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Obstructive urolithiasis in buffalo calves and surgical management using tube cystostomy technique: Assessment of oxidative stress and antioxidant enzyme

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Abstract

Obstructive urolithiasis is a serious problem that negatively affects the quality of life in buffalo calves. The present study aims to evaluate the relationship between oxidative stress, antioxidant enzyme activity, and obstructive urolithiasis in buffalo calves and tube cystostomy as a surgical technique to treat such conditions. Seventy-seven non-castrated male buffalo calves aged 3-10 months were admitted to the Faculty of Veterinary Medicine clinic, Zagazig University, Egypt, with a history of anuria. Based on clinical, ultrasonographic, and biochemical examination, the calves were diagnosed with either intact bladder (n=32 calves) or ruptured bladder (n=45 calves). Ten healthy buffalo calves of the same age were used as a control for a reference range of biochemical analysis. Serum levels of malondialdehyde (MDA) significantly increased in all affected calves, especially those with ruptured bladders. In addition, a significant decrease in glutathione peroxidase (GSH) was reported in all affected calves, with lower values in calves with ruptured bladders. The tube cystostomy technique succeeded in 95.95% of the operated cases, and only 4.05% had complications. In conclusion, there is a relation between oxidative stress, antioxidant activity, and obstructive urolithiasis in buffalo calves with useful management using the tube cystostomy technique.

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Introduction

In veterinary medicine, obstructive urolithiasis is one of the most frequent diseases requiring an examination, particularly in indoor breeding buffalo calves. In ruminants, it indicates the presence of calculi, which typically lodge at the sigmoid flexure (1). It was discovered to be among the main health issues negatively impacting buffalo calves' quality of life (2). Because the male urinary tract is anatomically structured, it is more common in men than women. Furthermore, buffalo male calves are more vulnerable than cow calves (3). When the bladder is intact, obstructive urolithiasis may develop, necessitating immediate surgical intervention to prevent complications (4), or when the bladder ruptures, urine flows into the peritoneum, causing uremia and peritonitis (5), diagnosis of such disorder based on ultrasonography, laboratory, clinical, and historical data. The most non-invasive imaging method for urolithiasis diagnosis and prognosis is ultrasound (6). Surgery is the primary treatment for this illness (7). Perineal urethrostomy (8), urinary bladder marsupialization, penile catheterization and amputation (9), and tube cystostomy (10) are examples of surgical intervention. A surgical procedure known as a tube cystostomy allows urine to run freely through a tube fixed in the bladder. It is followed by the chemical dissolving of calculi, which has great results (11-13). The imbalance between the generation of pro-oxidants and their removal by antioxidant activity is known as

oxidative stress. Thus, oxidative stress, caused by increased pro-oxidants such as reactive oxygen species (ROS) and free radicals inside the cells, causes tissue damage (14). Oxidative stress is given more attention in animals than in humans. The most common causes of oxidative stress in veterinary medicine have been metabolic and inflammatory disorders and environmental factors, including stress and diet (15). Understanding the pathology of oxidative stress in the bladder is crucial for understanding the causes of diseases and treatment approaches, as oxidative stress and pathological mechanisms of urinary bladder dysfunction are intimately associated with humans (14,16). Numerous enzymes, including glutathione peroxidase (GSH), catalase (CAT), and superoxidase dismutase (SOD), are part of the antioxidant defense system, which neutralizes excess oxygen species and guards against damaging processes brought on by oxidative stress (17). Partial bladder outlet obstruction (PBOO) has been shown to enhance ROS generation, which in turn causes an increase in malondialdehyde (MDA) and a decrease in SOD (18). Antioxidants work together to protect against oxidative stress in pathological situations. For instance, SOD transformed ROS to hydrogen peroxide, whereas CAT and GPX broke down hydrogen peroxide into water and oxygen (19). Understanding the pathological aspects of urinary bladder dysfunction requires assessing the state of oxidative stress and the biological activities of antioxidants in systemic or bladder tissues.

Therefore, the present study evaluated the relation between oxidative stress, antioxidant enzyme activity, and obstructive urolithiasis in buffalo calves and the tube cystostomy technique as a surgical method to treat such a problem.

Materials and methods

Ethical approval

The animal handling and surgical procedures were performed according to the guidance of Zagazig University's animal ethics committee (ZU-IACUC/2/F/158/2024).

