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EXPERIMENTAL EVALUATION OF AUTOLOGOUS GRAFTING USING TUNICA VAGINALIS PROPRIA FOR URINARY BLADDER AUGMENTATION IN DOGS

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ABSTRACT

A total number of 18 apparently healthy adult male mongrel dogs were used to evaluate tunica vaginalis propria as an autologus graft for bladder augmentation. Dogs were assigned into two groups. The first one, (control group), was exposed to primary incisional closure of the bladder without wall augmentation. The second group, in which the bladder was reconstructed with a sheet of the tunica vaginalis propria. The results of clinical examination revealed that all cases appeared normal with the presence of frequent urination in the control group. Five cases were died (three from control group and two cases from augmented group). Cystography showed small size of the bladder in the control group, while normal size in the augmented group. Grossly, decreased diameter of the graft indicated its shrinkage. It changed into remnant at 12weeks postoperatively. Microscopically, all layers of the bladder wall were observed in the augmented portion at 12 weeks postoperatively. It could be concluded that, the using of tunica vaginalis propria in male dogs has the advantage of using readily available autologous graft for bladder wall reconstruction.

Key words: *Autologous grafting, Tunica vaginalis, bladder*

INTRODUCTION

Augmentation of urinary bladder is a procedure in which a piece of tissue is used to enlarge the bladder capacity, maintain bladder wall integrity, and decrease intravesical pressure. This tissue should be biocompatible and be able to serve as a scaffold for the regeneration of all three layers of the urinary bladder. This process is used in conditions with severe damage or loss of the bladder such as cancer, trauma, infection, inflammation, innervation defects and congenital abnormalities (Parsons and Toozs-Hobson 2005; Eldaharawy *et al.*, 2006 and Wongsetthachai *et al.*, 2011).

Various grafts are used in the reconstruction of urinary bladder. Tunica vaginalis is one of these grafts. Tunica vaginalis is derived from the peritoneum which is composed of mesothelium and connective tissue (Wrobel, 1998). Peritoneal flap has been used successfully as a bladder wall substitute in animal models (Tsuji *et al.*, 1963; Hutschenreiter *et al.*, 1978 and Sadove *et al.*, 1993). Wongsetthachai *et al.* (2011) used autologus tunica vaginalis for bladder substitution in male dogs. On the other hand, Theodorescu *et al.* (1998); Calado *et al.* (2005) and Leslie *et al.* (2009) reported successful urethroplasty with tunica vaginalis in rabbit models. Gajda (2006) used tunica albuginea for ureteral reconstruction in dogs.

The aim of the current study is to assess the changes of the urinary bladder augmented with tunica vaginalis propria in male dogs, based on clinical findings, cystography and histopathological findings.

MATERIALS and METHODS

Eighteen apparently healthy adult male mongrel dogs 2-3 years old, weighing 12-15 kg were used in this study at Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Suez Canal University. Dogs were assigned into two groups each one consists of 9 individuals. The first one, (control group), was subjected to primary incisional closure of the bladder without wall augmentation. The second group, in which the bladder was reconstructed with a sheet of the tunica vaginalis propria.

Dogs were fasted for 12 hrs before the surgical procedure. Each dog was premedicated with intramuscular injection of chloropromazine hydrochloride in a dose rate of 1 mg/kg, 15 min prior to the induction of general anesthesia (Hall *et al.*, 2001). The surgical site was then clipped, shaved and disinfected with povidone iodine solution (Betadine: Povidone- Iodine U.S.P. 10%, El Nile-Co.). General anesthesia was conducted by intravenous injection of thiopental sodium 2.5% until the main reflexes were

abolished. The whole animal, except the sites of operation, was draped with sterile towel. Balanced electrolyte (0.9% Sodium chloride) solution was administered (10 ml/kg/hr) during surgery. Two surgical procedures were performed in each dog. The first procedure was closed orchidectomy. The separated tunica vaginalis was harvested into a circular sheet 3 cm in diameter, and placed in an aseptic petri dish containing lactated Ringer's solution until used for grafting (Fig. 1). The second surgical procedure was performed to expose the urinary bladder. The bladder was approached through a lower ventral midline incision (Fig. 2). 30% full thickness piece from the dome of the bladder wall was excised. In control group, closure of the bladder without wall augmentation was accomplished with simple continuous sutures pattern using 3-0 polyglactin 910 (Vicryl: Manufactured Johnson & Johnson Jutl). In reconstructed dogs, a sheet of the prepared tunica vaginalis was sutured to the cut edge of the native bladder using a simple continuous suture pattern using 3-0 polyglactin 910 (Fig.3). The bladder was refilled by saline and small leaks were repaired (Fig.4). Omentum was then placed over the operated area in all dogs and secured to the bladder with a simple interrupted suture of the 3-0 polyglactin 910 material before the abdominal incisions were routinely closed. The linea alba and subcutaneous tissue were closed using polyglactin 910 No. 0 in a simple continuous pattern. Skin edges were sutured using silk No. 0 in a simple interrupted pattern.

