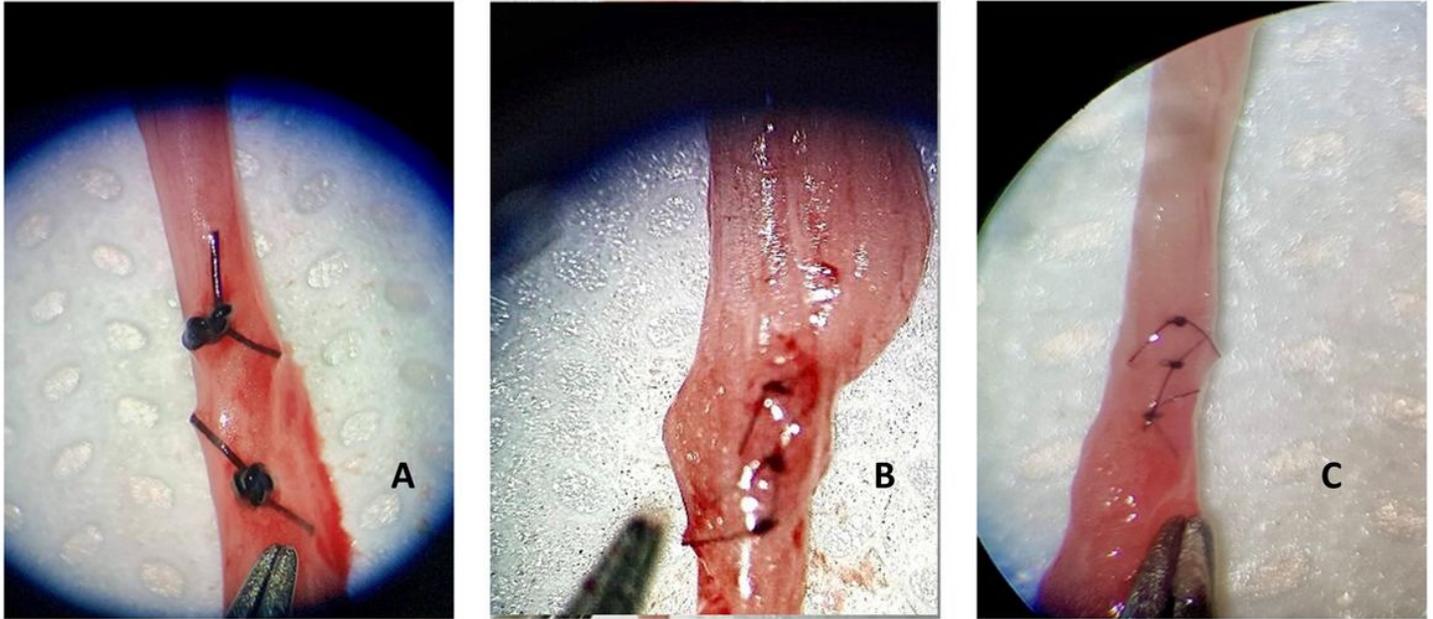
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