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URINARY BLADDER RECONSTRUCTION USING FRESH AND FORMALIN-PRESERVED BOVINE AMNION IN DOGS

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ABSTRACT

The use of bovine amnion in the urinary tract for reconstructive purposes following ablative surgery in cases of trauma, cancer or infection is now a common practice in urological surgery.

To evaluate urinary bladder reconstruction with bovine amniotic membrane (BAM), ten healthy mongrel dogs of either sex weighing 10-40 kg were used. The animals were randomly divided into two groups of five animals each. A piece of the cranial wall of the bladder 5 cm in diameter was resected and replaced with fresh and formalin-preserved BAM respectively. The graft compatibility was evaluated on the basis of clinical, biochemical ultrasonographical, radiological and histopathological changes.

Clinically all of the dogs were dull and depressed with blood tinged urine for the first few post-operative days. The biochemical parameters didn't show any significant changes in BUN and creatinine. Ultrasonographic findings consisted of floating masses in the bladder lumen (40%), chronic cystitis (10%), bladder adhesion with adjacent tissues (90%) and radiological findings were lack of normal distension of the graft site (100%) and filling defect (30%). No inflammatory responses and leakage were observed.

The regeneration of uroepithelium, and proliferation of granulation tissue, infiltration of lymphoid cells, degenerative changes at the junction of the bladder and graft and heterotopic bone formation were observed. Keeping in view the compatibility of the fresh and preserved BAM, this study showed that it can act as a scaffold for repairing urinary bladder defects in dogs.

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INTRODUCTION

Different pathological conditions of the urinary bladder, such as congenital, acquired, and benign and malignant disease may require reconstructive surgery. Attempts to replace a bladder defect using biodegradable grafts of natural tissue such as autogenous fascia, alcohol preserved fascia, human dura, preserved bladder allograft, pericardium,[11] collagen films, human-placental membrane and other tissues have been reported.[13,14,20] The primary aim of cystoplasty is to convert a high pressure,[11,12,15,16] noncompliant and small capacity bladder to an organ of urinary storage at low pressure. Furthermore, urinary bladder reconstruction should aim to provide a suitable environment for regeneration of bladder tissue so as to develop it into a functional organ. Therefore, there is a need for the investigation of novel graft materials with minimum side effects and post-operative complications in order to improve the functional status fol-

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lowing reconstruction of the urinary bladder.[2,3,5,20]

MATERIALS AND METHODS

Experimental animals

The study was conducted on ten healthy mongrel dogs of either sex weighing 10-40 kg and aged between 7 to 30 months. The animals were kept under standard managemental condition, with free access to food and water. They were dewormed and vaccinated before the experiments.

Experimental design

The animals were randomly divided into two groups of 5 animals each. In the first group after removal of a 5 cm piece of bladder tissue, BAM was used for reconstruction, whereas in group II animals formalin-preserved BAM was used (Fig. 1). The animals were fasted for 24 hours prior to surgery. On the day of the experiment, premedication was given by intramuscular injection of acepromazin maleate (0.1 mg/kg), and anesthesia was induced and maintained with sodium thiopental (20 mg/kg). The animals were positioned on dorsal recumbency and after preparation of the surgical site, a 10 cm midline incision was given caudal to the umbilicus.

Urinary catheterization was done before surgery and the catheter was fixed to the external orifice of the prepuce and remained until the seventh post-operative day.

Surgical procedure

A piece 5 cm in diameter was resected from the cranial wall of the bladder and replaced with fresh (group I) or formalin-preserved (group II) bovine amniotic membrane (Figs. 1,2). The graft was sutured using silk in a simple continuous layer. Leakage was checked by means of normal saline injection through the catheter (Fig. 3). The incision site was sutured in routine method. Parenteral antibiotics (cephalothin sodium 20 mg/kg and gentamicin 2 mg/kg) were administered once a day to all dogs for 5 post-operative days.

The compatibility of the graft was evaluated on the basis of clinical (general condition of animals, CBC, urine analysis, infection), biochemical (BUN, creatinine), ultrasonographical, radiographical and histopathological examinations.

Table I. Mean values of white blood cell counts (Mean±SE).

