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EXPERIMENTAL STUDY OF AUTOGRAFT AND ALLOGRAFT FOR REPAIRING URINARY BLADDER DEFECT IN DOGS

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SUMMARY

The experiment was carried out on 30 healthy mongrel dogs allocated into two groups 15 dog for each. A defined 3x3cm2 patch in the craniodorsal surface of the bladder was exised and resutured again in the autografted group and replaced by double layer of twice the size of the excised area using preserved amniotic membrane collected from pregnant dogs. Plain and contrast radiography, ultrasonography, and urine and serum analysis as well as histopathologic examination were conducted during three months period.

INTRODACTION

Several years ago a lot of urinary bladder diseases including congenital abnormalities, cancer, trauma, infection, inflammation, neural defects and iatrogenic injuries or conditions of severe damage to the bladder have been reported, most of these affections may require bladder wall substitution (Wongsetthachai, Pramatwinai, Banlunara and Kalpravidh, 2010). Most of the attempts of urinary bladder substitutes using both natural and synthetic biodegradable materials have failed due to mechanical, structural, functional or biocompatibility problems (Baumert, Baumert, Simon, Hekmati Fromont, Levy, Balaton, Molinié and Malavaud,2007). The idea of the substitution is to maintain bladder wall integrity, enhance the bladder capacity and decrease intravesical pressure. Amniotic membrane as a substitute for the urinary bladder of dogs is beneficial in regenerative therapy. The biocompatible substitute biomaterial is able to serve as a scaffold for the regeneration of all layers of the urinary bladder (Elbahnasy, Shalhav, Hoenig, Figenshau and Clayman, 1998 and Ismail, Marcos, Sherif, Thabet, El ghor, Ishac, Barsoun, Chowdhury and Selim, 2009). The present study is aimed to investigate the utilization of dog's amniotic membrane as a bladder wall substitute compared with a patch of bladder wall. Evaluations of both implantations by several diagnostic aids including clinico-pathological, ultrasonographic, radiological and histopathologic examinations have been adopted.

MATERIALS AND METHODS

The current investigation was carried out on 30 healthy mongrel dogs of both sexes, varied in ages from 2 to 5 years and weighed from 15 to 20 kg. The animals were allocated into two groups; 15 dogs for autografted group and 15 dogs for allografted group, additional 5 full-term pregnant bitches for harvesting the amniotic membrane were used. The pregnant bitches were verified by ultrasonographic examination according to Aissi and Slimani (2008) and subjected to selective laparo-hysterotomy for harvesting, preserving and banking of the amniotic membrane. The obtained placentas were immersed in one liter normal saline, containing 100 U/ml penicillin and 0.2 mg/ml streptomycin and 0.025mg/ml amphotericin B according to (Lee and Tseng, 1997; Sharifiaghdas et al., 2007 and Vongsakul et al., 2009). The amniotic membrane was then harvested by blunt dissection from chorion, molded on a sterile sheet of nitrocellulose and preserved in temperature below freezing -80ċ according to (Lee and Tseng, 1997; Kruse et al., 2000 and Vongsakul et al 2009). Urine and blood samples were collected before surgery, 1, 2 and 3 months postoperatively, urine examined for Physical, and Microscopical charcters and BUN and creatinine were estimated in serum samples.

A. Laparo-cystoplasty

The selected dogs were subjected to laparo-surgeries under the effect of general anesthesia. Premedication was adopted by injecting Atropine sulphate (Atropine sulphate 1%®, Adwia co.S.A.E- Egypt 0.05-0.1mg/kg.b.wt) and xylazine (Xyla-Ject 2%®, Adwia co.S.A.E- Egypt 1mg/kg.b.wt.), then anaesthesia was induced using ketamine HCL (Ketamine®, Sigma-Tec, Egypt 10-15mg/kg.b.wt.) and maintained by intermittent doses to effect of sodium thiopental 2.5% (Thiopental sodium®; 20-30mg/kg.b.wt, Epico Pharma - Egypt.). A ventral midline approach in females and ventral midline - paramedian approach in males were adopted for partial laparocystectomy. In the autografted group a defined 3x3 cm^2 patch in the dorsal surface of the bladder was excised and immediately reimplanted in situ by water tight continuous sutures using vicryl 3/0 (Surgi Sorb®, Suture LTD, U.K). In the

allografted group a defined 3x3 cm^2 patch in the dorsal surface of the bladder is resected and replaced by double layers of 4cm×5cm preserved amniotic membrane (fig.3&4). The first layer is sutured to the bladder wound with simple continuous sutures using vicryl 3/0 and the other layer is applied using surgical glue (3M Vetbond™; n-butyl cyanoacrylate).