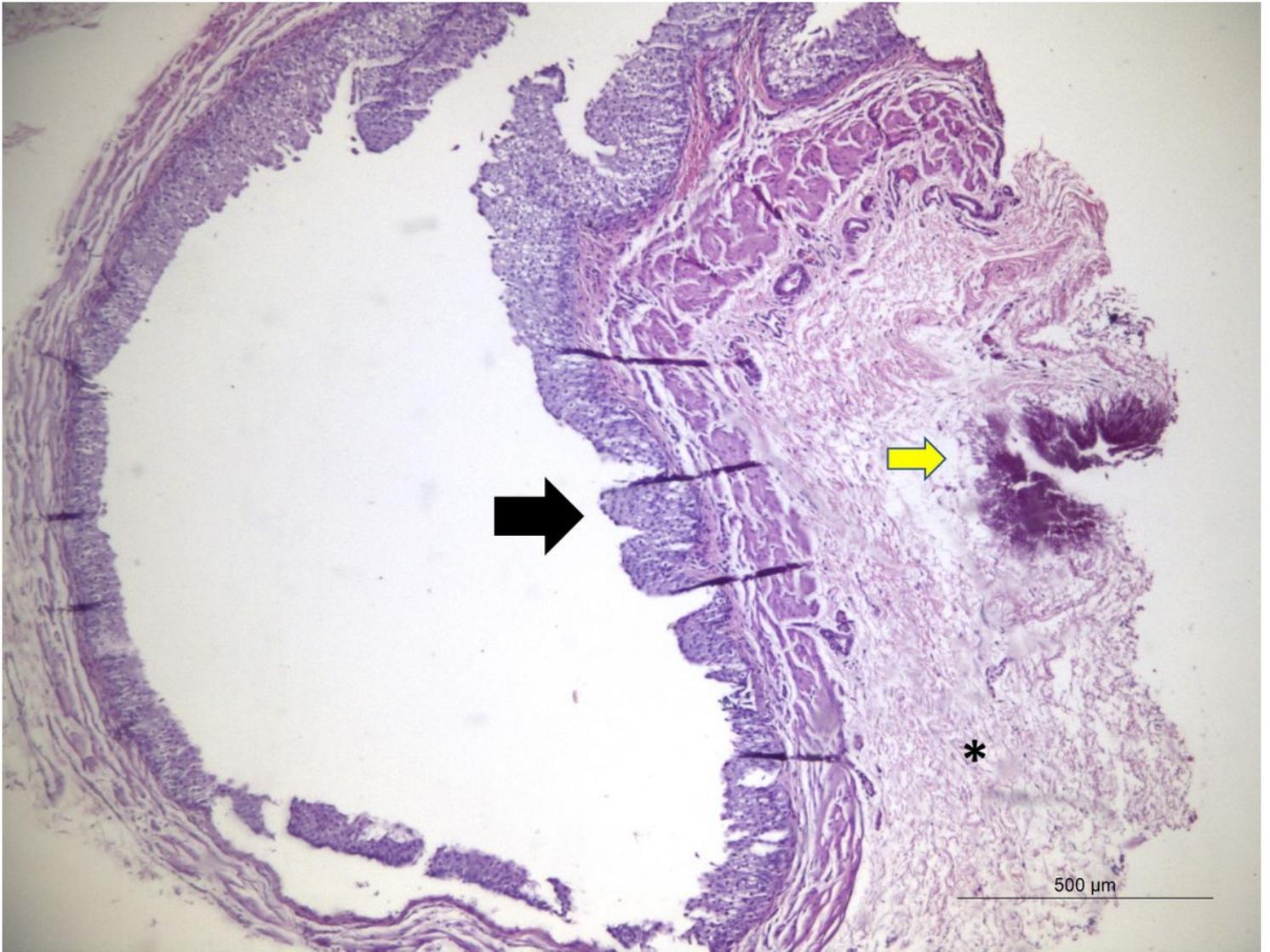
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## Figures