Groups	Day 0	Day 1	Day 3	Day 7	Day 60
I	5990 ±502	11550 ±1917	10220 .±423	8660 ±423	7520 ±1388
II	10710 ±1454	11380 ±1269	10790 ±2105	8200 ±200	8380 ±562

WBC mean values did not show significant changes ($p>0.05$) in either group

RESULTS

During or immediately after parturition, bovine amnion sacs were collected under strict sterile conditions for grafts. Anesthesia was given effectively in both groups of animals. Application of the amniotic sac as a graft was easier in animals of group II as compared with group I. A simple continuous inverting suture with silk (3/0) had satisfactory results in all animals of both groups, while catheterization of the bladder would minimize pressure on the graft site and prevent accumulation of urine until removal of the catheter.

Clinically, the animals were depressed and anorectic for a few days after surgery, and the urine was blood tinged for the first 4 days after surgery.

The temperature, pulse and respiration rate did not show any significant changes except in two animals of both groups (one in each) due to post-operative infections, either from graft materials or from septic conditions during surgical grafting.

Cell blood counts (white blood cell, hematocrit, neutrophils, lymphocytes, eosinophils, monocytes and band cells) showed significant changes in the first few post-operative days in all animals (Table I). There were significant changes in one animal in each group which were infected. The volume of urination was increased in all animals after taking food and water from the second day of operation.

The biochemical estimations revealed a slight increase in serum urea nitrogen and creatinine, which returned towards base levels after the 5th post-operative day (Table II). Ultrasonographic studies revealed floating masses in the bladder lumen (40%), chronic cystitis (10%), and bladder adhesion of the graft site with adjacent tissues (90%) (Figs. 5-6). The lack of distension at the graft site (100%), filling defect (30%), along with no inflammatory responses or leakage were observed in a series of radiographs obtained postoperatively (Fig. 4).

Gross observation of the graft site on postoperative days 30 and 60 showed adhesion at the graft site (100%), floating graft within the lumen of the bladder (40%), good attachment of the graft to the urinary bladder, and no evidence of leakage or fistulization.

Histopathological examinations revealed regeneration of uroepithelium and a few smooth muscle cells in the graft site. Other important findings were proliferation of granulation tissue, infiltration of lymphoid cells, degenerative changes at the junction of bladder and graft and heterotopic bone formation. In addition, congestion, edema, and inflammatory cell infiltration were also seen in two cases (Figs. 7-12).

On the basis of these results, in spite of complications such as infection, detachment of amnion from the bladder, lack of normal distension and adhesions of the urinary bladder at the graft site, it may be concluded that fresh and pre-

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Table II. Mean values of serum BUN and creatinine levels (mg/dL).

Group	Day 0		Day 1		Day 3		Day 5		Day 7		Day 60	
	BUN	Cr	BUN	Cr	BUN	Cr	BUN	Cr	BUN	Cr	BUN	Cr
I	17.08 ±5.39	1.40 ±1.01	20.36 ±513	1.43 ±1	16.34 ±2.57	1.46 ±0.24	19.12 ±3.82	1.56 ±0.42	19.52 ±6.17	1.63 ±0.54	14.94 ±4.31	1.02 ±0.25
II	19.34 ±7.23	1.69 ±0.3	22.92 ±4.05	1.92 ±1.62	23.48 ±4.50	1.55 ±0.52	22.67 ±6.51	1.15 ±0.15	17.00 ±5.29	1.05 ±0.34	16.08 ±6.44	1.03 ±0.44

Cr= Creatinine

BUN= Blood Urea Nitrogen

BUN and creatinine levels did not show significant changes in either group ($p>0.05$).

served bovine amnion acts as a scaffold for repair of the urinary bladder defect in dogs.