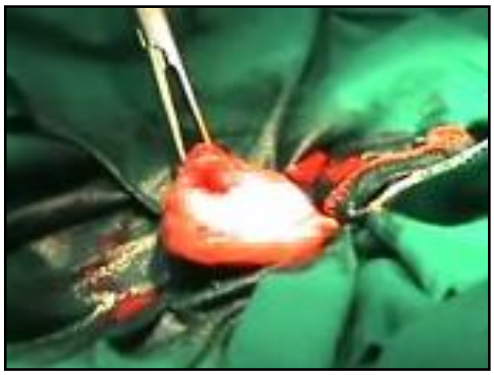

Fig. (1): A defined 3x3 cm patch of the recipient bladder.

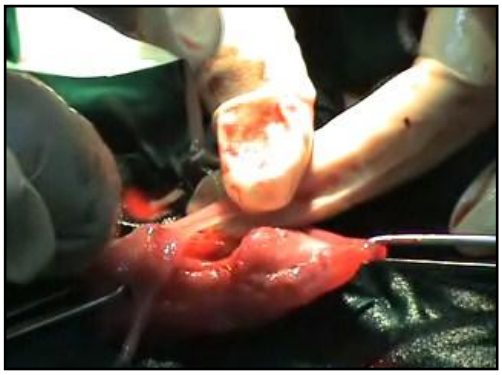

Fig. (2): Application of the amniotic membrane.

Following augmentation of the amniotic membrane, the augmented bladder was filled with 20-30 ml normal saline via a previously applied polyethylene urinary catheter to test the efficiency of the graft suturing. Intra-abdominal Pen & strept® antibiotic was applied, prior to opposing the laparotomy wound layer by layer using vicryl 2/0. Each five dogs of both groups were traced and euthanatized after 1, 2 and 3 months according to Portis et al., (2000).Daily wound care was focused and the skin sutures were removed 10 days post-operation. Systemic antibiotic ceftriaxone® (ceftriaxone sodium 50mg/kg., IM, Novartis Pharma S.A.E. Egypt) was daily injected. Dogs were provided with daily balanced diet and fresh clean water.

B. Ultrasonographic examinations

Ultrasonographic scanning was applied using B-mode transducers 5.0 MHz convex and 7.5 MHz micro-convex according to Finn-Bodner (1995) and Nyland et al (1995).

C. Radiographic examinations

Conventional and Contrast radiography were also performed. Urographine 76% and air for double contrast and air alone for negative contrast cystogram were employed. Four projections were applied for each animal including lateral, ventro-dorsal and two oblique projections according to Mahaffy and Barber (1992); Han et al (1994) and Farrow (2003).

D. Histopathologic procedures

Postmortem examination of the bladder was recorded and resection of piece of the dorsal surfaces of the bladders including the graft took place immediately after euthanasia. The resected specimens fixed in formol saline 10% then washed, dehydrated, cleared, embedded in paraffin and sectioned then stained according to Bancroft, Cook, Stirling and Turner (1996). The stained sections were examined microscopically.

E. Statistical analysis

All the data of the study were analyzed statistically using Student paired t-test between two variants (Petrie and Watson, 2006).

RESULTS

All the operated dogs were recovered and surgical glue proved no infection, leakage and tearing. Also, it plays a role together with the implanted membrane to arrest hemorrhage of the bladder wound edges. Absence of leakage is proved after infusion of the augmented bladder with saline. Hematuria was evidenced in some cases during the first few days.

Urine analyses

Urine analyses of autografted and allografted dogs at one, two and three month in comparison to preoperative values showed slight changes in the physical, chemical characters and the microscopical evaluation.

Serum analysis

Regarding BUN, comparing the post autografted samples at one month (14.27 ± 5.6), two months (12.82 ± 4.8) and three months (13.09 ± 4.8) to preoperative samples (12.28 ± 2.19), there was no significant

change. The postallografted samples at one month (13.79 ± 4.88), two months (15.31 ± 6.43) and three months (13.09 ± 3.83) in comparison to preoperative samples (15.09 ± 6.02), there was no significant change (table1). Regarding the serum creatinine, comparing the post autografted samples at one month (0.613 ± 0.076), two months (0.531 ± 0.081) and three months (1.4 ± 0.7) to presurgical samples (0.50 ± 0.113), there was no significant change. As regard the postallografted samples at one month (0.68 ± 0.297), two months (0.68 ± 0.208) and three months (0.66 ± 0.14) in comparison to presurgical samples (0.67 ± 0.19), there was no significant change (table - 1).

