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Preparation and Clinical Evaluation of a Decellularized Buffalo Ruminal Extracellular Matrix Scaffold Compared with Polypropylene Mesh for Hernioplasty in Calves

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Abstract:

Background

Large abdominal wall hernias in calves may require prosthetic reinforcement because primary herniorrhaphy can create excessive tension and increase the risk of recurrence when the hernial ring exceeds 8 cm. Polypropylene mesh is widely used, while biological extracellular matrix (ECM) scaffolds are being investigated as alternatives. This study compared a decellularized buffalo ruminal acellular matrix (ACM) with polypropylene mesh for repair of large abdominal hernias in calves.

Methods

Twenty female cattle calves with abdominal hernias and hernial-ring diameters greater than 8 cm were randomly allocated into two groups of 10 animals each. Group A underwent hernioplasty using polypropylene mesh, whereas Group B underwent hernioplasty using a decellularized buffalo ruminal acellular matrix (ACM) prepared from fresh buffalo rumen obtained from a local abattoir using a sodium deoxycholate-based decellularization protocol. The effectiveness of decellularization was evaluated by histological examination and DNA quantification. Physiological parameters, serum immunoglobulin G (IgG) concentrations, and clinical outcomes were assessed before surgery and on postoperative days 7 and 15. Clinical outcomes were evaluated based on physiological, haemato-biochemical, Immunological and clinical parameters.

Results

Histological examination and DNA analysis indicated removal of cellular material while the extracellular-matrix architecture was retained. Physiological parameters remained within normal ranges, with no significant between-group differences ($P > 0.05$). Serum IgG concentrations increased slightly after surgery, but the changes were not statistically significant. No hernia recurrence was observed during the one-month follow-up. Mean skin-healing time was shorter in the ACM group than in the polypropylene-mesh group (11.5 ± 0.31 vs. 14.5 ± 0.36 days; $P < 0.05$). Other clinical outcomes, including swelling, pain, and exudation, did not differ significantly between groups.

Conclusions

Both polypropylene mesh and buffalo ruminal acellular matrix were effective for the surgical repair of large abdominal hernias in calves. However, the buffalo ruminal ACM demonstrated superior biocompatibility, reduced postoperative tissue reaction, and faster wound healing, indicating that it is a promising biological scaffold for buffalo hernioplasty and may represent a cost-effective alternative to synthetic mesh.

Keywords: Abdominal hernia; buffalo; decellularization; extracellular matrix; hernioplasty; polypropylene mesh; ruminal acellular matrix; tissue engineering

Background:

Abdominal hernia is one of the most frequently encountered surgical conditions in bovine practice and is characterized by the protrusion of abdominal contents through a congenital or acquired defect in the abdominal wall (Nienhuijs *et al.,* 2008). Congenital umbilical hernia is the most common abdominal wall defect in cattle, whereas acquired hernias usually result from trauma, increased intra-abdominal pressure, surgical incisions, or weakening of the abdominal musculature (Ladurner *et al.,* 2001). If left untreated, hernias may progress to incarceration, strangulation, intestinal obstruction, tissue necrosis, and reduced productivity, resulting in considerable economic losses to livestock owners **(**Weaver *et al.,* 2022).

The choice of surgical repair depends primarily on the size of the hernial defect. Primary herniorrhaphy is generally suitable for small defects (Ober et al., 2008); however, closure of large abdominal wall defects creates excessive tension on the suture line, increasing the risk of wound dehiscence and recurrence. Therefore, prosthetic reinforcement is

recommended for hernial rings larger than 8 cm in cattle to achieve tension-free repair and improve long-term surgical outcomes (Vilar *et al.,* 2011). Polypropylene mesh is the most widely used prosthetic material because of its high tensile strength, ease of handling, and long-term durability. Nevertheless, synthetic meshes are associated with several postoperative complications, including surgical site infection, chronic inflammation, seroma formation, fistula development, mesh extrusion, intra-abdominal adhesions, intestinal obstruction, and chronic postoperative pain (Singh *et al.,* 2012**).** Recent studies have further demonstrated that the foreign-body response induced by synthetic prostheses may impair tissue remodeling and contribute to long-term postoperative complications (Deeken and Matthews, 2020).

Biological extracellular matrix (ECM) scaffolds have emerged as promising alternatives for abdominal wall reconstruction. Acellular matrices derived from allogeneic or xenogeneic tissues preserve the native extracellular matrix architecture while removing cellular components responsible for immunogenicity (Badylak, 2014). The preserved ECM provides a biologically active scaffold that promotes host cell migration, angiogenesis, collagen deposition, and constructive tissue remodeling, thereby facilitating functional tissue regeneration rather than fibrotic encapsulation (Badylak, 2007; Butler, 2006). Recent advances in regenerative medicine have demonstrated that decellularized extracellular matrices retain bioactive molecules capable of modulating macrophage polarization, enhancing vascularization, and improving tissue integration after implantation (Dziki *et al.,* 2018; Hussey *et al.,* 2018; Crapo *et al.,* 2021).