DISCUSSION

Uroepithelium is known for its great regenerative property.[1] Remodelling of fibrous scar, hypertrophic tissue, proliferation of smooth muscles and stretching of the bladder remnant also occur following bladder resection.[9] Therefore, the primary objective of urinary bladder reconstruction is to provide a suitable regenerative bed for normal bladder tissue to expand further and regain its ability to be a functional organ for urinary storage. All animals of both groups passed blood tinged urine after full recovery. The presence of blood in urine could be attributed to surgical trauma. Similar findings have been reported by Fishman et al.,[5] Light[10] and Shivaprakash.[18]

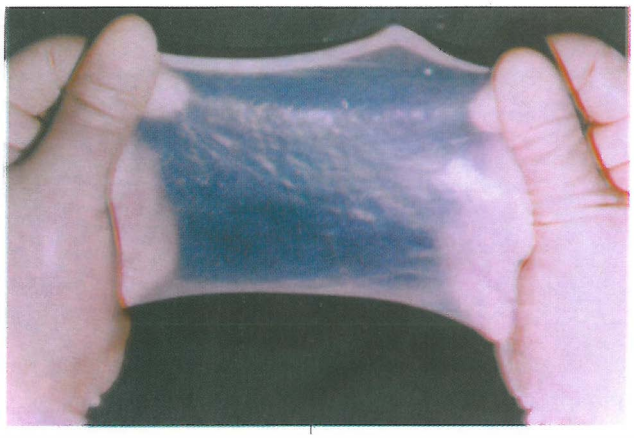


Fig. 1. Fresh bovine amniotic membrane.

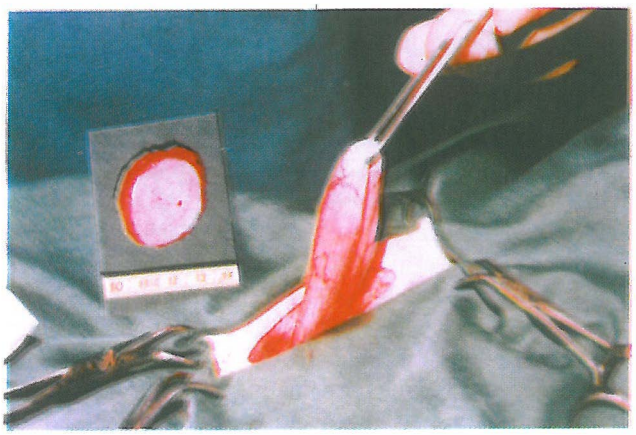


Fig. 2. Exposure of urinary bladder and subsequent resection of a piece of bladder tissue 5 cm in diameter.

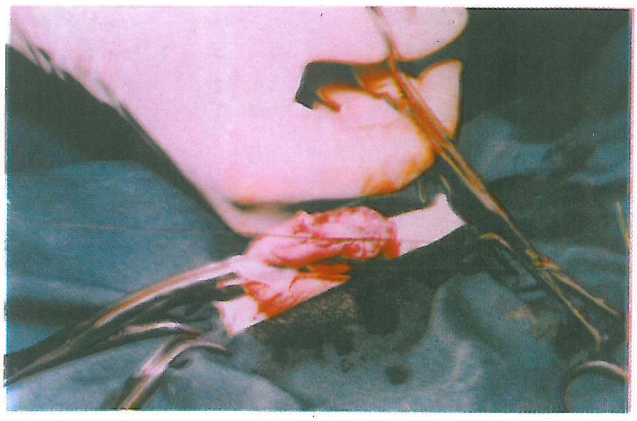


Fig. 3. Suturing BAM to the bladder.

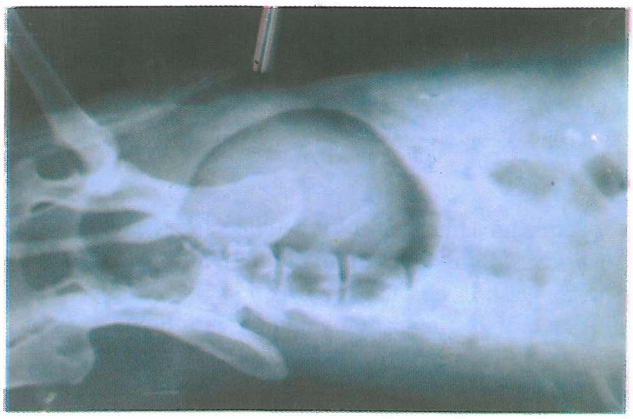


Fig. 4. Site of graft after full distention of the bladder.

