Research Article

Clinical and Macroscopical Evaluation the Effects of Acellular Tunica vaginalis and Acellular Dermal Matrix on Reconstruction of Abdominal Wall Hernia in Bucks

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Abstract | Biological hernioplasty of the abdominal wall more benefit than prosthetic meshes which is completely absorbed by the body and replaced by host tissue the purpose of the present study was designed to assess the efficacy of acellular bovine tunica vaginalis and caprine acellular dermal matrix clinically and macroscopically, in reconstructing abdominal walls defects in Iraqi bucks. In 18 Iraqi bucks, ventro-lateral abdominal walls hernias measuring (6x6)cm were induced experimentally. Thirty days after creation of hernias, the animals were split randomly into two main equal groups (9 Bucks/ group). The hernias of group (A) were treated by sublay grafting of bovine tunica vaginalis sheets after decellularized chemically whereas, the hernias of group (B) were treated by sublay grafting of caprine acellular dermal matrix sheets. The clinical findings after treatment by either bovine tunica vaginalis or caprine acellular dermal matrix were similar, except seroma which was recorded in caprine acellular dermal matrix more than bovine tunica vaginalis group. While, the macroscopic estimation of implantation site revealed that neovascularization was distributed in group (B) more than that in group (A) while, the deposition of fibrous connective tissue in group (A) treatment group was denser than that in group (B). Highly incorporation between the sheet and surrounding tissue was observed in group (A) more than that in group (B). At the same time, the macroscopic changes in the caprine acellular dermal matrix treatment group included decreasing the thickness of a sheet and partial degradation of implants in the site of implantation as well as, thinning of white fibrous connective tissue over the sheet. depending on the clinical and macroscopical outcomes, it can be concluded that bovine tunica vaginalis sheets were better than caprine acellular dermal matrixes for repairing of large ventro-lateral abdominal wall hernia in bucks.

Keywords | Bovine Tunica Vaginalis, Caprine Acellular Dermal Matrix, Ventral-lateral Abdominal Wall Hernias, Sublay Implantation, Iraq.

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INTRODUCTION

The function of abdominal wall is to protect the vital intra-abdominal organs so, the reconstruction of abdominal wall defects like hernia, in particular, big abdominal wall hernias provide a surgical challenge and are frequently encountered by surgeons with recurrences being a common result associated with high morbidity rate compared to other cutaneous affections (Burger et al., 2004; Hummadi and AL-Asadi, 2011). Therefore, the effective treatment for hernial defect is a reconstructive surgery (hernioplasty) to re-establish integrity of the abdominal wall (Mohammad and Al-Ebadi, 2023). Repairing of large abdominal wall defects can be done by mesh, which has two types; synthetic mesh and biological mesh (Peeters et al., 2013). Many studies have confirmed that using of bi-

ological materials have effective effect on acceleration of hernia healing (Al-ebadi and Al-Bayati et al., 2019). Depending on the area of collection (dermis, pericardium, or small intestine submucosa), the biologic products come in different forms and can be obtained from both allogeneic and xenogeneic sources (Porcine and Bovine) (Hussein et al., 2016). Regarding the restoration of abdominal wall defects, biological biomaterials offer a number of advantages over synthetic prosthetic materials. Their multidirectional fibrous structure, which is completely absorbed by the body and replaced by host tissue, provides several benefits, including less adhesion development, an improved framework for fibroblast proliferation and neovascularization and improvement the suture retention (Bellows et al., 2008; Smart et al., 2012). Tunica vaginalis is one of biological materials can be obtained from the parietal layer of peritoneum and can be used as a mesh or sheet for repairing of large abdominal wall hernia (Pratummintra et al., 2012). The host immune system appears to tolerate bovine tunica vaginalis, which is also cheap, readily integrating to host tissue and resistant the infection or fragmentation (Yusof and Yusof, 2002). While, caprine acellular dermal matrix are soft tissue grafts made from tissue that has been decellularized, leaving the extracellular matrix. The matrix functions as a scaffold for the patient's own cells to proliferate, revascularization the graft and adding a layer of new tissue (Logan et al., 2016). The role of bovine tunica vaginalis and caprine acellular dermal matrix needs more studies to evaluate their effect in healing of large abdominal hernia so, the current study was designed to compare between the efficiency of bovine acellular tunica vaginalis and caprine acellular dermal matrix in the reconstruction of experimental induced large lateroventral hernia in bucks through the following parameters: (clinical, macroscopical and microscopical evaluation).

