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RADIOGRAPHIC STUDY OF CYSTOPLASTY USING ACELLULAR BOVINE PERICARDIUM REINFORCED WITH TOPICAL APPLICATION OF AUTOGENOUS PLASMA-RICH FIBRIN IN DOGS

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ABSTRACT

This work was designated to reconstruct the experimental bladder defect using acellular bovine pericardium, reinforced with topical application of autogenous plasma-rich fibrin in dogs. A circular defect was induced in the UB of twelve adult stray dogs undergoing a protocol of sedation and anaesthesia. The animals were divided randomly into two groups of 6 each. In the first group, the defect was reconstructed with 4 cm of acellular bovine pericardium (ABP), which was previously prepared. In the second group, cystoplasty was done, and the site of operation was wrapped with previously prepared autogenous platelet-rich fibrin (PRF). Clinical observation was recorded for a few days postoperatively, and radiographic investigation at 7-, 15-, 30-, and 45-days post-surgery was recorded. The radiographic changes in the first group from 7 to 45 days post-surgery showed a distended UB with a consistent pear shape. There were depression irregularities at the upper anterior area of the bladder at the implanted site. No filling defects and no out-bulging in the wall. There was no leakage of urine. In the second group, the radiographic changes at 7, 15, and 30 days indicated that the UB is semi-distended with an altered pear shape and configuration, whereas after 45 days post-treatment, the UB was well-distended with a consistent pear shape, uniform wall, and smooth configuration. No diverticulum nor leakage were recorded. In conclusion, radiographic evaluation showed that using ABP supported with PRF reveals an improvement in the healing process of experimentally induced bladder defects.

Keywords: Acellular pericardium, Plasma rich fibrin, Cystoplasty, Contrast Radiography.

INTRODUCTION

Cystorrhaphy and cystoplasty are indicated in many disorders in dogs and other animals, such as inflammatory diseases of the urinary tract, congenital illnesses, nerve injury space occupying the lesion, accidental damage, and patent urachus, which may cause bladder injury or rupture (Granger *et al*., 2020; Alhamdany and Alkattan, 2019; Brehmer *et al*., 2007). Urinary bladder defects are represented as challenges that need surgical interventions to avoid critical complications, such as uroabdomen and obstruction of the lower urinary tract (Nguyen and Mitchell, 1991).

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These abnormal defects or injuries, whether acquired or congenital, make the bladder a non-suitable environment for urine storage and emptying (Ekder and Mahdi, 2021).

The bioactive agents must be biocompatible and contribute to reconstruction of the defective bladder (Ajalloueian *et al*., 2018). Many bioscaffolds were used successfully to reconstruct the defective urinary bladder as tunica vaginalis grafts (Ahmed *et al*., 2022; Ekder and Mahdi, 2021). Processed bovine pericardium (PBP) is termed as a particularly important bioactive material, which was used to improve the healing process of different defects that need surgical interventions. PBP represented one of the most bioactive materials, which was used with varying degrees in different defects to improve regenerative processes in the surgery field (Van Rijn *et al*., 2016), as duraplasty (Filippi *et al*., 2001), urethroplasty in dogs (Lara *et al*., 2004), repairing defective tendons, and reconstruction of defective abdominal walls in dogs (Pokrywczynska *et al*., 2022). The acellular pericardium was used with reliable results without tissue reaction and was biocompatible with the recipient tissue. It was compatible with defective tissue with low cost (Singh *et al*., 2022).

Plasma-rich fibrin (PRF) is considered a biomaterial that contains high white blood cells, especially leukocytes, and plays an important role in angiogenesis. It contains many important growth factors, such as fibroblast growth factor basic (FGFb), angiopoietin, platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) (Caruana *et al*., 2019). These substances act as antimicrobial agents used as gelatinous agents (Gollapudi *et al*., 2022; Allawi *et al*., 2019) and are easily applicable, mainly in veterinary medicine, to enhance healing of soft tissue after hernioplasty in rams (Zedan *et al*., 2022).
It has several applications in veterinary medicine, especially in maxillofacial and oral surgery, intra-bone defects, gingival recession, alveolar filling after extraction, and sinus lifting. PRF bioactive material increases deposition and regeneration of bone because it contains cytokines and growth factors (Atiyah and Alkattan, 2024). The aim of this study was to evaluate using acellular bovine pericardium supported with autogenous plasma-rich fibrin in improving the repair of experimentally induced urinary bladder defects in dogs.

