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Ajit Kumar Singh
Division of Surgery, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Naveen Kumar
Division of Surgery, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Sameer Srivastava
Division of Surgery, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

KP Singh
Division of Surgery, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

S Dey
Division of Surgery, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

PDS Raghuwansi
Division of Surgery, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

AP Singh
Division of Surgery, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Correspondence
Ajit Kumar Singh
Division of Surgery, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Evaluation of the efficacy of bubaline rumen derived extracellular matrix in umbilical hernioplasty in buffalo calves

Ajit Kumar Singh, Naveen Kumar, Sameer Srivastava, KP Singh, S Dey, PDS Raghuwansi and AP Singh

Abstract
Large umbilical hernias of buffalo calves pose a clinical challenge to the surgeons due to their large sized rings and immense distortion of hernial margins. If not managed timely, the deformity gets even worse because of inadequate tissue at the site to enable a satisfactory closure. In such cases, hernioplasty with implantation of synthetic or animal origin biomaterials is required. In the present study, a biomaterial *i.e.* bubaline rumen derived extracellular matrix was prepared and evaluated in umbilical hernioplasty in buffalo calves (n=26). The bubaline rumen derived extracellular matrix allowed a tension free repair of hernias and significant reduction in postoperative pain, length of recovery period and recurrence.

Keywords: Bubaline rumen, Extracellular matrix, Hernioplasty and Buffalo calves

Introduction
Umbilical hernia is the most common abdominal defect in calves. It occurs due genes carried by animal, weakening of abdominal wall caused by abscess or infection, cutting the umbilicus off close to the body wall and excessive traction applied to an oversized fetus during delivery. Small umbilical hernias with smaller ring and reducible contents can be repaired by primary closure but repair of large hernias poses clinical challenge to the surgeons due to large sized hernial rings and immense distortion of hernial margin. Recurrence in these cases occurs frequently and the deformity gets even worse because of inadequate tissue at the site to enable a satisfactory closure (Mohsina *et al.,* 2014) [15]. In such cases, hernioplasty with implantation of synthetic or animal origin biomaterials is indicated (Park and Lakes, 1992) [18]. The use of biomaterials allows a tension free repair of hernias and significant reduction in postoperative pain, length of recovery period and the number of recurrence (Amid, 1997) [2]. Biomaterials must be able to interact with living tissue, immunocompetent, absorbed naturally by the body and eliminated by biological processes or become a permanent part of the surrounding tissue (Kartinof, 2005) [10]. The frequently used synthetic biomaterials like nylon, polypropylene and Teflon mesh are costlier (Marques *et al.,* 1995) [14] and may lead to complications such as fistula formation wound infection, mesh extrusion, seroma and adhesions (Molloy *et al.,* 1991) [16]. Sometimes they do not provided sufficient strength during the degradation process, which is the most frequent complication leading to implant failure and recurrence is infection of the surgical site. Biomaterials of animal origin have shown several advantages over synthetic biomaterials as they are inexpensive, safe and can replace the commonly used highly expensive synthetic biomaterials (Karrouf *et al.,* 2016) [9]. Furthermore, the higher elasticity of biomaterials from animal origin make the site of implantation feel more pliable and increase the easiness in reconstruction of hernia (Abouelnasr *et al.,* 2014) [1]. Biomaterials from animal origin like bovine pericardium and tunica vaginalis have been used in reconstruction of large sized abdominal defect (Karrouf *et al.,* 2016) [9]. Acellular dermal grafts have been used successfully for the reconstruction of hernial defects in rats (Chung *et al.,* 2003) [5], rabbits (Gangwar *et al.,* 2006) [8] and in horses (Kumar *et al.,* 2013a). Although the results of the preclinical animal studies have been promising, studies on the use of bubaline rumen derived extracellular matrix grafts for the repair of hernias in clinical situations are not available. Therefore, the present study was undertaken to evaluate the efficacy of bovine rumen derived extracellular matrix for the repair of the umbilical hernias in clinical cases using inlay technique.

Materials and methods

Harvest and preparation of bovine rumen derived extracellular matrix (R-ECM):

Fresh rumen tissue (Fig. 1) of water buffalo (*Bubalus bubalis*) was procured from the local abattoir and immediately preserved in chilled 1x phosphate buffer saline (PBS), (pH 7.4) solution containing 0.1% amikacin and 0.02% EDTA. Ruminal tissue was thoroughly washed with PBS to remove adhered ruminal contents. Further, ruminal tissue was treated with ionic decellularizing chemical solution under constant agitation (180 rpm/min) at room temperature. Decellularizing chemical solution was changed at every 24 h. Decellularization protocol was optimized based on the principle of maximum removal of cellular contents with minimum damage to basic tissue architecture. The acellularity of the prepared matrix was confirmed microscopically using hematoxylin and eosin staining (Fig. 2-3). After decellularization, the prepared R-ECM (Fig. 4) was washed six times (2 hours each) with sterile PBS on the orbital shaker to remove the residual chemicals and then stored in PBS solution containing 0.1% amikacin solution at -20 ^{0}C until use.