Highlight: Both sheets were obtained from western sources following the slaughtering. These products were subsequently utilized in the treatment of large abdominal wall hernias (more that 6cm in diameter), they were processed and employed for reconstruction purposes. Notably, these sheets exhibited rapid absorption and facilitated the restoration of normal abdominal wall strength. Moreover, it is worth highlighting that these biological sheets provide numerous advantages in comparison to their synthetic counterparts.

MATERIALS AND METHODS

In the current study, 18 healthy adult local breed bucks, ages one to one and a half year, were used. Before the experiment began, all animals underwent a clinical examination and were kept in the University of Baghdad's College of Veterinary Medicine farm animals for a period of two weeks. Under effect of sedation and local anesthesia, latero-ventral hernias (6X6)cm in diameter were created in the right lower flank of all experimental animals through the circular resection of abdominal wall muscles. Thirty days after the operation, the animals were arbitrarily split into two equal groups (9 Bucks/group). The hernias in group A were treated by sublay implantation of bovine tunica vaginalis. In contrast, sublay implantation of caprine acellular dermal matrix was used to treat the hernias in group B. Post-treatment of hernias, all animals were clinically evaluated every day in the first week and then weekly for 16 week to monitoring the sites of operation for monitoring the presence of local or systemic complications, such as infection, fistula, seroma, hematoma formation or recurrence of hernia, and resolution of the signs of hernia while, the macroscopical examinations were performed at two, eight, and sixteen-weeks post-treatment, to detect the presence of neovascularization, incorporation of implant into the surrounding tissues, implant degradation, closing of hernia opening and the presence of infection or implants rejection. The site of implantation was expose by create a U shape skin incision around the hernia and the flap including the skin and subcutaneous tissue will be reflected-up for visual examination of implantation site.

Preparation of Bovine Tunica Vaginalis

Immediately after slaughtering, tunica vaginalis was removed from the scrotum of male bulls (1-2 years old). After rinsing the scrotum in sterile distilled water, a sterile surgical knife was used to carefully remove the skin, smooth muscles and tunica darts. The sac was opened by making a longitudinal incision parallel to the epididymal connection to the testis, and then, the connected fascia and blood were removed. After that, the sheet was immersed in a solution containing 0.1% peracetic acid (PAA) and 4% ethanol on a shaker for two hours in order to decellularize and sterilize the residual sub-mucosal layer. The pH is lowered following the treatment by the additional peracetic acids. Thus, all residues of peracetic acids were eliminated in order to get it back to about 7.4 pH. The extra cellular matrix was cleaned with phosphate buffer saline (PBS) solution. After that, it was shaken again and rinsed twice in water (15 minutes each time). All these operations were completed at natural temperature. After being decellularized, the extra cellular matrix scaffolds were immersed in a 0.1% PAA solution titrated to pH 7.0 at normal temperature for five hours in order to terminally sterilize them (Rosario et al., 2008; Al-Bayati et al., 2016; Taher, 2020).

Preparation of Caprine Acellular Dermal Matrix

Skin of cows was gathered from the nearby abattoir in Baghdad city and stored in ice-cold sterile PBS (pH7.4) that contained 0.25% ethylenediaminetetraacetic acid and

0.1% amikacin. To get rid of all adherent blood and debris and then, the specimens were shaved and cleaned in sterile PBS. For de-epithelialization, the specimen was placed in a solution containing, 0.25% trypsin and a 2M sodium chloride in 100ml of PBS for eight hours. Following de-epithelialization, 2% sodium deoxycholate was used for 48 hours to decellularize the dermis. To improve tissue interaction with chemicals, the samples underwent continuous agitation in a horizontal orbital shaker at a rate of 180 (rotations/min) during the de-epithelialization and decellularization processes. The decellularized acellular dermal matrix was washed six times for two hours each with sterile PBS to eliminate any remaining chemicals. Finally, the prepared caprine acellular dermal matrix was kept at -20°C in PBS solution containing 0.1% Amikacin (Kumar et al., 2013).

