



Efficacy of Cat-fish Acellular Dermal Matrices in Repairing of The Induced Ventro-lateral Hernias in Bucks

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Abstract

The study was aimed to evaluate the efficacy of cat fish acellular dermal matrices in reconstruction of induced ventro-lateral hernias in bucks. Twenty-four bucks were enrolled for this purpose and divided into two equal groups. All bucks were subjected to surgical induced 7 cm diameter ventro-lateral hernia in the right lower flank under sedation and local anesthesia. After one month from creation of hernia, hernioplasty was performed in G1 by (on-lay) fenestrated cat-fish acellular dermal matrices (ADMs) and in G2 by (on-lay) non-fenestrated cat-fish ADMs technique. The defects were secured with No.1 polypropylene suture. The skin and subcutaneous tissue were re-approximated with silk suture No.1 by interrupted horizontal mattress suture. To evaluate the efficacy of hernioplasty, clinical follow-up was performed weekly for 12 weeks, and a strips (7 cm length and 2 cm width) from reconstructed site were collected at 4, 8 and 12 weeks post-hernioplasty for determination of tensile strength. Clinically the hernias in in both groups were disappeared, few complications were noticed represented by seroma in three animals (n=3) (all responded to treatment) and hernia recurrence in one animal (n=1). There was significant decrement in tensile strength at the 4th week in both groups, and then the tensile strength increased gradually with time starting from (8th-12th week). Tensiometric evaluation declared, significant ($p \leq 0.05$) differences between two groups in all periods of study, and the tensile strength of G2 is better than that of G1. The study revealed that catfish ADMs serve as a good hernioplastic patch, having good tensile strength and accelerate the repair of hernias in bucks. The tensile strength of G2 is better than that of G1 and this exceed the normal values of tensile strength of the abdominal muscles up to 50%.

Key words: Ventral hernia, Fish acellular dermal matrices, Tensile strength, Buck.

Introduction

Hernia means an exit of part of abdominal organ or tissue through the cavity that contain it (1). Hernias can be congenital or acquired. Ventral hernias in farm animals are acquired. It might be due to severe trauma (2). The most

common herniated organs of the ventral hernia are the loops of intestine, part of omentum or both. Hernia consists of three parts (ring, content and sac). Swelling is the classical sign of ventral hernia (3). Diagnosis of hernia



depends on palpation, clinical signs or sonography (3, 4). There are different techniques of treatments for hernia. When it is small and reducible it is corrected surgically (herniorrhaphy) using various types of suture materials to oppose and close the hernia defects. However, such reconstruction often associated with high reoccurrence rate (5, 6). To avoid such complications hernioplasty using prosthetic meshes are the treatment of choice for large defects. This also accompanied with several complications such as infection, adhesions, fistula formation, mesh extrusion (7, 8). To overcome these mesh-related complications, nowadays, biomaterials derived from animal sources may be preferred for the surgical repair of abdominal wall defects such as ADMs (9), acellular bovine pericardium and urinary bladder sub-mucosa matrices (10). Recently biological matrices such as fish ADMs (11) freshwater and marine fish ADMs (12) had been used for reconstruction of different size abdominal hernia in bucks. Due to limited references about the uses of fish ADMs, therefore the present study was undertaken to evaluate the efficacy of cat-fish ADMs for repairing of induced ventro- lateral hernias in bucks.

Methodology

Animals

Twenty-four apparently healthy bucks had been used in the current study, weighing (28-32) Kg and aged (2-3) years. They were acclimatized for two weeks in the field prior to initiation of research.