Each animal was injected postoperatively with 1 g of amoxicillin intramuscularly (E.Mox: Egyptian International Pharm. Industries Company, A. R.E) and dipyrone (Analgin 50%: El-Nasr Pharmaceutical Chemicals co., Egypt.) in a dose of 10 mg/kg I.M. for 3 days.

Dogs were monitored for appetite, urine output, urine abnormalities, abdominal distention, abdominal pain and body temperature. The morphologic-functional assessment of the augmented bladder was performed through cystography before operation and at time of the scarification. Cystography was performed by insertion of a catheter gently through the urethra. All urine was aspirated out of the bladder then infused slowly positive contrast medium (tri-iodinated) diluted with saline (1:1) into the bladder until its full capacity. Lateral and ventrodorsal radiographs of the caudal abdomen were taken.

Dogs were euthanized at 4, 8 and 12 weeks postoperatively using an overdose of thiopental sodium solution. The abdominal incision, peritoneal cavity, and all abdominal viscera were evaluated for adhesions and any other post surgical changes. The specimens were collected and fixed in 10% neutral buffered formalin, embedded in paraffin, and stained with haematoxylin & eosin and Masson's trichrome (Bancroft *et al.*, 1990).

RESULTS

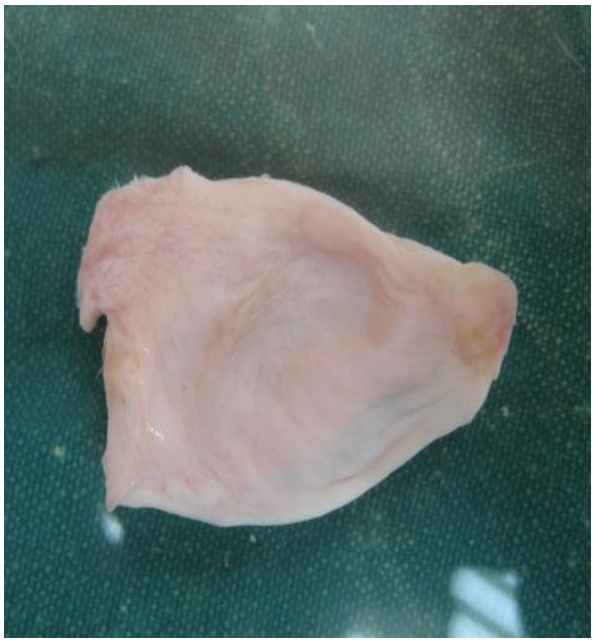
In the control group, six dogs appeared normal with the presence of frequent urination. The other three dogs died 10 days postoperatively. These cases showed inappetence, dullness and increase body temperature. In the augmented group, all cases appeared in good health conditions. Urine output appeared to be normal till the time of sacrificing except two cases died two week postoperatively. These cases suffered from inappetence, dullness, increase body temperature, abdominal distention, abdominal pain, difficult and decreased urine output.

Cystography showed more or less oval urinary bladder preoperatively in all cases. Cystography showed small size bladder in the control group postoperatively. Cystography of augmented group at 4, 8 and 12 weeks postoperatively, showed an elongated, irregular contour of the bladder that was returned nearly to the same size (Figs. 5&6).