MATERIALS AND METHODS

Ethical approval

This work was approved by the Ethical Committee of the College of Veterinary Medicine, University of Mosul, NO.UM.VET.2023.069.

Experimental design

This work was carried out on twelve adult stray dogs with (mean ±SE) weight (18 ±1.9) months and (mean ±SE) age (25 ±1.1) kg. Animals were randomly allotted to two equal groups. In the first control group, the urinary bladder defect was reconstructed using acellular bovine pericardium. In the second treated group, the same procedure as in the first group was done, but the implanted tissue was supported with PRF. Acellular bovine pericardium prepared according to (Narayanaswamy *et al*., 2023; Singh *et al*., 2022). To prepare PRF, 10 cm of blood was collected aseptically from the jugular vein in a tube without anticoagulant, then centrifuged and prepared according to (Dohan *et al*., 2006).

Surgical operation

The animal was prepared aseptically, and a regime of general anaesthesia was advised as a mixture of ketamine HCL 5% (Dutchfarm—Holland) with xylazine HCL 2% (Interchemi—Holland) at a dose of 15 and 3 mg/kg B.W., respectively (Alkattan and Helal, 2013).

The animal was restrained in lateral recumbency, and pre-pubic celiotomy was performed under strict aseptic conditions. The urinary bladder was prepared for partial cystectomy. A urethral catheter was

introduced via the urethra to evacuate the bladder of urine. The bladder was exteriorized and fixed by using two stay stitches of 3-0 polyglactin 910 (Yangzhou Super Union, China). A 3-cm full-thickness circular defect was induced at the dorsal aspect of the bladder.

In the first group, the prepared 4 cm bovine pericardium was implanted on the defect and fixed firmly by 3-0 polygalactin 910 using Lembert and Cushing suture techniques. Whereas in the second group, the same repair procedure was done with the addition of PRF to augment the implanted pericardium. The skin and abdominal incision were closed routinely in three layers: parietal peritoneum and muscles in simple continuous using 2-0 polyglactin 910, subcutis in simple continuous using 2-0 polyglactin 910, and skin in simple interrupted using 2-0 silk suture material (Yangzhou Super Union, China).

Healing was evaluated radiographically by cystography by using an X-ray machine (Shimadzu, Japan) accompanied by the digital wireless detector (Italray, Italy) and 10 ml of organic iodinated positive contrast media (Turkey-kopak). The contrast medium was diluted with 40 ml normal saline and injected retrograde into the bladder cavity by urethral catheter. The exposure factors were set as 60 kV and 0.02 mAs at ventro-dorsal and lateral views to exhibit the integrity of the bladder wall during the intervals of 7-, 14-, 30-, and 45-days post-treatment.

RESULTS

In the first treatment group with acellular bovine pericardium, the clinical results exhibited no adverse complications and no signs of rejection or immune reaction during the period of the experiment.
Two dogs in this group presented some clinical signs, such as pain during urination, haematuria and loss of appetite, incontinence, and arching of the back. These signs gradually subsided for a few days post-surgery, and the animals returned to normal activity and behaviour during urination.

Radiographic investigation at 7 days post-surgery, the bladder was well distended with a consistent pear shape. It had a uniform wall and smooth configuration, no filling defects, no out bulging (diverticulum) in the wall, and no leakage (Fig. 1). At 15 days post-surgery, the bladder was distended with a consistent pear shape. The dorsal cranial area of the bladder wall (site of the graft) was seen as an irregular depression, with no filling defects, no out bulging (diverticulum) in the wall, and no urine leakage (Fig. 2). At 30 and 45 days, changes exhibited by semi-distention of the UB wall with a constricted pear shape. However, there was depression with irregularities at the dome (site of the graft), no filling defects, and no leakage (Figs. 3 and 4).

In the second group, at 7, 15, and 30 days, the UB was semi-distended with an altered pear shape and configuration. However, there was a depression with irregularities at the dome (site of the graft), no filling defects, no wall bulging (diverticulum), and no leakage (Fig. 5).