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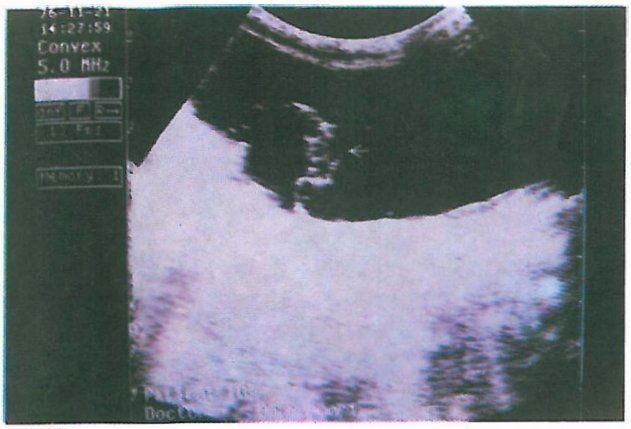


Fig. 5. Floating mass in saggital scanning with ultrasonography.

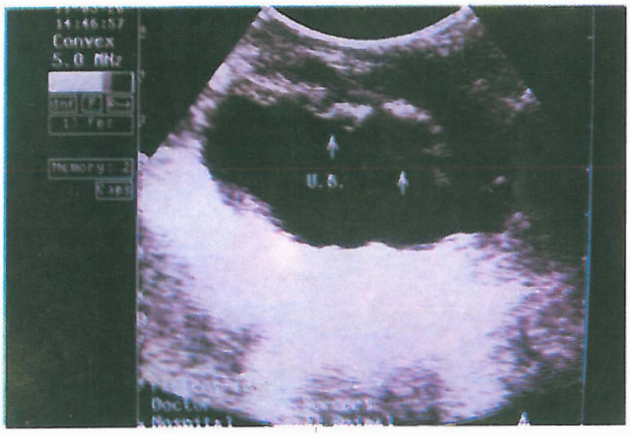


Fig. 6. Visualisation of graft sites through transverse ultrasonographic scanning.

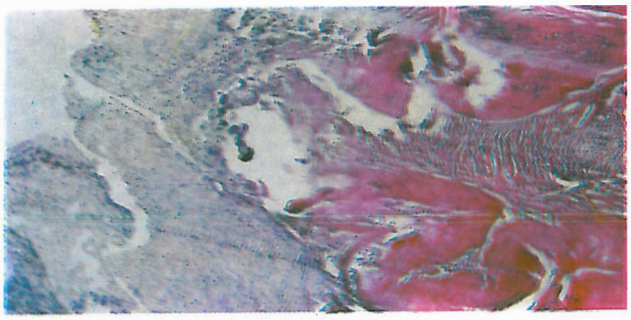


Fig. 7. Histomorphological changes at the site of graft showing granulation tissue and transitional epithelium (reddish part). H&E, ×160.

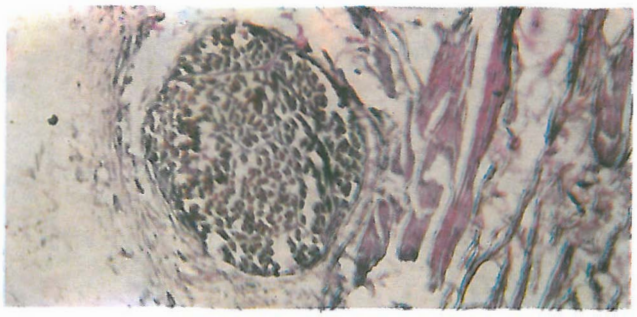


Fig. 8. Histomorphological changes at the suturing site.

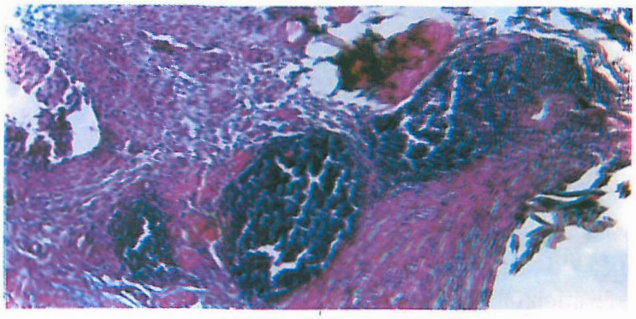


Fig. 9. Lymphoid cells in granulation tissue (H&E, × 100).