The clinical success of ECM scaffolds depends largely on effective decellularization, which removes cellular and nuclear components while preserving the structural integrity, biochemical composition, and mechanical properties of the extracellular matrix (Gilbert et al., 2006). Preservation of the native collagen architecture minimizes the host immune response and improves scaffold biocompatibility, vascular integration, and constructive remodeling (Schmidt and Baier, 2000**).** Current evidence indicates that decellularized biological scaffolds reduce postoperative inflammation, promote angiogenesis, and accelerate wound healing compared with conventional synthetic implants (Liu *et al.,* 2023). Although decellularized extracellular matrices have been investigated using several mammalian tissues, information regarding the application of buffalo ruminal acellular matrix for repair of large abdominal wall defects in cattle remains limited.

The ruminal extracellular matrix has a collagen-rich connective-tissue framework that may provide a scaffold for tissue repair. This study aimed to prepare and characterize a decellularized buffalo ruminal acellular matrix and compare its short-term clinical outcomes with those of polypropylene mesh for repair of large abdominal hernias in cattle. We hypothesized that the ACM would be associated with comparable short-term clinical outcomes and reduced postoperative reactions. Mechanical equivalence was not tested in this study and therefore cannot be concluded from the present data.

Materials and Methods

Study design and ethical approval

This prospective, randomized comparative clinical study was conducted on 20 female cattle calves presented to the Department of Veterinary Surgery and Radiology with abdominal hernias. Animals with a hernial ring diameter greater than 8 cm were eligible for inclusion. The study population comprised female 13 crossbred, 4 Sahiwal, and 3 Hariana cattle calves, ranging in age from 2 to 12 months and in body weight from 45 to 200 kg. The diagnosis of abdominal hernia was established by clinical examination and confirmed using radiographic and ultrasonographic evaluations.

Animals with severe concurrent systemic illness or recurrent hernias requiring emergency intervention were excluded. The sample size was determined by the availability of eligible clinical cases during the study period; no formal sample-size calculation was performed. The study protocol was approved by the Institutional Animal Ethics Committee (Approval No. IAEC/BVC/2024/15), and written informed consent was obtained from all animal owners before enrolment. All methods were performed in accordance with the relevant institutional guidelines and regulations for animal research.

Animals were randomly allocated into two treatment groups (n = 10 per group) using a computer-generated randomization sequence. Group A underwent hernioplasty using polypropylene mesh, whereas Group B received a decellularized buffalo ruminal acellular matrix (ACM). Surgeons were aware of treatment allocation because of the nature of the intervention, whereas postoperative clinical assessment and statistical analyses were performed by investigators blinded to treatment allocation.

Preparation of buffalo ruminal acellular matrix

Fresh buffalo rumen was collected immediately after slaughter of healthy buffalo for food production at a local abattoir using aseptic technique. The tissue was placed in a sterile, leak-proof container, transported to the laboratory at approximately 4°C, and processed within 1 h of collection. Upon arrival, the tissue was rinsed thoroughly with running water to remove gross contaminants and residual ruminal contents (Fig 1a). It was then immersed in sterile saline containing 0.02% ethylenediaminetetraacetic acid (EDTA) and 0.1% amikacin for 1 h (Fig. 1b), followed by 4–5 rinses with phosphate-buffered saline (PBS; pH 7.4, Fig 1c).The keratinized mucosal layer (Fig 1d) and serosa were carefully removed to expose the connective tissue matrix (Fig 1e). The remaining tissue was cut into appropriately sized grafts and subjected to decellularization using 1% sodium deoxycholate (Fig 1 f) under continuous agitation at 180 rpm and room temperature for 72 h (Fig. 1g). Following decellularization, the grafts were thoroughly rinsed with PBS, immersed in absolute ethanol for 4 h (Fig.1h), and washed again with PBS. The processed acellular matrix (ACM) was stored at −20°C in PBS containing 0.1% amikacin and 0.1% sodium azide until implantation (Fig. 1i). Before implantation, the grafts were rinsed 4–5 times with PBS to remove residual storage solution. Decellularization was evaluated by histological examination of hematoxylin-and-eosin-stained sections (Fig. 2a-2b) and quantitative DNA analysis (Fig. 3a-3b).