Ethical Statement

The experimental design and procedures carried out in this study were reviewed and approved in accordance with animal welfare ethical standards by the Research Ethics Committee at the University of Baghdad's College of Veterinary Medicine with ethics number 1807/P.G. dated on September 5, 2023.

RESULTS

In clinical following-up, all bucks were monitored over the duration of the study to document their general well-being, behavior, and alertness. The clinical and physical examination in animals post inducing of hernia showed the presence of swelling, redness in the site of operation for 1 to 2 days, lethargy and pain for three days. The surgical wounds appeared have no complications; no signs of bleeding or hematoma, no infection or stitches abscess were recorded throughout the periods of follow-up until the hernias were treated. These inflammatory signs were disappeared gradually after 4 to 5 days except the presences of ventro-lateral hernial sac which easily detected through the presence of bulging in the right lower flank of abdominal wall and observed directly after inducing of hernia and 30 days after that (Fig. 1A,B). Clinical outcomes in animals after treatment by either bovine tunica vaginalis and caprine acellular dermal matrix were similar which associated with hernial sac and hernial ring that disappeared directly after treatment and returned hernial site to the normal abdominal wall strength (Fig. 1C). In the present study, (20-50) ml of serous fluid was collected from the hernial site in group (A) and (10-120) ml in group (B) which was disappeared three days post treatment. The same clinical outcomes which were recorded post-inducing of hernia were also noticed in animals after treatment by either BTV and ADM associated with the presence of hernial sac and palpated hernial ring that disappeared directly after treatment and returned the abdominal wall to it normal strength at the site of hernia at the end of study.

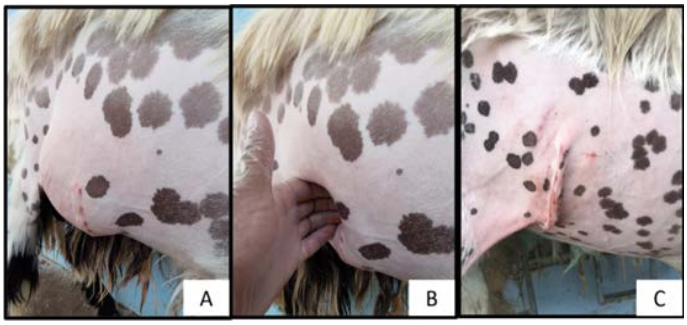

Figure 1: Shows; the detection of ventro-lateral abdominal wall hernia, 30 days post-inducing by the presence of hernial sac (A) and hernial ring (B) and the disappearance of hernia sac directly post-treatment (C).

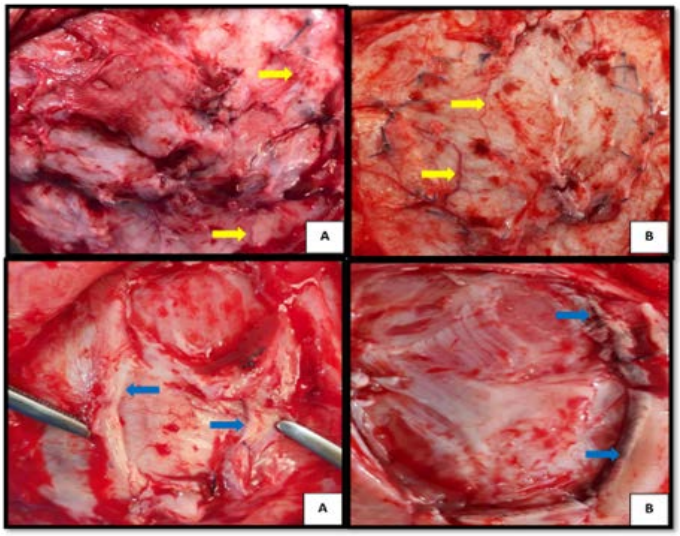

Figure 2: Macroscopic appearance of implantation sites, 2 weeks post-treatment of BTV treated hernias (A), and caprine ADM treated hernias (B), shows: numerous blood vessels (yellow arrows), deposition of white fibrous connective tissue in BTV more than caprine ADM (black arrows), incorporation of mesh with host tissue (blue arrows).