Ethics statement

This study was in full compliance with Institutional Animal Ethics Committee of the Indian Veterinary Research Institute.

Animals

A total of 26 buffalo calves (n=26) aged between 3 and 27 months and weighing 50-115 kg, suffering umbilical hernia with an average diameter of hernial ring ranged from (6-12.5) cm diagnosed at the Veterinary Polyclinic of ICAR-Indian veterinary research institute were included in this study. Physical examination was done to check the status of calves with hernia, a painless, reducible soft swelling was observed at the umbilicus (Fig. 5). Multiple loops of bowel could be palpated traversing the hernial ring. At the time of presentation the animals had normal temperature, respiration, and pulse rates. In each case, before implantation of the prepared R-ECM scaffolds, the owner of the animal was informed and written consent was taken.

Anesthetic considerations:

The calves were sedated using Xylazine HCL @ 0.1 mg/kg for and then circular infiltration analgesia was applied using 2% lidocaine Hcl ***in the region of hernial swelling***. The calves were controlled in lateral or dorsal recumbency as per the convenience.

Surgical technique

The aim of surgery was to reduce the contents and close the abdominal defect so that herniation could not recur. The prosthetic material was thawed and prepared according to the size of the hernial ring. The sites of operation were prepared for aseptic surgery. An elliptical skin incision was performed to expose the hernial sac which spanned the length of the hernia and extended 2 cm beyond the cranial and caudal margins of the hernial ring. Forcipressure was used to control subcutaneous hemorrhage during the operation. The hernial sac was dissected from the overlying skin, and dissection was continued laterally to expose the hernial ring and the external sheath of the rectus abdominis muscle. The fibrous adhesions were dissected and the devitalized tissues were removed and the hernial sac was opened and the contents were reduced. Underlay technique was used for the implantation of R-ECM. The R-ECM exceeding the defect by 1.5 cm in all directions was adjusted for adequate closure of the hernial ring. An appropriately sized piece of R-ECM with a pre-placed horizontal mattress suture of number 2 surgical black braided silk with long ends, attached to its cranial, caudal, and mid-lateral edges, was introduced into the abdomen through the hernial ring. The R-ECM was oriented within the abdomen and the suture ends retrieved using a non traumatic needle. Each of the sutures was tied, with the knots resting on the external sheath of the rectus abdominis muscle, thus temporarily securing the R-ECM to the internal sheath of the rectus abdominis muscle (Fig. 6-7). While the R-ECM was being implanted, the surgical site was lavaged periodically with physiological saline, containing 0.1 % amikacin. Excess skin was excised off, and the subcutaneous tissues were closed using number 2-0 polyglactin 910 placed in a simple continuous suture pattern. The skin incision was then closed using number 2 braided silk in a horizontal mattress suture pattern (Fig. 8).

Post operative care

Postoperatively, the animals were administered with amoxicillin-cloxacillin antibiotic combination (500 mg total dose in buffalo calves) by intramuscular route for 5 days and analgesic meloxicam (0.5 mg/Kg) for 3 days. The skin wounds were dressed routinely with 0.1 % povidone iodine (Betadine-topical antiseptic) for one week. Skin sutures were removed on postoperative day 12. The operated animals were followed either by regular visits or calling the owner during one year post surgery.

SDS-PAGE analysis

From each calf, 5 ml blood was collected from the jugular vein before implantation and on days 15 and 30 post-implantation. Serum was harvested and stored at -20 °C until use**.** Serum samples were mixed with equal volumes of sample buffer (1×sample buffer: 62.5 mM Tris, 2 % SDS, 10 % glycerol, 0.0125 % bromophenol blue, pH 6.8). Samples were heat-denatured for 5 minutes at 90°C and were subjected to SDS-PAGE analysis, as described by Laemmli (Laemmli, 1970), using 4 % stacking gels and 10 % resolving gels in a Mini Protean II unit (Bio- Rad Laboratories, Hercules, CA, USA) at 50 mA/gel. After fractionation, the gel was stained in a staining solution (50 % methanol, 10 % glacial acetic acid, 0.25 % Coomassie Brilliant Blue R-250) for 10 minutes. Next, the gel was destained in a solution containing 250 ml methanol, 100 ml acetic acid, and 650 ml distilled water, until protein fractions appeared clear.