Surgical procedure

Feed was withheld for 24 h and water for 12 h before surgery. Epidural anaesthesia was achieved using 6 mL of 2% lignocaine hydrochloride, while sedation was induced with xylazine hydrochloride at 0.1 mg/kg body weight administered intramuscularly (Fig. 4a). Following routine aseptic preparation of the surgical site, an elliptical skin incision was made, extending approximately 2 cm beyond the cranial and caudal margins of the hernial ring (Fig. 4b). The hernial sac was carefully dissected from the surrounding tissues and opened. The herniated abdominal contents were reduced into the abdominal cavity, and the hernial-ring dimensions were measured intraoperatively to confirm eligibility for inclusion in the study. Only animals with ventral abdominal hernias having a hernial-ring diameter greater than 8 cm were included.

In Group A, a sterile polypropylene mesh (Fig. 4d) was placed using an underlay technique and secured to the surrounding healthy abdominal wall tissues with polypropylene sutures (Fig. 4e). In Group B, the prepared buffalo ruminal acellular matrix (ACM) was implanted using the same underlay technique and fixed with polypropylene sutures to maintain

uniformity of the fixation method between the treatment groups (Fig. 5a–e). The dimensions of the implants were individually determined according to the size of the hernial defect. In both groups, the implant extended more than 2 cm beyond the margins of the hernial ring to provide adequate coverage of the defect. Following implant placement, the peritoneum was sutured to restore continuity of the peritoneal lining and separate the abdominal cavity from the implant and overlying surgical tissues. The surgical site was irrigated with sterile physiological saline containing 0.1% amikacin before routine closure. The subcutaneous tissues were opposed using polyglactin 910 in a simple continuous pattern, whereas the skin was closed with polyamide sutures using a horizontal mattress pattern (Figs. 4f and 5f).

Postoperative management

Following recovery from anaesthesia, the animals were returned to their respective owners' farms, as they originated from different farms. Postoperative analgesia consisted of meloxicam administered intramuscularly at 0.2 mg/kg on the day of surgery, followed by 0.1 mg/kg once daily for 3–5 days. Amoxicillin–sulbactam (10 mg/kg body weight, intramuscularly) was administered for five consecutive days.

The animals were maintained under their respective farms' routine husbandry conditions, with free access to drinking water. Feed intake was gradually increased from a restricted diet during the first postoperative week to the usual maintenance ration. Skin sutures were removed between postoperative days 10 and 15, depending on wound healing. All animals were monitored by the study team throughout the predefined observation period, and postoperative findings were recorded as described in the study protocol.

Clinical evaluation

Clinical evaluation was performed before surgery (day 0) and on postoperative days 7 and 15 by the operating veterinarian. Physiological parameters, including rectal temperature, heart rate, and respiratory rate, were recorded at each scheduled examination. Rectal temperature was measured using a digital thermometer, heart rate was determined by auscultation using a stethoscope, and respiratory rate was assessed by counting thoracic movements.

Venous blood samples were collected on days 0, 7, and 15 for haematological and biochemical evaluation. Haematological parameters included haemoglobin concentration (Hb), total leukocyte count (TLC), and differential leukocyte count (DLC). Biochemical assessments included kidney function tests (KFT), liver function tests (LFT), total protein, albumin, and globulin. For immunological evaluation, serum was separated and stored at −20°C until analysis. Serum immunoglobulin G (IgG) concentrations were measured using a commercially available cattle enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions.

Postoperative clinical outcomes included wound swelling, pain, exudation, skin healing time, postoperative complications, and hernia recurrence. The degree of swelling at the site of reconstruction was graded on 1-4 scale as per method of Schnell and Tonn, (2017). Were as degree of exudation at the site of repair was graded on 1-4 scale as per method of Bigbie *et al.* (1991) and The degree of pain was assessed by gently pressing the operation site and was graded on 1-4 scale as per method of Dufrane *et al.* (2008). Clinical scoring was performed using predefined criteria. Between scheduled veterinary examinations, the animals remained at their owners' farms and were monitored by their owners. The operating veterinarian examined the animals on postoperative days 7 and 15, and findings were recorded during these visits.

Postoperative clinical outcomes included wound swelling, pain, exudation, skin healing time, postoperative complications, and hernia recurrence. Clinical scoring was performed using predefined criteria. The degree of swelling at the reconstruction site was graded on 1- 4-point scale according to Schnell and Tonn (2017), whereas exudation at the repair site was graded on a 1-4-point scale according to Bigbie *et al.* (1991). Pain was assessed by gently pressing the operative site and graded on 1- 4-point scale according to Bussières, et *al.* (2008). Skin healing time was recorded postoperatively, and postoperative complications and hernia recurrence were monitored during the observation period. The operating veterinarian examined the animals on postoperative days 7 and 15, and clinical findings were recorded during these visits. Between scheduled veterinary examinations, the animals remained at their owners' farms and were monitored by their owners. Hernia recurrence was recorded during the postoperative observation period of up to 6 month.