Animals and study design

Seventy-seven non-castrated male buffalo calves aged 3-10 months were admitted to the Faculty of Veterinary Medicine clinic, Zagazig University, Egypt, from October 2022 to October 2023, with a history of anuria. Ten healthy buffalo calves of the same age were used as a control.

Clinical examination

All calves were subjected to thorough clinical examination (1). Data regarding age, duration of retention, and treatment trials were reported. Body temperature, heart rate, and respiration rate were reported. The degree of dehydration through the skin tent test and the color of the mucus membrane was evaluated. Fluid thrilling and distention of the abdomen were examined. Abdominocentesis was performed in animals with urinary bladder rupture just behind the umbilicus under aseptic precautions using a sterile 18-gauge needle.

Biochemical analysis

Whole blood samples from the jugular vein of each animal were collected in sterile tubes without anticoagulant to obtain sera. Serum levels of calcium (Ca), inorganic phosphorus (P), blood urea nitrogen (BUN), and creatinine were measured using diagnostic Zrt. Commercial kits, Biomerieux. Serum levels of MDA and GSH were measured using ELISA kits (Cat No. CSB-E12144r).

Ultrasonographic examination

The urinary bladder and urethra of the affected calves were examined ultrasonographically using an ultrasound machine (Sonoscape A5V, China) connected with a 5 MHz transducer for transabdominal examination just cranial to the pelvic rim in a standing position and a 6 MHz transducer for transrectal examination.

Surgical management

The affected animals with good health (n=74) were treated surgically using tube cystostomy in dorsal recumbency at the left paramedian aspect under local infiltration anesthesia as described previously (2-5). Briefly, linear infiltration anesthesia using lidocaine HCL 2% (Debocaine®, the Arab Company, Obour City, Egypt) was performed by extending 10 cm cranial to the rudimentary teat at the left paramedian region after aseptic preparation. Highly movable animals during securing were sedated using Xylazine HCL 2% (Xyla-Ject®, ADWIA Pharmaceuticals Co., 10th of Ramadan City, Egypt) at a 0.2 mg/kg dose. A 10 cm laparotomy incision at the site of anesthesia was performed parallel to the prepuce. After abdominal exploration, an 18 French Foley catheter was implanted into the urinary bladder through a new abdominal wall stab incision (Figure 1). Then, the catheter was fixed into the abdominal wall using a stitch. Cystorrhaphy after removal of cystic calculi and debridement of necrotic parts was performed before catheter implantation in calves with bladder rupture. Abdominal lavage using sterile 0.9% NaCl saline solution was performed before laparotomy incision site closure as routinely performed.

Postoperatively, Intramuscular injection of a broad-spectrum antibiotic (Pen&Strep®, Norbrook Co., N. Ireland, 1 mL/25 kg) and anti-inflammatory (flunixin meglumine, Flunixin®, Norbrook Co., N. Ireland, 2.2 mg/kg) were performed for 5 and 3 days, respectively. Ammonium chloride at a dose rate of 200 mg/Kg body weight. dissolved in 40 mL water was orally administered for 20 days to dissolve calculi. The Foley catheter was plugged intermittently for 30 min/3 times/day until normal urination occurred.

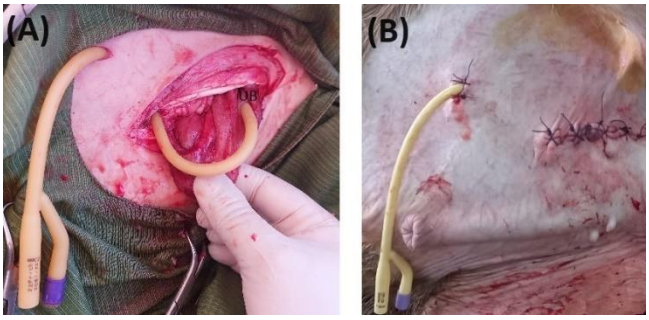

Figure 1: Urinary bladder catheter implantation. (A) The Foley catheter is implanted inside the urinary bladder in a buffalo calf through an abdominal incision. (B) The catheter was fixed outside at the abdominal wall with a stitch—UB: Urinary bladder.