**Figure 1**

Comparison of sutures in different groups.



**Figure 2**

Ureteral dilation in a group N8 rabbit.

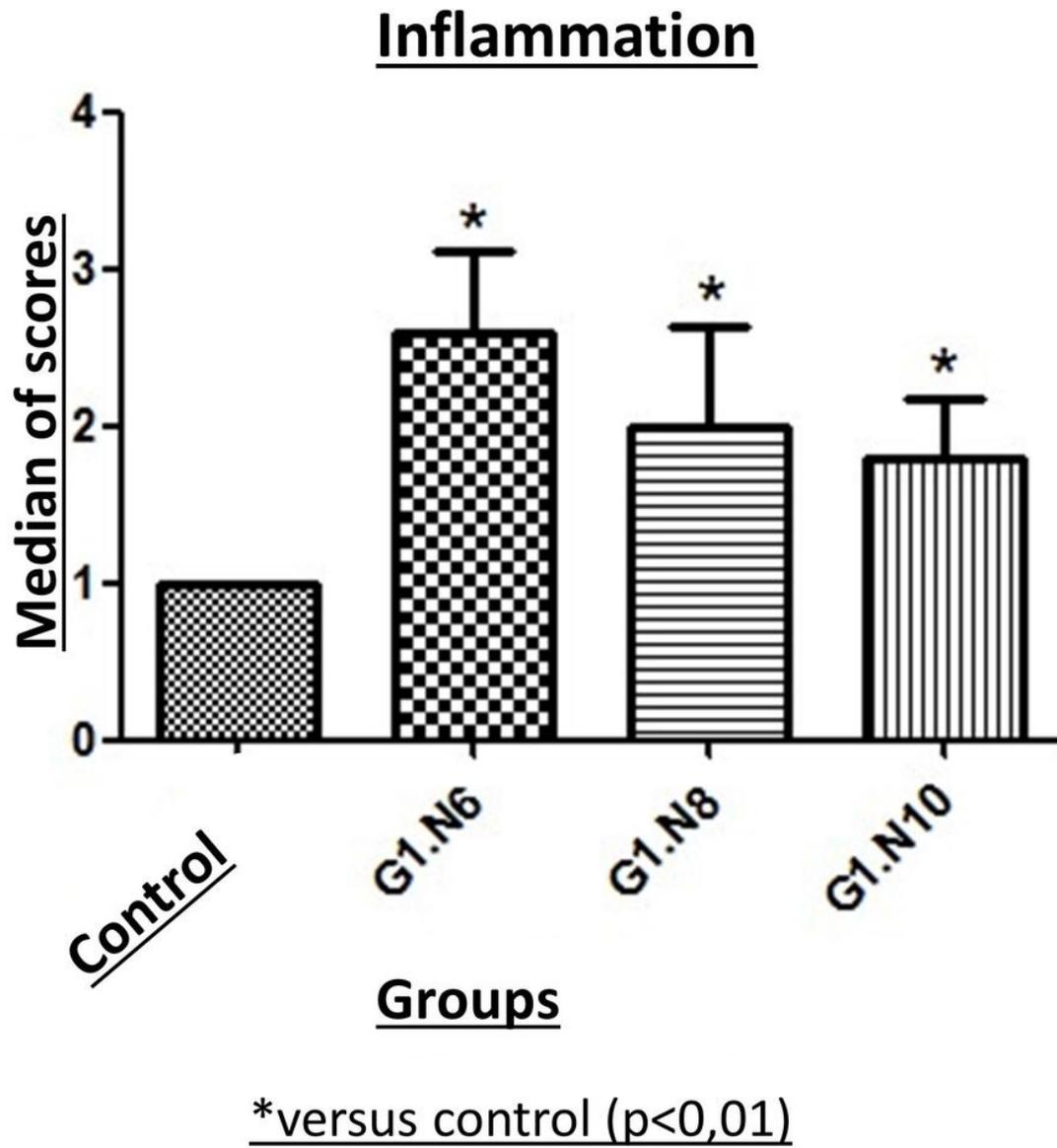
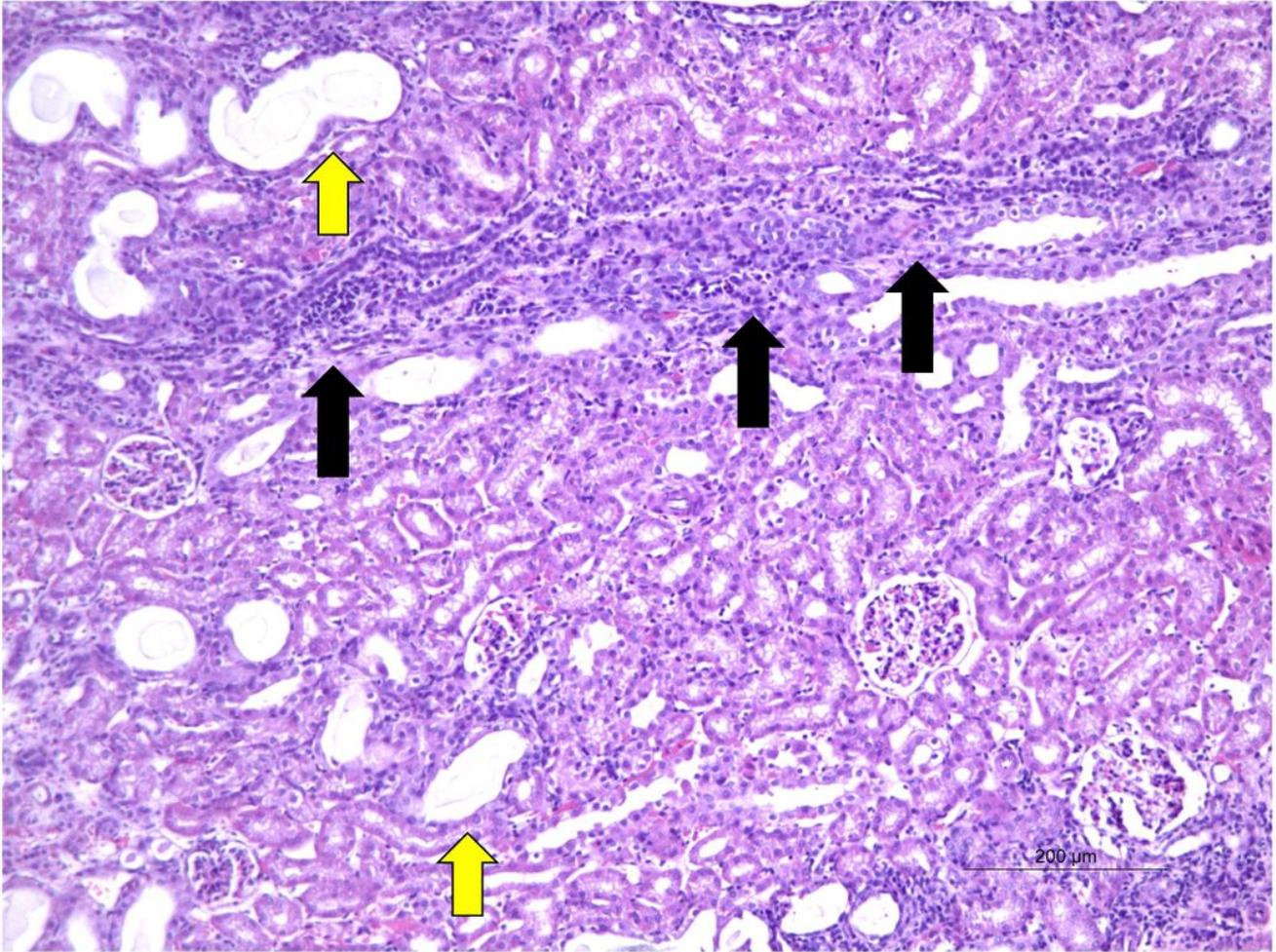