Statistical analysis

Data were analysed using IBM SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA). Data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene's test. Normally distributed continuous variables are presented as mean ± standard error (SE). Between-group comparisons were performed using the independent-samples t-test, whereas changes over time within groups were analysed using one-way analysis of variance (ANOVA). Variables that did not satisfy assumptions of normality were analysed using the Wilcoxon signed-rank test. Statistical significance was defined as $P < 0.05$.

Results

Characterization of the buffalo ruminal acellular matrix

The buffalo ruminal acellular matrix was successfully prepared using the sodium deoxycholate-based decellularization protocol. Histological examination of native ruminal tissue demonstrated intact epithelial and stromal cellular components (Figure 7), whereas decellularized tissues showed complete removal of cellular and nuclear material while preserving the three-dimensional collagen architecture of the extracellular matrix (Figure 8). The collagen fibres remained well organized following decellularization, indicating that the structural integrity of the extracellular matrix was maintained. DNA quantification further confirmed the effectiveness of the decellularization procedure. A significant reduction in residual DNA content was observed in decellularized ruminal tissues compared with native tissue (Figures 9 and 10), demonstrating efficient removal of cellular material while preserving the extracellular matrix scaffold.

Physiological parameters

Rectal temperature, heart rate, and respiratory rate remained within the normal physiological range throughout the study period in both treatment groups (Table 1). No significant differences were observed either between groups or across postoperative observation periods ($P > 0.05$), indicating that implantation of either polypropylene mesh or buffalo ruminal acellular matrix did not adversely affect the general physiological status of the animals.

Haemato-biochemical parameters

Haematological and biochemical parameters, including haemoglobin concentration, total and differential leukocyte counts, kidney and liver function indices, total protein, albumin, and globulin, were evaluated on postoperative days 0, 7, and 15. No statistically significant changes were observed in these parameters during the observation period except neutrophil and lymphocyte ($P > 0.05$).

Immunological response

Serum immunoglobulin G (IgG) concentrations showed a slight increase following surgery in both treatment groups (Table 2). However, neither the within-group changes over time nor the between-group comparisons reached statistical significance ($P > 0.05$). These findings suggest that implantation of the buffalo ruminal acellular matrix did not induce a greater systemic immune response than polypropylene mesh.

Clinical outcomes

Mild postoperative swelling and pain were observed in animals of both groups during the first postoperative week. These inflammatory signs gradually subsided with progression of wound healing, and no significant differences were detected between the polypropylene mesh group and the buffalo ruminal acellular matrix group ($P > 0.05$) (Table 3). Similarly, postoperative wound exudation was minimal in both treatment groups. Although exudation scores were numerically lower in the buffalo ruminal acellular matrix group, the difference between groups was not statistically significant ($P > 0.05$) (Table 3). The mean skin healing time was significantly shorter in animals treated with the buffalo ruminal acellular matrix than in those treated with polypropylene mesh (11.5 ± 0.31 days versus 14.5 ± 0.36 days, respectively; Table 4; $P < 0.05$), indicating more rapid wound healing following implantation of the biological scaffold.

Postoperative complications

No major postoperative complications, including wound dehiscence, graft rejection, surgical site infection requiring graft removal, or hernia recurrence, were observed in either treatment group during the one-month follow-up period. All animals recovered uneventfully and completed the study (Fig 6a-c & Fig. 7a-c). Minor wound-related complications occurred in a small number of animals treated with polypropylene mesh. These complications were

successfully managed with routine wound care and extended antimicrobial therapy, and no additional surgical intervention was required. Overall, both treatment techniques resulted in successful abdominal wall reconstruction. However, implantation of the buffalo ruminal acellular matrix was associated with faster wound healing and favourable postoperative clinical outcomes while maintaining comparable physiological stability and immunological responses to polypropylene mesh.

Discussion:

Reconstructive surgery remains the treatment of choice for large abdominal wall defects because it restores abdominal wall integrity, prevents visceral incarceration and strangulation, and improves long-term clinical outcomes (Ober *et al.,* 2008; Rathore *et al.,* 2019). Clinical management of abdominal wall defects depends primarily on the size of the hernial ring. Herniorrhaphy is generally suitable for small defects, whereas prosthetic reinforcement becomes necessary when the hernial ring exceeds 3 cm in small animals and 8 cm in large animals to achieve a tension-free repair and reduce recurrence (Vilar *et al.,* 2011). Recent studies have further emphasized that tension-free hernioplasty using biological scaffolds improves tissue integration while minimizing postoperative complications associated with excessive suture tension (Deeken and Matthews, 2020).