Macroscopic examination of implantation sites was carried out at 2, 8, and 16 weeks after the treatment, has showed that both sheets were presence in the situation and react to the surrounding tissues after implantation without any complications like; infection, signs of implant rejection, necrosis or gangrene. Additionally, it was evident that the implants had been incorporated with the host tissues. New vascularization was distributed in group (A) more than that in group (B) which characterized by the presence of fine and numerous blood vessels extended from host tissues to the implant (Fig. 2A,B). The host-graft junctions were highly deposited with fibrous connective tissue fibrous connective tissue which extended from the surrounding tissues toward the center of implantation site. In addition,

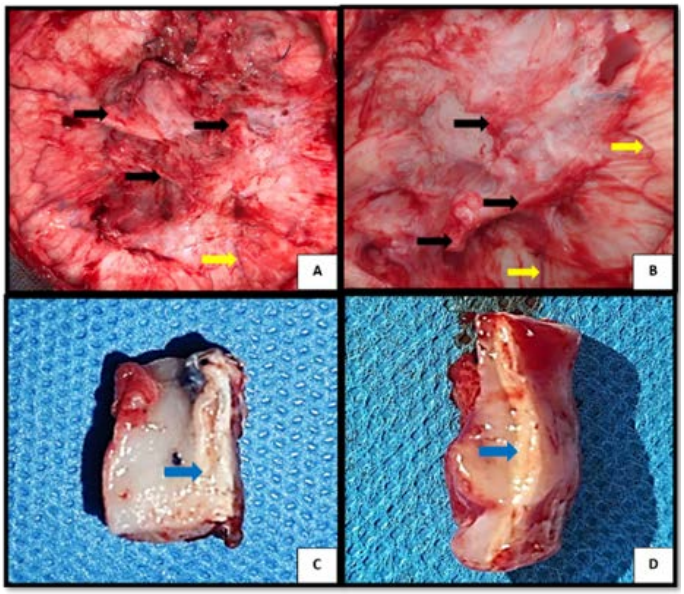

Figure 3: Macroscopic appearance of implantation site **8 weeks** post-treatment of **BTV** (A), and caprine **ADM** (B) shows; deposition of white fibrous connective tissue at the center of implantation (black arrows), sprouting blood vessels (yellow arrows), while, good incorporation between sheet and surrounding tissue (blue arrows)(C) and (D).

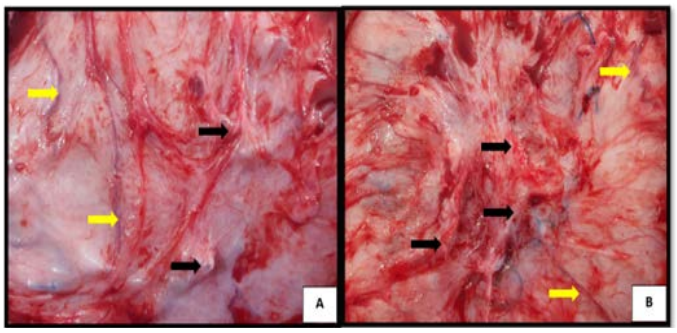

Figure 4: Macroscopic appearance of implantation site 8 weeks post-treatment of BTV (A), and caprine ADM (B) shows; deposition of white fibrous connective tissue at the center of implantation (black arrows), sprouting blood vessels (yellow arrows)