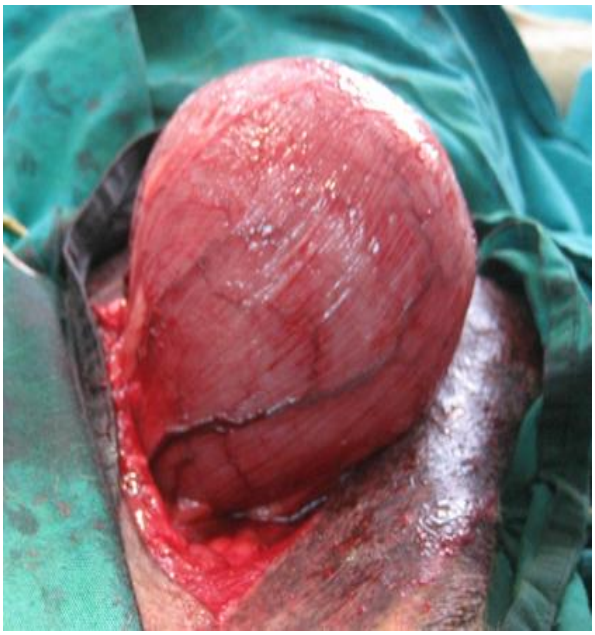
Grossly in six dogs of control group, there were adhesions between the bladder wall and omentum. The others three dogs showed wound dehiscence and urine leakage at 10 days postoperatively. In the augmented group at the time of sacrifice, the urinary bladder region showed weak adhesions between bladder wall and omentum (Fig. 7). These could easily be dissected by blunt dissection with gauze in the seven cases. No calcification of wall or stone formation was shown inside the urinary bladder in any of these dogs. The urinary bladder distended normally after filling with saline. There was no leak in any of the operated dogs. A decreased diameter of the graft indicated its shrinkage. It changed into remnant at 12 weeks postoperatively. The demarcation over anastomotic line between bladder wall and the graft was difficult to differentiate and internal surface of the augmented urinary bladder covered with apparently healthy mucosa at 12 weeks postoperatively (Fig.8). The bladder of the two died dogs showed perforation within the graft at two weeks postoperatively.

Microscopically, in the control group, all three layers of normal urinary bladder wall were observed at the closure areas. Plasma cells and lymphocytes were noted in the submucosa and serosa of the closure areas during the experimental periods. In the augmented group at 4 weeks, the two cut borders of the native bladder were joined with fibrous connective tissue containing newly formed blood capillaries (granulation tissue) (Fig. 9). Moderate numbers of lymphocytes and plasma cells were detected within the grafted region (Fig. 10). After 8 weeks, the native and grafted regions were in a good continuity and adhered well with fibrous connective tissue bands extending between both of them admixed with strands of smooth muscle, in addition to some inflammatory cells mostly lymphocytes

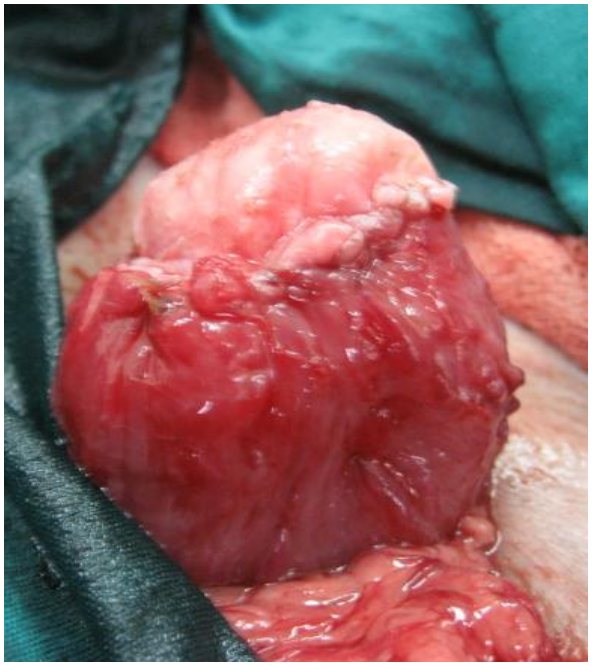
(Figs. 11-13). 12 weeks postoperatively, the grafted area showed full thickness that was evident by presence of well-organized dense fibrous connective tissue containing minimal inflammatory cells and remnant of suture material. The mucosal surface of the graft was covered with a mildly hyperplastic urothelium (2-4 layers). Focal aggregation of lymphocytes was noted underneath the transitional epithelium (Figs. 13&14). The tunica musclaris was present connected the graft from both sides with the native bladder which was stained with Masson's trichrome staining (Fig. 16).