The present study demonstrated that buffalo rumen incubated in 1% sodium deoxycholate for 72 h under continuous agitation was successfully decellularized while preserving the native extracellular matrix architecture. Histological examination confirmed complete removal of cellular components with preservation of the three-dimensional collagen network, and DNA quantification further verified the efficacy of the decellularization protocol. Preservation of the extracellular matrix ultrastructure is particularly important because it maintains the biomechanical properties of the scaffold while retaining bioactive molecules that regulate cell migration, angiogenesis, and tissue remodeling (Crapo *et al.,* 2011; Badylak, 2014**).** Similar findings were reported by Singh *et al.* (2022), who successfully prepared decellularized buffalo ruminal matrices using 1% sodium deoxycholate for 72 h, and by Kumar *et al*. (2015), who achieved complete decellularization of bubaline diaphragm using 2% sodium deoxycholate.

The prepared buffalo ruminal acellular matrix provided satisfactory reinforcement of abdominal wall defects without graft rejection or hernia recurrence throughout the observation

period. Comparable clinical outcomes have been reported following implantation of decellularized dermal matrices in goats (Kumar *et al.,* 2013), decellularized bubaline diaphragm in buffaloes (Mathew *et al.,* 2014), and decellularized ruminal matrices in buffalo calves (Singh *et al.,* 2022). Unlike synthetic prosthetic materials, biological extracellular matrix scaffolds undergo gradual remodeling and replacement by host tissue, thereby reducing chronic foreign-body reactions and promoting constructive tissue regeneration rather than fibrotic encapsulation (Badylak, 2014; Dziki *et al.,* 2017).Most clinical cases included in the present study involved younger animals, whereas comparatively fewer cases were observed in older cattle. This finding agrees with Rings (1995), who reported that abdominal hernias are most frequently encountered in young calves. Similarly, Brem *et al.* (1985) and Singh (2018) observed a higher incidence of umbilical hernias in female calves than in males, which is consistent with the present observations.

Rectal temperature, heart rate, and respiratory rate remained within normal physiological limits throughout the postoperative period in both treatment groups. Similar findings have been reported following implantation of acellular biological matrices (Kumar *et al.,* 2013) and polypropylene mesh (Kumar *et al.,* 2020). Maintenance of normal physiological parameters indicates that neither polypropylene mesh nor buffalo ruminal acellular matrix induced clinically significant systemic adverse effects following implantation, supporting the overall biocompatibility of both graft materials.

The haematological and biochemical parameters remained within the normal physiological range, with no significant changes observed following hernioplasty. The non-significant variations in haemoglobin, PCV, TLC, differential leukocyte counts, BUN, creatinine, liver enzymes, total protein, albumin, and globulin indicate that surgical intervention did not produce marked alterations in haematological status or biochemical homeostasis. The transient changes in neutrophil and lymphocyte counts on postoperative day 7 may reflect the normal inflammatory and immune responses associated with surgical trauma and tissue healing. These findings are consistent with previous studies by Rathore *et al.* (2019) and Asodiya *et al*. (2020).

Serum IgG concentrations showed only a slight, non-significant increase following surgery in both treatment groups. These findings suggest that implantation of the buffalo ruminal acellular matrix did not induce an exaggerated systemic immune response. Similar observations have been reported following implantation of bovine pericardium, porcine

acellular dermal matrix, and other decellularized biological scaffolds (Frascino *et al.,* 2021; Sun *et al.,* 2020). Meyer et al. (2009) also demonstrated that the host response to xenogeneic extracellular matrices is characterized primarily by constructive remodeling rather than graft rejection. This favorable immunological response is attributed to effective removal of cellular antigens during decellularization while preserving the extracellular matrix proteins that promote macrophage polarization toward a pro-regenerative (M2) phenotype, thereby reducing chronic inflammation and facilitating tissue repair (Brown *et al.,* 2012; Badylak, 2014).

Both polypropylene mesh and buffalo ruminal acellular matrix produced mild to moderate postoperative swelling during the early healing phase. The inflammatory response was greatest during the first postoperative week and gradually subsided thereafter. Although swelling was observed in both groups, it was less pronounced in animals treated with the acellular matrix. Similar findings have been reported in calves (Singh *et al.,* 2018) and pigs (Rathore *et al.,* 2019). The reduced inflammatory response observed following implantation of the biological scaffold is likely related to its superior biocompatibility and its ability to support early cellular infiltration and neovascularization while minimizing the foreign-body reaction commonly associated with synthetic prosthetic materials**.**Pain scores followed a similar pattern, increasing during the first postoperative week and progressively declining as tissue healing advanced. Animals treated with buffalo ruminal acellular matrix consistently exhibited lower pain scores than those treated with polypropylene mesh, suggesting reduced local inflammation and improved tissue integration. Similar observations have been reported by Kumar *et al*. (2015) and Singh *et al.* (2018). Mild wound exudation was observed between postoperative days 5 and 7 in both groups and gradually resolved as healing progressed. Comparable findings have also been reported following hernioplasty in calves (Rathore *et al.,* 2019).