in week 2nd post-treatment, it was noticed the deposition of fibrous connective tissue in group (A) was thicker than that in group (B) (Fig. 2A,B). While, in week 8th, the macroscopic observation of the implantation sites in BTV treatment group appeared highly deposition of white fibrous connective tissue at the center of implantation as well as, highly incorporation between sheet and surrounding tissue associated with high neovascularization (Fig. 3A). While, the hernias of group (B), showed mild deposition of white fibrous connective tissue at the center of implantation associated with the incorporation between the sheet and surrounding tissues with clear neovascularization and decreasing the thickness of sheet (Fig. 3B). At weeks 16th post treatment, the macroscopic changes in group (A) were included lack of white fibrous connective tissue, with clear neovascularization (Fig. 4A), partial degradation in tunica vaginalis, and incorporation of a sheet with surrounding tissue and new formation of some muscle bundles (Fig. 5A). At the same time, the macroscopic changes in the hernias of group (B) were included decreasing the thickness of a sheet in site of implantation (Fig. 5B), as well as, thinning of white F.C.T over the sheet (Fig. 4B).

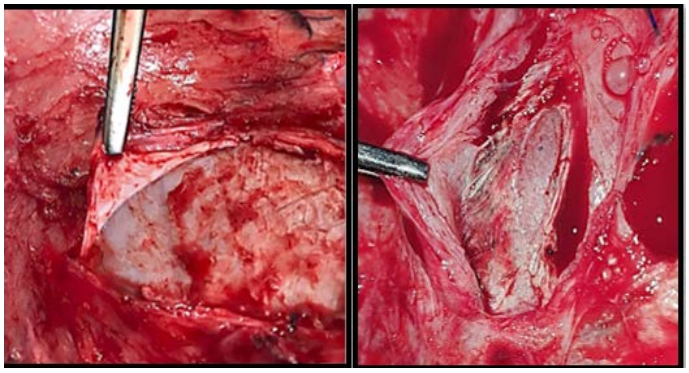

Figure 5: Macroscopic appearance of implantation site 16 week post-treatment of BTV (A) and caprine ADM(B) shows; decrease in thickness of meshes due to degradation (yellow arrows).

DISCUSSION

The same clinical outcomes which recorded post-inducing of hernia were also noticed after treatment by either bovine tunica vaginalis and caprine acellular dermal matrix associated with the presence of hernial sac and palpated hernial ring that disappeared directly after treatment and at the end of the study, returned the abdominal wall to it normal strength at the site of hernia (Fig. 1C). Similar signs were recorded in the study of Al-Sadi et al. (2005), who treated the umbilical hernias of buffalo calves with an acellular dermal graft, tunica vaginalis and pericardium, as allograft, for hernioplasty in sheep. In the present study, (20-50) ml of seroma fluid was collected from the hernial site in group (A) and (10-120) ml in group (B) and completely disappeared three days post-treatment. The same outcomes were recorded by other studies that used different types of biological materials, as in the study of Eberli et al. (2010) and Asodiya et al. (2020), whom explained that caprine acellular dermal matrix has ability to stimulate the rapid neovascularization during tissue healing.

Seroma is a collection of serum that builds up under the surface of the skin and they can develop post-surgical incisions which were made in the body or where bodily tissue was removed. In addition, the reasons behind seroma could be due to disruption of lymphatic and vascular drainage through extensive soft tissue dissection which lead to accumulate of serum in dead space. Srivastava et al. (2012), indicated that seroma commonly occurs also after a significant surgical procedure such as a hernia repair. While,

Hafeez et al. (2005), observed that postoperative seroma is caused by a host inflammatory reaction to the implanted bovine tunica vaginalis with the presence of dead space that formed between the implant and host tissue. Gangwar et al. (2006), referred that mild seroma investigated in day 3rd and disappeared in day 6th post-treatment by caprine acellular dermal matrix for the restoration of rabbits' malformed abdominal walls. They explained that the formation of local seroma may be attributed to inflammation in response to surgical trauma. Mahdi and AL-Bayati (2018), referred that the production of serous fluid is due to the manipulation of tissues at the implantation site which can result in aseptic inflammation and dissections that create a division between skin and subcutaneous tissues.