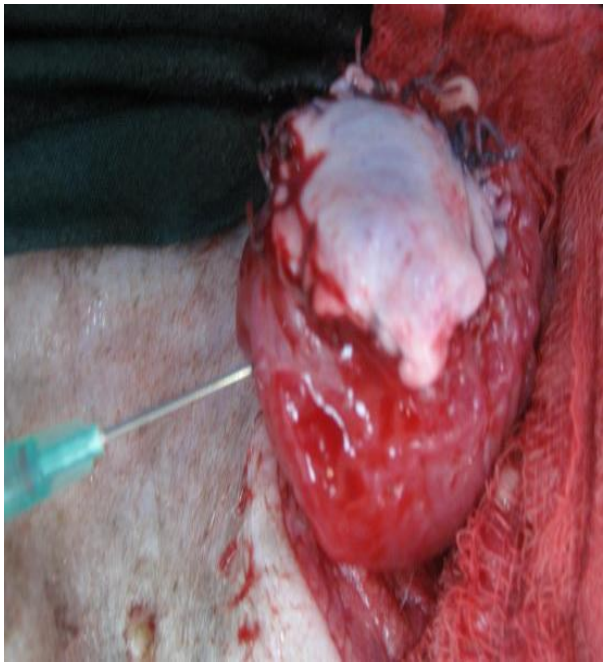
(Fig. 1): A tunica vaginalis sheet placed in an aseptic petri dish.



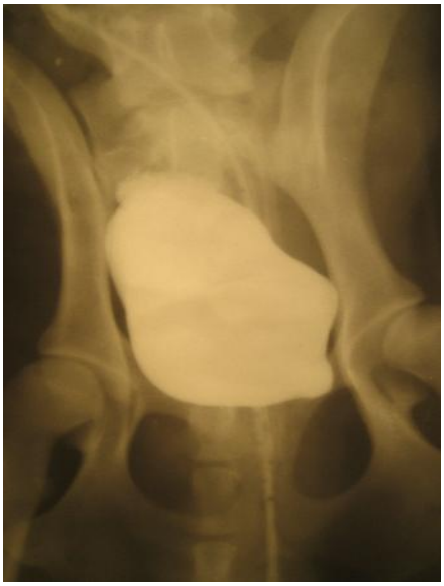
(Fig. 2): The urinary bladder exteriorized after opening of the peritoneum.



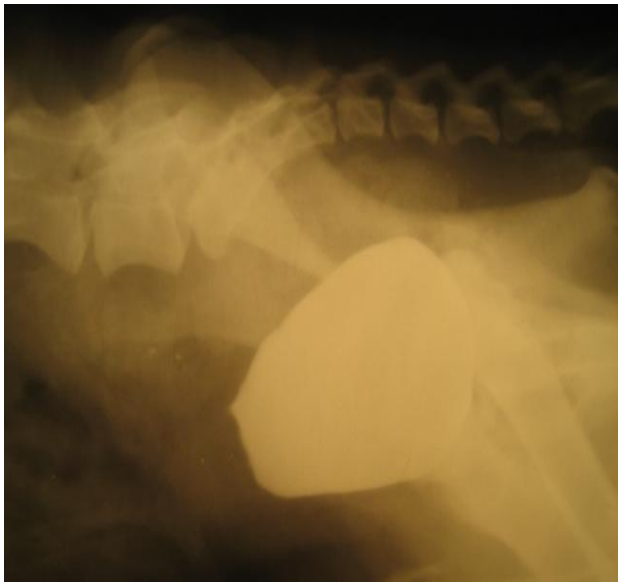
(Fig.3): A sheet of the prepared tunica vaginalis was sutured to the cut edge of the native bladder in a reconstructed dog.



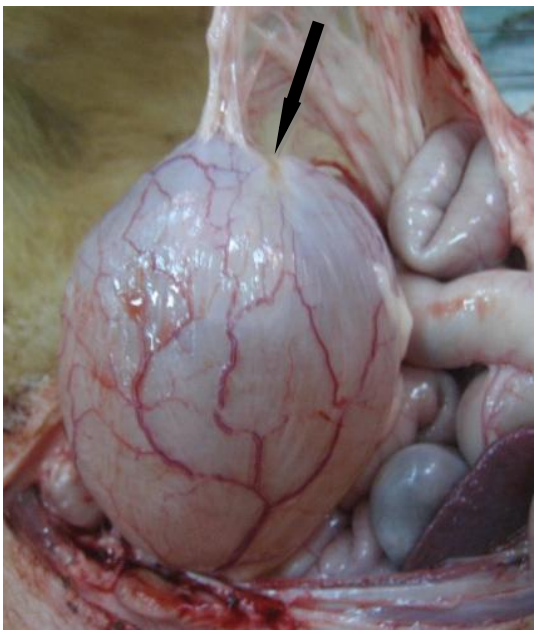
(Fig.4): The bladder was refilled by saline.