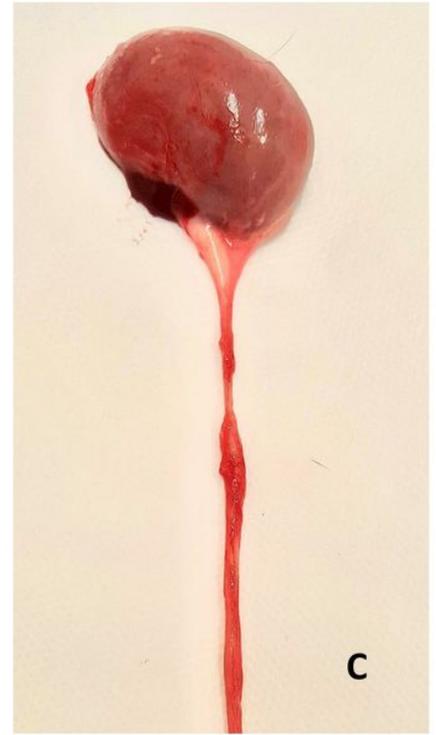
Figure 3

Degree of inflammation at the surgical site in the different groups.



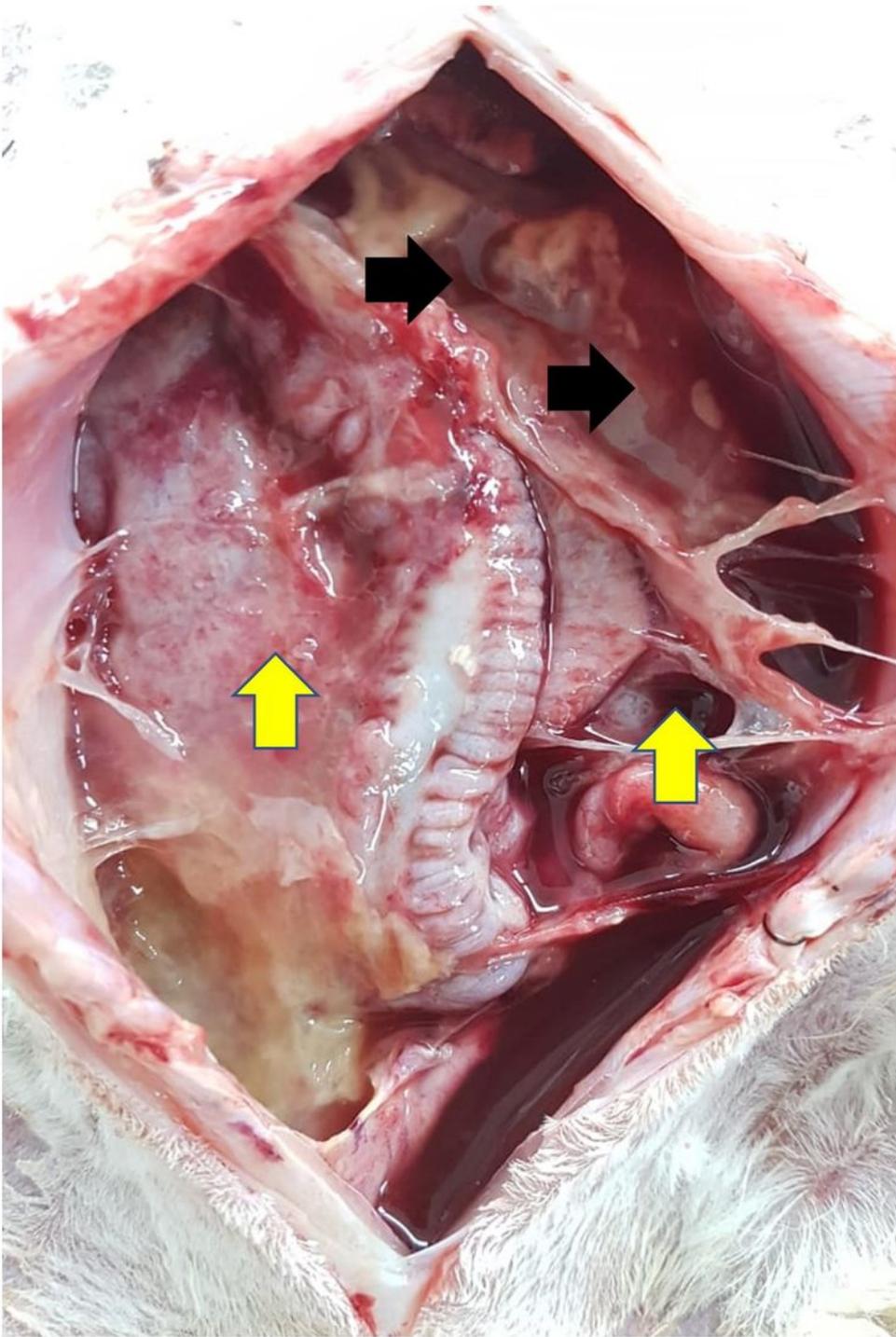
**Figure 4**

Microscopic analysis of renal morphological alterations in group N6.