Preparation of catfish acellular dermal matrix (ADM)

The preparation was done according to the method described by (13). The ADM of catfish was prepared after the skin obtained by skinning with a surgical blade. To completely overcome the hernia defect (9x9) cm catfish skin was prepared. It was thoroughly cleaning with running tap water to get-rid any debris, then immediately preserved in cold PBS containing 0.1 percent Amikacin. In a hypertonic solution comprising tris base (605 mg), sodium chloride (4 g), and ethylene diamine tetra acetic acid (EDTA) (202.5 mg) in 100 mL PBS, the skin sections were de-epithelialized for two hours followed by de-cellularization for 12 hours with 1 percent sodium deoxycholate then rinsed in sterile PBS and stored at -4°C in PBS containing 0.1 percent amikacin and 0.1 percent sodium azide. The skin pieces were agitated constantly on an orbital shaker during the de-epithelization and de-cellularization processes at (150 rpm). Twelve Catfish ADMs patches were manually fenestrated by rounded sharp pin (3mm in diameter) and spaced 10 mm apart between each hole for use in G1, and the other remain non-fenestrated to use in G2.

Pre-Operative considerations

Bucks were off-feed 12 hrs. and 6hrs. water withheld prior to surgery. Broad spectrum antibiotic, Pencillin-Streptomycine (Interchemie, Holland) 1ml / 10kg B.W. was injected I.M. one hour before surgery. The right flank was prepared for aseptic surgery. Animals were sedated with 2% xylazine HCl (Interchemie, Holland) in a dose 0.2 mg/kg I.M., then local anesthesia was performed by linear S/C infiltration of 2% lidocaine HCl



(Johnlee pharmaceuticals, India) at the intended site of incision in a dose of 10 mg/kg (14).

Technique to induce hernia:

The animals were submitted to surgical procedure to provoke a standard opening (artificial hernia) in the ventro-lateral abdominal wall. Under aseptic condition a right low flank cutaneous incision was made 10 cm in length and bleeding was carefully arrested. The S/C tissue was dissected to identify the abdominal muscles. which were opened bluntly, and then part of them was cut and discarded to create a hernial ring in a diameter of (7 cm). The skin and S/C tissue were re-stitched with non-absorbable suture material (silk no.1) by interrupted horizontal mattress suture and the hernia formed directly after recovered from analgesia and become more obvious and increased in size when bucks were standing (figure 1).

Experimental design

The bucks were allocated to two treatment groups equally G1 and G2. Hernias in G1 were repaired via fenestrated cat-fish ADMs by (on-lay technique). While G2 were repaired with non-fenestrated cat-fish ADMs by the same technique.

Surgical repair of hernias (hernioplasty)

After one month post creation of the hernia, the operative site was prepared aseptically. The animal restrained in dorso-lateral recumbence

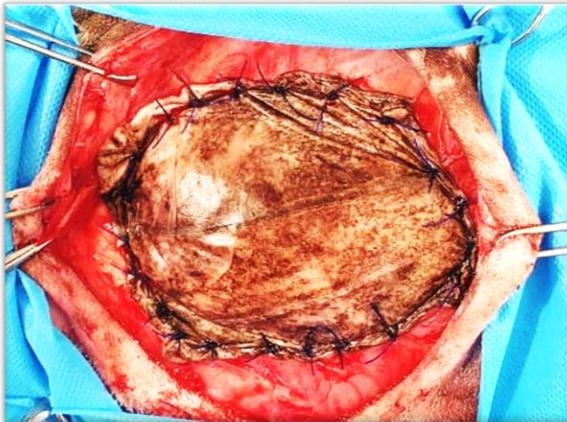
on a surgical table, and after sedation and local anesthesia, skin incision with sufficient length parallel to the first one had been done directly over the hernia. The skin edges were reflected laterally and separated from the peritoneal sac up to hernia ring by blunt dissection to expose the circumference of the abdominal wall defect. Once this has been achieved, The sac with its content push back again to the abdominal cavity. Hernioplasty was performed in G1 via on-lay fenestrated fish ADMs (figure 2 A), and G2 was repaired by on-lay non-fenestrated ADMs (figure 2 B). In both groups, the matrices put circumferentially 2cm; far from the hernial ring and were secured with sutures of polypropylene (no.1) using interrupted U-shape mattress suture pattern (1cm apart). The subcutaneous tissue and skin were re-approximated with non-absorbable suture materials (silk no.1) by interrupted horizontal mattress pattern. Thus hernia swelling was disappeared with normal appearance of abdominal wall (figure 3).