Ultrasonographic findings

The ultrasonographs of the urinary bladders of the control group were seen as uniform, pear-shaped structures. Scanning at the longitudinal plane of the urinary bladders in the autografted dogs after one month revealed presence of echogenic area at Scaning at the longitudinal plane of the urinary bladders in the autografted dogs after one month revealed presence of echo genic area at the cranio-dorsal wall of the bladder while there was irregularity of craniodorsal wall of the bladder of the allografted dogs after one month (fig – 3a &b).

Table 1. Blood urea nitrogen (BUN) and creatinine values of the experimental groups.

Kidney function	Pre auto-grafted dogs (M± SD)	Post autografted dogs (M± SD)			Pre allografted dogs (M± SD)	Post allografted dogs (M± SD)		
		One month	Two months	Three months		One month	Two month	Three Month
(BUN) (mg/dl)	12..28 ± 2.19	14..27 ±5.6	12..82 ±2.38	13..09 ±4.80	15..09 ±6.02	13.79 ±4.88	15..31 ±6.43	13..09 ±3.83
Creatinine (mg/dl)	0.5 ±0.113	0.613 ±0.076	0.531 ±0.081	0.640 ±0.070	o.67 ±0.19	0.68 ±0.297	0.68 ±0.208	0.66 ±0.14

M = mean SE = Standard Deviation P ˉ = > 0.05 P * = < 0.05 P ** = < 0.01

Two months postoperation the autografted dogs revealed homogenous appearance of the dorsal wall and ill demarkated grafted part from the original bladder wall. The allografted dogs after two months, showed presence of small hypoechoic thickening at the craniodorsal wall of the bladder (fig.4).Three months postoperation, the autografted and allografted dogs showed normal homogenous smooth bladder wall appearance with additional presence of double hyperechoic dots at the edges of graft (allografted dogs) indicating remnants of the graft .

Radiological findings

The plain radiographs of the urinary bladders were seen as soft tissue dense,pear-shaped structures in the caudoventeral abdomen. The mucosal margins were not identified.The negative contrast cystography showed normal bladder thickness in control dogs (fig.6a). One month post surgery, u7the autografted and allografted dogs showed mild wall thickness.

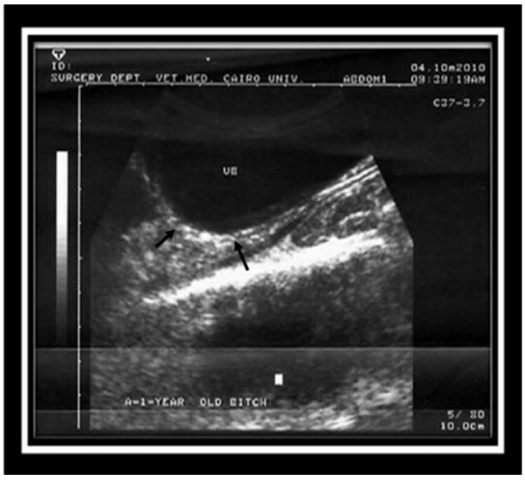

Fig (3a): longitudinal plane of an Auto-grafted dog after one month showing echogenic craniodorsal area of the urinary bladder (arrows).

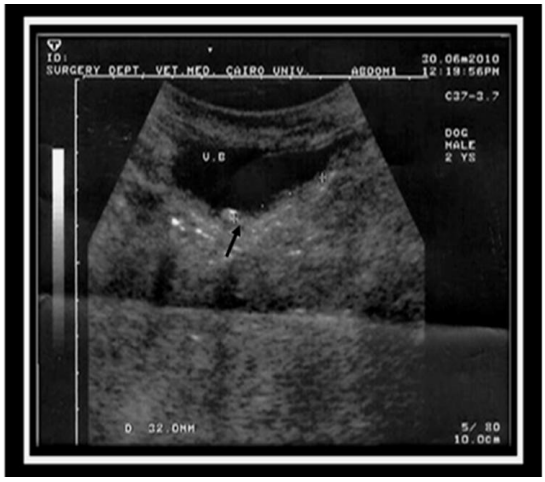

Fig (3b): Longitudinal plan of allogfted dog after one month showing irrigularities at the craniodorsal wall of bladder

At three months the autografted dogs (fig. 6c), and allografted dogs (fig 6d) showed normal bladder wall thickening.at the dorsal surface (fig.6b) which became milder at two months. At three months, normal bladder.