Retrograde cystogram at 45 days after surgery: the UB was well distended with a consistent pear shape, had a uniform wall and a smooth configuration, no filling defects, no diverticulum, and no leakage.

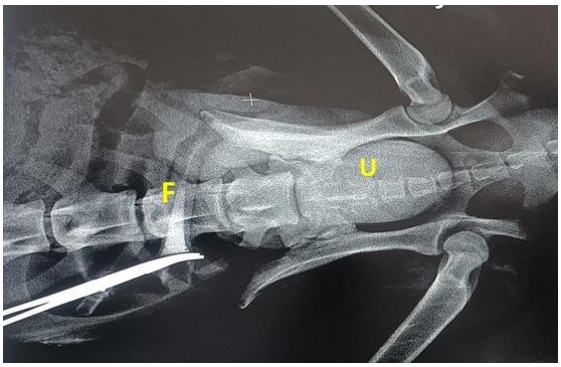

Figure 1: Ventro-dorsal radiograph of the pelvis at 7 days post-surgery in a dog of the first group (F=catheter, U=urinary bladder).

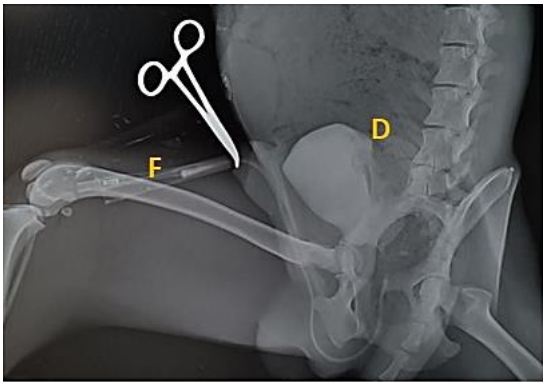

Figure 2: Lateral radiograph of the pelvis at 15 days post-surgery in a dog of the first group (F=catheter, D=site of urinary bladder defect).

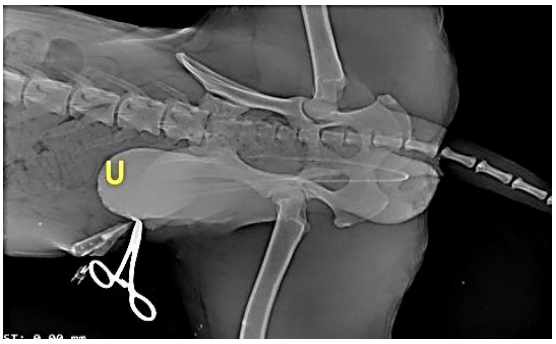

Figure 3: Ventro-dorsal radiograph of the pelvis at 30 days post-surgery in a dog of the first group (U=urinary bladder).

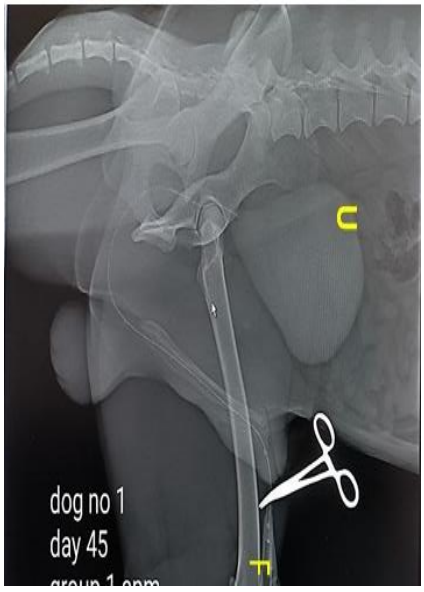

Figure 4: Lateral radiograph of the pelvis at 45 days post-surgery in a dog of the first group (F=catheter, U=urinary bladder).