Post-operative cares

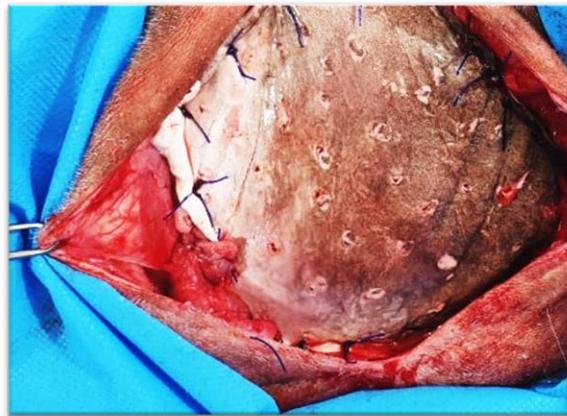
Operated animals were injected with broad spectrum antibiotic for five successive days to overcome infections. Furthermore, analgen (Metalgin 1%) was injected IM; as analgesic at a dose rate of 1 mg/kg B.W., for the first three days post-surgery. The skin stitches were taken off after 10-14 days post repair.



Fig. 1. Photograph showing artificial ventro-lateral hernia in buck (arrow).



(A)



(B)

Fig. 2. Hernia repaired via on-lay fenestrated (A), and non-fenestrated (B) with catfish ADMs by using interrupted horizontal mattress suture pattern.



Fig. 3. Disappearance of hernia after hernioplasty.

Assessment of study parameters

1. Clinical follow-up information

Visual inspection was performed daily in the first week post-surgery to observe any complications which may happen such as gross swelling (seroma or hematoma formation), infection as well as re-herniation, and then the inspection was done every week for the following 12 weeks' time of experiment.

2. Tensile strength test

Strips (7 cm length and 2 cm width) of reconstructed site of abdominal wall and the surrounding tissue were collected at 4, 8 and 12 weeks post-hernioplasty, and immediately preserved in PBS solution for biomechanical assay. The strips were placed firmly from (proximal and distal ends) in metal screw

clamps beside sand paper covering the clamp ends of the tensiometer, to avoid slipping and stretched automatically at a rate of 10mm/min (15). Values of tensile strength were determined and regested in Newton.

Ethical approval:

The local Committee for Animal Care and Use at the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, reviewed and approved all procedures. involved in the current study.

Statistical analysis

Mean values of tensile strength test (Newton) were statistically analyzed by Two-way Analysis of Variance (ANOVA) and Least Significant Differences (LSD). The $P \leq 0.05$ is considered statistically significant (16).

Results

Clinical findings

Secondary complications were observed represented by seromas (n=3) and recurrence of

hernia (n=1). Seroma were occurred in two bucks (out of 12) in G1 and one buck (out of 12)



in G2 during the first week following repair. Seroma was small in size in G2 and resolved spontaneously within three days post treatments. Re-herniation was noticed in one buck (out of 12) in G2 two weeks post hernioplasty. This buck was discarded after failure of second operation and replaced by a new one. Surgery was performing with non-fenestrated ADM with perfect outcome.

Tensile strength

Data fixed in table (1) revealed significant differences ($p \leq 0.05$) between G1 and G2 and animals related to the same group among all periods. At zero time the value of the G1 was lower (115.50 ± 2.52 N) compared with G2

(179.68 ± 2.60 N). During 4th week, the values declined significantly ($p \leq 0.05$) in two groups. It recorded (43.06 ± 1.18 N) in G1 and (88.00 ± 1.63) in G2. The tensile strength increased gradually with the advancement of experimental time starting 8th week to reach (52.00 ± 0.88 N) in G1 and (100.91 ± 1.80 N) in G2. The highest value of tensile strength were noticed at 12th week post-treatment and it was (59.18 ± 1.44 N) or (51%) in G1 (116.43 ± 1.62 N) or (64%) in G2 in comparing with original values (zero time), this means that the tensile strength of G2 is better than that of G1 and this exceed the normal values of tensile strength of the abdominal muscles (50%).

Table 1. Mean values of tensile strength test (Newton) of the repaired hernia in both groups.