The double contrast cystography showed positive contrast formed a puddle in the dependent portion of the bladder and an identified and uniform normal bladder wall thickness in the control dogs (fig. 7a). One and two months after the autografted and the allografted dogs, showed mild bladder wall thickening (fig7b). After three months, identical bladder wall thickness in the auto grafted dogs (fig. 7c), and the allografted dogs that show adhesion to the momentum (fig. 7d).

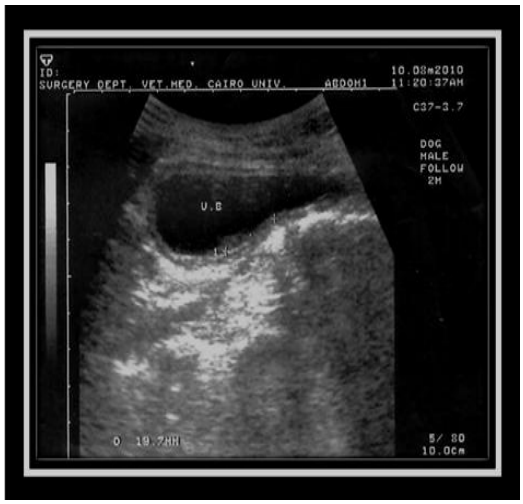

Fig (4): longitudinal plane of allografted bladder after two months showing small hypoechoic thickening remnants of amniotic membrane (arrows).

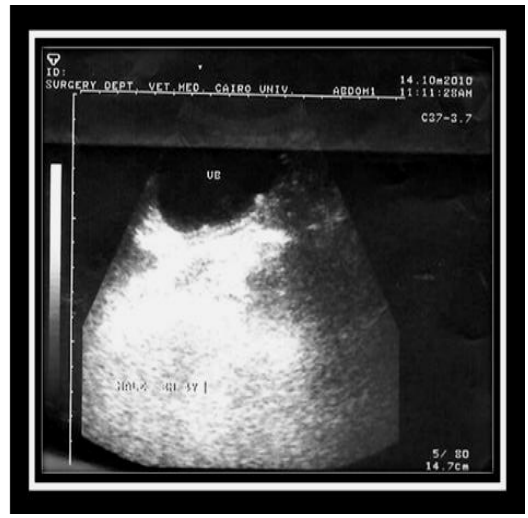

Fig (5): Lomgitudinal plan of allografted bladder after three months showing double hyperechoic dots indicting of craniodorsal wall of the urinary bladder

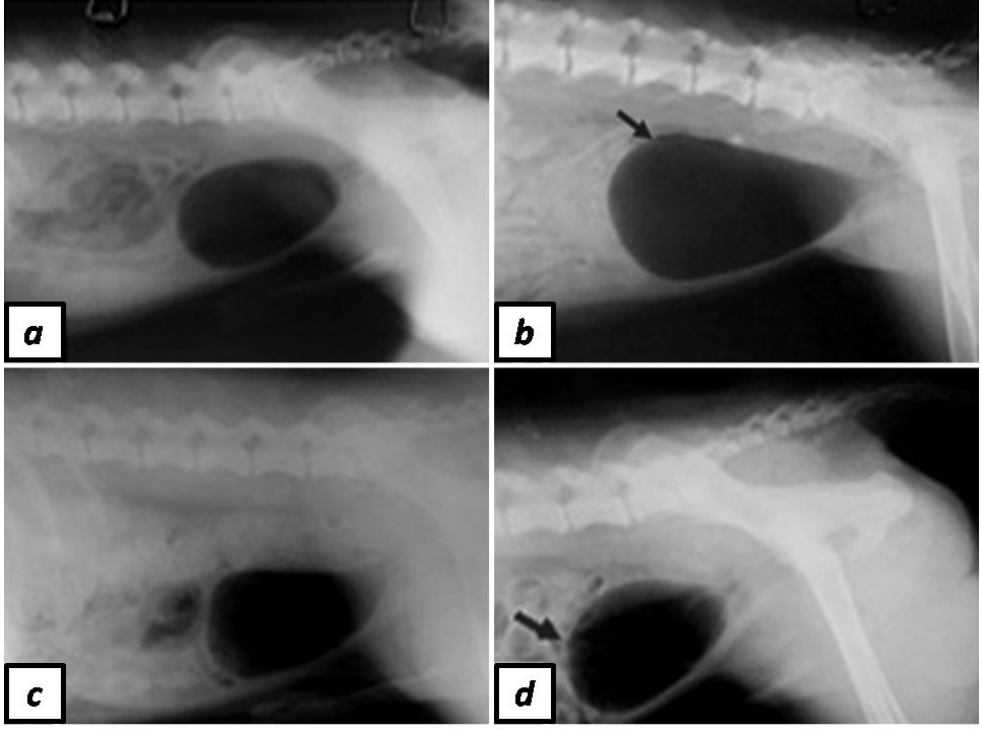

Fig. 6: Radiological findings of lateral views of negative contrast in different groups.