**Figure 5**

Macroscopic analysis of the ureteral surgical site.



**Figure 6**

Abdominal macroscopic changes observed in the N6 group necropsy.

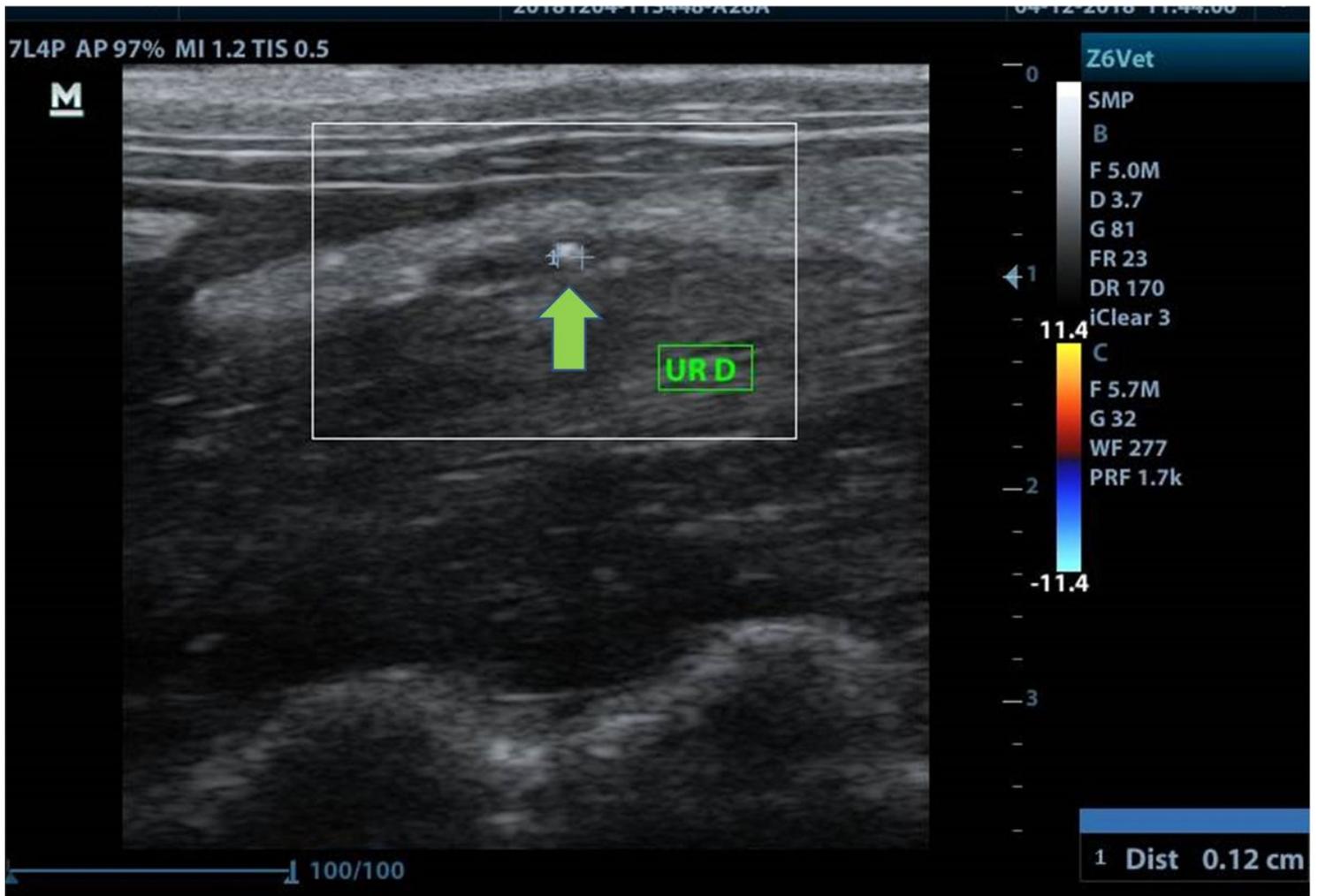


Figure 7

Ureterolith at the suture site.