Statistical analysis

Results in the present study were reported as mean ± SEM (Standard Error of Mean) and analyzed using Statistical Package for Social Sciences version 24.0 (SPSS, IBM Corp., Armonk, NY). The statistical differences between the serum levels of MDH, GSH, Ca, P, urea, and creatinine in the control and affected calves were investigated using a one-way variance analysis (ANOVA) followed by Tukey's multiple comparisons test as a post hoc test. Statistical significance was set at $P<0.05$.

Results

Clinical findings

After thorough clinical and ultrasonographic examinations, the affected calves were classified into calves with intact bladder (n=32 calves) and calves with ruptured bladder (n=45 calves). The clinical findings of affected calves with obstructive urolithiasis are summarized in (Table 1). Calves with intact bladder showed signs of stranguria or anuria, inappetence, reluctance to walk, abdominal pain, and raised tail. The most observed signs were abdominal pain 90.63%, decrease or absence of ruminal motility 87.5%, and urethral pulsation 87.5%. The most observed signs of the ruptured bladder were a pear-shaped abdomen 82.2%, abdominal thrilling 95.56%, and uremic breathing 88.9%. Among calves with ruptured bladder, 3 animals (6.7%) were recumbent and had bradycardia with a louder heart sound. Rectal temperatures of all calves with intact bladder 100% were within the normal reference values (38-38.8ºC). In comparison, rectal temperatures of the calves with ruptured bladder ranged from 37.8 to 38.5˚C in 42 calves (93.3%) and 36.5 to 37.5°C in 3 calves (6.7%). Ruptured bladder calves were severely dull and depressed with sunken eyes and rough shingles hair coats.

Biochemical findings

The biochemical findings are summarized in (Table 2) and revealed a significant increase in serum levels of MDA in all affected calves, with higher levels in calves with ruptured bladders compared to the control animals. There was a significant decrease in serum level of GSH in all affected calves, with a higher decrease in ruptured bladder calves compared to the control animals. The serum calcium level in all affected calves was significantly lower than in the control ones. In contrast, the serum levels of urea and creatinine in all affected calves were significantly higher than the control ones, with a significant increase in calves with ruptured bladder.

Table 1: Clinical findings of calves suffering obstructive urolithiasis

Items	With intact bladder		With bladder rupture	
	Number (n=32)	Percentage (%)	Number (n=45)	Percentage (%)
Alert and restlessness	32	100%	8	17.8%
Dullness	2	6.25%	37	82.2%
Off food	23	71.8%	36	80%
Alteration of vital signs	16	50%	38	84.4%
Congested conjunctival mucosa	25	78.13%	33	73.3%
Decrease or absent rumen motility	28	87.5%	39	86.6%
Pain in abdomen	29	90.63%	-	-
Penile body twitching	30	93.75%	-	-
Pear shaped abdomen	-	-	37	82.2
Thrilling	-	-	43	95.56%
Rough coat	22	68.75%	39	86.7%
Bran-like scale on the skin	20	62.5%	35	77.78%
Uremic breath	-	-	40	88.9%
Urethral pulsation	28	87.5%	-	-
Rectal prolapse	1	3%	-	-
Recumbency	0	-	3	6.7%

Table 2: Mean ± SEM of biochemical parameters in control and affected calves

Items	Control	Calves suffering obstructive urolithiasis		P value
		with intact bladder	with bladder rupture	
MDH	0.56±0.048[c]	1.43±0.085[b]	6.53±0.357[a]	<0.0001
GSH	222±6.309[a]	104.90±5.086[b]	28.73±0.562[c]	<0.0001
Ca	8.11±0.180[a]	7.69±0.107[a]	4.98±0.0562[b]	<0.0001
P	4.23±0.064[c]	5.99±0.0369[b]	7.16±0.0302[a]	<0.0001
Urea	14.12±0.187[c]	37.07±0.459[b]	124.44±0.388[a]	<0.0001
Creatinine	0.78±0.014[c]	1.91±0.024[b]	4.81±0.088[a]	<0.0001

[abc] Means within the same row carrying different superscripts are significantly different (P < 0.05).

Ultrasonographic findings

Ultrasonography of calves with intact bladder showed a distended anechoic bladder sac with circumscribed hyperechoic contour inside the abdomen in transabdominal examination and distended pelvic urethra when examined transrectally. While ultrasonography of calves with ruptured bladder showed free anechoic fluid inside the abdomen with echogenic fibrinous jelly-like material in transabdominal examination and shrinkage of thickened wall bladder when examined transrectally (Figure 2).