a. Preoperative rdiograph showing normal bladder appearance with a well identified wall thickness.

b. Autografted dog after two month showing mild wall thickening at the craniodorsal surface (arrow).

c. An autografted dog after three months showing normal bladder appearance.

d. An allografted dog after three months showing normal bladder appearance with adhesions to omentum (arrow).

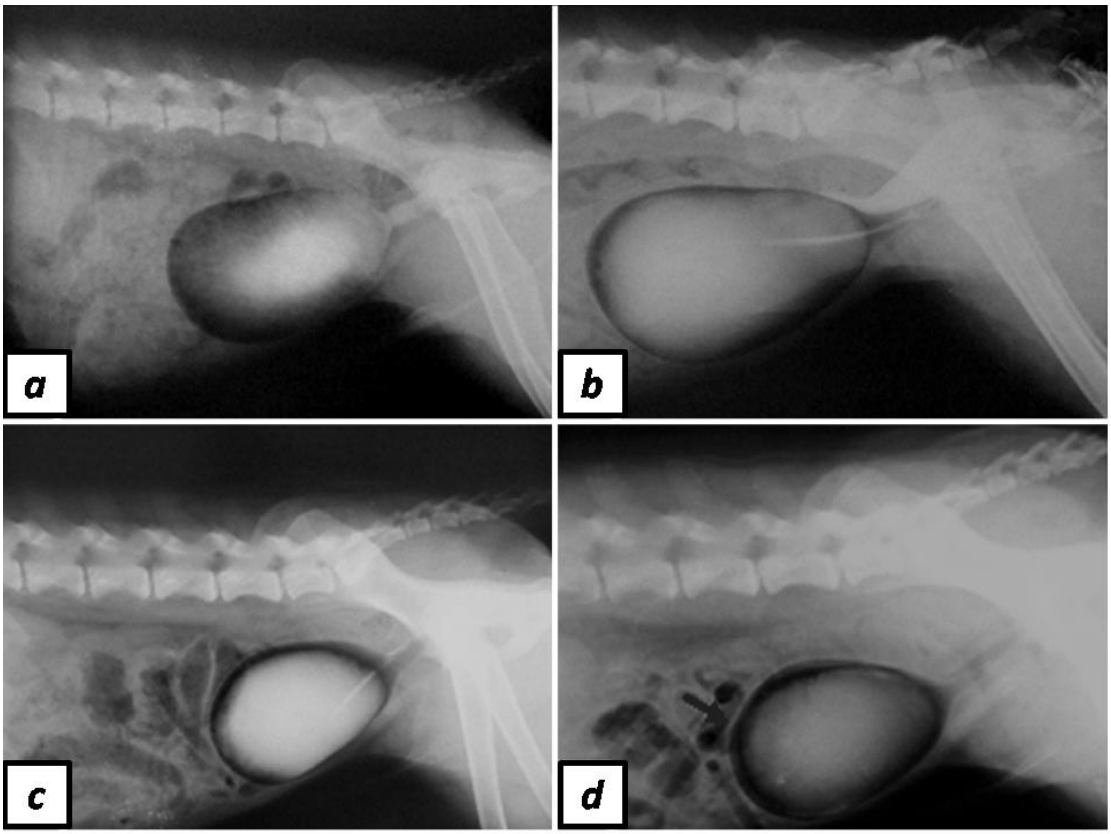

Fig. (7): Radiological findings of lateral view of double contrast in different groups.

a. Preoperative radiograph showing positive contrast formed a puddle in the dependent portion of the bladder and the wall thickness was identified and uniform.
b. An Allografted dog after one months showing uniform wall thickness.
c. An autografted dog after two months showing uniform wall thickness.
d. An allogrfted radiograph after three months showing a uniform wall thickness except at the cranil part due to adhesion.