The macroscopic investigation by the present study, appeared the deposition of white fibrous connective tissue at the sites of implantation with incorporation of implant into the surrounding tissues associated with highly neovascularization which were confirmed by many studies post-using of tunica vaginalis in repairing the defects of abdominal wall. Abass (2008), observed after using of bovine tunica vaginalis in hernioplasty of umbilical hernias in sheep, that the deposition of white fibrous connective tissue associated with good interposition and noticeable vascularization. Al Sadi et al. (2005) and Ayele et al. (2011), observed, after using of allogeneic pericardium and tunica vaginalis for hernioplasty in sheep, a good incorporation between mesh and surrounding tissues with the formation of dense fibrous tissue and formation of new blood vessels which invaded the graft particularly. Yusof and Yusof (2002), noticed that the batches become stronger since collagenous connective tissue and muscle fibers infiltrate into the biomaterial after using of processed bovine tunica vaginalis for repairing of major abdominal wall defects. El-Husseiny (2019), approved that the implantation site of animals after slaughtering at week 4th post-treatment, have gradual increasing the deposition of connective tissue and neovascularization which became more prominent at weeks 8th and 12th post-treatment.

The present study confirmed that both sheets were played a good role in the acceleration and enhancing the healing of injured tissues with non significant differences between them but, bovine tunica vaginalis has a superior effect than caprine acellular dermal matrix. Both decellularized sheets in the present study are a matrices composed, as mentioned by Qing, (2017), from collagen network and growth factors that secreted during hernia repair to facilitate native tissue healing and incorporation with surrounding tissues. In general, all stages of healing process are controlled by a wide variety of different growth factors and cytokines. The main growth factors involved; vascular endothelial growth factor (VEGF) fibroblasts growth factor (FGF), transforming growth factor (TGF) and keratinocytes growth factor (KGF) (Koveker, 2000; Grazul-Bilska et al., 2003). The main goal can be obtained from that is to provide the extracellular components necessary to complete healing allow for the reconstruction of new and healthy tissue and restore mechanical and functional integrity to the abdominal wall. As a result, the formation of new blood vessels in the current results in group (A) could be clearly seen more than that seen in group (B). This may be related to the releasing of many growth factors from these bioimplants especially, VEGF and PDGF during degradation which responsible to form new blood vessels (Xin et al., 2019; Ptrie et al. 2022), whom mentioned that caprine acellular dermal matrix has growth factor receptors and vascular channels even after chemical decellularized process. These growth factors promote the migration of inflammatory cells and neovascularization after implantation, because they act as signaling molecules between cells and contribute the regulation of cellular processes through; encouraging cellular proliferation, differentiation and maturation, in addition to angiogenesis and degradation of extra cellular matrix.

Secondly, collagen network of these sheets plays main role in the healing process of injured tissues which depend on thickness, architecture of collagen network and number and size of pores of a sheet. These factors allow for an appropriate cell seeding density within the scaffold, adequate porosity with interconnected pores is required to support cell proliferation and differentiation by facilitating the movement of nutrients and oxygen into and out of the scaffold (Goddard and Hotchkiss, 2007; Asodiya et al. 2020). They indicated that the ideal size of acellular dermal matrix pores is generally between 100 and 200µm and the pores with a diameter of 166.9µm showed the highest number of adherent and proliferating cells while, the size of bovine tunica vaginalis pores is (200) µm, which make the processed bovine tunica vaginalis appeared to be more able to support the inflammatory reaction and then healing process (El-Husseiny et al., 2023). The differences in pores size of each scaffold may explained the differences in the outcomes between them. Scaffold need to have high porosity and contain interconnected pores with suitable pore size in order to provide an ideal physical environment for growing and proliferation of the cells to distribute uniformly and to support neovascularization (Lim et al., 2019). A previous studies were demonstrated that permeability increases with increasing pore size due to a reduction in specific surface area (O'Brien et al., 2007). If pores are too small, cell migration is limited, resulting in the formation of a cellular capsule around the edges of the scaffold. This in turn can limit the distribution of nutrients and removal of waste products resulting in necrotic regions within the construct. Conversely if pores are too large there is a decrease in specific surface area available limiting cell

attachment (Murphy and O'Brien, 2010).