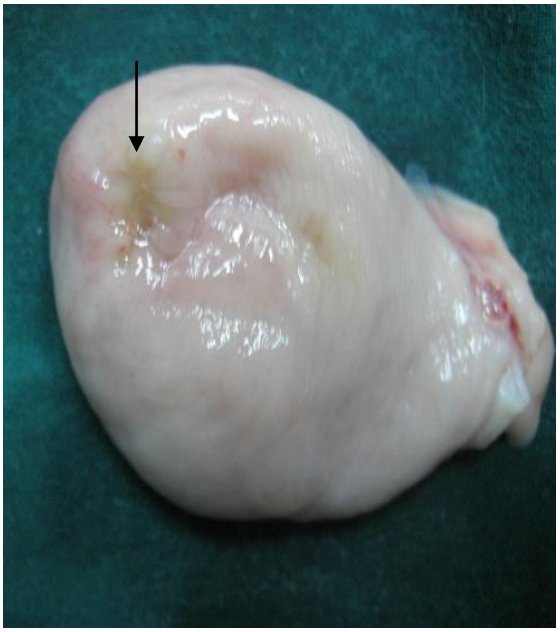
(Fig.5): Ventrodorsal view of positive contrast medium cystogram showing an elongated and irregular contour of the augmented urinary bladder at 12 weeks postoperatively**.**



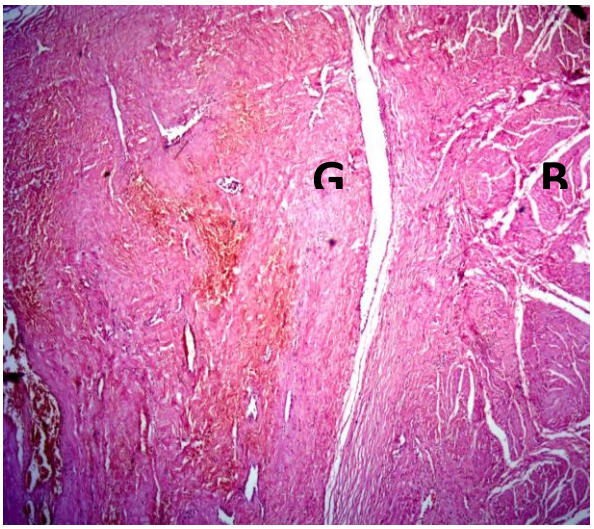
(Fig. 6): Lateral view of a dog of Fig.7, showing elongated and an irregular contour of augmented bladder dome.



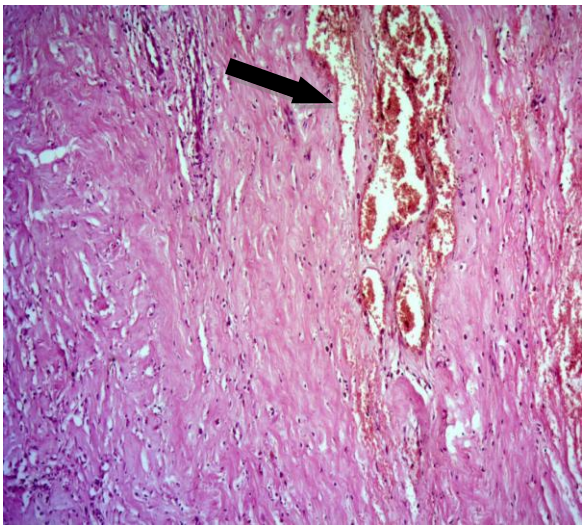
(Fig.7): The augmented urinary bladder showed adhesion with omentum with a remnant of the graft at 12 weeks postoperatively (arrow).