Pathological findings

1. Postmortem findings

In the autografted and allogrfted dogs after one month there was inflamed mucosal lining and thickening of the bladder wall), after two months there was thickened wall of the autograft and after three

months, normal appearance of the autografted and allografted bladders were detected (fig 8Cand 9C). There was adhesion of the allograft to the omentum in most cases.

2. Histopathologic results:

One month post operations, The autografted and allografted bladder showed regenerating transitional epithelium and sub epithelial fibrous connective tissue proliferation as well as regenerating smooth muscle bundles. In the auto grafted specimens there was infiltration of the mucosa and sub mucosa with inflammatory cells and highly vascularized submucosal granulation tissue (fig 8a) which was characterized by mild inflammatory reaction in the allografted samples ((fig.9a).

After two months the autografted specimens showed complete proliferated transitional epithelial lining, with regenerated submucosal fibrous connective tissue and full thickness muscle layer and sub mucosal highly vascuolarized connective tissue (fig 8b).The allografted bladder showed regenerating transitional epithelium over intact amniotic membrane and regenerating smooth muscle bundles fig (fig.9b). After three months the autografted and allografted bladder revealed regenerating transitional epith-elium, and full thickness regeneration of the bladder. Presence of remnant of amniotic graft in the allografted group was detected (fig. 8c &9c).

DISCUSSION

Treatment of bladder diseases relies greatly on early detection and aggressive surgical management. (Atala, 2001; El Sebaie et al., 2005 and Atala et al., 2006). Several urologists have long been searching for alloplastic grafts or a synthetic biodegradable material suitable for use as a urinary bladder replacement (Elbahnasy et al, 1998; Shokeir, 2002; Atala, 2004 and Baumert, et al, 2007). Elective caesarian section of full term pregnant bitches is the best for production of aseptic amniotic membrane, (Malak and Bell, 1994). The use of amniotic membrane as urinary bladder graft without any component of the placenta is must as the chorion provokes neovascularization and inflammatory reaction in

the host tissue which eventually causes rejection phenomenon (Avanoglu et al., 1994). The characteristic features of the amniotic membrane are it's easy handle, adhesion to the surface of the organs on which it is applied, its cheaper cost than other commercial product (Avanoglu et al., 1994).

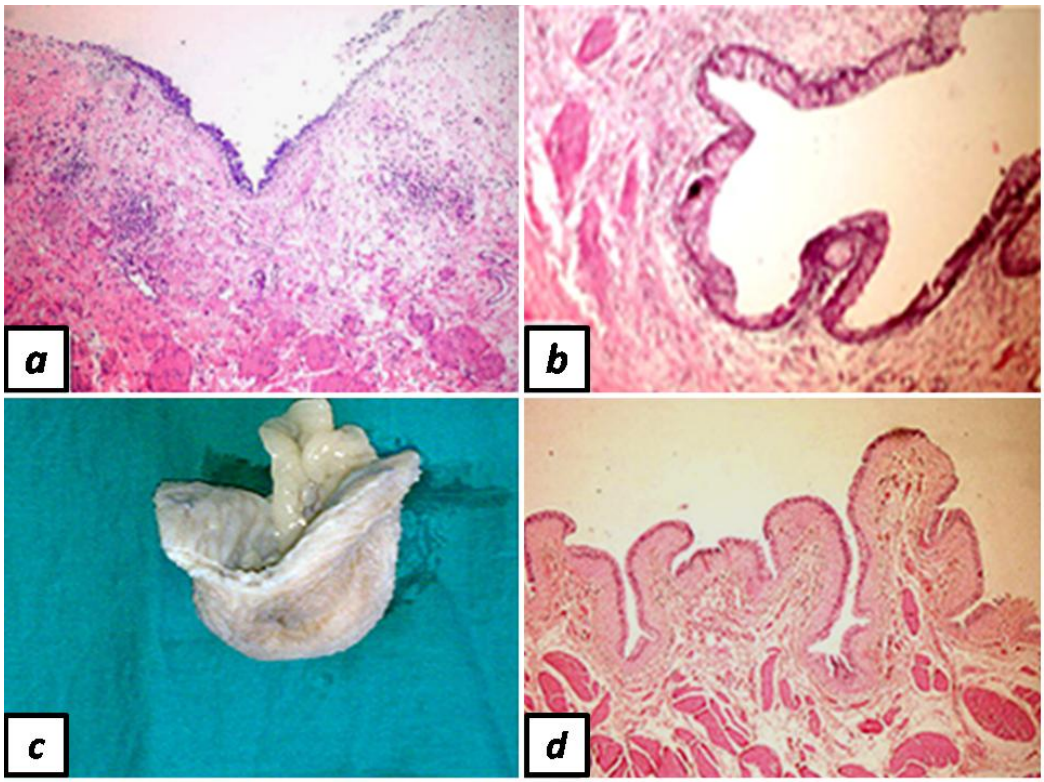

Fig. (8): Histopathologic and PM findings of the urinary bladder of autografted dogs.