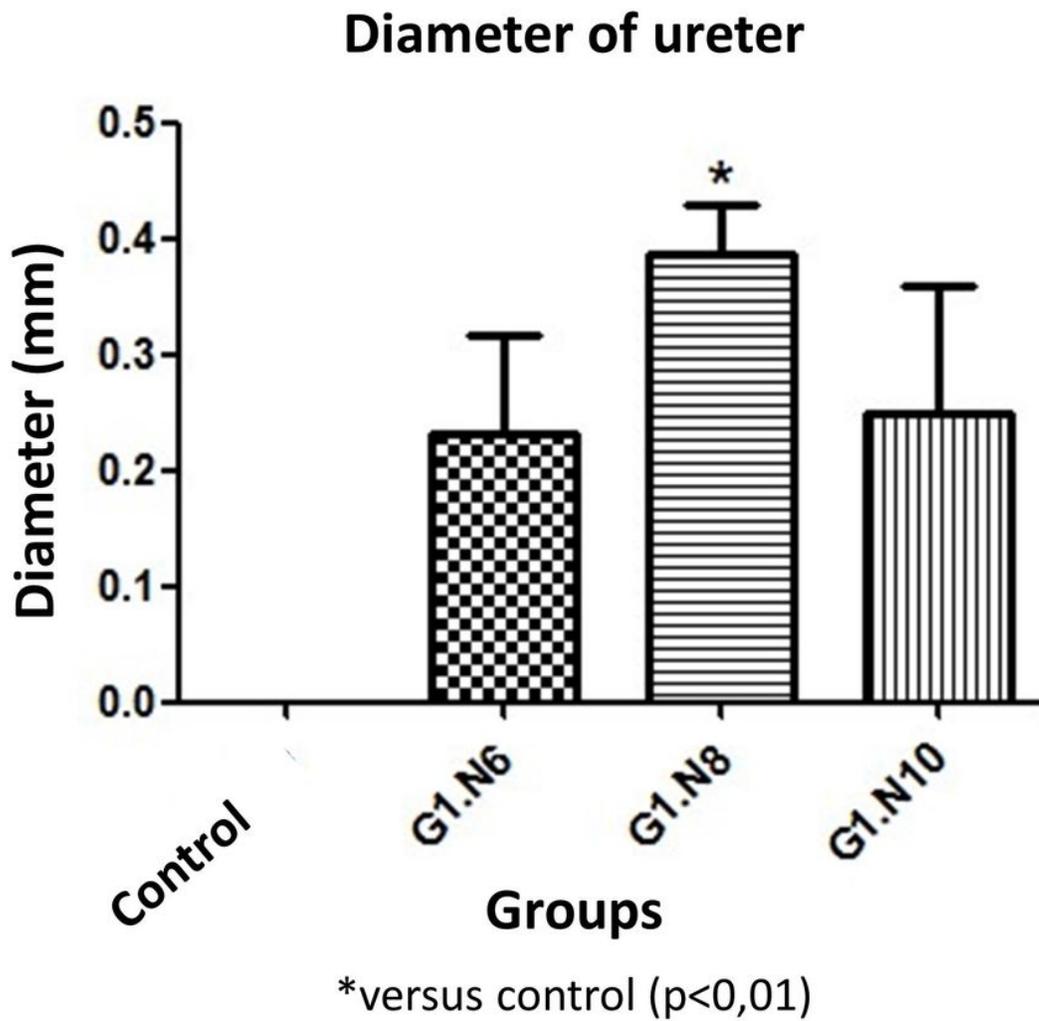


Figure 8

Acute change in ureteral diameter at the surgical site.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [ARRIVEGuidelinesChecklistTMBP.pdf](#)