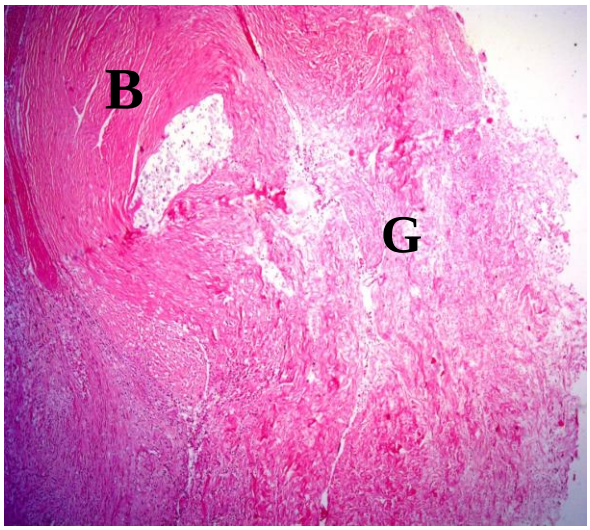
(Fig. 8): The mucosal surface of the augmented urinary bladder covered with apparently healthy mucosa with a remnant of the graft at 12 weeks postoperatively (arrow).



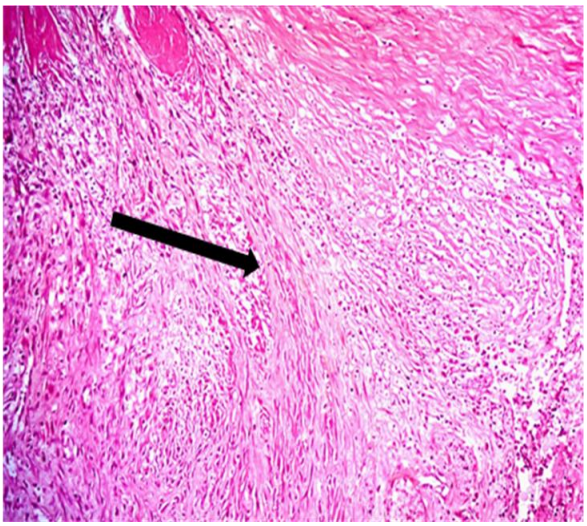
(Fig. 9): Augmented urinary bladder at 4 weeks postoperatively showing native bladder (B) joined to the graft (G). H&E 40X.



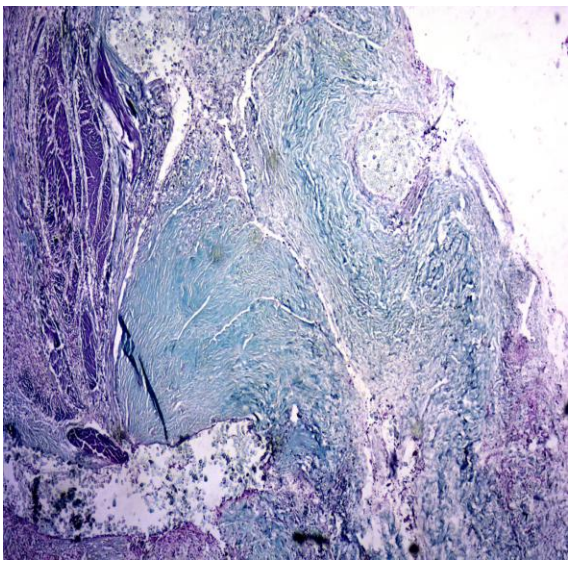
(Fig.10): Higher magnification of the previous figure revealing the presence of newly formed capillaries (arrow) and fibrous connective within the graft. H&E. 100X



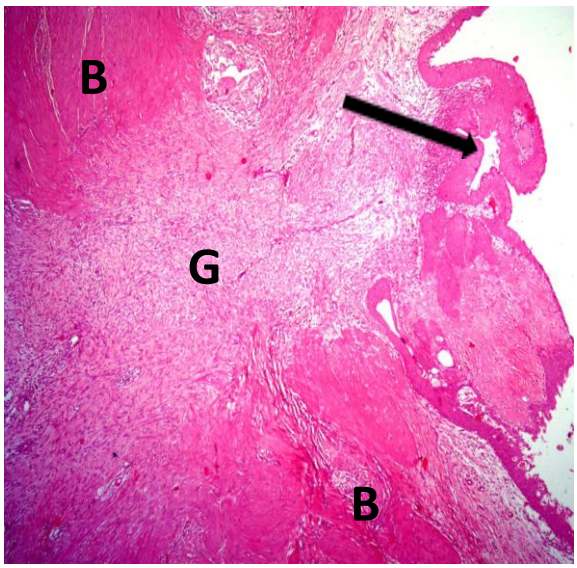
(Fig. 11): Augmented urinary bladder at 8 weeks postoperatively showing good adhesion between the native bladder (B) and the graft (G). H&E 40X.