Furthermore, Ayele et al. (2011) and Bakar et al. (2011), indicated that the cross sectioned of bovine tunica vaginalis appeared it has thick collagen bundle mainly type-I and few type-III and elastic fibers with sufficient and large porosity while, Asodiya et al. (2020), indicated that acellular dermal matrix has thick and more compact collagen fibers type I and III. Xue and Jackson, (2015), referred that collagen type-I is a stronger type of collagen and most durable one. Moreover, since collagen is highly hydrophilic, it can improve the interaction of cells with the scaffolds. It also has the ability to trigger biological signals to support cell adhesion and proliferation. In addition, Rose et al. (2016) and Doungel et al. (2021), mentioned that the thickness of caprine acellular dermal matrix is thicker (860 to 218) µm than the thickness of bovine tunica vaginalis (75.1 ± 6.29) µm from the left tests and was (125.25 ± 0.77) µm from right one an bovine tunica vaginalis which will delay the infiltration of inflammatory cells in caprine acellular dermal matrix treatment hernias and subsequently leading to a delayed graft degradation which will delay the liberation of growth factors, compare to the bovine tunica vaginalis treatment group. This fact supports nearly the superiority of bovine tunica vaginalis compare to caprine acellular dermal matrix in enhancing the reconstruction of abdominal wall defects.

In the current study, the biologic materials, like tunica vaginalis, may be useful for repairing of hernia due to their ability to incorporate with surrounding tissues without rejection because it is derived from the sero-fibrous membrane that formerly lined the abdominal cavity wall (peritoneum), thus, it mimics the characteristics of the abdominal wall's natural. The same results were obtained by Yusof and Yusof (2002) and Ayele et al. (2011). Karrouf et al. (2016), Asodiya et al. (2020) and Ahmed et al. (2022), indicated that the main reason for using of caprine acellular dermal matrix in the reconstruction of injured tissues may be due to high biocompatibility of this scaffold without complications due to their poor immunological reaction because of its low antigenicity. They added, the low antigenicity of this bioscaffold makes it degrade very slow. At the same time, bovine tunica vaginalis appears to be a good biomaterial for fixing defects in the abdominal wall since it is readily available, reasonable, highly integrating with the host tissue, immune system-accepting, and not easily fragmented or infected. Gangwar et al. (2006) and Abass, (2008), indicated that the absence of graft rejection and complications in treatment groups come are agree with several studies which confirmed that there were no signs of wound infections or adverse reactions without bad odors, discharge or itching after using of bovine tunica vaginalis and caprine acellular dermal matrix in hernioplasty. The success of decellularization protocol used in the current study played an important role in the absence of graft rejection. The study. Kumar et al. (2013), explained that the production of caprine acellular dermal matrix involves a regulated procedure that eliminates the cells from the dermis and the epidermis will maintaining the integrity of the basement membrane and extra cellular matrix. The same outcomes were obtained by Eberli et al. (2010) and Abouelnasr et al. (2017). In addition, the absence of graft rejection in present study may be related to the using of sublay fixation technique of sheet which, regarded by Kumar et al. (2013), Saeed et al. (2014) and Ahmed and Mehboob (2019), is best hernioplasty technique than another technique of fixation like; onlay, inlay and underlay, because sublay technique has more advantages than others ones such as; less seroma formation, less wound infection and less recurrence rate.

CONCLUSION

Based on the data obtained from the current study, it can be concluded that both caprine acellular dermal matrix and bovine tunica vaginalis are successful biological materials to be used for reconstruction of abdominal wall defects and have ability to produce the supporting to abdominal wall following hernioplasty without rejection or complications, in associated with no significant differences between their role in acceleration and enhancement the healing process of abdominal wall hernias, with a superiority of bovine tunica vaginalis.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

NOVELTY STATEMENT

The novelty of this study is the comparison between bovine tunica vaginalis and caprine acellular dermal matrix in reconstruction of the return of normal strength of ventro-lateral abdominal wall hernia and the ability of these biological materials to integrate with host tissue.

AUTHOR CONTRIBUTION

These authors each contributed equally.

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