a) Photomicrograph showing highly vascularised submucosal granulation tisues at one month postoperation (H&E, X100).
b) Photomicrograph showing hyperplastic transitional epithelium with smooth muscle bundles with normal urothelium two month postoperation (H&E, X100).
c) Gross appearance, normal wall thickness of the autografted bladder three months postoperation.
d) Photomicrograph showing regenerating transitional epithelium three month postoperation(H&E, X100).

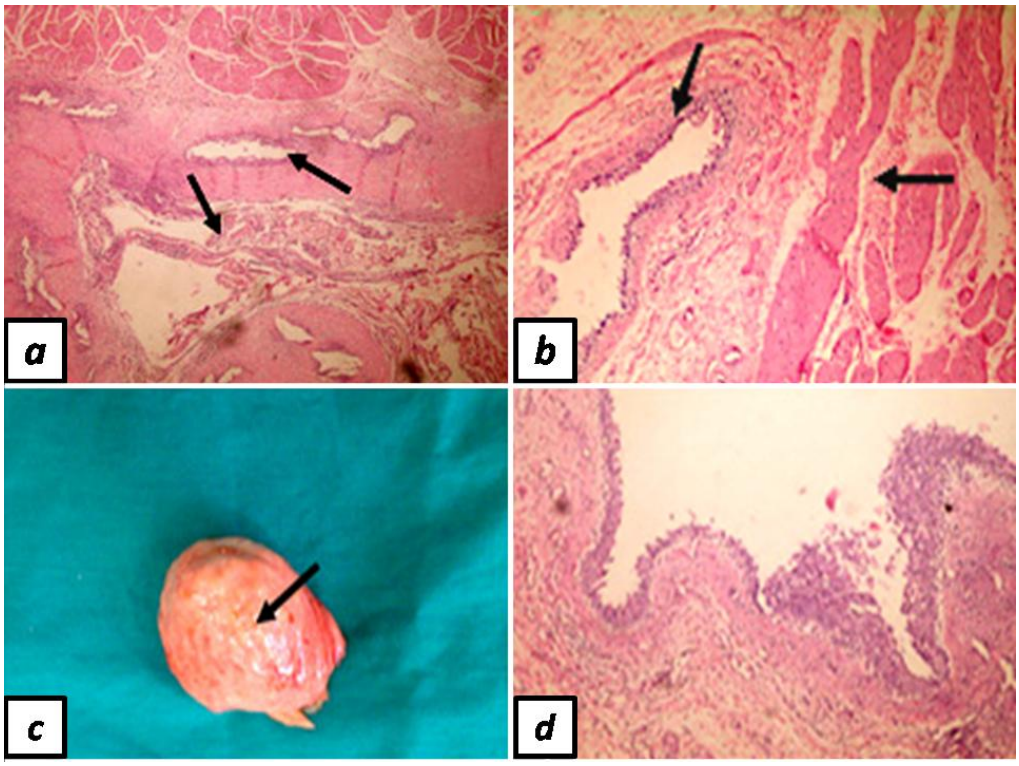

Fig. (9): Histopathological and PM findings of the urinary bladder of allografted dogs.

a) Photomicrograph showing multi layer regenerating transitional epithelium (arrows) and smooth muscle bundles one month post implantation. (H&E, X100).
b) Photomicrograph showing regenerating transitional epithelium, sub epithelial connective tissue proliferation with regenerating smooth muscle bundles (arrows) two months post implantation (H&E, X100).
c) Gross appearance, mild thickening and mild inflamed mucosal surface three months post implantation.
d) Photomicrograph showing hyperplastic regenerated transitional epithelium with subepithelial inflamed fibrous connective tissue months post implantation (H&E, X100).

The preserved amniotic membrane plays its role in early healing process due to its viable matrix which is more effective than viable epithelial cells as stated by (Sotozono, et al, 1994; Li and Tseng, 1995; Kilby, et al, 1996; Shimazaki et al., 1997 and Kruse et al., 2000). Several growth factors liberate from damaged amnion cells and serve as a feeder layer (Rheinwald and Green, 1975; Keelan, et al, 1998;; Kruse et al., 1999 and Kruse et al., 2000). It is recognized in several studies that stromal matrix contains specific compounds that suppress TGF-beta (Transforming Growth Factor) signaling, proliferation and myofibroblast differentiation and the basement side of the membrane has proved to be an ideal substrate for epithelial progenitor cells (Dekaris et al., 2001).