Results

Harvest and preparation of bovine rumen derived extracellular matrix (R-ECM)

A histological photograph of the rumen, with no treatment, showed cellularity (Fig. 2). The ruminal tissue decellularized by ionic decellularizing chemical solution showed thin, loose arranged collagen fibers with very high porosity. No cellular debris was evident. The collagen fibers were loosely arranged as compared to the native tissue. Histological analysis of the acellular matrix showed that the cells had been completely removed and the collagen fibers were arranged order (Fig. 3).

Gross and clinical observations

Umbilical hernias were diagnosed in 26 buffalo calves. The hernial swellings were reducible in animals presented with congenital hernias while in the acquired ones there were varying degrees of irreducibility and adhesions. Wound dehiscence and hernia recurrence were not recorded in any of the cases. All animals showed normal behavior and growth rate during the 12 months follow up and the hernia repaired without any complications. The hernial ring size varied between 6 and 12.5 cm in diameter. Hernial contents were variable and consisted of the omentum or small intestine, and they were located within the hernial sac. The fibrosed edges of the hernial rings were refreshed before implantation of the prosthetic materials in order to facilitate a better mesh incorporation with the host tissue (Attinger *et al.*, 2000) [3]. Temperature, pulse and respiration rates were increased during the first 48 h post-implantation. These signs gradually subsided, and became normal within 1 week. Signs of mild pain on palpation were observed in all the animals during the first 3-4 post implantation days. These signs were satisfactorily managed with analgesics. Mild inflammatory oedema developed during the first week after surgery. The edema reduced gradually, until complete resolution in all cases between 1 and 2 weeks after surgery. The use of non-absorbable silk suture materials provided no complications. At the end of the first post-implantation week, a thick and slightly hard mass resembling the size of implanted R-ECM was palpated at the site of the hernioplasty. The thick and hard mass of tissue at the site of implantation was palpable until the end of the second post-implantation week. These masses subsided by the end of the third post-implantation week. After the fifth post-implantation week the masses become thinner and fibrous, and were difficult to feel on palpation. The umbilical region looked normal on inspection. The skin over the R-ECM adhered firmly to the underlying tissue and was healthy and non-painful on firm and deep palpation. All the animals recovered uneventfully. Clinical signs like wound dehiscence, infections, or hernial recurrence were not recorded in any of the cases. All the animals were observed up to 12 months after hernioplasty and the R-ECM was found to be suitable for hernial repair.

SDS-PAGE analysis

The serum protein distribution pattern on post-implantation day 15 was found different in all samples as compared to day 0 and became normal on post-implantation day 30 (Fig. 10).

Fig 1: Rumen tissue of buffalo origin

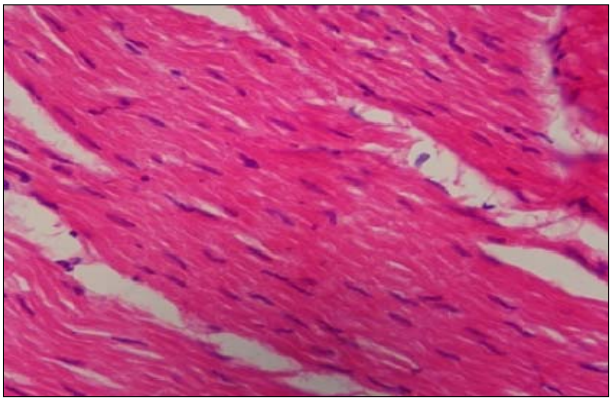

Fig 2: Native bubaline rumen matrix (H&E, 200X)

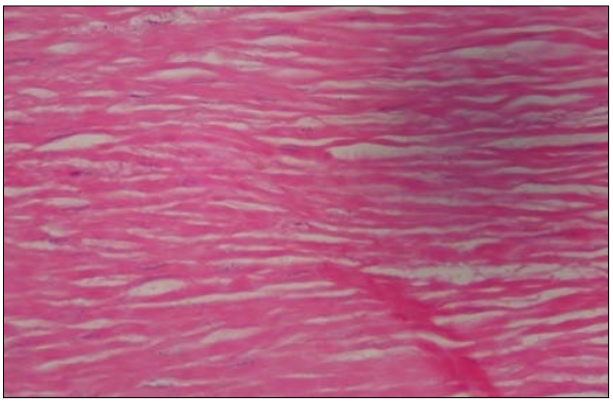

Fig 3: Decellurized bubaline rumen matrix (H&E, 200X)