Groups	Time			
	Zero	4 wks.	8 wks.	12 wks.
G1	A115.50±2.52b	D43.06±1.18b	C52.00±0.88b	B59.18±1.44b
G2	A179.68±2.60a	D88.00±1.63a	C100.91±1.80a	B116.43±1.62a
LSD	5.32			

Zero = before placement of ADMs

Means with a different vertical small letter referred to significantly different ($p \leq 0.05$) between two groups.

Means with a different horizontal capital letter referred to significantly different ($p \leq 0.05$) among animals of the same group.

Discussion

Clinical outcome of the present study showing **seromas** in three out of 24 bucks. The authors ascribed these phenomena to two main causes. First; inadequate hemostasis while repairing the hernia and second; excessive dissection to separate the hernia sac from the subcutaneous tissue to expose the ring which left dead space, this hypothesis is consistent

with the earlier report (17), who indicate that hernioplasty result in excessive dead space. Furthermore, use of large amount of foreign materials within the wound, all elicits non-septic inflammation capable of causing seroma. Also current interruptions were come in line with (18) who mentioned that seroma formation occurs due to detach of considerable amount of



subcutaneous tissue resulting in dead-space formation in addition to injury of the blood vessels during dissection. A study of (19) indicated that the abdominal wall approaches lead to accumulation of blood and serum among the various layers of wound resulting in hematoma or seroma or dead space. All three cases of seromas were respond to treatment applied in this study which was similar to what mentioned by (20, 21, and 22). Re-herniation was noticed in one buck of G2, two weeks post-hernioplasty. We thought that the etiology of recurrence was related to exposure of this buck to trauma from another buck (horn thrust) at the operative site and there are clear contusions in the area. This may cause weakening (laxity) of the abdominal muscles which cannot hold the sutures. This means that recurrence happen due to poor tissue healing. These interpretation corroborated by the findings of (23, 24). While (25) revealed that technical failures, such as the use of incorrect suture materials, in addition to decrease in laxity of the tissue surrounding the hernia which is influenced by retraction of muscles and inadequate nutrition of the exposed tissue may be important factors for recurrence. Tensiometric evaluation, declared significant difference in tensile strength between G1 and G2 matrices at different times of the present study. This outcome was in accordance with a research by (26) who compared of bovine pericardium (BP) implant with mesh to repair abdominal wall defects in dogs and their results indicated obvious differences between the two materials at all-time intervals. The tensile strength was significantly decreased at four weeks post repair in both fenestrated (G1) and

non-fenestrated (G2) matrices; this may contribute to irregular and less formation of collagen fibers at this period. Similar findings were recorded by (27) who found that the tensile strength values of (BP) declined in the 1st month after implantation then they increased again after subsided the inflammatory reactions. While (28) used porcine matrices for hernia repair in pigs and recorded no significant differences at one month in tensile strengths of the matrices this may be due to its type (non-fenestrated versus fenestrated) or due to the placement location (sub-lay or in-lay). Moreover, current study revealed that the tensile strength of both matrices increased gradually at 8th and 12th weeks after implantation this may be attributed to adequate deposition of collagen fibers that differed in quantity and quality, in addition to depress in the host inflammatory reactions. This interpretation come in contact with a study by (29) that used tunica vaginalis and pericardium as allografts for hernia repair in small ruminant and noticed that the tensile strength slightly increased starting from 10th week post-repair. The maximum tensile strength of repair site in the present study occurs approximately 12th week after repair this may be ascribed to maturation and organization of collagen fibers and regain the tensile strength up to 51% and 64% in fenestrated (G1) and non-fenestrated (G2) matrices respectively although it still remained less than original value comparing with pre-injury time. These percent are exceeding to that recorded by Paul (30) who evaluated the tensile strength of the ventral abdominal wall in baboon and goat and found



that the tensiometric value ranged from (40-50 N), these differences may be related to the site or location of the muscles harvested. A study by (31) stated that the maturation phase is occurred when wound bed slowly strengthens and gains flexibility and reached up to 60% at 3 months after repairing abdominal wall defect.

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