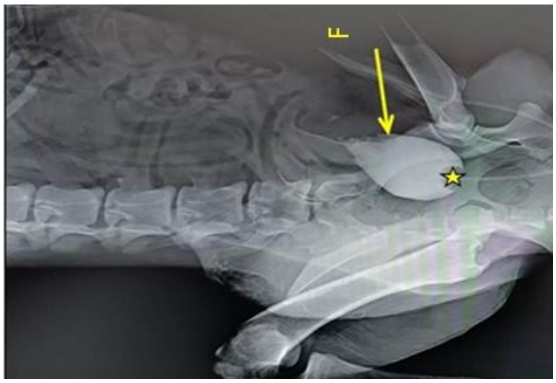

Figure 5: Ventro-dorsal radiograph of the pelvis at 7 days post-surgery in a dog of the second group (F=catheter, Yellow Arrow=site of urinary bladder defect, Yellow Star=balloon of the Foley catheter).

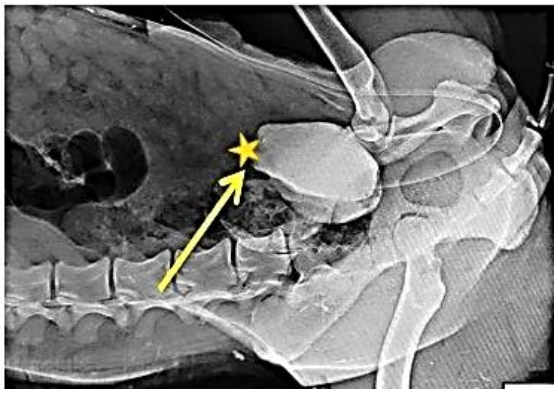

Figure 6: Lateral radiograph of the pelvis at 15 days post-surgery in a dog of the second group (yellow arrow = site of urinary bladder defect, yellow star = balloon of the Foley catheter).

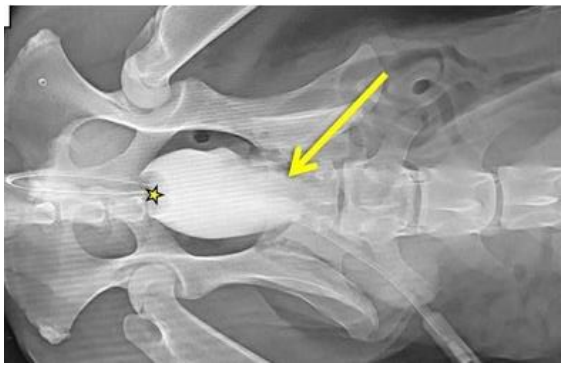

Figure 7: Ventro-dorsal radiograph of the pelvis at 30 days post-surgery in a dog of the second group (yellow arrow = site of urinary bladder defect, yellow star = balloon of the Foley catheter).

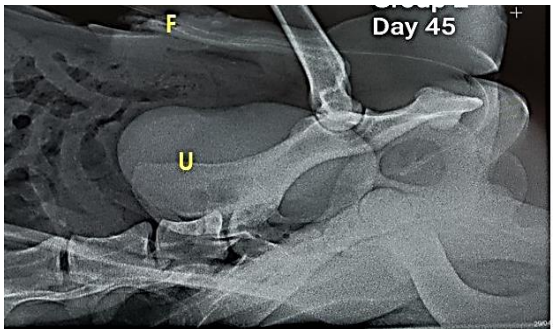

Figure 8: Ventro-dorsal radiograph of the pelvis at 45 days post-surgery in a dog of the second group (F=catheter, U=urinary bladder).

DISCUSSION

The acellular bovine pericardial graft is considered a bioactive material. It is a biodegradable agent, directly compatible with the urinary bladder, and contributes to the healing of this organ. In this study, this organ gradually restores its normal function and capacity, as in another study (Salehipour *et al*., 2016). In concern of haematuria, this sign occurred due to trauma and manipulation during surgery of the bladder. These signs and abnormal behaviour during urination subsided during the first few days post-surgery in both groups. These results agree with those of other studies (Alhamdany and Alkattan, 2019; El‑Taji *et al*., 2015; Broen, 2002) and were attributed to trauma at the surgical site of operation. Concerning the signs of incontinence and arching of the back during micturition, these signs were due to pain and abdominal muscle stress. These signs subsided gradually by 48 hours post-operation (Alhamdany and Alkattan, 2019; Al-Asadi and Khwaf, 2014). In this trial positive cystography has beneficial value to exhibit the irregularities in the mucosal surface and the conformation in the bladder structure inside the abdominal wall. The closure of the bladder defect was secure without leakage of urine intraperitoneally (Gallatti and Iwasaki, 2004).