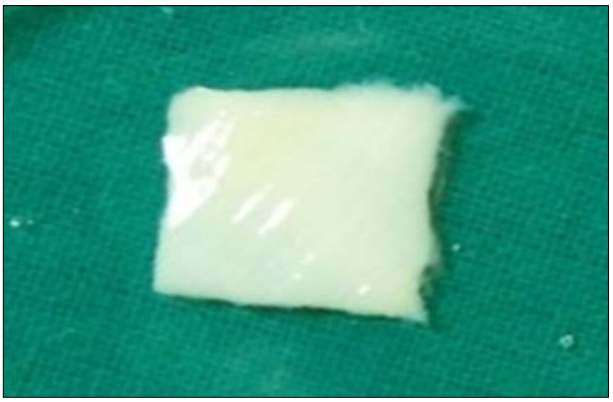

Fig 4: Decellurized bubaline rumen matrix

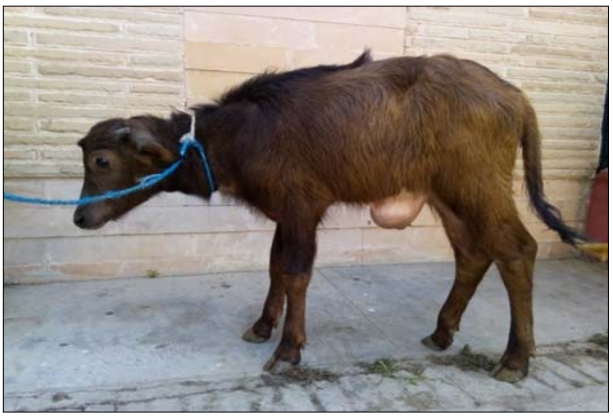

Fig 5: Buffalo calf presented with umbilical hernia

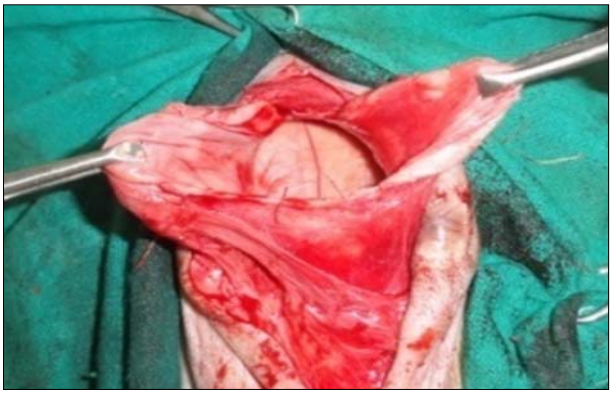

Fig 6: Hernial ring observed intraoperatively

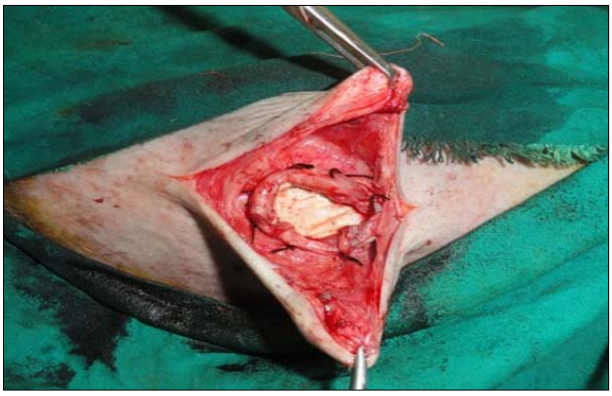

Fig 7: Repair of umbilical hernia with bubaline rumen extracellular matrix

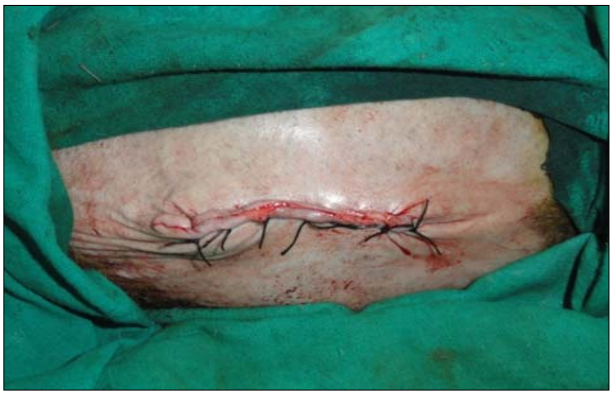

Fig 8: Repaired hernial defect and skin sutures.