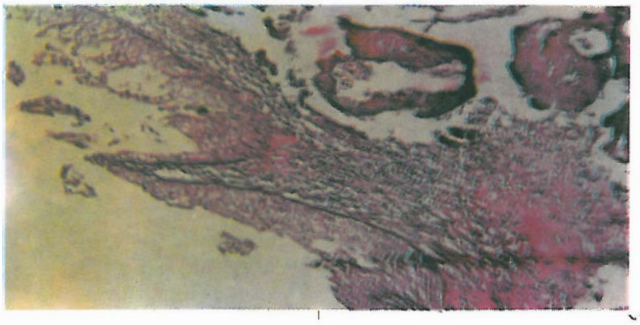


Fig. 10. Transitional epithelium (*), infiltration of mononuclear cells beneath the epithelium (**) and heterotopic bone formation (***). H&E, × 160.

Serum urea nitrogen and creatinine increased gradually, but remained however within normal limits as reported by Shivaprakash in goats.[18] Other workers have reported significant changes in serum urea nitrogen following bladder reconstruction with different types of graft materials.[2,7,12,15]

The use of air as a negative contrast agent for visualization of the urinary bladder is well documented.[17] Other workers have also used positive as well as double contrast cystograms for the diagnosis of lesions in the urinary bladder.[12]

This study confirmed the findings of Fishman et al.[5] radiologically, and floating masses in the bladder lumen, chronic cystitis, bladder adhesions at the graft site reported by Hertzberg et al.[8] ultrasonographically.

The regenerative ability of urinary bladder has been documented by Bohne et al.[1] and Stanley et al.[19] as they reported regeneration of epithelial cells and smooth muscle. Taguchi et al.[20] observed the development of granulation tissue during regeneration of the bladder. Gera et al.[7] reported complete detachment of the formalin preserved allograft within 60 days. In this study regeneration of urothelium and the presence of smooth muscle were observed which was similar to their findings.

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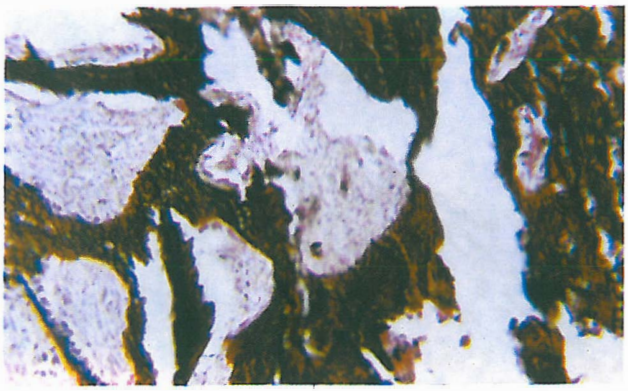


Fig. 11. The brownish black parts show heterotopic bone formation (von Kossa staining, × 400).

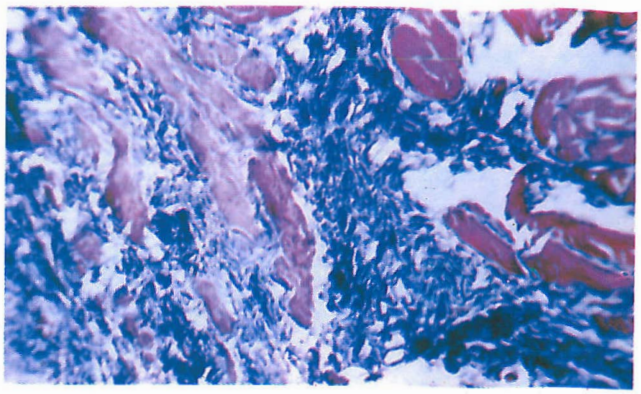


Fig. 12. Abundant proliferation of granulation tissue (bluish part) and presence of a few smooth muscle cells (reddish part). (Masson's trichrome staining, × 400).

Regeneration of urothelium and the presence of smooth muscle in microscopical sections and lack of important complications that may occur with enterocystoplasty may encourage use of bovine amniotic membrane for urinary bladder reconstruction.[6,21] However, long term studies are needed to assess other clinical and laboratory findings before its recommendation for clinical use.[22]

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