One of the most important findings of the present study was the significantly shorter healing time observed in cattle treated with buffalo ruminal acellular matrix compared with polypropylene mesh. The accelerated healing observed in the acellular matrix group is likely attributable to preservation of the native extracellular matrix, which serves as a biologically active scaffold supporting fibroblast migration, collagen deposition, angiogenesis, and constructive tissue remodeling. In addition, extracellular matrices retain endogenous growth factors, including fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and transforming growth factor-β (TGF-β), which collectively promote tissue regeneration and wound healing (Badylak, 2014; Hussey *et al.,* 2018). In contrast, polypropylene mesh primarily

provides mechanical support and may provoke a more pronounced foreign-body inflammatory response, thereby delaying complete tissue integration (Singh *et al.,* 2018).

No hernia recurrence or graft rejection was observed in either treatment group during the one-month postoperative follow-up period. Although one calf treated with polypropylene mesh developed mild wound discharge, the condition resolved with appropriate wound management and antimicrobial therapy. Similar postoperative outcomes have been reported following polypropylene mesh hernioplasty (Malik *et al.,* 2012; Nath *et al.,* 2013) and implantation of biological acellular grafts in bovines and goats (Kumar *et al.,* 2014). These findings further support the clinical safety and biocompatibility of decellularized buffalo ruminal extracellular matrix as a biological scaffold for abdominal wall reconstruction. The present study has several limitations. The sample size was relatively small, which may have limited the statistical power of the analysis. Furthermore, postoperative follow-up was restricted to one month and therefore long-term graft remodeling, biomechanical performance, and recurrence could not be evaluated. Animals were maintained under field conditions at their owners' farms, introducing unavoidable variation in husbandry and postoperative management. Future studies involving larger populations, longer follow-up periods, histological evaluation of implanted grafts, and biomechanical testing are warranted to further validate the long-term clinical efficacy of buffalo ruminal acellular matrix for hernioplasty in cattle.

Conclusion:

Processing buffalo rumen with 1% sodium deoxycholate (SDC) for 72 hours resulted in effective decellularization while preserving the native collagen architecture of the extracellular matrix. Both polypropylene mesh and buffalo ruminal acellular matrix were effective for hernioplasty in cattle; however, the acellular matrix was associated with fewer postoperative complications, faster wound healing, and better tissue integration. Buffalo ruminal acellular matrix was well accepted by the host tissue and proved to be a biocompatible, inexpensive, and readily available alternative to polypropylene mesh. These findings suggest that buffalo ruminal acellular matrix has considerable potential as a biological scaffold for abdominal wall reconstruction in cattle.

Ethics approval and consent to participate

The study protocol was approved by the Institutional Animal Ethics Committee of Bihar Animal Sciences University, Patna (Approval No. IAEC/BVC/2024/15). Written informed consent was obtained from the owners of all animals before enrolment in the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Pankaj Kumar, Rajesh Kumar: Conceptualization, methodology, investigation, formal analysis, writing original draft, Aakanksha, Ramesh Tiwary**:** Investigation, data collection, validation, Arjana kumari, M. Kumar, Sanjiv Kumar**:** Histopathological analysis, methodology, G.D. Singh, Ajeet Kumar: Statistical analysis, data interpretation,Pallav Shekhar, N.K. Singh: Supervision, project administration, writing review and editing. All authors read and approved the final manuscript.

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Table 1: Physiological parameters (mean ± SE) in cattle receiving polypropylene mesh (Group A) and buffalo ruminal ACM (Group B) before surgery and on postoperative days 7 and 15.

Parameters	Groups	Before surgery	7 day	15 Day
Temperature (°F)	A	101.58± 0.18	101.88± 0.14	101.50± 0.16

	B	101.07± 0.30	101.60± 0.19	101.32± 0.32
Heart Rate (per minute)	A	79.17± 2.87	82.00± 3.02	80.33± 2.96
	B	78.17± 2.33	81.17± 2.46	79.17± 2.24
Respiratory Rate (per minute)	A	23.83± 0.60	25.00± 0.45	23.67± 0.56
	B	23.33± 0.76	25.83± 0.60	23.83± 1.14

Mean ± SE differ non-significantly (P<0.05) within the group and between the groups

Table 2: Serum IgG concentration (mean ± SE; [AUTHOR TO ADD UNITS]) in cattle receiving polypropylene mesh (Group A) and buffalo ruminal ACM (Group B) before surgery and on postoperative days 7 and 15.