During amniotic membrane implantation procedures; the sutured graft might be water tight, as reported in other studies (Fishman et al., 1987 and Ismail et al., 2009). The use of surgical glue as a substitute for suturing the second layer of the membrane is concurrent with (Szurman et al., 2006 and Niknejad et al, 2008), Moreover, the use of double layer is influencing the healing process as reported by (Rodriguez-Ares et al., 2004). This is attributed to the use of multilayer membrane amplifying the concentration of useful factors secreted from the stroma (Dekaris et al., 2001; Prabhasawat, et al, 2001; Solomon, et al, 2002; Rodriguez-Ares et al., 2004 and Kalpravidh et al., 2009).

The urine analyses of both autografted and allografted dogs during the experiment showed no significant changes in comparison to the preoperative values. The blood urea nitrogen (BUN) and creatinine of the preoperative autografted and allografted groups compared to the postoperative values at one, two and three months revealed insignificant changes. This result are agreed with that mentioned by (Vaden et al., 2009). In comprasion to other used grafts, urinary system impairment have been recorded, as, reported by (McDougal, 1992; Kropp et al., 1995 ; Elbahnasy et al., 1998 and Shakeri et al., 2008).

As regard the diagnostic imaging, after elapse of one month it is shown in the autografted dogs that there is echogenic area at the craniodorsal

bladder wall corresponding to thickening of the wall that was proved by the negative and double contrasts. The previous findings are supported by the postmortum inflamed and thickened wall as well as the histopathologic findings that revealed the presence of inflammatory cells infiltration and highly vascularized submucosal granulation tissue. Negative contrast may be adequate for detection of most mass lesions and double contrast makes more defined wall thickness, mucosal margins and luminal contents (Mahaffy and Barber, 1992; Han et al., 1994 and Farrow, 2003). On the other hand after one month in allografted dogs there are irregularities of craniodorsal part of the wall. Two months post-operation; the autografted animals displayeed homogenous appearance of the dorsal wall of urinary bladder and the grafted part is proven by mild thickening of the bladder wall as shown in the negative and double contrasts. These findings are supported by the histopathologic findings that revealed a well formed bladder showing complete proliferated transitional epithelial lining, with regenerated submucosal fibrous connective tissue and full thickness muscle layer. On the other hand the allografted dogs displayed small hypoechoic thickening of the craniodorsal part that is proven by slight thickening at the craniodorsal surface with adhesions to omentum in the negative and double contrasts. This was confirmed by the histopathologic finding indicated regeneration of the transitional epithelium.

Three months post-autograft, the bladder displayed normal characteristics after ultrasonographic examination which proved similar to normal bladder appearance as shown in the negative and double contrasts. The histopathological findings revealed complete and full thickness regeneration of the bladder. Meanwhile, the allografted dogs displayed normal homogenous smooth bladder wall with presence of double hyperechoic dots at the edges of graft. This indicates remnants of the graft which confirmed by histopathological finding which demonstrate regenerating, hyperplastic transitional epithelium. Presence of a remnant of amniotic graft support the fact that the membrane acts as scaffold till bladder wall regeneration (Kruse, et al, 1998; Portis et al., 2000 and Wongsetthachai, et al., 2010). These findings reflect that

the bladder of both autografted and allografted dogs recover successfully to the normal structural, functional and contractile layers without any growth abnormalities

The stromal matrix of canine amniotic membrane contains proteinase inhibitors that promote healing of the epithelium and reduce the inflammation (Kim et al., 2000 and Kalpravidh et al., 2009). Moreover, the microscopic comparison of autografts and allografts at one month proved that the inflammatory cell infiltrations are more abundant in autografts than allografts. This suggests that the amniotic membrane suppresses inflammation rather than the rational autografting (Park and Tseng, 2000 and Kalpravidh et al., 2009)

CONCLUSION

From the present study it could be concluded that preparation and application of the amniotic membrane is a simple method. The use of surgical glue facilitates sealing of the minute pores of suture bites and limits the thin membrane suturing. Determinations of the graft thickness can be monitored using ultrasonography and contrast radiography. The urinary bladder wall gets its normal appearance three months post graft application.

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