The retrograde cystography of the first group exhibited a smooth configuration with an irregular pear shape of the urinary bladder without leakage at 7-, 15-, and 30-day post-surgery. Whereas the radiographic changes at 45 days exhibited a regular pear shape, indicated good healing, and the implanted bovine pericardium merged completely with the host tissue. There were cross-links between bladder and bovine pericardium collagen fibers similar to the results of other studies (Singh *et al*., 2021; Spiliopoulos *et al*., 2015). The bovine pericardium is rich in collagen type 1, cytokines, and growth factors, so this implant succeeds in filling the defective bladder without rejection. These results agreed with those of another study (Al-Hyani and Al-Hasan 2019).

In the second group, the augmentation occurs in the bladder after implantation with bovine pericardium, which is reinforced with PRF. The PRF acts as a bioactive material that contributes to healing, enhancement, and improved augmentation of defective bladders. This enhancement in healing was attributed to its content of growth factors and cytokines. Radiographic changes in this group exhibited a regular smooth structure configuration of the bladder without leakage. The good response, especially in this group, is due to adding PRF, which plays an important role in accelerating the healing process of the implanted bladder. Similar results in other studies that used PRF for improving bone gaps in dogs (Atiyah *et al*., 2024; Al-Saiegh *et al.*, 2024) supported the current results.

CONCLUSION

The results of the current investigation demonstrated that bladder augmentation cystoplasty (AC) was successful, and the function of the urinary system was restored in the ABP + PRF group and the ABP group.

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Conflict of interest

The authors declare there are no conflicts of interest.

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دراسة شعاعية لاستئصال المثانة باستخدام غشاء التامور البقري اللاخلوي المعزز بالتطبيق الموضعي للفيبرين الغني بالبلازما الذاتية في الكلاب

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الخلاصة

تم تصميم هذا العمل لإعادة بناء عيب المثانة التجريبي باستخدام التامور البقري اللاخلوي، المعزز بالتطبيق الموضعي للفيبرين الغني بالبلازما الذاتية في الكلاب. تم إحداث عيب دائري في المثانة لاثني عشر كلبًا ضالًا بالغًا يخضعون لبروتوكول التهدئة والتخدير. تم تقسيم الحيوانات عشوائيًا إلى مجموعتين من ٦ حيوانات لكل منهما. في مجموعة العلاج الأولى، أعيد بناء العيب باستخدام ٤ سم من التامور البقري اللاخلوي والذي تم تحضيره مسبقًا. في المجموعة الثانية، تم إجراء رأب المثانة كما في المجموعة الأولى، وتم لف موقع العملية بالفيبرين الغني بالصفائح الدموية المحضر مسبقًا. تم تسجيل الملاحظات السريرية لبضعة أيام بعد الجراحة، وتم تسجيل الفحص الشعاعي في الأيام ٧ و١٥ و٣٠ و٤٥ بعد الجراحة. أظهرت التغيرات الشعاعية في المجموعة الأولى من ٧ إلى ٤٥ يومًا بعد الجراحة أن المثانه متمددًا مع شكل كمثرى ثابت. كانت هناك منخفضات غير منتظمة في المنطقة الأمامية العلوية للمثانة في موقع الزرع. لا توجد عيوب في الملء ولا انتفاخ في الجدار. لم يكن هناك تسرب للبول. في المجموعة الثانية، أشارت التغييرات الشعاعية في ٧ و١٥ و٣٠ يومًا إلى أن المثانة متمددة جزئيًا مع شكل وتكوين كمثرى متغيرين، بينما بعد ٤٥ يومًا من العلاج، كانت المثانة متمددًتا جيدًا مع شكل كمثرى ثابت وجدار موحد وتكوين ناعم. لم يتم تسجيل أي رتج أو تسرب. وخلصت نتائج هذه الدراسة، اعتمادًا على التقييم الشعاعي أن استخدام غشاء التامور البقري المدعوم بـالفبرين الغني بالبلازما, أدى إلى تحسن في عملية الشفاء من عيوب المثانة المستحثة تجريبيًا.