Group	0 Day	7 Day	15 Day
Group A	15.22±0.25	15.52±0.35	15.70±0.32
Group B	15.31±0.16	15.74±0.24	15.90±0.22

Mean ± SE differ non-significantly (P<0.05) within the group and between the groups

Table 3. Clinical scores for swelling, pain, and exudation in cattle receiving polypropylene mesh (Group A) and buffalo ruminal ACM (Group B) before surgery and on postoperative days 7 and 15.

Parameters	Groups	Before surgery	7 day	15 Day
Swelling Score	A	1.00 ± 0.00^{a}	2.50 ± 0.22^{b}	2.00 ± 0.17^{c}
	B	1.00 ± 0.00^{a}	2.00 ± 0.00^{b}	$1.5\pm0.22^{b, a}$
Degree of Pain	A	1.00 ± 0.00^{a}	3.00 ± 0.21^{b}	2.00 ± 0.21^{c}
	B	1.00 ± 0.00^{a}	2.00 ± 0.17^{b}	1.50 ± 0.22^{c}
Degree of exudation	A	1.00±.00	1.50±.22	1.00±.00
	B	1.00±.00	1.00±.21	1.00±.00

Mean ± SE differ non-significantly (P<0.05) within the group and between the groups

Table 4: Skin-healing time (days; mean ± SE) in cattle receiving polypropylene mesh (Group A) and buffalo ruminal ACM (Group B).

Treatment	**Healing time(days)**

Group A	14.5 ± 0.36^{A}
Group B	11 ± 0.31^{B}

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Fig1a. Washed with tap water



Fig1b. Kept in 0.02% EDTA & 0.1% Amikacin saline solution



Fig1c. Washed with PBS solution

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Fig d. Removal of inner keratinized layer



Fig 1e. Peeled off serosal layer

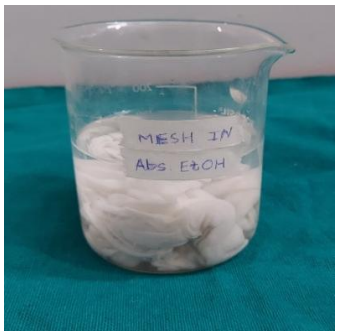


Fig 1f Cellular collagenous biomaterials dipped in 1% SDC solution

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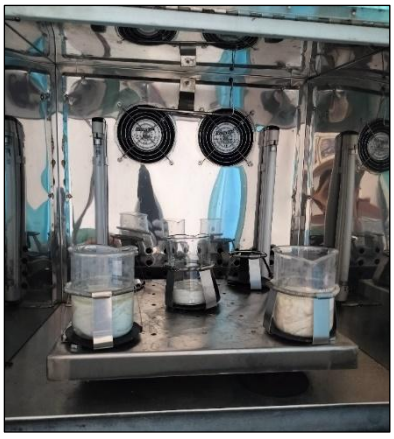


Fig 1g. Sodium deoxycholate (1%) treatment on an orbital shaker



Fig 1h. Tissues treated with absolute ethanol;

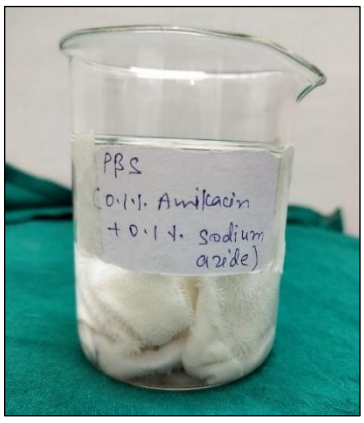
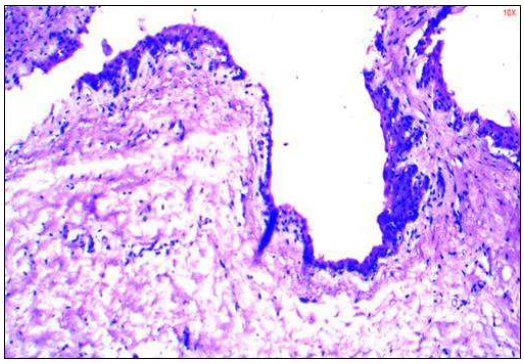


Fig 1i. Stored in PBS solution containing 0.1% amikacin and 0.1% sodium azide at -20°C for clinical use.