Surgical findings and outcomes

The surgical procedure was performed for 32 (100%) calves with intact bladder and 42 (93.3%) calves with bladder rupture. In contrast, the remaining 3 cases with bladder rupture were excluded due to bad health conditions with recumbency. During surgery, the bladder condition was evaluated. In calves suffering retained urine with intact bladder, it was found that 32 (100%) cases had smooth and pinkish to bluish-coloured bladder. In calves suffering retained urine with ruptured bladder, it was found that 26 cases (61.9%) had thick, small-sized rosey red bladder, while 13 cases (31%) had moderately necrosed walls, and the remaining 3 cases (7.1%) had severely necrosed wall. The site of rupture was at the dorsal aspect in 27 cases (64.3%), the ventral aspect in 6 cases (14.3%), and subserosal rupture in the remaining 9 cases (21.4%). Interestingly, the bladder wall was adhered to omentum, mesentery, peritoneum, or intestine in 18 calves with ruptured bladder. The operated calves (n=74 cases) were discharged in a standing position after surgery and started feeding within 6 hours after surgery. The calves had recovered uneventfully without complications in 71 out of 74 cases (95.95%), while 3 out of 74 cases (4.05%) had complications; 1 had catheter failure, and 2 had digestive disorders. The operated animals returned normal urination within 3, 7, 14, 21, and 27 days after surgery in 9 (12.68%), 22 (30.99%), 26 (36.62%), 11 (15.49%), and 3 (4.22%) cases, respectively. The Foley catheter was removed after resuming normal urination from the external urethral orifice. Pursuing the operated calves for 7 months revealed normal urination without recurrence.

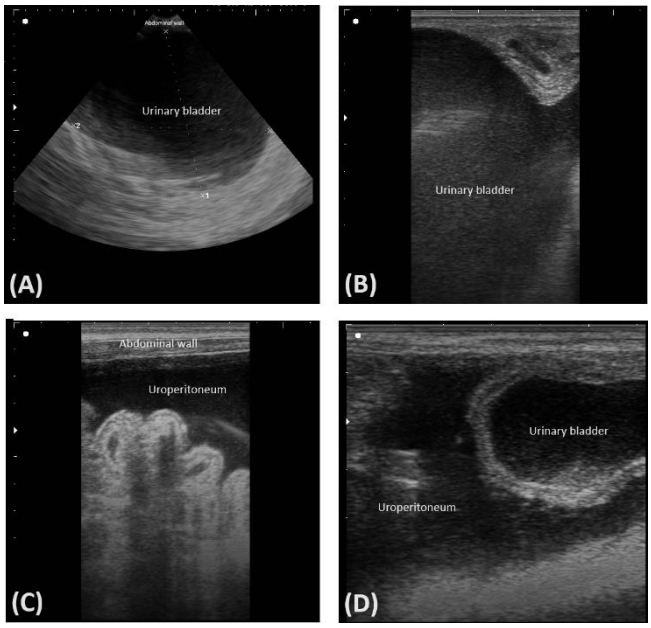

Figure 2: Ultrasonography of calves with obstructive urolithiasis. (A) Ultrasonographic image of the distended urinary bladder when scanned in front of the scrotum using a transabdominal probe in a 5-month-old male buffalo calf. (B) Ultrasonographic image of the distended urinary bladder when scanned transrectally in the same buffalo calf. The urinary bladder appeared as a distended fluid-filled anechoic sac with a hyperechoic wall. (C) Ultrasonographic image of the abdomen in a 6-month-old male buffalo calf with the ruptured bladder at the caudal abdomen using a linear probe, note free anechoic fluid inside the abdomen (Uroperitoneum). (D) Ultrasonographic image of the urinary bladder when scanned transrectally in the same buffalo calf with ruptured bladder. The urinary bladder appeared as a small anechoic sac with a thickened wall and uroperitoneum.

Discussion

Urolithiasis is a worldwide disorder affecting all species of domestic animals and is extensively reported in ruminants (6). It was reported in buffalo calves more than cow calves (7-9). In the present study, the age of the affected calves was

between 3 to 10 months. Similar findings were reported previously, where they reported that the age of the affected calves was 3-6 months (9), 3-7 months (4), 4-7 months (5), and 3-11 months (10). The incidence of the disease in young calves might be attributed to sudden diet changes to highly concentrated rations after weaning and lack of water intake in the winter season (4,11,20). In addition, the lack of testosterone in younger animals causes narrowing of the urethral diameter, and expelling the calculus will be difficult (9).