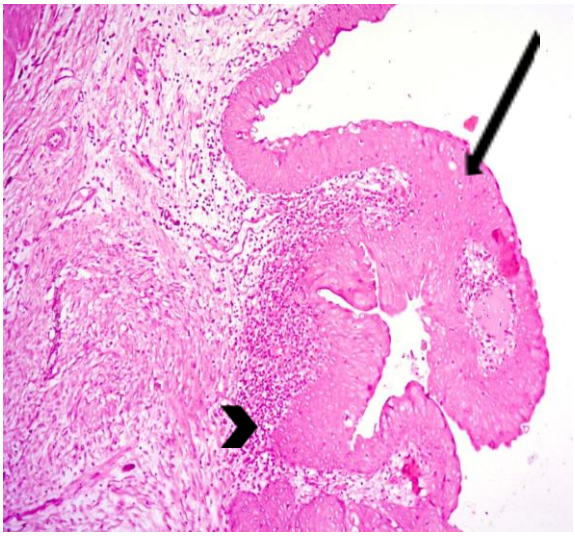
(Fig. 12): Higher magnification of the fig. 15 demonstrating fibrous connective tissue bundles joining the native and grafted regions. H&E. 100X



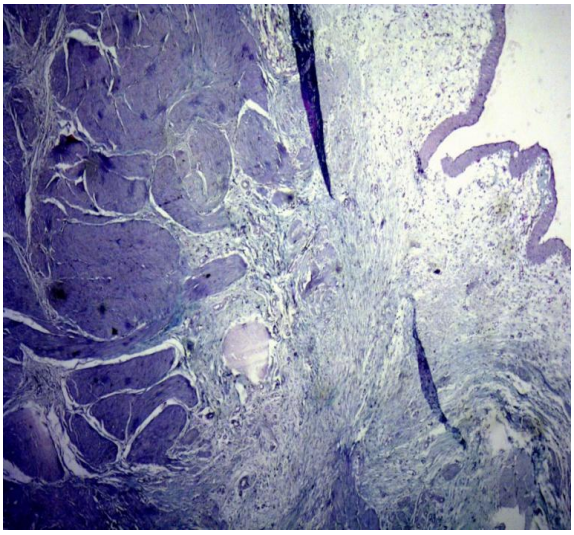
(Fig.13): Augmented urinary bladder at 8 weeks postoperatively showing stands of smooth muscle extending into the graft. Masson's trichrome 40x.



(Fig. 14): Augmented urinary bladder at 12 weeks postoperatively showing well-organized graf (G) covered with urothelium joining the two cut borders of the native bladder (B) H&E 40X.



(Fig. 15): The graft is covered by a mildly hyperplastic epihtlium (arrow) and focal aggregation of lymphocyte is present underneath it (arrow head). H&E. 100X



(Fig. 16): Augmented urinary bladder at 12 weeks postoperatively showing formed tunica musclaris at the graft. Masson's trichrome 40x

DISCUSSION

Autologous tunica vaginalis grafts have the advantage of easy preparation, no need for preservation, less expense, highly vascularity, and no graft rejection. Use of this kind of the tissue graft is useful for maintaining bladder wall integrity especially in an emergency situation where a massive loss of the bladder wall has occurred (Sadove *et al.,* 1993 and Wongsetthachai *et al.,* 2011).

Our results revealed that six dogs in control group suffered from frequent urination. This symptom may due to bladder inflammation and decrease bladder capacity after partial cystectomy. In the augmented group, bladder began to regain normal capacity at 12 weeks postoperatively. The time is necessary for returning bladder capacity. The cystography and histopathological findings enforced this result. Similar result was obtained by Kropp *et al.* (1996) who reported that duration of 14 months is required to regain normal bladder capacity after the resected wall had been substituted with porcine small intestine submucosa. On the other hand, decreased bladder capacity was reported by Wongsetthachai *et al.* (2011) who mentioned that the shrinkage of the substituted portions of the bladder was resulting from inflammation, fibrosis, dystrophic calcification, and bone metaplasia within the grafted portions.

Three dogs from control group died 10 days postoperatively. The main cause was peritonitis due to urine leakage resulting in wound dehiscence and sepsis. Similar complications were obtained by Vilar *et al.* (2004).