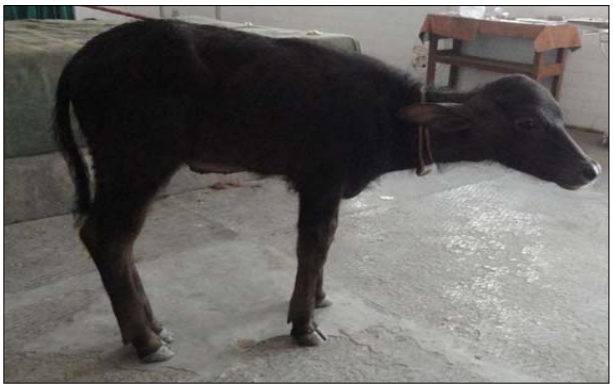

Fig 9: Animal after correction of hernia on the day of suture removal.

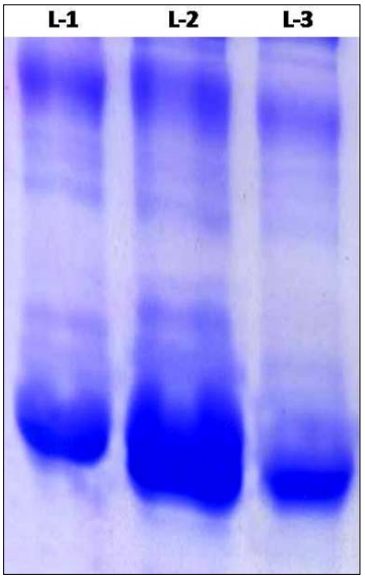

Fig 10: Sodium dodecyl sulfate polyacrylamide gel electrophoresis of calf serum collected at intervals after surgical implantation of R-ECM to repair a umbilical hernia. Lane 1, day 0; lane 2, day 15; lane 3, day 30.

Discussion

Decellularization of the biomaterials is most important factor for the implantation without immergence of adverse inflammatory and immune responses. In the present study, rumen of the buffalo origin was decellularized using an ionic biological detergent solution and results demonstrated that decellularized R-ECM retained the distinctive, natural, three-dimensional collagen structures. After decellularization rumen matrices were used for umbilical hernioplasty in buffalo calves. Further, the results of this study demonstrated the uncomplicated healing of the repaired area, without hernial recurrence or rejection of R-ECM in any of the calves. Similar findings were reported in other studies in which acellular dermal matrix had been successfully used for the reconstruction of hernias in goats (Gangwar *et al.*, 2004) [7] and cellular aortic matrix was used for the reconstruction of abdominal hernias in calves (Kumar *et al.*, 2013b). Decellularized collagenous materials of animal origin (extracellular matrices) are preferred in hernial repair because of their inherent low antigenecity and ability to integrate with surrounding tissue. Furthermore, these matrices are biocompatible, slowly degraded upon implantation and are replaced and remodeled by the extracellular matrix proteins, synthesized and secreted by ingrowing host cells (Pariente *et al.*, 2001) [17]. In the present study, underlay technique of implantation was used which is considered to be the best method due to the position of the implant behind the rectus muscles where the force of abdominal pressure holds the prosthesis against the deep surface of the abdominal muscle wall. Other techniques have a disadvantage of lacking fixation of the implant by intra-abdominal pressure due to minimal surface area of contact between the implant and the adjacent tissue which leads to high hernial recurrence rates.

However, in the preset study, no post-operative complications were observed after implantation of R-ECM in any calves during the observation period after the hernioplasty. Similar results were reported in bubaline (Kumar *et al.*, 2014) studies. The Decellularized collagen matrices have appropriate mechanical properties that support cell ingrowth and induce

interaction with the host cells that results in functional tissues tissue regeneration (Badylak, 2005) [4]. SDS-PAGE analysis of the serum of buffalo calves with R-ECM matrices showed changed protein distribution patterns on day 15 as compared to days 0 and 30. This might be attributed to inflammatory reactions in response to surgical trauma and the R-ECM implantation. These changed protein distribution patterns might be due to the increased production of plasma proteins by the liver during inflammation, fibroblastic proliferation, neovascularization, and collagen synthesis in the matrix (Schreiber *et al.*, 1982) [20].

Conclusion

The promising results were obtained from the clinical use of R-ECM in terms of body tolerance, availability and its economic effectiveness. The findings of the present study suggest that R-ECM produced by decellularization may be used safely for umbilical hernioplasty in buffalo calves and is at the forefront with respect to the conventional suture repair techniques. Although, further research is warranted in order to verify the immunogenic properties of this biomaterial.

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Conflict of interest

The authors declare that they have no conflict of interest.

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