The main clinical findings reported in the affected calves with intact bladder included inappetence, anuria, restlessness, forceful attempts to urinate, grinding of teeth, reluctance to move, arched back, tachycardia, abdominal colic, urethral pulsation, raised tail, constipation and to and from penile movements. In bladder rupture, the main clinical signs were anorexia, dehydration, sunken eyes and congested conjunctival mucous membrane, dullness, distended abdomen, thrilling movement on abdominal percussion, uremic breath, hypothermia, and recumbency. Previous studies reported similar findings (4,9,10,12,13).

Ultrasonography is a non-invasive diagnostic method for obstructive urolithiasis, urethral calculi localization, as well as diagnosis of cystitis, urethritis, dilated urethra, and rupture of urethra or the urinary bladder (14,15). Ultrasonography is suitable for examination of small-sized urinary bladder, as it cannot be detected by abdominal palpation or radiography (16). Trans-abdominal ultrasonography is a quick and easy diagnostic modality that allows the visualization of the distended urinary bladder, uroperitoneum, hydronephrosis, and uroliths; this modality is sufficient for the confirmatory diagnosis of obstructive urolithiasis (13). Ultrasonography helps assess the urinary bladder status, especially in calves, where rectal examination is impossible (9). Ultrasonography is a decision-making tool for planning surgical interventions for obstructive urolithiasis in buffalo calves (17).

The significant increase in the level of urea and creatinine in all affected calves compared to the control ones might be due to the reduction of glomerular filtration rate resulting from back pressure on the kidney in intact bladder animals and increased leakage of urea and creatinine from the peritoneal cavity in ruptured bladder (18). These findings agreed with previous studies (4,5,10,19,21,22). The highest level of phosphorus was observed in ruptured bladder cases, and this increase is the main cause of secondary hypocalcemia by increased urinary calcium excretion (10,21,22). Hypocalcemia also might be attributed to feeding mostly the concentrate ration in most animals, which is generally low in calcium and potassium and rich in phosphorus and magnesium (23). Further, anorexia and inappetence, recorded in most animals, could also have reduced oral intake and absorption of calcium from the gastrointestinal tract (24). Obstructive urolithiasis in ruminants is frequently associated with metabolic alkalosis and subsequent reduction in the proportion of the ionized serum calcium, which might also have been responsible for the lower serum calcium levels (21). Most of the affected calves under the study had a history of grain-based concentrate ration, with a limited quantity of roughages, which might have developed struvite and calcium phosphate calculi.

In this study, a significant increase in serum levels of MDH in all affected calves was observed, with higher levels in calves with ruptured bladder. Our results agreed with animal studies (25) and some human studies (26). This has occurred because of severe inflammatory reactions, especially in ruptured bladder, that induce oxidative stress. On the other hand, there was a significant decrease in GSH in all affected animals compared to the control ones, as observed previously (25). This might be because the GSH enzyme represents the major antioxidant defense component in protecting the cells against increased ROS (27) by metabolizing hydrogen peroxide into water and oxygen (28).

Intraoperatively, the urinary bladder was distended with the intact contour in 32 animals and shrinkage with the ruptured contour in 42 calves. The bladder rupture was mainly found at the dorsal aspect 64.3%, followed by subserous rupture 21.4% and at the ventral aspect 14.3%. The previous studies reported that the bladder rupture was mostly at the dorsal aspect (4,5,29). This might be due to muscular weakness and transitional epithelium at the dorsum of the bladder, while the subserous rupture might be due to muscular separation rather than tearing (4). The smooth surface of the bladder with discoloration from pinkish to bluish in intact bladder cases might be due to venous congestion resulting from overstretching. On the other hand, the bladder wall was thick, corrugated, and necrosed in cases of ruptured bladder; this might be due to bladder wall contraction after rupture (4).