In the augmented group, dogs appeared with normal urine output till the time of sacrificing. Cystography showed integration of the graft into the bladder that was returned nearly to the same size. These results were supported in dogs by Elkoushy (2003); Vilar *et al.* (2004) and Eldaharawy *et al.* (2006). Two dogs from this group, suffered from abdominal distention, abdominal pain, inappetence, increase body temperature, difficult and decreased urine output after two weeks postoperatively. The bladder showed perforation and adhesions between the bladder wall, and omentum. A number of factors may be proposed to be etiologic including chronic urinary tract infection, adhesions and over distention which results in areas of ischemia and contractions. Adhesions result in a fixed configuration that may result in sheering with emptying and filling. These causes were supported by Crane *et al.* (1991) and Bauer *et al.* (1992).

The regeneration time of the bladder wall depended on graft type and the animal species in addition to the extent of the bladder wall defect. Epithelialization of the augmented bladder in the present study completed at 12 weeks. Nearly similar result was mentioned by Wongsetthachai *et al.* (2011) who observed epitheliazation earlier than the 12 weeks. On the other hand, Hutschenreiter *et al.* (1978) and Pope *et al.* (1997) reported that, complete epithelialization was observed at 5 and 3–4 weeks after the use of free peritoneal grafts in rabbits and porcine small intestine submucosa in dogs respectively. Smooth muscle regeneration was present at 8 weeks in this study. This result was agreed by Wongsetthachai *et al.* (2011) who showed smooth muscle regeneration at 6 weeks postoperatively. On contrast, regeneration of smooth muscle after substitution with a free peritoneal graft in rabbits (Hutschenreiter *et al.,* 1978) and a pedicled peritoneal flap in dogs (Tsuji *et al.,* 1963) occurred at 10 and 12 weeks, respectively.

Bone metaplasia is not detected in our study. On contrast, Wongsetthachai *et al.* (2011) found bone metaplasia at 8 and 10 weeks following bladder wall substitution with tunica vaginalis in male dogs. Bone metaplasia was also reported when the porcine small intestine submucosa was used as a bladder wall substitute in dogs (Pope *et al.,* 1997). Bone metaplasia was not observed when the peritoneum was used as a pedicled flap in dogs (Tsuji *et al.,* 1963) and as a free graft in rabbits (Hutschenreiter *et al.,* 1978). The exact cause of the metaplasia is unknown. Variations in graft preparation and processing may contribute to these contrasting findings.

It could be concluded that the use of tunica vaginalis propria in male dogs has the advantage as a readily available autologous graft for bladder wall reconstruction. It is a simple and less expense method with a reliable regenerative capacity. The disadvantage is that it is only applicable to the male intact dog which is the self donor. Use of tunica vaginalis as an allograft in either sex of dogs requires further investigation and a search for the proper method of the preparation and preservation of the graft.

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التقييم التجريبي للطعمة الذاتية باستخدام الغلالة الغمدية الحقيقية لزيادة حجم المثانة البولية في الكلاب

محمد حسين الدهراوى ، عبد العظيم منير ابراهيم

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تمت الدراسة على 18 من ذكور الكلاب الهجينة لتقييم الطعمة الذاتية للغلالة الغمدية الحقيقية لزيادة حجم المثانة البولية. تم تقسيم الكلاب إلى مجموعتين. المجموعة الأولى (الضابطة) تم الأغلاق الجراحي فقط للمثانة بعد استئصال ثلثها الأمامى. المجموعة الثانية تم فيها أعادة بناء المثانة بواسطة طعمة من الغلالة الغمدية الحقيقية بقطر 3 سم .وقد أظهرت نتائج الفحص الأكلينيكى أن جميع الحالات بدت بصورة طبيعية مع وجود بول متكرر فى حالات المجموعة الضابطة توفى خمسة كلاب خلال التجربة ثلاثة من المجموعة الضابطة واثنين من المجموعة الاخرى . كما أظهرت صور الأشعة صغر حجم المثانة فى المجموعة الضابطة بينما كان لها نفس الحجم فى المجموعة الثانية وأما بالفحص بالعين المجردة لوحظ وجود التصاقات بين المثانة والثرب فى جميع الحالات وقد أظهر الفحص المجهري طبقات جدار المثانة عند مكان الطعمة مصحوبا بانكماش الطعمة عند الأسبوع الثانى عشر بعد الجراحة. وقد خلصت هذه الدراسة إلى أن طعمة الغلالة الغمدية الحقيقية في ذكور الكلاب يسهل الحصول عليها لاستخدمها كطعمه ذاتيه بنجاح لزيادة حجم المثانة البولية.