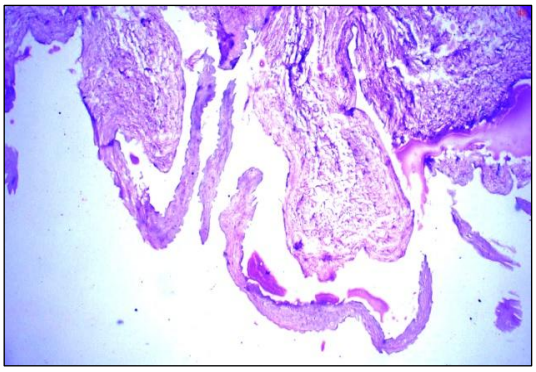


Fig.2a Histological examination before decellurization

Fig.2b Histological examination after decellurization



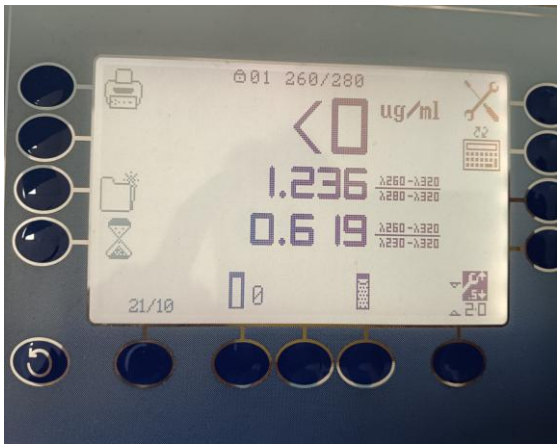
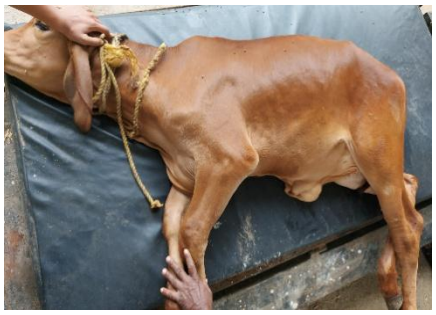


Fig 3a. DNA quantification before decellurization

Fig 3b. DNA quantification after decellurization

Group A (Fig.4 a-f)





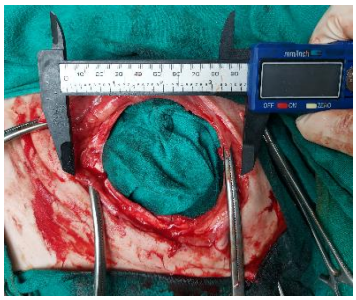
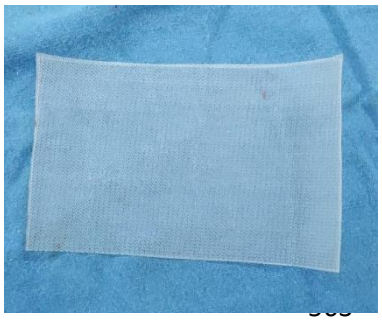


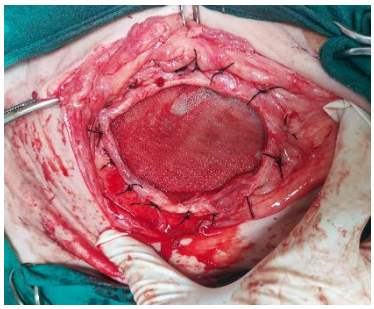
Fig 4a. Clinical case of hernia

Fig 4b. Elliptical incision

Fig 4c. Measurement of hernial ring

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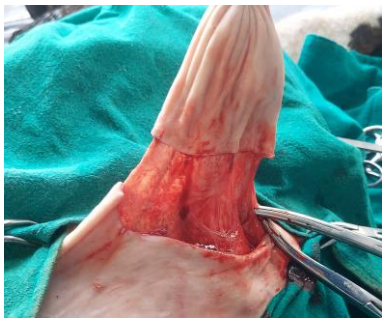


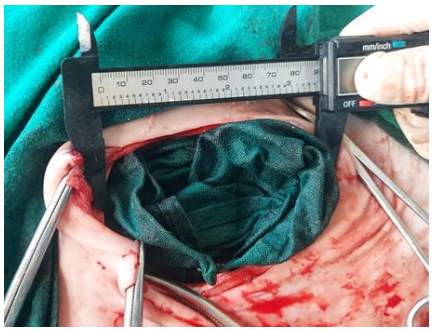


564 Fig 4d. Synthetic propylene mesh Fig.4e closing of hernial ring Fig 4f. Skin suture

565 **Group B (Fig.5 a-**f)







566

567 Fig 5a. Clinical case of hernia Fig 5b. Elliptical incision Fig 5c. Measurement of

568







570 Fig 5d. synthetic propylene mesh Fig.5e closing of hernial ring Fig 5f. Skin suture

571 **Recovery in group A (Fig. 6 a-c)**







572

573 Fig 6a. Complete recovery Fig b. complete recovery Fig c. complete recovery

574 **Recovery in group B (Fig. 7 a-c)**







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576 Fig 7a. Complete recovery Fig 7b. Complete recovery Fig 7c. Complete recovery

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Supplementary Files

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