Obstructive urolithiasis is a surgically treated disorder in male calves (30). In the present study, the tube cystostomy technique was successful and efficient in 95.95% of the treated cases with minimal complications 4.05%. Similar findings were reported previously (3-5,22,31). It was reported that the blockade of the Foley catheter was 12% in the treated animals (32). Also, it was reported that 2.7% of the treated cases with tube cystostomy had complications such as catheter loosening and catheter obstruction (4). The catheter obstruction was mainly caused by mucus shreds, blood clots, and tiny calculi, while the loosening of the catheter may be due to the deflation of the bulb. The tube cystostomy technique has been more advantageous than other surgical procedures in preserving the breeding purposes of the animals and urinary continence (33).

The operated animals resumed normal urination through the external urethral orifice within 3-27 days after surgery. The percentages of animals to resume normal urination were 12.68, 30.99, 36.62, 15.49, and 4.22% at 3, 7, 14, 21 and 28 days after surgery, respectively. These findings agreed with the previous studies (3,4,9,34,35). It was reported that 95.3%

of calves treated with tube cystostomy returned their normal urination within 7-14 days postoperatively (4), 3-14 days (5), 16 days (35), and 5-15 days (9). The free flow of urine through normal opening is possibly due to reduced inflammation of urinary conducts and urethral spasm, drying up of urinary calculi, dissolution of urethral calculi by the agents used, and oral administration of ammonium chloride and sodium chloride (36). Follow-up of the treated calves for 7 months after surgery revealed normal urination without recurrence of obstruction. Similar findings were reported (4) where recurrence was not recorded after 5 months of follow-up of the treated cases. It was reported that the surgical procedure was successful when the obstruction recurrence did not recur (37).

Conclusion

Obstructive urolithiasis is a significant clinical disorder affecting young male buffalo calves. It also initiates the oxidative stress process in the animal's body or body cells, especially those with bladder rupture, with increased serum MDA levels and lowered GSH levels. Surgical management of such a condition using tube cystostomy was successful and efficient with minimal complications, and it plays a crucial role in preserving the breeding status of the animals.

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Conflict of interest

The authors declare that there is no conflict of interest.

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احتباس البول الانسدادي في عجول الجاموس والتدخل الجراحي باستخدام تقنية فغر المثانة الأنبوبي: تقييم الإجهاد التأكسدي والإنزيمات المضادة للأكسدة

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الخلاصة

يعد احتباس البول الانسدادي مشكلة خطيرة ذات أثار سلبية على حياة عجول الجاموس. هدفت الدراسة الحالية إلى تقييم العلاقة بين الإجهاد التأكسدي ونشاط الإنزيمات المضادة للأكسدة والاحتباس البولي الانسدادي في عجول الجاموس وتقنية فغر المثانة الأنبوبي كتقنية جراحية لعلاج مثل هذه الحالة. في هذه الدراسة تم استخدام سبعة وسبعون عجول جاموس ذكر غير مخصي تتراوح أعمارهم بين ٣-١٠ أشهر تم دخولهم إلى عيادة كلية الطب البيطري، جامعة الزقازيق، مصر بتاريخ مرضى وهو انقطاع البول. تم تشخيص العجول إما بمثانة سليمة (ن = ٣٢ عجلاً) أو تمزق المثانة (ن = ٤٥ عجلاً) بناءً على الفحص السريري والموجات فوق الصوتية والكيمياء الحيوية. بالإضافة الى ذلك تم استخدام عشرة عجول جاموس صحية ظاهريًا في نفس العمر كمجموعة تحكم لاستخدامها كنطاق مرجعي للتحاليل الكيميائية. ارتفعت مستويات المالوندالديهايد في مصل الدم في جميع العجول المصابة وخاصة المصابة بتمزق المثانة مقارنة بمجموعة التحكم. بالإضافة إلى ذلك، تم تسجيل انخفاض معنوي في هرمون الكلوتاثيون في جميع العجول المصابة وظهر الانخفاض بشكل أكبر في العجول المصابة بتمزق المثانة مقارنة بمجموعة التحكم. نجحت تقنية فغر المثانة الأنبوبي في ٩٥,٩٥٪ من الحالات التي أجريت لها العمليات الجراحية و٤,٠٥٪ فقط من الحالات حدثت لها بعض المضاعفات. نستنتج من ذلك أن هناك علاقة بين الإجهاد التأكسدي ونشاط مضادات الأكسدة والاحتباس البولي الانسدادي في عجول الجاموس مع الاستخدام الصحيح لعملية فغر المثانة الأنبوبي.

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